Cladocopium community divergence in two *Acropora* coral hosts across multiple spatial scales

Davies SW^{1,2*}, Moreland K², Wham DC³, Kanke MR⁴ and MV Matz²

¹ Boston University, Department of Biology, Boston, MA

² The University of Texas at Austin, Department of Integrative Biology, Austin, TX

³ Pennsylvania State University, Department of Biology, University Park, PA

⁴ Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University,
Ithaca, New York 14853

*Corresponding author: Sarah W. Davies

Department of Biology, 5 Cummington Mall, Boston University, Boston, MA 02215, tel-617-353-
8980, fax-617-353-6340, email: daviessw@bu.edu

Running Title: *Cladocopium* community divergence in corals

Abstract

Many broadly-dispersing corals acquire their algal symbionts (Symbiodiniaceae) ‘horizontally’ from their environment upon recruitment. Horizontal transmission could promote coral fitness across diverse environments provided that corals can associate with divergent algae across their range and that these symbionts exhibit reduced dispersal potential. Here we quantified community divergence of *Cladocopium* algal symbionts in two coral host species (*Acropora hyacinthus*, *Acropora digitifera*) across two spatial scales (reefs on the same island, and between islands) across the Micronesian archipelago using microsatellites. We find that both hosts associated with two genetically distinct *Cladocopium* lineages (C40, C21), confirming that *Acropora* coral hosts associate with a range of *Cladocopium* symbionts across this region. Both C40 and C21 exhibited extensive clonality. Clones not only existed across host conspecifics living on the same reef, but also spanned host species, reef sites within islands, and even different islands. Both *Cladocopium* lineages exhibited moderate host specialization and divergence across islands. In addition, within every island, algal symbiont communities were significantly clustered by both host species and reef site, highlighting that coral-associated *Cladocopium* communities are structured across small spatial scales and within hosts on the same reef. This is in stark contrast to their coral hosts, which never exhibited significant genetic divergence between reefs on the same island. These results support the view that horizontal transmission could improve local fitness for broadly dispersing *Acropora* coral species.

Keywords: coral, Symbiodiniaceae, *Cladocopium*, community divergence, *Acropora*, symbiosis, horizontal transmission, clonality

Introduction

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

Associations with algal symbionts in the family Symbiodiniaceae are obligatory for the majority of shallow water tropical corals since they rely on photosynthetic byproducts from the algae for energy in oligotrophic waters. In turn, the algae benefit from a protected and light-exposed residence as well as inorganic nutrients and CO₂ concentration mechanisms provided by

the host (Barott, Venn, Perez, Tambutte, & Tresguerres, 2015; Muscatine, 1990; Muscatine & Cernichiar, 1969; Trench & Blank, 1987). Coral symbiosis, like many other ecologically important symbioses, is endosymbiotic (occur within cells) and can establish by two fundamentally different modes of transmission: vertical (symbiont inheritance from mother) and horizontal (symbiont from environmental, free-living sources) (Harrison & Wallace, 1990). Vertically transmitting corals guarantee the maintenance of symbiosis in their offspring, however if larvae encounter novel environments, their symbiont composition may be suboptimal resulting in reduced fitness (Byler, Carmi-Veal, Fine, & Goulet, 2013; Douglas, 1998; Wilkinson & Sherratt, 2001). During horizontal transmission, aposymbiotic larvae have flexibility in symbiont acquisition and upon arrival to new environments, they can uptake novel symbionts not present in parental populations (Abrego, van Oppen, & Willis, 2009; Ali et al., 2019; Gómez-Cabrera, Ortiz, Loh, Ward, & Hoegh-Guldberg, 2008; Little, van Oppen, & Willis, 2004), but availability of symbionts upon arrival is not guaranteed.

Given the obligatory nature of this symbiosis for the host, it is somewhat surprising that in the majority of coral species (~85%), algal symbionts must be acquired by the juvenile coral from its local environment post settlement (Baird, Guest, & Willis, 2009; Fadlallah, 1983; Harrison & Wallace, 1990; Hartmann, Baird, Knowlton, & Huang, 2017). However, this prevalence of horizontal transmission in coral-algal symbiosis is consistent with a recent meta-analysis on transmission modes in bacteria-eukaryotes being dominated by horizontal transmission in marine environments (Russell, 2019). One possible benefit to this horizontal transmission strategy in marine environments is that these aposymbiotic coral larvae can disperse great distances with ocean currents (Davies, Treml, Kenkel, & Matz, 2015; Foster et al., 2012; Rippe et al., 2017; van

Oppen, Peplow, Kininmonth, & Berkelmans, 2011). Yet, coral larvae can encounter a great variety of reef habitats (Gorospe & Karl, 2011), and therefore conditions on the reef where they eventually settle can be very different from their natal reef (Baird, Cumbo, Leggat, & Rodriguez-Lanetty, 2007; LaJeunesse et al., 2004). To improve their chance of survival in this novel environment, corals could potentially associate with locally available, and putatively ecologically specialized, algal strains (Byler et al., 2013; Howells et al., 2012; Rowan & Knowlton, 1995).

Indeed, the diversity of algal symbionts in the family Symbiodiniaceae is rich (LaJeunesse et al., 2018) and specific coral-algae associations have been suggested to play pivotal roles in holobiont adaptation to climate change (Berkelmans & van Oppen, 2006; Howells et al., 2012). The genus *Cladocopium* (formerly clade C *Symbiodinium*; LaJeunesse et al., 2018) originated and diversified most recently among Symbiodiniaceae, and has achieved the highest diversity of all lineages (Lesser, Stat, & Gates, 2013; Pochon & Gates, 2010; Pochon, Montoya-Burgos, Stadelmann, & Pawlowski, 2006; Thornhill, Howells, Wham, Steury, & Santos, 2017; Thornhill, Lewis, Wham, & LaJeunesse, 2014). This diversity has been associated with functional variation in symbiont thermal performance across reefs (Davies, Ries, Marchetti, & Castillo, 2018; Howells et al., 2012) as well as with functional differences in gene expression between reef zones (Barfield, Aglyamova, Bay, & Matz, 2018; Davies et al., 2018), lending support for the potential for reef-specific symbiont communities. In addition, the draft genome of *Cladocopium goreau* confirm the divergence of this genus from other Symbiodiniaceae genera and specifically highlight that gene families related to the establishment and maintenance of symbiosis (photosynthesis, host-symbiont interactions, nutrient exchange) were under positive selection (Liu et al., 2018).

However, much less is known about the population biology of *Cladocopium* spp. algal

104 symbionts, including how their populations are structured in comparison to their coral hosts.
 105 Understanding the relative importance of reef environment, coral host, and geographical distance
 106 in structuring coral-associated algal symbiont communities is essential to identifying the adaptive
 107 capacity of this symbiosis. However, thus far there are only a handful of population genetic studies
 108 of Symbiodiniaceae based on multilocus markers, none of which address all of the above-
 109 mentioned potential sources of genetic variation. Here, using microsatellites, we examined the
 110 community divergence of *Cladocopium* spp. algal symbionts hosted by two common, co-occurring
 111 species of *Acropora* corals—*A. hyacinthus* and *A. digitifera*—collected from the same reef locations
 112 across the Micronesian Pacific (Fig 1A, B). We explore this community divergence across several
 113 ecological scales including host species, islands across the Micronesian archipelago, and unique
 114 sites within each island. We then contrast results for the algal symbionts to the previously
 115 published population genetic structure of their coral hosts (based on a subset of the exact same
 116 coral samples), which demonstrated that both coral species exhibited extensive genetic
 117 connectivity and their genetic structure was well explained by the biophysical connectivity
 118 between sites (Davies et al., 2015).

Fig 1: Locations where coral samples were collected and overall DAPC *Cladocopium* community divergence. (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. Additional site information can be found in Table 1. (C). DAPC assignments for *Cladocopium* at an optimal cluster number 2, corresponding to C40 (Dark Green) and C21 (light Green). On panel C, color bars below assignment plot indicate coral host species (see legend) and shades of grey correspond to different sites within each island.

Materials and Methods

Sampling of coral-associated algal symbionts

This study comprised a subset of samples previously analyzed for coral host genetics in Davies et al. (2015) (Table 1, Fig 1A). Twenty-five individuals of each coral host species (*Acropora hyacinthus* and *Acropora digitifera*) were examined at two reef sites within each of seven islands (Fig 1B). There were two exceptions to this sampling design. First, at Ngulu only one site was visited and only *A. hyacinthus* was collected. Second, at Guam, no *A. hyacinthus* was found on either of the sampled reefs, so only *A. digitifera* was collected. In total, 13 reef sites were included in this experimental design. All samples were collected between 3–7 m depth, all colonies were >2 m apart, and all samples from both species at a given site were collected at the same approximate GPS coordinates (Table 1).

Table 1: Reef Site Collections

Site, main island group, GPS coordinates, number of *Acropora digitifera* and *Acropora hyacinthus* hosts genotyped. The first value is the number of individuals successfully genotyped, which were included in the first discrimination analysis (Fig 1C). The second value corresponds to the number of individuals that were successfully discriminated between C3 and C40 at an assignment rate of >0.9 (C40: 172, C3: 388; Fig 1C). Numbers in brackets correspond to the number of individuals hosting unique *Cladocopium* with clones removed, which were included in all downstream analyses (Total: C40: 127, C3: 328; Fig 3, 4). Site letters corresponds to island insets in Figure 1B.

Site	Island	GPS	<i>A. digitifera</i>	<i>A. hyacinthus</i>
WC.2: West Channel	Palau	7°31'55.7 N, 134°29'42.8 E	25, 25 (16,0)	25, 24 (13,1)
LH.1: Lighthouse Reef	Palau	7°16'62.4 N, 134°27'61.9 E	24, 24 (19,0)	25, 25 (18,0)
NG1: Ngulu	Ngulu Atoll	8°18'12.0 N, 137°29'18.7 E	0 ₁	42, 42 (28,0)
ST.1: South Tip Reef	Yap	9°26'05.4 N, 138°02'10.4 E	25, 24 (1,23)	25, 25 (0,20)
GO.2: Goofnuw Channel	Yap	9°34'26.4 N, 138°12'19.2 E	24, 24 (17,5)	25, 25 (0,24)
PB.1: Pago Bay	Guam	13°25'66.6 N, 144°47'94.3 E	26, 26 (0,20)	0*
TG.2: Tanguisson	Guam	13°32'61.1 N, 144°48'52.6 E	23, 20 (0,17)	0*
WP.1: West Polle	Chuuk	7°19'69.7 N, 151°33'21.1E	16, 15 (2,11)	24, 23 (1,22)
SE.2: South East Pass	Chuuk	7°14'60.3 N, 152°01'29.1 E	21, 21 (1,20)	23, 23 (2,13)
AN.1: Ant Atoll (East)	Pohnpei	6°47'42.3 N, 158°01'20.7 E	24, 24 (0,17)	24, 23 (3,13)
RO.2: Roj	Pohnpei	6°46'37.7 N, 158°12'24.1 E	24, 24 (0,21)	24, 24 (1,21)
CG.1: Coral Garden	Kosrae	5°18'47.2 N, 162°53'01.8 E	25, 24 (1,19)	25, 25 (2,20)
HP.2: Hiroshi Point	Kosrae	5°15'88.0 N, 162°59'01.8 E	25, 25 (2,14)	25, 25 (0,22)
TOTAL			282 (73,203)	287 (99,185)

* indicates no individuals of this host species were found

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

Laboratory procedures

DNA was isolated following Davies et al. (2013). Microsatellite primers consisted of five previously described *Cladocopium*-specific loci (previously described as clade C *Symbiodinium*) (Bay, Howells, & van Oppen, 2009; Wham, Carmichael, & LaJeunesse, 2014) and one novel locus mined using MsatCommander (Faircloth, 2008) from nucleotide EST data for *Cladocopium* lineage C3 (Leggat, Hoegh-Guldberg, Dove, & Yellowlees, 2007), for a total of six loci (Table S1). Loci were multiplexed in 20 µl polymerase chain reaction (PCR) mixtures containing 10 ng of template DNA, 0.1 µM of each forward and reverse primers, 0.2 mM dNTP, 1X *ExTaq* buffer, 0.025 U *ExTaq* Polymerase (Takara) and 0.0125 U *Pfu* Polymerase (Promega). Amplifications began at 94°C for 5 min, followed by 35 cycles of 94°C for 40 s, annealing temperature for 120 s, and 72°C for 60 s and a 10 minute 72°C extension period. Molecular weights were analyzed using the ABI 3130XL capillary sequencer. Data were binned by repeat size and individuals failing to amplify at ≥ 3 loci were excluded from downstream analyses.

Analyses of allele presence-absence data

Although Symbiodiniaceae *in hospite* are assumed to be haploid (Santos & Coffroth, 2003), the genus *Cladocopium* are generally observed to have two copies of every allele (Thornhill et al., 2014; Wham et al., 2014; Wham & LaJeunesse, 2016). This apparent genome duplication may or may not correspond to a change in chromosome number, or the actual diploid state (Wham & LaJeunesse, 2016), and it has been previously suggested that these lineages should be scored as if they were effectively diploid (i.e. with the expectation of two alleles per locus) to appropriately construct multilocus genotypes (MLGs) from samples (LaJeunesse et al., 2014; Pettay, Wham,

Smith, Iglesias-Prieto, & LaJeunesse, 2015; Thornhill et al., 2014; Wham et al., 2014; Wham & LaJeunesse, 2016). However, ploidy of the *Cladocopium* samples in our study is unknown, and a single coral could potentially contain several genetically distinct *Cladocopium* clones. Therefore, data were analyzed as binary (allele presence/absence) values for each sample. This conservative approach allowed us to retain all individuals in analyses (569 total: 282 *A. digitifera* and 287 *A. hyacinthus*), with the drawback that it confounds genetic divergence and community divergence in cases of multiple strains per host. However, since multiple-strain infections are rare in *Cladocopium* (Thornhill et al., 2017), genetic divergence is expected to be the major contributor to our distance measures. Still, we chose to refer to our distances as “*Cladocopium* community divergence” throughout this manuscript, to ensure that there is no confusion with true genetic distances.

First, all binary allele data (N=569 samples: Supplemental File S1) were converted into a *genind* object with allele presence/absence data using *adeigenet* 2.0.0 (Jombart, Devillard, & Balloux, 2010) in R (R Development Core Team, 2018). Next, discriminant analysis of principal components (DAPC) was implemented, which classifies samples into user-defined groups based on their coordinates in principle components’ space. Because DAPC does not rely on traditional population genetics models, it is free from Hardy-Weinberg equilibrium and linkage disequilibrium assumptions and thus is considered to be applicable across organisms regardless of their ploidy and genetic recombination rates (Jombart et al., 2010). Here, identification of clusters was achieved using the *find.clusters* function with a maximum number of 40 clusters. Eighteen principle components (PCs) were maintained and the Bayesian Information Criterion (BIC) indicated that two clusters were optimal in our data. In this initial analysis of all data, samples

exhibited strong assignments into two highly supported clusters - Light green and Dark green (Fig 1C). These data were therefore split into two subsets, corresponding to these two clusters, for downstream analyses. Only samples assigned to one of the two clusters with a probability >0.9 were retained, resulting in N=388 for the Light green cluster and N=172 for the Dark green cluster (Table 1, Supplemental Files S2 and S3).

Sequencing analysis of Cladocopium psbA_{ncr}

To confirm phylogenetic affiliation of the two highly-supported *Cladocopium* clusters, the non-coding region of the psbA chloroplast gene (psbA_{ncr}) was amplified in representative samples. The psbA_{ncr} region was chosen because of its utility for differentiating species of Symbiodiniaceae (i.e. Lewis, Chan, & LaJeunesse, 2019). Amplifications were conducted using the primers and settings described by Moore et al. (2003). Amplified products were directly sequenced using the reverse primer. Phylogenetic analysis was conducted using PAUP Version 4.4a147 (Swofford, 2014) using psbA_{ncr} reference sequences for C40 (from various scleractinians) and C21 (from Acropora) provided by the LaJeunesse lab. The nexus file used to generate the phylogenetic tree containing these reference sequences is included as Supplemental File S4. An unrooted distance tree was constructed to demonstrate that the representative samples from the two highly-supported clusters are separated by large differences in sequence divergence. These results indicated that the two major clusters by our genetic data were C40 (*sensu* LaJeunesse et al., 2004) and C21 (*sensu* Thornhill et al., 2014), respectively. Further community divergence analysis of these data were completed for each cluster separately and these lineages are referred to as *Cladocopium* C40 and *Cladocopium* C21 throughout the rest of the paper.

215

216 *Clonal analyses within each cluster*

217 To determine rates of clonality within *Cladocopium* C40 and C21, individuals with
 218 matching MLGs were investigated. Clonal analyses for C40 and C21 were conducted
 219 independently using the following steps. Singleton alleles were removed (12 alleles in C40; 10
 220 alleles in C21), after which a hierarchical clustering tree was constructed in R (R Development
 221 Core Team, 2018) using the *vegdist*(*x*, *binary*=*T*, *method*="manhattan") function from the *vegan*
 222 package (Oksanen et al., 2013) and processed with the function *hclust*(*x*). Manhattan distance was
 223 chosen for this analysis because it corresponds to the total number of non-shared alleles between
 224 two MLGs, which should be zero for identical MLGs (clones). Samples sharing identical MLGs
 225 were then identified using the function *cutree*(*x*, *h*=0.2), which grouped individuals with less than
 226 one (i.e., zero) non-shared alleles. The probability of chance occurrence of matching MLGs was
 227 assessed by a resampling simulation in R. 100,000 artificial MLGs were generated using 44 non-
 228 singleton alleles in C40 and 46 non-singleton alleles in C21, by resampling the vector of presences-
 229 absences of each allele across individuals. The probabilities of identical genotype groups occurring
 230 by chance were calculated using the formula $\left(\frac{a}{100,000}\right)^{b-1}$, where *a* is how often the MLG was
 231 observed in the simulation and *b* is how often it was observed in the actual data. For downstream
 232 DAPC analyses, we have created dataframes including only a single representative of each MLG
 233 within a site within the same host species (Supplemental Files S5 and S6 for C40 and C21
 234 respectively).

235 The geographical distance spanned by MLGs was investigated by calculating a distance
 236 matrix from reef site coordinates, in decimal degrees, using the *dist* function in R. The *dms2dec*

function from Zanolli et al. (2018) was used to convert degrees minutes seconds to decimal degree format. The largest distance was taken for MLGs spanning more than one site. Distances were converted to kilometers using the National Hurricane Center and Central Pacific Hurricane Center's calculator (<https://www.nhc.noaa.gov/gccalc.shtml>). Genotype diversity, the probability that two randomly sampled MLGs were different, was calculated using the formula $1 - \sum_i (\frac{n_i}{N})^2$, where n_i is the number of individuals representing a i 'th repeated MLG and N is the total number of individuals. Genotypic diversity differences between C40 and C21, and between host species within C40 and C21 were tested using $t.test()$ in R.

Within-cluster analyses across coral hosts, islands and sites within islands

To visualize *Cladocopium* community divergence between host species, between islands, and between sites and host species within each island, assignment of samples to genetic clusters with prior grouping of island/host/site was performed in R (R Development Core Team, 2018) using DAPC (Jombart, 2008; Jombart et al., 2010) separately for C40 and C21. Successful reassignment, indicated by a high proportion of samples correctly assigning back to their *a priori* group, indicates that these user-defined groups are distinct, which in our case implies divergence between hosted *Cladocopium* communities. Here, data were converted into principal components (PCs) and then a-scores were computed to determine the optimal number of PCs to retain. A-scores determine the proportion of successful reassignment corrected for the number of PCs retained and protect against model overfitting (Jombart et al., 2010). Assignment rates, PCs and DFs retained, and the overall proportion of variance explained by each of the models are included in Table S2. Proportion of successful assignments within each model are shown on all figures (Fig 3, 4).

259

260 *Unconstrained Cladocopium community analyses*

261 Because DAPC analyses aim to maximize variation between pre-defined groups, we also
 262 visualized all C40 and C21 data independently using a principal coordinate analysis of allele
 263 presence/absence data using the *vegdist* (*x*, *method*="bray") function implemented in the *vegan*
 264 package in R (Oksanen et al., 2013). *Cladocopium* community divergence between host species,
 265 islands and host species and sites within islands were then tested using the *vegan::adonis* function
 266 (*method*="bray").

267

268 *Data and code availability*

269 All data and code used for all analyses and figure generation are publicly available at
 270 https://github.com/daviessw/Cladocopium_Micronesia.

271

272 **Results**

273 *Two clusters of Cladocopium symbionts observed in Micronesian acroporids*

274 Across the two coral host species in Micronesia (Fig 1A, B), two distinct *Cladocopium*
 275 clusters were observed with 98.4% of samples (560/569) strongly assigning to one of the two
 276 clusters (Fig 1C). Sequencing of the *psbA_{ncr}* gene from representative samples from each cluster
 277 identified them as *Cladocopium* C40 and C21 (LaJeunesse et al., 2004; LaJeunesse & Thornhill,
 278 2011; Thornhill et al., 2014) (Fig S1). It is important to note that the possible presence of other
 279 background Symbiodiniaceae genera would not affect *Cladocopium* genotyping results since our
 280 microsatellite assays are genus-specific (Bay, Howells, & van Oppen, 2009; Wham, Carmichael,

& LaJeunesse, 2014). Corals of both *Acropora* species from Palau and Ngulu were found to almost exclusively host *Cladocopium* C40 (Fig 1C, dark green bars). C40 was also prevalent in *A. digitifera* at one reef site on Yap (Goofnuw Channel: GO.2) and was occasionally found in *A. digitifera* across the rest of Micronesia (Fig 1C). All other *Acropora* hosts across the spatial gradient associated with *Cladocopium* C21 (Fig 1C, light green bars). Both *Cladocopium* lineages possessed high allelic diversity, with a total of 44 unique alleles in C40 (N=127 corals) and 49 unique alleles in C21 (N=328 corals).

Clonality of Cladocopium symbionts

Overall, clonality was high in both C40 (51.7%; N=127) and C21 (34.0%; N=328), however rates of clonality in C40 were significantly higher than C21 even though more corals hosted C21 across the archipelago ($p=0.04514$; Fig 2A-C). C40 was represented by a total of 105 unique MLGs, 22 of which were found in multiple individuals (Fig 2A). In C21 there were 309 unique MLGs, 53 of which occurred repeatedly (Fig 2B). 16/22 C40 MLGs and 53/53 C21 repeatedly occurring MLGs were supported with high confidence ($p<0.001$) as clonal groups via simulation. Six repeated MLGs in C40 were less robustly supported (group 12: $p=0.0166$, N=2; group 14: $p=0.0044$, N=2; group 32: $p=0.0014$, N=2; group 35: $p=0.0173$, N=2; group 81: $p=0.0395$, N=2; group 87: $p=0.0017$, N=2), but still all passed the $p<0.05$ significance threshold.

Notably, repeated MLGs were not restricted to a specific host species or reef site. Many spanned host species, reef sites, and even islands (Fig 2A,B). Summaries of clonal rates for each reef site are shown in Fig 2 I-J. MLG group size was on average 4.05 for C40 and 2.49 for C21, ranging up to 14 in C40 and 5 in C21 (Fig 2C,D). Consistent with larger MLG group sizes in C40

relative to C21 ($p=0.04514$; Fig 2C), C40 also exhibited lower overall genotype diversity than C21 ($p=0.01321$; Fig 2F). Notably, larger MLG group size in C40 did not translate into larger geographic distance spanned by an MLG (Fig. 2E). The largest distance spanned by C40 MLGs was between Goofnuw Channel (GO.2), Yap and Lighthouse Reef (LH.1), Palau (~578 km), while the largest distance spanned by C21 MLGs was between South Tip (ST.1), Yap and Hiroshi Point (HP.2), Kosrae (~3714 km) (Fig 2E). Rates of MLGs spanning host species also varied between C40 and C21. 36.4% of MLGs spanned host species in C40 (Fig 2A) and 20.8% of MLGs spanned host species in C21 (Fig 2B). There was no significant difference in genotype diversity between algal symbionts hosted by different host species for either C40 ($p=0.3403$; Fig 2G) or C21 ($p=0.1619$; Fig 2H).

Fig 2: Rates of *Cladocopium* clonality within C40 and C21

Fan trees of *Cladocopium* C40 (A) and *Cladocopium* C21 (B) MLGs. Host species, *A. hyacinthus* and *A. digitifera*, are color-coded on the inside of the tree and the seven islands in Micronesia are indicated in the ring around the tree. (C) Histogram of MLG group sizes. C40 tends to have larger MLG groups than C21. (D) Frequencies of MLGs spanning host species, binned by MLG group size. The number indicates the total number of MLG groups in the size bin. There is no clear difference in the proportion of host-spanning MLGs between C40 and C21. (E) Greatest geographical distance spanned by a MLG group of a given size. C21 MLGs span considerably larger distances than C40 MLGs. (F-H) Violin plots of genotype diversity (probability that the two randomly picked MLGs are different) for C40 and C21 (F) and for each cluster among two host species (G, H). Each point represents a reef site. (I-J) Clonality levels at each reef site in each host species. Bar colors correspond to host species, where faded bar segments represent unique MLGs and bright bar segments represent repeated MLGs. h, *A. hyacinthus*; d, *A. digitifera*. Reef site colors correspond to Fig 1 and Table 1.

Cladocopium community divergence by coral host species, islands, and local reef environments

Discriminant analysis of principal components (DAPC) strongly differentiated between host species for both *Cladocopium* C40 and C21 (Table S2, Fig 3A,B), with assignment rates ranging from 0.75 (*A. hyacinthus* hosting C21) to 0.93 (*A. digitifera* hosting C40). In addition, unconstrained analyses confirmed these results with significant differences observed in both C40 (*adonis* $p < 0.001$) and C21 (*adonis* $p < 0.001$) (Fig S2A,B). These results confirm that host species play a role in structuring *Cladocopium* populations across Micronesia. However, both coral hosts were capable of maintaining symbiosis with multiple Symbiodiniaceae lineages (C40 or C21) across this region. In addition, DAPC demonstrated clustering among islands for each *Cladocopium* species irrespective of host species: generally high per-island assignment rates were obtained both for C40 (Fig 3C, 0.61-0.91) and C21 (Fig 3D, 0.53-0.88), which were also confirmed with significant differences for both C40 (*adonis* $p < 0.001$) and C21 (*adonis* $p < 0.001$) across islands using unconstrained analyses (Fig S2C,D). Notably, algal symbionts from Yap consistently showed some of the lowest assignment rates for both C40 (0.61) and C21 (0.63). Another notable

observation was that algal symbionts from Ngulu and Kosrae were strongly separated from other algal communities, suggesting the possibility of additional *Cladocopium* lineages existing there (Fig 3C,D), which was not further explored here.

Fig 3: DAPC of binary MLG data for *Cladocopium* C40 and C21 by host species and islands
Discriminant analysis of principal components (DAPC) of binary MLG data for *Cladocopium* C40 and C21 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 C40 (A) and C21 (B). Numbers overlaying the curves indicate proportions of correctly assigned samples. DAPC scatter plot for individual samples from C40 (C) and C21 (D) 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 S2.

Fig 4: DAPC of *Cladocopium* C40 and C21 hosted by *Acropora hyacinthus* and *Acropora digitifera* at twelve reef sites across six islands in Micronesia.

Discriminant analysis of principal components (DAPC) of binary MLG data for *Cladocopium* C40 and C21 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 and site assignments. DAPC scatter plots for individual samples from within (A) Palau for C40, (B) Yap for C21, (C) Chuuk for C21, (D). Pohnpei for C21, and (E) Kosrae for C21. Density plots are shown for the two sites in Guam for C21 for *A. digitifera* hosts only (purple distributions) (F). Proportions of correct assignments are indicated in the clusters and information on the DAPC models can be found in Table S2.

When clustering was performed within islands for C40 (Palau) and C21 (Yap, Chuuk, Pohnpei, Kosrae, Guam), of the two top eigenvalues in DAPC analysis, generally one discriminant function (DF) explained divergence by host species while the other DF explained differences between reef sites (Fig 4). Unconstrained analyses corroborated this result: *Cladocopium* communities were always significantly different between coral host species and sites within islands (Fig S3). One instance where DAPC clustering and unconstrained analysis by sites and host species within island showed weaker support was C21 from Yap (Figs 4B, S3B), however, the sample size of *A. digitifera* symbionts at Goofnuw (GO.2) was low (N=5). This low sample size was due to *A. digitifera* hosts at Goofnuw largely associated with C40 (N=17), while the majority of other *A. digitifera* hosts across Yap associated with C21 (Fig 1C), which may have led to lack of power to detect clustering. The strongest separation between host:site groups were observed at Chuuk (Figs 4D, S3D) and at Kosrae (Figs 4E, S3E).

Discussion

Acropora corals establish symbiosis with distinct *Cladocopium* communities

Across the Micronesian Pacific (Fig 1A), both *Acropora* coral hosts associated with two distinct lineages of *Cladocopium* (Fig 1C), which were identified as C40 and C21 (Fig S1), with

potentially additional species present (i.e. Ngulu *Cladocopium* C21; Fig 3C). This observation suggests that both coral hosts show considerable flexibility in their symbiotic associations with *Cladocopium* across their range and within their specific environments (Abrego et al., 2009; Berkelmans & van Oppen, 2006). This association with *Cladocopium* is consistent with the wealth of previous comprehensive community composition studies suggesting that Indo-Pacific acroporids are generally dominated by algal symbionts in this genus (i.e. LaJeunesse et al., 2004; LaJeunesse et al., 2003; Thornhill et al., 2014). While initial symbiont infection is likely determined by local availability of free-living symbionts and made possible by the flexibility of arriving coral recruits (Abrego et al., 2009; Ali et al., 2019; Cumbo, Baird, & van Oppen, 2013; Little et al., 2004), a winnowing process involving selection, competition, immune system response, and differing rates of asexual proliferation likely play roles in the eventual dominance of a unique algal community in the majority of coral hosts in their respective habitat (Rowan, Knowlton, Baker, & Jara, 1997; Thornhill et al., 2017).

Strict associations with a single Symbiodiniaceae algal lineage have been observed across a variety of coral hosts and Symbiodiniaceae genera (Baums, Devlin-Durante, & LaJeunesse, 2014; Pinzón, Devlin-Durante, Weber, Baums, & LaJeunesse, 2011; Thornhill et al., 2014), however this strict association is not always the case (see Howells, van Oppen, & Bay, 2009; Howells, Willis, Bay, & van Oppen, 2013). In our study, it is also important to acknowledge that we only explored community divergence patterns within *Cladocopium* because we leveraged *Cladocopium*-specific microsatellite loci (Bay, Howells, & van Oppen, 2009; Wham, Carmichael, & LaJeunesse, 2014). This genus is most commonly known to associate with *Acropora* in this region, which is consistent with our previous ITS2 metabarcoding results on the same coral

samples from Palaun reefs, which showed that *Acropora* hosts strictly associated with one of two *Cladocopium* symbiont haplotypes (Quigley et al., 2014). Here, we tested several samples (N=4) for community level algal species identification (Fig S1), which confirmed C40 and C21 designations, however these more coarse-grained genus-level analyses were not performed on samples from across the range. Therefore, we are unable to comment on other algal genera known to inhabit corals at background levels (Silverstein, Correa, & Baker, 2012; Ziegler, Stone, Colman, Takacs-Vesbach, & Shepherd, 2018).

Cladocopium C40 and C21 exhibit extensive clonality

Research leveraging microsatellite loci in Symbiodiniaceae demonstrate that rates of clonality can substantially differ between Symbiodiniaceae genera, between lineages within a genus, and between regions (Thornhill et al., 2017). For example, work on *D. trenchii* hosted by *Acropora* colonies found very low rates of shared clones between colonies (Hoadley et al., 2019), and similarly low clonality rates have been observed in *Dusurdinium* from *Galaxea fascicularis* from the South China Sea (Chen et al., 2020). However, Pettay et al. (2011) found that unique *Pocillopora* hosts frequently associated with the same *S. glynni* clone. Here we find high frequencies of clonality in *Cladocopium* symbionts in *Acropora* hosts with 51.7% of corals hosting C40 sharing the same MLG (Fig 2A) and 34% in C3 (Fig 2B), which cannot be explained by clonality of coral hosts (Davies et al., 2015). While few studies have investigated clonality in Pacific *Cladocopium*, Howells et al. (2013) found that only 13% of *A. millepora* from the Great Barrier Reef hosted the same MLGs. However, clonality rates appear to be different across *Cladocopium* lineages. For example, Thornhill et al. (2014) observed that C3 hosted by

Siderastrea siderea exhibited 17% clonality, C7 hosted by *Orbicella* spp. exhibited 70% clonality, and C7a/C12 hosted by *Orbicella* spp. exhibited 47% clonality. In light of these data, our observed clonality rates for C40 and C21 are well within previously published estimates.

Interestingly, we found that *Cladocopium* clones were not only shared across conspecifics on the same reef, but also across different host species, different reefs on the same island, and even between host species on different islands (Fig 2A,B). Given that unique MLG genotypes have been shown to exhibit functional variation both in culture (i.e. *S. psygmophilum*, Parkinson et al., 2016) and in hospite (Davies et al., 2018; Howells et al., 2012), these results are counter intuitive for several reasons. First, it is difficult to imagine how clones are distributed across such wide spatial scales, which was especially evident in C21 (Fig 2E), given that the majority of symbioses in corals are horizontal (Baird et al., 2009) and Symbiodiniaceae are expected to have low dispersal potential (reviewed in Thornhill et al., 2017). Secondly, it seems unlikely the same C40 or C21 clone would be successful across both host species and across different environments given that coral-associated symbiont distributions have been proposed to correlate with depth (Andras, Kirk, & Harvell, 2011; Kirk, Andras, Harvell, Santos, & Coffroth, 2009), temperature (Baums et al., 2014; Hume et al., 2016; LaJeunesse et al., 2014), PAR (Rowan et al., 1997), and host species (Thornhill et al., 2017; Thornhill et al., 2014). Another interesting discussion point is that C21 clones appear to be more broadly distributed across the seascape than C40 clones, suggesting that C21 may have higher dispersal potential than C40. Clearly, more intensive sampling of hosts associated with *Cladocopium* across additional host species and sites will shed light on the population biology of these generalist symbionts.

Cladocopium C40 and C21 exhibit imperfect host specificity

The majority of reef-building coral species associate with a specific Symbiodiniaceae type, which have traditionally been coarsely defined based on ribosomal and/or chloroplast markers (Fabina, Putnam, Franklin, Stat, & Gates, 2013; Rodriguez-Lanetty, Krupp, & Weis, 2004; Thornhill et al., 2014; Weis, Reynolds, deBoer, & Krupp, 2001). Previous Symbiodiniaceae multilocus genotyping studies revealed that each of these symbiont types can harbor within-type diversity, both at genetic and functional levels (Howells et al., 2012; Howells et al., 2009; Santos, Shearer, Hannes, & Coffroth, 2004). Here we observe significant divergence between *Cladocopium* communities among two different host species in both C40 and C21 across the Micronesian Pacific (Fig 3A,B; Fig S2A,B), and this pattern of host specificity consistently holds between host species on the same reef (Fig 4; Fig S3). Previous work on octocorals similarly observed significant host differentiation among algal symbionts, however they found that this genetic divergence was driven by different aged cohorts and depth in their system (Andras et al., 2011). Here, host habitat depth or age class is not relevant for the host specificity observed given that specific attention was paid to collecting colonies located at similar depths and of similar size classes. Instead, our data suggest that for both C40 and C21, local association of hosts and symbionts within the same cluster is due to host specificity in *Cladocopium* (Fig 4; Fig S3), which has been previously proposed in symbionts hosted by *Pocillopora* in the south Pacific (Magalon, Baudry, Husté, Adjero, & Veuille, 2006). Since our study rigorously sampled two coral host species across several spatial scales, we also detected that this specificity is imperfect: at every location, there were symbionts in one host species that would have been assigned to another coral host based on their MLG (Fig 4, Fig S3). In fact, there were MLGs both within C40 and C21 that

were shared across hosts at the same site and across different islands (Fig 2A,B), further highlighting that this host specificity is imperfect. Overall, these patterns suggest that host specialization in *Cladocopium* is present, however the boundary between hosts appears permeable in *A. hyacinthus* and *A. digitifera* across the spatial scale investigated here.

Divergent Cladocopium communities within islands

Within each island and sympatric host species, all *Cladocopium* pairwise comparisons exhibit high assignment rates back to their *a priori* groups (Fig 4), which demonstrates significant community divergence between closely located reef sites (Fig S3). It is tempting to speculate that *Cladocopium* community divergence among individual reefs might be due not only to dispersal limitation, but also to spatially varying selection, implying environmental specialization (i.e. local adaptation) in the symbionts. Unfortunately, these islands are remote and understudied and therefore we cannot provide further support for this claim as we did not measure environmental parameters and did not assess symbionts' fitness across environments. Among factors that might contribute to genetic subdivision among reefs irrespective of distance is high variation in reproductive success among *Cladocopium* clones on a local scale, which would elevate divergence due to spatial discordance of short-term allele frequency fluctuations (Thornhill et al., 2017). Yet, previous work has demonstrated that other *Cladocopium* symbiont populations have exhibited classic signals of local adaptation (Howells et al., 2012), and therefore reef sites investigated here offer an excellent study system for investigating the fine-scale local adaptation potential of *Cladocopium*. If these algal symbionts are indeed locally adapted, this would ensure that horizontally transmitting coral hosts increase their local fitness by associating with local

symbionts. To confirm this hypothesis, future work is required to experimentally demonstrate that these symbionts are achieving their maximum fitness in their local reef environment (Kawecki & Ebert, 2004).

Cladocopium communities are more spatially structured than their coral hosts

With our conservative approach to analysis of our symbiont genetic data we cannot directly compare the divergence of symbiont communities to the previously published genetic structure of their coral hosts (Davies et al., 2015). Still, we can compare these results qualitatively. For symbiont communities hosted by the same coral species, we consistently find significant divergence between different sites within the same island (Fig 4; Fig S3). In contrast, no significant within-island genetic divergence was ever detected for either host species, using the exact same coral samples (Davies et al., 2015). This indicates that C40 and C21 algal symbiont communities are more spatially structured than their coral hosts across the same spatial scale.

Strong community divergence in *Cladocopium* was not surprising given the prevailing view of their life cycle. It involves symbiotic existence in sedentary hosts alternating with a short-term free-living form that largely exists in the benthos. The opportunity for *Cladocopium* dispersal by ocean currents is therefore limited, and the primary role of the free-living stage is to invade novel hosts (Fitt, Chang, & Trench, 1981; Fitt & Trench, 1983; Littman, van Oppen, & Willis, 2008; Magalon et al., 2006; Yacobovitch, Benayahu, & Weis, 2004). Our data support this hypothesis with the observation of significant clustering between all pairs of sampled sites within islands in both C40 and C21 lineages (Fig 4, Fig S3), which was never observed in the coral host (Davies et al., 2015). Overall our data support the prevailing view that Symbiodiniaceae dispersal

517 is limited, especially relative to their coral hosts, across the seascape. Still, the fact that several
518 MLGs spanned reef sites and even islands highlights the potential for occasional long-range
519 dispersal in *Cladocopium*, especially in C21 (Fig 2E).

520

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 statistical analyses; DCW, MRK and KM participated in data analysis, statistical analyses and interpretation; All authors gave final approval for publication.

Acknowledgements

Thanks to field assistants Carly Kenkel, Tim Keitt, Irina Yakushenok and David Stump. Nida Khawaja Rahman contributed to molecular work and James Derry assisted with data management. We acknowledge the Federated States of Micronesia Department of Resources and Development (#11-27-09-01, #11-27-09-02), Guam permit authorities, and the Republic of Palau Bureau of Marine Resources and Koror State Government (Marine Research permit: #09-201; Marine Resource Export Certification #RE-09-23) for all collection permits and CITES export permits. Todd LaJeunesse helped classify *Cladocopium* lineages and provided useful advice on results. We are grateful to Ulrich Mueller, Dan Thornhill, Emily Howells, and the many reviewers who have offered their critical feedback through several sets of revisions throughout the publication process.

Funding

This study was supported by the Coral Reef Conservation program of the National Oceanic and Atmospheric Administration (NA05OAR4301108) to M.V.M. DCW was funded in part by

543 Pennsylvania State University and grants from the National Science Foundation (OCE-0928764
544 and IOS-1258058).

545

546 **Data Accessibility**

547 All data are available in Supplementary files 1-6 and all data and code used for all analyses and
548 figure generation are publicly available at https://github.com/daviessw/Cladocopium_Micronesia.

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. (2019). Recruit symbiosis establishment and Symbiodiniaceae composition influenced by adult corals and reef sediment. *Coral Reefs*, 38, 405-415.
- 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: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:10.1146/Annurev.Ecolsys.110308.120220
- Barfield, S. J., Aglyamova, G. A., Bay, L. K., & Matz, M. V. (2018). Contrasting effects of *Symbiodinium* identity on coral host transcriptional profiles across latitudes. *Molecular Ecology*, 27(15), 3103-3115.
- 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: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
- Chen, B., Yu, K., Qin, Z., Liang, J., Wang, G., Huang, X., . . . Jiang, L. (2020). Dispersal, genetic variation, and symbiont interaction network of heat-tolerant endosymbiont *Durussdinium trenchii*: Insights into the adaptive potential of coral to climate change. *Science of the Total Environment*, 723, 138026.

- 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., Buschiazzi, 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: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
- Douglas, A. E. (1998). Host benefit and the evolution of specialization in symbiosis. *Heredity*, 81, 599-603.
- 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: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: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: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:10.1111/J.1365-294x.2012.05455.X
- Gómez-Cabrera, M. d. C., Ortiz, J. C., Loh, W. K. W., Ward, S., & Hoegh-Guldberg, O. (2008). Acquisition of symbiotic dinoflagellates (Symbiodinium) by juveniles of the coral *Acropora longicyathus*. *Coral Reefs*, 27(1), 219-226.

- 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., Halanach, 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
- Hoadley, K. D., Lewis, A. M., Wham, D. C., Pettay, D. T., Grasso, C., Smith, R., . . . Warner, M. E. (2019). Host-symbiont combinations dictate the photo-physiological response of reef-building corals to thermal stress. *Sci Rep*, 9(1), 9985. doi:10.1038/s41598-019-46412-4
- 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
- Hume, B. C. C., Voolstra, C. R., Arif, C., D'Angelo, C., Burt, J. A., Eyal, G., . . . Wiedenmann, J. (2016). Ancestral genetic diversity associated with the rapid spread of stress-tolerant coral symbionts in response to Holocene climate change. *Proceedings of the National Academy of Sciences of the United States of America*, 113(16), 4416-4421. doi:10.1073/pnas.1601910113
- 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
- Kirk, N. L., Andras, J. P., Harvell, C. D., Santos, S. R., & Coffroth, M. A. (2009). Population structure of Symbiodinium sp associated with the common sea fan, *Gorgonia ventalina*, in the Florida Keys across distance, depth, and time. *Marine Biology*, 156(8), 1609-1623. doi:10.1007/S00227-009-1196-Z
- 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: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.

Lewis, A. M., Chan, A. N., & LaJeunesse, T. C. (2019). New Species of Closely Related Endosymbiotic Dinoflagellates in the Greater Caribbean have Niches Corresponding to Host Coral Phylogeny. *Journal of Eukaryotic Microbiology*, 66, 469-482.

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:10.1016/J.Jembe.2008.06.034

Liu, H., Stephens, T. G., Gonzalez-Pech, R. A., Beltran, V. H., Lapeyre, B., Bongaerts, P., . . . Chan, C. X. (2018). Symbiodinium genomes reveal adaptive evolution of functions related to coral-dinoflagellate symbiosis. *Commun Biol*, 1, 95. doi:10.1038/s42003-018-0098-3

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

Moore, R., Ferguson, K., Loh, W., Hoegh-Guldberg, O., & Carter, D. (2003). Highly organized structure in the non-coding region of the psbA minicircle from clade C Symbiodinium. *International journal of systematic and evolutionary microbiology*, 53.

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

- 721 Nakabachi, A., Ishida, K., Hongoh, Y., Ohkuma, M., & Miyagishima, S. Y. (2014). Aphid gene
722 of bacterial origin encodes a protein transported to an obligate endosymbiont. *Current*
723 *Biology*, 24(14), R640-641. doi:10.1016/j.cub.2014.06.038
- 724 Oksanen, J. F., Blanchet, G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., . . . Wagner,
725 H. (2013). *vegan: Community Ecology Package*. R package version 2.0-7.
- 726 Parkinson, J. E., Baumgarten, S., Michell, C. T., Baums, I. B., LaJeunesse, T. C., & Voolstra, C.
727 R. (2016). Gene Expression Variation Resolves Species and Individual Strains among
728 Coral-Associated Dinoflagellates within the Genus *Symbiodinium*. *Genome Biology and*
729 *Evolution*, 8(3), 665-680. doi:10.1093/gbe/evw019
- 730 Peksa, O., & Skaloud, P. (2011). Do photobionts influence the ecology of lichens? A case study
731 of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxioophyceae).
732 *Molecular Ecology*, 20(18), 3936-3948. doi:10.1111/j.1365-294X.2011.05168.x
- 733 Pettay, D. T., Wham, D. C., Pinzon, J. H., & LaJeunesse, T. C. (2011). Genotypic diversity and
734 spatial-temporal distribution of *Symbiodinium* clones in an abundant reef coral. *Molecular*
735 *Ecology*, 20(24), 5197-5212. doi:10.1111/j.1365-294X.2011.05357.x
- 736 Pettay, D. T., Wham, D. C., Smith, R. T., Iglesias-Prieto, R., & LaJeunesse, T. C. (2015). Microbial
737 invasion of the Caribbean by an Indo-Pacific coral zooxanthella. *Proceedings of the*
738 *National Academy of Sciences of the United States of America*, 112(24), 7513-7518.
739 doi:10.1073/pnas.1502283112
- 740 Pinzón, J. H., Devlin-Durante, M. K., Weber, M. X., Baums, I. B., & LaJeunesse, T. C. (2011).
741 Microsatellite loci for *Symbiodinium* A3 (*S. fitti*) a common algal symbiont among
742 Caribbean *Acropora* (stony corals) and Indo-Pacific giant clams (*Tridacna*). *Conservation*
743 *Genetics Resources*, 3(1), 45-47. doi:10.1007/s12686-010-9283-5
- 744 Pochon, X., & Gates, R. D. (2010). A new *Symbiodinium* clade (Dinophyceae) from soritid
745 foraminifera in Hawai'i. *Mol Phylogenet Evol*, 56(1), 492-497.
746 doi:10.1016/j.ympev.2010.03.040
- 747 Pochon, X., Montoya-Burgos, J. I., Stadelmann, B., & Pawlowski, J. (2006). Molecular phylogeny,
748 evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus
749 *Symbiodinium*. *Mol Phylogenet Evol*, 38(1), 20-30. doi:10.1016/j.ympev.2005.04.028
- 750 Quigley, K. M., Davies, S. W., Kenkel, C. D., Willis, B. L., Matz, M. V., & Bay, L. K. (2014).
751 Deep-Sequencing Method for Quantifying Background Abundances of *Symbiodinium*
752 Types: Exploring the Rare *Symbiodinium* Biosphere in Reef-Building Corals. *Plos One*,
753 9(4). doi:ARTN e94297
754 10.1371/journal.pone.0094297
- 755 R Development Core Team. (2018). *R: A language and environment for statistical computing*.
756 Vienna, Austria: R Foundation for Statistical Computing. Retrieved from [http://www.R-](http://www.R-project.org/)
757 [project.org/](http://www.R-project.org/).
- 758 Rippe, J. P., Matz, M. V., Green, E. A., Medina, M., Khawaja, N. Z., Pongwarin, T., . . . Davies,
759 S. W. (2017). Population structure and connectivity of the mountainous star coral,
760 *Orbicella faveolata*, throughout the wider Caribbean region. *Ecology and Evolution*, 7(22),
761 9234-9246. doi:10.1002/ece3.3448
- 762 Rodriguez-Lanetty, M., Krupp, D. A., & Weis, V. M. (2004). Distinct ITS types of *Symbiodinium*
763 in Clade C correlate with cnidarian/dinoflagellate specificity during onset of symbiosis.
764 *Marine Ecology Progress Series*, 275(97-102).

- 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
- Russell, S. L. (2019). Transmission mode is associated with environment type and taxa across bacteria-eukaryote symbioses: a systematic review and meta-analysis. *FEMS Microbiology Letters*, 336(3).
- 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.
- 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
- Silverstein, R. N., Correa, A. M. S., & Baker, A. C. (2012). Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. *Proceedings of the Royal Society B-Biological Sciences*, 279(1738), 2609-2618. doi:10.1098/Rspb.2012.0055
- Swofford, D. L. (2014). *PAUP* Phylogenetic Analysis Using Parsimony (*and other methods)*. Sunderland, Massachusetts: Sinauer Associates.
- 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* Freudenthal, *S. Goreau* sp. nov., *S. Kawaguti* 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:10.1111/J.1365-294X.2011.05328.X

- 807 Weis, V. M., Reynolds, W. S., deBoer, M. D., & Krupp, D. A. (2001). Host-symbiont specificity
808 during onset of symbiosis between the dinoflagellates *Symbiodinium* spp. and planula
809 larvae of the scleractinian coral *Fungia scutaria*. *Coral Reefs*, 20(3), 301-308.
- 810 Wham, D. C., Carmichael, M., & LaJeunesse, T. C. (2014). Microsatellite loci for *Symbiodinium*
811 *goreaui* and other Clade C *Symbiodinium*. *Conservation Genetics Resources*, 6(1), 127-
812 129.
- 813 Wham, D. C., & LaJeunesse, T. C. (2016). *Symbiodinium* population genetics: Testing for cryptic
814 species and analyzing samples with mixed genotypes. *Molecular Ecology*, in press.
- 815 Wilkinson, D. M., & Sherratt, T. N. (2001). Horizontally acquired mutualisms, an unsolved
816 problem in ecology? *Oikos*, 92(2), 377-384.
- 817 Yacobovitch, T., Benayahu, Y., & Weis, V. M. (2004). Motility of zooxanthellae isolated from the
818 Red Sea soft coral *Heteroxenia fuscescens* (Cnidaria). *Journal of Experimental Marine*
819 *Biology and Ecology*, 298(1), 35-48. doi:Doi 10.1016/J.Jembe.2003.08.003
- 820 Zanolli, M., Altamirano, M., Carmona, R., De la Rosa, J., Souza-Egipsy, V., Sherwood, A., . . .
821 Andreakis, N. (2018). Assessing global range expansion in a cryptic species complex:
822 insights from the red seaweed genus *Asparagopsis* (Florideophyceae). *Journal of*
823 *Phycology*, 54(1), 12-24. doi:10.1111/jpy.12598
- 824 Ziegler, M., Stone, E., Colman, D., Takacs-Vesbach, C., & Shepherd, U. (2018). Patterns of
825 *Symbiodinium* (Dinophyceae) diversity and assemblages among diverse hosts and the
826 coral reef environment of Lizard Island, Australia. *Journal of Phycology*, 54(4), 447-460.
827 doi:10.1111/jpy.12749
- 828
- 829

Supplementary Information for: *Cladocopium* community divergence in two *Acropora* coral

hosts across multiple spatial scales

Davies SW^{1,2*}, Moreland K², Wham DC³, Kanke MR⁴ and MV Matz²

Supplementary Figures

Fig S1

Fig S1: Phylogenetic analysis of *psbA_{ancr}* sequences for *Cladocopium*

Phylogenetic analysis of *psbA_{ancr}* sequences from representative samples from this study (indicated by colored boxes) along with publicly available *psbA_{ancr}* sequences from known *Cladocopium* species identifies the presence of two lineages (C40: Dark green and C21: Light green). The nexus file used to generate the phylogenetic tree is included as Supplemental File S4.

Fig S2

Fig S2: Principle Component Analysis (PCA) of *Cladocopium* binary alleles hosted by *Acropora hyacinthus* and *Acropora digitifera* across seven islands in Micronesia. PCA scatter plots for presence/absence data for all individual samples showing differences between host species in C40 (A), differences between host species in C21 (B), differences between islands in C40 (C), and differences between islands in C21 (D). Sample clustering was significantly different (*adonis*) for host species and islands as indicated by p-values on each panel.

Fig S3

856 **Fig S3: Principle Component Analysis (PCA) of *Cladocopium* binary alleles hosted by**
 857 ***Acropora hyacinthus* and *Acropora digitifera* at twelve reef sites across six islands in**
 858 **Micronesia.** PCA scatter plots for presence/absence data for all individual samples showing
 859 community clustering within Palau C40 (A), Yap C21 (B), Chuuk C21 (C), Pohnpei C21 (D),
 860 Kosrae C21 (E), and Guam C21 (*A. digitifera* hosts only) (F). Sample clustering was
 861 significantly different (*adonis*) for host species and site on every island as indicated by p-values
 862 on each panel.

Supplementary Tables

Table S1: Microsatellite loci summary

Summary of six polymorphic microsatellite loci used to assess genetic variation in *Cladocodium* spp. hosted by *A. hyacinthus* and *A. digitifera* and their corresponding multiplexing groups.

Multiplex Group	Locus (Repeat)	Primer Sequence 5'-3'	Repeat	Annealing Temperature	Source
A	<i>SgrSpl_30</i>	F: FAM-ccgaactaccttggtaac R: aaaagacaaggacatctcgg	TA	53	Wham et al., 2014
B	<i>SgrSpl_78</i>	F: FAM-tgaaattcgggttcattgt R:ctcagatgtttccgacgagt	TA	54	Wham et al., 2014
	<i>Sgr_21</i>	F: HEX-tgctgagtgccgtgtatc R: tgatggtacttgatggtg	TCA	54	Wham et al., 2014
	<i>Spl_33</i>	F: HEX-acttgcaaagtcgaagatcg R: gaacggtgaaaggaaaatga	CAT	54	Wham et al., 2014
C	<i>C784</i>	F: Hadp-ctccttaggactggactcgc R: agaagtcgaatcgaccatcg	ATC	60	This Study
D	<i>C105</i>	F: FAM-tttcgtgttgacattgtatg R: ggactgaaaggtgcttgagg	complex	55	Bay et al., 2009

Hadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter tag sequence: HEX: tcgtcgttgagtcacgtta

Table S2: 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 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	16	1	0.914	0.811
C21	17	1	0.915	0.838
B. Islands	PC	DF	var	assign
C40	17	5	0.924	0.850
C21	11	5	0.804	0.704
C. Within Island	PC	DF	var	assign
Palau C40	14	3	0.923	0.697
Yap C21	16	3	0.961	0.833
Guam C21	3	3	0.522	0.810
Chuuk C21	10	3	0.823	0.815
Pohnpei C21	9	3	0.814	0.846
Kosrae C21	7	3	0.792	0.840