

Ecological factors rather than barriers to dispersal shape genetic structure of algal symbionts in horizontally-transmitting corals

Davies SW<sup>1,2\*</sup>, Wham FC<sup>3</sup>, Kanke MR<sup>1,4</sup> and MV Matz<sup>1</sup>

<sup>1</sup> The University of Texas at Austin, Department of Integrative Biology, Austin, TX

<sup>2</sup> The University of North Carolina at Chapel Hill, Department of Marine Sciences, Chapel Hill, NC

<sup>3</sup> Pennsylvania State University, Department of Biology, University Park, PA

<sup>4</sup> The University of North Carolina at Chapel Hill, Department of Genetics, Chapel Hill, NC

\* Corresponding author:

Sarah W. Davies, 512-609-9134. [daivessw@gmail.com](mailto:daivessw@gmail.com)

Department of Marine Sciences, 123 South Road, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3300

KEYWORDS: coral, symbiosis, *Symbiodinium*, population structure, local adaptation, host-specificity, ecological partitioning

## Abstract

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

## 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 lead to their deep integration at biochemical and genomic levels (i.e. Buchnera in aphids (1, 2)). The result of such symbiosis is essentially a novel composite organism. In other types of symbioses, the association between partners must be newly established in every generation (horizontal transmission), which allows for the maintenance of each partner's species identity. 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 (3) or allow a fungal partner to expand its geographic range across a broader climatic envelope (4). Similarly, in aphids, association with various horizontally transmitted bacterial symbionts allows these insects to colonize novel host plants across climatic zones (5). Considering these and other examples of ecological adaptation based on varying symbiotic associations, it has been argued that the joint genomic content of symbiotic systems should be studied as a single unit of evolution, the 'hologenome' (6, 7). However, the usefulness of this concept depends on whether the evolutionary trajectories of both symbiotic partners are sufficiently aligned to present a unified target of selection (8). Here, we explore this question in the symbiosis between a horizontally transmitting reef-building coral and dinoflagellate algae of the genus *Symbiodinium* (9).

Association with *Symbiodinium* is obligatory for many coral hosts that rely on algal photosynthesis for energy, while the algae benefit from protected and light-exposed

residence as well as inorganic nutrients and CO<sub>2</sub> concentration regulatory mechanisms provided by the host (10-13). Given the obligatory nature of this symbiosis for the host, it is somewhat surprising that in the majority of coral species (~85%) *Symbiodinium* are not transmitted vertically, but rather must be acquired by the juvenile coral from its local environment (14, 15). One possible explanation is that dispersal ranges of aposymbiotic coral larvae typically extend over hundreds of kilometers (i.e. (16), while the environmental variation corals must deal with exists on much smaller spatial scales: reef environments with varying light, thermal and nutrient conditions can be separated by meter-scale distances (i.e. (17). Under such circumstances, coral hosts would benefit from the mixing and matching strategy, improving their fitness by associating with the locally available, and putatively ecologically specialized, algal strains (18-20). Establishing the relative roles of these symbiotic partners in adaptation to variable environments is essential for better prediction of coral reefs' future under climate change (i.e. (21).

The working hypothesis for this study, the 'global host, local symbiont' hypothesis, posits that (i) coral host disperses widely and is able to establish symbiosis with diverse algal strains across locations; (ii) *Symbiodinium* algae are poor dispersers (i.e., 21), which results in strong divergence among locations aligning with physical barriers and facilitates their local environmental specialization; (iii) local *Symbiodinium* strains also diverge with respect to the host species as a result of selective pressure towards higher host specificity (22, 23). To validate all three components of our hypothesis, we examined multi-locus genotypes (MLG) of clade C *Symbiodinium* in two species of *Acropora* – *A. hyacinthus* and *A. digitifera* – collected from the same reef

locations across the Micronesian Pacific (Fig. 1A). Our previous work has shown that both host species exhibit extensive genetic connectivity and their genetic structure is nearly perfectly explained by the patterns of regional surface currents (16). By using two coral species that co-occur across the same locations as well as local reef environments we aimed to disentangle the roles of environmental specialization, host specialization, and physical barriers to dispersal in driving the fine-scale genetic structure of *Symbiodinium*.

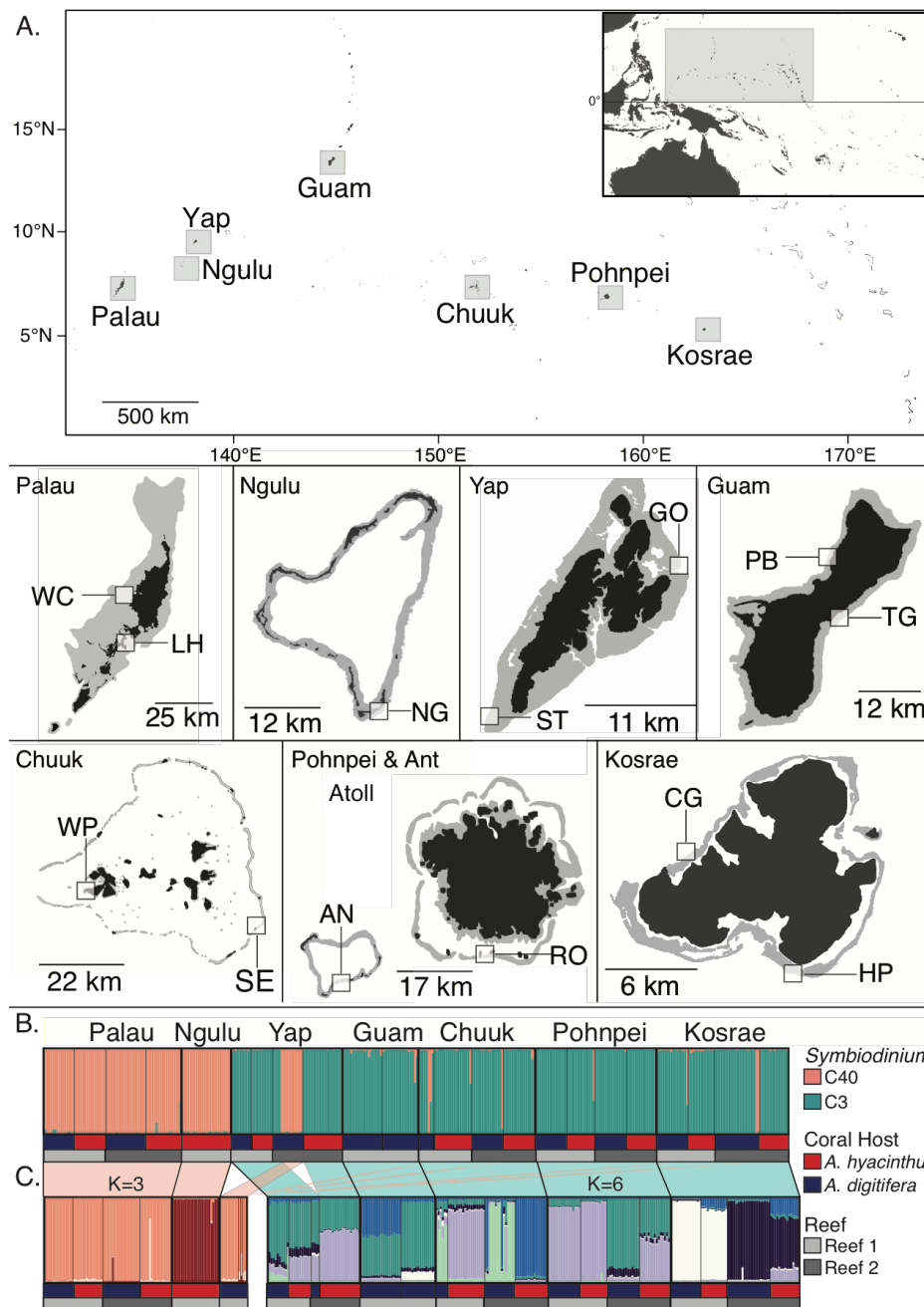


Figure 1: Geographic location of the Micronesian islands where *Acropora hyacinthus* and *A. digitifera* coral samples were collected (A). Top: Map of the Micronesian Pacific with an inset of the Pacific Ocean for reference. Islands where samples were collected and analyzed for *Symbiodinium* genetics are designated with grey boxes. Detailed information on each sampling site is located in Table S3. No *A. hyacinthus* were present in Guam and *A. digitifera* were not collected in Ngulu. Analyses of microsatellite loci data for *Symbiodinium* hosted by *Acropora hyacinthus* and *Acropora digitifera* using multilocus genotyping data. B. STRUCTURE population assignment for *Symbiodinium* from two *Acropora* host species across greater Micronesia at an optimal population number ( $K=2$ ), corresponding to C40 (pink) and C3 (turquoise) clade C subclades. Colors in the bottom panels correspond to host species and shades of grey correspond to different sites within each island. C. Within-subclade STRUCTURE analysis.

## Results

### Two *Symbiodinium* clade C lineages

To enable standard population genetic analysis, data analysis was restricted to corals hosting a single diploid *Symbiodinium* clone, which encompassed the majority (69% *A. digitifera* and 64% *A. hyacinthus*) of our samples. Across the two coral species in Micronesia (Fig. 1A), two distinct *Symbiodinium* lineages were observed, which were most clearly discriminated by two of the six microsatellite loci (Fig. S1). Sequencing of the *psbA<sub>ncr</sub>* gene for a subset samples classified these lineages as C40 and C3 (39, (24) (Fig. S2). Corals of both species from Palau and Ngulu hosted exclusively C40 (Fig. 1B, pink bars). This lineage was also prevalent in *A. digitifera* at one reef site on Yap and was rarely found in *A. digitifera* across the rest of Micronesia. All other coral hosts of either species associated with C3 (Fig. 1B, turquoise bars). Both *Symbiodinium* lineages possessed high genetic diversity, with a total of 70 unique alleles across three islands in C40 (Table S1A) and 130 unique alleles across five islands in C3 (Table S1B). Mean numbers of alleles per island for each locus for C40 ranged from 3.00-4.67 with the highest numbers of private alleles in Palau (N=7) (Table S1A). Mean allele numbers per island for each locus for C3 ranged from 3.83-5.17 and numbers of private alleles ranged from 1-4 (Table S1B).

### *Symbiodinium* genetic structure

All pairwise between-island  $F_{ST}$ 's for C40 were significant (Table 1A). C3 had one non-significant  $F_{ST}$  (Yap-Pohnpei) while all others were significant and ranged from 0.058 (Guam-Chuuk) to 0.078 (Yap-Kosrae) (Table 1B). Notably, *Symbiodinium* C3

differentiation did not show the isolation-by-distance (IBD) pattern observed in both coral hosts (Fig. 2). IBD could not be computed for C40 due to too few between-island comparisons. C3  $F_{ST}$  values generally exceeded  $F_{ST}$  values for the host (Fig. 2A), however, this result did not imply stronger symbiont genetic differentiation: higher  $F_{ST}$  in the symbionts was a consequence of higher mean heterozygosity in *Symbiodinium* markers (e.g. (25). Indeed, an alternative measure of genetic differentiation controlling for mean heterozygosity - Jost's D (26) - demonstrated that symbiont genetic divergence was in fact intermediate between the two hosts (Fig. 2B).

Table 1. Summary of pairwise  $F_{ST}$  values between all islands for *Symbiodinium* C40 (A) and C3 (B). Permutations were run 9999 times. All significant comparisons are shaded in grey.

A. *Symbiodinium* C40

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

B. *Symbiodinium* C3

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

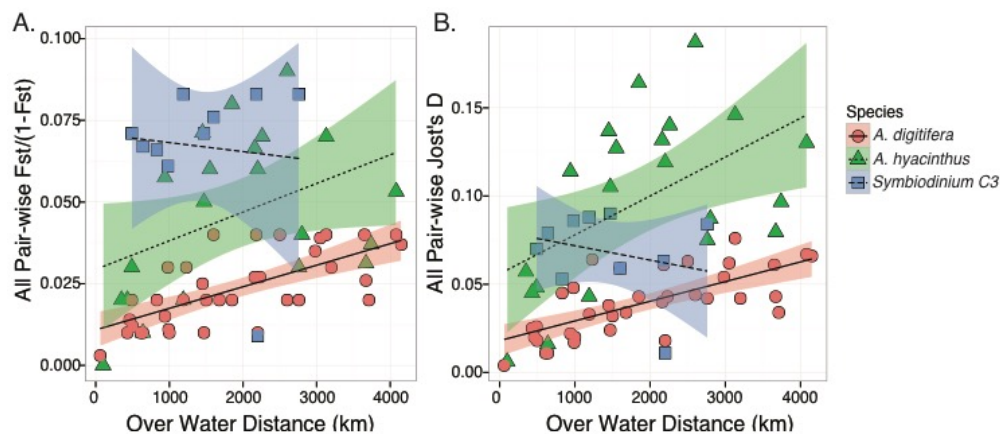


Figure 2: Comparison of two host species and *Symbiodinium* C3 differentiation. A. Pairwise genetic differentiation [ $F_{ST} / (1 - F_{ST})$ ] of two species of *Acropora* coral and *Symbiodinium* C3 across linear distances (km) demonstrating significant isolation by distance for the two host species but no correlation for the symbiont. B. Pairwise Jost's D for the same two host species and *Symbiodinium* C3 across linear distances (km) demonstrating no isolation by distance and no difference in overall divergence between the host and symbiont.



## Host specificity and environmental specialization

Discriminant analysis of principal components (DAPC) strongly differentiated between host species for both *Symbiodinium* C40 and C3 (Table S2, Fig. S3A, B), suggesting that host specificity drives symbiont diversity. In addition, DAPC demonstrated consistent differences among islands for each *Symbiodinium* lineage irrespective of host species: strong per-island assignment proportions were observed for C40 (Fig. S3C, 82-100%) and C3 (Fig. S3D, 51-98%), consistent with both STRUCTURE (Fig. 1B) and  $F_{ST}$  results (Table 1). Moreover, nearly all pairwise  $F_{ST}$  values between different reef sites within islands were significant for both lineages (Table 2), suggesting environmental partitioning of symbionts. In accord with these results, of the two top eigenvalues in DAPC of individual within islands, one explained divergence by host species (host specificity) and the other explained differences between reef sites (environmental specialization) (Fig. 3).

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

### A. *Symbiodinium* C40

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

### B. *Symbiodinium* C3

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

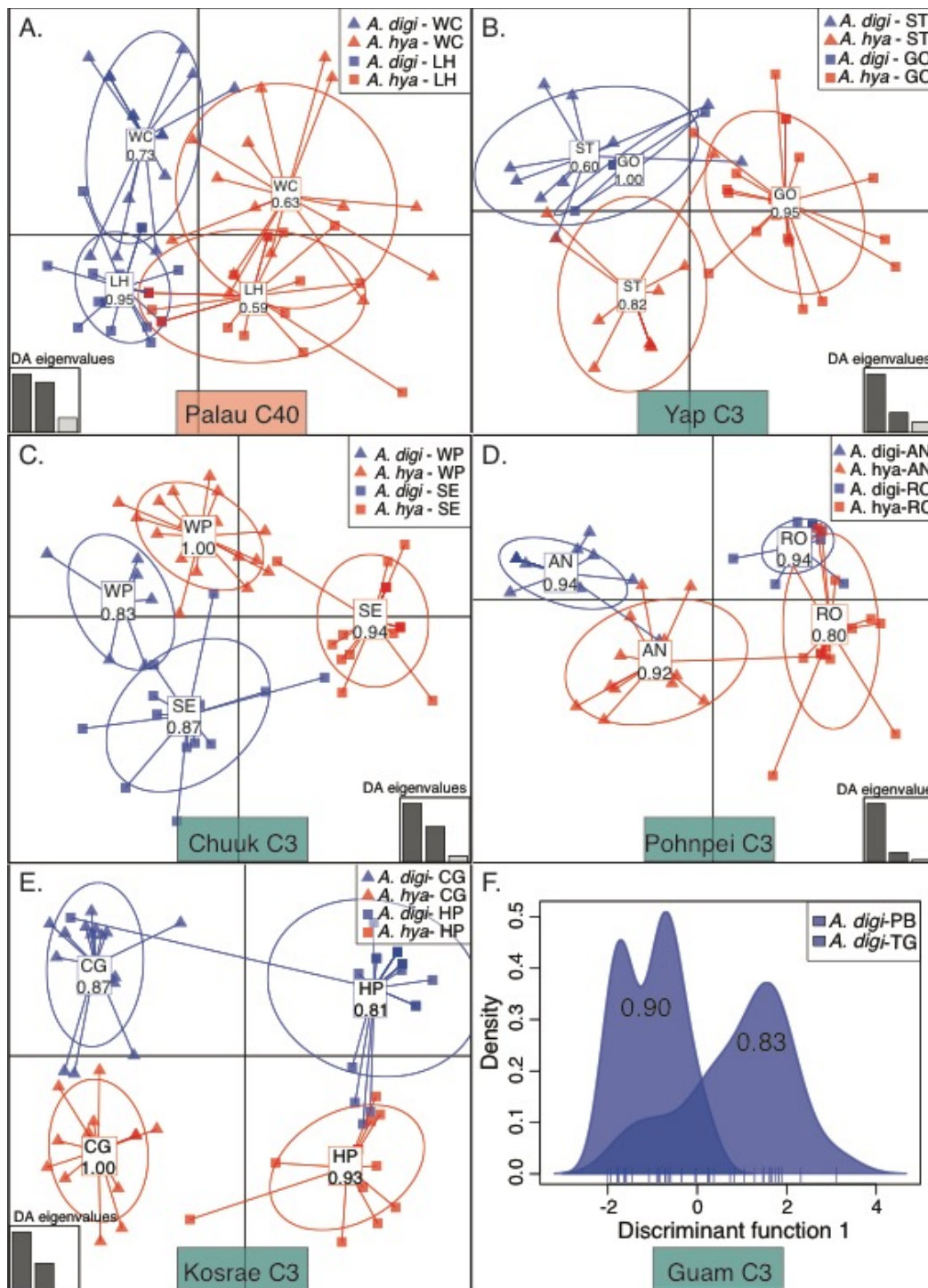


Figure 3: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium* C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at twelve sites across six islands (Ngulu not included) in Micronesia. DAPC analysis on two discriminant functions demonstrating host species assignments and site assignments. DAPC scatter plots for individual samples from within A. Palau for *Symbiodinium* C40. B. Yap for *Symbiodinium* C3, C. Chuuk for *Symbiodinium* C3, D. Pohnpei for *Symbiodinium* C3, E. Kosrae for *Symbiodinium* C3, and F. Guam for *Symbiodinium* C3 for *A. digitifera* hosts only (1 DF axis). Proportions of assignments are indicated in the clusters. Information on the DAPC models can be found in Table S2.

## Discussion

### *Promiscuity of the coral host*

Clade C *Symbiodinium* are considered to be the most derived lineage within the genus *Symbiodinium* and exhibit significantly higher within-clade diversity when compared to other, more basal, clades (9, 27, 28). Across the Micronesian Pacific (Fig. 1A), both coral hosts associated exclusively with clade C *Symbiodinium*, which was nevertheless represented by two distinct lineages— C40 and C3 (Fig. S2). This observation confirms the first prediction of the ‘global host, local symbiont’ hypothesis: coral hosts show considerable flexibility in their symbiotic association across their range and within their habitat.

### *Lack of dispersal limitation in Symbiodinium across Micronesia*

Initially, we expected to find strong isolation by distance in *Symbiodinium*, since the prevailing view of the *Symbiodinium* life cycle involves symbiotic existence in sedentary hosts alternating with a free-living form that largely exists in the benthos where dispersal by ocean currents must be limited (29-32). In contrast, we observed that genetic differentiation between islands of *Symbiodinium* C3 across Micronesia did not exceed  $F_{ST} = 0.078$ , which contrasts studies from other locations reporting symbiont  $F_{ST}$  as high as 0.54 in clade A (33) and  $\Phi_{ST}$  as high as 0.468 in clade C (34). This lack of physical dispersal limitation is best demonstrated by comparing *Symbiodinium* C3 divergence to divergence of their coral hosts, which has been shown to nearly perfectly follow biophysical barriers (16). If *Symbiodinium* dispersal were limited by the same physical barriers and to a greater extent, as our hypothesis posited, we would have observed steeper increases in divergence with distance relative to the host; instead, no increase of

$F_{ST}$  with distance was observed (Fig. 2A). Moreover, after correcting for mean heterozygosity, genetic divergence (Jost's D) in *Symbiodinium* C3 was found to be intermediate between *A. hyacinthus* and *A. digitifera* (Fig. 2B), once again demonstrating that symbiont dispersal is less physically limited than their hosts. This finding contradicts the second prediction of our 'global host, local symbiont' hypothesis and is contrary to our expectations based on earlier  $F_{ST}$ -based reports of stronger *Symbiodinium* differentiation compared to hosts (20-22, 34).

### *Host specificity*

The majority of reef-building coral species associate with a specific strain (termed "clade" or "subclade") of *Symbiodinium* broadly defined based on ribosomal and/or chloroplast markers (22, 35-37). Previous *Symbiodinium* multilocus genotyping studies revealed that each of these strains harbors greater diversity, both in terms of genetic and functional diversity (20, 34, 38). Our data indicate that the local association of hosts and symbionts of the same genotypic cluster is due to pervasive evolution of host specificity in *Symbiodinium* (Fig. S3 & 3). As our study includes two coral species, we also observe that this specificity is not perfect: at every location there were symbionts that would have been assigned to another coral host based on their multilocus genotype (Fig. 3). This suggests that host specialization in *Symbiodinium* arises in the face of considerable exchange between symbiont communities hosted by different coral species at the same location.

## Environmental partitioning

The most ecologically relevant postulate of the ‘global host, local symbiont’ hypothesis is that locally available symbionts are also locally adapted, giving rise to a locally adapted holobiont. Recurrent genetic divergence of symbionts between reef sites within the same island (Fig. 3) despite the lack of physical dispersal limitation across much larger distances (Fig. 2) suggests that symbiont genetic divergence is likely due to poor survival of immigrants rather than to physical barriers to migration. Given that hosts are available at every site, this leaves other ecological parameters of local reef environment as the most likely barrier-forming force, preventing survival of immigrants adapted to a different environment— a situation termed “phenotype-environment mismatch” (39). This in turn implies environmental specialization (i.e., local adaptation) in the symbionts, which supports our hypothesis. Interestingly, just like host specificity, this environmental specialization is imperfect: there are several *Symbiodinium* genotypes that appear to be successful migrants between reef sites (particularly clearly visible for Kosrae, Fig. 3E), indicating that environmental partitioning also arises in the face of considerable gene flow.

## Conclusions

Both coral host species associated with two divergent *Symbiodinium* lineages, indicating that coral hosts are promiscuous in their symbiont associations across the seascape. In contrast, within each *Symbiodinium* lineage, strong associations with particular host species were observed, suggesting that host-specificity is an important driver of *Symbiodinium* diversification. Unexpectedly, *Symbiodinium* genetic divergence did not

correlate with physical distance between islands, but was driven by the combined effects of local (within-island) environment and host identity. This indicates that *Symbiodinium* assemblages across Micronesia are comprised by broadly dispersing lineages dynamically adapting to specific coral hosts and reef environments across their range. Assuming that host species can be regarded as part of *Symbiodinium*'s environment, this pattern clearly follows the classical hypothesis of Baas Becking (originally proposed for soil microbes): "Everything is everywhere, but the environment selects" (40). Overall, our observations support the view that horizontal transmission might be a strategy for broadly dispersing coral hosts to improve their local fitness by associating with locally available, environmentally specialized, *Symbiodinium*. Finally, dramatic differences in factors that structure genetic diversity in the coral host and *Symbiodinium* indicate that viewing their combined genomes as a single 'hologenome' would not be useful in the context of their evolution and adaptation.

## Materials and Methods

**Sampling:** This study comprised a subset of samples previously analyzed for coral host genetics in Davies *et al.* (16) (Table S3, Fig. 1A). Twenty-five individuals of each coral host species (*Acropora hyacinthus* and *A. digitifera*) were examined at two reef sites within seven islands, with the exception of Ngulu (only collected *A. hyacinthus*) and Guam (no *A. hyacinthus* found), for a total of thirteen sites.

**Laboratory Procedures:** Holobiont DNA was isolated following Davies *et al.* (41). Microsatellite primers consisted of six previously published clade C loci (42, 43) and one

novel locus mined using Msatcommander (44) from nucleotide EST data for *Symbiodinium* sp. clade C3 (45) (Table S4). Loci were multiplexed in 20  $\mu$ l polymerase chain reaction (PCR) mixtures containing 10 ng of template, 0.1  $\mu$ M of each forward and reverse primers, 0.2 mM dNTP, 1  $\mu$ l 10X *ExTaq* buffer, 0.025 U *ExTaq* Polymerase and 0.0125 U *Pfu* Polymerase. 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  $\geq 3$  loci were excluded from analyses.

**Data Analysis:** *Symbiodinium* were scored as diploid based on available information on their ploidy (22, 43, 46) Since each host could potentially contain populations of genetically distinct *Symbiodinium*, multi-locus genotypes (MLG) were only considered if they contained two or fewer alleles across all loci, suggesting that all alleles originate from a single, clonally replicated, genome (46), which was the case for 190 of 277 *A. digitifera* hosts and 178 of 278 *A. hyacinthus* hosts. STRUCTURE v2.3.3 (47) assigned similar MLGs to populations ( $10^6$  iterations, burn in = 300,000) across ten replicate runs for each K (1-10) using an admixture model with no location prior. Following (48),  $\Delta K$  was calculated in STRUCTURE Harvester (49) and CLUMPP (50) and DISTRUCT (51) produced all graphics. We observed high percent assignment to two clusters among sympatric individuals. Following (46), we investigated the relationship between these assignments and allele identities and found strong relationships between assignments and allele identity, particularly at Sgr\_21 and Spl\_33 (Fig. S1), and we concluded that two



distinct *Symbiodinium* lineages were present. MLG data were then split into lineages and additional STRUCTURE runs were completed.

Partitioned lineage data were investigated for genetic divergences among islands and sites using  $F_{ST}$  (AMOVA, 9999 permutations in GENALEX v6.5, (52). Pairwise differentiations ( $F_{ST}$  and Jost's D) and pairwise island distances were correlated to test for isolation by distance (IBD) in *Symbiodinium* C3 (insufficient C40 data) and these trends were compared to results from the host species (53). Mean allelic diversities per island and numbers of private alleles per island were also calculated in GENALEX v6.5.

To resolve differences between islands, between host species and between sites and host species within each island, assignment of samples to genetic clusters using discriminant analysis of principal components (DAPC) was performed in R (54) using the ADEGENET package (55, 56). Data were converted into principle components (PCs) and then a-scores were used to determine trade-offs between power of discrimination and model over-fitting. Relationships were examined by DAPC and model information are listed in Table S2.

### **Sequencing Analysis of *Symbiodinium* psbA<sup>ncr</sup>**

To confirm individual assignments to different *Symbiodinium* lineages, the non-coding region of the circular plastid (psbA<sup>ncr</sup>) was amplified in a subset of samples using the methods described by (24). Amplified products were directly sequenced and aligned with ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) to clade C psbA<sup>ncr</sup> sequences from common Indo-Pacific *Symbiodinium* types (Table S5). The phylogeny was reconstructed using the default settings of Phylogeny.fr (57) and identities



of *Symbiodinium* types were interpreted based on percent sequence identity with previously identified *Symbiodinium* types.

## Acknowledgements

Thanks to field assistants Carly Kenkel, Tim Kielt, Irina Yakushenok and David Stump. Nida Khawaja Rahman was integral in molecular work and James Derry assisted with data management. We are grateful to Ulrich Mueller for numerous suggestions. We acknowledge all permit authorities. This study was supported by the Coral Reef Conservation program of the National Oceanic and Atmospheric Administration (administered through the Hawaiian Undersea Research Laboratory) and the National Science Foundation grant DEB-1054766 to M.V.M. DW was funded in part by Pennsylvania State University and grants from the National Science Foundation (OCE-0928764 and IOS-1258058).

## References

1. Nakabachi A, Ishida K, Hongoh Y, Ohkuma M, & Miyagishima SY (2014) Aphid gene of bacterial origin encodes a protein transported to an obligate endosymbiont. *Curr Biol* 24(14):R640-641.
2. Shigenobu S & Wilson AC (2011) Genomic revelations of a mutualism: the pea aphid and its obligate bacterial symbiont. *Cellular and molecular life sciences : CMLS* 68(8):1297-1309.
3. Peksa O & Skaloud P (2011) Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Mol Ecol* 20(18):3936-3948.
4. Fernandez-Mendoza F, *et al.* (2011) Population structure of mycobionts and photobionts of the widespread lichen *Cetraria aculeata*. *Mol Ecol* 20(6):1208-1232.
5. Henry LM, *et al.* (2013) Horizontally transmitted symbionts and host colonization of ecological niches. *Curr Biol* 23(17):1713-1717.
6. Brucker RM & Bordenstein SR (2014) Response to Comment on "The hologenomic basis of speciation: Gut bacteria cause hybrid lethality in the genus *Nasonia*". *Science* 345(6200).
7. Zilber-Rosenberg I & Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *Fems Microbiol Rev* 32(5):723-735.
8. Moran NA & Sloan DB (2015) The Hologenome Concept: Helpful or Hollow? *PLoS biology* 13(12):e1002311.
9. Lesser MP, Stat M, & Gates RD (2013) The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of corals are parasites and mutualists. *Coral Reefs*:603-611.
10. Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. *Ecosystems of the World* 25:75-87.
11. Barott KL, Venn AA, Perez SO, 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.
12. Muscatine L & Cernichiaro E (1969) Assimilation of photosynthetic products of zooxanthellae by a reef coral. *The Biological Bulletin* 137(3):506-523.
13. Trench RK & Blank RJ (1987) *Symbiodinium microadriaticum* Fredenthal, S. Goreau sp. nov., S. Kawaguti sp. nov., and S. pilosum sp. nov.: Gymnodinoid dinoflagellate symbionts of marine invertebrates. *J Phycol* 23(3):469-481.
14. Fadlallah YH (1983) Sexual reproduction, development and larval biology of scleractinian corals. A review. *Coral Reefs* 2:129-150.
15. Harrison PLW, C.C. (1990) *Reproduction, dispersal and recruitment of scleractinian corals* (elsevier Science Publications Amsterdam).
16. Davies SW, Treml EA, Kenkel CD, & Matz MV (2015) Exploring the role of Micronesian islands in the maintenance of coral genetic diversity in the Pacific Ocean. *Mol Ecol* 24(1):70-82.
17. Gorospe KD & Karl SA (2011) Small-Scale Spatial Analysis of In Situ Sea Temperature throughout a Single Coral Patch Reef. *Journal of Marine Biology* 2011:12.

- 379 18. Byler KA, Carmi-Veal M, Fine M, & Goulet TL (2013) Multiple symbiont  
380 acquisition strategies as an adaptive mechanism in the coral *Stylophora pistillata*.  
381 *Plos One* 8(3):e59596.
- 382 19. Rowan R & Knowlton N (1995) Intraspecific diversity and ecological zonation in  
383 coral-algal symbiosis. *Proc Natl Acad Sci U S A* 92(7):2850-2853.
- 384 20. Howells EJ, *et al.* (2012) Coral thermal tolerance shaped by local adaptation of  
385 photosymbionts. *Nat Clim Change* 2:116-120.
- 386 21. Baums IB, Devlin-Durante MK, & LaJeunesse TC (2014) New insights into the  
387 dynamics between reef corals and their associated dinoflagellate endosymbionts  
388 from population genetic studies. *Mol Ecol* 23(17):4203-4215.
- 389 22. Thornhill DJ, Lewis AM, Wham DC, & LaJeunesse TC (2014) Host-specialist  
390 lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution*  
391 68(2):352-367.
- 392 23. Coffroth MA, Santos SR, & Goulet TL (2001) Early ontogenetic expression of  
393 specificity in a cnidarian–algal symbiosis. *Mar Ecol Prog Ser* 222:85-96.
- 394 24. LaJeunesse TC & Thornhill DJ (2011) Improved resolution of reef-coral  
395 endosymbiont (Symbiodinium) species diversity, ecology, and evolution through  
396 psbA non-coding region genotyping. *Plos One* 6(12):e29013.
- 397 25. Jakobsson M, Edge MD, & Rosenberg NA (2013) The Relationship Between F-  
398 ST and the Frequency of the Most Frequent Allele. *Genetics* 193(2):515-528.
- 399 26. Jost L (2008) G(ST) and its relatives do not measure differentiation. *Mol Ecol*  
400 17(18):4015-4026.
- 401 27. Pochon X & Gates RD (2010) A new Symbiodinium clade (Dinophyceae) from  
402 soritid foraminifera in Hawai'i. *Molecular phylogenetics and evolution* 56(1):492-  
403 497.
- 404 28. Pochon X, Montoya-Burgos JI, Stadelmann B, & Pawlowski J (2006) Molecular  
405 phylogeny, evolutionary rates, and divergence timing of the symbiotic  
406 dinoflagellate genus Symbiodinium. *Molecular phylogenetics and evolution*  
407 38(1):20-30.
- 408 29. Fitt WK, Chang SS, & Trench RK (1981) Motility Patterns of Different Strains of  
409 the Symbiotic Dinoflagellate Symbiodinium (=Gymnodinium) Microadriaticum  
410 (Freudenthal) in Culture. *Bulletin of Marine Science* 31(2):436-443.
- 411 30. Fitt WK & Trench RK (1983) The Relation of Diel Patterns of Cell-Division to  
412 Diel Patterns of Motility in the Symbiotic Dinoflagellate Symbiodinium-  
413 Microadriaticum Freudenthal in Culture. *New Phytol* 94(3):421-432.
- 414 31. Yacobovitch T, Benayahu Y, & Weis VM (2004) Motility of zooxanthellae  
415 isolated from the Red Sea soft coral *Heteroxenia fuscescens* (Cnidaria). *J Exp*  
416 *Mar Biol Ecol* 298(1):35-48.
- 417 32. Littman RA, van Oppen MJH, & Willis BL (2008) Methods for sampling free-  
418 living Symbiodinium (zooxanthellae) and their distribution and abundance at  
419 Lizard Island (Great Barrier Reef). *J Exp Mar Biol Ecol* 364(1):48-53.
- 420 33. Andras JP, Kirk NL, & Harvell CD (2011) Range-wide population genetic  
421 structure of Symbiodinium associated with the Caribbean Sea fan coral, *Gorgonia*  
422 *ventalina*. *Mol Ecol* 20(12):2525-2542.

- 423 34. Howells EJ, van Oppen MJ, & Bay LK (2009) High genetic differentiation and  
424 cross-shelf patterns of genetic diversity among Great Barrier Reef populations of  
425 Symbiodinium. *Coral Reefs* 28(1):215-225.
- 426 35. Fabina NS, Putnam HM, Franklin EC, Stat M, & Gates RD (2013) Symbiotic  
427 specificity, association patterns, and function determine community responses to  
428 global changes: defining critical research areas for coral-Symbiodinium  
429 symbioses. *Global Change Biol* 19(11):3306-3316.
- 430 36. Rodriguez-Lanetty M, Krupp DA, & Weis VM (2004) Distinct ITS types of  
431 Symbiodinium in Clade C correlate with cnidarian/dinoflagellate specificity  
432 during onset of symbiosis. *Mar Ecol Prog Ser* 275(97-102).
- 433 37. Weis VM, Reynolds WS, deBoer MD, & Krupp DA (2001) Host-symbiont  
434 specificity during onset of symbiosis between the dinoflagellates Symbiodinium  
435 spp. and planula larvae of the scleractinian coral *Fungia scutaria*. *Coral Reefs*  
436 20(3):301-308.
- 437 38. Santos SR, Shearer TL, Hannes AR, & Coffroth MA (2004) Fine-scale diversity  
438 and specificity in the most prevalent lineage of symbiotic dinoflagellates  
439 (Symbiodinium, Dinophyceae) of the Caribbean. *Mol Ecol* 13(2):459-469.
- 440 39. Marshall DJ, Monro K, Bode M, Keough MJ, & Swearer S (2010) Phenotype-  
441 environment mismatches reduce connectivity in the sea. *Ecol Lett* 13(1):128-140.
- 442 40. Baas Becking LGM (1934) Geobiologie of inleiding tot de milieukunde. *Den*  
443 *Haag [Netherlands]: W.P. Van Stockum & Zoon, N. V.*
- 444 41. Davies SW, *et al.* (2013) Novel polymorphic microsatellite markers for  
445 population genetics of the endangered Caribbean star coral, *Montastraea*  
446 *faveolata*. *Mar Biodivers* 43(2):167-172.
- 447 42. Bay LK, Howells EJ, & van Oppen MJ (2009) Isolation, characterisation and  
448 cross amplification of thirteen microsatellite loci for coral endo-symbiotic  
449 dinoflagellates (Symbiodinium clade C). *Conserv Genet Resour* 1(1):199-203.
- 450 43. Wham DC, Carmichael M, & LaJeunesse TC (2014) Microsatellite loci for  
451 Symbiodinium goreau and other Clade C Symbiodinium. *Conserv Genet Resour*  
452 6(1):127-129.
- 453 44. Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat  
454 arrays and automated, locus-specific primer design. *Molecular ecology resources*  
455 8(1):92-94.
- 456 45. Leggat W, Hoegh-Guldberg O, Dove S, & Yellowlees D (2007) Analysis of an  
457 EST library from the dinoflagellate (Symbiodinium sp.) symbiont of reef-building  
458 corals. *J Phycol* 43(5):1010-1021.
- 459 46. Traylor-Knowles N, *et al.* (2011) Production of a reference transcriptome and  
460 transcriptomic database (PocilloporaBase) for the cauliflower coral, *Pocillopora*  
461 *damicornis*. *BMC genomics* 12:585.
- 462 47. Pritchard JK, Stephens M, & Donnelly P (2000) Inference of population structure  
463 using multilocus genotype data. *Genetics* 155(2):945-959.
- 464 48. Evanno G, Regnaut S, & Goudet J (2005) Detecting the number of clusters of  
465 individuals using the software STRUCTURE: a simulation study. *Mol Ecol*  
466 14(8):2611-2620.

- 467 49. Earl DA & Vonholdt BM (2012) STRUCTURE HARVESTER: a website and  
468 program for visualizing STRUCTURE output and implementing the Evanno  
469 method. *Conserv Genet Resour* 4(2):359-361.
- 470 50. Jakobsson M & Rosenberg NA (2007) CLUMPP: a cluster matching and  
471 permutation program for dealing with label switching and multimodality in  
472 analysis of population structure. *Bioinformatics* 23(14):1801-1806.
- 473 51. Rosenberg NA (2004) DISTRUCT: a program for the graphical display of  
474 population structure. *Mol Ecol Notes* 4(1):137-138.
- 475 52. Peakall R & Smouse PE (2006) GENALEX 6: genetic analysis in Excel.  
476 Population genetic software for teaching and research. *Mol Ecol Notes* 6(1):288-  
477 295.
- 478 53. Davies SW, Treml EA, Kenkel CD, & Matz MV (2015) Exploring the role of  
479 Micronesian islands in the maintenance of coral genetic diversity in the Pacific  
480 Ocean. *Mol Ecol* 24(1):70-82.
- 481 54. R Development Core Team (2015) R: A language and environment for statistical  
482 computing. (R Foundation for Statistical Computing, Vienna, Austria).
- 483 55. Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic  
484 markers. *Bioinformatics* 24(11):1403-1405.
- 485 56. Jombart T, Devillard S, & Balloux F (2010) Discriminant analysis of principal  
486 components: a new method for the analysis of genetically structured populations.  
487 *BMC genetics* 11:94.
- 488 57. Dereeper A, *et al.* (2008) Phylogeny.fr: robust phylogenetic analysis for the non-  
489 specialist. *Nucleic Acids Res* 36(Web Server issue):W465-469.
- 490
- 491

## Supporting Information

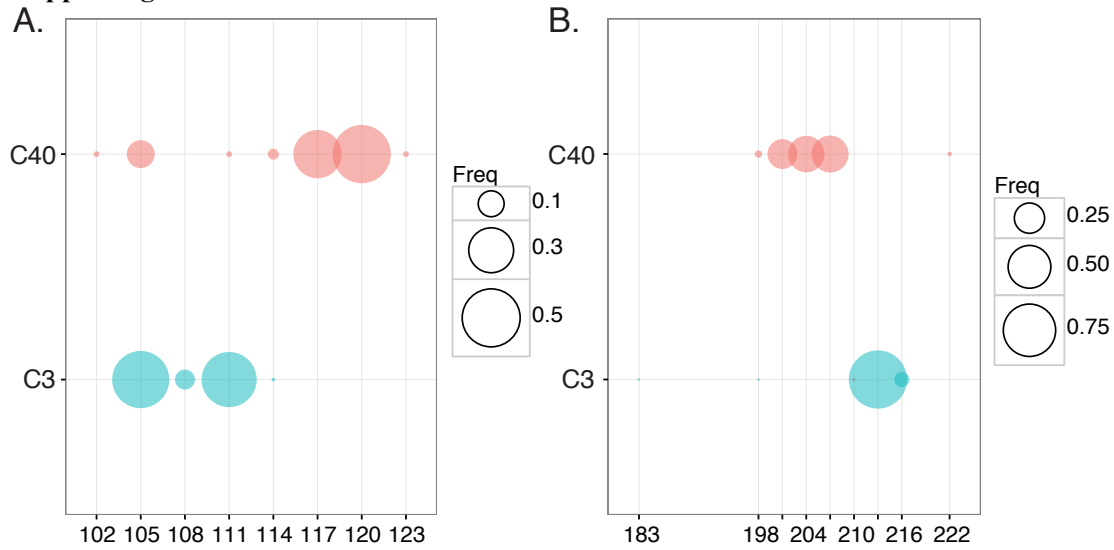


Figure S1: Bubble plot of the SSR allele frequencies of C3 (red) and C40 (blue) at the two loci that showed the largest discrimination power, Sgr\_21 (A) and Spl\_33 (B). The size of each bubble is proportional to the allele frequency within each *Symbiodinium* type.

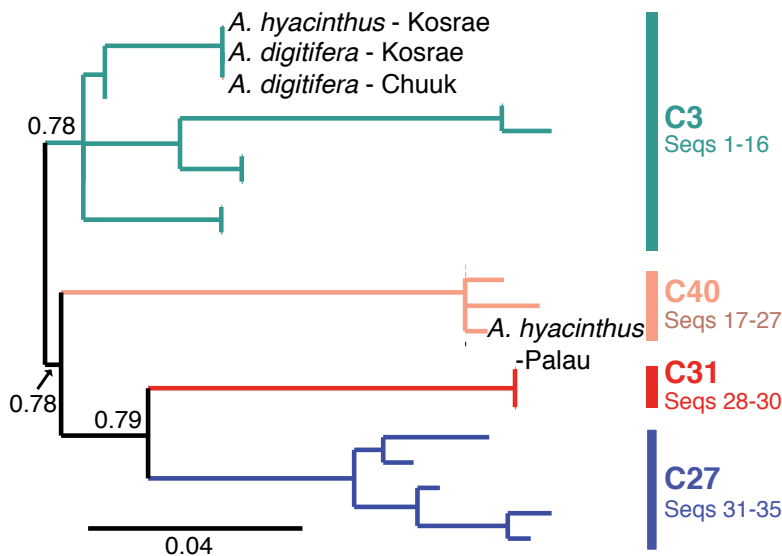


Figure S2. Phylogenetic analysis of *psbA<sup>ncr</sup>* sequences from representative samples from this study (labeled branch tips) along with publically available *psbA<sup>ncr</sup>* sequences from known *Symbiodinium* subclades identifies two lineages present (C3 and C40). Bootstrap support values are shown at the partitions that define known subclades. Scale bar: replacements per nucleotide site. Sequence accession numbers for reference sequences are given in Table S1.



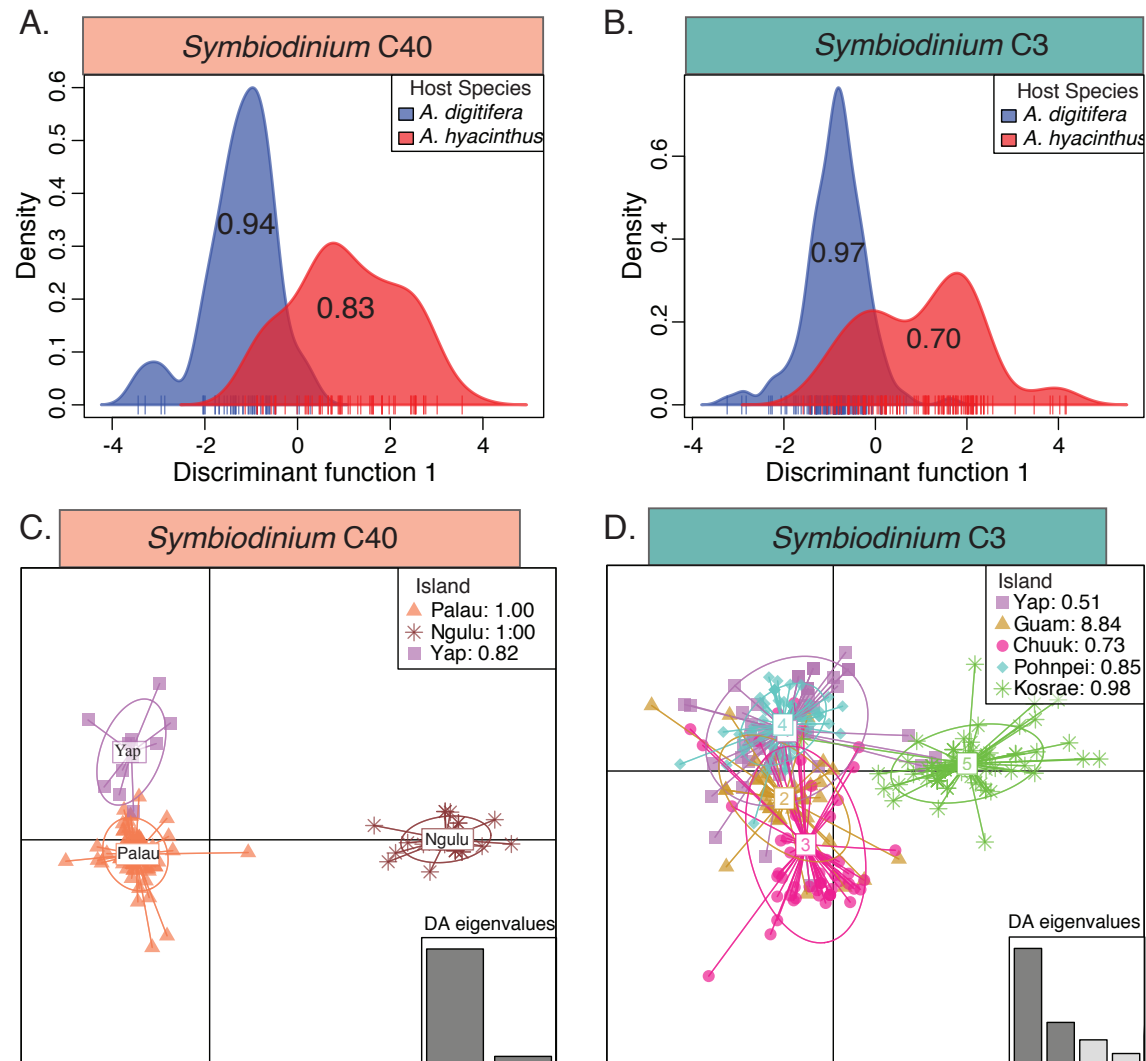


Figure S3: Discriminant analysis of principal components (DAPC) of MLG data for *Symbiodinium* C40 and C3 hosted by *Acropora hyacinthus* and *Acropora digitifera* at thirteen sites across seven islands in Micronesia. A. DAPC analysis on two discriminant functions demonstrating strong host species assignments across all islands for *Symbiodinium* C40 and B. *Symbiodinium* C3. Numbers overlaying the curves indicate successfully assigned fraction of samples. C. DAPC scatter plot for individual samples from *Symbiodinium* C40 represented by colored dots clustered by islands. D. DAPC scatter plot for individual samples from *Symbiodinium* C3 represented by colored dots clustered by islands. Proportions of assignments are indicated in the clusters or in the legends. Information on the DAPC models can be found in Table S2.



Table S1: Summary of allelic diversity measures for *Symbiodinium* C40 (A) and *Symbiodinium* C3 (B). Na: number of alleles, He: expected heterozygosity, Ho: observed heterozygosity, PA: number of private alleles

**A. *Symbiodinium* C40**

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

**B. *Symbiodinium* C3**

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

Table S2: 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 *Symbiodinium* from different host species, B. islands, and C. sites and host species within each island.

Model Information

A. Host Species	PC	DF	var	assign
<i>Symbiodinium</i> C40	7	1	0.814	0.879
<i>Symbiodinium</i> C3	14	1	0.922	0.846
B. Islands	PC	DF	var	assign
<i>Symbiodinium</i> C40	16	4	0.970	0.981
<i>Symbiodinium</i> 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 S3: Reef Site Collections. GPS coordinates, main island group, number of *A. digitifera* and *A. hyacinthus* hosts genotyped. The numbers in brackets are individuals hosting a single *Symbiodinium* MLG, which were included in all analyses presented here. Site letter corresponds to island insets in Figure 1A.

Site	Island	GPS	<i>A. digitifera</i>	<i>A. hyacinthus</i>
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 <sup>1</sup>	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) <sup>m</sup>	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°33'21.1 E	15 (6) <sup>m</sup>	24 (18)
SE. South East Pass	Chuuk	7°14'60.3 N, 152°01'29.1 E	21 (17) <sup>m</sup>	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) <sup>m</sup>	24 (13)
HP. Hiroshi Point	Kosrae	5°15'88.0 N, 162°59'01.8 E	25 (23) <sup>m</sup>	25 (14)
TOTAL			<b>277 (190)</b>	<b>278 (178)</b>

\* indicates that no individuals of this species were found

<sup>1</sup> indicates that individuals were not collected from this site but are likely present

<sup>m</sup> indicated sites where multiple *Symbiodinium* species were detected

Table S4: Summary of six polymorphic microsatellite loci used to assess genetic variation *Symbiodinium* clade C 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-ccgaactaccttggtcaac R: aaaagacaaggacatctcgg	TA	53	Wham et al., 2014
B	<i>SgrSpl_78</i>	F: FAM-tgaattcgggtgttcattgt R: ctcagatgttccgacgagt	TA	54	Wham et al., 2014
	<i>Sgr_21</i>	F: HEX-tgctgagtggtgttatatc R: tgatggtactgtgatggtg	TCA	54	Wham et al., 2014
	<i>Spl_33</i>	F: HEX-acttgcaaaagccaagatcg R: gaacggtgaaaggaaaatga	CAT	54	Wham et al., 2014
C	<i>C784</i>	F: Hadp-ctccttaggactggactcgc R: agaagtcaaatcgtcaccatcg	ATC	60	This Study
D	<i>C105</i>	F: FAM-tttcgttggtgacattgttatg R: ggactgaaagtgcttgagg	complex	55	Bay et al., 2009

Fadp- labeled primers were indirectly labeled in each PCR reaction with an additional FAM labeled adapter tag sequence: FAM: agcagcgaactcagtagaca

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

544 Table S5: Accession number and corresponding Clade C *Symbiodinium* lineage for each unique  
545 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

546