

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

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13

## 14 **Abstract**

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

## 37 **Introduction**

38 Obligate symbioses, relationships in which two or more organisms depend on one  
39 another for nutrition and survival, occur globally. Such symbioses are ubiquitous in plants and  
40 Mycorrhiza [1], ants and bacteria [2], and lichens [3]. The effects of climate change are expected  
41 to disrupt proper functioning of many symbioses, including that of reef-building corals [4-6].  
42 The success of coral reefs worldwide depends on the symbiosis between the coral host and  
43 photosynthetic algae of the genus *Symbiodinium* [7-9]. Under stressful conditions this coral-  
44 *Symbiodinium* relationship breaks down, resulting in the loss of endosymbiont cells and/or  
45 photosynthetic pigments from the coral tissue in a process known as ‘coral bleaching’ [10]. Coral  
46 bleaching is most commonly associated with thermal stress [11-15] and is predicted to increase  
47 in frequency and severity as the world’s climate continues to change [5, 16-21]. Increased  
48 thermal stress resulting from climate change combined with other local stressors such as  
49 eutrophication, habitat destruction, and overfishing has created an uncertain future for coral reefs  
50 [6, 13, 22]. In the Caribbean Sea, warming rates are higher than in any other tropical basin [23]  
51 and coral cover has declined by as much as 80% in recent decades [24]. It has been predicted that  
52 Caribbean coral reefs may suffer biannual bleaching events within the next 20-30 years [17] and  
53 annual bleaching by 2040 [25].

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

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

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

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

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

98         *Symbiodinium* communities have been shown to vary regionally [between reef systems;  
99 59, 68, 69], locally [within a reef system; 68], temporally [across time on the same reef; 70], and  
100 within a colony [69]. Studies of this variation have revealed geographically endemic lineages of  
101 *Symbiodinium* which may play a significant role in local and regional scale coral survival and  
102 stress tolerance [38, 69, 71]. While temperature stress may play a role in structuring  
103 *Symbiodinium* communities [72], variations in other environmental factors have also been shown  
104 to drive *Symbiodinium* community composition. For example, physical processes and total  
105 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<sub>TP</sub>, mod<sub>TP</sub>, high<sub>TP</sub>) and exhibited variations in coral species diversity and

129 richness [81], were selected. High<sub>TP</sub> sites (inshore) were characterized by larger annual  
130 temperature variation, higher annual maximum temperatures, and are exposed to temperatures  
131 above the regional bleaching threshold of 29.7°C (Aronson et al., 2002) more often than mod<sub>TP</sub>  
132 sites (mid-channel reefs) and low<sub>TP</sub> sites (offshore) [81]. High<sub>TP</sub> sites were dominated by stress  
133 tolerant and weedy coral species while corals representing all four coral life histories [stress  
134 tolerant, weedy, competitive, and generalist; 80] were present in low<sub>TP</sub> and mod<sub>TP</sub> sites [81].

135

### 136 *Sample Collection*

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

152 ice at  $-20^{\circ}\text{C}$ . Preserved microsamples were transported on ice to the coral ecophysiology lab at  
153 the University of North Carolina at Chapel Hill and stored at  $-20^{\circ}\text{C}$  until DNA isolation.

154

### 155 *Sea Surface Temperature*

156 Daily 1-km horizontal resolution sea surface temperature (SST) estimates were acquired  
157 from the NASA Jet Propulsion Laboratory's Multi-Scale High Resolution SST (JPL MUR SST)  
158 product via NOAA Environmental Research Division's Data Access Program (ERDDAP-  
159 <https://coastwatch.pfeg.noaa.gov/erddap/index.html>) [82] and analyzed following Baumann et al  
160 [81]. Briefly, SST data were binned by latitude and longitude for each site and annual values for  
161 maximum temperature, temperature range, number of days above the regional bleaching  
162 threshold ( $29.7^{\circ}\text{C}$ , Aronson et al. [83]), and longest streak of consecutive days above the  
163 bleaching threshold were calculated for each site and for the entire MBRS reef area. Using  
164 standard deviations from the mean for all four parameters, sites were classified as low<sub>TP</sub>, mod<sub>TP</sub>,  
165 and high<sub>TP</sub> [81]. Several additional temperature parameters were taken into account for this  
166 study, including: annual degree heating days (similar to degree heating weeks, as per Gleeson  
167 and Strong [84]), annual minimum temperature, annual average temperature, annual winter  
168 average temperature, and annual summer average temperature. Values for these parameters  
169 within the three thermal regimes are reported in Table S1.

170

### 171 *DNA Extraction*

172 Coral holobiont (coral, algae, and microbiome) DNA was isolated from each sample  
173 following a modified phenol-chloroform [83, 85, 86] method described in detail by Davies et al  
174 (2013). Briefly, DNA was isolated by immersing the tissue in digest buffer (100 mM NaCl,

175 10mM Tris-Cl pH 8.0, 25 mM EDTA pH 9.0, 0.5% SDS, 0.1 mgml<sup>-1</sup> Proteinase K, and 1 µgml<sup>-1</sup>  
176 RNaseA) for 1 h at 42°C followed by a standard phenol-chloroform extraction. Extracted DNA  
177 was confirmed on an agarose gel and quantified using a Nanodrop 2000 Spectrophotometer  
178 (Thermo Scientific).

179

### 180 *PCR amplification and metabarcoding*

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

192 Each 20µL PCR reaction contained 5-100 ng DNA template, 12.4 µL MilliQ H<sub>2</sub>O, 0.2  
193 µM dNTPs, 1µM forward and 1µM reverse primers, 1X *Extaq* buffer, and 0.5 U (units) *Extaq*  
194 polymerase (Takara Biotechnology). PCR cycles were run for all samples using the following  
195 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  
196 elongation step of 72°C for 7 min. The optimal number of PCR cycles for each sample was  
197 determined from visualization of a faint band on a 2% agarose gel (usually between 22 and 28

198 cycles) as per Quigley et al. (2014). PCR products were cleaned using GeneJET PCR  
199 purification kits (Fermentas Life Sciences) and then a second PCR reaction was performed to  
200 incorporate custom barcode-primer sequences [63] modified for Illumina Miseq as in Klepac et  
201 al. [88]. Custom barcode primer sequences included 5'-*Illumina adaptor* + 6 bp **barcode**  
202 **sequence** + one of two universal linkers-3' (e.g.: 5'- *CAAGCAGAAGACGGCATAACGAGAT* +  
203 **GTATAG** + *GTCTCGTGGGCTCGG*-3', or 5'- *AATGATACGGCGACCACCGAGATCTACAC*  
204 + **AGTCAA** + *TCGTCGGCAGCGTC*-3'). These universal linking barcoded adapters can be  
205 used to target any loci and therefore significantly reduce costs associated with Miseq sequencing  
206 of multiple loci. Following barcoding, PCR samples were visualized on a 2% agarose gel and  
207 pooled based on band intensity (to ensure equal contributions of each sample in the pool). The  
208 resulting pool was run on a 1% SYBR Green (Invitrogen) stained gel for 60 minutes at 90 volts  
209 and 120 mAmps. The target band was excised, soaked in 30 uL of milli-Q water overnight at  
210 4°C, and the supernatant was submitted for sequencing to the University of North Carolina at  
211 Chapel Hill High Throughput Sequencing Facility across two lanes of Illumina MiSeq (one  
212 2x250, one 2x300). The two lanes produced similar mapping efficiencies (73% and 73%,  
213 respectively; Table S3).

214

### 215 *Bioinformatic Pipeline*

216 The bioinformatic pipeline used here builds upon previous work by Quigley et al. [63]  
217 and Green et al. [71]. Raw sequences were renamed to retain sample information and then all  
218 forward (R1) and reverse (R2) sequences were concatenated into two files, which were processed  
219 using CD-HIT-OTU[89]. CD-HIT-OTU clusters concatenated reads into identical groups at  
220 100% similarity for identification of operational taxonomic units (OTUs). Each sample was then

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

225 The phylogeny of representative sequences of each distinct *Symbiodinium* OUT was  
226 constructed using the PhyML tool [90, 91] within Geneious version 10.0.5 (<http://geneious.com>)  
227 [92]. PhyML was run using the GTR+I model (chosen based on delta AIC values produced from  
228 jModelTest [90, 93]) to determine the maximum likelihood tree. The TreeDyn tool in  
229 Phylogeny.fr was used to view the tree (Fig 2) [94-96]. The reference sequences included in the  
230 phylogeny were accessed from GenBank (Table S6).

231

### 232 *Statistical Analysis*

233 OTU count analysis used the R [R Core97] package *MCMC.OTU* and followed methods  
234 described in Green et al. [71]. First, outlier samples with low sequence coverage (total log counts  
235  $\geq 2.5$  standard deviations below the mean of all samples) were identified and removed, which  
236 removed 3 samples. Next, rare OTUs ( $< 0.1\%$  of the global sum of counts [as per 63]) were  
237 identified and discarded leaving 56 of the original 5,132 OTUs. Many remaining OTUs were  
238 identified as having the same *Symbiodinium* lineage (i.e., C1 or D1a) and these OTUs were  
239 regressed against one another. Positive correlations between OTUs within a lineage may indicate  
240 paralogous loci from the same genome [36, 71]. As a result, reads from OTUs within the same  
241 lineage that showed a positive  $R^2$  and significant  $p$ -value following linear regression were pooled  
242 in order to control for possible overestimation of biodiversity [98]. Pooling resulted in a final  
243 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<sub>TP</sub> which were collected in 2015; Fig 3). *Symbiodinium* D1a (*S.*  
269 *trenchi*) was most abundant in *S. siderea* at low<sub>TP</sub> sites, particularly the low<sub>TP</sub> 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<sub>TP</sub> 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<sub>TP</sub> 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<sub>TP</sub> and high<sub>TP</sub> sites and C1.II was the  
283 second most abundant lineage in the low<sub>TP</sub> 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<sub>TP</sub> sites had higher *chl-a* than low<sub>TP</sub> 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 et 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<sub>TP</sub> and mod<sub>TP</sub> 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

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

399

## 400 **Conclusion**

401 This study demonstrates that *Symbiodinium* communities associated with corals in Belize  
402 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<sub>TP</sub> sites indicates thermal  
410 tolerance at these sites must be conferred through alternative mechanisms, such as local  
411 adaptation.

412

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769 **Tables and Figures**

770 Table 1: Sampling locations and microsamples information for *S. siderea* (SSID), *S. radians* (SRAD), and *P.*  
771 *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

772

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

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

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## 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<sub>TP</sub>, mod<sub>TP</sub>, and  
781 high<sub>TP</sub> 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<sub>TP</sub> sites exhibit the lowest values for all parameters measured and high<sub>TP</sub> 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<sub>TP</sub> sites, green boxes indicate mod<sub>TP</sub> sites, and red boxes indicates high<sub>TP</sub> 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



Kilometers



Belize

Belize City

★ Sampling Sites

- LOW<sub>TP</sub>
- Mod<sub>TP</sub>
- High<sub>TP</sub>

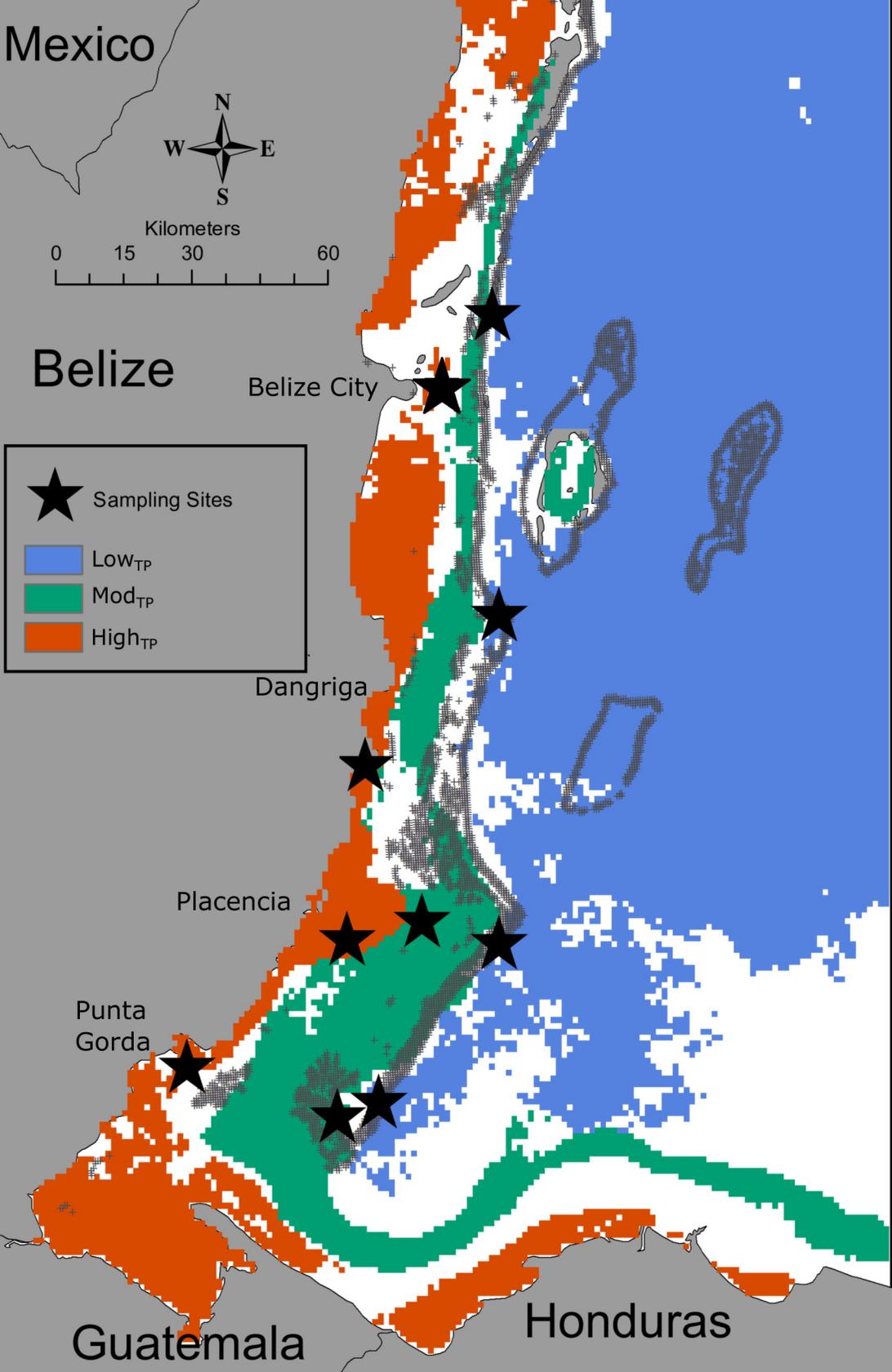
Dangriga

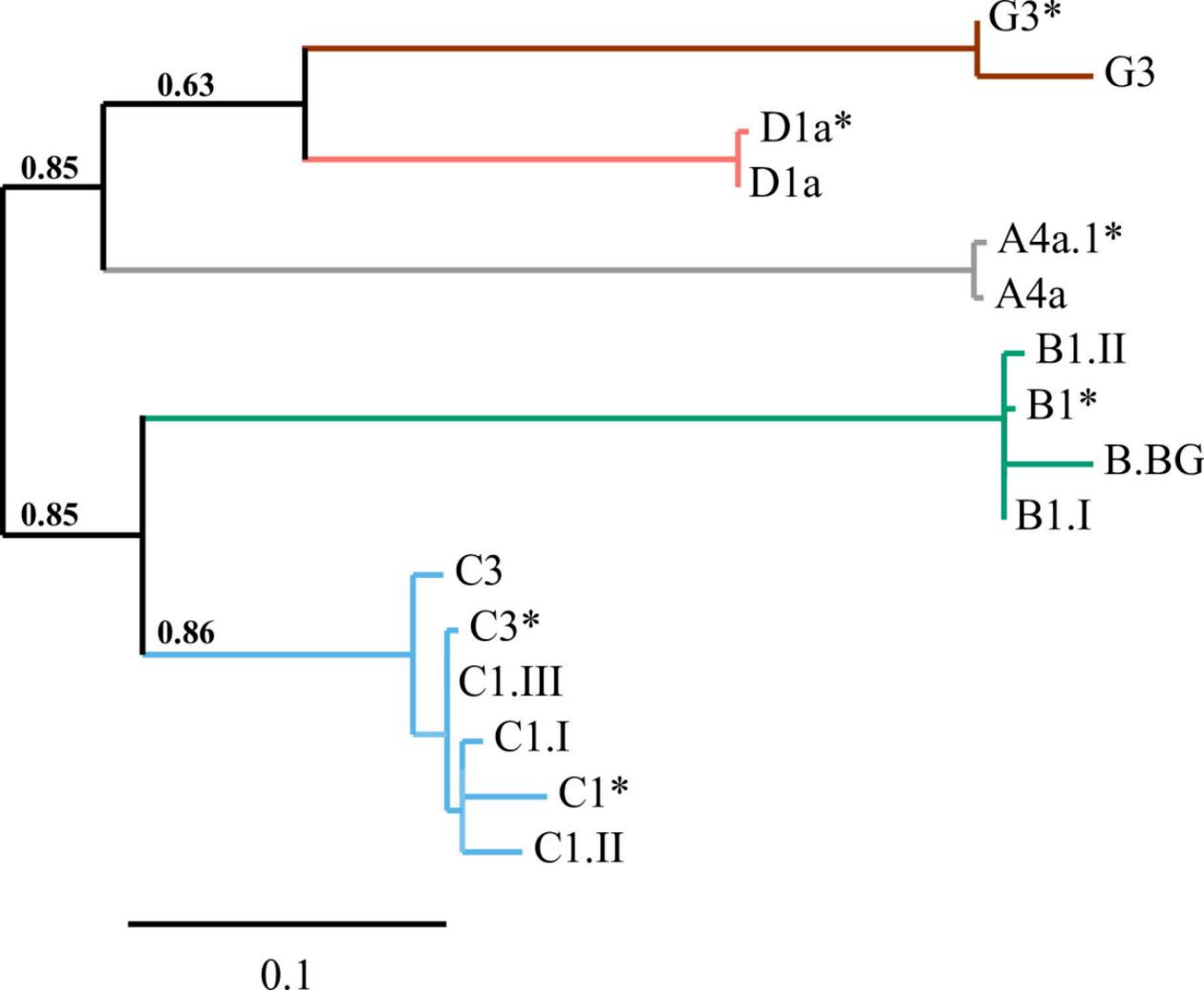
Placencia

Punta Gorda

Guatemala

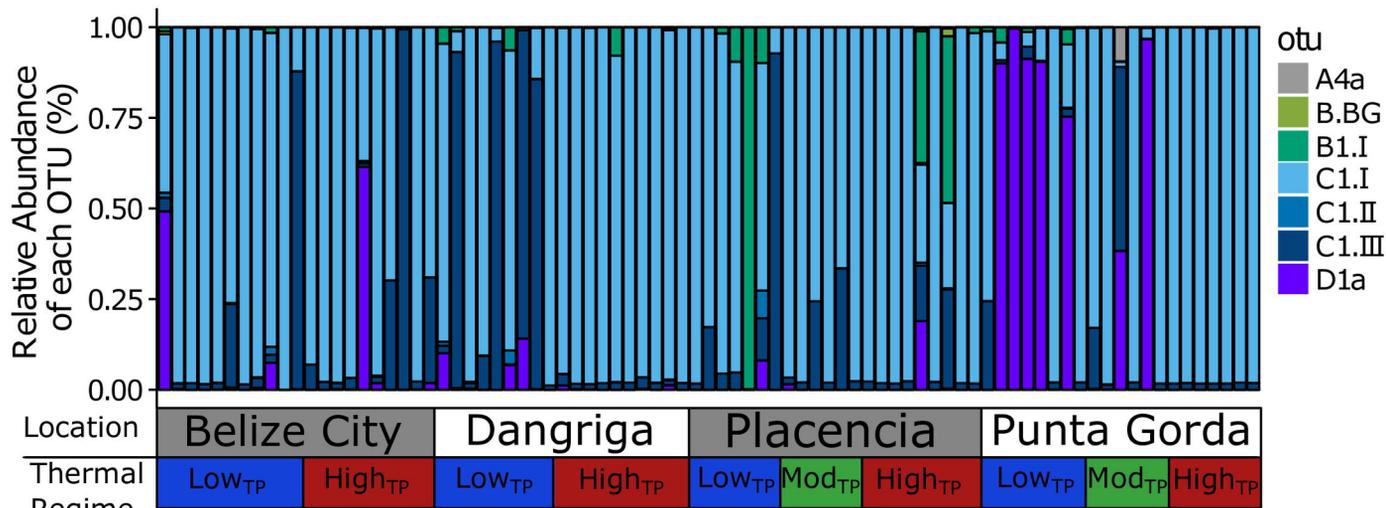
Honduras



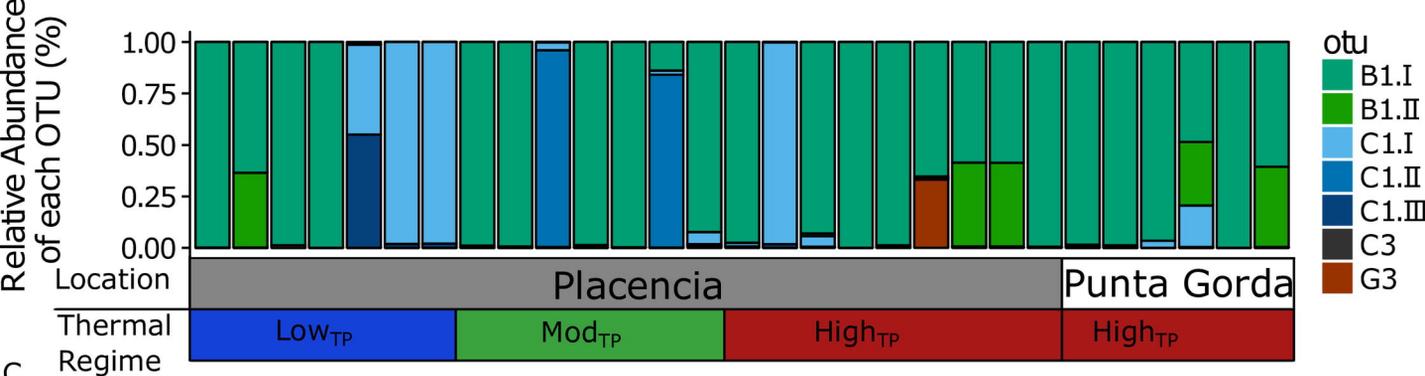


Latitude: North ← → South

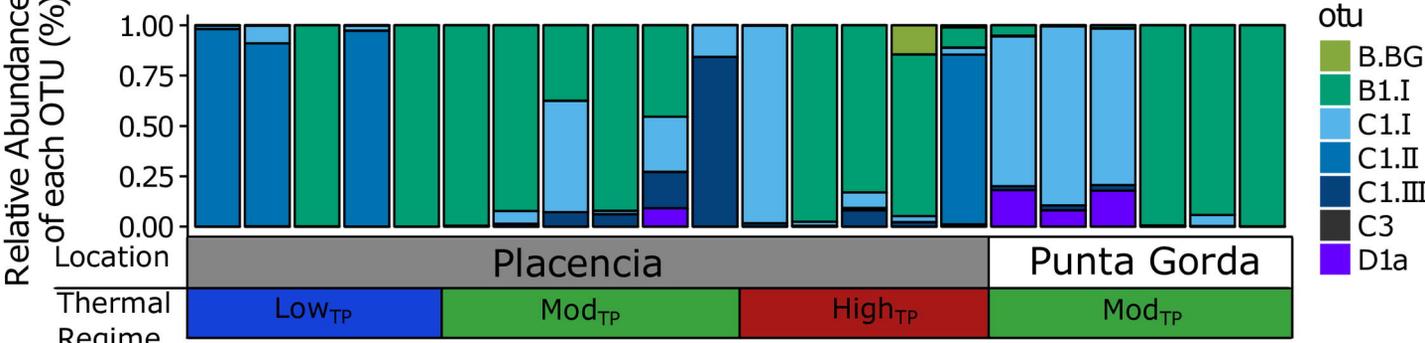
A.

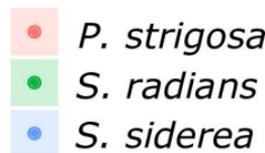
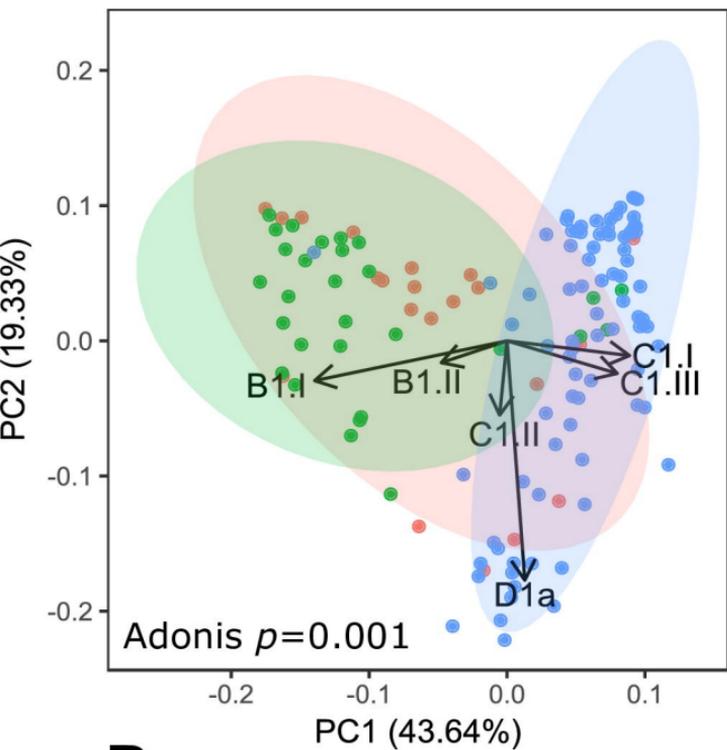


B.



C.



**A.****B.**