Coral *Symbiodinium* community composition across the Belize Mesoamerican Barrier Reef System is driven by host species and environmental variability

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Abstract

Reef-building corals maintain a symbiotic relationship with dinoflagellate algae of the genus *Symbiodinium* and this symbiosis is vital for the survival of the coral holobiont. *Symbiodinium* community composition within the coral host has been shown to influence a coral's ability to resist and recover from stress. A multitude of stressors including ocean warming, ocean acidification, and eutrophication have been linked to global scale coral decline in coral health and cover in recent decades. Three distinct thermal regimes ($high_{TP}$, mod_{TP}, and low_{TP}) following an inshore-offshore gradient of declining average temperatures and thermal variation were identified on the Belize Mesoamerican Barrier Reef System (MBRS). Quantitative metabarcoding of the ITS-2 locus was employed to investigate differences and similarities in *Symbiodinium* genetic diversity of the Caribbean corals *Siderastrea siderea*, *S. radians*, and *Pseudodiploria strigosa* between the three thermal regimes. *Siderastrea siderea* associated with distinct *Symbiodinium* communities when compared to their congener *S. radians* as well as *P. strigosa*, demonstrating host-specificity of *Symbiodinium* along the MBRS. *Symbiodinium* community differences were only detected across thermal regimes for *S. siderea*; however, thermal parameters influenced *Symbiodinium* communities in all coral species investigated. Interestingly, *Symbiodinium trenchi*, a symbiont known to confer thermal tolerance, was dominant only in *S. siderea* at one sampled offshore site and was rare inshore, suggesting that coral thermal tolerance in more thermally variable inshore habitats is achieved through alternative mechanisms. Overall, thermal parameters alone were not the primary drivers of *Symbiodinium* community composition, suggesting that environmental variables unrelated to temperature (i.e., light availability, or nutrients) may play key roles in structuring coral-algal communities in Belize.

Introduction

Obligate symbioses, relationships in which two or more organisms depend on one another for nutrition and survival, occur globally. Such symbioses are ubiquitous in plants and Mycorrhiza [1], ants and bacteria [2], and lichens [3]. The effects of climate change are expected to disrupt proper functioning of many symbioses, including that of reef-building corals [4-6]. The success of coral reefs worldwide depends on the symbiosis between the coral host and photosynthetic algae of the genus *Symbiodinium* [7-9]. Under stressful conditions this coral-*Symbiodinium* relationship breaks down, resulting in the loss of endosymbiont cells and/or photosynthetic pigments from the coral tissue in a process known as 'coral bleaching' [10]. Coral bleaching is most commonly associated with thermal stress [11-15] and is predicted to increase in frequency and severity as the world's climate continues to change [5, 16-21]. Increased thermal stress resulting from climate change combined with other local stressors such as eutrophication, habitat destruction, and overfishing has created an uncertain future for coral reefs [6, 13, 22]. In the Caribbean Sea, warming rates are higher than in any other tropical basin [23] and coral cover has declined by as much as 80% in recent decades [24]. It has been predicted that Caribbean coral reefs may suffer biannual bleaching events within the next 20-30 years [17] and annual bleaching by 2040 [25].

In the face of a changing climate and widespread reef declines, corals will need to rapidly increase their thermal tolerance in order to persist in their current form [18, 26]. Coral thermal tolerance has been shown to be influenced by a coral's thermal history, which among other factors includes average environmental temperature and extent of thermal variability [27, 28]. On average, corals previously exposed to warmer temperatures show decreased mortality during bleaching events [29] and more stable growth patterns [30] compared with corals exposed to

cooler temperatures, which exhibit greater mortality during heat stress and declining growth rates with increased temperatures [29, 30]. Exposure to greater daily thermal variation has also been shown to increase coral thermal tolerance [31] and has been associated with higher coral cover and slower mortality rates when compared to reefs exposed to less thermal variation [32]. Coral thermal tolerance is also heritable with larvae from parent colonies on lower-latitude (warmer) reefs showing a 10-fold increase in survival under heat stress when compared to larvae from cooler reefs locations [33]. A growing body of evidence suggests that the coral host plays a significant role in thermal tolerance [34-37], however, plasticity or specificity of coral-associated *Symbiodinium* communities also plays a significant role in overall thermal tolerance [38-41].

The clades, lineages, or species of *Symbiodinium* hosted by a coral are critical to its survival and resilience to stress. The genus *Symbiodinium* is genetically diverse and comprises at least nine divergent clades [clades A-I; 42]. These clades can be further broken down into lineages, corresponding approximately to species level diversity [43], with some species conferring variable benefits [38, 42, 44]. In particular, some *Symbiodinium* are more thermally tolerant than others [9, 38, 45], specifically *Symbiodinium* clade D [46]. In contrast, clade C is more thermally sensitive [47-49], yet it includes *Symbiodinium thermophilum*, a thermally tolerant species within clade C endemic to the Red Sea [50]. This example illustrates that making clade level generalizations is problematic due to the physiological diversity within a single *Symbiodinium* clade [51]. Specific lineages within clades can also confer various advantages. For example, C1 enhances growth rate [52], *S. thermophilum* confers heat tolerance [50], and B2 confers cold tolerance [53]. Additionally, species D1a (*Symbiodinium trenchi)* has been shown to be both heat tolerant [54, 55], and cold tolerant [45]. However, the increased thermal tolerance of a coral which predominantly hosts clade D *Symbiodinium* appears to come at a cost of lower

lipid stores, reproductive potential, growth, and carbon fixation rates compared with corals that host other clades [56-59]. Due to the high levels of variation in coral host-*Symbiodinium* interactions, it is essential to identify which lineages are present in order to help predict how a coral may respond to environmental stressors.

The majority of coral species host one dominant *Symbiodinium* lineage [42, 60, 61] along with several non-dominant lineages [62], each proliferating primarily by asexual cloning [51]. However, other corals can host multiple dominant lineages or clades [38, 51]. Recent advances in genetic techniques, especially next-generation sequencing (NGS), have allowed researchers to identify cryptic and low-abundance symbionts comprising 0.1% or more of the total *Symbiodinium* community within a host [36, 63]. It is important to understand these low-abundance *Symbiodinium*, as they have the potential to play important roles in coral-algal holobiont physiology under ambient and stressful conditions [64-66, but see also 67]. Identifying trends in *Symbiodinium* community variation (including cryptic or low abundance lineages) within and between species across a coral reef may allow for a better understanding of the role of *Symbiodinium* communities in modulating coral response to environmental variation.

Symbiodinium communities have been shown to vary regionally [between reef systems; 59, 68, 69], locally [within a reef system; 68], temporally [across time on the same reef; 70], and within a colony [69]. Studies of this variation have revealed geographically endemic lineages of *Symbiodinium* which may play a significant role in local and regional scale coral survival and stress tolerance [38, 69, 71]. While temperature stress may play a role in structuring *Symbiodinium* communities [72], variations in other environmental factors have also been shown to drive *Symbiodinium* community composition. For example, physical processes and total suspended solids (a proxy for nutrients and flow) drive *Symbiodinium* associations within the

106 *Orbicella annularis* species complex in Belize and Panama [68]; however, on a regional scale
107 (e.g., the entire Caribbean Sea), *O. annularis Symbiodinium* communities differed based on
108 patterns of chronic thermal stress [73]. Additionally, the presence of several subclades of
109 *Symbiodinium* correlated with other environmental parameters, such as cooler summers, nutrient
110 loading, and turbidity [73]. Taken together, these studies demonstrate that variation in
111 *Symbiodinium* communities can be driven by a variety of environmental parameters and may be
112 specific to each coral species in each specific environment.

113 The majority of Caribbean *Symbiodinium* biogeography studies have focused on the
114 *Orbicella* species complex [68, 69, 73] as *Orbicella* spp. has experienced significant declines
115 over the last two decades [74] and is now listed as 'threatened' under the Endangered Species
116 Act. However, the variation in *Symbiodinium* communities of other more stress tolerant corals,
117 such as *Sidereastrea siderea* and *S. radians* [75-80], remain relatively understudied. Here, we
118 assess *Symbiodinium* community composition in three species of ubiquitous Caribbean corals
119 (*Siderastrea siderea*, *S. radians*, and *Pseudodiploria strigosa*) across three distinct thermal
120 regimes along the Belize Mesoamerican Barrier Reef System (MBRS) previously shown to
121 influence coral community composition [81]. Coral-associated *Symbiodinium* communities were
122 examined across an inshore-offshore thermal gradient and a latitudinal gradient to elucidate the
123 role that coral species, local habitat, and a suite of thermal parameters play in structuring
124 *Symbiodinium* communities in the western Caribbean Sea.

125 **Methods:**

126 *Site selection and characteristics*

127 Ten sites along the Belize MBRS, that were previously characterized into three thermally
128 distinct regimes (low_{TP}, mod_{TP}, $high_{TP}$) and exhibited variations in coral species diversity and

richness [81], were selected. $High_{TP}$ sites (inshore) were characterized by larger annual temperature variation, higher annual maximum temperatures, and are exposed to temperatures above the regional bleaching threshold of 29.7°C (Aronson et al., 2002) more often than mod_{TP} sites (mid-channel reefs) and low_{TP} sites (offshore) [81]. $High_{TP}$ sites were dominated by stress tolerant and weedy coral species while corals representing all four coral life histories [stress tolerant, weedy, competitive, and generalist; 80] were present in low_{TP} and mod_{TP} sites [81].

Sample Collection

In November 2014, five to ten (quantity depended on local availability) coral tissue microsamples (approx. 2 mm diameter) were collected at 3 to 5 m depth from three coral species (*Siderastrea siderea, S. radians, and Pseudodiploria strigosa*) at nine sites across four latitudes along the Belize MBRS (Fig 1; Table 1). Each latitudinal transect contained a low_{TP}, mod_{TP}, and $high_{TP}$ site. The transects from north to south were: Belize City, Dangriga, Placencia, and Punta Gorda (Fig 1). All three sites within the Punta Gorda and Placencia transects were sampled, but only the low_{TP} and $high_{TP}$ sites were sampled along the Belize City and Dangriga transects due to time constraints. Samples collected at the Belize City $high_{TP}$ site were collected in October 2015, as no corals were located in the area in 2014, but patch reefs were located in 2015. Coral microsamples were collected using a hammer and chisel and sampled colonies were separated by at least 1m to randomize micro-environmental and host genetic effects in order to attain more site-specific representative samples. Microsamples were collected from colony edges to avoid unnecessary damage to the larger colony and to limit effects of *Symbiodinium* zonation within an individual [69]. Tissue microsamples were placed on ice immediately following collection for transport to mainland Belize. Microsamples were then preserved in 96% ethanol and stored on

ice at –20°C. Preserved microsamples were transported on ice to the coral ecophysiology lab at the University of North Carolina at Chapel Hill and stored at −20°C until DNA isolation.

Sea Surface Temperature

Daily 1-km horizontal resolution sea surface temperature (SST) estimates were acquired from the NASA Jet Propulsion Laboratory's Multi-Scale High Resolution SST (JPL MUR SST) product via NOAA Environmental Research Division's Data Access Program (ERDDAP- https://coastwatch.pfeg.noaa.gov/erddap/index.html) [82] and analyzed following Baumann et al [81]. Briefly, SST data were binned by latitude and longitude for each site and annual values for maximum temperature, temperature range, number of days above the regional bleaching threshold (29.7°C, Aronson et al. [83]), and longest streak of consecutive days above the bleaching threshold were calculated for each site and for the entire MBRS reef area. Using standard deviations from the mean for all four parameters, sites were classified as low_{TP}, mod_{TP}, and $high_{TP}$ [81]. Several additional temperature parameters were taken into account for this study, including: annual degree heating days (similar to degree heating weeks, as per Gleeson and Strong [84]), annual minimum temperature, annual average temperature, annual winter average temperature, and annual summer average temperature. Values for these parameters within the three thermal regimes are reported in Table S1.

DNA Extraction

Coral holobiont (coral, algae, and microbiome) DNA was isolated from each sample following a modified phenol-chloroform [83, 85, 86] method described in detail by Davies et al (2013). Briefly, DNA was isolated by immersing the tissue in digest buffer (100 mM NaCL,

10mM Tris-Cl pH 8.0, 25 mM EDTA pH 9.0, 0.5% SDS, 0.1 $mgml^{-1}$ Proteinase K, and 1 μgml^{-1} RNaseA) for 1 h at 42°C followed by a standard phenol-chloroform extraction. Extracted DNA was confirmed on an agarose gel and quantified using a Nanodrop 2000 Spectrophotometer (Thermo Scientific).

PCR amplification and metabarcoding

The ITS-2 region (350 bp) was targeted and amplified in each sample using custom primers that incorporated *Symbiodinium* specific ITS-2-dino-forward and its2rev2-reverse regions [63, 71, 87]. Each primer was constructed with a universal linker, which allowed for the downstream incorporation of Illumina specific adapters and barcodes during the second PCR as well as four degenerative bases whose function was to increase the complexity of library composition. The forward primer was 5'-<u>GTCTCGTCGGCTCGG</u> + *AGATGTGTATAAGAGACAG* + NNNN + **CCTCCGCTTACTTATATGCTT**-3' where the underlined bases are the 5'- universal linker, italicized bases indicate spacer sequences, N's denote degenerative bases and the bold bases are the ITS-2-dino. The reverse primer was 5'-<u>TCGTCGGCAGCGTCA</u> + *AGATGTGTATAAGAGACAG* + NNNN + **GTGAATTGCAGAACTCGTG**-3'.

Each 20uL PCR reaction contained 5-100 ng DNA template, 12.4 µL MilliQ H_2O, 0.2 µM dNTPs, 1µM forward and 1µM reverse primers, 1X *Extaq* buffer, and 0.5 U (units) *Extaq* polymerase (Takara Biotechnology). PCR cycles were run for all samples using the following PCR profile: 95°C for 5 min, 95°C for 40 s, 59°C for 2 min, 72°C for 1 min per cycle and a final elongation step of 72°C for 7 min. The optimal number of PCR cycles for each sample was determined from visualization of a faint band on a 2% agarose gel (usually between 22 and 28

cycles) as per Quigley et al. (2014). PCR products were cleaned using GeneJET PCR purification kits (Fermentas Life Sciences) and then a second PCR reaction was performed to incorporate custom barcode-primer sequences [63] modified for Illumina Miseq as in Klepac et al. [88]. Custom barcode primer sequences included 5'-*Illumina adaptor* + 6 bp **barcode sequence** + one of two universal linkers-3' (e.g.: 5'- *CAAGCAGAAGACGGCATACGAGAT* + **GTATAG** + GTCTCGTGGGCTCGG-3', or 5'- *AATGATACGGCGACCACCGAGATCTACAC* + **AGTCAA** + TCGTCGGCAGCGTC-3'). These universal linking barcoded adapters can be used to target any loci and therefore significantly reduce costs associated with Miseq sequencing of multiple loci. Following barcoding, PCR samples were visualized on a 2% agarose gel and pooled based on band intensity (to ensure equal contributions of each sample in the pool). The resulting pool was run on a 1% SYBR Green (Invitrogen) stained gel for 60 minutes at 90 volts and 120 mAmps. The target band was excised, soaked in 30 uL of milli-Q water overnight at 4°C, and the supernatant was submitted for sequencing to the University of North Carolina at Chapel Hill High Throughput Sequencing Facility across two lanes of Illumina MiSeq (one 2x250, one 2x300). The two lanes produced similar mapping efficiencies (73% and 73%, respectively; Table S3).

Bioinformatic Pipeline

The bioinformatic pipeline used here builds upon previous work by Quigley et al. [63] and Green et al. [71]. Raw sequences were renamed to retain sample information and then all forward (R1) and reverse (R2) sequences were concatenated into two files, which were processed using CD-HIT-OTU[89]. CD-HIT-OTU clusters concatenated reads into identical groups at 100% similarity for identification of operational taxonomic units (OTUs). Each sample was then

mapped back to the resulting reference OTUs and a counts table for each sample across all OTUs was produced. A BLASTn search of each reference OTU was then run against the GenBank (NCBI) nucleotide reference collection using the representative sequence from each OTU to identify which *Symbiodinium* lineage was represented by each OTU (Table S2).

The phylogeny of representative sequences of each distinct *Symbiodinium* OUT was constructed using the PhyML tool [90, 91] within Geneious version 10.0.5 (http://geneious.com) [92]. PhyML was run using the GTR+I model (chosen based on delta AIC values produced from jModelTest [90, 93]) to determine the maximum likelihood tree. The TreeDyn tool in Phylogeny.fr was used to view the tree (Fig 2) [94-96]. The reference sequences included in the phylogeny were accessed from GenBank (Table S6).

Statistical Analysis

OTU count analysis used the R [R Core97] package *MCMC.OTU* and followed methods described in Green et al. [71]. First, outlier samples with low sequence coverage (total log counts ≥2.5 standard deviations below the mean of all samples) were identified and removed, which removed 3 samples. Next, rare OTUs (<0.1% of the global sum of counts [as per 63]) were identified and discarded leaving 56 of the original 5,132 OTUs. Many remaining OTUs were identified as having the same *Symbiodinium* lineage (i.e., C1 or D1a) and these OTUs were regressed against one another. Positive correlations between OTUs within a lineage may indicate paralogous loci from the same genome [36, 71]. As a result, reads from OTUs within the same lineage that showed a positive R^2 and significant *p*-value following linear regression were pooled in order to control for possible overestimation of biodiversity [98]. Pooling resulted in a final OTU table containing ten OTUs (Table S2). Raw reads, trimmed reads, mapped reads, and

244 percentage of reads mapped per species were calculated and reported in Table 2. Final pooled
245 OTUs were run through the MCMC.OTU package in R and fit to a model that included fixed
246 effect for host species, collection site, and thermal regime (Table S4). Differences between fixed
247 effects were calculated based on their sampled posterior distributions and statistical significance
248 was calculated as per Matz et al. [99]. OTU count data were converted to relative abundances
249 (%), which were used to generate Fig 3 (Table S5).

250 To visualize differences in symbiont communities between temperature regimes, latitude,
251 and species, principal component analyses (PCA) were performed on all OTUs that passed
252 filtering using the *vegan* package in R [100]. Count data were transformed using Bray-Curtis
253 similarity and were used as input for PCA. PERMANOVA was carried out on each PCA using
254 the *adonis* function of the *vegan* package in R [100]. Canonical Correlation Analysis (CCA),
255 which is widely used for ecological applications [101], was undertaken using the *cca* function of
256 the *vegan* package in R. CCA was used to measure associations between *Symbiodinium*
257 communities within a species and temperature variables (Table S1) at each temperature regime.
258

259 **Results**

260 *Symbiodinium diversity and abundance across the Belize MBRS*

261 Our analysis produced 118,834 unique sequences of which 89,211 mapped to 10 OTUs
262 (Table 1). The dominant OTU (hereafter referred to as lineage) in *S. siderea* was C1.I (74.39%),
263 while B1.I dominated *S. radians* (70.31%) and *P. strigosa* (51.74%) samples (Table S5, Fig 3).
264 Nine out of ten *Symbiodinium* lineages were present in *S. siderea* and *P. strigosa* while all ten
265 were present in *S. radians* (Table S5). The four most abundant lineages in *S. siderea* were C1.I,
266 C1.III, D1a, and B1.I (74.39%, 12.94%, 9.29%, and 2.94%, respectively; Table S5, Fig 3A) and

267 date of collection did impact the dominate *Symbiodinium* lineages (all samples collected in 2014
268 except for Belize City high$_{TP}$ which were collected in 2015; Fig 3). *Symbiodinium* D1a (*S.*
269 *trenchi*) was most abundant in *S. siderea* at low$_{TP}$ sites, particularly the low$_{TP}$ site along the most
270 southern Punta Gorda transect (Table S5, Fig 3A) while lineage C1.II is more abundant in central
271 and northern Belize (Belize City and Dangriga transects; Figs 1, 2).

272 The four most abundant lineages in *S. radians* were B1.I, C1.I, B1.II, and C1.II (70.31%,
273 13.41%, 6.54%, and 2.19% respectively; Table S5, Fig 3B). B1.I was the dominant symbiont
274 across all thermal regimes and all latitudes, but C1.I and C1.II were the most abundant
275 *Symbiodinium* lineages in several samples from the central Placencia transect (Table S5, Fig 3B).
276 Lineage C1.II was only present in proportions above 1% in 2 samples, both from the mod$_{TP}$ site
277 along the Placencia transect (Table S5, Fig 3B). D1a (*S. trenchi*) was only present in low
278 abundance in *S. radians* (Table S5, Fig 3B).

279 The four most abundant lineages in *P. strigosa* were B1.I, C1.I, C1.II, and C1.III
280 (51.74%, 21.87%, 16.92%, and 6.24%, respectively). C1.II was the most abundant lineage at the
281 low$_{TP}$ site in the Placencia transect, but B1.I was most abundant at all other sites (Table S5, Fig
282 3). C1.I was the second most abundant lineage in mod$_{TP}$ and high$_{TP}$ sites and C1.II was the
283 second most abundant lineage in the low$_{TP}$ site (Table S5, Fig 3C). D1a (*S. trenchi*) was only
284 present in low abundance in *P. strigosa* (Table S5, Fig 3C).

285

286 *Host species specificity in Symbiodinium community composition*

287 *Symbiodinium* communities differed significantly between *S. siderea* and the other two
288 coral host species (Table S4, Fig 4A, *p*-value=0.001). This difference appears to be driven by
289 higher relative abundances of C1.I and D1a (*S. trenchi*) in *S. siderea* compared to *P. strigosa* and

290 *S. radians* (Fig 3A). Within *S. siderea*, *Symbiodinium* communities varied by thermal regime and
291 site, but not by latitude (Table S4, Fig 4B). *Symbiodinium* communities in *S. radians* and *P.*
292 *strigosa* did not differ significantly by thermal regime, site, or latitude (Table S4). As
293 *Symbiodinium* communities did not differ significantly by latitude in any of the three coral
294 species (Table S4), there does not appear to be a significant effect of Illumina lane (northern and
295 southern latitudes were run on separate lanes; Table 1) on dominant *Symbiodinium* lineages.
296
297 *Correlation of Symbiodinium community structure with temperature parameters*
298 Canonical correlation analysis (CCA) revealed that thermal parameters measured in this
299 study correlated with 29.6% of the variance in *Symbiodinium* communities within *S. siderea*,
300 11.5% of the variance in *S. radians*, and 28.4% of the variance in *P. strigosa* (Fig 5). 17.2% of
301 total variation in *S. siderea* symbiont community is explained by CCA axis 1 and 8.9% is
302 explained by CCA axis 2 (Fig 5A). CCA axis 1 explained 7.6% of the variance in *S. radians* and
303 19.3% of the variance in *P. strigosa*; while CCA axis 2 explained 2.4% and 8.2% of the variation
304 in *S. radians* and *P. strigosa*, respectively (Figs 5B, C). Average annual temperature, average
305 annual minimum temperature, annual temperature range, degree heating days, and summer
306 average temperature all appear to influence variation in *Symbiodinium* communities in *S. siderea*
307 (Fig 5A); while summer average temperature, annual average temperature, and annual minimum
308 temperatures appeared to play principal roles in *S. radians* (Fig 5B). Average annual
309 temperature, annual days above the bleaching threshold, and the annual longest streak of days
310 above the bleaching threshold best explained variation within *P. strigosa* (Fig 5C).
311
312 **Discussion**

313 *Host-specificity drives Symbiodinium community composition*

314 This study indicates that *Siderastrea siderea* hosts significantly different *Symbiodinium*
315 communities than *S. radians* and *P. strigosa* on the Belize MBRS (Table S5, Fig 3), providing
316 evidence to support previous findings of host-specific *Symbiodinium* associations [51, 102]. The
317 three coral species studied here were found to be dominated by the two most abundant
318 *Symbiodinium* clades in the Caribbean [103]: B1 in *S. radians* and *P. strigosa* colonies and C1 in
319 *S. siderea* (Table S5, Fig 3). These associations are consistent with previous studies that
320 identified the same dominant *Symbiodinium* in these species on the Belize MBRS [102]; but
321 contrast with findings of other studies on the same species elsewhere in the Caribbean, that
322 identified other dominant *Symbiodinium* lineages in these species [102, 104, 105], supporting
323 previous evidence for regional endemism within the Caribbean Sea and specificity of the coral-
324 algal symbiosis [102, 106]. Differences in *Symbiodinium* communities between coral host
325 species appear to be driven by the relative abundance of B1 and C1 as well as the presence or
326 absence of D1a (Fig 4A). Interestingly, *Symbiodinium* communities appear more similar between
327 *S. radians* and *P. strigosa* than between *S. radians* and *S. siderea*, indicating that members of the
328 same coral genus do not necessarily share a common dominant *Symbiodinium* partner. Presence
329 of multiple lineages of C1 and B1 (Table S2, Table S5) support previous evidence of
330 phylogenetic partitioning, or highly specific lineages, in clades B and C [69, 102, 107, 108].
331 Differences in *Symbiodinium* communities between *S. siderea* and *S.* radians/ P. *strigosa* is
332 suggestive that corals species are differentially affected by the environmental gradients sampled
333 here.
334

335 *Temperature parameters shape Symbiodinium community composition in S. siderea, but not*
336 *other species*

337 *Symbiodinium* communities varied significantly across thermal regimes in *S. siderea*
338 (Table S4, Fig 4B), supporting previous evidence that habitat type [109] and temperature [110]
339 are correlated with differences in *Symbiodinium* associations. *Symbiodinium* communities did not
340 differ significantly across thermal regimes in *S. radians* or *P. strigosa*, possibly due to low
341 sample size at each sampling site for these two coral species (Table 1; Fig 3). While
342 *Symbiodinium* communities did not differ between thermal regimes in *S. radians* or *P. strigosa*
343 (Table S4), temperature parameters accounted for a proportion of the variation in these species
344 (approx. 10% and 28%, respectively; Fig 5B, C). Thermal parameters also explained up to 30%
345 of the variation in *Symbiodinium* communities in *S. siderea*, with temperature range and degree
346 heating days playing the largest roles (Fig 5A). This finding supports evidence from previous
347 studies that temperature, and more specifically chronic thermal stress (degree heating days and
348 days above bleaching threshold; Fig 5A, C), can be important drivers of *Symbiodinium*
349 associations in some, but not all coral species [73, 110]. In this study, the role of temperature
350 parameters in influencing *Symbiodinium* associations varied based on coral host species and was
351 most significant for *S. siderea*. However, temperature parameters did not account for all of the
352 variance in *Symbiodinium* communities for any coral host species investigated in the current
353 study (Fig 5), indicating that other local factors, such as nutrients, light availability, and/or
354 sedimentation may play a role [46, 111-115].

355

356 *Role of local impacts on Symbiodinium communities*

357 It has previously been shown that prevalence of specific *Symbiodinium* types within a
358 coral host species can differ based on local scale environmental parameters such as nutrient
359 loading and turbidity [73]. While these variables were not quantified in this study, chlorophyll-a
360 (*chl-a*), a proxy for nutrient input, has previously been shown to be positively correlated with
361 thermal regime in Belize. Specifically, $high_{TP}$ sites had higher *chl-a* than low_{TP} sites across the
362 Belize MBRS [81]. Therefore, a PERMANOVA that shows significant differences in
363 *Symbiodinium* communities between thermal regimes includes a confounding effect of nutrient
364 input (Table S4). Since significant differences in *Symbiodinium* communities occurred between
365 thermal regimes in *S. siderea* only, it is possible that nutrient loading or turbidity played a role in
366 *Symbiodinium* variation within *S. siderea*, but may not have significantly influenced
367 *Symbiodinium* communities in *S. radians* or *P. strigosa*. However, the magnitude of this
368 influence cannot be teased apart from the effect of thermal regime without extensive
369 quantification of nutrient concentrations across the Belize MBRS.
370
371 *Coral host plays a significant role in thermal tolerance*
372 In this study, the relative abundance of thermally tolerant *Symbiodinium* D1a (*S. trenchi*)
373 was not associated with inshore reefs as in Toller at al. [116], marginal reefs as in Hennige et al.
374 [117] and LaJeunesse et al. [103], sites exposed to the highest temperatures as in Baker et al.
375 [46], or sites exposed to the widest range of thermal fluctuations as in Abrego et al. [118],
376 Fabricius et al. [119], and LaJeunesse et al. [39, 120]. Instead, *S. trenchi* was most prevalent at
377 the southern Punta Gorda low_{TP} and mod_{TP} sites (Table S1, S5, Fig 3). Since *S. trenchi* is often
378 associated with recently bleached and/or recovering corals [46, 121], but can be replaced or
379 outcompeted following recovery [105], it could be possible that a recent bleaching event may

have occurred at these sites, however these data are not available. In summer 2014, temperatures at all sites in this study exceeded the published local bleaching threshold of 29.7°C [83] (Fig S1), yet *S. trenchi* was only the dominant symbiotic partner in eight *S. siderea* samples, all of which were from the same two sites (Punta Gorda low_{TP} and mod_{TP}; Fig 3). The presence of *S. trenchi* in several *P. strigosa* corals taken from the Punta Gorda mod_{TP} site provides additional evidence of temperature stress at these sites (Punta Gorda low_{TP} and mod_{TP}). However, no evidence of mass bleaching was observed at any of the study sites during collection in Nov 2014 or Oct 2015 so corals at these sites had either bleached recently or retained *S. trenchi* as a dominant symbiont following bleaching, possibly as a way to increase thermal tolerance. Lower thermal tolerance has been proposed previously at these sites (Punta Gorda low_{TP} and mod_{TP}) and may be due to nutrients and sediments exported from Guatemala and Honduras by currents that wash over this area of the Belize MBRS [122-124]. Low abundances of *S. trenchi* at other low_{TP} and mod_{TP} sites corroborates this hypothesis, as estimated thermal stress occurred at all latitudes at roughly the same magnitude (Fig S1). Overall, lack of *S. trenchi* in $high_{TP}$ sites indicates that regardless of warmer and more variable conditions, these three coral species do not associate with this thermally tolerant symbiont. Therefore, presumed increased thermal tolerance at high TP sites may be due to local adaptation of the coral host [36, 125] or strains of *Symbiodinium* [126, 127]. Further research into coral host and symbiont local adaptation would be needed to confirm this hypothesis.

Conclusion

This study demonstrates that *Symbiodinium* communities associated with corals in Belize are dependent on both host species as well as environmental variables. *S. siderea Symbiodinium*

403 communities were divergent from *S. radians* and *P. strigosa* (Fig 3; Fig 4A). Temperature
404 parameters played a role in driving *Symbiodinium* community composition in all three coral host
405 species, but overall significant differences across thermal regimes were only detected in *S.*
406 *siderea*. Temperature parameters did not account for all of the variation in *Symbiodinium*
407 communities within any of the three coral host species, suggesting that local impacts such as
408 nutrients, sediment, or light availability may influence *Symbiodinium* communities on the Belize
409 MBRS. Additionally, low abundance of *S. trenchi* in inshore high$_{TP}$ sites indicates thermal
410 tolerance at these sites must be conferred through alternative mechanisms, such as local
411 adaptation.
412

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Tables and Figures

Table 1: Sampling locations and microsamples information for *S. siderea* (SSID), *S. radians* (SRAD), and *P. strigosa* (PSTR). Locations are listed in order of descending latitude (Northernmost to Southernmost).

Transect	Thermal regime	Collection Date	Illumina Lane	Lat (°N)	Long (°W)	SSID	SRAD	PSTR
Belize City	Low	Nov 2014	2	17.64363	88.0264	n=10	n=0	n=0
Belize City	High	Oct 2015	2	17.48685	88.1207	n=10	n=0	n=0
Dangriga	Low	Nov 2014	2	17.078	88.01285	n=9	n=0	n=0
Dangriga	High	Nov 2014	2	16.79491	88.27699	n=10	n=0	n=0
Placencia	Low	Nov 2014	1	16.45816	88.01295	n=7	n=7	n=5
Placencia	Mod	Nov 2014	1	16.49995	88.16527	n=6	n=7	n=6
Placencia	High	Nov 2014	1	16.4654	88.31315	n=9	n=9	n=5
Sapodilla	Low	Nov 2014	1	16.15729	88.25073	n=8	n=0	n=0
Sapodilla	Mod	Nov 2014	1	16.13013	88.33234	n=6	n=0	n=6
Sapodilla	High	Nov 2014	1	16.2245	88.62943	n=8	n=6	n=0

Table 2: Average number of raw reads, trimmed reads, and mapped reads including mapping efficiency (% of trimmed reads that mapped) for each species.

Species	Raw reads	Trimmed reads	Mapped reads	Mapping efficiency
S. siderea	46161	28453	22048	73%
S. radians	51081	46812	35290	75%
P. strigosa	88888	43928	31873	69%
Total	186130	118834	89211	75%

775

776

777

778 **Figure Legends**

779 Fig 1: Thermal regime designations for sampling sites on the Belize MBRS [81]. Stars indicate sites
780 where coral tissue samples were collected for *Symbiodinium* community analysis. Low_{TP}, mod_{TP}, and
781 $high_{TP}$ are defined based on combined averages of annual maximum temperature, annual temperature
782 range, annual days above the bleaching threshold, and annual longest streak of consecutive days above
783 the bleaching threshold. Low_{TP} sites exhibit the lowest values for all parameters measured and $high_{TP}$ sites
784 exhibit the highest. A more detailed description of classification of these thermal regimes can be found in
785 Baumann et al. [81].

786

787 Fig 2: Phylogenetic analysis of ITS-2 sequences of representative OTUs from this study in addition to
788 reference sequences for each clade (indicated by *). Branch support values are shown on the branches at
789 divisions between distinct clades. The scale bar represents replacements per nucleotide site.

790

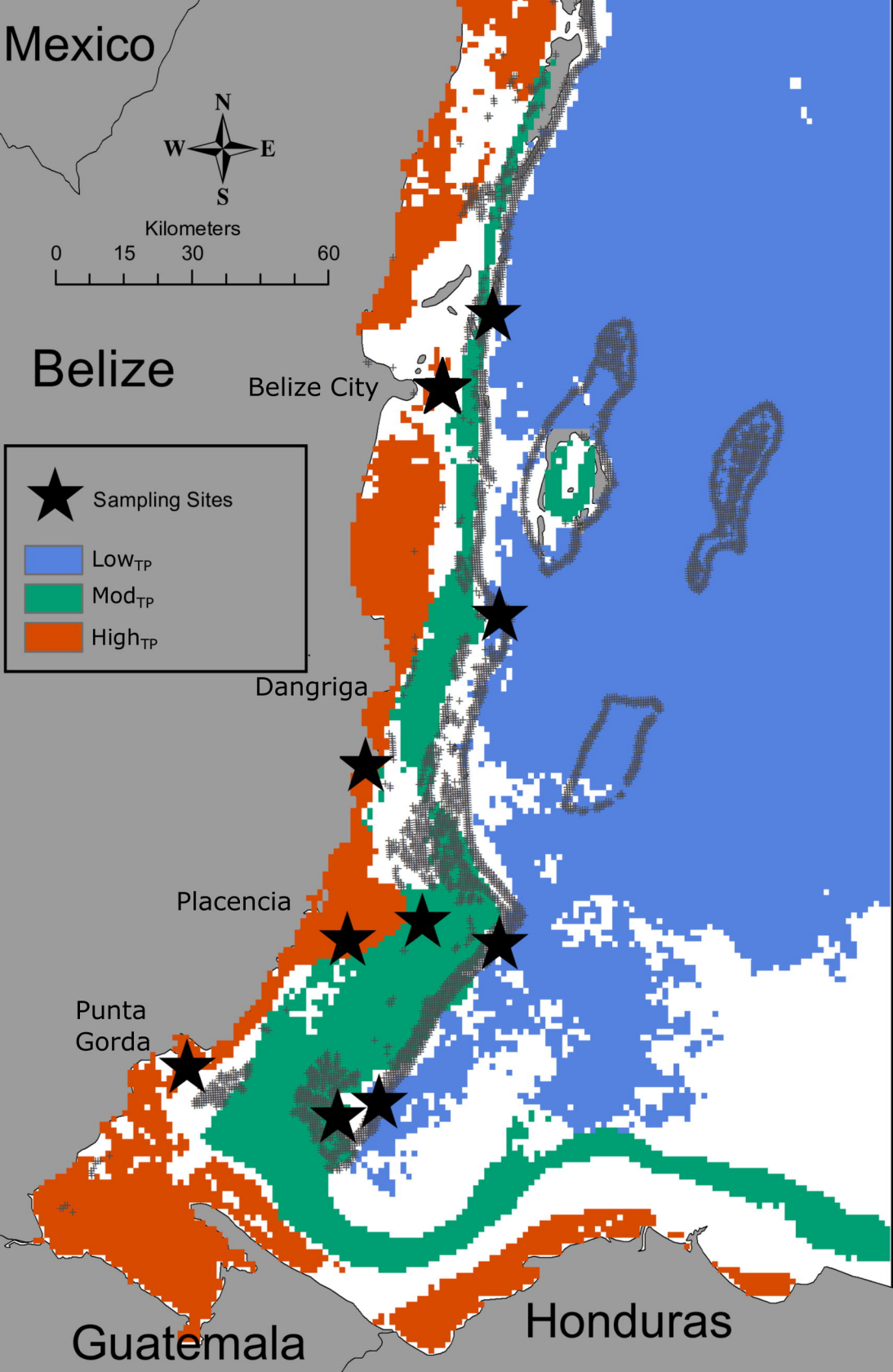
791 Fig 3. Relative abundance (%) of each OTU (lineage) in *S. siderea* (A), *S. radians* (B), and *P. strigosa*
792 (C). Each column represents an individual sample. Columns are arranged by latitudinal transect (as
793 indicated by site names in alternating gray and white boxes) and then by thermal regime (blue boxes
794 indicate low_{TP} sites, green boxes indicate mod_{TP} sites, and red boxes indicates $high_{TP}$ sites.

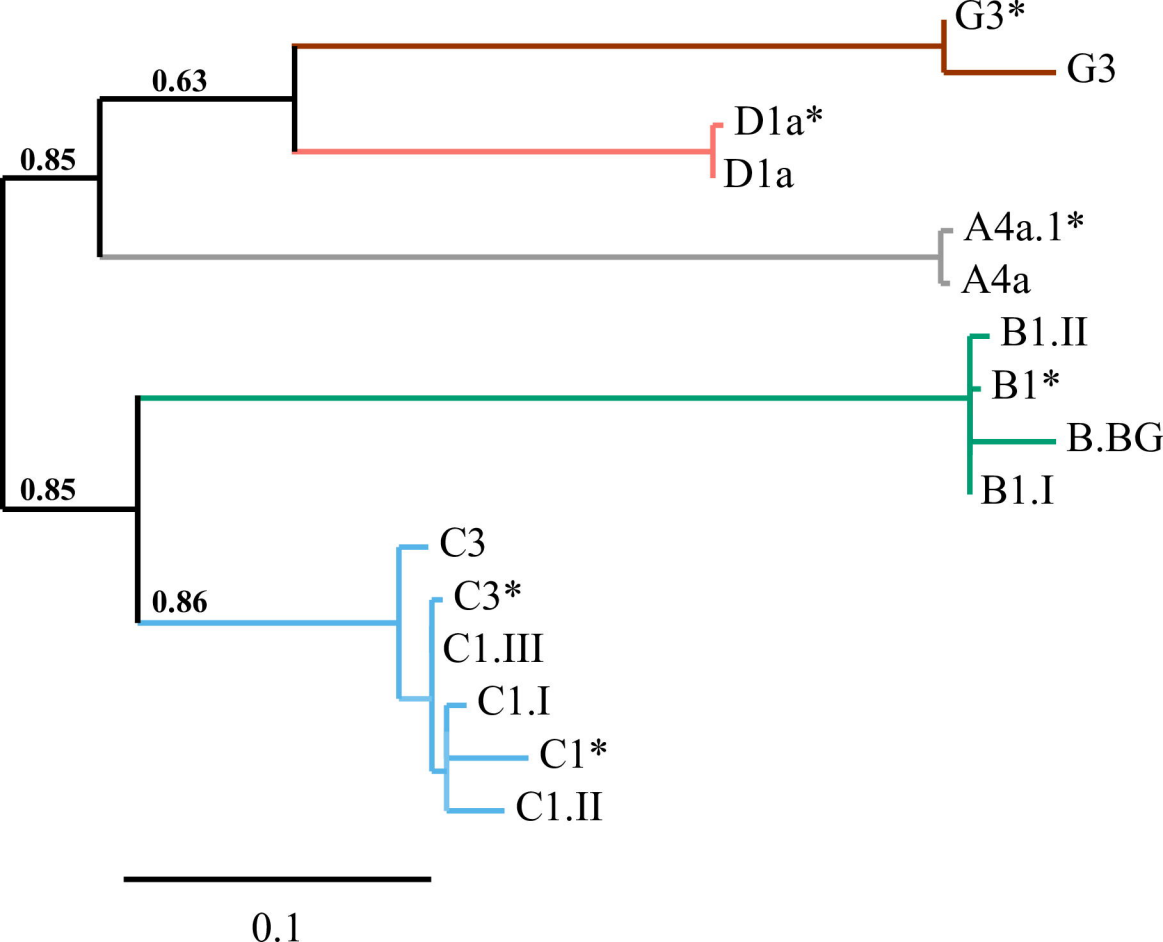
795

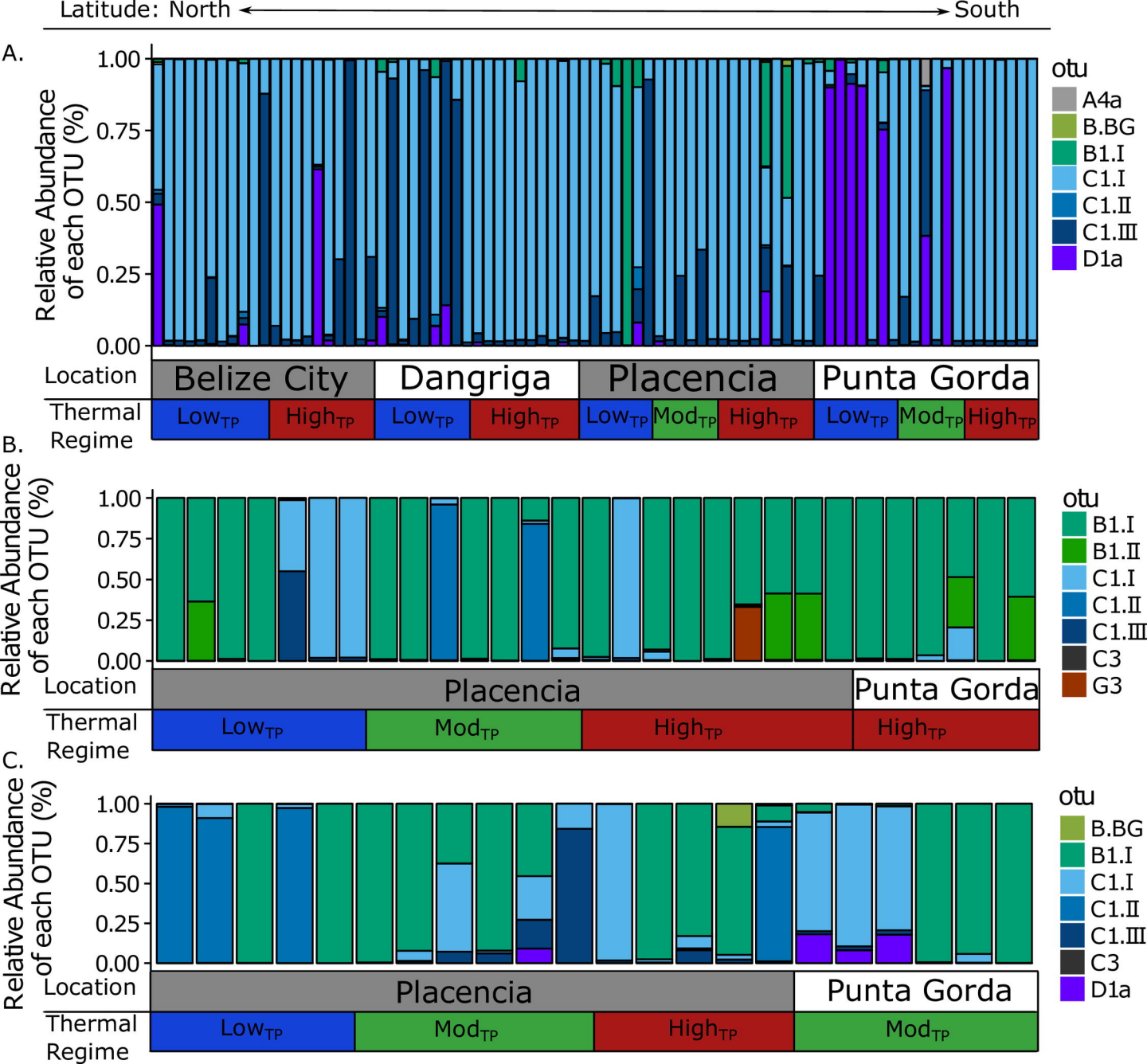
796 Fig 4. Principal component analysis (PCA) plots of *Symbiodinium* communities by species (A) and by
797 thermal regime for *S. siderea* (B). Percentages on each axis indicate the amount of variation explained by
798 each axis. Adonis *p-values* indicate significant results of PERMANOVA tests. See Table S4 for
799 additional PERMANOVA results. Black arrows indicate loadings showing the magnitude and direction of
800 the effect of each OTU on the total variance. Colored ellipses indicate 95% confidence intervals.

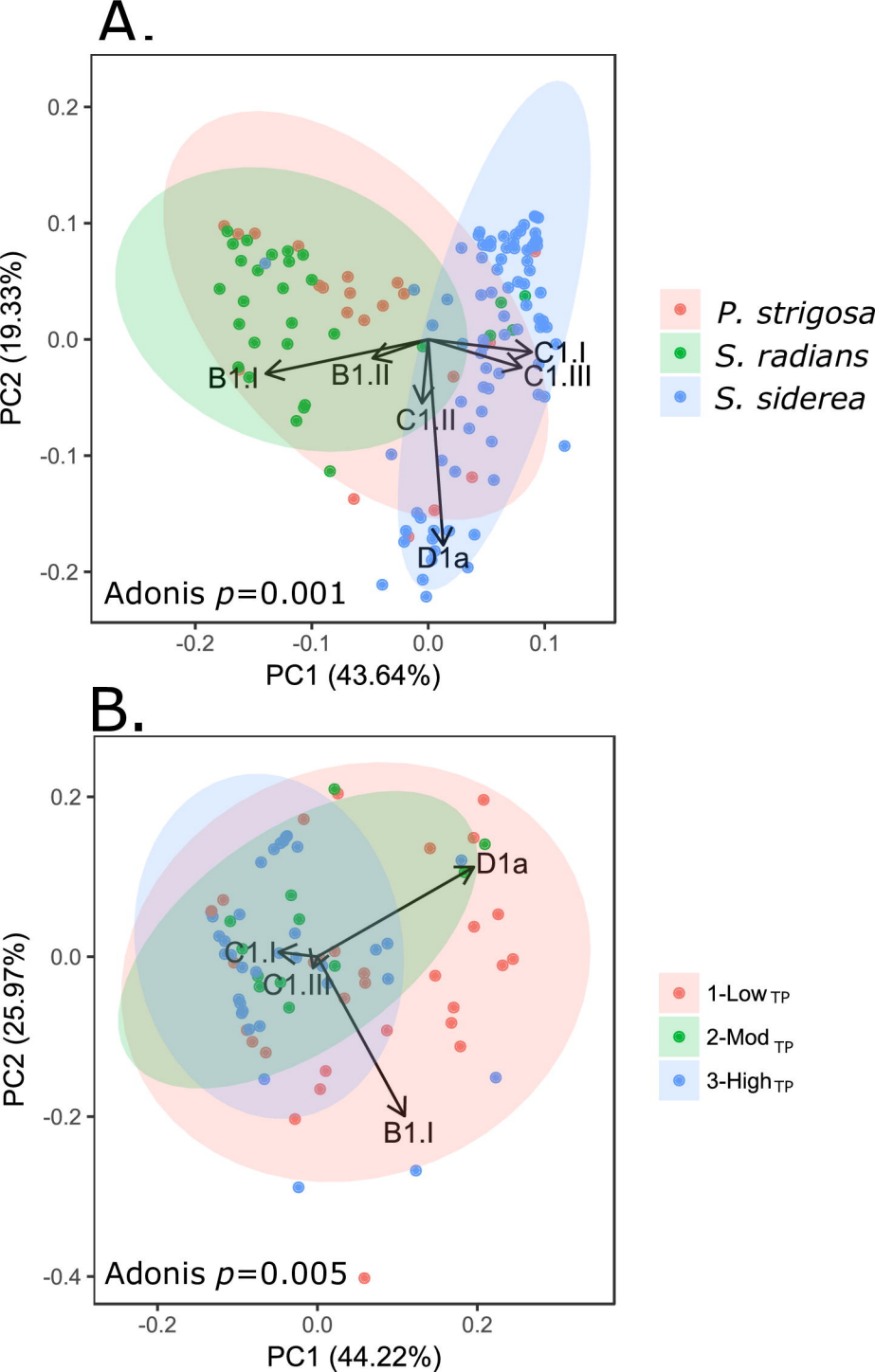
801

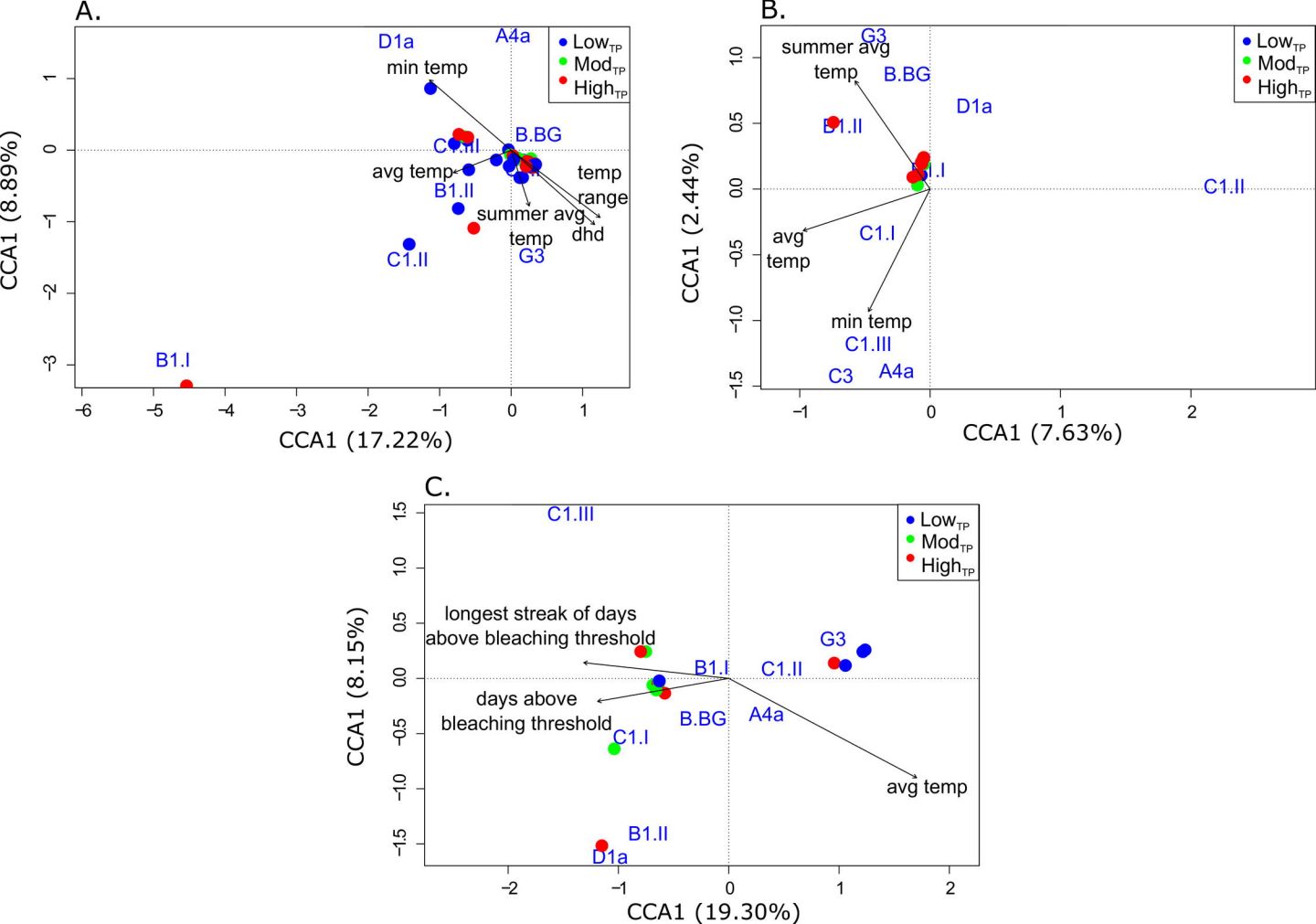
802 Fig 5. Canonical correlation analysis (CCA) showing relationship between thermal parameters (Table S1),
803 *Symbiodinium* lineages, and *Symbiodinium* communities within *S. siderea* (A), *S. radians* (B), and *P.*
804 *strigosa* (C). CCA scores for each sample are represented by a filled circle (colored by thermal regime)
805 and scores for each lineage are denoted by the name of each lineage. Percentages on each axis indicate the
806 total variation explained by that axis.

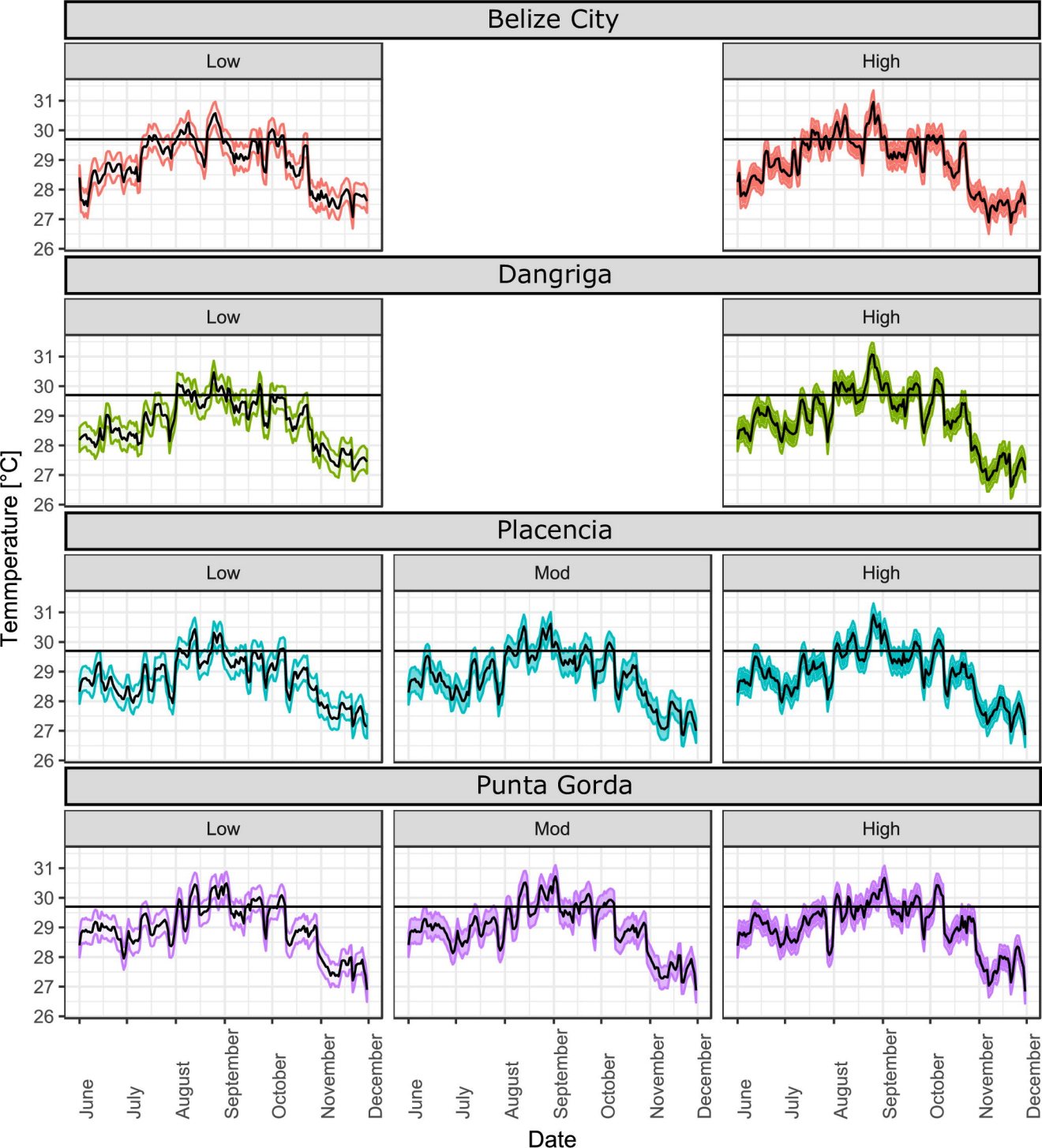

Mexico
N
W
E
S
Kilometers
0
15
30
60
Belize
Belize City
Sampling Sites
Low$_{TP}$
Mod$_{TP}$
High$_{TP}$
Dangriga
Placencia
Punta
Gorda
Guatemala
Honduras


G3*
G3
0.63
D1a*
D1a
0.85
A4a.1*
A4a
B1.II
B1*
B.BG
B1.I
0.85
C3
0.86
C3*
C1.III
C1.I
C1*
C1.II
0.1


Latitude: North
South
A.
Relative Abundance of each OTU (%)
1.00
0.75
0.50
0.25
0.00
otu
A4a
B.BG
B1.I
C1.I
C1.II
C1.III
D1a
Location
Belize City
Dangriga
Placencia
Punta Gorda
Thermal Regime
Low TP
High TP
Low TP
High TP
Low TP
Mod TP
High TP
Low TP
Mod TP
High TP
B.
Relative Abundance of each OTU (%)
1.00
0.75
0.50
0.25
0.00
otu
B1.I
B1.II
C1.I
C1.II
C1.III
C3
G3
Location
Placencia
Punta Gorda
Thermal Regime
Low TP
Mod TP
High TP
High TP
C.
Relative Abundance of each OTU (%)
1.00
0.75
0.50
0.25
0.00
otu
B.BG
B1.I
C1.I
C1.II
C1.III
C3
D1a
Location
Placencia
Punta Gorda
Thermal Regime
Low TP
Mod TP
High TP
Mod TP


A.
PC2 (19.33%)
PC1 (43.64%)
B1.I
B1.II
C1.I
C1.II
C1.III
D1a
Adonis p=0.001
P. strigosa
S. radians
S. siderea
B.
PC2 (25.97%)
PC1 (44.22%)
D1a
C1.I
C1.III
B1.I
Adonis p=0.005
1-Low TP
2-Mod TP
3-High TP


A.
D1a
A4a
min temp
Low$_{TP}$
Mod$_{TP}$
High$_{TP}$
B.BG
C1.III
avg temp
temp
range
B1.II
summer avg
temp
dhd
C1.II
G3
B1.I
CCA1 (8.89%)
CCA1 (17.22%)
B.
G3
summer avg
temp
B.BG
D1a
B1.II
C1.II
avg
temp
C1.I
min temp
C1.III
C3
A4a
CCA1 (2.44%)
CCA1 (7.63%)
C.
C1.III
longest streak of days
above bleaching threshold
G3
B1.I
C1.II
days above
bleaching threshold
B.BG
A4a
C1.I
avg temp
B1.II
D1a
CCA1 (8.15%)
CCA1 (19.30%)


Belize City
Low
High
Dangriga
Low
High
Placencia
Low
Mod
High
Punta Gorda
Low
Mod
High
Temmperature [°C]
31
30
29
28
27
26
June
July
August
September
October
November
December
Date