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Fine-Scale Biogeographical Boundary Delineation and Sub-population Resolution in the *Symbiodinium thermophilum* Coral Symbiont Group From the Persian/Arabian Gulf and Gulf of Oman

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The adaptation of tropical coral communities to the world's hottest sea, the Persian/Arabian Gulf (PAG), has recently been associated with ecological selection acting on a group of coral-associated algal symbionts, the *Symbiodinium thermophilum* group. Previous studies have shown that considerable genetic diversity exists within the group and that group members found within the PAG are significantly differentiated from those found externally, in the Gulf of Oman and wider waters. However, little is known about this genetic diversity. As an initial step towards understanding whether this diversity could represent niche adapted, selectable populations within the *S. thermophilum* group that may act as natural sources of stress tolerant associations to Indo-Pacific reefs, we investigate whether the diversity is structured between populations and where the location of the internal-external genetic partition lies. We use regions of the nuclear ribosomal DNA (ITS1-5.8S-ITS2) and chloroplastic *psbA* gene (non-coding region) from >100 *S. thermophilum* group-harbouring *Porites* spp. (*P. lobata, P. lutea, and P. harrisoni*) sampled across steep temperature and salinity gradients to conduct analyses of variance and create maximum parsimony networks to assess genetic structure and (dis)similarity within and between populations of *S. thermophilum* found within the PAG and externally in the Gulf of Oman. Our analyses resolve a sharp genetic boundary between *Symbiodinium* populations in the western Strait of Hormuz and identify significant genetic structure between populations with as little as 20 km between them demonstrating that differentiation between populations is likely due to factors other

than limited connectivity. Further, we hypothesize that genotypes identified outside of the PAG in the Gulf of Oman existing in near-oceanic salinities, yet thermally challenging waters, putatively represent candidates for stress-tolerant symbionts that could act as natural seed populations of stress tolerant genotypes to the wider indo-Pacific.

Keywords: adaptation, biogeography, climate change, coral reefs, ITS2, Persian/Arabian Gulf, salinity, thermal tolerance

INTRODUCTION

The Persian/Arabian Gulf (PAG) is a thermally extreme and hypersaline body of water with temperatures regularly exceeding 34°C during summer in its southern and hottest part (Hume et al., 2013; Shuail et al., 2016; **Figure 1**, Figure S1), temperatures that prove fatal to most tropical corals (Coles and Riegl, 2013; Hume et al., 2013). Yet, thriving coral communities are found along its coastline (Coles, 2003). These exceptional coral communities are gaining attention as model systems to inform on how corals globally might adapt to increasing sea surface temperatures (SSTs; Hume et al., 2013; Burt et al., 2014; D'Angelo et al., 2015). Such increases are causing more frequent and severe coral bleaching events (Logan et al., 2014) during which the obligate symbiotic relationship between coral and algal symbiont (genus *Symbiodinium*) breaks down leading to symbiont loss, and if recovery is not timely, death of the host (Hughes et al., 2003). Temperature thresholds at which these bleaching events occur are dependent on host and symbiont genotypes but typically lie 1°C above the average maximum summer temperature for a reef (Goreau and Hayes, 1994; Shuail et al., 2016).

Given that the maximum mean temperature of the majority of coral reefs is ≤30°C (Hume et al., 2013), the ability of corals from the southern PAG to withstand temperatures in excess of 35°C without bleaching is truly exceptional (Coles and Riegl, 2013; Hume et al., 2013; Shuail et al., 2016). This exceptional thermal tolerance may be in part due to their algal associations given that hosting certain *Symbiodinium* types may confer an increased thermal tolerance to the coral host (LaJeunesse et al., 2010; Silverstein et al., 2015). Specifically, corals of the southern PAG predominantly form year-round associations with the recently described *Symbiodinium thermophilum* (Hume et al., 2015). The prevalence of this symbiont within PAG corals suggests it is at least partially responsible for their thermotolerance (D'Angelo et al., 2015; Hume et al., 2015, 2016).

S. thermophilum was defined using four genetic markers including specific sequences of the mitochondrial *cytochrome b* (*cob*; K234522) and chloroplastic ribosomal 23S (cp23S; KP234523) (Hume et al., 2015). In addition, genetic characteristics of the nrDNA second internal transcribed spacer regions (ITS2) were used. This non-coding region is multicopy in character with multiple genetic variants of different but closely related sequences present in an individual *Symbiodinium* genome (Thornhill et al., 2007; Arif et al., 2014). Within *S. thermophilum,* an 8bp duplication insert (called *S. thermo.*-indel) in a minority proportion of intragenomic ITS2 variants (∼15%) in an otherwise C3 type sequence (KP234524 & KM487748) was described and its presence has been used as an effective means of screening for this symbiont species (D'Angelo et al., 2015; Hume et al., 2016). The last genetic marker used in the description, the highly variable chloroplastic *psbA* non-coding region (*psbA^{ncr}*), resolves at a subspecies level in the *Symbiodinium* genus (Thornhill et al., 2014). As such, multiple haplotypes were recovered from the *S. thermophilum* symbionts (Hume et al., 2015). Critically, all sequences resolved within a monophyletic grouping separated from other ITS2 C3 type symbionts by considerable genetic distance (Hume et al., 2015). Since the formal description of *S. thermophilum*, additional samples containing the characteristic ITS2 *S. thermo.*-indel have been genotyped using the psbA^{ncr} (D'Angelo et al., 2015; Hume et al., 2016). These recovered psbA^{ncr} sequences showed a considerably larger diversity than the diversity seen in the original *sensu strictu S. thermophilum* definition. Hence, these symbionts are identified as members of the "*S. thermophilum* group" (D'Angelo et al., 2015).

The extreme conditions and exceptional coral-algal associations found in the PAG are a product of the regions unique geography. The PAG is connected to the adjacent Gulf of Oman by the ∼40 km wide Strait of Hormuz that navigates the Musandam Peninsula and is fed by a dominant surface inflow of near-oceanic salinity water (**Figure 1**). Due to high evaporation rates and minimal freshwater input, the PAG is characterised by a net inflow with only a minor deep water outflow of heavier, more saline water through the Strait (Yao and Johns, 2010). This surface inflow is strongest in summer month with the cooler, lower salinity surface water moving northwest towards Qatar or south into the main basin forming an overall cyclonic circulation in the southern Gulf (Yao and Johns, 2010). Despite this lower salinity inflow, salinities in the southern PAG remain high year round (>41.5) resulting in a gradual decrease in salinity from the southern PAG towards the tip of the Musandam Peninsula into the Gulf of Oman (D'Angelo et al., 2015; **Figure 1a**). Temperature gradients along the same coast are seasonally dependent. In summer, the inflow has a cooling effect and the resultant temperature gradient decreases from the southern PAG to the Gulf of Oman whereas in winter month the southern PAG is considerably cooler (winter averages ∼21°C) than the inflowing water (∼24°C; **Figures 1a,b**).

Although cooler than the PAG, the coral-harbouring waters immediately external to it, in the Gulf of Oman, represent an environment with salinities closer to those of the wider Indo-Pacific but with thermal regimes still greater than are found in the majority of coral-containing waters globally. In addition to the relatively large annual thermal ranges of the region (max ∼31°C, min ∼23°C), coral communities in these waters may experience acute hypothermal shocks due to local upwelling events (Coles,

2003). As such the coral communities of these waters should also be considered extraordinarily stress tolerant.

Recently, the abundance of *S. thermophilum* group symbionts was investigated at a range of sites spanning from within the PAG to Muscat, in the neighbouring Gulf of Oman. Along the southern coast of the PAG from Dalma to Ras Al-Khaimah, the association of *Porties* spp. corals with members of the *S. thermophilum* group was complete (100% of associations) despite considerable changes in salinity and temperature (D'Angelo et al., 2015; Hume et al., 2015, 2016). However, immediately external to the PAG off the coast of the Musandam Peninsula in the Strait of Hormuz an increasing number of the *Porites* spp. corals were found in association with symbiont types other than *S. thermophilum*, namely ITS2 type C15 and *S. trenchii* (ITS2 type D1a or D1-4). This decrease in *S. thermophilum* group associations continues with increasing distance from the PAG until associations are almost undetectable in reefs off Muscat. Interestingly, this low level abundance of *S. thermophilum* group associations has been documented as widely distributed as the northern Red Sea (Hume et al., 2016).

Phylogenetic analyses conducted as part of these studies using the psbA^{ncr} revealed a greater genetic diversity of *S. thermophilum* group symbionts external to the PAG than within (Hume et al., 2016). Investigation into the age of this diversity (>several million years), and the group members themselves (~13 Mya), demonstrated them to be considerably older than the age of the PAG (~12.5 kya; Purkis et al., 2010; D'Angelo et al., 2015; Hume et al., 2016). These findings led to the hypothesis that positive selection for thermal and salinity tolerance acting on the diversity of group members found external to the PAG, facilitated the colonisation of the physically extreme PAG by a subset of Indo-Pacific corals. Other ecological mechanisms including founder or bottleneck effects were also considered in the explanation of the group's prevalence and relative genetic homogeneity in the PAG. However, the PAG's dominant inflow (Yao and Johns, 2010), the presence of several other thermally tolerant symbiont types within the PAG (Baker et al., 2004; LaJeunesse et al., 2014; Hume et al., 2015) and the fact that *Symbiodinium* can disperse rapidly over large distances (Baird et al., 2009; Pettay et al., 2015) make these mechanisms unlikely.

In the same way that members of the *S. thermophilum* group underwent ecological selection to proliferate in the extreme conditions of the PAG, these members may be selected to enrich reefs in the Gulf of Oman and wider Indo-Pacific with tolerant symbionts. Such an enrichment may afford these reefs greater resilience to perturbations that may arise from predicted increases in SSTs. However, to better understand the capacity for such ecological selection to occur we must first better understand the genetic diversity that exists within the *S. thermophilum* group to know which populations are available for selection. Here, we specifically investigate whether the genetic diversity of the *S. thermophilum* group is structured between populations as a first indication towards whether niche adapted, selectable populations exist. To assess for genetic structure within the group, we use a novel approach analysing the diversity of intragenomic variants in the ITS1-5.8S-ITS2 region of the nrDNA as well as the psbA^{ncr} to compute ϕst indices and create maximum parsimony networks to identify population groupings and assess genetic (dis)similarity within and between individual populations. Our findings identify significant genetic differentiation between *S. thermophilum* group populations and further resolve the coral-symbiont biogeographical boundary in the Strait of Hormuz.

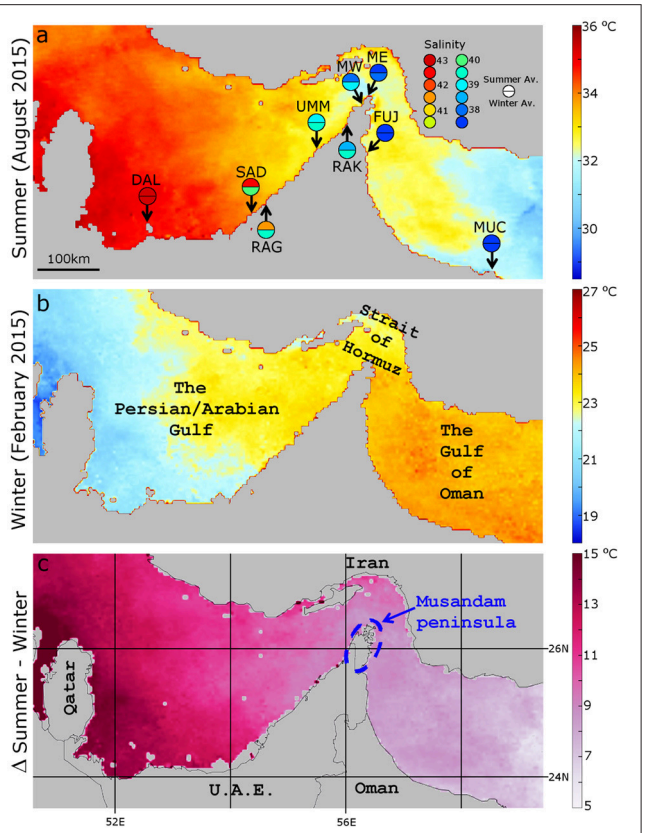

FIGURE 1 | Sampling sites across temperature and salinity gradients of the Persian/Arabian Gulf, Strait of Hormuz, and Gulf of Oman. **(a)** Monthly average (August 2015; warmest month) sea surface temperatures (SSTs) representative of a summer thermal profile. Sampling locations are denoted according to three or two letter codes: DAL, Dalma Island; SAD, Saadiyat reef; RAG, Ras Ghanada; UMM, Umm Al-Quwain; RAK, Ras Al-Khaimah; MW, Musandam peninsula West; ME, Musandam peninsula East; FUJ, Fujairah; & MUC, Muscat. Modelled seasonal (summer, J-A-S; winter, J-F-M) salinities from Yao and Johns (2010) for sampling sites are denoted. **(b)** Monthly average (February 2015; coolest month) SSTs representative of a winter thermal profile. **(c)** Difference in monthly average SSTs between **(a** and **b)** representative of a regular seasonal temperature range. Remotely sensed SST data of **(a,b)** are Aqua MODIS Sea Surface Temperature (11 μ daytime), monthly, 4 km resolution accessed through the level 3 data browser of OceanColor Web (oceancolor.gsfc.nasa.gov/cgi/l3). netCDF libraries were viewed and images exported using Panoply 4.3 (gis.nasa.gov/tools/panoply). Panel **(c)** was created using Panoply's combine plot function subtracting the winter profile from the summer.

MATERIALS AND METHODS

Sample Collection and Genotyping by nrDNA and psbA^{ncr}

S. thermophilum group-containing coral samples of *P. lobata*, *P. lutea,* and *P. harrisoni* were collected and underwent nrDNA (ITS1-5.8S-ITS2) and psbA^{ncr} genotyping as part of D'Angelo et al. (2015). Field identification relied on local experts to

differentiate between *Porites* spp. The relatively low diversity of *Porites* spp. found in the PAG and Gulf of Oman simplified the identification (Bauman et al., 2013). Fragments of *P. lobata* and *P. lutea* were bleached in sodium hypochlorite solution and inspected under a microscope (MZ10, Leica). Analysis of the corallite structure (Veron, 2000) confirmed the field identification of these samples. The *P. lobata* samples were distinguishable from *P. lutea* by the lack of a protruding ring of five tall pali, and from *P. solida* by the lack of a flattened columella. However, it should be noted that *Porites* spp. corals are notoriously difficult to identify from skeletal morphology alone (Forsman et al., 2009). *P. harrisoni* was differentiated from *P. lobata* and *P. lutea* by its gross morphology being more columnar in form. Unfortunately for samples collected at Ras Al Khaimah (RAK) and the one sample collected in Muscat, field identification by an expert was not available. Rather than omit these samples from the study they were identified conservatively as *Porites* spp.

In summary, corals were sampled at between 2 and 7 m in September 2012 and March 2013 from sites within the PAG, the Strait of Hormuz and the Gulf of Oman (**Figure 1**; Figure S2). Throughout this study two or three character length codes (e.g., DAL—Dalma) will be used to refer to sampling sites as denoted in **Figure 1a**. Genomic DNA extractions were performed using a CTAB protocol and the PCR amplification of the nrDNA and psbA^{ncr} markers was achieved using the SYM_VAR_FWD/SYM_VAR_REV and psbAFor_1/psbA_Rev_1 primer pairs. nrDNA amplicons were cloned using a StrataClone PCR cloning kit before sequencing (universal primer T7) whilst psbA^{ncr} amplicons were directly sequenced using the internal primer psbA_int_Fwd. Sequences were aligned in MEGA 6 (Tamura et al., 2013) with nrDNA sequences' unsupported nucleotides (nucleotides divergent from the alignment consensus that were not found in more than one PCR amplicon library) reverted to the consensus to minimise incorporation of genetic diversity generated through PCR error. A full list of psbA^{ncr} sequences used in this study may be found in Table S1, Supplementary Material (106 sequences). The number of colonies nrDNA sequenced as well as the average sequencing depth can be found in Table S2, Supplementary Material. A multiple sequence alignment of the ITS1-5.8S-ITS2 nrDNA sequences used in this study has been submitted to the Dryad Repository (675 sequences).

Assessment of Host-Effect on *Symbiodinium* Sequence

Porites spp. abundance between sites was heterogeneous and uneven sampling across sites was unavoidable (Figure S2). To assess whether any signature of genetic structure found in this study might be an artefact caused by host species distribution due to different species of *Porites* hosting different *Symbiodinium* types, rather than other factors (e.g., environmental conditions), cluster-based correlation analysis between *Symbiodinium* sequence recovered and host species was conducted. For both markers, genetic distances between all unique sequences were calculated in MOTHUR 1.36.1 (Schloss et al., 2009) using the "pairwise.seqs" function. Column-based distances were converted to matrices using a custom PYTHON script before being plotted in two dimensional space through multidimensional scaling using the R package bios2mds. K-means clustering was conducted in R (3 clusters; one per species) using the function "kmeans" with the initial seed set to 20 and with an nstart equalling 50.

If host species had no effect on sequence recovered, sequences should be randomly distributed between the three groups irrespective of host. A two-way table (species, cluster) and chi-square test were employed to test if there was a significant difference between the distribution of sequences amongst the three k-means determined clusters according to either: observed distribution by species, or expected random distribution disregarding species. The counts of sequences from each of the three identified species (unidentified *Porites* sp. were discounted from the analysis) associated within each of the determined clusters were calculated alongside frequencies representative of the null hypothesis of no association between species and cluster. A chi-squared test was performed and results were compared to critical values at 4 degrees of freedom (3 species, 3 clusters).

Inclusion of Its1 and 5.8S nrDNA Regions in Genetic Analyses

Biogeographical and phylogenetic analyses of *Symbiodinium* populations often use only one region of the nrDNA, commonly the ITS2. To maximise possible inferences on genetic structure and investigate possible improvements in phylogenetic resolution within the *S. thermophilum* group the ITS1 and 5.8S regions of the nrDNA were included in all analyses in this study.

Population Genetic Structure Inference by Analysis of Variance

To offer an improved resolution of the previously described biogeographic boundary (located in the Strait of Hormuz) to *S. thermophilum* group-containing coral populations (D'Angelo et al., 2015; Hume et al., 2016) three group configurations were tested for variance. Specifically, two groups of populations were defined as "internal" and "external" (approximate to PAG and Gulf of Oman populations). Within these groupings the sites, Dalma, Saadiyat, Ras Ghanada, Umm Al-Quwain, and Ras Al-Khaimah were always placed in the internal groups whilst Fujairah and Muscat were always in the external group. The three permutations of these groups with the addition or lack of one or both of the Musandam West or Musandam East sites were then tested for among group variance in WinArl 3.5.1.3 (Excoffier and Lischer, 2010) using the complete ITS1-5.8S-ITS2 region of the nrDNA (611 bp) and the partial psbA^{ncr} (495 bp). By this means, the two populations in proximity to the Strait with the greatest haplotypic difference may be identified. These two populations will be the most easterly and westerly population in the internal and external groupings that have largest among group variance.

In addition to the calculation of among groups variance (Va), among populations within groups (Vb), and within populations variances (Vc) were calculated along with their associated fixation

indices (φ_{CT}, $\varphi_{SC,}$ and φ_{ST}, respectively). In this analytical scenario, the among group variance (Va) represents the variance between individual gene sequences that are grouped as belonging to either an internal or external site. When calculating the among group variance, the population to which a sequence belongs is not taken into account. That is, individual gene comparisons are only constrained by which group they belong to. The among populations within groups variance (Vb) represents the average variance found amongst populations that belong to the same group. Here, population comparisons are nested hierarchically within the groups so that only gene sequences found within populations that are found within the same groups will be compared. Within population variance (Vc) represents the variance found between sequences for each population. Here, which group the population belongs to is not taken into account and one Vc value is calculated for each population (i.e., sequences from different populations are not compared). For each of the comparison types listed above, the respective fixation indices are calculated using the same grouping constraints. These fixation indices represent a measure of population differentiation due to genetic structure (i.e., gene flow isolation) with a value closer to 1 representing greater isolation and therefore structure.

Population Genetic Structure Inference by Population Pairwise Distance

To test for genetic dissimilarity between populations, population pairwise genetic distances were also calculated in WINARL in the form of pairwise ϕst values. A null distribution of pairwise ϕst under the hypothesis of no difference between the populations is obtained by permuting haplotypes between populations as part of WINARL's "compute pairwise difference" function. The outputted probability values represent the proportion of permutations leading to a ϕst value larger or equal to the observed one (Excoffier and Lischer, 2010). Whilst one psbA^{ncr} sequence per sample was used in the pairwise analyses, further considerations were taken in the appropriate use of the nrDNA sequences. Whilst not a traditional genetic marker used in population biology, the nrDNA's heritability and complex character states (in the form of multiple intagenomic variants due to the nrDNA's multicopy character) enable its use as such a marker (Sunnucks, 2000). In fact, given that approximations to ecologically relevant species level descriptions in *Symbiodinium* are commonly made according to the sequence and predominance of intragenomic variants of the nrDNA (LaJeunesse et al., 2010; Arif et al., 2014), quantifying dissimilarity between individuals and populations according to these intragenomic abundances is likely an effective means of identifying ecologically significant units. Thus, to ensure the consideration of all available nrDNA intragenomic variants and their frequencies within individual corals and populations each sequence was treated as an individual rather than creating a single "inclusivity consensus sequence" (Barshis et al., 2010) by incorporating IUPAC ambiguity nucleotides representative of polymorphisms detected within clone libraries into the consensus sequence from that individual; a method that cannot take into consideration the frequency of polymorphism-containing intragenomic variants as previously discussed by Barshis et al. (2010). Additionally, the treatment of intragenomic variants as individuals enables the incorporation of samples that are represented by a considerable number of clones but from a low number of individuals (e.g., Muscat site; only one sample with 14 nrDNA clones). Whilst such a low sampling is not ideal, further sampling is limited by the rarity of *S. thermophilum* group external to the PAG. The single Muscat sample is potentially highly informative given its rarity and the considerable distance to its next nearest sampling site (Fujairah 363 km). As such Muscat was included in the pairwise ϕst analyses using the nrDNA marker but excluded using the psbA^{ncr} marker due to the direct sequencing approach used (i.e., returning only a single sequence). Pairwise differences were considered significant at $P < 0.05$.

To test for significant correlation between distances and pairwise genetic dissimilarities between populations a Mantel test (test of correlation between two matrices) was additionally calculated using WINARL (the distance matrix may be found in Table S3).

Creation of Maximum Parsimony Networks

Maximum parsimony networks were created using the ITS2 and the complete ITS1-5.8S-ITS2 region of the nrDNA and the partial psbA^{ncr} using TCS (Clement et al., 2000). For the nrDNA networks, sequences with frequency of 1 were not plotted. TCSbu (Múrias dos Santos et al., 2015) was used to aesthetically arrange the network before further manual manipulation for aesthetic purposes only. To account for extreme variability in the psbA^{ncr}, sequences were clustered to a distance of 0.04 (empirically determined to cluster groups of sequences separated by large mutational distances) using the *cluster* command and average neighbour algorithm in MOTHUR 1.36.1 (Schloss et al., 2009).

Script Access

Access to R and PYTHON scripts used in this study may be found in Code S1 in Supplementary Material.

RESULTS

Assessment of Host-Effect on *Symbiodinium* Sequence

To test for an effect of host species on *Symbiodinium* sequence recovered, a χ^2 analysis was conducted to assess whether sequences were distributed independent of host species amongst three k-means clustered groupings on a 2D plot of sequence pairwise distances. A non-significant interaction was found between species and cluster [$\chi^2_{(4)} = 5.94, p > 0.05$] for the nrDNA regions. In contrast, a significant interaction between species and cluster was identified [$\chi^2_{(4)} = 21.99$, $p < 0.05$] for the psbA^{ncr} marker. However, 80.4% of the divergence from expected was attributable to a single species, *P. harrisoni,* with a single cluster representing 55.9% of this variance.

TABLE 1 | Pairwise population ϕst *P*-values.

	Internal sites					External sites			
	DAL	**SAD**	**RAG**	**UMM**	**RAK**	**MW**	**ME**	**FUJ**	**MUC**
DAL		0.000	0.000	0.000	0.000	0.000	0.000	0.027	
SAD	0.180		0.612	0.198	0.432	0.018	0.000	0.009	
RAG	0.207	0.000		0.198	0.216	0.045	0.000	0.000	
UMM	0.063	0.018	0.702		0.838	0.000	0.000	0.018	
RAK	0.225	0.126	0.333	0.297		0.000	0.000	0.000	
MW	0.036	0.000	0.279	0.649	0.189		0.000	0.000	
ME	0.000	0.000	0.000	0.000	0.000	0.000		0.216	
FUJ	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
MUC	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.072	

Significant difference at $P < 0.05$ is denoted by an underlined value. Top right = psbA^{ncr}; bottom left = nrDNA.

Population Genetic Structure Inference

To determine into which population groupings (internal or external) the Musandam sites were best allocated to maximise between group variance, multiple combinations were assessed. Allocation of the Musandam West and Musandam East populations to the internal and external groups, respectively, were supported by the highest "among group" (Va and ϕct) and lowest "among population within group" (Vb and ϕsc) variances and fixation indices (Va = 11%, 29%, ϕct = 0.108, 0.294; Vb = 3%, 7%, ϕsc = 0.039, 0.097; nrDNA and psbA^{ncr}, respectively). Pairwise ϕst analysis of the 9 populations corroborated the strong genetic dissimilarity between the external (ME, FUJ, and MUC; **Table 1**) and internal (all other populations) populations in both genetic markers.

For both markers, "among individuals within populations" (population = site) accounted for the majority of variance (86 and 63% for the nrDNA and psbA^{ncr}, respectively) whilst relatively low "among populations/within groups" (3 and 7%) and relatively high "among group" (11 and 29%; group = internal or external) variances supported the groupings of internal and external populations as characterised by the associated high and low fixation indices (ϕct = 0.108, 0.294; ϕsc = 0.039, 0.097). Inclusion of MW within the external grouping did not increase "among group" variance (5 vs. 11 and 9 vs. 29% for the nrDNA and psbA^{ncr} markers, respectively).

Despite the well-supported genetic disparity between internal and external populations and relatively low ϕsc indices, some populations within each group were found to have significant genetic disparity (**Table 1**) from each other. Within the external grouping, disparity was seen amongst the majority of sites. Within the internal grouping, MW showed disparity to all populations with the psbA^{ncr} marker but to only two in the nrDNA marker whilst DAL showed complete disparity to all sites with the psbA^{ncr} and SAD demonstrated dissimilarity to neighbouring DAL, RAG, and UMM with the nrDNA marker.

Mantel tests for both genetic markers recovered insignificant P (rand. >= obs.) values >> than 0.05; 0.222 and 0.219, nrDNA and psbA^{ncr}, respectively.

The maximum parsimony networks for both markers resolved phylogenetic groupings that corresponded well to the pairwise ϕst matrices and population groupings (**Figures 2**, **3**). Within the nrDNA network the external sequences shared no haplotypes with internal sequences except for sequences containing the 8bp *S. thermo*.-indel mutation (**Figure 3A**). In addition, the strong genetic disparity between the DAL population and all other populations is exemplified by a monophyletic grouping of approximately half of the DAL sequences in the psbA^{ncr} phylogeny. MW sequences grouped exclusively with high frequency haplotypes comprising internal and external sequences within the nrDNA phylogeny and yet with the psbA^{ncr} sequences resolved at considerable distance from the main internal grouping, although some haplotypes were shared.

Maximum parsimony networks of the nrDNA regions were clearly divided into two groupings; those with and those without the 8bp *S. thermo*.-indel insert (**Figure 3**). The inclusion of the ITS1 and 5.8S regions of the nrDNA greatly improved phylogenetic resolution within the *S. thermophilum* group samples evidenced by the further separation of a single large haplotype in the ITS2 only network into 6 haplotypes with further minor satellite haplotypes in the combined ITS1-5.8S-ITS2 analysis (**Figure 3**).

DISCUSSION

Host-Effect on *Symbiodinium* Sequence

To assess whether any signature of genetic structure found in this study might be an artefact of host species distribution due to different species of *Porites* hosting different *Symbiodinium* types, cluster-based correlation analysis between *Symbiodinium* sequence recovered and host species was conducted. Whilst a significant result of the psbA^{ncr} chi-squared test suggests a significant interaction between species and cluster for this marker it should be noted that the majority of the divergence was driven by the lower than expected abundance of *P. harrisoni* in the most populace cluster (55.9% of divergence). The large difference between the expected and observed values in this cluster is likely an artefact caused by the high sampling frequency of *P. lutea* (raising the expected value in the cluster in question) and the smaller number of more western sites at which *P. harrisoni* was sampled (lowering the recovered genetic diversity for *P. harrisoni*; *P. harrisoni* sampled at 3 vs., 6 and 5 sites for *P. lutea* and *P. lobata*, respectively; Figure S2) rather than a true species effect of *P. harrisoni* on sequence recovered. Given the likely artefactual cause of this significant result for the psbA^{ncr} marker and the lack of significant correlation between coral host and sequence for the nrDNA marker, *Symbiodinium* sequences and any associated genetic structure identified in this study will be discussed independent of host-species.

Genetic Diversity of the *S. thermophilum* Group

S. thermophilum sensu stricto was originally described from *Porites* spp. samples collected from the DAL, SAD and UMM regions of the southern PAG (Hume et al., 2015). Since this initial description considerable additional genetic diversity has been uncovered from samples collected outside of Southern PAG. This increase in genetic diversity has

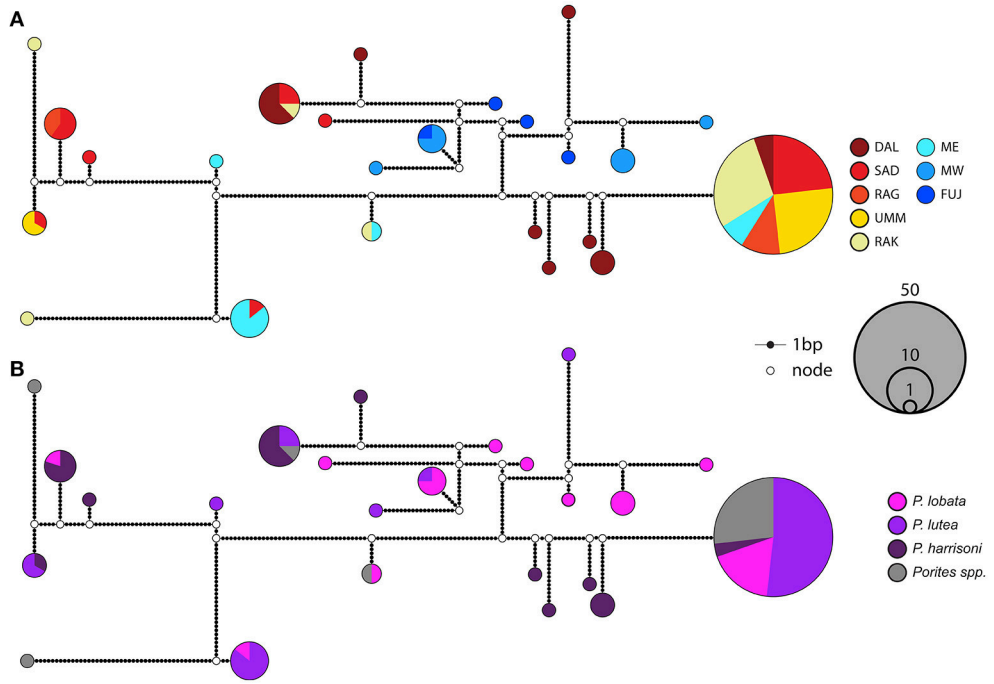

FIGURE 2 | Maximum parsimony network using the psbA^{ncr} genetic marker. Populated nodes are representative sequences of operational taxonomic units (OTUs) created by clustering at a distance of 0.04. Nodes are colored according to both site **(A)** and host species **(B)**.

led to the use of the term *S. thermophilum* group due to the fact that such diversity may be indicative of additional species.

Whilst all of the samples used in the initial *S. thermophilum* description resolved with a single dominant ITS2 type C3 sequence, a range of psbA^{ncr} haplotypes were recovered (Hume et al., 2015). The majority of these psbA^{ncr} sequences (17/22) cluster within the largest OTU of the psbA^{ncr} maximum parsimony network created in this study with the remaining 5 clustering amongst the monophyletic DAL grouping also from this study (**Figure 2**). The majority of internal samples (from DAL, SAD, RAG, UMM, RAK, and MW) analysed in this study also cluster within the same psbA^{ncr} OTU and display a limited diversity of closely related intragenomic ITS1-5.8S-ITS2 variants that share only 3 out of 38 haplotypes with external samples (ME, FUJ, MUC; **Figures 2**, **3**). Taken together, these findings suggest that the majority of *S. thermophilum* group symbionts found within the southern PAG are *S. thermophilum sensu stricto* but that additional *S. thermophilum* group diversity is also present, such as the PAG *Acropora* spp.—specific symbiont (C3V1; KM487747) identified in Hume et al. (2015). In contrast, whilst this study's external *S. thermophilum* group populations also return ITS2 type C3 and *S. thermo*-indel containing sequences characteristic of the *S. thermophilum* group, their lack of shared haplotypes with internal PAG symbionts (both non-*S. thermo*-indel-containing nrDNA and psbA^{ncr} sequences) suggest that they are either a genetically distinct subpopulation of *S. thermophilum* or represent a separate species. Indeed, the quantification of genetic distances between members within the group by Hume et al. (2016) as comparable to those observed between other *Symbiodinium* types estimated to have diverged more than 10 Mya would also support the likely existence of multiple subpopulations within the group.

Genetic Resolution of *S. thermophilum* Sub-taxa

The consideration of nrDNA intragenomic sequence variants in *Symbiodinium* ITS2 phylogenetics has enabled the further resolution of types originally designated only according to their predominant ITS2 sequence, for example the distinction between D1, D1-4 (*S. trenchii*) and D1-4-6 (LaJeunesse et al., 2010). Whilst the consideration of such intragenomic variants has enabled the distinction of *S. thermophilum* group symbionts from other ITS2 type C3 symbionts, neither the presence nor frequency of specific intragenomic variants has yet been able to resolve different populations of *S. thermophilum* group symbionts to the same extent as the inclusion of additional regions of the nrDNA has in this study (**Figure 3A** vs. **Figure 3B**). This inclusion of the 5.8S and ITS1 region has effectively brought the resolution of the rDNA marker closer to that of the psbAncr marker, a marker generally considered hypervariable (Thornhill et al., 2014). However, by sequencing nrDNA ITS2 PCR amplicons to a greater depth using NGS technologies (*sensu* (Arif et al., 2014), Smith et al., 2017) fine scale assessment of differences in the presence and proportion of intragenomic sequence variants will likely result in a significant increase in the resolving power of this marker.

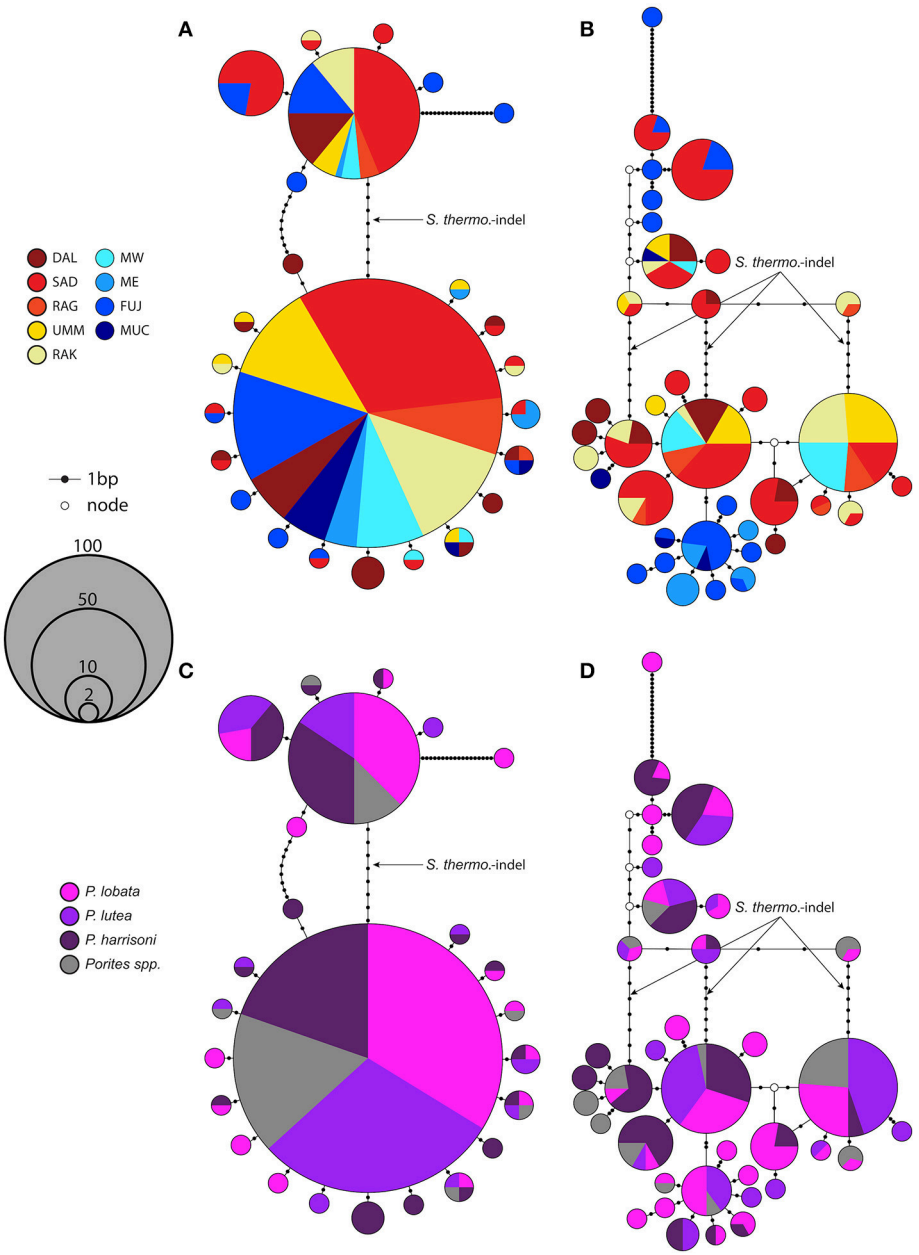

FIGURE 3 | Maximum parsimony networks using the nrDNA. Populated nodes are representative of nrDNA sequences of either the ITS2 region only **(A + C)** or the complete ITS1-5.8S-ITS2 region **(B + D)**. Nodes supported by only a single sequence are not displayed. Nodes are colored by site **(A + B)** and host-species **(C + D)**. The link representing the 8 bp *S. thermo*.-indel is annotated.

Identification of *S. thermophilum* Sub-populations

Whilst *S. thermophilum* group symbionts have been documented throughout reefs on the south coast of the PAG and to a lesser extent externally in the Strait of Hormuz and Gulf of Oman (D'Angelo et al., 2015) it is unclear whether the diversity of physical environments over which they are found has led to a range of genetically disparate sub-populations. Here we further resolve the previously described biogeographical boundary to coral holobiont distribution found in the Strait of Hormuz and identify genetically disparate populations either side of it.

D'Angelo et al. (2015) first documented the host-symbiont biogeographical boundary at the Strait of Hormuz through noting a gradual decrease in the predominance of *S. thermophilum* group associations moving from the western Strait of Hormuz out into the Gulf of Oman. The significant effect of this boundary upon a large number of taxa is qualified by the limited diversity found within the PAG compared to the

wider Indo-Pacific (Coles, 2003; D'Angelo et al., 2015). Given the large among group variance between internal and external populations, location either side of the boundary, rather than distance between study sites, would be expected to explain genetic disparity. As such, insignificant results from the mantel test also corroborates the presence of this boundary.

The genetic structure identified in this study would suggest that the western edge of the Strait of Hormuz (i.e., site MW) may represent a specific area where the decrease in genetic diversity from the Gulf of Oman to the PAG is greatest. This is demonstrated through the significant genetic difference of MW to all outer sites in concert with an only mixed differentiation from internal sites (**Table 1**). In addition, the fact that the two significantly differentiated Musandam sites (i.e., MW, ME) have only ∼20 km between them may point to an sudden spatial divide between the internal and external symbiont biomes in this region. Such a small distance between genetically differentiated sites could suggest that local adaptation to specific local conditions, rather than allopatric diversification due to limited connectivity, is responsible for the genetic heterogeneity observed.

Given that the surface inflow into the PAG effectively acts to buffer thermal extremes by cooling in the summer and warming in the winter, a gradient of seasonal thermal range exists that correlates with the penetrative distance of this inflow (**Figure 1c**). As such, coral communities within the southern and more westerly PAG experience the coolest winters and warmest summers in conjunction with the highest year round salinities (∼42; **Figure 1**). Remotely sensed estimated upper bleaching thresholds of reefs in this southern region reflect this with a difference of more than 0.5°C between DAL and SAD (35.05 and 34.53°C, respectively; Shuail et al., 2016). The genetic disparity seen between DAL and to a lesser extent SAD with other internal sites may therefore be due to local adaptation to different thermal histories. However, further population genetic data would be required to verify this.

S. thermophilum Group Symbionts as Stress Tolerant Genetic Resources

As coral reef ecosystems become increasingly under threat so do the natural systems and human populations that rely upon them (Hughes et al., 2003). Coupled with the advanced state of reef degradation these ecosystems are therefore increasingly the focus of conservation efforts (Burt et al., 2014). With their extraordinary stress tolerance, coral associations of the PAG have been suggested as potential candidates to seed thermally degraded reefs in order to accelerate reef recovery and increase future stress tolerance through the integration of genetic resources (Coles and Riegl, 2013). However, mesocosm-based studies by D'Angelo et al. (2015) demonstrated that whilst some *S. thermophilum*-associated corals from the PAG were able to survive in waters of normal oceanic salinity, their growth was reduced and their exceptional thermal tolerance lost. As such, due to the trade-offs associated with the local adaptations to the higher salinity of the PAG, corals from this waterbody appear to have a small potential to colonise reefs elsewhere that are characterised by a more oceanic salinity.

However, the presence of genetically distinct sub populations of the *S. thermophilum* group identified in this study would suggest that a considerable pool of group member symbionts upon which natural selection could act exist. Specifically, group members in the Gulf of Oman surviving in waters characterised as thermally challenging yet with oceanic-like salinities, would appear to be model candidates to act as natural sources of stress tolerant associations to Indo-Pacific reefs. The modification of symbiont associations by corals to those of a more stress tolerant type has been demonstrated (Ortiz et al., 2013; Silverstein et al., 2015) and such mechanisms could allow for the ecological proliferation and integration of *S. thermophilum* group symbionts amongst reefs, especially considering the broad range of cnidarians *S. thermophilum* group symbionts have been identified in association with (more than 40 genera; Hume et al., 2016). Indeed, the spread of a stress-tolerant coral generalist *Symbiodinium trenchii* throughout Caribbean reefs in a period of possibly only several decades has recently been documented and would imply that such an ecological proliferation could occur in a time frame relevant to the slowing of further reef degradation due to future warming (Pettay et al., 2015). Nevertheless, the almost undetectable abundance of *S. thermophilum* group symbionts external to the PAG (found in ∼4% of corals in the Red Sea and Gulf of Oman, often at low within coral abundances; Hume et al., 2016) would suggest that *S. thermophilum* group associations are not competitive given current environmental conditions and extant symbiont populations.

As coral reef ecosystems continue to be degraded by increasingly stressful conditions, and typical associations begin to breakdown, stress tolerant *S. thermophilum* group associations may become more competitive conferring increased tolerances to reefs in future conditions (Pettay et al., 2015). However, many other biotic and abiotic factors that will influence whether these symbionts will be able to acquire a niche and thus proliferate must be taken into account. In particular, trade-offs such as reduced calcification rates that can be associated with thermal tolerance must also be considered (Jones and Berkelmans, 2010). The ability of *S. thermophilum* group associations to be able to confer increased tolerances will be dependent on the maximal retention of genotypes within the group from which better adapted and resilient symbionts may gain predominance and enable reef adaptation to warming waters. As such, reduction of any reef stressors other than rising SSTs that may negatively affect this biodiversity must be prioritised in conservation strategies (D'Angelo and Wiedenmann, 2014).

DATA ACCESSIBILITY

Genbank accession numbers for psbA^{ncr} sequences analysed as part of this study are listed in Table S1. A multiple sequence alignment of the ITS1-5.8S-ITS2 nrDNA sequences used in this study has been submitted to the Dryad Repository. MOTHUR, R, and PYTHON scripts used in this study are available at https://github.com/didillysquat/PAG-Code.

AUTHOR CONTRIBUTIONS

BH: Conceived and conducted the analysis; BH, CD, and JW: Planned and conceived the project; BH: Wrote

the initial report; BH, CD, and JW: Revised final versions of the report. JB: Sponsored fieldwork to collect samples and along with JW contributed resources to the analysis.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00138/full#supplementary-material

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