MISS SARAH WHITENEY DAVIES (Orcid ID : 0000-0002-1620-2278)

Article type : Original Article

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

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

[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 a variety of multilocus genotypes (MLG) within 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 included multiple asexual lineages bearing identical MLGs, many of which spanned host species, reef sites within islands, and even different islands. Both C40 and C21 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, asexual lineages

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 CO_2 concentration mechanisms provided by the host (Barott, Venn, Perez, Tambutte, & Tresguerres, 2015; Muscatine, 1990; Muscatine & Cernichiari, 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. This study demonstrated that horizontal transmission was the dominant transmission mode 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 goreaui* 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 symbionts, including how their populations are structured in comparison to their coral hosts. Understanding the relative importance of reef environment, coral host, and geographical distance in structuring coral-associated algal symbiont communities is essential to identifying the adaptive capacity of this symbiosis. However, thus far there are only a handful of population genetic studies of Symbiodiniaceae based on multilocus markers, none of which address all of the above-mentioned potential sources of genetic variation. Here, using microsatellites, we examined the community divergence of *Cladocopium* spp. algal symbionts hosted by two common, co-occurring species of *Acropora* corals– *A. hyacinthus* and *A. digitifera* – collected from the same reef locations across the Micronesian Pacific (Fig 1A, B). We explore this community divergence across several ecological scales including host species, islands across the Micronesian archipelago, and unique sites within each island. We then discuss these results for the algal symbionts to the previously published population genetic structure of their coral hosts (based on a subset of the exact same coral samples), which demonstrated that both coral species exhibited extensive genetic connectivity and their genetic structure was well explained by the biophysical connectivity between sites (Davies et al., 2015).

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).

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 "communities of alleles", i.e., binary (allele presence/absence) values for each sample. This conservative approach recognizes that each multilocus genotype (MLG) could represent multiple genomes from mixed *Cladocopium* lineages and allowed us to retain all individuals in analyses (569 total: 282 *A. digitifera* and 287 *A. hyacinthus*). The drawback of this approach is 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, 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 *adegenet* 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 assigning 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 of psbA^{ncr} reference sequences for C40 (from various scleractinians) and C21 (from *Acropora*), provided by the LaJeunesse lab, was conducted using PAUP Version 4.4a147 (Swofford, 2014) using maximum parsimony. Statistical significance was confirmed via bootstrapping (based on 1000 replicates). A nexus file (Supplemental File S4) was used to generate an unrooted phylogenetic tree 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.

Analyses of asexual lineages within each cluster

To determine the prevalence of identical asexual lineages within *Cladocopium* C40 and C21, individuals with matching MLGs were investigated. Singleton alleles were removed (12 alleles in C40; 10 alleles in C21). Genotypic identity, the probability that two MLGs sampled without replacement from the dataset were identical, was calculated as $G_I = \sum_i p_i^2$, where p_i is the frequency of the i'th repeated MLG. Genotypic diversity, the probability that two MLGs sampled without replacement from the dataset were different, is the complement of genotypic identity: $G_D = 1 - G_I$. Hierarchical clustering tree was constructed in R (R Development Core Team, 2018) using the *vegdist(x, binary=T, method="manhattan")* function from the *vegan* package (Oksanen et al., 2013) and processed with the function *hclust(x)*. Manhattan distance was chosen for this analysis because it corresponds to the total number of non-shared alleles between two MLGs, which is zero for identical MLGs. Samples sharing identical MLGs were then identified using the function *cutree(x, h=0.2)*, which grouped samples with less than one (i.e., zero) non-shared alleles. The probability of chance occurrence of identical MLGs was assessed by a resampling simulation in R. To create simulated MLGs assuming random sorting of alleles, we first created a matrix of allele presence-absences, where rows were samples and columns were alleles using 44 non-singleton alleles in C40 and 46 non-singleton alleles in C21. In each column, 1 marked the presence and 0 marked the absence of an allele. Then, 100,000 simulated MLGs were created by taking a single random draw from each column. In this way, the probability of sampling an allele is equal to its frequency in the total population, and multiple alleles per locus can be sampled since the allele presence-absence matrix did not contain locus information. The probability that *n* MLGs in the dataset were identical by chance was calculated using the formula $\left(\frac{a}{100,000}\right)^{n-1}$a, where *a* is the number of times the MLG was observed in the simulation and *n* is the number of times it was observed in the actual data. For downstream DAPC analyses, we have created dataframes including only a single representative of each MLG within a site within the same host species (Supplemental Files S5 and S6 for C40 and C21 respectively).

The geographical distances spanned by MLGs were investigated by calculating a distance matrix from reef site coordinates, in decimal degrees, using the *dist* function in R. The *dms2dec* function from Zanolla 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). Differences in per-site genotypic diversity between C40 and C21 and between coral host species were tested using *t.test()* function in R. Differences between frequencies of repeated MLGs between C40 and C21 was tested using the function *chisq.test()* based on Monte Carlo simulations with 10,000 replicates (Hope, 1968).

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 *in hospite 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 discriminant functions (DF) 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.

Unconstrained Cladocopium *community analyses*

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

Data and code availability

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

Results

Two clusters of Cladocopium *symbionts observed in Micronesian acroporids*

Across the two coral host species in Micronesia (Fig 1A, B), two distinct *Cladocopium* clusters were observed with 98.4% of samples (560/569) strongly assigning to one of the two clusters (Fig 1C). Sequencing of the psbA^{ncr} gene from representative samples from each cluster identified them as *Cladocopium* C40 and C21 (LaJeunesse et al., 2004; LaJeunesse & Thornhill, 2011; Thornhill et al., 2014) (Fig S1). It is important to note that the possible presence of other background Symbiodiniaceae genera would not affect *Cladocopium* genotyping results since our 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* throughout Micronesia (Fig 1C). All other *Acropora* hosts 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).

Asexual lineages in Cladocopium *symbionts*

C40 comprised a total of 105 unique MLGs, 22 of which were found more than once (Fig 2A). In C21 there were 309 unique MLGs, 53 of which were found more than once (Fig 2B). Using resampling simulations, we determined that 16 out of 22 repeated MLGs in C40 and all 53 repeated MLGs in C21 were unlikely to occur due to random assortment of microsatellite alleles ($p<0.001$). Six repeated MLGs in C40 were less robustly supported (p-values ranging from

0.0014 to 0.0395), but all still passed the $p<0.05$ significance threshold. We therefore posit that repeated MLGs constitute evidence of identity by descent, i.e., represent lineages descending by asexual reproduction from a common MLG ancestor.

Asexual lineage group size was on average 4.05 for C40 and 2.49 for C21, ranging up to 14 in C40 and 5 in C21. This difference was significant (*p*=0.013, Fig 2C,D). For the whole dataset, the genotypic identity level (probability that two randomly sampled MLGs are identical) in C40 was almost tenfold higher than in C21 (0.0204 vs. 0.0024), but this difference was not readily apparent when per-reef measures of genotypic diversity were compared (*p*=0.13; Fig S2A). There was also no significant difference in overall genotypic diversity of algal symbionts (of any type) hosted by the two coral species (*p*=0.9; Fig S2B). Summaries of proportions of repeated MLGs for each reef site are shown in Figure 2F,G.

Notably, many asexual lineages spanned host species, reef sites, and even islands (Fig 2A, B). Larger group size in C40 compared to C21 did not translate into larger geographic distance spanned by an asexual lineage (Fig 2E). The largest distance spanned by C40 lineages was between Goofnuw Channel (GO.2), Yap and Lighthouse Reef (LH.1), Palau (~578 km), while the largest distance spanned by C21 lineages was between South Tip (ST.1), Yap and Hiroshi Point (HP.2), Kosrae (~3714 km) (Fig 2E). Proportions of asexual lineages spanning host species also differed between C40 and C21: 36.4% of them spanned host species in C40 (Fig 2A), compared to 20.8% in C21 (Fig 2B).

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

Unlike MLGs that occurred repeatedly and thereby could be attributed to individual asexual lineages, singleton MLGs could represent individual symbiont genotypes or mixtures of genotypes hosted by the same coral. Therefore, all MLGs were analyzed as “communities of alleles”, making no genetic assumptions. 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 that distinction between host species was significant for both C40 and C21 (PERMANOVA $p<0.001$) (Fig S3A,B). These results confirm that host species play a role in structuring *Cladocopium* communities across

Micronesia. 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, 0.54-0.88), which were also confirmed using unconstrained analyses for both C40 and C21 (PERMANOVA p<0.001, Fig S3C,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 fact was that algal symbiont communities from Ngulu and Kosrae were highly distinct, suggesting the possibility of additional *Cladocopium* lineages (besides C40 and C21) existing there (Fig 3C), which was not further explored here.

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 *Cladocopium* community 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 S4). There was only one instance when DAPC and unconstrained analysis did not show strong support for clustering by sites and host species within island: C21 from Yap (Figs 4B, S4B). This is likely due to unbalanced sampling of site:symbiont groups for *A. digitifera*: this species showed high prevalence of C21 relative to C40 across all Yap sites except Goofnuw channel, where C40 was more prevalent (Fig 1C). The strongest separation between host:site groups was observed at Chuuk (Figs 4D, S4D) and at Kosrae (Figs 4E, S4E).

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 the potential for additional species present (e.g. the highly distinct C21 from Ngulu, Fig 3C). This observation suggests that both coral hosts show 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 community composition studies suggesting that Indo-Pacific acroporids are dominated by algal symbionts in this genus (i.e. LaJeunesse et al., 2004; LaJeunesse et al., 2003; Thornhill et al., 2014). Initial symbiont infection is likely determined by local availability of symbionts, either free-living or, those that have been recently evacuated from local coral hosts (Thornhill et al., 2017). Diverse infections are 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). After infection, a winnowing process - competition between symbiont strains modulated both by the host and by the environment - leads to the eventual dominance of a single asexual lineage of symbionts in a single host colony and distinct symbiont communities across coral hosts in a specific habitat (Rowan, Knowlton, Baker, & Jara, 1997; Thornhill et al., 2017).

Strict associations of a single coral with a single Symbiodiniaceae asexual lineage have been observed across a variety of coral species and Symbiodiniaceae genera (Baums, Devlin-Durante, & LaJeunesse, 2014; Pinzón, Devlin-Durante, Weber, Baums, & LaJeunesse, 2011; Thornhill et al., 2014), however this 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 Palau 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).

Distinct asexual lineages within Cladocopium *C40 and C21*

We posit that symbiont MLGs shared between coral colonies represent asexual lineages descending from the same MLG ancestor, because, as we demonstrate through resampling simulations, repeated occurrence of an MLG through random sorting of alleles is highly unlikely.

Note that we call groups of shared MLGs "asexual lineages" rather than "clones", to recognize that their representatives might have accumulated mutations throughout their genomes since their divergence from the common ancestor, despite retaining the ancestral MLG at the six microsatellite loci analyzed here. Previous Symbiodiniaceae studies based on microsatellite loci demonstrated that rates of MLGs sharing can differ substantially 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 MLGs between colonies (Hoadley et al., 2019), and similarly low 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* MLG. Here we find 22 repeated MLGs in C40 and 53 in C21, which account for 51.7% of C40 corals and 34% of C3 corals (Fig 2 A,B). Unlike Caribbean *Acropora* (Baums et al 2014), all coral hosts analyzed here represent distinct genets (i.e. the small proportion of clones detected in Davies et al., 2015 were avoided) and therefore sharing of symbiont MLGs cannot be attributed to clonality of their hosts. While few studies have investigated MLG sharing in Pacific *Cladocopium,* Howells et al. (2013) found that only 13% of *A. millepora* from the Great Barrier Reef hosted identical MLGs. However, rates of MLG sharing appear to be different across *Cladocopium* species. For example, Thornhill et al. (2014) observed that 17% of C3 hosted by *Siderastrea siderea*, 70% of C7 hosted by *Orbicella* spp, and 47% of C7a/C12 hosted by *Orbicella* spp. represented shared MLGs. In light of these data, the prevalence of asexual lineages that we have observed are well within previously published estimates.

Interestingly, we found that *Cladocopium* asexual lineages 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 MLGs 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 an asexual lineage can disperse across such distances, which was especially evident in C21 (Fig 2E), given that the majority of symbioses in corals involve horizontal transmission (Baird et al., 2009) and free-living Symbiodiniaceae are expected to have low dispersal potential (reviewed in Thornhill et al., 2017). Secondly, it is surprising that the same asexual lineage 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 asexual lineages appear to be more broadly distributed across the seascape than C40 (Fig 2E), suggesting that C21 may have higher dispersal potential than C40. If so, this might explain larger group size in C40 compared to C21: since less dispersal implies less mixing of asexual lineages across locations, the symbiont with less dispersal would be more likely to have larger same-MLG groups detected at any given location. An alternative explanation of the difference between MLG group sizes in C40 and C21 is higher variance in the rates of asexual reproduction among C40 genotypes compared to C21 genotypes (Thornhill et al, 2017).

It is important to note that we are likely underestimating the frequencies of identical asexual lineages given the complexities of peak calling in microsatellite analyses and error rates associated with PCR-based analyses of repeated loci. Our results highlight the urgent need for in-depth population genomic studies of Symbiodiniaceae, which would allow for the investigation of evolution within and among asexual lineages, local adaptation, emergence of novel symbiont-host associations, and interactions between all of these aspects. An effective approach for Symbiodiniaceae genomics would be the recently introduced expression exome capture sequencing (eecSeq, Puritz and Lotterhos, 2018), which would provide a cost-efficient solution to the problem of pervasive host DNA contamination. Intensive sampling of hosts associated with *Cladocopium* across additional host species and sites coupled with sequencing deeper coverage across the genome will undoubtedly 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 S3A,B), and this pattern of host specificity consistently holds between host species on the same reef (Fig 4; Fig S4). 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 S4), which has been previously proposed in symbionts hosted by *Pocillopora* in the south Pacific (Magalon, Baudry, Husté, Adjeroud, & 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 S4). In fact, there were multiple 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 S4). 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. However, 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* asexual lineages 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 S4). 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 that 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 S4), which was never observed in the coral host (Davies et al., 2015). Overall our data support the prevailing view that Symbiodiniaceae dispersal is limited, especially relative to their coral hosts, across the seascape. Still, the fact that several asexual lineages spanned reef sites and even islands highlights the potential for occasional long-range dispersal in *Cladocopium*, especially in C21 (Fig 2E).

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 and the biology of *Cladocopium*. 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

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

Data Accessibility

All data are available in Supplementary files 1-6 and all data and code used for all analyses and 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:Doi 10.1111/J.1365-294x.2011.05115.X

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

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

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

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

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

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

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:Doi 10.1111/Gcb.12320

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

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

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

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

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

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

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

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

Hope, A.C.A. (1968) A simplified Monte Carlo significance test procedure. *Jounral of the Royal Statistical Society Series B,* 30, 582-598.

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

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

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

Parkinson, J. E., Baumgarten, S., Michell, C. T., Baums, I. B., LaJeunesse, T. C., & Voolstra, C. R. (2016). Gene Expression Variation Resolves Species and Individual Strains among Coral-Associated Dinoflagellates within the Genus Symbiodinium. *Genome Biology and Evolution, 8*(3), 665-680. doi:10.1093/gbe/evw019

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

Pettay, D. T., Wham, D. C., Pinzon, J. H., & LaJeunesse, T. C. (2011). Genotypic diversity and spatial-temporal distribution of Symbiodinium clones in an abundant reef coral. *Molecular Ecology, 20*(24), 5197-5212. doi:10.1111/j.1365-294X.2011.05357.x

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

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

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

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

Puritz, J. B. & Lotterhos, K. E. (2018). Expressed exome capture sequencing: A method for cost-effective exome sequencing for all organisms. *Molecular Ecology Resources*. Wiley Online Library, 18, pp. 1209–1222.

Quigley, K. M., Davies, S. W., Kenkel, C. D., Willis, B. L., Matz, M. V., & Bay, L. K. (2014). Deep-Sequencing Method for Quantifying Background Abundances of Symbiodinium Types: Exploring the Rare Symbiodinium Biosphere in Reef-Building Corals. *Plos One, 9*(4). doi:ARTN e94297 10.1371/journal.pone.0094297

R Development Core Team. (2018). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/.

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

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

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: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 Fredenthal, S. Goreau sp. nov., S. Kawagut2 sp. nov., and S. pilosum sp. nov.: Gymnodinoid dinoflagellate symbionts of marine invertebrates. *Journal of Phycology, 23*(3), 469-481.

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

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

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

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

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

Wilkinson, D. M., & Sherratt, T. N. (2001). Horizontally acquired mutualisms, an unsolved problem in ecology? *Oikos, 92*(2), 377-384.

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

Zanolla, M., Altamirano, M., Carmona, R., De la Rosa, J., Souza-Egipsy, V., Sherwood, A., . . . Andreakis, N. (2018). Assessing global range expansion in a cryptic species complex: insights from the red seaweed genus Asparagopsis (Florideophyceae). *Journal of Phycology, 54*(1), 12-24. doi:10.1111/jpy.12598

Ziegler, M., Stone, E., Colman, D., Takacs-Vesbach, C., & Shepherd, U. (2018). Patterns of Symbiodinium (Dinophyceae) diversity and assemblages among diverse hosts and the coral reef environment of Lizard Island, Australia. *Journal of Phycology, 54*(4), 447-460. doi:10.1111/jpy.12749

Figure Legends

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.

Fig 2: Repeated MLGs (asexual lineages) in *Cladocopium.*

Fan trees of *Cladocopium* C40 (A) and 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) Frequencies of repeated MLG group sizes. C40 has larger repeated MLG groups than C21. (D) Frequencies of repeated MLGs spanning hosts, 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, G) Proportions of coral colonies hosting repeated MLGs 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 identical MLGs. h, *A. hyacinthus*; d, *A. digitifera*. Reef site colors correspond to Figure 1 and Table 1.

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.

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 identical MLG 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	*A. digitifera*	*A. hyacinthus*
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*

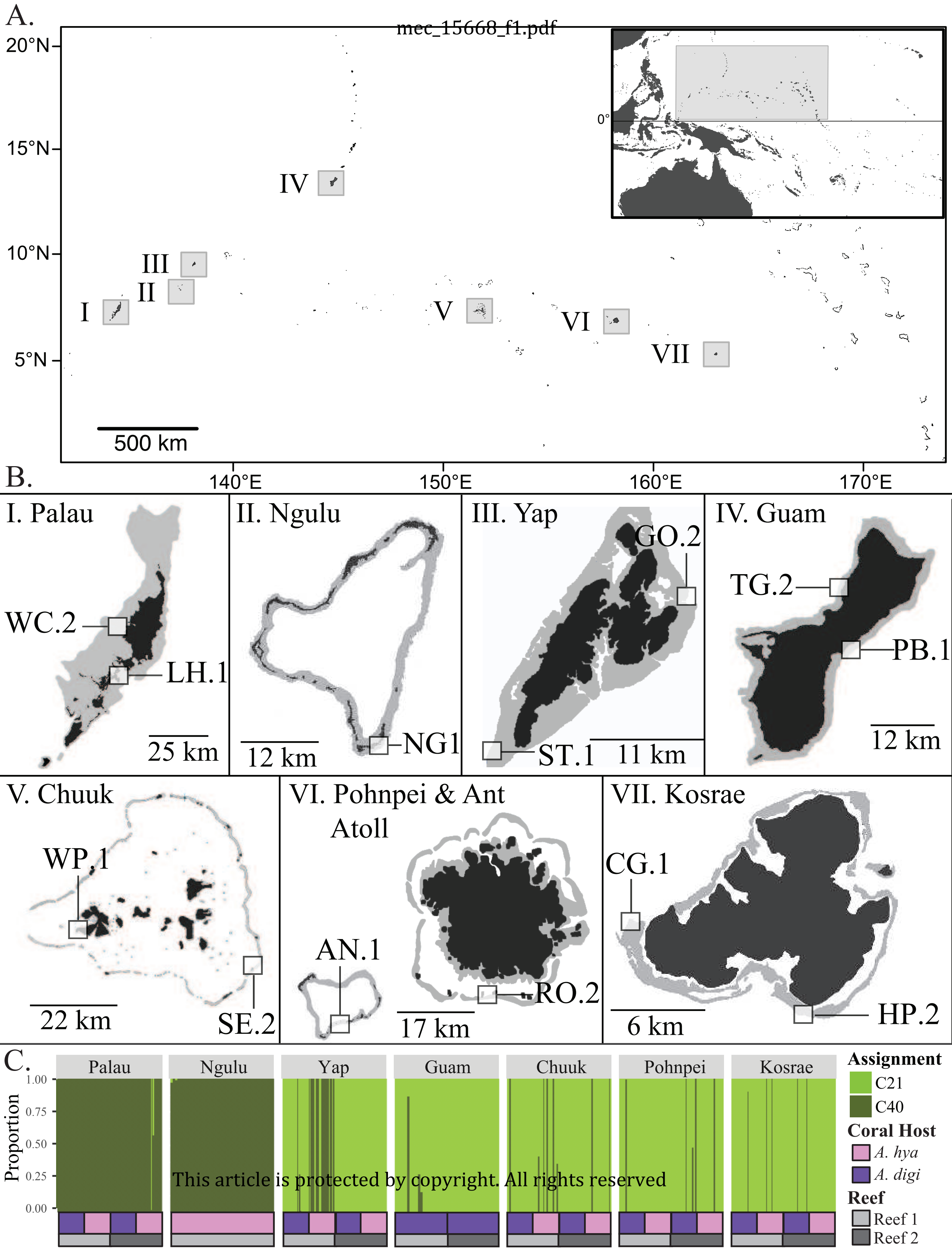
TG.2: Tanguisson	Guam	13°32’61.1 N, 144°48’52.6 E	23, 20 (0,17)	0*
WP.1: West Polle	Chuuk	7°19’69.7 N, 151°33’21.1E	16, 15 (2,11)	24, 23 (1,22)
SE.2: South East Pass	Chuuk	7°14’60.3 N, 152°01’29.1 E	21, 21 (1,20)	23, 23 (2,13)
AN.1: Ant Atoll (East)	Pohnpei	6°47’42.3 N, 158°01’20.7 E	24, 24 (0,17)	24, 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)**

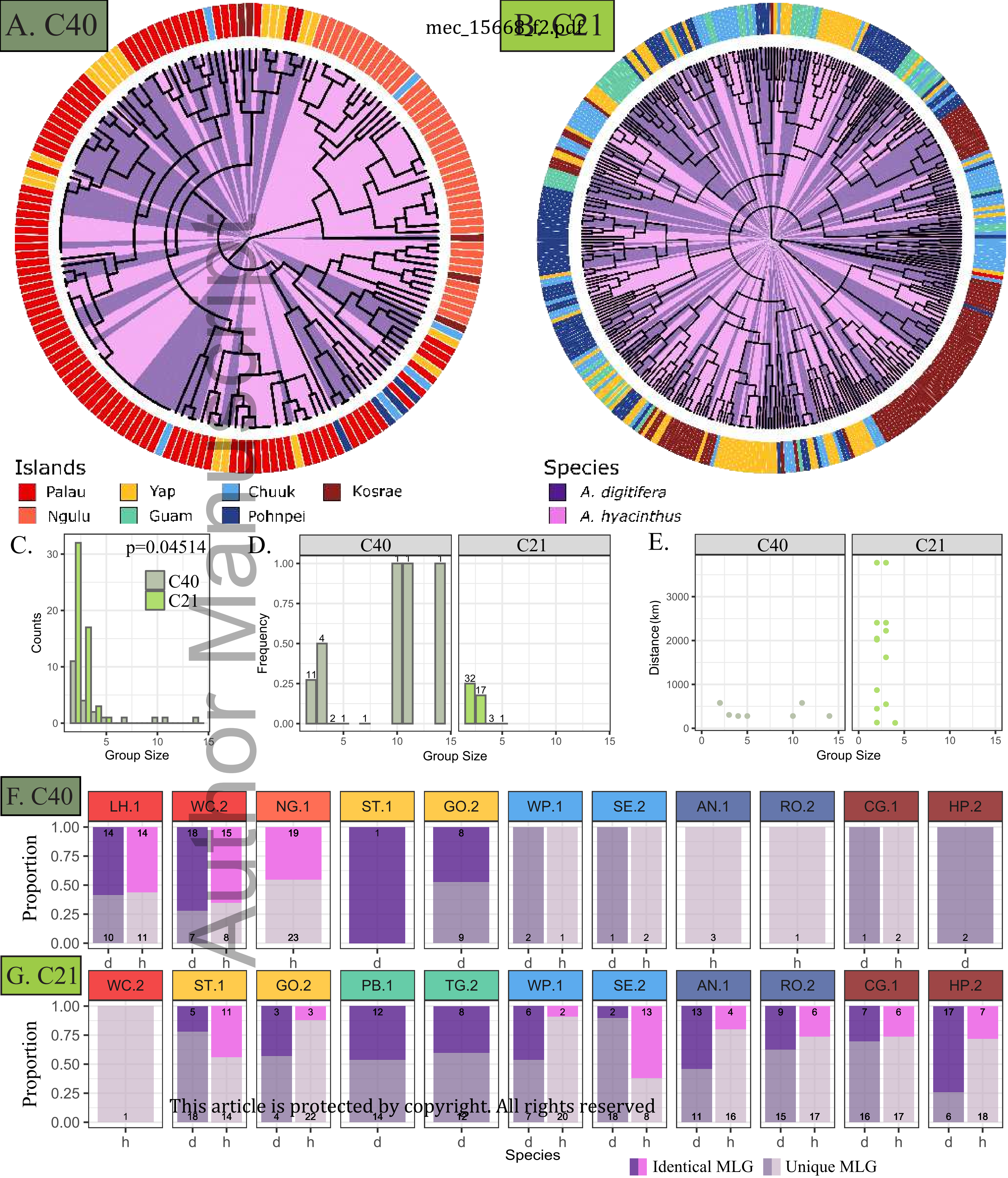
---

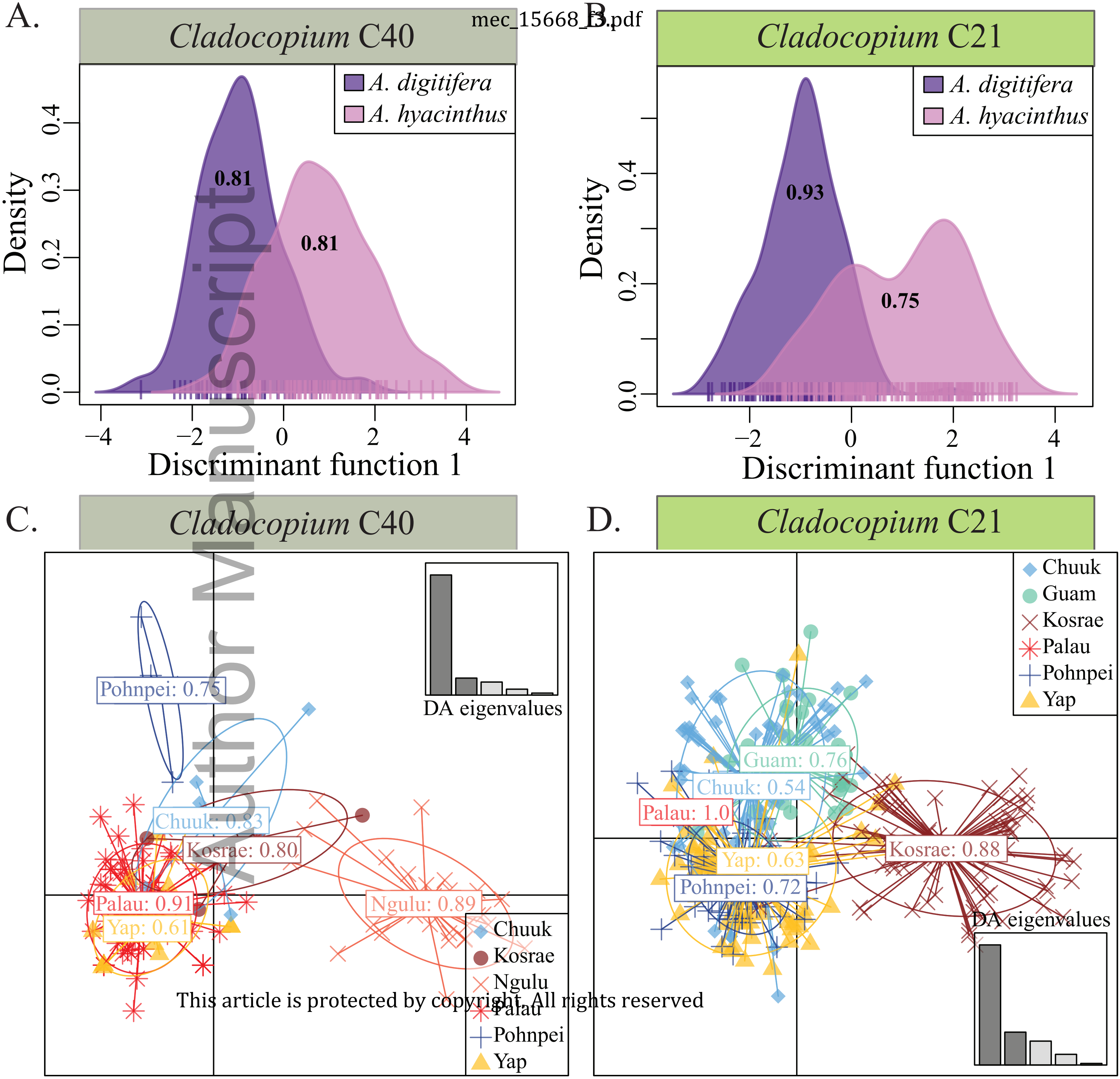
* indicates no individuals of this host species were found

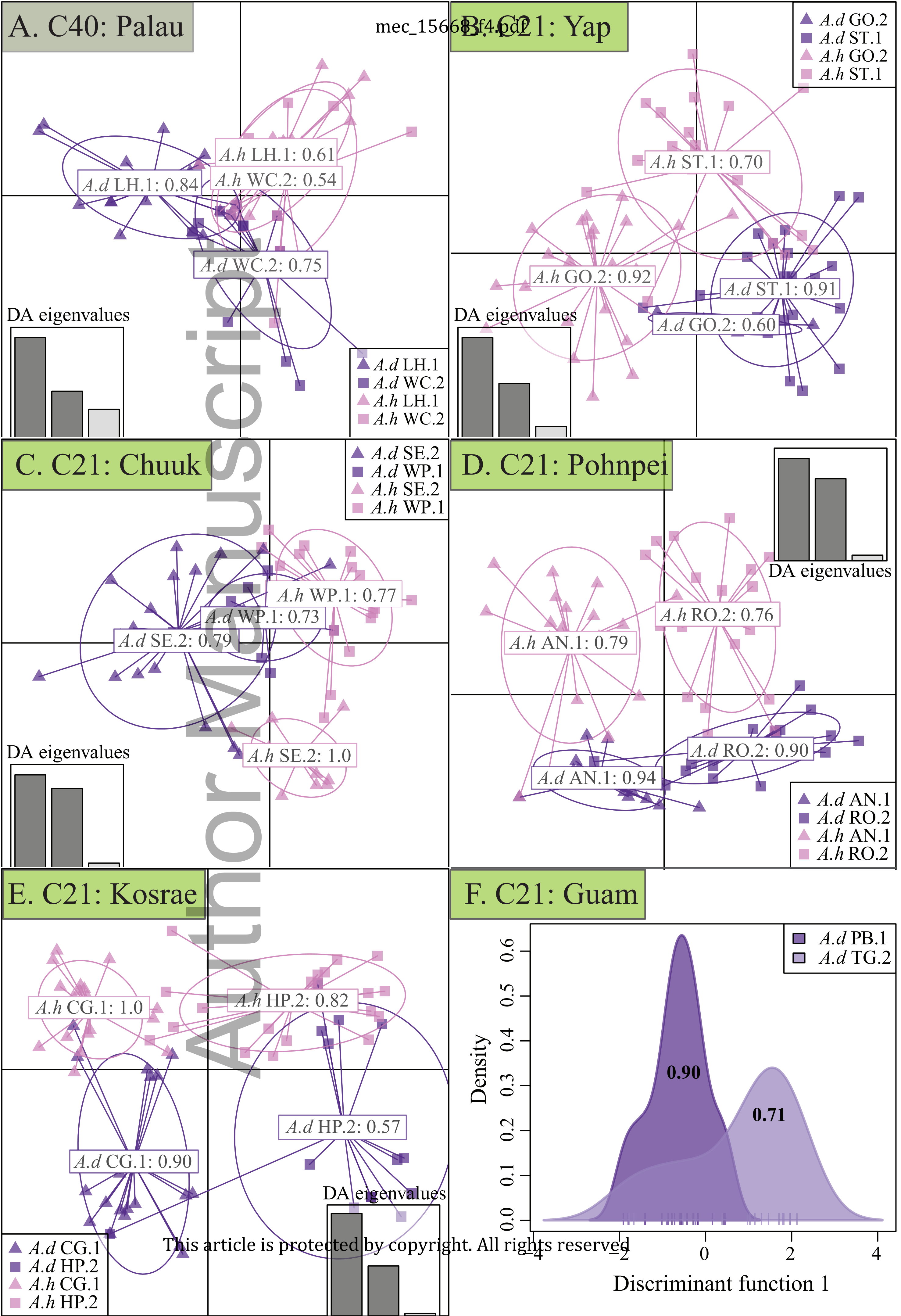
[1] indicates individuals were not collected from this site but are likely present

mec_15668_f1.pdf




A. C40
B. C21
Islands
🟥 Palau
🟥 Ngulu
🟨 Yap
🟩 Guam
🟦 Chuuk
🟦 Pohnpei
🟥 Kosrae
Species
🟪 *A. digitifera*
🟪 *A. hyacinthus*
C.
p=0.04514
C40
C21
Counts
0
10
20
30
5
10
15
Group Size
D.
C40
C21
Frequency
0.00
0.25
0.50
0.75
1.00
11
4
2
1
1
1
1
1
32
17
3
1
5
10
15
Group Size
E.
C40
C21
Distance (km)
0
1000
2000
3000
0
5
10
15
Group Size
F. C40
LH.1
WC.2
NG.1
ST.1
GO.2
WP.1
SE.2
AN.1
RO.2
CG.1
HP.2
Proportion
0.00
0.25
0.50
0.75
1.00
14
14
18
15
19
1
8
10
11
7
8
23
9
2
1
1
2
3
1
1
2
2
d
h
d
h
h
d
d
d
h
d
h
h
h
d
h
d
G. C21
WC.2
ST.1
GO.2
PB.1
TG.2
WP.1
SE.2
AN.1
RO.2
CG.1
HP.2
Proportion
0.00
0.25
0.50
0.75
1.00
5
11
3
3
12
8
6
2
2
13
13
4
9
6
7
6
17
7
1
18
14
4
22
14
12
7
20
18
8
11
16
15
17
16
17
6
18
h
d
h
d
h
d
d
d
h
d
h
d
h
d
h
d
h
d
h
Species
Identical MLG
Unique MLG


A.
Cladocopium C40
A. digitifera
A. hyacinthus
0.81
0.81
Density
0.0
0.1
0.2
0.3
0.4
−4
−2
0
2
4
Discriminant function 1
B.
Cladocopium C21
A. digitifera
A. hyacinthus
0.93
0.75
Density
0.0
0.2
0.4
−2
0
2
4
Discriminant function 1
C.
Cladocopium C40
DA eigenvalues
Pohnpei: 0.75
Chuuk: 0.83
Kosrae: 0.80
Ngulu: 0.89
Palau: 0.91
Yap: 0.61
Chuuk
Kosrae
Ngulu
Palau
Pohnpei
Yap
D.
Cladocopium C21
Chuuk
Guam
Kosrae
Palau
Pohnpei
Yap
Guam: 0.76
Chuuk: 0.54
Palau: 1.0
Kosrae: 0.88
Yap: 0.63
Pohnpei: 0.72
DA eigenvalues


A. C40: Palau
A.d LH.1: 0.84
A.h LH.1: 0.61
A.h WC.2: 0.54
A.d WC.2: 0.75
DA eigenvalues
A.d LH.1
A.d WC.2
A.h LH.1
A.h WC.2
B. C21: Yap
A.d GO.2
A.d ST.1
A.h GO.2
A.h ST.1
A.h ST.1: 0.70
A.h GO.2: 0.92
A.d ST.1: 0.91
A.d GO.2: 0.60
DA eigenvalues
C. C21: Chuuk
A.d SE.2
A.d WP.1
A.h SE.2
A.h WP.1
A.h WP.1: 0.77
A.d WP.1: 0.73
A.d SE.2: 0.79
A.h SE.2: 1.0
DA eigenvalues
D. C21: Pohnpei
DA eigenvalues
A.h RO.2: 0.76
A.h AN.1: 0.79
A.d RO.2: 0.90
A.d AN.1: 0.94
A.d AN.1
A.d RO.2
A.h AN.1
A.h RO.2
E. C21: Kosrae
A.h CG.1: 1.0
A.h HP.2: 0.82
A.d HP.2: 0.57
A.d CG.1: 0.90
DA eigenvalues
A.d CG.1
A.d HP.2
A.h CG.1
A.h HP.2
F. C21: Guam
A.d PB.1
A.d TG.2
Density
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.90
0.71
-4
-2
0
2
4
Discriminant function 1