# Contrasting population genetic structure in *Acropora* coral hosts and their algal symbionts across multiple spatial scales

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Running Title: Coral-associated symbiont genetic structure

**Abstract**

Many broadly-dispersing corals acquire their algal symbionts (Symbiodiniaceae) from their environment upon recruitment. This horizontal transmission strategy has the potential to promote coral fitness across diverse environments provided that corals can associate with diverse algae across their range and that these symbionts exhibit reduced dispersal potential. Here we quantified genetic structure of algal symbionts in symbiosis with two hosts (*Acropora hyacinthus, Acropora digitifera*) across two spatial scales (across islands, within islands) in Micronesia using microsatellites. We contrast these symbiont genetic structures to previously published coral host structures across the same spatial gradient. We find that both hosts associated with two genetically distinct *Cladocopium* lineages (C3, C40), confirming that *Acropora* coral hosts associate with diverse algae across this range. In addition, each *Cladocopium* lineage exhibited signatures of moderate host specialization. Across locations, algal populations were significantly more genetically structured compared to their hosts: they demonstrated higher $F_{ST}$ among islands and often exhibited significant divergence among reefs on the same island, which was never observed in coral hosts. While *Cladocopium* C3, like their hosts, broadly followed an isolation-by-distance pattern across islands, there was one notable case of no genetic structure across more than 2,000 km (between Yap and Pohnpei), demonstrating that *Cladocopium* genetic structure can be more complex than their hosts. Overall, our results support the view that horizontal transmission – establishing novel symbioses with local symbionts - has the potential to facilitate local fitness for broadly dispersing coral species, and highlight the complexity of factors affecting the population biology of *Cladocopium*.

**Keywords:** coral, Symbiodiniaceae*, Cladocopium,* population genetics, *Acropora,* isolation by distance, symbiosis, horizontal transmission

41 **Introduction**

42 Many well-known symbioses involve the passing of symbionts from parents to offspring (vertical
43 transmission), fully aligning the evolutionary trajectories of symbiotic partners and typically
44 leading to their deep integration at biochemical and genomic levels (i.e. Buchnera in aphids
45 (Nakabachi, Ishida, Hongoh, Ohkuma, & Miyagishima, 2014; Shigenobu & Wilson, 2011)). The
46 result of such symbiosis is essentially a novel composite organism, often called the 'holobiont',
47 upon which selection can act (Bordenstein & Theis, 2015). In other types of symbioses, the
48 association between partners must be established anew each generation (horizontal transmission),
49 which offers the host's offspring the opportunity to sample a variety of symbiont lineages and
50 select partners that potentially confer some sort of local advantage (Hilario et al., 2011; Schwarz,
51 Krupp, & Weis, 1999; Usher, Bergman, & Raven, 2007). In theory, this kind of relationship should
52 generate novel ecological opportunities for both symbiotic partners through their mixing and
53 matching across environments. For example, association with ecologically specialized algal
54 photobionts can lead to distinct ecological guilds of lichens (Peksa & Skaloud, 2011) or allow a
55 fungal partner to expand its geographic range across a more broad climatic envelope (Fernandez-
56 Mendoza et al., 2011). Similarly, in aphids, association with various horizontally transmitted
57 bacterial symbionts allows these insects to colonize novel host plants across climatic zones (Henry
58 et al., 2013).

59 Reef building corals are no exception, and associations with algal symbionts in the family
60 Symbiodiniaceae is obligatory for the majority of tropical corals since they rely on photosynthetic
61 byproducts from the algae for energy in oligotrophic waters and, in turn, the algae benefit from a
62 protected and light-exposed residence as well as inorganic nutrients and $CO_2$ concentration

63 mechanisms provided by the host (Barott, Venn, Perez, Tambutte, & Tresguerres, 2015;
64 Muscatine, 1990; Muscatine & Cernichiari, 1969; Trench & Blank, 1987). Given the obligatory
65 nature of this symbiosis for the host, it is somewhat surprising that in the majority of coral species
66 (~85%), algal symbionts are not transmitted vertically, but rather must be acquired by the juvenile
67 coral from its local environment post settlement (Baird, Guest, & Willis, 2009; Fadlallah, 1983;
68 Harrison & Wallace, 1990; Hartmann, Baird, Knowlton, & Huang, 2017). One possible benefit to
69 this horizontal transmission strategy is that aposymbiotic coral larvae are highly dispersive (S. W.
70 Davies, Treml, Kenkel, & Matz, 2015; Foster et al., 2012; Rippe et al., 2017; van Oppen, Peplow,
71 Kininmonth, & Berkelmans, 2011), but the within-reef environmental variation that corals
72 experience is highly variable (Gorospe & Karl, 2011) and conditions within the coral's new reef
73 environment are likely to be ecologically distinct from conditions on their natal reef (Baird,
74 Cumbo, Leggat, & Rodriguez-Lanetty, 2007; LaJeunesse et al., 2004). In this way, corals can
75 potentially improve their fitness by associating with locally available, and putatively ecologically
76 specialized, algal strains (Byler, Carmi-Veal, Fine, & Goulet, 2013; E.J. Howells et al., 2012;
77 Rowan & Knowlton, 1995).

78 Indeed, the diversity of algal symbionts in the family Symbiodiniaceae is rich (LaJeunesse
79 et al., 2018) and specific coral-algae associations have been suggested to play pivotal roles in
80 holobiont adaptation to climate change (Berkelmans & van Oppen, 2006; E.J. Howells et al.,
81 2012). The genus *Cladocopium* (formerly clade C *Symbiodinium*; (LaJeunesse et al., 2018))
82 originated and diversified most recently among Symbiodiniaceae, and has achieved the highest
83 diversity of all Symbiodiniaceae lineages (Lesser, Stat, & Gates, 2013; Pochon & Gates, 2010;
84 Pochon, Montoya-Burgos, Stadelmann, & Pawlowski, 2006; Thornhill, Howells, Wham, Steury,

85 & Santos, 2017; Thornhill, Lewis, Wham, & LaJeunesse, 2014). This diversity has been associated
86 with functional variation in symbiont thermal performance across reefs (Sarah W. Davies, Ries,
87 Marchetti, & Castillo, 2018; E.J. Howells et al., 2012) as well as with functional differences in
88 gene expression between reef zones (Sarah W. Davies et al., 2018), lending support for the
89 potential for reef-specific symbiont communities.

90 However, little is known about the population biology of *Cladocopium spp.* algal
91 symbionts, including how their populations are structured relative to their coral hosts. Comparing
92 the dispersive capacities of each of these symbiotic partners across diverse environments is an
93 essential component to better understanding this symbiosis. Here, using microsatellites, we
94 examined multilocus genotypes (MLG) of *Cladocopium spp.* algal symbionts hosted by two
95 species of *Acropora* coral hosts – *A. hyacinthus* and *A. digitifera* – collected from the same reef
96 locations across the Micronesian Pacific (Fig 1A, B). Our previous work demonstrated that both
97 host species exhibited extensive genetic connectivity and their genetic structure was well explained
98 by the patterns of regional surface currents and geographic distance in a classic isolation by
99 distance pattern (S. W. Davies et al., 2015). Here, by analyzing two coral species that co-occur
100 across the same islands as well as local reef environments, we aimed to investigate the relative
101 roles of geographic distance and host species in driving the genetic structure of algal symbiont
102 populations in the genus *Cladocopium* and to compare the structure of the algal symbiont
103 populations to their coral hosts.

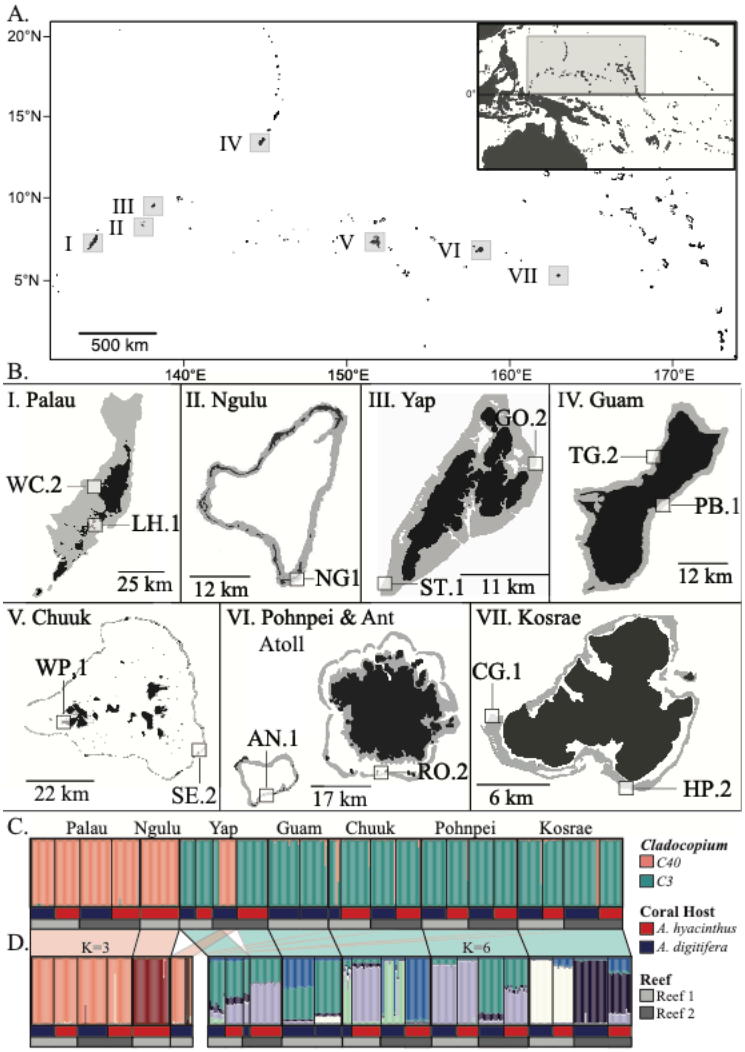


**Fig 1: Locations where coral samples were collected and observed *Cladocopium* genetic structure**. (A) Sampled islands in Micronesia, with an inset of the Pacific Ocean for reference. (B) Sampled locations within each island. Locations were chosen to potentially maximize within-island divergence. (C). STRUCTURE assignments for *Cladocopium* at an optimal population number (K=2), corresponding to C40 (pink) and C3 (turquoise) lineages. (D). Within-lineage STRUCTURE analysis. On panels C and D, color bars below the STRUCTURE plot indicate coral host species (see legend) and shades of grey correspond to different sites within each island.

## Materials and Methods

### *Sampling*

This study comprised a subset of samples previously analyzed for coral host genetics in Davies *et*

116 *al.* (2015) (Table S1, Fig 1A). Twenty-five individuals of each coral host species (*Acropora*
117 *hyacinthus* and *Acropora digitifera*) were examined at two reef sites (Fig 1B) within each of seven
118 islands, with the exception of Ngulu (where only *A. hyacinthus* was collected and only one site
119 was visited) and Guam (where there were no identified *A. hyacinthus*), for a total of 13 reef sites.
120

121 *Laboratory Procedures*

122 DNA was isolated following Davies *et al.* (2013). Microsatellite primers consisted of five
123 previously described *Cladocopium*-specific loci (previously described as clade C *Symbiodinium*)
124 (Bay, Howells, & van Oppen, 2009; Wham, Carmichael, & LaJeunesse, 2014) and one novel locus
125 mined using MsatCommander (Faircloth, 2008) from nucleotide EST data for lineage C3 (Leggat,
126 Hoegh-Guldberg, Dove, & Yellowlees, 2007), for a total of six loci (Table S2). Loci were
127 multiplexed in 20 µl polymerase chain reaction (PCR) mixtures containing 10 ng of template DNA,
128 0.1 µM of each forward and reverse primers, 0.2 mM dNTP, 1X *ExTaq* buffer, 0.025 U *ExTaq*
129 Polymerase (Takara) and 0.0125 U *Pfu* Polymerase (Promega). Amplifications began at 94°C for
130 5 min, followed by 35 cycles of 94°C for 40 s, annealing temperature for 120 s, and 72°C for 60 s
131 and a 10 minute 72°C extension period. Molecular weights were analyzed using the ABI 3130XL
132 capillary sequencer. Data were binned by repeat size and individuals failing to amplify at ≥3 loci
133 were excluded from downstream analyses.
134

135 *Data Analysis*

136 Although Symbiodiniaceae *in hospite* are assumed to be haploid (Santos & Coffroth, 2003), the
137 genus *Cladocopium* are generally observed to have two copies of every allele (Thornhill et al.,

138 2014; Wham et al., 2014; Wham & LaJeunesse, 2016). This apparent genome duplication may or
139 may not correspond to a change in chromosome number, or the actual diploid state (Wham &
140 LaJeunesse, 2016), and it has been suggested that these lineages should be scored as if they were
141 effectively diploid (i.e. with the expectation of two alleles per locus) to appropriately construct
142 multilocus genotypes (MLGs) from samples (LaJeunesse et al., 2014; Pettay, Wham, Smith,
143 Iglesias-Prieto, & LaJeunesse, 2015; Thornhill et al., 2014; Wham et al., 2014; Wham &
144 LaJeunesse, 2016). However, given that the ploidy of the *Cladocopium* samples in our study is
145 unknown and because a single coral host could potentially contain several genetically distinct
146 *Cladocopium* clones, data were analyzed in two ways. In the first analysis, MLGs were only
147 included if they contained two or fewer alleles across all loci, which would suggest that all alleles
148 originated from a diploid *Cladocopium* clone or a haploid clone with a duplicated genome (Wham
149 et al., 2014). This MLG dataset consisted of 190 of 277 (69%) *A. digitifera* samples and 178 of
150 278 (64%) *A. hyacinthus* samples. A second analysis made no ploidy assumptions and alleles were
151 simply designated a binary presence/absence values for each individual, thereby retaining all
152 alleles and all individuals in the analysis. This analysis is described in detail below under the
153 'additional unconstrained community analyses' section.

154 For the first analysis, STRUCTURE v2.3.3 (Pritchard, Stephens, & Donnelly, 2000) was used
155 to assign similar MLGs to clusters ($10^6$ iterations, burn in = 300,000) across ten replicate runs for
156 each K (1-10) using an admixture model with no location prior. Following Evanno *et al.* (Evanno,
157 Regnaut, & Goudet, 2005), ΔK was calculated in STRUCTURE Harvester (Earl & Vonholdt, 2012)
158 and CLUMPP (Jakobsson & Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004) produced all
159 graphics. In this initial analysis, all samples exhibited strong assignments into two highly

160 supported clusters. Following Wham and LaJeunesse (2016), we investigated the relationship
161 between these cluster assignments and their allele identities and found strong relationships
162 between assignments and allele identity, particularly at two loci (Sgr_21 and Spl_33; Fig S1), and
163 therefore concluded that two distinct *Cladocopium* lineages were present. Additional analysis of
164 psbA$^{ncr}$ locus, described below, indicated that these two lineages were C3 *sensu* (LaJeunesse et
165 al., 2003) and C40 *sensu* (LaJeunesse et al., 2004).

166 MLG data were then split into initial STRUCTURE lineages using the BAYES ALLELE
167 script from Wham and LaJeunesse (2016) and additional STRUCTURE runs were completed on these
168 lineage subsets. All C40 MLG data and C3 MLG data are available in the supplementary
169 information (Supplemental files 1-2). Partitioned lineage data were investigated for genetic
170 divergences among host species, islands and sites and hosts within islands using $F_{ST}$ (AMOVA,
171 9999 permutations in GENALEX v6.5, (Peakall & Smouse, 2006)).

172 Pairwise differentiation ($F_{ST}$) and pairwise island distances (km) were correlated to test for
173 isolation by distance (IBD) in *Cladocopium* C3 (data were insufficient for C40) using Mantel's
174 test implemented in the *vegan* package in R (Oksanen et al., 2013). Two matrices were tested, the
175 full $F_{ST}$ dataset and an $F_{ST}$ data subset from which a single non-significant pairwise $F_{ST}$ (Yap-
176 Pohnpei) was excluded. These trends were compared to our previously published results from the
177 two host species (S. W. Davies et al., 2015). Mean allelic diversities per island and numbers of
178 private alleles per island were also calculated in GENALEX v6.5.

179 To visualize differences within C3 and C40 between host species, between islands, and
180 between sites and host species within each island, assignment of samples to genetic clusters with
181 prior grouping of island/host/site was performed in R (R Development Core Team, 2015) using

182 discriminant analysis of principal components (DAPC) in the ADEGENET package (Jombart,
183 2008; Jombart, Devillard, & Balloux, 2010). DAPC is a machine-learning algorithm aiming to
184 classify samples into user-defined groups based on their coordinates in the principle components'
185 space and because DAPC does not rely on a traditional population genetics model, it is free from
186 Hardy-Weinberg equilibrium and linkage disequilibrium assumptions and thus is considered to be
187 useful across organisms regardless of their ploidy and genetic recombination rates (Jombart et al.
188 2010). Successful reassignment, indicated by a high proportion of samples correctly assigning to
189 their group, indicates that user-defined groups are distinct, which in our case implies genetic
190 divergence. Here, data were converted into principal components (PCs) and then a-scores were
191 computed to determine the optimal number of PCs to retain. A-scores determine the proportion of
192 successful reassignment corrected for the number of PCs retained and ensure that model overfitting
193 is not occurring (Jombart et al., 2010). Assignment rates, PCs and DFs retained, and the proportion
194 of variance explained by the model are included in Tables S4. To further substantiate genetic
195 divergence of our populations, we computed $F_{ST}$ for each DAPC clustering analysis.
196
197 *Sequencing Analysis of Cladocopium psbA$^{ncr}$*
198 To confirm phylogenetic affiliation of the two detected *Cladocopium* lineages, the non-
199 coding region of the circular plastid (psbA$^{ncr}$) was amplified in four representative samples (two
200 for each lineage) using methods described by LaJeunesse and Thornhill (LaJeunesse & Thornhill,
201 2011). Amplified products were directly sequenced and aligned with ClustalW2
202 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) to *Cladocopium* psbA$^{ncr}$ sequences from common
203 Indo-Pacific lineages (Table S5). The phylogeny was reconstructed using the default settings of

204 Phylogeny.fr (Dereeper et al., 2008) and identities of lineages were interpreted based on percent
205 sequence identity with previously identified *Cladocopium* lineages.
206
207 *Additional unconstrained community analyses*
208 Since the ploidy of *Cladocopium* remains debated and only four *Cladocopium* samples
209 were confirmed via psbA$^{ncr}$ sequencing, we elected to employ a second analysis of DAPC to
210 confirm the population structure results we detected using the analyses that assume diploidy. All
211 samples were included regardless of allele number at each locus and data were converted to a
212 binary table of allele presence/absences within each sample (Supplementary File 3). Genetic
213 clusters were then explored using DAPC with group priors as described above. In addition, because
214 DAPC analyses maximize variation between groups, we also visualized data using a principle
215 component analysis of binary allele data using the *rda(x, binary=TRUE)* function implemented in
216 the *vegan* package in R (Oksanen et al., 2013).
217
218 **Results**
219 *Two* Cladocopium *algal symbiont lineages observed in Micronesian acroporids*
220 To enable standard population genetic analyses, data were restricted to corals hosting a single
221 multilocus genotype (MLG), which encompassed 69% of *A. digitifera* and 64% of *A. hyacinthus*
222 samples. Across the two coral species in Micronesia (Fig 1A, B), two distinct *Cladocopium*
223 lineages were observed, which were most clearly discriminated by two of the six microsatellite
224 loci (Fig S1). Sequencing of the psbA$^{ncr}$ gene for two representative samples from each lineage
225 identified these lineages as *Cladocopium* C40 and C3 (LaJeunesse & Thornhill, 2011) (Fig S2). It

226 is important to note that the possible presence of other genera of Symbiodiniaceae in the
227 background would not affect *Cladocopium* genotyping results since our microsatellite assays are
228 highly genus-specific. Within the single MLG data subset, corals of both *Acropora* species from
229 Palau and Ngulu were found to exclusively host *Cladocopium* C40 (Fig 1C, pink bars). C40 was
230 also prevalent in *A. digitifera* at one reef site on Yap (Goofnuw Channel) and was occasionally
231 found in *A. digitifera* across the rest of Micronesia (Fig 1C). All other *Acropora* samples across
232 the spatial gradient explored associated with lineage C3 (Fig 1C, turquoise bars). Both
233 *Cladocopium* lineages possessed high genetic diversity, with a total of 70 unique alleles across
234 three islands in C40 (Table S3A) and 130 unique alleles across five islands in C3 (Table S3B).
235 Mean numbers of alleles per island for each locus for C40 ranged from 3.00-4.67 with the highest
236 number of private alleles observed in Palau (N=7) (Table S3A). Mean C3 allele numbers per island
237 for each locus ranged from 3.83 to 5.17 and numbers of private alleles ranged from one to four
238 (Table S3B).
239
240 Cladocopium *C3 are more genetically structured than their coral hosts*
241 Overall *Cladocopium* genetic divergence between the two host species across the entire dataset
242 was significant for both C40 ($F_{ST}$=0.092) and C3 ($F_{ST}$=0.037), confirming that host species play a
243 role in structuring *Cladocopium* populations across Micronesia (Fig 2A, B). All pairwise between-
244 island $F_{ST}$'s for C40 were significant (Table 1A) and in fact, the high $F_{ST}$ values for Palau and Yap
245 C40 relative to C40 from Ngulu suggest the potential for a third lineage of *Cladocopium* (Fig 2C),
246 although this pattern is not further explored here. C3 exhibited one surprisingly non-significant
247 $F_{ST}$ comparison (Yap-Pohnpei, 0.009) with all other island pairs exhibiting significant $F_{ST}$ values,

which ranged from 0.058 (Guam-Chuuk) to 0.078 (Yap-Kosrae) (Table 1B). Notably, when all pairwise comparisons were included, C3 differentiation did not show significant isolation-by-distance (IBD), which had been previously observed for both coral hosts over this range (S. W. Davies et al., 2015) (Mantel's r=0.1161, p=0.1809, Fig 3A). However, when the single non-significant Yap-Pohnpei $F_{ST}$ comparison was removed, significant IBD was observed for C3 (Mantel's r=0.4082, p=0.0277, Fig 3B). IBD could not be computed for C40 due to too few between-island comparisons. With the exception of the Yap-Pohnpei comparison, C3 $F_{ST}$ values between islands exceeded those for both host species and the slope of $F_{ST}$ ~ distance dependence was parallel to the slope observed in both coral hosts (Fig 3B). Overall, with the exception of C3 collected on Yap, all $F_{ST}$ values between reefs located within the same island (regardless of host species) were significant and ranged from 0.025 (C3 Guam) to 0.183 (C3 Kosrae) (Table 2). This result is in stark contrast to both coral host species, where no significant within-island genetic divergence was detected (S. W. Davies et al., 2015).

**Table 1.** Summary of pairwise $F_{ST}$ values and associated p-values between all islands for *Cladocopium* C40 (A) and C3 (B). Permutations were run 9999 times. All significant comparisons are shaded in grey. ***=p<0.001, **=p<0.01, *=p<0.05

A. C40

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

B. 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 |

**Table 2.** Summary of pairwise $F_{ST}$ values and associated p-values for *Cladocopium* C40 (A) and C3 (B) between all sites pooled across host species. Permutations were run 9999 times. All significant comparisons are shaded in grey and those within an island are in bold. ***=p<0.001, **=p<0.01, *=p<0.05

A. 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 |

B. 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 |

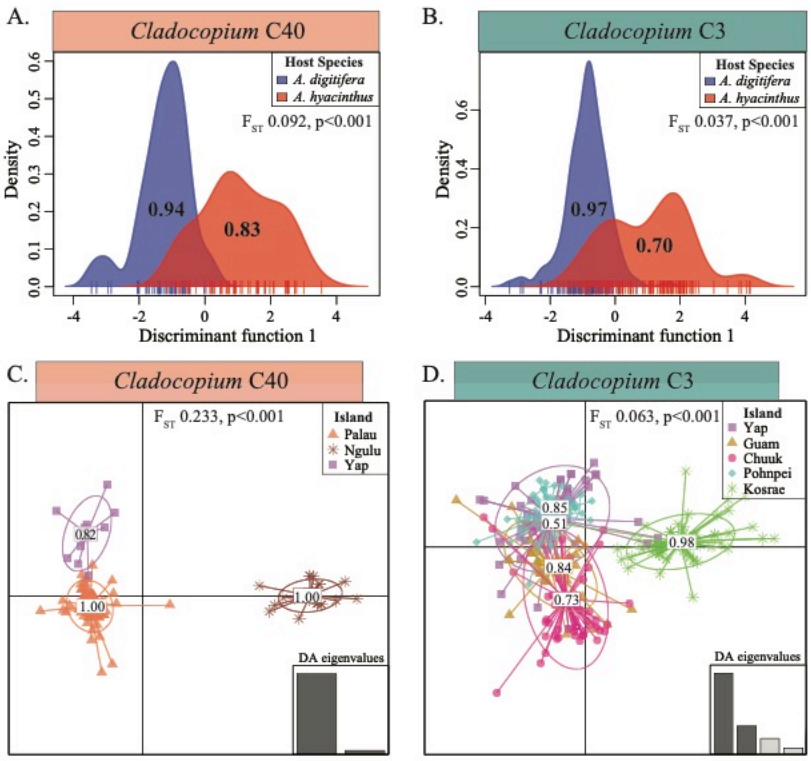


**Fig 2: DAPC of MLG data for *Cladocopium* C40 and C3 by host species and islands**
Discriminant analysis of principal components (DAPC) of MLG data for *Cladocopium* C40 and C3 hosted by *Acropora hyacinthus* and *A. digitifera* at thirteen sites across seven islands in Micronesia. DAPC analysis on two discriminant functions demonstrating strong host species assignments across all islands for *Cladocopium* C40 (A) and *Cladocopium* C3 (B) along with associated $F_{ST}$ values. Numbers overlaying the curves indicate proportion of correctly assigned samples. C. DAPC scatter plot for individual samples from *Cladocopium* C40 represented by colored dots clustered by islands. D. DAPC scatter plot for individual samples from *Cladocopium* C3 represented by colored dots clustered by islands. Proportions of correct assignments are indicated within the clusters. Information on the DAPC models can be found in Table S4.

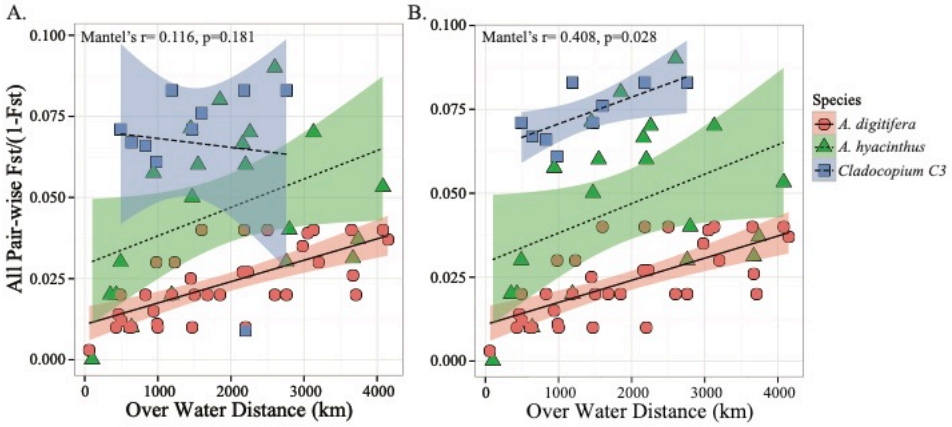


**Fig 3: Comparison of genetic differentiation with respect to distance for two coral host species and *Cladocopium* C3**

(A) Pairwise genetic differentiation [($F_{ST}$ /(1-$F_{ST}$)] of two species of *Acropora* coral hosts and *Cladocopium* C3 across overwater distances (km) demonstrating significant isolation by distance for the two coral host species but no correlation with distance for the symbiont. (B) The same pairwise genetic differentiation [($F_{ST}$ /(1-$F_{ST}$)] shown in A with one insignificant outlier (Yap-Pohnpei) removed demonstrating strong isolation by distance for the algal symbiont.

*Algal symbiont genetic differentiation by coral host species and local reef environment*

Discriminant analysis of principal components (DAPC) strongly differentiated between host species for both *Cladocopium* C40 and C3 (Table S4, Fig 2A, B), corroborating $F_{ST}$ estimates and suggesting that host specificity drives symbiont genetic diversity. However, both *Acroporia* hosts were capable of maintaining symbiosis with the same Symbiodiniaceae lineages across Micronesia, demonstrating that these hosts have flexibility across the spatial range investigated here. In addition, DAPC demonstrated differences among islands for each *Cladocopium* lineage irrespective of host species: generally high per-island assignment rates were obtained for C40 (Fig 2C, 82-100%) and C3 (Fig 2D, 51-98%), consistent with both STRUCTURE (Fig 1C, D) and $F_{ST}$ results (Table 1). Notably, algal symbionts from Yap consistently showed the lowest assignments rates for both C3 and C40. Moreover, nearly all pairwise $F_{ST}$ values between unique host-environment pairings on each island were significant for both lineages (Table 3), suggesting that

309 some combination of host specialization, environmental partitioning and/or strong dispersal
310 limitation across space is responsible for algal symbiont genetic differentiation. In accord with
311 these results, of the two top eigenvalues in DAPC analysis within islands, one discriminant
312 function (DF) explained divergence by host species and the other DF explained differences
313 between reef sites (Fig 4). However, there were several examples of non-significant pairwise $F_{ST}$
314 comparisons within the dataset (Table 3). These included *A. hyacinthus* symbionts across sites
315 within Palau, *A. digitigera* symbionts within Guam, *A. digitifera* symbionts across sites within Yap
316 and also *A. hyacinthus* and *A. digitifera* symbionts within Goofnuw on Yap (Table 3). However,
317 it is important to note that the sample size of *A. digitifera* symbionts at Goofnuw was low (N=4)
318 given that many of these hosts associated with C40, while the rest of *A. digitifera* symbionts on
319 Yap associated with C3 (Fig 1C, D) and this may explain the lack of significance observed.
320 Interestingly the strongest pattern of separation within host-environment pairings was observed
321 for Kosrae (Fig 4E), suggesting that whatever processes are responsible for symbiont genetic
322 divergence are strongest in Kosrae.

**Table 3.** Summary of pairwise $F_{ST}$ values for *Cladocopium* from the islands of (A) Palau, (B) Yap, (C) Chuuk, (D) Pohnpei, (E) Kosrae and (F) Guam between all host species and site combinations. Permutations were run 9999 times. All significant comparisons are shaded in grey. ***=p<0.001, **=p<0.01, *=p<0.05

A. Palau C40

| | Ad_LH | Ah_LH | Ad_WC | Ah_WC |
|---|---|---|---|---|
| Ad_LH | | ** | ** | *** |
| Ah_LH | 0.115 | | *** | NA |
| Ad_WC | 0.051 | 0.150 | | ** |
| Ah_WC | 0.105 | 0.018 | 0.081 | |

B. Yap C3

| | Ad_ST | Ah_ST | Ad_GF | Ah_GF |
|---|---|---|---|---|
| Ad_ST | | * | NA | ** |
| Ah_ST | 0.063 | | NA | NA |
| Ad_GF | 0.000 | 0.088 | | * |
| Ah_GF | 0.063 | 0.021 | 0.092 | |

C. Chuuk C3

| | Ad_WP | Ah_WP | Ad_SE | Ah_SE |
|---|---|---|---|---|
| Ad_WP | | ** | ** | *** |
| Ah_WP | 0.073 | | *** | *** |
| Ad_SE | 0.056 | 0.112 | | *** |
| Ah_SE | 0.275 | 0.167 | 0.219 | |

D. Pohnpei C3

| | Ad_AA | Ah_AA | Ad_RO | Ah_RO |
|---|---|---|---|---|
| Ad_AA | | ** | ** | *** |
| Ah_AA | 0.071 | | ** | * |
| Ad_RO | 0.104 | 0.092 | | ** |
| Ah_RO | 0.123 | 0.057 | 0.058 | |

E. Kosrae C3

| | Ad_CG | Ah_CG | Ad_HP | Ah_HP |
|---|---|---|---|---|
| Ad_CG | | *** | *** | *** |
| Ah_CG | 0.184 | | *** | *** |
| Ad_HP | 0.232 | 0.317 | | *** |
| Ah_HP | 0.334 | 0.167 | 0.191 | |

F. Guam C3

| | Ad_PB | Ad_TG |
|---|---|---|
| Ad_PB | | NA |
| Ad_TG | 0.025 | |

Lastly, to confirm that the patterns we observed were not simply an artifact of selecting only individuals with two or fewer alleles per locus, we performed additional unconstrained

342 community analyses on all data in binary format for each island. We conducted both DAPC and
343 Principle Component Analyses (PCA) and the general patterns were upheld: there was evidence
344 of both genetic divergence by host species and reef environment within each island (Fig. S3, S4).

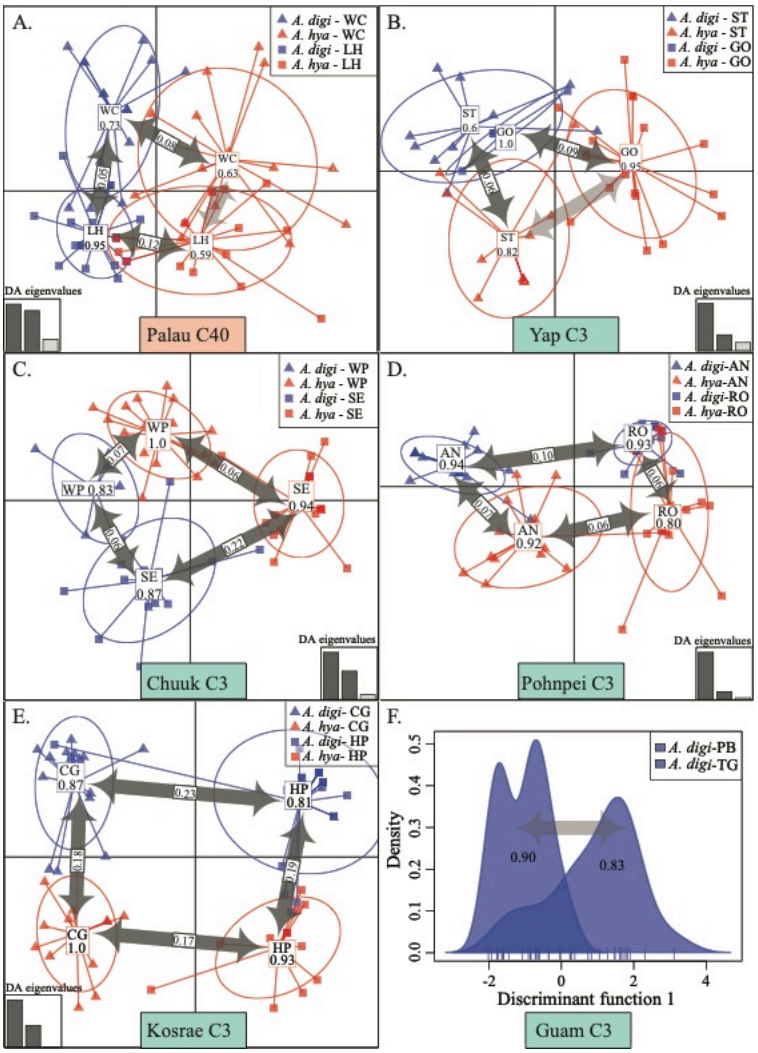


**Fig 4: DAPC of *Cladocopium* genotypes hosted by *Acropora hyacinthus* and *Acropora digitifera* at twelve reef sites across six islands in Micronesia.**
Discriminant analysis of principal components (DAPC) of MLG data for *Cladocopium* C40 and C3 hosted by *A. hyacinthus* and *A. digitifera* at two sites within each island in Micronesia. The first two discriminant functions are shown, which generally correspond to host species assignments and site assignments. DAPC scatter plots for individual samples from within (A) Palau for C40, (B) Yap for C3, (C) Chuuk for C3, (D). Pohnpei for C3, (E) Kosrae for C3, and (F) Guam for C3 for *A. digitifera* hosts only (hence a single discriminant function axis). Proportions of correct assignments are indicated in the clusters and dark grey arrows between clusters designate significant corresponding $F_{ST}$ values. Light grey arrows indicate that there was no significant differentiation. All pairwise $F_{ST}$ data are also shown in Table 3 and information on the DAPC models can be found in Table S4.

357 **Discussion**

358 Acropora *corals establish symbiosis with diverse* Cladocopium *lineages*

359 Across the Micronesian Pacific (Fig 1A, B), both *Acropora* coral hosts associated with
360 *Cladocopium* algal symbionts, which were represented by two distinct lineages – C40 and C3 (Fig
361 1C; Fig S2), with potentially more diverse cryptic lineages present (i.e. Ngulu *Cladocopium;* Fig
362 2C). This observation demonstrates that both hosts show considerable flexibility in their symbiotic
363 associations across their range and within their specific environments (Abrego, van Oppen, &
364 Willis, 2009; Berkelmans & van Oppen, 2006). This specificity at the *Cladocopium* genus level is
365 consistent with the wealth of previous comprehensive community composition studies suggesting
366 that Indo-Pacific acroporids are generally dominated by algal symbionts in this genus (i.e.
367 LaJeunesse et al., 2004; LaJeunesse et al., 2003). While initial algal symbiont infection is likely
368 determined by the local availability of free-living symbionts and made possible by the flexibility
369 of arriving coral recruits (Abrego et al., 2009; Cumbo, Baird, & van Oppen, 2013; Little, van
370 Oppen, & Willis, 2004), a winnowing process involving selection, competition, immune system
371 response, and differing rates of asexual proliferation likely plays a role in the eventual dominance
372 of a single MLG in the majority of coral hosts in their respective habitat (Rowan, Knowlton, Baker,
373 & Jara, 1997; Thornhill et al., 2017). Strict associations with a single MLG have been observed
374 across a variety of coral hosts and Symbiodiniaceae genera (Baums, Devlin-Durante, &
375 LaJeunesse, 2014; Pettay & Lajeunesse, 2013; Pinzón, Devlin-Durante, Weber, Baums, &
376 LaJeunesse, 2011; Thornhill et al., 2014), however this strict association is not always the case
377 (see E.J. Howells, van Oppen, & Bay, 2009; E. J. Howells, Willis, Bay, & van Oppen, 2013). Here,
378 we observed that the majority of corals contained no more than two *Cladocopium* alleles per locus,

379 which can be interpreted as hosting a single *Cladocopium* clone (*A. digitifera:* 69%, *A. hyacinthus:*
380 64%), corroborating previous works on Symbiodiniaceae genetics (reviewed in Thornhill et al.,
381 2017). However, it is also important to note that our study only explicitly explored population
382 genetic patterns in *Cladocopium,* the symbiont genus most commonly known to associate with
383 *Acropora* in this region, by targeting *Cladocopium*-specific loci. Only a few samples (N=4) were
384 specifically tested here for community level algal species identification, however our research
385 group had previously characterized community level Symbiodiniaceae communities on Palau
386 using ITS2 metabarcoding and determined that all hosts associated with one of two *Cladocopium*
387 algal symbiont haplotypes (Quigley at al., 2014). However, these community level analyses were
388 not performed on the other islands explored here and therefore, we are unable to comment on the
389 genetic patterns of other algal genera known to inhabit corals in background amounts (Silverstein
390 et al. 2012, Kennedy et al. 2015, Lee et al. 2016, Ziegler et al 2018).
391
392 Cladocopium *C3 exhibits IBD and stronger genetic structure that coral hosts across Micronesia*
393 We expected to observe strong genetic differentiation in *Cladocopium*, since the prevailing view
394 of their life cycle involves symbiotic existence in sedentary hosts alternating with a short-lived
395 free-living form that largely exists in the benthos where dispersal by ocean currents is limited and
396 their primary role is to invade novel hosts (Fitt, Chang, & Trench, 1981; Fitt & Trench, 1983;
397 Littman, van Oppen, & Willis, 2008; Magalon, Baudry, Husté, Adjeroud, & Veuille, 2006;
398 Yacobovitch, Benayahu, & Weis, 2004; Ali et al. *in press*). Our data supported this hypothesis:
399 with one notable exception of the Yap-Pohnpei comparison, we observed significant $F_{ST}$ between
400 all pairs of sampled islands in both C3 and C40 lineages. Our $F_{ST}$ estimates for the more broadly

401 distributed lineage (i.e. generalist), C3, did not exceed 0.078 (Table 1B) which is markedly lower
402 than previously published estimates from other locations (Andras, Kirk, & Harvell, 2011; E.J.
403 Howells et al., 2009). This could be attributable to the use of different markers and analytical
404 approaches, differences in the spatial scales explored, or differences between generalist/specialist
405 algal symbionts. In addition, exceptionally high genetic divergence values might be the result of
406 the presence of additional Symbiodiniaceae lineages within the same genus. Here, as an example,
407 we observed that C40 $F_{ST}$ values involving Ngulu were higher than any of those observed in C3
408 even though C40-populated islands were only separated by 100's, not 1000's, of kilometers (Palau-
409 Ngulu = 0.279, Yap-Ngulu = 0.326) (Table 1A). These results may suggest the presence of an
410 additional *Cladocopium* lineage present in *Acropora hyacinthus* on Ngulu. Regardless of the
411 magnitude, significant C3 and C40 $F_{ST}$ estimates support the prevailing view that Symbiodiniaceae
412 dispersal is limited across the seascape, although there are occasional cases of high genetic
413 similarity over very long distances (C3, Yap-Pohnpei).

414 Our working hypothesis predicted stronger symbiont $F_{ST}$ values compared to coral hosts.
415 It also predicted that *Cladocopium* algal symbionts would follow isolation by distance (IBD)
416 patterns, which was previously shown for both coral host species (S. W. Davies et al., 2015). Here,
417 we were only able to investigate IBD in *Cladocopium* C3 given that C40 was only observed in
418 high proportions at three islands (Fig 1B). Indeed, *Cladocopium* C3 $F_{ST}$ values were nearly always
419 greater than host $F_{ST}$ values, however, when all pairwise island comparisons were included
420 significant C3 IBD was not detected (Fig 3A). This lack of IBD was driven by one pairwise
421 comparison between Yap and Pohnpei, which was the only insignificant $F_{ST}$ across more than
422 2000 km distance (Table 1). When this outlier was removed, we observed significant IBD for

423 *Cladocopium* C3 ($r^2$=0.4082, p=0.0277) with higher values than their hosts (Fig 3B), which is
424 consistent with other studies investigating host-symbiont population genetics (i.e. (Baums et al.,
425 2014)). Similar levels of distance-dependent $F_{ST}$ in *Symbiodinium* have also been observed in C1
426 in *Pocillopora meandrina* in the South Pacific Islands (Magalon et al., 2006) and B1 in *Gorgonia*
427 *ventallina* (Andras et al., 2011). This lack of genetic differentiation between Yap-Pohnpei C3 (Fig
428 2D and Fig 3C) suggest that there is potential for occasional long-range dispersal in *Cladocopium*
429 C3 symbionts and that their population genetics, and perhaps population genetics of other
430 generalist symbionts, can exhibit complexities that cannot be predicted by traditional distance-
431 based dispersal limitation (Thornhill et al., 2017; Thornhill et al., 2014).
432
433 Cladocopium *C3 and C40 exhibit imperfect host specificity*
434 The majority of reef-building coral species associate with a specific Symbiodiniaceae type, which
435 have traditionally been broadly defined based on ribosomal and/or chloroplast markers (Fabina,
436 Putnam, Franklin, Stat, & Gates, 2013; Rodriguez-Lanetty, Krupp, & Weis, 2004; Thornhill et al.,
437 2014; Weis, Reynolds, deBoer, & Krupp, 2001). Previous Symbiodiniaceae multilocus genotyping
438 (MLG) studies revealed that each of these symbiont types can harbor within-type diversity, both
439 at the genetic and functional levels (E.J. Howells et al., 2012; E.J. Howells et al., 2009; Santos,
440 Shearer, Hannes, & Coffroth, 2004). Here we observe significant *Cladocopium* population
441 structure among two different host species within each site (Fig 4; Table 3). Previous work on
442 octocorals similarly observed significant host differentiation among algal symbionts, however they
443 found that this genetic divergence was driven by different aged cohorts and depth in their system
444 (Andras et al., 2011). However, host habitat depth or age class cannot directly explain the host

445 specificity observed in our study given that specific attention was payed to collecting colonies
446 located at similar depths and of similar size classes. Instead, our data suggest that the
447 local association of hosts and symbionts within the same genotypic cluster is due to host specificity
448 in *Cladocopium* (Fig 3, 4), which has been previously proposed in symbionts hosted by
449 *Pocillopora* in the south Pacific (Magalon et al., 2006). Since our study sampled two coral host
450 species, we also detected that this specificity is imperfect: at every location, there were symbionts
451 in one host species that would have been assigned to another coral host based on their MLG (Fig
452 4). This pattern suggests that *Cladocopium's* host specialization is present, however there is
453 genetic exchange between these host-specific symbiont assemblages within a location.
454
455 *Genetic subdivision of* Cladocopium *within islands*
456 Within each island and sympatric host species, the majority of *Cladocopium* pairwise comparisons
457 exhibit significant genetic divergence between closely located reef sites (Fig 4; Table 3) suggesting
458 either that symbiont dispersal is very limited or spatially varying selection among reef sites is at
459 play. Genetic subdivision between reef sites at the same island is the best demonstration that
460 symbionts exhibit stronger genetic structure than their coral hosts, which never showed significant
461 substructure within islands (Davies et al., 2015). Notably, in some cases these within-island $F_{ST}$
462 values are higher than those observed between islands, which are much greater distance apart
463 (Tables 1-3). Given this pattern, it is tempting to speculate that genetic divergence among
464 individual reefs might be due not only to dispersal limitation, but also to spatially varying selection,
465 implying environmental specialization (i.e. local adaptation) in the symbionts. Unfortunately,
466 these islands are remote and understudied and therefore we lack further support for this claim as

467 we did not measure environmental parameters and did not assess symbionts' fitness across
468 environments. Among factors that might contribute to genetic subdivision among reefs irrespective
469 of distance is high variation in reproductive success among *Cladocopium* clones on a local scale,
470 which would elevate $F_{ST}$ due to spatial discordance of short-term allele frequency fluctuations
471 (Thornhill et al., 2017). Yet, previous work has demonstrated that other *Cladocopium* symbiont
472 populations have exhibited classic signals of local adaptation (E.J. Howells et al., 2012), and
473 therefore reef sites investigated here offer an excellent study system for investigating the fine-scale
474 local adaptation potential of *Cladocopium*. If these algal symbionts are indeed locally adapted, this
475 would ensure that horizontally transmitting coral hosts increase their local fitness by associating
476 with local symbionts. To confirm this hypothesis, much future work is required to experimentally
477 demonstrate that these symbionts are achieving their maximum fitness in their local reef
478 environment (Kawecki & Ebert, 2004).

**Authors' Contributions**

SWD and MVM conceived of the study, designed the study, coordinated the study and drafted the manuscript. SWD, MRK and MVM collected coral samples. SWD carried out molecular lab work, participated in data analysis, and carried out the statistical analyses; DCW participated in data analysis and interpretation; MRK participated in statistical analyses and carried out sequence alignments; All authors gave final approval for publication.

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501 **Data Accessibility**

502 All multilocus genotyping (MLG) data and binary presence/absence data for *Cladocopium* are
503 available in the supplementary files 1-3.

**References**

Abrego, D., van Oppen, M. J., & Willis, B. (2009). Onset of algal endosymbiont specificity varies among closely related species of Acropora corals during early ontogeny. *Molecular Ecology, 18*(16), 3532-3543.

Ali, A., Kriefall, N.G., Emery, L.E., Kenkel, C.D., Matz M.V., & Davies, S.W. (in press) Recruit symbiosis establishment and Symbiodiniaceae composition influenced by adult corals and reef sediment. *Coral Reefs*

Andras, J. P., Kirk, N. L., & Harvell, C. D. (2011). Range-wide population genetic structure of Symbiodinium associated with the Caribbean Sea fan coral, Gorgonia ventalina. *Molecular Ecology, 20*(12), 2525-2542. doi:Doi 10.1111/J.1365-294x.2011.05115.X

Baird, A. H., Cumbo, V. R., Leggat, W., & Rodriguez-Lanetty, M. (2007). Fidelity and flexibility in coral symbioses. *Marine Ecology Progress Series, 347*, 307-309. doi:10.3354/meps07220

Baird, A. H., Guest, J. R., & Willis, B. L. (2009). Systematic and Biogeographical Patterns in the Reproductive Biology of Scleractinian Corals. *Annual Review of Ecology Evolution and Systematics, 40*, 551-571. doi:Doi 10.1146/Annurev.Ecolsys.110308.120220

Barott, K. L., Venn, A. A., Perez, S. O., Tambutte, S., & Tresguerres, M. (2015). Coral host cells acidify symbiotic algal microenvironment to promote photosynthesis. *Proc Natl Acad Sci U S A, 112*(2), 607-612. doi:10.1073/pnas.1413483112

Baums, I. B., Devlin-Durante, M. K., & LaJeunesse, T. C. (2014). New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. *Molecular Ecology, 23*(17), 4203-4215. doi:10.1111/mec.12788

Bay, L. K., Howells, E. J., & van Oppen, M. J. (2009). Isolation, characterisation and cross amplification of thirteen microsatellite loci for coral endo-symbiotic dinoflagellates (Symbiodinium clade C). *Conservation Genetics Resources, 1*(1), 199-203.

Berkelmans, R., & van Oppen, M. J. H. (2006). The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society B-Biological Sciences, 273*(1599), 2305-2312. doi:Doi 10.1098/Rspb.2006.3567

Bordenstein, S. R., & Theis, K. R. (2015). Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. *PLoS Biol, 13*(8), e1002226. doi:10.1371/journal.pbio.1002226

Byler, K. A., Carmi-Veal, M., Fine, M., & Goulet, T. L. (2013). Multiple symbiont acquisition strategies as an adaptive mechanism in the coral Stylophora pistillata. *Plos One, 8*(3), e59596. doi:10.1371/journal.pone.0059596

Cumbo, V. R., Baird, A. H., & van Oppen, M. J. H. (2013). The promiscuous larvae: flexibility in the establishment of symbiosis in corals. *Coral Reefs, 32*(1), 111-120. doi:10.1007/s00338-012-0951-7

Davies, S. W., Rahman, M., Meyer, E., Green, E. A., Buschiazzo, E., Medina, M., & Matz, M. V. (2013). Novel polymorphic microsatellite markers for population genetics of the endangered Caribbean star coral, Montastraea faveolata. *Marine Biodiversity, 43*(2), 167-172. doi:Doi 10.1007/S12526-012-0133-4

Davies, S. W., Ries, J. B., Marchetti, A., & Castillo, K. D. (2018). Symbiodinium Functional Diversity in the Coral Siderastrea siderea Is Influenced by Thermal Stress and Reef

Environment, but Not Ocean Acidification. *Frontiers in Marine Science, 5*(150). doi:10.3389/fmars.2018.00150

Davies, S. W., Treml, E. A., Kenkel, C. D., & Matz, M. V. (2015). Exploring the role of Micronesian islands in the maintenance of coral genetic diversity in the Pacific Ocean. *Molecular Ecology, 24*(1), 70-82. doi:10.1111/mec.13005

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., . . . Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research, 36*(Web Server issue), W465-469. doi:10.1093/nar/gkn180

Earl, D. A., & Vonholdt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources, 4*(2), 359-361. doi:Doi 10.1007/S12686-011-9548-7

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology, 14*(8), 2611-2620. doi:Doi 10.1111/J.1365-294x.2005.02553.X

Fabina, N. S., Putnam, H. M., Franklin, E. C., Stat, M., & Gates, R. D. (2013). Symbiotic specificity, association patterns, and function determine community responses to global changes: defining critical research areas for coral-Symbiodinium symbioses. *Global Change Biology, 19*(11), 3306-3316. doi:Doi 10.1111/Gcb.12320

Fadlallah, Y. H. (1983). Sexual reproduction, development and larval biology of scleractinian corals. A review. *Coral Reefs, 2*, 129-150.

Faircloth, B. C. (2008). MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Mol Ecol Resour, 8*(1), 92-94. doi:Doi 10.1111/J.1471-8286.2007.01884.X

Fernandez-Mendoza, F., Domaschke, S., Garcia, M. A., Jordan, P., Martin, M. P., & Printzen, C. (2011). Population structure of mycobionts and photobionts of the widespread lichen Cetraria aculeata. *Molecular Ecology, 20*(6), 1208-1232. doi:10.1111/j.1365-294X.2010.04993.x

Fitt, W. K., Chang, S. S., & Trench, R. K. (1981). Motility Patterns of Different Strains of the Symbiotic Dinoflagellate Symbiodinium (=Gymnodinium) Microadriaticum (Freudenthal) in Culture. *Bulletin of Marine Science, 31*(2), 436-443.

Fitt, W. K., & Trench, R. K. (1983). The Relation of Diel Patterns of Cell-Division to Diel Patterns of Motility in the Symbiotic Dinoflagellate Symbiodinium-Microadriaticum Freudenthal in Culture. *New Phytologist, 94*(3), 421-432. doi:Doi 10.1111/J.1469-8137.1983.Tb03456.X

Foster, N. L., Paris, C. B., Kool, J. T., Baums, I. B., Stevens, J. R., Sanchez, J. A., . . . Mumby, P. J. (2012). Connectivity of Caribbean coral populations: complementary insights from empirical and modelled gene flow. *Molecular Ecology, 21*(5), 1143-1157. doi:Doi 10.1111/J.1365-294x.2012.05455.X

Gorospe, K. D., & Karl, S. A. (2011). Small-Scale Spatial Analysis of In Situ Sea Temperature throughout a Single Coral Patch Reef. *Journal of Marine Biology, 2011*, 12. doi:10.1155/2011/719580

Harrison, P. L., & Wallace, C. C. (1990). *Reproduction, dispersal and recruitment of scleractinian corals* (Z. Dubinsky Ed. Vol. 25). Amsterdam: elsevier Science Publications

Hartmann, A. C., Baird, A. H., Knowlton, N., & Huang, D. (2017). The Paradox of Environmental Symbiont Acquisition in Obligate Mutualisms. *Current Biology, 27*(23), 3711-+. doi:10.1016/j.cub.2017.10.036

Henry, L. M., Peccoud, J., Simon, J. C., Hadfield, J. D., Maiden, M. J., Ferrari, J., & Godfray, H. C. (2013). Horizontally transmitted symbionts and host colonization of ecological niches. *Current Biology, 23*(17), 1713-1717. doi:10.1016/j.cub.2013.07.029

Hilario, A., Capa, M., Dahlgren, T. G., Halanych, K. M., Little, C. T., Thornhill, D. J., . . . Glover, A. G. (2011). New perspectives on the ecology and evolution of siboglinid tubeworms. *Plos One, 6*(2), e16309. doi:10.1371/journal.pone.0016309

Howells, E. J., Beltran, V. H., Larsen, N. W., Bay, L. K., Willis, B., & van Oppen, M. J. (2012). Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change, 2*, 116-120.

Howells, E. J., van Oppen, M. J., & Bay, L. K. (2009). High genetic differentiation and cross-shelf patterns of genetic diversity among Great Barrier Reef populations of Symbiodinium. *Coral Reefs, 28*(1), 215-225.

Howells, E. J., Willis, B. L., Bay, L. K., & van Oppen, M. J. (2013). Spatial and temporal genetic structure of Symbiodinium populations within a common reef-building coral on the Great Barrier Reef. *Molecular Ecology, 22*(14), 3693-3708. doi:10.1111/mec.12342

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics, 23*(14), 1801-1806. doi:Doi 10.1093/Bioinformatics/Btm233

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics, 24*(11), 1403-1405. doi:10.1093/bioinformatics/btn129

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet, 11*, 94. doi:10.1186/1471-2156-11-94

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters, 7*(12), 1225-1241. doi:10.1111/j.1461-0248.2004.00684.x

LaJeunesse, T. C., Bhagooli, R., Hidaka, M., DeVantier, L., Done, T., Schmidt, G. W., . . . Hoegh-Guldberg, O. (2004). Closely related Symbiodinium spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. *Marine Ecology Progress Series, 284*, 147-161. doi:Doi 10.3354/Meps284147

LaJeunesse, T. C., Loh, W. K. W., van Woesik, R., Hoegh-Guldberg, O., Schmidt, G. W., & Fitt, W. K. (2003). Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Limnology and Oceanography, 48*(5), 2046-2054.

LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., & Santos, S. R. (2018). Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology, 28*(16), 2570-+. doi:10.1016/j.cub.2018.07.008

LaJeunesse, T. C., & Thornhill, D. J. (2011). Improved resolution of reef-coral endosymbiont (Symbiodinium) species diversity, ecology, and evolution through psbA non-coding region genotyping. *Plos One, 6*(12), e29013. doi:10.1371/journal.pone.0029013

LaJeunesse, T. C., Wham, D. C., Pettay, D. T., Parkinson, J. E., Keshavmurthy, S., & Chen, C. A. (2014). Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate

genus Symbiodinium (Dinophyceae) Clade D are different species. *Phycologia, 53*(4), 305-319. doi:10.2216/13-186.1

Leggat, W., Hoegh-Guldberg, O., Dove, S., & Yellowlees, D. (2007). Analysis of an EST library from the dinoflagellate (Symbiodinium sp.) symbiont of reef-building corals. *Journal of Phycology, 43*(5), 1010-1021.

Lesser, M. P., Stat, M., & Gates, R. D. (2013). The endosymbiotic dinoflagellates (Symbiodinium sp.) of corals are parasites and mutualists. *Coral Reefs*, 603-611.

Little, A. F., van Oppen, M. J., & Willis, B. L. (2004). Flexibility in algal endosymbioses shapes growth in reef corals. *Science, 304*(5676), 1492-1494. doi:10.1126/science.1095733

Littman, R. A., van Oppen, M. J. H., & Willis, B. L. (2008). Methods for sampling free-living Symbiodinium (zooxanthellae) and their distribution and abundance at Lizard Island (Great Barrier Reef). *Journal of Experimental Marine Biology and Ecology, 364*(1), 48-53. doi:Doi 10.1016/J.Jembe.2008.06.034

Magalon, H., Baudry, E., Husté, A., Adjeroud, M., & Veuille, M. (2006). High genetic diversity of the symbiotic dinoflagellates in the coral Pocillopora meandrina from the South Pacific. *Marine Biology, 148*(5), 913-922. doi:10.1007/s00227-005-0133-z

Muscatine, L. (1990). The role of symbiotic algae in carbon and energy flux in reef corals. *Ecosystems of the World, 25*, 75-87.

Muscatine, L., & Cernichiari, E. (1969). Assimilation of photosynthetic products of zooxanthellae by a reef coral. *The Biological Bulletin, 137*(3), 506-523.

Nakabachi, A., Ishida, K., Hongoh, Y., Ohkuma, M., & Miyagishima, S. Y. (2014). Aphid gene of bacterial origin encodes a protein transported to an obligate endosymbiont. *Current Biology, 24*(14), R640-641. doi:10.1016/j.cub.2014.06.038

Oksanen, J. F., Blanchet, G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., . . . Wagner, H. (2013). vegan: Community Ecology Package. R package version 2.0-7.

Peakall, R., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes, 6*(1), 288-295. doi:Doi 10.1111/J.1471-8286.2005.01155.X

Peksa, O., & Skaloud, P. (2011). Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga Asterochloris (Trebouxiophyceae). *Molecular Ecology, 20*(18), 3936-3948. doi:10.1111/j.1365-294X.2011.05168.x

Pettay, D. T., & Lajeunesse, T. C. (2013). Long-range dispersal and high-latitude environments influence the population structure of a "stress-tolerant" dinoflagellate endosymbiont. *Plos One, 8*(11), e79208. doi:10.1371/journal.pone.0079208

Pettay, D. T., Wham, D. C., Smith, R. T., Iglesias-Prieto, R., & LaJeunesse, T. C. (2015). Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthella. *Proceedings of the National Academy of Sciences of the United States of America, 112*(24), 7513-7518. doi:10.1073/pnas.1502283112

Pinzón, J. H., Devlin-Durante, M. K., Weber, M. X., Baums, I. B., & LaJeunesse, T. C. (2011). Microsatellite loci for Symbiodinium A3 (S. fitti) a common algal symbiont among Caribbean Acropora (stony corals) and Indo-Pacific giant clams (Tridacna). *Conservation Genetics Resources, 3*(1), 45-47. doi:10.1007/s12686-010-9283-5

Pochon, X., & Gates, R. D. (2010). A new Symbiodinium clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Mol Phylogenet Evol, 56*(1), 492-497. doi:10.1016/j.ympev.2010.03.040

Pochon, X., Montoya-Burgos, J. I., Stadelmann, B., & Pawlowski, J. (2006). Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus Symbiodinium. *Mol Phylogenet Evol, 38*(1), 20-30. doi:10.1016/j.ympev.2005.04.028

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics, 155*(2), 945-959.

R Development Core Team. (2015). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/.

Rippe, J. P., Matz, M. V., Green, E. A., Medina, M., Khawaja, N. Z., Pongwarin, T., . . . Davies, S. W. (2017). Population structure and connectivity of the mountainous star coral, Orbicella faveolata, throughout the wider Caribbean region. *Ecology and Evolution, 7*(22), 9234-9246. doi:10.1002/ece3.3448

Rodriguez-Lanetty, M., Krupp, D. A., & Weis, V. M. (2004). Distinct ITS types of Symbiodinium in Clade C correlate with cnidarian/dinoflagellate specificity during onset of symbiosis. *Marine Ecology Progress Series, 275*(97-102).

Rosenberg, N. A. (2004). DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes, 4*(1), 137-138. doi:Doi 10.1046/J.1471-8286.2003.00566.X

Rowan, R., & Knowlton, N. (1995). Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proc Natl Acad Sci U S A, 92*(7), 2850-2853.

Rowan, R., Knowlton, N., Baker, A., & Jara, J. (1997). Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature, 388*(6639), 265-269. doi:10.1038/40843

Santos, S. R., & Coffroth, M. A. (2003). Molecular genetic evidence that dinoflagellates belonging to the genus Symbiodinium freudenthal are haploid. *Biological Bulletin, 204*(1), 10-20. doi:Doi 10.2307/1543491

Santos, S. R., Shearer, T. L., Hannes, A. R., & Coffroth, M. A. (2004). Fine-scale diversity and specificity in the most prevalent lineage of symbiotic dinoflagellates (Symbiodinium, Dinophyceae) of the Caribbean. *Molecular Ecology, 13*(2), 459-469.

Schwarz, J. A., Krupp, D. A., & Weis, V. M. (1999). Late Larval Development and Onset of Symbiosis in the Scleractinian Coral Fungia scutaria. *Biological Bulletin, 196*(1), 70-79. doi:10.2307/1543169

Shigenobu, S., & Wilson, A. C. (2011). Genomic revelations of a mutualism: the pea aphid and its obligate bacterial symbiont. *Cell Mol Life Sci, 68*(8), 1297-1309. doi:10.1007/s00018-011-0645-2

Thornhill, D. J., Howells, E. J., Wham, D. C., Steury, T. D., & Santos, S. R. (2017). Population genetics of reef coral endosymbionts (Symbiodinium, Dinophyceae). *Molecular Ecology*. doi:10.1111/mec.14055

Thornhill, D. J., Lewis, A. M., Wham, D. C., & LaJeunesse, T. C. (2014). Host-specialist lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution, 68*(2), 352-367. doi:10.1111/evo.12270

Trench, R. K., & Blank, R. J. (1987). Symbiodinium microadriaticum Fredenthal, S. Goreau sp. nov., S. Kawagut2 sp. nov., and S. pilosum sp. nov.: Gymnodinoid dinoflagellate symbionts of marine invertebrates. *Journal of Phycology, 23*(3), 469-481.

Usher, K. M., Bergman, B., & Raven, J. A. (2007). Exploring cyanobacterial mutualisms. *Annual Review of Ecology Evolution and Systematics, 38*, 255-273. doi:10.1146/annurev.ecolsys.38.091206.095641

van Oppen, M. J. H., Peplow, L. M., Kininmonth, S., & Berkelmans, R. (2011). Historical and contemporary factors shape the population genetic structure of the broadcast spawning coral, Acropora millepora, on the Great Barrier Reef. *Molecular Ecology, 20*(23), 4899-4914. doi:Doi 10.1111/J.1365-294x.2011.05328.X

Weis, V. M., Reynolds, W. S., deBoer, M. D., & Krupp, D. A. (2001). Host-symbiont specificity during onset of symbiosis between the dinoflagellates Symbiodinium spp. and planula larvae of the scleractinian coral Fungia scutaria. *Coral Reefs, 20*(3), 301-308.

Wham, D. C., Carmichael, M., & LaJeunesse, T. C. (2014). Microsatellite loci for Symbiodinium goreaui and other Clade C Symbiodinium. *Conservation Genetics Resources, 6*(1), 127-129.

Wham, D. C., & LaJeunesse, T. C. (2016). *Symbiodinium* population genetics: Testing for cryptic species and analyzing samples with mixed genotypes. *Molecular Ecology, in press*.

Yacobovitch, T., Benayahu, Y., & Weis, V. M. (2004). Motility of zooxanthellae isolated from the Red Sea soft coral Heteroxenia fuscescens (Cnidaria). *Journal of Experimental Marine Biology and Ecology, 298*(1), 35-48. doi:Doi 10.1016/J.Jembe.2003.08.003

743 **Supplementary Information for:** Contrasting population genetic structure in *Acropora* coral
744 hosts and their algal symbionts across multiple spatial scales

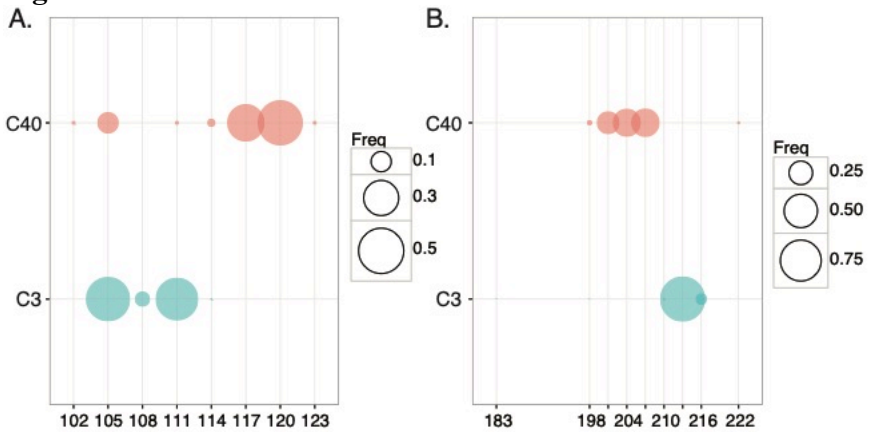
745

746 Davies SW[1,2*], Wham DC[3], Kanke MR[4] and MV Matz[2]

747

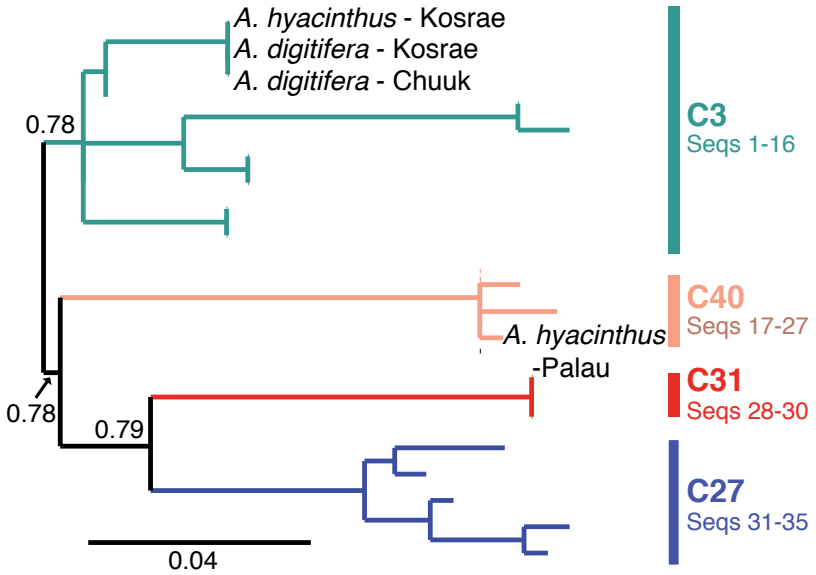
748 **Supplementary Figures**

749 **Fig S1**




750
751 **Fig S1: SSR allele frequencies of *Cladocopium* C3 and C40**
752 Bubble plots of the SSR allele frequencies of C3 (pink) and C40 (green) at the two loci that showed
753 the largest discrimination power, Sgr_21 (A) and Spl_33 (B). The size of each bubble is
754 proportional to the allele frequency within each *Cladocopium* lineage.

755 **Fig S2**




756
757 **Fig S2: Phylogenetic analysis of psbA$^{ncr}$ sequences for *Cladocopium***
758 Phylogenetic analysis of psbA$^{ncr}$ sequences from representative samples from this study (labeled
759 branch tips) along with publicly available psbA$^{ncr}$ sequences from known *Cladocopium* subclades
760 identifies two lineages present (C3 and C40). Bootstrap support values are shown at the partitions
761 that define known subclades. Scale bar: replacements per nucleotide site. Sequence accession
762 numbers for reference sequences are given in Table S5.

**Fig S3**

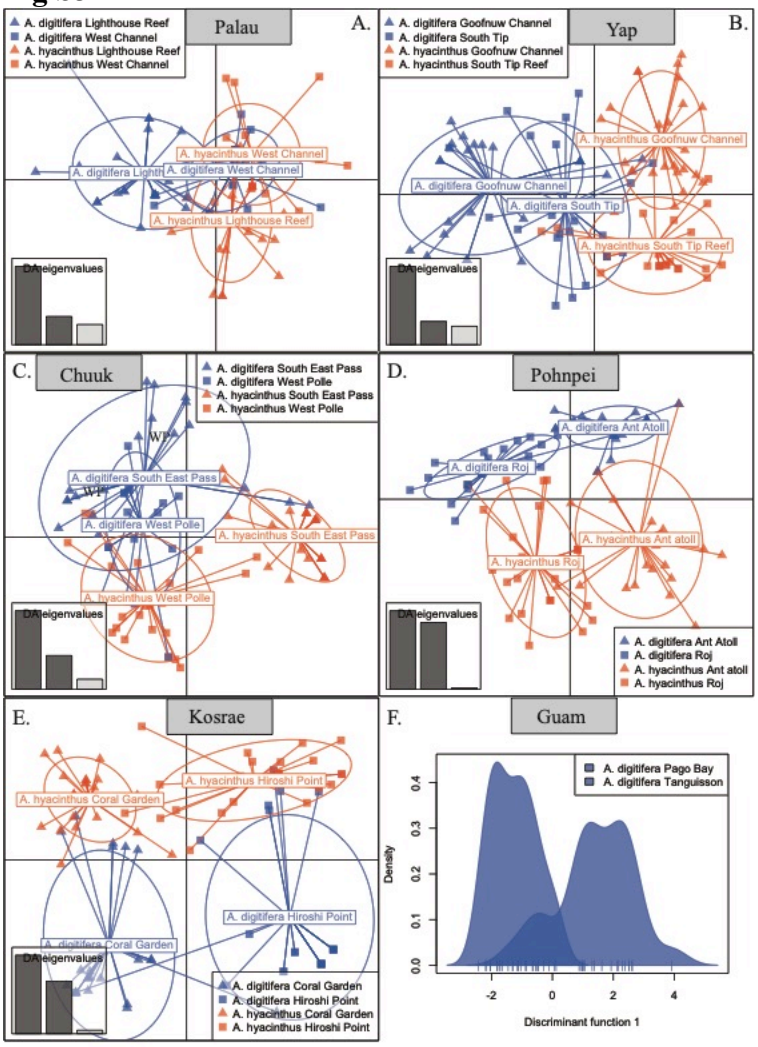



**Fig S3: Discriminant analysis of principal components (DAPC) of *Cladocopium* binary alleles hosted by *Acropora hyacinthus* and *Acropora digitifera* at twelve reef sites across six islands in Micronesia.**

DAPC scatter plots for presence/absence data for all individual samples from within (A) Palau, (B) Yap, (C) Chuuk, (D). Pohnpei, (E) Kosrae, and (F) Guam (*A. digitifera* hosts only, hence a single discriminant function axis). First two discriminant functions are shown, which tended to correspond to host species assignments and site assignments, although this trend is less clear than in the diploi-only MLG dataset.

**Fig S4**

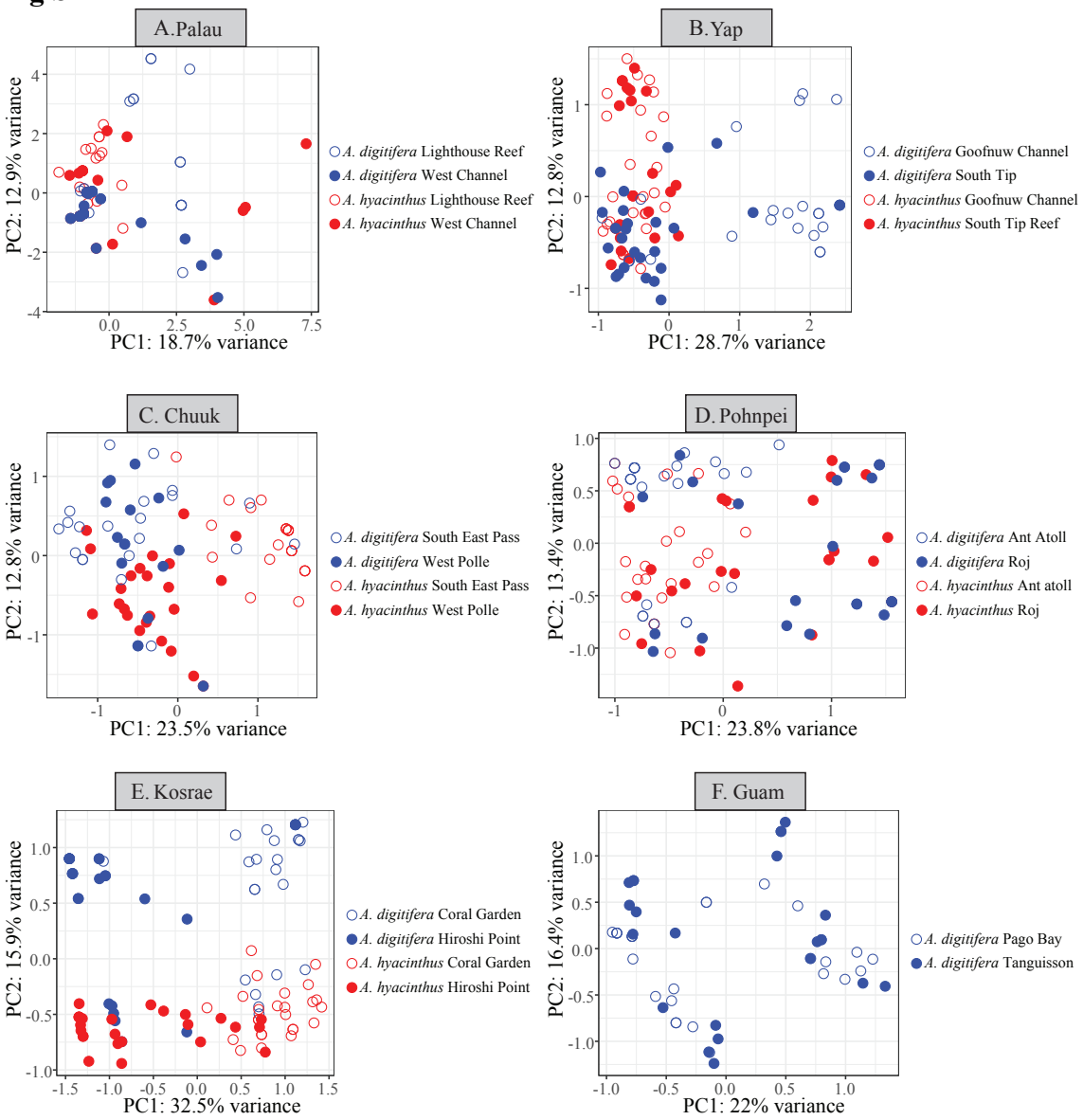



**Fig S4: Principle Component Analysis (PCA) of *Cladocopium* binary alleles hosted by *Acropora hyacinthus* and *Acropora digitifera* at twelve reef sites across six islands in Micronesia.** PCA scatter plots for presence/absence data for all individual samples from within (A) Palau, (B) Yap, (C) Chuuk, (D). Pohnpei, (E) Kosrae, and (F) Guam for *A. digitifera* hosts only. Clusters of samples tended to correspond to host species and site location.

## Supplementary Tables

### Table S1: Reef Site Collections

GPS coordinates, main island group, number of *Acropora digitifera* and *Acropora hyacinthus* hosts genotyped. Numbers in brackets are individuals hosting a single *Cladocopium* multi-locus genotype (MLG), which were included in all analyses presented in the main paper. All individuals were included in the binary analyses included in the supplementary materials. Site letters corresponds to island insets in Figure 1B.

| 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)** |

* indicates no individuals of this host species were found

[1] indicates individuals were not collected from this site but are likely present

[m] indicated sites where multiple *Cladocopium* species were detected

790 **Table S2: Microsatellite loci summary**
791 Summary of six polymorphic microsatellite loci used to assess genetic variation in *Cladocopium spp.* hosted
792 by *A. hyacinthus* and *A. digitifera* and their corresponding 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 |

793 Fadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter tag
794 sequence: FAM: agcagcgaactcagtacaaca
795 Hadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter tag
796 sequence: HEX: tcgtcgcttgagtcatcgtta

**Table S3: Summary of allelic diversity measures for *Cladocopium***

Summary of allelic diversity measures for C40 (A) and C3 (B). Na: number of alleles, He: expected heterozygosity, Ho: observed heterozygosity, PA: number of private alleles

A. 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 |

B. 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 |

**Table S4: Discriminant analysis of principle component (DAPC) model information**

Discriminant analysis of principle component (DAPC) model information including the number of principle components ("PC") and discriminant functions ("DF") retained, the proportion of conserved variance by the clustering model ("var"), and the overall assignment proportions across the model ("assign"). A. DAPC information for *Cladocopium* from different host species, B. islands, and C. sites and host species within each island.

Model Information

| **A. Host Species** | **PC** | **DF** | **var** | **assign** |
|---|---|---|---|---|
| C40 | 7 | 1 | 0.814 | 0.879 |
| C3 | 14 | 1 | 0.922 | 0.846 |
| **B. Islands** | **PC** | **DF** | **var** | **assign** |
| C40 | 16 | 4 | 0.970 | 0.981 |
| 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 |

**Table S5: *Cladocopium* accession numbers**

Accession numbers and corresponding *Cladocopium* (previously clade C *Symbiodinium*) lineage for each unique sequence identifier from Figure S2.

| 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 |