Algal symbioses with fire corals demonstrate host genotype specificity and niche adaptation at subspecies resolution

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Abstract

Corals share an intimate relationship with photosynthetic dinoflagellates that contribute to the biology of the emerging metaorganism. While many coral-algal associations exhibit high host fidelity, the extent of this specificity under environmental change remains to be fully understood and is a prerequisite to forecasting the adaptive potential of this obligate symbiosis. Here, we disentangled the contribution of host genotype and environment on governing coral-algae associations by working at subspecies resolution. We used fine-scale genotyping of algal symbionts from 198 fire coral colonies (*Millepora* cf. *platyphylla)* that map to ten distinct sexually produced clonal host genotypes across three environmentally distinct reef habitats. Based on microalgal ITS2 genotyping, we show that algal-host specificity extends down to the Symbiodiniaceae subspecies level in a natural reef environment. Closely related *Symbiodinium* (A7)-dominated algal assemblages almost perfectly mapped to fire coral host genotype. Furthermore, identification of host genotype- and habitat-specific *Symbiodinium* alga suggest the presence of algal phenotypic diversity even at this taxonomic resolution (i.e., within *Symbiodinium* A7), which may aid environmental niche adaptation of the metaorganism. Our results suggest that the here-identified *Millepora*-*Symbiodinium* associations are co-evolved to match their prevailing environment. Thus, despite the presence of rarer host generalist *Cladocopium* algae, scope for environmentally induced modification of the cnidarian-algal association is likely constrained by host genotype.

Introduction

Symbiotic microorganisms are increasingly recognized as foundational components of animal hosts, thereby impacting the ecology and physiology of their hosts[1]. Reef-building corals (among other cnidarians) form an obligate endosymbiosis with dinoflagellate algae of the family Symbiodiniaceae[2]. The algal symbionts satisfy up to 95% of their host energetic requirements[3,4], while the coral host provides a protected light-rich environment and supply of nutrients[5]. This symbiosis creates the foundation of coral reef ecosystems and is one of the oldest, most widespread, and ecologically successful known mutualisms found in nature[6]. The rapid pace of environmental change caused by climate warming and other stressors is now posing a serious threat to coral reef ecosystems and has been linked to numerous occurrences of coral bleaching (severe loss of algal symbionts under stressful conditions)[7,8].

Algal identity is an important factor contributing to variability of stress tolerance in corals[9-12]. This variability is likely in part due to the considerable genetic and phenotypic diversity found within each of the most common coral-associating algal genera (*Symbiodinium*, *Breviolum*, *Cladocopium*, and *Durusdinium*[13]) characterized by their own physiological and ecological traits[2,14-16]. Such phenotypic diversity means that certain assemblages are better suited for specific prevailing environments than others, as evidenced by the observed environmental structuring of coral-algal associations[17-20]. Changing algal associations may therefore act as a mechanism of ecological adaptation/acclimatization. However, the degree to which corals are able to modify their dominant algal symbiont association appears dependent on the host and algal genotype. Notably, host and symbiont specialists and generalists exist[18,21-23]. There are many examples of coral colonies exhibiting fixed algal associations under environmental change [24-27]. Conversely, environmentally induced changes from a predominant alga of one genus to a different genus are documented and are exemplified by the appearance of the stress-

72 tolerant *Durusdinum trenchii*[12,28]. However, those changes are typically transient and revert
73 back to the original association when stress subsides[14,27,29]. These contrasting examples
74 highlight that the identity of a given coral-algal assemblage is therefore a consequence of
75 host genotype and environment.
76
77 Disentangling the influence of host genotype and environment on coral-algal associations
78 is challenging, since host genotype needs to be controlled for in the sampling design
79 and/or experimental setup. Further, methods used for algal genotyping must be able to
80 resolve differences at the desired taxonomic resolution[30]. To date, host genotype has often
81 been controlled for at the species level or even higher (e.g., genus), demonstrating host-
82 specificity in most cases[31-33]. However, the relevance of any conclusions on host-specificity
83 by such studies is limited to the resolution at which the host has been resolved. Thus, to
84 investigate whether host fidelity can also be observed below the species level (i.e.,
85 genotype), subspecies resolution of the host is required. Further, individuals harboring
86 the same genotype must be distributed and assessed across different environments to
87 disentangle the relative contribution of host genotype and environment to prevalent host-
88 algal associations. This has been addressed by investigating algal associations of clonal
89 coral replicates transplanted across environments or captively reared, showing that
90 Symbiodiniaceae assemblages are highly host genotype-specific[21,34,35].
91
92 Here, we ITS2-genotyped the algal assemblage of 198 hydrocoral samples (*Millepora* cf.
93 *platyphylla*) previously characterized to belong to ten different clonal genotypes with
94 members of each genotype distributed across at least two environmentally distinct reef
95 habitats. In doing so, we could assess algal associations across environments in
96 dependence on host genotype in a natural population at a subspecies level. We
97 demonstrate that algal-host specificity extends down to the subspecies level of host and
98 symbiont. This argues for phenotypic diversity even between highly similar algae and

99 implies that host-algal symbiotic associations are likely constrained at the level of
100 individual genotypes.
101

102 **Results**

103 **Fire coral clones are exposed to distinct environmental conditions**

104 To discriminate the relative contribution of host genotype and environment on
105 structuring coral-algal associations, we used microalgal ITS2 genotyping from ten clonal
106 genotypes of the fire coral *M.* cf. *platyphylla* distributed across three reef habitats (mid
107 slope, upper slope, back reef) that exhibit contrasting environmental conditions, as shown
108 by temperature and light data obtained from *in situ* deployed loggers (Fig. 1). Although
109 temperature profiles showed a similar daily mean water temperature across the three
110 habitats (back reef, BR: 26.89 ± 0.07°C, upper slope, UP: 26.79 ± 0.05°C, mid slope, MD:
111 26.74 ± 0.04°C), they exhibited a three- to four-fold greater diel amplitude at the back reef
112 (1.37 ± 0.43°C) compared to both fore reef habitats (UP: 0.45 ± 0.18°C, MD: 0.34 ± 0.15°C).
113 Daily maximum temperatures were significantly higher at the back reef (BR: 27.71 ±
114 0.08°C, UP: 27.05 ± 0.06°C, MD: 26.92 ± 0.05°C, Kruskal-Wallis, $P < 0.001$) (Supplementary
115 Fig. 2). Light intensity profiles revealed a similar pattern with significantly higher daily
116 mean and maximum irradiance at the back reef (446.28 ± 20.99 and 2 271.69 ± 63.80 µmol
117 photons/m^2/s, respectively) compared to the upper slope (266.57 ± 13.36 and 1 371.83 ±
118 46.54 µmol photons/m^2/s) and the mid slope (137.50 ± 6.82 and 726.38 ± 22.55 µmol
119 photons/m^2/s, Kruskal-Wallis, $P < 0.001$) (Supplementary Fig. 2).

120 **Symbiodiniaceae ITS2 sequence assemblage**

121 From the 198 fire coral samples, a total of 419 post-MED Symbiodiniaceae sequences were
122 retrieved from the sequencing of the ITS2 amplicons that belonged to six genera:
123 *Symbiodinium* (n = 107), *Cladocopium* (n = 276), *Durusdinium* (n = 2), *Gerakladium* (n = 27),
124 *Fugacium* (n = 4), and *Halluxium* (n = 3) (corresponding to previously described clades A,

125 C, D, G, F, and H, respectively)[4] (Supplementary Data 2). Eight fire coral samples
126 contained sequences representing at least three genera, 47 contained sequences
127 representing two genera (44 associations of *Symbiodinium*-*Cladocopium* and 3 of
128 *Symbiodinium*-*Gerakladium*), and 143 samples contained sequences from a single genus (of
129 which all were from *Symbiodinium*). The majority of sequences were from *Symbiodinium*
130 (Fig. 2A), accounting for 99.8% of the total Symbiodiniaceae reads and for at least 92% of
131 reads per sample. Six (out of 107) *Symbiodinium* sequences occurred in all 198 samples: A7
132 (the most abundant across all samples ranging from 30 to 59% of reads per sample), A16d
133 (7–27%), A3 (1–9%), A3a (1–8%), A7b (1–6%), and A7a (1–5%) (Fig. 2B). *Cladocopium*
134 sequences were present in 52 of the 198 samples (26%) and in those samples accounted
135 for at most 8% of reads. The two most common *Cladocopium* sequences, C15 and C116,
136 occurred at a maximum relative abundance of 5% and 3% of Symbiodiniaceae reads,
137 respectively. Differences in the rDNA copy number between different taxa may confound
138 the translation of Symbiodiniaceae ITS2 counts to actual relative abundances of algal cells
139 – especially for inter-genera comparisons. Current estimates suggest *Cladocopium* has a
140 higher copy number than *Symbiodinium* (means of 2119 ± 217 and 1721 ± 125, taken
141 respectively from Saad et al.[36]). As such, the greater abundance of *Symbiodinium* compared
142 to *Cladocopium* ITS2 reads observed in our samples likely relate to a higher abundance of
143 *Symbiodinium* cells.
144
145 **Symbiodiniaceae ITS2 profiles**
146 SymPortal identified a total of 18 ITS2 profiles (used as a proxy for distinct
147 Symbiodiniaceae genotypes, i.e. genetically distinct entities putatively belonging to one
148 or more species within each of the genera) defined by 56 DIVs that belonged to three
149 Symbiodiniaceae genera: *Symbiodinium* (7 profiles), *Cladocopium* (10 profiles), and
150 *Gerakladium* (1 profile; Supplementary Data 3). The one *Gerakladium* profile was not
151 considered further in the analysis as it only occurred in a single sample at an abundance

152 < 0.01%. The fire corals in this study were dominated by *Symbiodinium* profiles with each
153 of the 198 samples predicted to contain only one of the identified *Symbiodinium* profiles
154 (Fig. 2C). *Symbiodinium* profiles were all highly similar with each of the above mentioned
155 six common ITS2 sequences (A7, A16d, A3, A3a, A7b, and A7a) found in at least two of
156 the profiles and with ITS2 sequences A7 and A16d found in all profiles. The profiles were
157 unevenly distributed across samples with three of the most similar profiles found in 82%
158 of the samples (Fig. 2C). In order of abundance, the five most abundant ITS2 profiles were
159 found in 88, 47, 30, 21, and 10 of the fire coral samples with the remaining two ITS2 profiles
160 (i.e., putative genotypes) found in only one fire coral sample each (Fig. 2C). Of the five
161 most abundant *Symbiodinium* profiles found in this study (Supplementary Data 3), only
162 one was found in other samples in the SymPortal remote database. These were two
163 *Millepora* cf. *platyphylla* samples collected in the same reef system as this study, but three
164 years later (November 2016), as part of the *Tara* Pacific Expedition[37]. Finally, the two ITS2
165 profiles found in only one sample from this study were found in 43 and 2 samples from
166 the SymPortal database also collected as part of the *Tara* Pacific Expedition in a separate
167 sampling effort by E. Boissin in November 2015 (*M.* cf. *platyphylla*; unpublished). While
168 the majority of *Tara* Pacific samples were collected in the same reef as this study's samples,
169 6 of the 43 were collected from reefs off the coast of Fiji over 3000 km away.
170
171 Ten unique *Cladocopium* profiles were found in 15 of the samples and were always below
172 9% relative abundance (Supplementary Fig. 3). Of these ten ITS2 profiles, six were defined
173 by only 1 or 2 DIVs indicating that SymPortal likely did not sample the *Cladocopium*
174 genotypes they represent a sufficient number of times (i.e., they were not present in a
175 sufficient number of samples) to be able to generate profiles with a greater number of
176 DIVs that would be more specific representatives of these genotypes. However, four of
177 the profiles (characterized by the C91, C15, C1, and C116 sequences as the respective most
178 abundant sequences) were defined by at least 4 DIVs suggesting that they were likely

179 specific representatives of the underlying *Cladocopium* genotypes. These *Cladocopium*
180 profiles were found in 4, 3, 1, and 1 of the samples from this study (Supplementary Data
181 3) and in 0, 2, 96, and 1 samples from other studies in the remote SymPortal database.
182 Unfortunately, only two of these samples from the other studies (both containing the C15
183 profile) are publicly available. These two samples derive from *Stylophora pistillata*, one
184 from the northern Red Sea and one from the central Red Sea[38].

185 **High fidelity of *Millepora* host genotypes and algal assemblages**

186 The explanatory power of host genotype and reef habitat variables on the variation in
187 Symbiodiniaceae community composition was assessed. Differences in ITS2 sequence
188 assemblage (i.e., total taxonomic composition) were predominantly explained by host
189 genotype (two-way PERMANOVA, F = 119.5, R^2 = 0.832, P < 0.001), but also by the
190 interaction of host genotype and reef habitat (F = 3.6, R^2 = 0.034, P < 0.001). Accordingly,
191 samples demonstrated a discrete grouping by host genotype in the PCoA with only three
192 exceptions: G4 samples grouped by host genotype and habitat, two G3 samples grouped
193 with G4 back reef and upper slope samples and with one G4 upper slope sample grouping
194 reciprocally, and the G6 and G8 samples overlapped in their ordination coordinates in the
195 first three PCs. However, G6 and G8 samples were clearly differentiated by the presence
196 of the A7q ITS2 sequence in all of only the G8 samples (Fig. 2B). Genotype-specific
197 *Symbiodinium* sequences were also identified (29 in total and ranging from 1 to 8 per
198 genotype; Supplementary Data 2). Of note, reef habitat was also significant in explaining
199 ITS2 sequence assemblage (i.e., total taxonomic composition) although with a much
200 weaker explanatory power than host genotype (two-way PERMANOVA result; F = 5.574,
201 R^2 = 0.009, P < 0.001).
202
203 In support of the ITS2 sequence-based analysis, the ITS2 profile analysis predicted
204 discrete profiles for all G10 samples (genotype-specific) and the G4 mid slope samples

205 (genotype- and habitat-specific with the unique DIV A7n) (Fig. 2B and 2C). However,
206 other abundant algal profiles were present in multiple host genotypes. The clear (host
207 genotype-specific ITS2 sequences) and consistent (found across multiple samples from
208 each host genotype) pattern of algal assemblage differentiation between members of
209 different host genotypes seen in the sequence-based analysis suggests that the process of
210 ITS2 profile prediction (in SymPortal) did not resolve the likely host-specific algal
211 genotypes.
212
213 Mantel tests further confirmed host genotype specific algal assemblages by revealing a
214 significant positive correlation between Symbiodiniaceae community composition
215 dissimilarities and host genetic distances ($r = 0.3258$, $P < 0.001$). In other words, genetically
216 more distant host genotypes were associated with more dissimilar Symbiodiniaceae
217 communities (Supplementary Fig. 4). For instance, genotypes G5 and G7 (34 samples)
218 were closely related genetically (Supplementary Fig. 1) and characterized by similar
219 Symbiodiniaceae communities (Fig. 3), including association with the same *Symbiodinium*
220 profiles (Fig. 2C). Correspondingly, genetically divergent genotype G10 (Supplementary
221 Fig. 1) was uniquely and solely associated with one ITS2 profile that was genetically
222 distinct based on average relative abundance of defining intragenomic variants (Fig. 2C).
223
224 **Symbiodiniaceae community dissimilarity correlates with fine-scale spatial distance of**
225 ***Millepora* colonies**
226 The sampling design with georeferenced clonal colonies enabled us to investigate
227 Symbiodiniaceae community composition of fire coral genotypes across small spatial
228 scales. From ten mantel tests performed for each of the ten host genotypes, a significant
229 positive correlation between the Symbiodiniaceae community composition dissimilarity
230 and the spatial distance between clones across reef habitats (Euclidean distance in meters
231 based on x and y spatial coordinates of each clone) was demonstrated for five of the

232 genotypes (G1, G3, G4, G6, G8). In other words, more spatially distant clones were
233 associated with more dissimilar Symbiodiniaceae communities (Supplementary Table 1).
234 However, because these tests contained clones distributed in multiple habitats, the effect
235 of spatial distance is confounded with the effects of reef habitat. To separate the effect of
236 spatial distance from reef habitat, we performed the same correlations, but for distinct
237 genotype-habitat combinations (Supplementary Table 1). Significant positive correlations
238 were returned for three of the twenty tests (Fig. 4) demonstrating fine-scale spatial
239 variation (distances ranging from 0.07 to 20 meters) in algal assemblage even between
240 clones of the same habitat.

241 **Discussion**

242 Identifying coral-algal symbiosis specificity and the extent to which the host and its
243 symbionts co-diversify in response to environmental change is essential for forecasting
244 how this obligate symbiosis may promote or restrict the potential for coral adaptation
245 under climate change[39]. Numerous studies have convincingly demonstrated the
246 importance of host identity on Symbiodiniaceae assemblage[31-33], including
247 environmentally induced variations in symbiont composition[40-42]. Yet studies that are able
248 to disentangle the relative influence of animal host and environment on partner pairing
249 dynamics below the species level by controlling for host genotype are missing at large.
250 Here, we assessed the extent of environmental-induced variations in Symbiodiniaceae
251 community composition in reef-building corals, while controlling for host genotype at a
252 clonal level without the manipulation of corals through transplantation or captive rearing.
253
254 Species definitions within the Symbiodiniaceae are becoming more common, and recent
255 definitions are starting to formally recognize the large extant algal richness. However, the
256 majority of coral-associating algal symbionts still lack a formal definition[30,43].
257 Unfortunately, many of the existing formal taxonomic descriptions lack information

258 regarding ITS2 sequences. For those definitions that do exist, they are often characterized
259 by on average one to three dominant ITS2 sequences (e.g., one or two dominant sequences
260 for *Symbiodinium*[44,45] and *Cladocopium*[46,47], and one to three for *Durusdinium*[48]). Thus,
261 characterization of our samples goes far beyond these two-sequence definitions.
262
263 Our study revealed that algal assemblage maps almost perfectly to host genotype, at a
264 subspecies host and algal resolution (Fig. 2 and 3). While we observed disagreement
265 between the ITS2 sequence- and profile-based approaches, this is likely due to the lack of
266 resolving power at this fine-scale level by SymPortal. Rather, our results suggest that a
267 combination of sequence-based and profile-based analyses as employed here in some
268 cases are preferable to yield the resolutive power needed to identify patterns at this fine-
269 scale resolution. Here we can show that the importance of host identity in structuring
270 coral-algal assemblages extends to the level of subspecies genotype. The strong host
271 genotype fidelity observed is consistent with previous reports showing that host identity
272 largely determines the physiological response of the symbiont, as well as the stability and
273 functionality of the symbiosis to changing environmental conditions[35,49]. Notably,
274 variations in the physiological response to increased temperature within a single
275 Symbiodiniaceae species or clonal host-symbiont combinations have been previously
276 described in temperate corals[16] and Aiptasia[49], but are rare for tropical corals[50].
277
278 Our study also showed that host genetic similarity aligns with Symbiodiniaceae
279 assemblage similarity, irrespective of host habitat, which is often expected for species that
280 acquire their symbionts via vertical transmission[51]. Unlike many broadcast spawning
281 corals, *Millepora* hydrocorals can vertically transmit their algal symbionts to their eggs[52],
282 thus exhibiting a mixed mode of symbiont acquisition. This may explain, at least to some
283 degree, the strong host genotype specificity observed for *Millepora*-associated
284 *Symbiodinium* algae. The establishment and maintenance of such specific coral-algae

285 associations likely involve cell-signaling and other inter-partner recognition mechanisms,
286 so that intracellular symbionts may co-evolve with the host to become particularly well
287 suited to meet the metabolic demands of their host's micro-environment. *Symbiodinium*
288 characterized with their most abundant ITS2 sequences of A7 (hereafter simply referred
289 to as *Symbiodinium* A7, and a format we will use for describing other alga associated to
290 specific ITS2 sequences) is known to associate with hydrozoans, but not with anthozoans
291 in the Pacific Ocean[53]. This suggests that this alga might be highly host-specialized due to
292 co-evolutionary processes with *Millepora* hydrocorals. Many members of the
293 *Symbiodinium* genus are hosted by corals found in variable environments[54] and are often
294 associated with increased tolerance to high irradiance and temperature[38,45]. Accordingly,
295 fire corals have been described as thermotolerant species during climate-driven marine
296 heat waves in French Polynesia, although many colonies bleached and died from the
297 recent massive bleaching event of 2019[52]. This thermal tolerance might be linked to their
298 partnership with specific *Symbiodinium* algae, as previously reported for bleaching-
299 resistant Caribbean *Millepora* species[55].
300
301 Despite a symbiont community dominated by *Symbiodinium*, our results revealed that *M.*
302 cf. *platyphylla* can also associate with a variety of *Cladocopium* algae, as seen previously in
303 the Pacific Ocean[31,56]. Samples from our study associated with *Cladocopium* C91, C15, C1,
304 and C116, while *Millepora* in the wider Pacific were reported to associate with C57 and
305 C66. The presence of these rarer *Cladocopium* algae may be explained by *Millepora*'s
306 uncommon life history trait combination of broadcast spawning of medusoids (which
307 then release sexual gametes) with a (non-strictly) vertically transmitting symbiosis. The
308 increased duration of time larvae spend in the water column may allow for an increased
309 exposure to exogenous algal symbionts compared to strictly vertically transmitting
310 symbiosis (typical of brooders). If host uptake mechanisms allow, this may enable the
311 larvae to associate with symbionts from the water column that are not present in the

312 maternal colony[57]. Of particular interest is the apparent cosmopolitan and host-generalist
313 nature of at least one (a *Cladocopium* C15), but possibly more of the *Cladocopium* algae that
314 *Millepora* associated with in this study (the genotypes represented by the *Cladocopium* C1
315 and C116 profiles were found in additional samples from the SymPortal database). These
316 associations represent a contrast to the apparent host-specific *Symbiodinium* algae that
317 otherwise dominated the *Millepora* assemblages. Association with *Cladocopium* algae may
318 allow fire corals to respond more promptly to changes in environmental conditions across
319 reefs through shuffling of background species[19,58,59] and an increase in the number of
320 different genera hosted by coral has been posited to be a possible response to climatic
321 perturbations[38].
322
323 Although host genotype was the main factor explaining variance in Symbiodiniaceae
324 assemblage structuring, the interaction of reef habitat with host genotype and reef habitat
325 alone returned significant results in statistical testing, although with a much-reduced
326 strength of correlation. In addition, our fine-scale mapping of fire corals on the reef
327 substratum revealed that for three of the genotypes (Fig. 4), colonies separated by smaller
328 spatial distances associate with more similar Symbiodiniaceae communities compared to
329 more distant colonies. This suggests that host micro-habitat may influence coral-algal
330 structuring, adding to the potential for fine-scale environmentally induced variations (i.e.,
331 isolation by distance (IBD) within reef habitat). In line with this environmental effect, we
332 identified one habitat-specific (i.e., endemic) *Symbiodinium* profile (with the unique DIV
333 A7n) in all clones belonging to the host genotype G4 from the mid slope, while those on
334 the upper slope and back reef were associated with other *Symbiodinium* profiles. This
335 result suggests a phenotypic diversity within these closely related algae upon which
336 natural selection may act, similar to previously reported within species phenotypic
337 diversity but at a more fine-scale resolution[56,60], enabling niche adaptation of the coral-
338 algal association. Specifically, this habitat-endemic *Symbiodinium* genotype may be

339 functionally distinct and adapted to the environmental conditions characterizing the mid
340 slope habitat, e.g. a lower irradiance, though detailed characterization of this functional
341 diversity to understand its potential role as a substrate for ecological adaptation requires
342 dedicated investigations characterizing the physiological performance of these host-algal
343 associations, such as light harvesting, carbon production, and nutrient translocation.
344 Interestingly, no *Symbiodinium* genotypes were uniquely associated with the back reef,
345 even though corals are exposed to warmer temperature and high irradiance than their
346 reef slope counterparts. Light and temperature are known to exert a strong selection
347 pressure on dinoflagellate algae, often leading to the zonation of Symbiodiniaceae
348 populations between reef habitats[42,61,62]. This was not the case in our study, suggesting that
349 such zonation may depend on the host species or geographic region.

350 **Conclusion**

351 With reef ecosystems under stress from climate change on a global scale, it is important
352 to consider how the host and environmental structuring we have identified here will
353 impact the adaptive potential of *Millepora* corals to future environmental change[39]. Our
354 data demonstrates high host-fidelity in the *Millepora-Symbiodinium* associations and a
355 limited effect of the environment. The relevance of these findings may be viewed from
356 two contrasting perspectives. It may be that the individual algal genotypes that are found
357 across the different reef habitats are sufficiently phenotypically plastic to form successful
358 associations with their genotype-specific coral hosts (i.e., able to survive in the long-term).
359 Conversely, the high fidelity of the *Millepora-Symbiodinium* associations may be viewed as
360 limiting, with the coral host unable to maintain alternative associations over the long
361 term, either due to a lack of alternative host-specific symbionts, or due to host-generalist
362 symbionts being incompatible. The former scenario is more parsimonious given the
363 documented selectable phenotypic diversity within the *Millepora*-associating
364 *Symbiodinium* algae and a weak but present host-independent effect of the environment

365 on algal assemblage (indicating the algal assemblage may be modified according to
366 environmental forcings at least to some degree). Furthermore, our identification of
367 (possibly multiple) cosmopolitan, host generalist *Cladocopium* algae suggest the ability of
368 the host to associate with generalist symbionts. However, their low overall abundance
369 indicates either that these symbionts are outperformed by host-specific algal associations
370 or that these symbionts are poorly suited to the prevailing environment. Taken together,
371 our results suggest that although the current *Millepora-Symbiodinium* associations may
372 well suit their environment, there is scope for putative adaptive, environmentally induced
373 algal assemblage modification, selecting upon the extant host-specific co-evolved
374 *Symbiodinium* diversity.
375

376 ## Methods

377 ### Fire coral sampling

378 Between May and September 2013, 198 fragments of the fire coral *M.* cf. *platyphylla* were
379 collected on the north shore of Moorea Island, French Polynesia (17.5267 S, 149.8348 W),
380 from three reef habitats, located in front of Papetoai village, that are in close proximity to
381 each other, the mid slope (13 m depth), upper slope (6 m depth), and back reef (< 1 m
382 depth), but presumably exhibit contrasting environmental conditions, including varying
383 exposure to temperature, solar irradiance, partial pressure of carbon dioxide, and
384 disturbances[63-65]. Our sampling design is described in detail in Dubé et al.[66], where fire
385 coral colonies were sampled to investigate the clonal structure between habitats on a
386 barrier reef system. Briefly, three 300 m-long by 10 m-wide belt transects were laid over
387 the reef within each habitat, parallel to the shore. All colonies of *M.* cf. *platyphylla* were
388 georeferenced by determining their position along the transect-line (0 to 300 m) and
389 straight-line distance from each side of the transect (0 to 5 m, totalling 10 m). From these
390 measures, each colony was mapped with x and y coordinates. The colony size (projected

391 surface) of each colony was estimated (in cm^2) from 2D photographs using ImageJ 1.4f[67].
392 Small fragments of tissue-covered skeleton (< 2 cm^3) were collected from each colony
393 using a hammer and a chisel, and were preserved in 80% ethanol for further molecular
394 analysis.
395
396 **Environmental conditions**
397 The temperature and light intensity were monitored over a one-month period (i.e., from
398 August 23 to September 26 2019) to assess environmental differences between the three
399 reef habitats at Papetoai. Temperature was recorded for each habitat in 60-sec intervals
400 using an *in situ* deployed HOBO Pendant Temperature Data Logger (Onset, USA), while
401 the light conditions were recorded in 90-sec intervals using a 2π PAR Loggers (Odyssey,
402 New Zealand). Differences in daily temperature and light intensity between reef habitats
403 were assessed using one-way ANOVA or Kruskal-Wallis tests (in cases where ANOVA
404 assumptions of normality and homoscedasticity were not met) with the package 'stats'
405 implemented in the R software v3.6.3[68], and the complemented post hoc pairwise
406 comparisons were also conducted.
407
408 **DNA isolation and fire coral genotyping**
409 The 198 colonies of *M.* cf. *platyphylla* were sampled and genotyped using microsatellite
410 markers (as described in Dubé et al.[66]). Briefly, DNA isolation was performed using a
411 QIAxtractor automated genomic DNA extraction instrument, according to
412 manufacturer's instructions, following an incubation at 55°C for 1 hr in 450 µl of lysis
413 buffer with proteinase K (QIAGEN, Hilden, Germany). Each colony was genotyped using
414 twelve microsatellite loci[69]. Samples were sent to the GenoScreen platform (Lille, France)
415 for fragment analysis on an Applied Biosystems 3730 Sequencer with the GeneScan 500
416 LIZ size standard. All alleles were scored and checked manually using GENEMAPPER
417 v4.0 (Applied Biosystems, Foster City CA, USA). Multilocus genotypes (MLGs) were

418 identified in GENCLONE v2.0[70]. To assess the discriminative power of the microsatellite
419 markers, the genotype probability (GP) was estimated at each locus and a combination of
420 all loci in GENALEX v6.5[71]. Colonies with the same alleles at all loci were assigned to the
421 same MLG (genet) and were considered as clone mates due to fragmentation. The 198
422 colonies belonged to ten different genotypes with at least four clonal replicates in at least
423 two of the three surveyed habitats, provide an ideal study system to examine variation in
424 Symbiodiniaceae communities among fire coral clones across distinct reef habitats (as a
425 proxy for different environments) (Fig. 1, see Supplementary Data 1 for sample overview).
426 A phylogenetic tree based on Nei's genetic distances between the ten genotypes was
427 inferred using the 'poppr' R package[72] with bootstrap support from 5000 replicates
428 (Supplementary Fig. 1A). A map with the x and y coordinates of each clonal genotype
429 was produced using the R package 'ggplot2'[73] (Supplementary Fig. 1B).
430

431 **Symbiodiniaceae ITS2 library preparation, sequencing, and SymPortal analysis**

432 The Symbiodiniaceae ITS2 region of the rDNA was amplified using the primers
433 SYM_VAR_5.8S2 and SYM_VAR_REV[74] with added sequencing adapters (forward: 5'–
434 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAATTGCAGAACTCCGTGAA
435 CC–3'; reverse: 5'–GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCGGGTTCW
436 CTTGTYTGACTTCATGC–3'; Illumina overhang adaptor sequences are underlined). Ten
437 µl PCRs containing 1 µl of template DNA (10–50 ng of DNA from each fire coral sample)
438 and 0.25 µM of each primer were run in triplicate per sample using the Multiplex PCR Kit
439 (QIAGEN, Hilden, Germany). Thermal cycler conditions for ITS2 PCR amplification were
440 95°C for 15 min, followed by 30 cycles of 95°C for 30 s, 56°C for 90 s, and 72°C for 30 s,
441 with a final extension time of 10 min at 72°C. Amplification success was verified by
442 running 5 µl of the PCR products on a 1 % agarose gel, and successful triplicate reactions
443 were pooled and cleaned using the Illustra ExoProStar PCR and Sequence Reaction Clean-
444 Up Kit (GE Healthcare Life Sciences, Pittsburgh PA, USA). Indexing adaptors were added

445 via PCR (8 cycles) according to the Nextera XT DNA library preparation protocol using
446 the Multiplex PCR Kit. Indexed PCR products were purified and normalized using the
447 SequalPrep Normalization Plate Kit (Invitrogen, Carlsbad CA, USA), subsequently
448 quantified using the Qubit dsDNA HS Kit (Invitrogen, Carlsbad CA, USA), and run on
449 the Agilent 2100 Bioanalyzer using the Agilent High Sensitivity DNA Kit (Agilent
450 Technologies, Santa Clara CA, USA) to confirm amplicon length and purity. The ITS2
451 gene amplicon library was sequenced at the KAUST BioScience Core Laboratory on the
452 Illumina HiSeq 2500 platform using the rapid-run mode with 2 x 250 bp overlapping
453 paired-end reads with a 10% phiX control. Determined sequencing data for this project
454 are available under NCBI BioProject PRJNA888138. Paired-end sequencing reads were
455 submitted to SymPortal for quality control and analysis at SymPortal.org[75]. Briefly,
456 sequence quality control was conducted as part of the SymPortal pipeline using mothur
457 v1.39.5[76], the BLAST+ suite of executables[77], and minimum entropy decomposition
458 (MED)[78]. ITS2 type profiles, representative of putative Symbiodiniaceae genotypes, were
459 predicted by searching for co-occurring sets of sequences and characterized by specific
460 sets of defining intragenomic ITS2 sequence variants (DIVs). Where ITS2 sequence
461 abundances are reported in this study, we refer to post-MED, rather than pre-MED,
462 abundances.
463
464 **Symbiodiniaceae community composition analysis**
465 To determine the relative contribution of host genetic background (represented by the ten
466 different host genotypes) and environment (represented by the three contrasting reef
467 habitats) on coral-algae symbioses, two different methods of assessing the
468 Symbiodiniaceae community composition were used: (1) ITS2 sequence assemblages
469 were assessed as a proxy for total Symbiodiniaceae taxonomic composition and (2)
470 predicted ITS2 profiles were assessed as a proxy for specific Symbiodiniaceae genotypes.
471 As such, ITS2 sequence (post-MED) and ITS2 type profile abundance count tables

472 (Supplementary Data 2 and 3), as output by SymPortal, were directly used to plot data
473 using the relative abundances for each sample, as well as the presence or absence of a
474 given sequence in a sample. Plots were drawn using ggplot2. To assess the explanatory
475 power of host genotype and reef habitat variables on the variation in Symbiodiniaceae
476 community composition, a two-way permutational multivariate analysis of variance
477 (PERMANOVA) was run with the adonis2 function in the 'vegan' R package[79].
478 Dissimilarities between samples were visualized using a principal coordinate analysis
479 (PCoA) using the 'ape' R package[80]. All statistical analyses were performed on Bray-Curtis
480 distances of square root transformed ITS2 sequence counts using the vegdist function in
481 vegan. We also analyzed our data to test for relationships between Symbiodiniaceae
482 taxonomic composition dissimilarity (Bray-Curtis matrix) and host genetic distances
483 (Euclidean matrix computed in GENODIVE v3.04)[81] as well as host spatial distances
484 (Euclidean matrix in meters computed in GENALEX v6.5) using Mantel tests and the
485 Spearman correlation method with 9999 permutations using the function mantel in vegan.
486 A hierarchical clustering analysis was performed on non-transformed ITS2 type profile
487 counts using the function hclust in the R package stats to establish dissimilarity between
488 the different identified *Symbiodinium* genotypes (i.e., sequences that belong to the
489 *Symbiodinium* genus, formerly clade A[2]). Scripts used for analysis and plotting are
490 deposited in a GitHub repository (https://github.com/CarolineDUBE).

491 **Data availability**

492 Sequencing data determined in this study are available under NCBI BioProject ID
493 PRJNA888138. Other data are available in the Supplementary Data files.

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747

748 **Acknowledgements**

749 We thank S. Planes for his contribution to the previous genotyping effort of *Millepora*
750 samples at Moorea and KAUST Bioscience Core Lab for the ITS2 sequencing. This work
751 was funded by the Laboratoire d'Excellence "CORAIL" project COMIC and KAUST
752 baseline funds to CRV.

753 **Contributions**

754 C.E.D and A.M collected the samples. C.E.D extracted the DNA and prepared the ITS2
755 libraries. C.E.D carried out the analyses with the contribution from B.C.C.H, A.M, and
756 M.Z. C.E.D, E.B and C.A.-F.B conceptualized the study. C.E.D and C.R.V supervised the
757 study. C.E.D, B.C.C.H and C.R.V wrote the manuscript with contribution from all the
758 authors.
759

760 **Figure captions**

761

762 **Fig. 1 | Schematic overview of the sampling design. A** On the left of the alluvial plot,
763 the ten sampled clonal genotypes are shown (G1 to G10) with clones naturally distributed
764 across the reef habitats on the north shore of Moorea, French Polynesia: the back reef (BR
765 at < 1m depth), the upper slope (UP at 6 m), and the mid slope (MD at 12 m), which are
766 illustrated on the right. Each genotype and habitat are represented by a unique color with
767 faded colors towards the middle where clones of a given genotype are aligned with the
768 habitat in which they were found. The thickness of each branch corresponds to the
769 number of replicates per genotype and habitat shown in parentheses. In the middle, the
770 thickness of each branch corresponds to the abundance of clones (n) for each genotype in
771 each of the three habitats. **B** Temperature profiles from each of the three habitats (BR =
772 black, UP = blue, MD = gold), based on 1 440 data points collected daily for a period of 29
773 days in the months of August to September. **C** Light profiles at each of the three habitats
774 based on 960 data points collected daily for a period of 29 days in the months of August
775 to September.

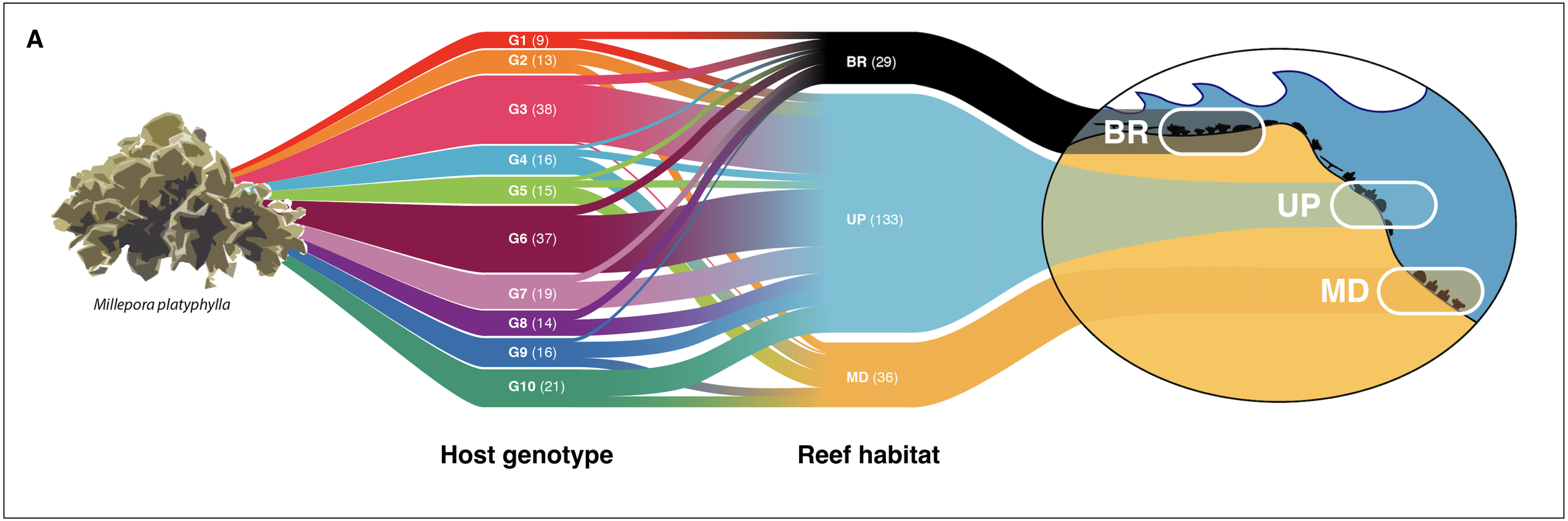
776

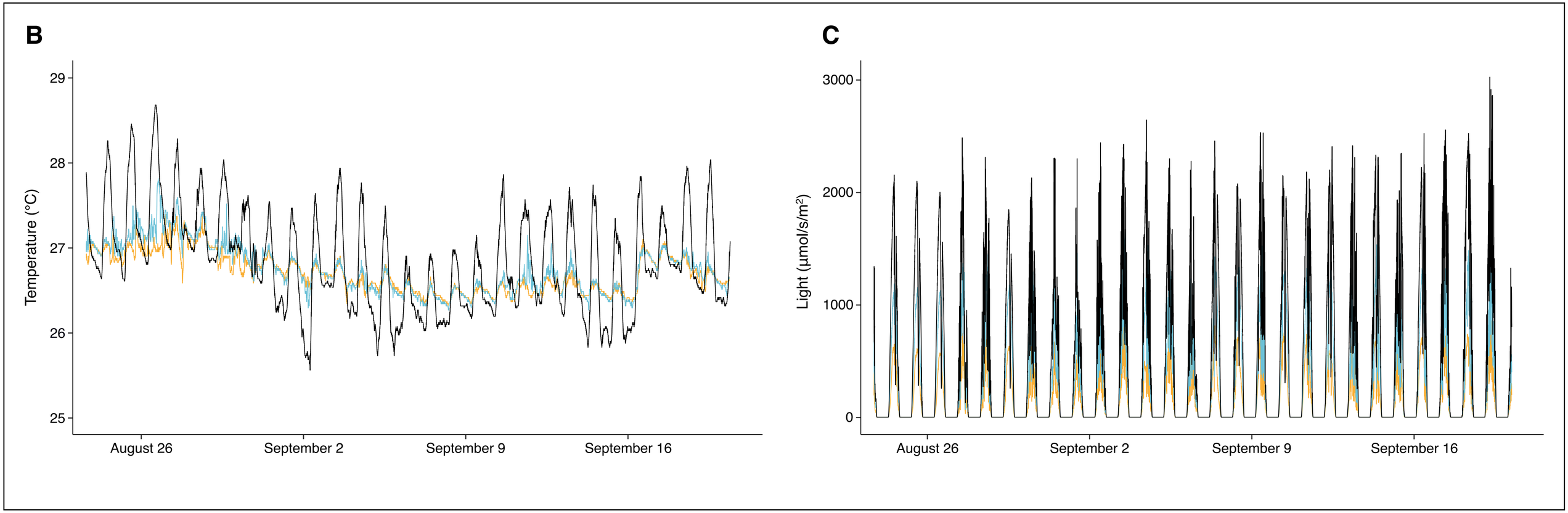
777 **Fig. 2 | Symbiodiniaceae community composition of 10 fire coral clones across 3**
778 **distinct reef habitats.** In each of the main figure sections (A-D), every sample is
779 represented as a single column with samples grouped by host genotype and reef habitat
780 denoted in **D. A** Relative abundance of post-MED ITS2 sequences (as a proxy for
781 Symbiodiniaceae taxonomic composition). **B** Presence or absence of ITS2 sequences
782 regardless of abundance. Missing bars represent sequences not present in the sample. For
783 both **A** and **B**, sequences are ordered from bottom to top by overall relative abundance
784 across all study samples. Sequences that were found in all samples with a relative
785 abundance > 2.5% are annotated in the legend. All other sequences are grouped as 'Other'
786 and are shown in light grey. **C** Predicted ITS2 type profiles (as a proxy for
787 Symbiodiniaceae genotypes). Profiles are annotated in the dendrogram on the bottom
788 right. The dendrogram shows the hierarchical clustering of Bray-Curtis-derived between-
789 profile distances calculated from the average relative abundance of defining intragenomic
790 variants for each of the profiles (from 0 to 1). Thickness of leaves corresponds to the
791 abundance of the respective *Symbiodinium* type profile. **D** Host genotype and reef habitat
792 of origin. Clonal genotypes are numbered from G1 to G10 and each genotype contains
793 multiple samples (n) that occur naturally in at least two of the three surveyed habitats (BR
794 = black, UP = blue, MD = gold).

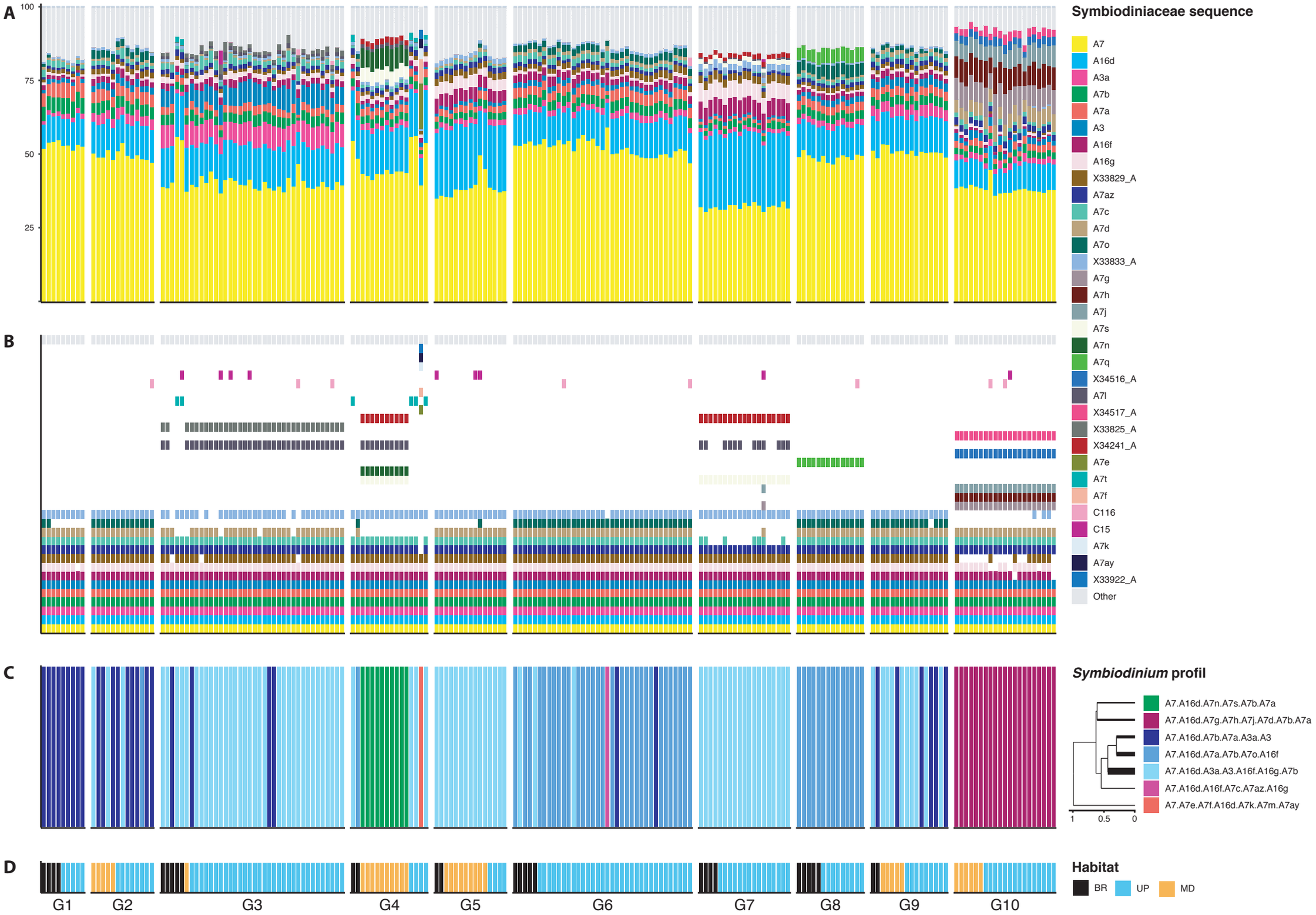
795

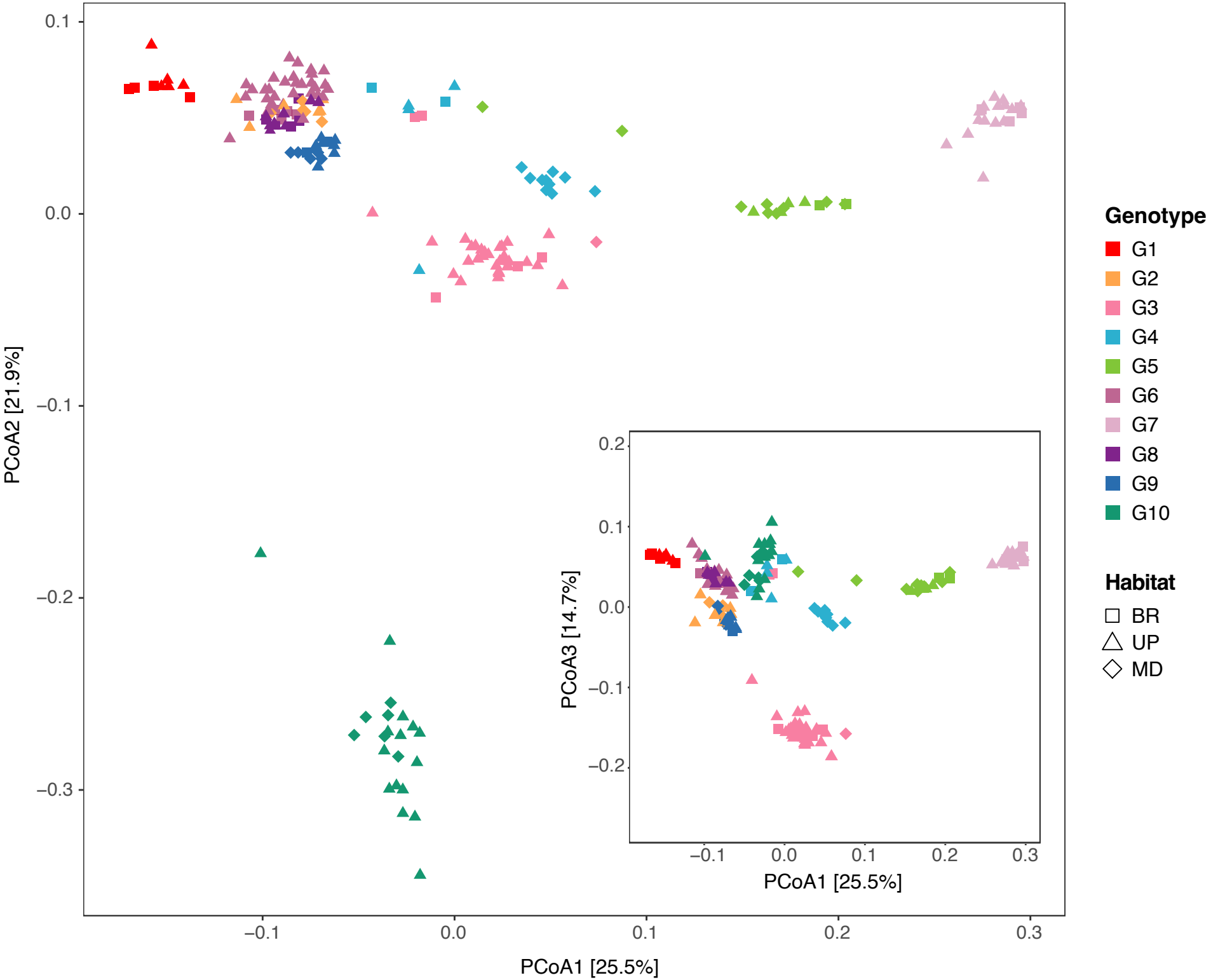
796 **Fig. 3 | Contribution of host genotype and habitat to *Symbiodinium* assemblage**. PCoA
797 of square root-transformed relative abundances of *Symbiodinium* (A-)sequences
798 constructed using Bray-Curtis distances. Each dot represents a colony, colors denote the
799 genotype, and symbols denote the reef habitat.
800

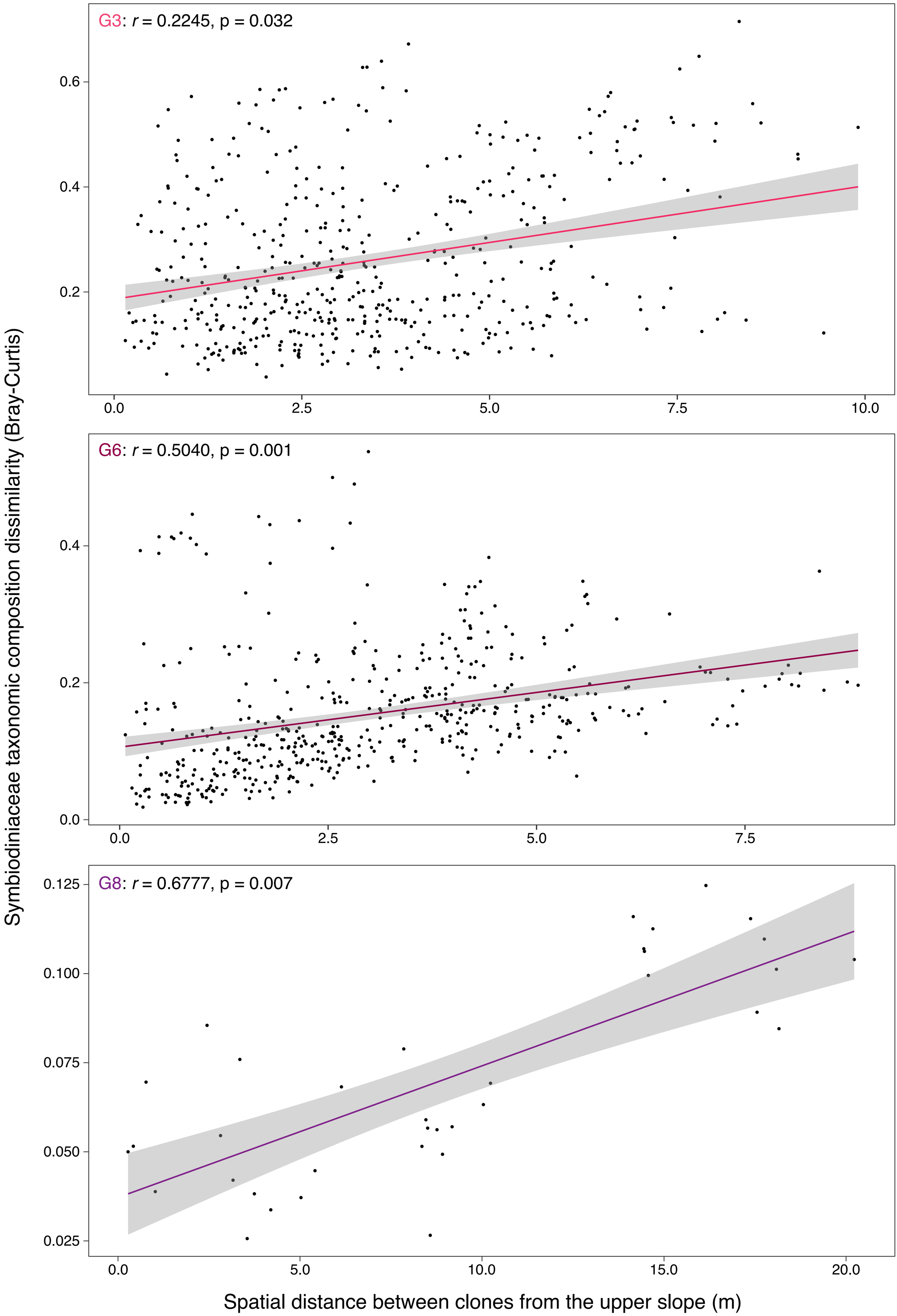
801 **Fig. 4 | Relationship between *Symbiodinium* ITS2 sequence assemblage dissimilarity**
802 **and spatial distance of clones in reef habitats.** Positive correlations between relative
803 abundances of *Symbiodinium* (A-)sequences constructed using Bray-Curtis and host
804 spatial distances (Euclidean matrix in meters). Only clones of the genotypes G3, G6, and
805 G8 originating from the upper slope are shown, with their respective Spearman's Rank
806 correlation coefficient alongside p-values estimated with a Mantel test. Correlations for
807 the other genotype x habitat combinations were not significant (Supplementary Table 1).


A
Millepora platyphylla
G1 (9)
G2 (13)
G3 (38)
G4 (16)
G5 (15)
G6 (37)
G7 (19)
G8 (14)
G9 (16)
G10 (21)
BR (29)
UP (133)
MD (36)
BR
UP
MD
Host genotype
Reef habitat


B
Temperature (°C)
29
28
27
26
25
August 26
September 2
September 9
September 16
C
Light (µmol/s/m²)
3000
2000
1000
0
August 26
September 2
September 9
September 16


A
100
75
50
25
B
C
D
G1
G2
G3
G4
G5
G6
G7
G8
G9
G10
Symbiodiniaceae sequence
A7
A16d
A3a
A7b
A7a
A3
A16f
A16g
X33829_A
A7az
A7c
A7d
A7o
X33833_A
A7g
A7h
A7j
A7s
A7n
A7q
X34516_A
A7l
X34517_A
X33825_A
X34241_A
A7e
A7t
A7f
C116
C15
A7k
A7ay
X33922_A
Other
Symbiodinium profil
A7.A16d.A7n.A7s.A7b.A7a
A7.A16d.A7g.A7h.A7j.A7d.A7b.A7a
A7.A16d.A7b.A7a.A3a.A3
A7.A16d.A7a.A7b.A7o.A16f
A7.A16d.A3a.A3.A16f.A16g.A7b
A7.A16d.A16f.A7c.A7az.A16g
A7.A7e.A7f.A16d.A7k.A7m.A7ay
1
0.5
0
Habitat
BR
UP
MD


PCoA2 [21.9%]
PCoA1 [25.5%]
0.1
0.0
−0.1
−0.2
−0.3
−0.1
0.0
0.1
0.2
0.3
PCoA3 [14.7%]
PCoA1 [25.5%]
Genotype
G1
G2
G3
G4
G5
G6
G7
G8
G9
G10
Habitat
BR
UP
MD


G3: r = 0.2245, p = 0.032
0.6
0.4
0.2
0.0
2.5
5.0
7.5
10.0
G6: r = 0.5040, p = 0.001
0.4
0.2
0.0
0.0
2.5
5.0
7.5
G8: r = 0.6777, p = 0.007
0.125
0.100
0.075
0.050
0.025
0.0
5.0
10.0
15.0
20.0
Symbiodiniaceae taxonomic composition dissimilarity (Bray-Curtis)
Spatial distance between clones from the upper slope (m)