1 Ecological factors rather than physical barriers to dispersal shape genetic structure of
2 algal symbionts in horizontally-transmitting corals
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23 divergence, dispersal, local adaptation, host-specificity, ecological partitioning, ecology

24 **Abstract**
25 Many reef-building corals acquire their algal symbionts (*Symbiodinium* sp.) from the
26 local environment upon recruitment. This horizontal transmission strategy where hosts
27 pair with locally available symbionts could serve to increase coral fitness across diverse
28 environments, as long as the host maintains high promiscuity and symbionts adapt
29 locally. Here, we tested this hypothesis in two coral host species by comparing host and
30 symbiont genetic structures across different spatial scales in Micronesia. Each host
31 species associated with two genetically distinct *Symbiodinium* lineages, confirming high
32 promiscuity in broadly dispersing hosts. However, contrary to our initial expectation,
33 symbiont genetic structure was independent of physical barriers to dispersal between
34 islands, unlike genetic structure of their hosts that was nearly perfectly explained by
35 oceanic currents. Instead, *Symbiodinium* consistently demonstrated genetic divergence
36 among local reefs and between the two host species at each island, although not
37 necessarily between distant islands. These observations indicate that *Symbiodinium*
38 disperse much more broadly than previously thought and continuously adapt to specific
39 hosts and reef environments across their range, following the classical Baas Becking's
40 hypothesis: "Everything is everywhere, but the environment selects". Overall, our
41 findings confirm horizontal transmission could be a mechanism for broadly dispersing
42 coral species to enhance their local fitness by associating with locally adapted symbionts.
43 Dramatic differences in factors driving the genetic structures of horizontally-transmitting
44 corals and their *Symbiodinium* imply that viewing their combined genomes as a single
45 evolving entity ('hologenome') would be misleading.
46

47    **Introduction**

48    Symbioses are ubiquitous in nature and the interactions between symbiotic partnerships

49    have been implicated in eukaryote evolutionary diversification (1-4) and in the origin of

50    eukaryotes (5). Many well-known symbioses involve the passing of symbionts from

51    parents to offspring (vertical transmission), fully aligning the evolutionary trajectories of

52    symbiotic partners and typically lead to their deep integration at biochemical and

53    genomic levels (i.e. Buchnera in aphids (6, 7)). The result of this symbiosis is essentially

54    a novel composite organism. In other types of symbioses, the association between

55    partners must be newly established in every generation (horizontal transmission), which

56    allows for the maintenance of each partner's species identity. In theory, this kind of

57    relationship should generate novel ecological opportunities for both symbiotic partners

58    through their mixing and matching across environments. For example, association with

59    ecologically specialized algal photobionts can lead to distinct ecological guilds of lichens

60    (8) or allow a fungal partner to expand its geographic range across a broader climatic

61    envelope (9). Similarly, in aphids, association with various horizontally transmitted

62    bacterial symbionts allows these insects to colonize novel host plants across climatic

63    zones (10). Considering these and other examples of ecological adaptation based on

64    varying symbiotic associations, it has been argued that the joint genomic content of

65    symbiotic systems should be considered as a single unit of evolution, the 'hologenome'

66    (11, 12). However, the usefulness of this concept depends on whether the evolutionary

67    trajectories of both symbiotic partners are sufficiently aligned to present a unified target

68    of selection (13). Here, we explore this question in the symbiosis between a horizontally

69    transmitting reef-building coral and dinoflagellate algae of the genus *Symbiodinium* (14).

70      Association with *Symbiodinium* is obligatory for many coral hosts that rely on

71  algal photosynthesis for energy, while the algae benefit from protected and light-exposed

72  residence as well as inorganic nutrients and $CO_2$ concentration regulatory mechanisms

73  provided by the host (15-18). Given the obligatory nature of this symbiosis for the host, it

74  is somewhat surprising that in the majority of coral species (~85%) *Symbiodinium* are not

75  transmitted vertically, but rather must be acquired by the juvenile coral from its local

76  environment (19, 20). One possible explanation is that dispersal ranges of aposymbiotic

77  coral larvae typically extend over hundreds of kilometers (i.e. 21), while the

78  environmental variation corals must deal with exists on much smaller spatial scales: reef

79  environments with varying light, thermal and nutrient conditions can be separated by

80  meter-scale distances (i.e. 22). Under such circumstances, coral hosts would benefit from

81  the mixing and matching strategy, improving their fitness by associating with the locally

82  available, and putatively ecologically specialized, algal strains (23-25). Establishing the

83  relative roles of these symbiotic partners in adaptation to variable environments is

84  essential for better prediction of coral reefs' future under climate change (i.e. 26).

85      Although symbiont-free coral larvae show practically unrestricted flexibility in

86  symbiont uptake (27-30), it nevertheless results in strong specificity of the resulting

87  coral-*Symbiodinium* association: the majority of coral species are found hosting a single

88  *Symbiodinium* subclade (26, 31-38). This specificity putatively arises from within-host

89  competition between algal strains after initial uptake (32). Selection for a better match

90  with the host is therefore expected to be another major evolutionary force affecting

91  *Symbiodinium* populations (3), in addition to environmental specialization.

92   The third force that would influence the dynamics of coral-*Symbiodinium*

93 symbiosis is dispersal. It is reasonable to hypothesize that local environmental

94 specialization of the symbionts would be most easily achieved if the symbionts were

95 dispersal-limited compared to the coral host. Baums *et al.,* (26) investigated within-

96 subclade genetic variation of Caribbean *Symbiodinium* in the larger context of the

97 genetics of the host (*Acropora palmata*) and determined that *Symbiodinium* exhibited

98 stronger genetic structure than the coral host, suggesting limitation of symbiont dispersal

99 relative to the host.

100   To integrate these three evolutionary forces, we formulated a working hypothesis

101 for this study, the 'global host, local symbiont' hypothesis. It posits that (i) coral hosts

102 disperse widely and are able to establish symbiosis with diverse algal strains across

103 locations; (ii) *Symbiodinium* algae are poor dispersers, which results in strong divergence

104 among locations aligning with physical barriers and facilitates their local environmental

105 specialization; (iii) local *Symbiodinium* strains also diverge with respect to the host

106 species as a result of selective pressure towards higher host specificity. To validate all

107 three components of our hypothesis, we examined multi-locus genotypes (MLG) of clade

108 C *Symbiodinium* in two species of *Acropora* – *A. hyacinthus* and *A. digitifera* – collected

109 from the same reef locations across the Micronesian Pacific (Fig. 1). Our previous work

110 has shown that both host species exhibit extensive genetic connectivity and their genetic

111 structure is nearly perfectly explained by the patterns of regional surface currents (21).

112 By using two coral species that co-occur across the same locations as well as local reef

113 environments we aimed to disentangle the roles of environmental specialization, host

114 specialization, and physical barriers to dispersal in driving the fine-scale genetic structure
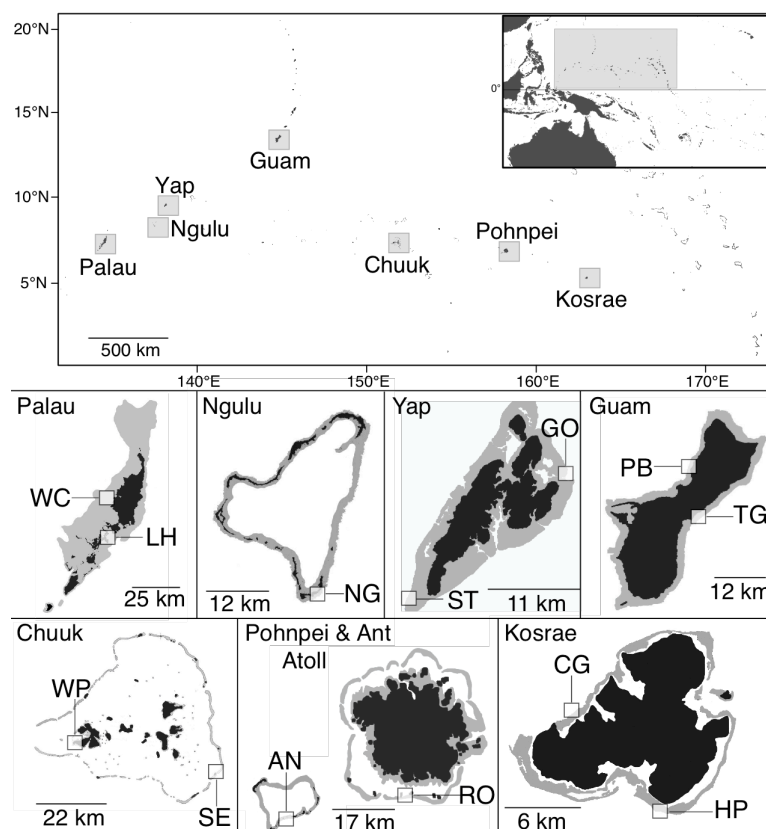
115    of *Symbiodinium*.



117    Figure 1: Geographic location of the Micronesian islands where *Acropora hyacinthus* and *A.*
118    *digitifera* coral samples were collected. Top: Map of the Micronesian Pacific with an inset of the
119    Pacific Ocean for reference. Islands where samples were collected and analyzed for *Symbiodnium*
120    genetics are designated with grey boxes. Detailed information on each sampling site is located in
121    Table 5. No *A. hyacinthus* were present in Guam and *A. digitifera* were not collected in Ngulu.
122

123    **Results**

124    **Two *Symbiodinium* clade C lineages**

125    To enable standard population genetic analysis, we restricted our study to only corals that

126    hosted a single diploid *Symbiodinium* clone (i.e., yielded a unique diploid *Symbiodinium*

127    genotype across six analyzed microsatellite loci), which encompassed the majority (69%

128    *A. digitifera* and 64% *A. hyacinthus*) of our samples. Across the two coral species in

129    Micronesia (Fig. 1), two distinct *Symbiodinium* lineages were observed, which were most

130    clearly discriminated by two of the six microsatellite loci (Fig. S1). Sequencing of the

131    psbA$_{ncr}$ gene for a subset samples classified these lineages as C40 and C3 *sensu*

132    Lajeunesse et al. (39) and LaJeunesse and Thornhill (40) (Fig. 2). Corals of both species

133    from Palau and Ngulu hosted exclusively C40 *Symbiodinium* (Fig. 3A, pink bars). This

134    lineage was also prevalent in *A. digitifera* at one reef site on Yap and was rarely found in

135    *A. digitifera* across the rest of Micronesia. All other coral hosts of either species

136    associated with *Symbiodinium* C3 (Fig. 3A, turquoise bars). Both *Symbiodinium* lineages

137    possessed high genetic diversity, with a total of 70 unique alleles across six SSR loci

138    observed in C40 across three islands (Table 1A) and 130 unique alleles across the same

139    six SSR loci in C3 across five islands (Table 1B).  Mean numbers of alleles per island for

140    each locus for C40 ranged from 3.00-4.67 with the highest numbers of private alleles in

141    Palau (N=7) (Table 1A). Mean allele numbers per island for each locus for C3 ranged

142    from 3.83-5.17 and numbers of private alleles ranged from 1-4 (Table 1B).

143

144    Table 1. Summary of allelic diversity measures for *Symbiodinium* C40 (A) and *Symbiodinium* C3
145    (B). Na: number of alleles, He: expected heterozygosity, Ho: observed heterozygosity, PA:
146    number of private alleles
147    A. *Symbiodinium* C40

|  | Na | He | Ho | PA |
|---|---|---|---|---|
| PAL | 4.67 | 0.52 | 0.64 | 7 |
| NGU | 4.00 | 0.42 | 0.55 | 6 |
| YAP | 3.00 | 0.43 | 0.51 | 2 |

148    B. *Symbiodinium* C3

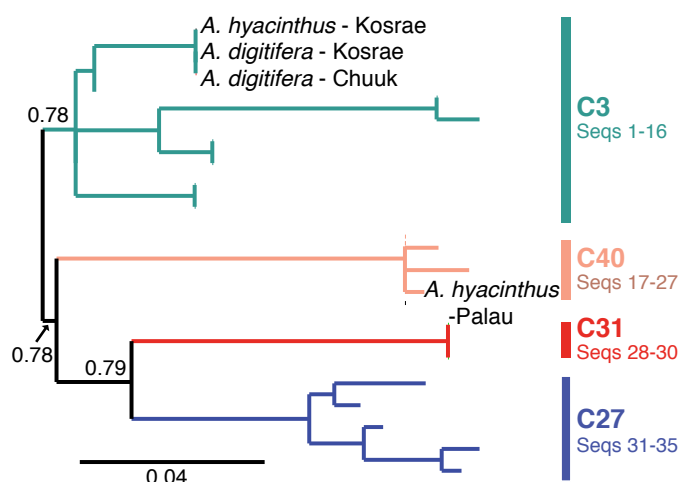|  | Na | He | Ho | PA |
|---|---|---|---|---|
| YAP | 4.33 | 0.47 | 0.56 | 1 |
| GUA | 3.83 | 0.42 | 0.46 | 3 |
| CHU | 5.17 | 0.58 | 0.51 | 3 |
| POH | 4.33 | 0.47 | 0.56 | 1 |
| KOS | 4.00 | 0.48 | 0.51 | 4 |

149

150 Figure 2. Phylogenetic analysis of $psbA^{ncr}$ sequences from representative samples from this
151 study (labeled branch tips) along with publically available $psbA^{ncr}$ sequences from known
152 *Symbiodinium* subclades identifies two lineages present (C3 and C40). Bootstrap support values
153 are shown at the partitions that define known subclades. Scale bar: replacements per nucleotide
154 site. Sequence accession numbers for reference sequences are given in Supplementary Table 1.
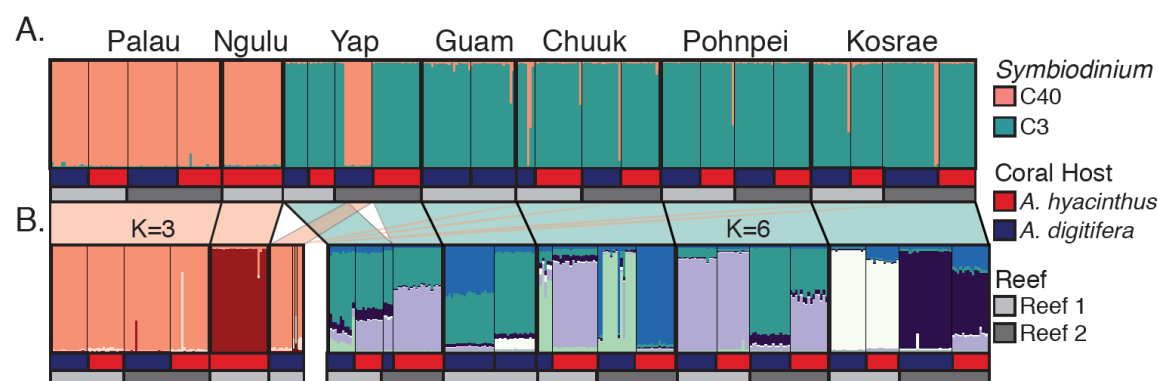
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157

158 Figure 3: Analyses of microsatellite loci data for *Symbiodinium* hosted by *Acropora hyacinthus*
159 and *Acropora digitifera* at thirteen sites across seven islands in Micronesia, using multilocus
160 genotyping data. A. STRUCTURE population assignment for *Symbiodinium* from two *Acropora*
161 host species across greater Micronesia at an optimal population number (K=2), corresponding to
162 C40 (pink) and C3 (turquoise) clade C subclades. Colors in the bottom panels correspond to host
163 species and shades of grey correspond to different sites within each island. B. Within-subclade
164 STRUCTURE analysis.

165

166 ***Symbiodinium* genetic structure**

167 All pairwise between-islands $F_{ST}$ for *Symbiodinium* C40 were significant (Table 2A).

168 *Symbiodinium* C3 had one non-significant $F_{ST}$ (Yap-Pohnpei) while all others were

9

169    significant and ranged from 0.058 (Guam-Chuuk) to 0.078 (Yap-Kosrae) (Table 2B).

170    Notably, *Symbiodinium* C3 differentiation did not show the isolation-by-distance pattern

171    observed in both coral hosts (Fig. 4). The correlation of $F_{ST}$ with distance could not be

172    computed for *Symbiodinium* C40 due to few between-island comparisons available. For

173    *Symbiodinium* C3 $F_{ST}$ values generally exceeded $F_{ST}$ values for the host (Fig. 4A),

174    however, this result did not imply stronger genetic differentiation in the symbionts:

175    higher $F_{ST}$ in the symbionts was a consequence of higher mean heterozygosity in

176    *Symbiodinium* markers (e.g. (41). Indeed, an alternative measure of genetic

177    differentiation controlling for mean heterozygosity - Jost's D (42) - demonstrated that

178    *Symbiodinium* C3 genetic divergence was in fact intermediate between the two hosts

179    (Fig. 4B).

180

181    Table 2. Summary of pairwise $F_{ST}$ values between all islands for *Symbiodinium* C40 (A) and C3
182    (B). Permutations were run 9999 times. All significant comparisons are shaded in grey.
183    A. *Symbiodinium* C40

|  | PAL | NGU | YAP |
|---|---|---|---|
| PAL | 0.000 | *** | 0.004 |
| NGU | 0.279 | 0.000 | *** |
| YAP | 0.056 | 0.326 | 0.000 |

184    B. *Symbiodinium* C3

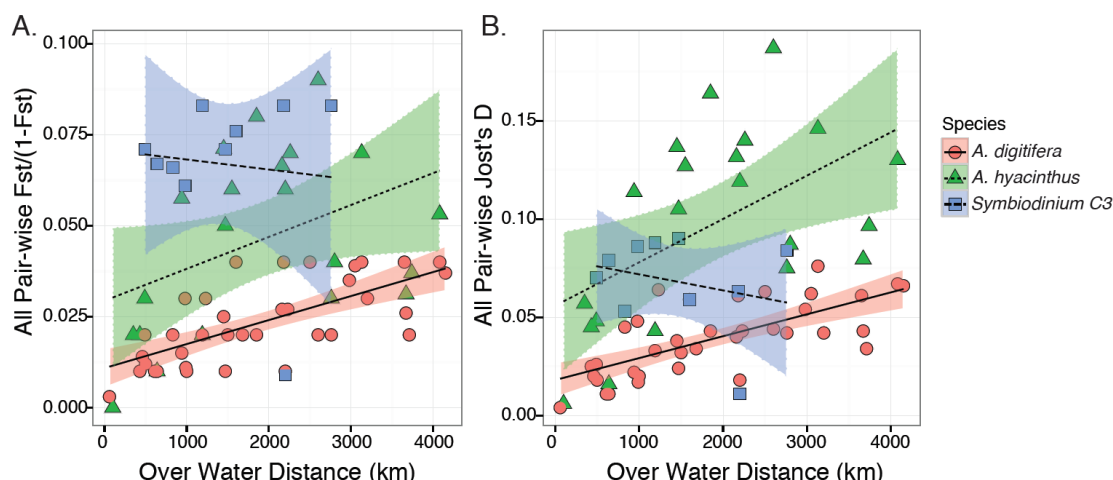|  | YAP | GUA | CHU | POH | KOS |
|---|---|---|---|---|---|
| YAP | 0.000 | *** | *** | 0.059 | *** |
| GUA | 0.062 | 0.000 | *** | *** | *** |
| CHU | 0.066 | 0.058 | 0.000 | *** | *** |
| POH | 0.009 | 0.071 | 0.063 | 0.000 | *** |
| KOS | 0.078 | 0.077 | 0.076 | 0.067 | 0.000 |

185

Figure 4: Comparison of two host species and *Symbiodinium* C3 differentiation. A. Pairwise genetic differentiation [($F_{ST}$ /(1-$F_{ST}$)] of two species of *Acropora* coral and *Symbiodinium* C3 across linear distances (km) demonstrating significant isolation by distance for the two host species but no correlation for the symbiont. B. Pairwise Jost's D for the same two host species and *Symbiodinium* C3 across linear distances (km) demonstrating no isolation by distance and no difference in overall divergence between the host and symbiont.

**Host specificity and environmental specialization**

Discriminant analysis of principal components (DAPC) strongly differentiated between host species for both *Symbiodinium* C40 and C3 (Table 3, Fig. 5A, B), suggesting that host specificity is an effective driver of symbiont diversity. In addition, DAPC analysis also demonstrated consistent differences among islands for each *Symbiodinium* type irrespective of host species: strong per-island assignment proportions were observed for C40 (Fig. 4C, 82-100%) and C3 (Fig. 5D, 51-98%), consistent with both STRUCTURE (Fig. 3) and $F_{ST}$ results (Table 1). Moreover, nearly all pairwise $F_{ST}$ values between different reef sites at the same island were significant for both *Symbiodinium* lineages (Table 4), suggesting environmental partitioning of symbionts. In accord with these results, of the two top eigenvalues in DAPC analyses within individual islands, one explained *Symbiodinium* genetic divergence by host species (host specificity) and the other corresponded to differences between reef sites (environmental specialization) (Fig.

208    6).

209    Table 3: Discriminant analysis of principle component (DAPC) model information including the
210    number of principle components ("PC") and discriminant functions ("DF") retained, the
211    proportion of conserved variance by the clustering model ("var"), and the overall assignment
212    proportions across the model ("assign"). A. DAPC information for *Symbiodinium* from different
213    host species, B. islands, and C. sites and host species within each island.
214
215    Model Information

| A. Host Species | PC | DF | var | assign |
|---|---|---|---|---|
| *Symbiodinium* C40 | 7 | 1 | 0.814 | 0.879 |
| *Symbiodinium* C3 | 14 | 1 | 0.922 | 0.846 |
| **B. Islands** | **PC** | **DF** | **var** | **assign** |
| *Symbiodinium* C40 | 16 | 4 | 0.970 | 0.981 |
| *Symbiodinium* C3 | 19 | 4 | 0.966 | 0.796 |
| **C. Within Island** | **PC** | **DF** | **var** | **assign** |
| Palau C40 | 14 | 3 | 0.984 | 0.735 |
| Yap C3 | 7 | 3 | 0.844 | 0.778 |
| Guam C3 | 3 | 3 | 0.647 | 0.860 |
| Chuuk C3 | 12 | 3 | 0.933 | 0.927 |
| Pohnpei C3 | 8 | 3 | 0.906 | 0.900 |
| Kosrae C3 | 7 | 3 | 0.878 | 0.888 |

216
217
218    Table 4. Summary of pairwise $F_{ST}$ values for *Symbiodinium* C40 (A) and C3 (B) between all sites
219    pooled across host species. Permutations were run 9999 times. All significant comparisons are
220    shaded in grey.
221    A. *Symbiodinium* C40

|  | PAL1 | PAL2 | NGU | YAP2 |
|---|---|---|---|---|
| **PAL1** | 0.000 | 0.009 | *** | 0.007 |
| **PAL2** | 0.031 | 0.000 | *** | 0.007 |
| **NGU** | 0.283 | 0.303 | 0.000 | *** |
| **YAP2** | 0.057 | 0.074 | 0.326 | 0.000 |

222    B. *Symbiodinium* C3

|  | YAP1 | YAP2 | GUA1 | GUA2 | CHU1 | CHU2 | POH1 | POH2 | KOS1 | KOS2 |
|---|---|---|---|---|---|---|---|---|---|---|
| **YAP1** | 0.000 | 0.139 | 0.002 | 0.009 | *** | *** | 0.002 | 0.055 | *** | *** |
| **YAP2** | 0.011 | 0.000 | *** | 0.003 | *** | *** | 0.027 | 0.064 | *** | *** |
| **GUA1** | 0.059 | 0.107 | 0.000 | 0.045 | *** | *** | *** | *** | *** | *** |
| **GUA2** | 0.045 | 0.062 | 0.025 | 0.000 | 0.002 | *** | *** | 0.008 | *** | *** |
| **CHU1** | 0.071 | 0.073 | 0.104 | 0.065 | 0.000 | *** | *** | *** | *** | *** |
| **CHU2** | 0.079 | 0.112 | 0.070 | 0.086 | 0.068 | 0.000 | *** | *** | *** | *** |
| **POH1** | 0.053 | 0.028 | 0.126 | 0.111 | 0.085 | 0.126 | 0.000 | *** | *** | *** |
| **POH2** | 0.018 | 0.018 | 0.093 | 0.039 | 0.067 | 0.092 | 0.063 | 0.000 | *** | *** |
| **KOS1** | 0.154 | 0.146 | 0.170 | 0.110 | 0.163 | 0.176 | 0.168 | 0.119 | 0.000 | *** |
| **KOS2** | 0.092 | 0.121 | 0.116 | 0.131 | 0.129 | 0.090 | 0.136 | 0.100 | 0.183 | 0.000 |

223
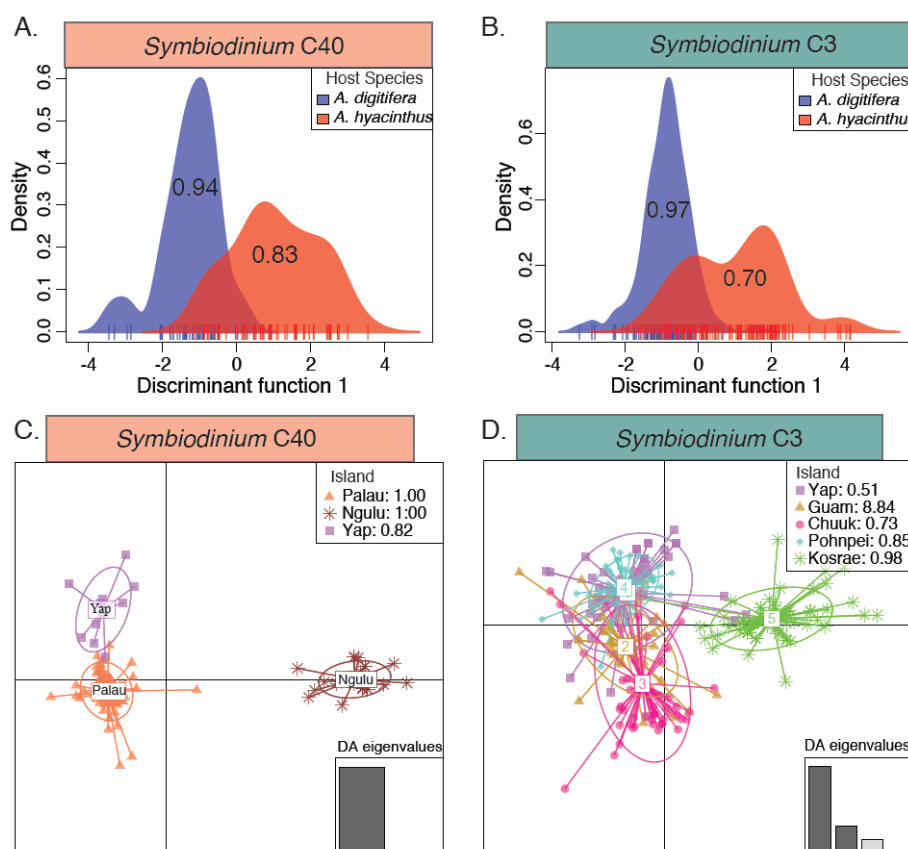
Figure 5: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium* C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at thirteen sites across seven islands in Micronesia. A. DAPC analysis on two discriminant functions demonstrating strong host species assignments across all islands for *Symbiodinium* C40 and B. *Symbiodinium* C3. Numbers overlaying the curves indicate successfully assigned fraction of samples. C. DAPC scatter plot for individual samples from *Symbiodinium* C40 represented by colored dots clustered by islands. D. DAPC scatter plot for individual samples from *Symbiodinium* C3 represented by colored dots clustered by islands. Proportions of assignments are indicated in the clusters or in the legends. Information on the DAPC models can be found in Table 3.
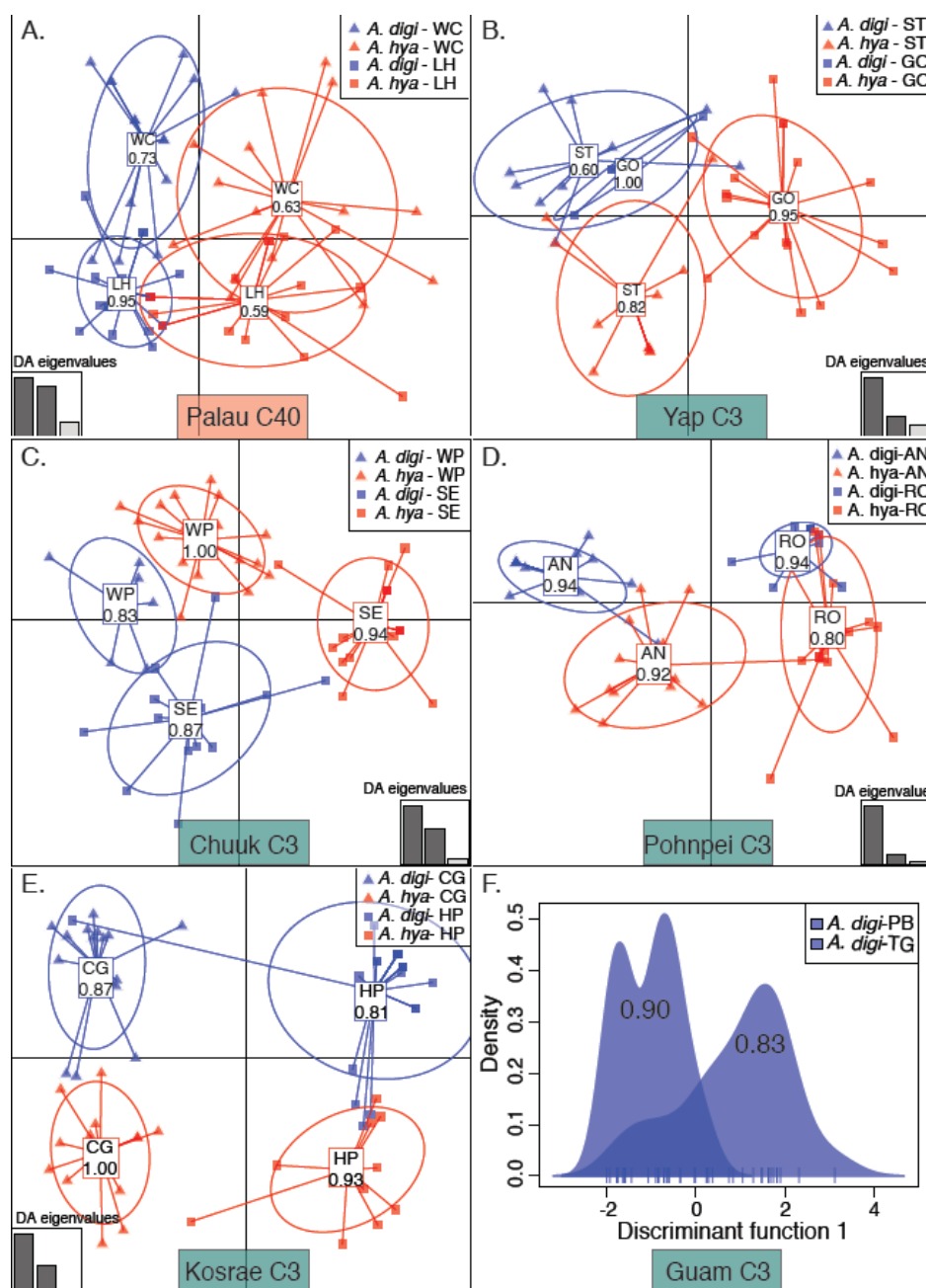
235
236 Figure 6: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium*
237 C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at twelve sites across six
238 islands (Ngulu not included) in Micronesia. DAPC analysis on two discriminant functions
239 demonstrating host species assignments and site assignments. DAPC scatter plots for individual
240 samples from within A. Palau for *Symbiodinium* C40. B. Yap for *Symbiodinium* C3, C. Chuuk for
241 *Symbiodinium* C3, D. Pohnpei for *Symbiodinium* C3, E. Kosrae for *Symbiodinium* C3, and F.
242 Guam for *Symbiodinium* C3 for *A. digitifera* hosts only (1 DF axis). Proportions of assignments
243 are indicated in the clusters. Information on the DAPC models can be found in Table 3.
244

245

246 **Discussion**

247 *Promiscuity of the coral host*

248 Clade C *Symbiodinium* are the dominant symbiont type found in Indo-Pacific reef-

249 building corals (43), presumably because this clade is capable of greater rate of carbon

250 fixation (44) and increased carbon translocation to hosts, which positively influences host

251 fitness (growth) when compared to other clades (44-46). Clade C *Symbiodinium* are

252 considered to be the most derived lineage within the genus *Symbiodinium* and exhibit

253 significantly higher within-clade diversity when compared to other, more basal, clades

254 (14, 47, 48). Across the Micronesian Pacific (Fig. 1), both coral hosts associated

255 exclusively with clade C *Symbiodinium*, which was represented by two distinct lineages–

256 C40 and C3, putatively corresponding to different species (Fig. 2). This observation

257 confirms the first prediction of the 'global host, local symbiont' hypothesis: coral hosts

258 show considerable flexibility in their symbiotic association across their range and within

259 their habitat.

260
261 *Lack of dispersal limitation in* Symbiodinium *across Micronesia*

262 Initially, we expected to find strong isolation by distance in *Symbiodinium*, since the

263 prevailing view of the *Symbiodinium* life cycle involves symbiotic existence in sedentary

264 hosts alternating with a free-living form that largely exists in the benthos where dispersal

265 by ocean currents must be limited (49-52). In contrast, we observed that genetic

266 differentiation between islands of *Symbiodinium* C3 across Micronesia did not exceed

267 $F_{ST} = 0.078$, which contrasts studies from other locations reporting symbiont $F_{ST}$ as high

268 as 0.54 in clade A (53) and $\Phi_{ST}$ as high as 0.468 in clade C (54). This lack of physical

269 dispersal limitation is best demonstrated by comparing *Symbiodinium* C3 divergence to

270    divergence of their coral hosts, which has been shown to nearly perfectly follow

271    biophysical barriers (21). If *Symbiodinium* dispersal were limited by the same physical

272    barriers and to a greater extent, as our hypothesis posited, we would have observed

273    steeper increases in divergence with distance relative to the host; instead, no increase of

274    $F_{ST}$ with distance was observed (Fig. 4A).   Moreover, after correcting for mean

275    heterozygosity, genetic divergence (Jost's D) in *Symbiodinium* C3 was found to be

276    intermediate between *A. hyacinthus* and *A. digitifera* (Fig. 4B), once again demonstrating

277    that symbiont dispersal is less limited than their hosts. This finding contradicts the second

278    prediction of our 'global host, local symbiont' hypothesis and is contrary to our

279    expectations based on earlier $F_{ST}$-based reports of stronger *Symbiodinium* differentiation

280    compared to hosts (3, 25, 26, 54).

281

282    *Host specificity*

283    The majority of reef-building coral species associate with a specific strain (termed

284    "clade" or "subclade") of *Symbiodinium* broadly defined based on mitochondrial and/or

285    chloroplast markers (3, 36, 38, 55). Previous *Symbiodinium* multilocus genotyping

286    studies revealed that each of these strains harbors greater diversity, both in terms of

287    genetic and functional diversity (25, 54, 56). Our data indicate that the local association

288    of hosts and symbionts of the same genotypic cluster is due to pervasive evolution of host

289    specificity in *Symbiodinium* (Fig. 5 & 6). As our study includes two coral species, we

290    also observe that this specificity is not perfect: at every location there were symbionts

291    that would have been assigned to another coral host based on their multilocus genotype

292    (Fig. 6). This suggests that host specialization in *Symbodinium* arises in the face of

293   considerable exchange between symbiont communities hosted by different coral species

294   at the same location. However, to which extent this between-host exchange contributes to

295   gene flow is unclear since it remains unknown if *Symbidinium* undergo sexual

296   reproduction within or outside the host.

297

298   *Environmental partitioning*

299   The most ecologically relevant postulate of the 'global host, local symbiont' hypothesis is

300   that locally available symbionts are also locally adapted, giving rise to a locally adapted

301   holobiont. Recurrent genetic divergence of symbionts between reef sites within the same

302   island (Fig. 6) despite the lack of physical dispersal limitation across much larger

303   distances (Fig. 4) suggests that symbiont genetic divergence is likely due to poor survival

304   of immigrants rather than to physical barriers to migration. Given that hosts are available

305   at every site, this leaves other ecological parameters of local reef environment as the most

306   likely barrier-forming force, preventing survival of immigrants adapted to a different

307   environment– a situation termed "phenotype-environment mismatch" (57). Thus,

308   although our study did not directly test for local adaptation of *Symbiodinium* as per

309   Kawecki and Ebert (58), genetic partitioning of symbionts among different reef

310   environments across small spatial scales suggests that their local adaptation does indeed

311   occur, thereby supporting our hypothesis. Notably, similar to the situation with host

312   specificity, there are several *Symbiodinium* genotypes that appear to be successful

313   migrants between reef sites (particularly clearly visible for Kosrae, Fig. 6E), indicating

314   that environmental partitioning is also incomplete and arises in the face of considerable

315   gene flow.

316    **Conclusions**

317    Across Micronesia, both coral host species associated with two divergent *Symbiodinium*

318    lineages (putatively corresponding to species), indicating high host promiscuity in

319    symbiont association. In contrast, within each *Symbiodinium* lineage, strong associations

320    with particular host species were observed, suggesting that host-specificity is an

321    important driver of *Symbiodinium* diversification. Unexpectedly, *Symbiodinium* genetic

322    divergence did not correlate with physical distance between islands, contrary to our initial

323    prediction that symbionts would exhibit a more pronounced isolation-by-distance pattern

324    than their hosts due to stronger barriers to dispersal. Instead, *Symbiodinium* genetic

325    structure was driven by the combined effects of local (within-island) environment and

326    host identity, suggesting that *Symbiodinium* assemblages across Micronesia are

327    comprised by broadly dispersing lineages dynamically adapting to specific coral hosts

328    and reef environments across their range. Notably, this pattern perfectly follows Baas

329    Becking's hypothesis for microbial communities: "Everything is everywhere, but the

330    environment selects" (59), assuming that host species themselves contribute to this

331    'environment'. Although this observation contradicts the original formulation of our

332    'global host, local symbiont' hypothesis, it supports the view that coral hosts could derive

333    fitness benefits from associating with locally available *Symbiodinium*. Finally, dramatic

334    differences in factors that structure genetic diversity in the coral host and their algal

335    symbionts render the '"hologenome"' concept irrelevant to horizontally-transmitting

336    coral-algal symbioses.

337

338

339    **Materials and Methods**

340    ***Sampling Locations and Methodology:*** This study comprised a subset of samples

341    previously analyzed for coral host genetics in Davies *et al.* (21) (Table 5, Fig. 1).

342    Twenty-five individuals of each coral host species (*Acropora hyacinthus* and *A.*

343    *digitifera*) were examined at two reef sites within seven islands, with the exception of

344    Ngulu (the only species collected was *A. hyacinthus*) and Guam (no *A. hyacinthus* were

345    found and only *A. digitifera* were collected), for a total of thirteen sites.

346

347    Table 5. Reef Site Collections. GPS coordinates, main island group, number of *A. digitifera* and
348    *A. hyacinthus* hosts genotyped. The numbers in brackets are individuals hosting a single
349    *Symbiodinium* MLG, which were included in all analyses presented here. Site letter corresponds
350    to island insets in Figure 1.

| Site | Island | GPS | *A. digitifera* | *A. hyacinthus* |
|---|---|---|---|---|
| WC. West Channel Reef | Palau | 7°31'55.7 N, 134°29'42.8 E | 24 (20) | 25 (17) |
| LH. Lighthouse Reef | Palau | 7°16'62.4 N, 134°27'61.9 E | 23 (15) | 24 (16) |
| NG. Ngulu | Ngulu Atoll | 8°18'12.0 N, 137°29'18.7 E | $0^1$ | 39 (25) |
| ST. South Tip Reef | Yap | 9°26'05.4 N, 138°02'10.4 E | 25 (10) | 25 (11) |
| GO. Goofnuw Channel | Yap | 9°34'26.4 N, 138°12'19.2 E | 24 (15) [m] | 25 (20) |
| PB. Pago Bay | Guam | 13°25'66.6 N, 144°47'94.3 E | 26 (20) | 0* |
| TG. Tanguisson | Guam | 13°32'61.1 N, 144°48'52.6 E | 21 (17) | 0* |
| WP. West Polle | Chuuk | 7°19'69.7 N, 151°33'21.1E | 15 (6) [m] | 24 (18) |
| SE. South East Pass | Chuuk | 7°14'60.3 N, 152°01'29.1 E | 21 (17) [m] | 22 (16) |
| AN. Ant Atoll (East) | Pohnpei | 6°47'42.3 N, 158°01'20.7 E | 24 (16) | 22 (13) |
| RO. Roj | Pohnpei | 6°46'37.7 N, 158°12'24.1 E | 24 (16) | 23 (15) |
| CG. Coral Garden | Kosrae | 5°18'47.2 N, 162°53'01.8 E | 25 (15) [m] | 24 (13) |
| HP. Hiroshi Point | Kosrae | 5°15'88.0 N, 162°59'01.8 E | 25 (23) [m] | 25 (14) |
| TOTAL | | | **277 (190)** | **278 (178)** |

351    * indicates that no individuals of this species were found
352    [1] indicates that individuals were not collected from this site but are likely present
353    [m] indicated sites where multiple *Symbiodinium* species were detected

354

355    ***Laboratory Procedures:*** Holobiont DNA was isolated following Davies *et al.* (60).

356    Microsatellite primers for this study consisted of six previously published clade C loci

357    (61, 62) and one novel locus mined using Msatcommander (63) from nucleotide EST data

358    for *Symbiodinium* sp. clade C3 in GenBank (64) (Table 6). Loci were multiplexed

359    according to annealing temperatures and fragment sizes. Each 20 µl polymerase chain

360    reaction (PCR) mixture contained 10 ng of DNA template, 0.1 µM of each forward

361    primer, 0.1 µM of each reverse primer, 0.2 mM dNTP, 1 µl 10X *ExTaq* buffer, 0.025 U

362    *ExTaq* Polymerase (Takara Biotechnology) and 0.0125 U *Pfu* Polymerase (Agilent

363    Technologies). Amplifications were performed using a DNA Engine Tetrad2 Thermal

364    Cycler (Bio-Rad, Hercules CA). Cycling began at 94°C for 5 min, followed by 35 cycles

365    of 94°C for 40 s, annealing temperature for 120 s, and 72°C for 60 s and a 10 minute

366    extension period at 72°C. Molecular weights were analyzed using the ABI 3130XL

367    capillary sequencer with an in-house ROX-labeled size standard. Data were binned by

368    repeat size and individuals failing to amplify at ≥3 loci were excluded from analyses.

369

370    Table 6: Summary of six polymorphic microsatellite loci used to assess genetic variation
371    *Symbiodinium* clade C hosted by *A. hyacinthus* and *A. digitifera* and their corresponding
372    multiplexing groups.

| Multiplex Group | Locus (Repeat) | Primer Sequence 5'-3' | Repeat | Annealing Temperature | Source |
|---|---|---|---|---|---|
| A | *SgrSpl_30* | F: FAM-ccgaactacctttggtcaac<br>R: aaaagacaaggacatctcgg | TA | 53 | Wham et al., 2014 |
| B | *SgrSpl_78* | F: FAM-tgaaattcggtgttcattgt<br>R:ctcagatgtttccgacgagt | TA | 54 | Wham et al., 2014 |
| | *Sgr_21* | F: HEX-tgctgagtggcgtgtatatc<br>R: tgatggtacttgatggtg | TCA | 54 | Wham et al., 2014 |
| | *Spl_33* | F: HEX-acttgcaaagtccaagatcg<br>R: gaacggtgaaaggaaaatga | CAT | 54 | Wham et al., 2014 |
| C | *C784* | F: Hadp-ctccttaggactggactcgc<br>R: agaagtcaaatcgtcaccatcg | ATC | 60 | This Study |
| D | *C105* | F: FAM-tttcgttgttggacattgttatg<br>R: ggactgaaaggtgcttgagg | complex | 55 | Bay *et al.,* 2009 |

373    Fadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter
374    tag sequence: FAM: agcagcgaactcagtacaaca
375    Hadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled
376    adapter tag sequence: HEX: tcgtcgcttgagtcatcgtta
377
378

379    ***Data Analysis:*** *Symbiodinium* in clade C microsatellite data were scored as diploid based

380    on available information on the ploidy of these organisms (3, 62, 65) Since each

381    individual host could potentially contain a population of genetically distinct

382    *Symbiodinium* individuals, multi-locus genotypes (MLG) were only considered in this

383    study if they contained two or fewer alleles across all loci, suggesting that all alelles

384    originate from a single, clonally replicated, genome (65), which was the case for a total of

385    190 out of 277 for *A. digitifera* hosts and 178 out of 278 for *A. hyacinthus* hosts. We first

386    applied a Bayesian approach implemented in STRUCTURE v2.3.3 (66). STRUCTURE uses a

387    Monte Carlo Markov chain (MCMC) clustering algorithm to assign individuals with

388    similar genotypes to populations. Mean and variance of log likelihood values of the

389    number of populations K (1-10) were inferred by STRUCTURE with $10^6$ iterations (burn in

390    = 300,000 iterations) in ten replicate runs for each K. An admixture model was

391    implemented with no location prior. Following the recommendations of (67), the ad hoc

392    statistic ΔK was calculated based on the rate of change of the log-likelihood between

393    consecutive K values, which is implemented in the program STRUCTURE Harvester (68).

394    CLUMPP (69) and DISTRUCT (70) were used to produce graphics. We observed high

395    percent assignment to two different clusters among sympatric individuals. Following the

396    methodology of Wham and LaJeunesse (65), we investigated the relationship between

397    these cluster assignments and allele identities. From this analysis we found a strong

398    relationship between cluster assignments and allele identity, particularly at Sgr_21 and

399    Spl_33 (Supplement Fig. S1A, B), and it became clear that two distinct *Symbiodinium*

400    lineages were present. MLG data were then split into *Symbiodinium* lineages based on the

401    probability of species assignment conditioned on their cluster assignment (65).

402    Additional STRUCTURE runs were completed following descriptions above with these

403    subsequent differences: 1) location prior was implemented and 2) instead of using the $\Delta K$

404    statistic, we presented the K that maximized the mean of the estimated probability of data

405    while minimizing the standard deviation.



406
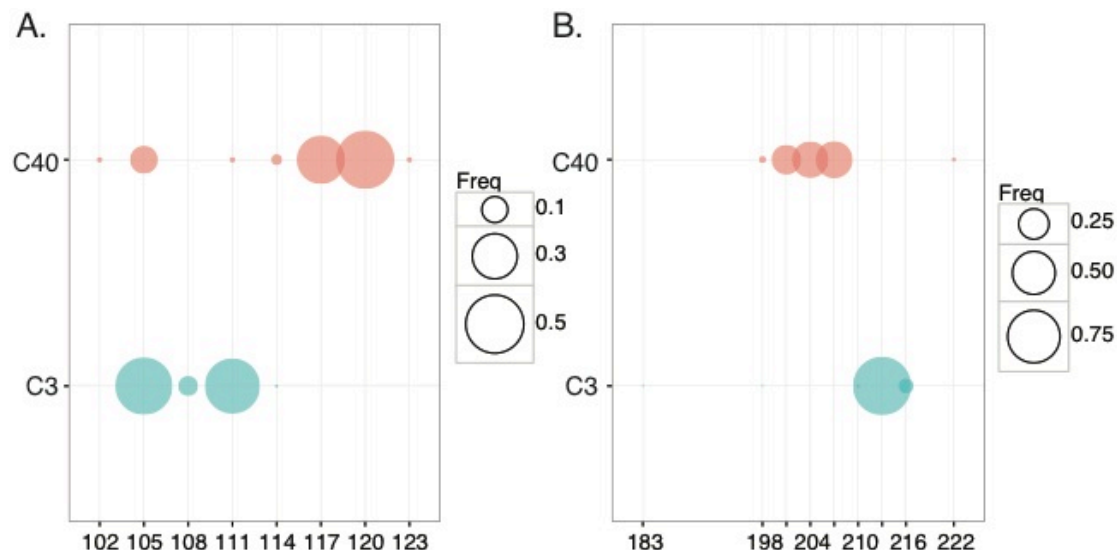
407 Supplementary Figure 1: Bubble plot of the SSR allele frequencies of C3 (red) and C40 (blue) at
408 the two loci that showed the largest discrimination power, Sgr_21 (A) and Spl_33 (B).
409 The size of each bubble is proportional to the allele frequency within each *Symbiodinium* type.
410

411    Once data were partitioned by *Symbiodinium* lineages, genetic divergences among

412    islands and sites were investigated using $F_{ST}$ (AMOVA, 9999 permutations in GENALEX

413    v6.5, (71)). Pairwise differentiations ($F_{ST}$ and Jost's D) and pairwise island distances

414    were then correlated to test for isolation by distance (IBD) in *Symbiodinium* C3 (not

415    sufficient data for C40) and these IBD trends were compared to results from the host

416    species (72). Mean allelic diversities per island and numbers of private alleles per island

417    were also calculated in GENALEX v6.5.

418    To resolve differences between islands, between host species and between sites

419    and host species within each island, assignment of samples to genetic clusters using

420  discriminant analysis of principal components (DAPC) was performed in R (73) using the

421  ADEGENET package (74, 75). Here, *Symbiodinium* data were converted into principle

422  components and then a-scores were used to determine trade-offs between power of

423  discrimination and model over-fitting. Relationships were examined by DAPC, which

424  optimizes variation among clusters by minimizing variation within clusters, while

425  retaining the optimal number of principle components and maximum number of

426  discriminant functions. All information on DAPC model parameters and results are

427  contained in Table 3. Cluster assignment patterns were compared across islands, host

428  species, and among host species and sites within each island.

429

430  **Sequencing Analysis of *Symbiodinium* psbA$^{ncr}$**

431  In order to confirm the assignments of individuals to different *Symbiodinium* lineages, we

432  selected a small subset of the full sample set and analyzed the non-coding region of the

433  circular plastid (psbA$^{ncr}$). We amplified the psbA$^{ncr}$ locus with the primers 7.4-Forw and

434  7.8-Rev following the methods described by LaJeunesse and Thornhill (40). The

435  amplified product was directly sequenced and the resulting sequences were aligned with

436  ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) to clade C psbA$^{ncr}$ sequences

437  from common *Symbiodinium* types found throughout the Indo-Pacific region

438  (Supplemental Table 1: JQ043587-JQ043676 from (40)). We reconstructed the

439  phylogeny of these samples using the default settings of Phylogeny.fr (76). The identities

440  of *Symbiodinium* types from our analysis were interpreted from their percent sequence

441  identity with these previously identified *Symbiodinium* types and their position on the

442  resulting phylogeny.

443

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454
455

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669 Supplemental Table 1: Accession number and corresponding Clade C *Symbiodinium* lineage for
670 each unique sequence identifier from Figure 2.
671

| Sequence ID | Accession Number | Lineage |
|---|---|---|
| 1 | JQ043642 | C3 |
| 2 | KF572372 | C3 |
| 3 | JQ043643 | C3 |
| 4 | JQ043641 | C3 |
| 5 | JQ043640 | C3 |
| 6 | JQ043644 | C3 |
| 7 | JQ043635 | C3 |
| 8 | JQ043638 | C3 |
| 9 | JQ043637 | C3 |
| 10 | JQ043636 | C3 |
| 11 | KF572331 | C3 |
| 12 | KF572332 | C3 |
| 13 | KF572318 | C3 |
| 14 | KF572319 | C3 |
| 15 | KF572373 | C3 |
| 16 | KF572374 | C3 |
| 17 | KF572358 | C3 |
| 18 | KF572368 | C40 |
| 19 | KF572359 | C40 |
| 20 | KF572367 | C40 |
| 21 | KF572366 | C40 |
| 22 | KF572360 | C40 |
| 23 | KF572357 | C40 |
| 24 | KF572363 | C40 |
| 25 | KF572365 | C40 |
| 26 | KF572364 | C40 |
| 27 | KF572370 | C40 |
| 28 | JQ043603 | C31 |
| 29 | JQ043599 | C31 |
| 30 | JQ043604 | C31 |
| 31 | JQ043671 | C27 |
| 32 | JQ043673 | C27 |
| 33 | JQ043670 | C27 |
| 34 | JQ043669 | C27 |
| 35 | JQ043676 | C27 |

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