From juveniles to giants: Drivers of holobiont assembly in massive Porites

Carly B Scott[1*], Raegen Schott[1], Mikhail V Matz[1]

[1]Department of Integrative Biology, University of Texas at Austin, Austin, TX
[*]Corresponding author: cbscott@utexas.edu

Abstract

The fate of coral reefs in response to climate change depends on their ability to adapt to new environments. The coral animal is buffered from environmental stress by its algal endosymbionts and microbial partners (together, the "holobiont"). However, the flexibility of holobiont community assembly in time and space is not well understood, making it difficult to estimate its contribution to coral adaptation. To clarify these processes at a centuries-long time scale, we compared the holobiont of massive Porites adults (>2m) to their juvenile (<30cm) counterparts across environments. We genotyped coral hosts from Orpheus Island, Australia and characterized their associated Symbiodiniaceae and bacterial communities. We recovered ten distinct genetic clusters of the coral host, reflecting the known difficulty in identifying species of massive *Porites* compounded by cryptic genetic structure. Within the largest of these clusters, we additionally found significant genetic separation between adult and juvenile corals. Host genetic structure was the primary driver of both Symbiodiniaceae (84% of variance explained) and microbial (60% of variance explained) community composition, indicating strong holobiont specificity in cryptic lineages. The relative role of environment and age was over two-fold higher for the microbial community compared to the Symbiodiniaceae community. Our results indicate local environmental factors and time influence the microbial community at a meaningful scale, but genetics trumps all for the vertically-transmitted Symbiodiniaceae community.

Keywords: Cnidarians, Community Ecology, Ecological Genetics, Microbial Biology, Population Genetics – Empirical, Algae

Introduction

The rapid rate of climate change poses an immediate threat to coral reefs globally. Environmental change acts on the entire coral holobiont, or the collective unit of the coral animal, its intracellular algal symbionts, and its associated microbial community (Goulet et al., 2020). Studies have posited that specific holobiont communities may increase the resilience of the coral to stress (Voolstra et al., 2021). Thus, corals may keep pace with climate change in one of two ways – through the evolution of the coral host animal and/or through holobiont community reassembly (potentially driven by host evolution). Given the acceleration of global change, acclimatization to future conditions through microbial community shifts has garnered significant attention as a hopeful mechanism for reef persistence (Berkelmans & van Oppen, 2006; Morikawa & Palumbi, 2019; Peixoto et al., 2017).

The underlying mechanisms which regulate coral holobiont assembly are still not fully understood. Without understanding the drivers of coral-associated communities, it is difficult to assess their acclimatization potential. It is likely that genetic, spatial, and temporal factors interact to drive the coral holobiont (Dunphy et al., 2019). Coral taxa exhibit distinct “core” holobiont members in the same environments, implying the functional importance of certain microbial species to the reef (Morrow et al., 2012; Pollock et al., 2018). Similarly, species exhibit geographic variability in their microbial and microalgal associations, indicating a degree of local acclimatization (Hernandez-Agreda et al., 2018).

Over-time studies of the holobiont response to stressors (e.g., bleaching) have yielded conflicting results. Stress events generally induce holobiont reassembly for both Symbiodiniaceae and microbial communities, but the degree and permanence of these shifts is highly variable (Baker, 2003; Glasl et al., 2019; McDevitt-Irwin et al., 2019; Zhu et al., 2022). For Symbiodiniaceae, studies have reported both stable switches to a new symbiont association (Claar, Starko, et al., 2020; Rouzé et al., 2019) and a return to the native state after the stress event (LaJeunesse et al., 2009; Thornhill et al., 2006). The degree of variability of the coral microbiome is also not well established, but the effect of time is often dwarfed by environmental and species-specific pressures (Dunphy et al., 2019; Epstein et al., 2019; Quek et al., 2023).

It has been proposed that coral holobiont dynamics are ungeneralizable and likely geographically and genotypically unique (Dunphy et al., 2019; Hoegh-Guldberg et al., 2007; Stat et al., 2009). While this is possible, most studies fail to integrate long-term (e.g., greater than 5 year) temporal data and individual-level genotyping to disentangle these effects. Thus, the relative roles of host genotype, environment, and time have yet to be resolved at ecologically relevant scales.

Here, we compared host-associated communities of juvenile and adult massive *Porites* spp. The massive *Porites* species complex forms the structure of many Pacific reefs, growing up to 17 meters in diameter and living over 500 years (Brown et al., 2009; Smith et al., 2021). Additionally, *Porites* spp. are broadcast spawning species which transmit their Symbiodiniaceae vertically through eggs and generally transmit their microbial community horizontally (Forsman

et al., 2020). Thus, we expect the Symbiodiniaceae community to be tightly associated with host genetics and the microbial community to be strongly affected by the local environment. With respect to age, we expect populations to acclimatize to environmental change in one of two ways: through changes in the host genome or through associations with different holobiont partners. Differences between age classes in the holobiont community could reflect novel environmental pressures now versus hundreds of years ago.

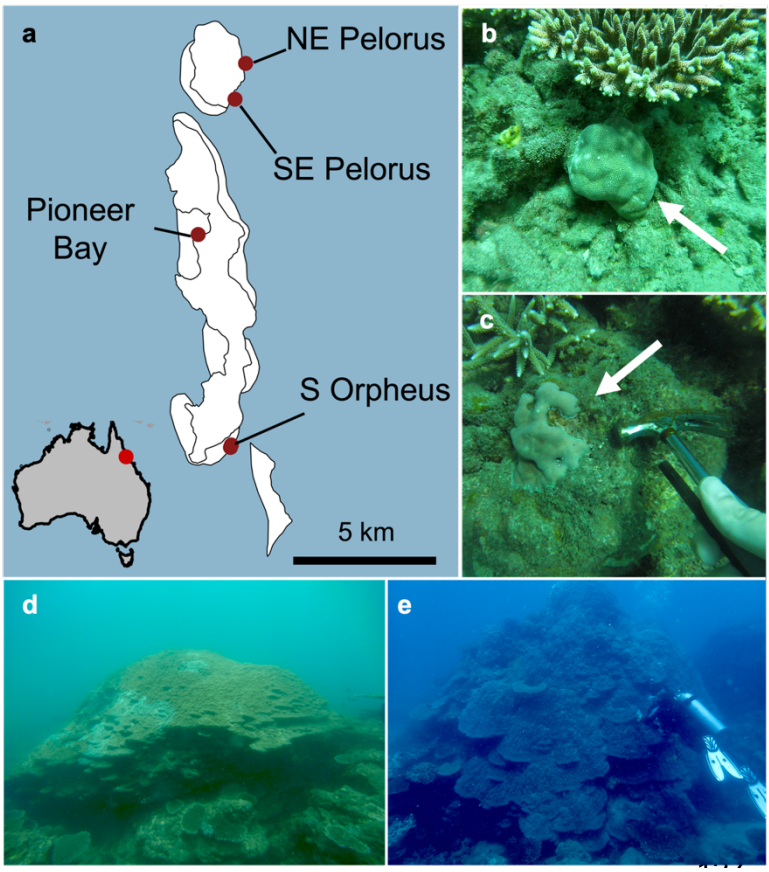

Figure 1. Sampling location and example *Porites* spp. adults and juveniles. (a) Map of Orpheus and Pelorus Islands, Australia. These islands are on inner Great Barrier Reef, and their relative location to Australia is marked on the map inset. (b, c) Examples of colonies we considered *Porites* spp. juveniles, less than 30 cm in diameter. (d, e) Individuals we considered *Porites* spp. adults, greater than 2 m in diameter. Individual colonies were not measured due to the large, visible size disparity between what we considered adults and juveniles.

We sampled juvenile and centuries-old massive *Porites* colonies in 2019 at four sites across Orpheus and Pelorus Islands, Australia. Despite their close proximity, these sites exhibit qualitatively different environments: NE and SE Pelorus are deeper sites with generally high visibility and high wave action, Pioneer Bay is a shallow, nearshore, and highly turbid site, and South Orpheus is also a shallow nearshore site but with fast currents due to its channel location. From this we aimed to, (i) investigate the genetic structure of the coral hosts, and (ii) estimate the relative importance of this genetic structure, site, and age class for the composition of the associated Symbiodiniaceae and microbial communities.

Methods

Sampling

Porites spp. were sampled around Orpheus and Pelorus Islands, Australia in November 2019. To estimate the effects of age on holobiont structure, we sampled massive adults (>2m diameter) and their juvenile counterparts (<30cm diameter; Figure 1). Three samples were taken across each massive individual (one central and two on opposing sides) and one sample was taken from each juvenile. Samples were collected from four sites around the island exhibiting different environments (Figure 1). In total, we collected 193 samples

from 90 colonies. Coral fragments were fixed immediately after sampling in 100% EtOH and subsequently stored at -80°C.

DNA Library Preparations

DNA was extracted from all samples using the CTAB method (see supplemental methods). 2bRAD genomic sequencing libraries (https://github.com/z0on/ 2bRAD_denovo) were constructed for each colony and sequenced using Illumina NextSeq 500 SR 75 at the Genomic Sequencing and Analysis Facility at UT Austin. Amplicon sequencing libraries were constructed for all samples. ITS2 amplification used primers from Hume et al., 2015 (SymVarF: CTACACGACGCTCTTCCGATCTGAATTGCAGAACTCCGTGAACC, SymVarR: CAGACGTGTGCTCTTCCGATCTCGGGTTCWCTTGTYTGACTTCATGC); 16S V3/V4 region was amplified using primers from Osman et al., 2020 (16S_341F_Truseq: CTACACGACGCTCTTCCGATCTCCTACGGGNGGCCTACGGGNGGCWGCAG, 16S_805R_Truseq: CAGACGTGTGCTCTTCCGATCTGACTACHVGGGTATCTAATCC). All amplicon sequencing libraries were amplified with 20-30 PCR cycles, depending on band intensity. Negative controls (MilliQ water) were run with each set of samples and amplification was never observed. Libraries were pooled according to visually assessed gel band brightness. Pooled amplicon libraries were sequenced on the MiSeq PE150.

Bioinformatic Pipeline

Reads were trimmed of adaptors and deduplicated using custom 2b-RAD scripts (https://github.com/z0on/2bRAD_denovo). Reads containing base calls with quality less than Q15 were removed using *cutadapt* (Martin, 2011). Cleaned reads were mapped to a combined reference of the *Porites lutea* genome (Robbins, 2019), *Symbiodinium* sp. (Shoguchi et al., 2018), *Breviolum minutum* (Shoguchi et al., 2013), *Cladocopium* sp. (Shoguchi et al., 2018), and *Durusdinium* sp. (Dougan et al., 2022) genomes using *bowtie2* with default local settings (Langmead & Salzberg, 2012). Mapped reads were filtered for a mapping quality >30 and genotyping rate >50%, and only reads which mapped to *Porites lutea* were retained. Between-sample genetic distances were calculated as identity-by-state (IBS) based on single-read resampling in ANGSD (Korneliussen et al., 2014). At this point we subset the data to remove technical replicates, colony replicates, and clones based on hierarchical clustering, retaining one individual per genotype (N=65). After genetically identical samples were removed, the genetic distances were calculated again in ANGSD with the same filtering scheme.

Host Genetic Analysis

Initial analysis revealed highly distinct genetic groups within *Porites* spp. To estimate the most likely number of clusters, we implemented a Bayesian model-based clustering algorithm from the 'mclust' R package (Scrucca et al., 2016). We chose to use the number of clusters (N=10) with the highest Bayesian Information Criterion in subsequent analysis. This visually corresponded well with the hierarchical clustering tree generated from the genetic distance matrix. Finally, we calculated pairwise F_{ST} between each genetic cluster using realSFS.

We used random forest and RDA/PERMANOVA analysis to determine the relative contribution of site and age to *Porites* spp. genetic structure. We implemented the random forest procedure using R package 'randomForest' to predict genetic cluster identity from sample age class and site (Liaw & Wiener, 2002). We split the individuals randomly into a training set (N=45) and test set (N=20). We ran the random forest on the training set with 1500 trees. We calculated overall model accuracy and p-value (probability that model accuracy is higher than the no information rate) using R package 'caret' (Kuhn, 2022). For the PERMANOVA analysis, we used the 'adonis2' function from the R package 'vegan' (Oskansen et al., 2022) to test for significance of age and site in predicting genetic distance between individuals (genetic distances ~ Site + Age).

Symbiont and Microbe ASV Analysis

We used the same basic computational workflow for both 16S and ITS2 sequences. Primers and adapters were trimmed from reads with cutadapt. For microbes, likely host contamination was removed from our samples using METAXA2 (Bengtsson-Palme et al., 2015). We only retained reads classified as bacterial or archaeal in origin. The DADA2 pipeline was then used on trimmed reads for denoising and ASV classification (see https://github.com/cb-scott/PoritesHolobiont_Final for full description of pipeline parameters). Samples with less than 50 total mapped reads and ASVs with less than 10 mapped reads across samples were discarded. The ASV abundance table was standardized using the 'vst' function from DESeq2 (McMurdie & Holmes, 2014). Any values <0 in the standardized count table were set to zero, as DESeq2's vst transformation will assign negative values to ASVs with less than one estimated read. From this, we calculated the Euclidean distance between samples using vegan's 'vegdist' command.

To partition the role of genetics, age, and site, we conducted separate RDA-forest and PERMANOVA analyses (Black et al., 2022). We first constructed an unconstrained PCoA from community distances using vegan's 'capscale' command. The first fifteen principal coordinates were used as the response matrix, and the predictor matrix included host genetic cluster, site, and age class. Categorical predictors with multiple levels (site and genetic cluster) were converted into dummy quantitative variables with values 1 and 0, with one variable per predictor level. Each random forest model used 1500 bootstrapped trees and max variable permutation level of $\log2(N_{Individuals} * 0.368/2)$. Then, for multi-level categorical predictors, we summed the weighted importance across dummy variables representing their levels to obtain overall importance. In the PERMANOVA analysis, we used the 'adonis2' function from the R package 'vegan' to test for the significance of linear association between site, age, and host genetic cluster and community distance between individuals (community distances ~ Genetic Cluster + Site + Age). Finally, we ran a Procrustes test between the genetic and symbiont/microbial community ordination objects to determine correlation between host genetic structure and holobiont community structure.

ASV Classification and Differential Abundance Analysis

We assigned taxa to ASVs in DADA2 using the 'assignTaxa' function. For the Symbiodiniacaea samples, we used the reference database associated with (Claar, Tietjen, et al., 2020). For the microbes, we used the Silva version 138.1 SSU reference database (DADA2 formatted database taken from: https://zenodo.org/records/4587955; Quast et al 2013). Differential abundance of microbes was calculated using DESeq2 with the experimental design, abundance~GeneticGroup + Site + Age. We designated $\alpha < 0.01$ as the cutoff to determine which taxa were significantly differentially abundant between comparisons.

All results were visualized in R with ggplot2. Scripts for replicating this analysis can be found at https://github.com/cb-scott/PoritesHolobiont_Final and archived on Zenodo (doi:10.5281/zenodo.10384279).

Results

Cryptic genetic lineages in Porites spp. structured by site

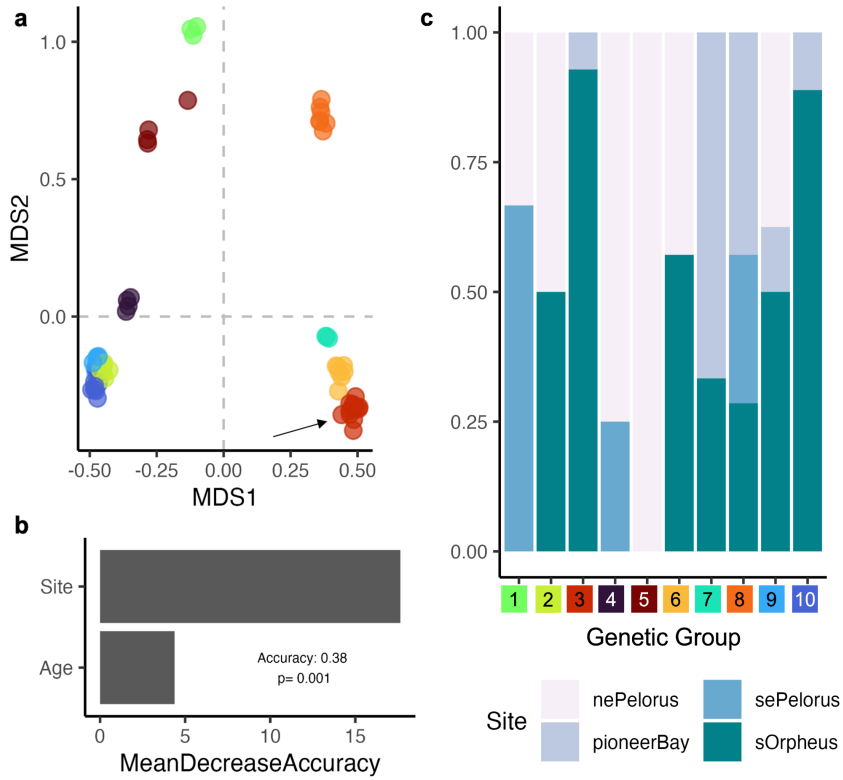

Figure 2. High degree of cryptic genetic structure within *Porites* spp. driven by site. (a) PCoA of *Porites* spp. individuals colored by assigned genetic cluster. "Genetic cluster 3", used in downstream analysis is marked on the PCoA with an arrow. (b) Genetic cluster identity is primarily predicted by sampling site, demonstrated by random forest. (c) Distribution of each genetic cluster across sites.

Initial population genetic analysis suggested a high degree of clustering/sub clustering (Figure 2a, Supplemental Figure 1). Bayesian model-based clustering returned the optimal number of clusters as ten (Supplemental Figure 2). These clusters correspond well visually to the hierarchical clustering tree generated from genomic distances between samples. Calculation of weighted pairwise F_{ST} further supported genetic distinction between these ten groups (minimum pairwise F_{ST}: 0.14, maximum: 0.55; Supplemental Figure 3).

To determine the drivers of these cryptic genetic clusters, we conducted a random forest analysis with our two measured traits: site and age. This model was both accurate and significant in

273 predicting genetic clusters (model prediction accuracy = 0.38, p = 0.001). Site was
274 approximately four times more important than age in improving genetic cluster prediction
275 accuracy (Figure 2b). A four-fold higher, significant role for site relative to age was confirmed
276 through PERMANOVA analysis (Supplemental Table 1).
277
278 *Symbiodiniaceae community more structured by host genetics than microbial community*
279
280 We estimated the relative importance of genetic cluster, age, and site in structuring the
281 Symbiodiniaceae and microbial community. For both holobiont members, the host's genetic
282 cluster primarily drove community variation, followed by site, then age class (Figure 3c).
283 Procrustes analysis confirmed the posited connection between host genetic structure and
284 Symbiodiniaceae community structure (Correlation with host genetics=0.470, $p<0.001$) and
285 microbial community structure (Correlation with host genetics=0.501, $p<0.001$). RDA-forest
286 supported a stronger link between the host's cryptic genetic group and Symbiodiniaceae
287 community than the microbial community. For the Symbiodiniacaea, of the total gradient forest
288 model importance (total importance = 0.200), 84% of the importance was generated by genetic
289 group (weighted variable importance = 0.169), 14% was generated by site (weighted variable
290 importance = 0.0280), and 1.6% was generated by age class (weighted variable importance =
291 0.0031). At the same time, for the microbial community, of the total gradient forest model
292 importance (total importance = 0.232), only 60% of the importance was generated by genetic
293 group (weighted variable importance = 0.139), 36% was generated by site (weighted variable
294 importance = 0.0836), and 4% was generated by age class (weighted variable importance =
295 0.010). These results were additionally supported by separate PERMANOVA analyses
296 (Supplemental Tables 2-3).

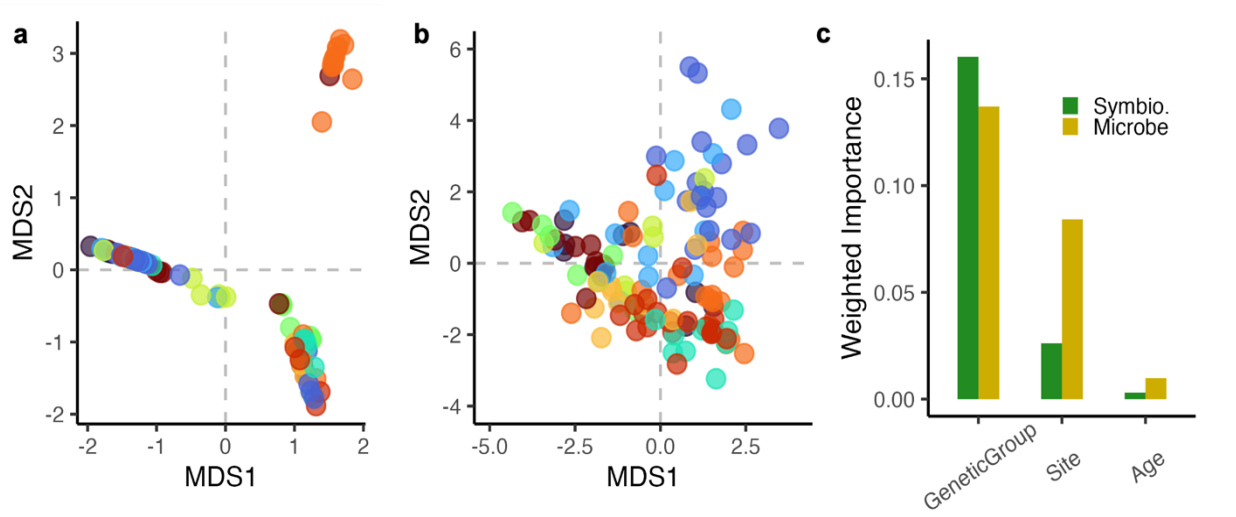

Figure 3. Microbial community is more dependent on site and age than Symbiodiniaceae community. PCoA colored by host genetic cluster assignment for the Symbiodiniaceae community (a) and microbial community (b). Gradient forest estimates that host genetic cluster is the most important predictor driving both algal and microbial communities (c). However, site and age are more important in determining microbial community assemblage compared to algal community assemblage. These results were confirmed by RDA analysis (Supplemental Tables 2-3).

Differentially abundant taxa between groups

All coral colonies but one hosted the same genus of Symbiodiniacaea (*Cladocopium* spp.), with background levels (<1%) of other genera (Supplemental Figure 4).

However, we did find 29 significantly differentially abundant bacterial taxa between adults and juveniles and/or between sites (Supplemental Figure 5-6). Most notably, *Vibrio* spp. and *Endozoicomonas* spp. were often differentially abundant between sites. Additionally, some strains of *Vibrio* spp. were differentially abundant between age cohorts.

Within-cluster genetic divergence between adults and juveniles

Subsequent within-group analysis was restricted to only genetic cluster three (N=14), as no other group retained a sample size greater than ten. Only one sample in this cluster was not from South Orpheus (Figure 2c), so we only evaluated the role of age in structuring host genetics. Age class (adult versus juvenile) was significantly correlated with within-cluster genetic subdivision (Figure 3a, PERMANOVA; R^2_{age}=0.083, p=0.014; Gradient forest weighted importance = 0.249), reflecting genetic divergence over the past 500 years (weighted F_{ST} = 0.070). Despite this genetic separation, we did not recover any SNPs under diverging selection using BayeScan, likely due to the lack of power because of low sample size.

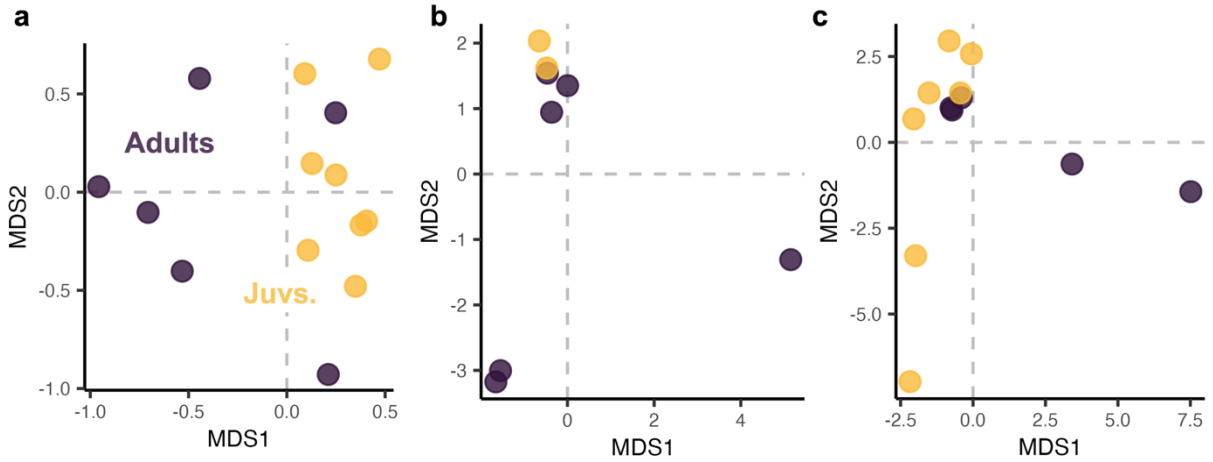

Figure 4. Massive Porites show significant genetic divergence over the past 500 years, but no significant holobiont separation. (a) PCoA plot showing separation between adult and juvenile *Porites* spp. individuals from the largest genetic cluster. Subsequent PERMANOVA analysis found this separation to be significant (R^2_{age}=0.083, p=0.014). (b) PCoA of symbiont community between adults and juveniles in this cluster (PERMANOVA; R^2_{age}=0.127; p=0.313). (c) PCoA of the microbial community between adults and juveniles in this cluster, colored by age (PERMANOVA; R^2_{age}=0.0969, p=0.208).

This genetic divergence did not correspond to significant differences between the holobiont of adults and juveniles (Figure 4b,c). For the Symbiodiniaceae, PERMANOVA did not identify any significant differences between adults and juveniles (R^2_{age}=0.127; p = 0.313) and a Procrustes analysis did not find a significant relationship between genetic structure and symbiont

community structure (correlation=0.685, *p*=0.291). Similarly for the microbial community, PERMANOVA did not indicate a significant relationship between age and community structure when replicate samples from the same colony were removed (R^2_{age}=0.0969, p=0.208). Procrustes analysis supported this result, and we did not find a significant correlation between genetic structure and microbial community structure (correlation=0.355, *p*=0.616).

Discussion

We found a high degree of cryptic genetic structure in massive Porites. This is unsurprising, as previous studies have revealed complex genetic substructure within the species complex (Forsman et al., 2020; Starko et al., 2023). We identified 10 distinct genetic groups in this study from just one island in the Indo Pacific. Likely, the distinct genetic clusters recovered reflect the sampling of multiple species of *Porites* spp., as they are notoriously difficult to identify in the field. Still, since there are only three named species of massive *Porites*, some of our 10 genetic lineages must reflect cryptic genetic diversity within the nominal species. Much like previous studies, these cryptic lineages are associated with specific geographical locations, hinting at an evolutionary mechanism linked to diverse environmental conditions.

Our results stress that failure to assess cryptic genetic structure may lead to the overestimation of holobiont flexibility. Cryptic species complexes are emerging as a common feature of scleractinian taxa and often segregate by site/environment (Fifer et al., 2022; Forsman et al., 2020; Ladner & Palumbi, 2012; Rippe et al., 2021). Thus, site-specific patterns in the holobiont may not reflect flexible environmental associations, but rather genetically determined, lineage-specific communities. It is imperative to genotype hosts in holobiont studies, as morphologically similar – but genetically divergent – sympatric corals are likely associated with unique microbial/microalgal communities.

The associated Symbiodiniaceae of massive Porites are most strongly influenced by the host's genetics (and not at all by age class). This advances evidence for long-term coevolution between *Porites* spp. and their symbiotic algae (Hoadley et al., 2021; Starko et al., 2023). However, prior studies have found that *Porites* spp. can change symbionts after a bleaching event (Coffroth et al., 2022; Starko et al., 2023). Our failure to find age-class specific holobionts suggests one of two things: that these changes do not persist at long-term scales, or that newly acquired Symbiodiniaceae are faithfully transmitted to offspring. Combined with the negligible role of local environment in determining the Symbiodiniaceae community compared to host genetics, we feel it is most likely that *Porites* spp. maintain genetically determined, centuries-long fidelity to a particular community.

Microbial communities, in comparison, are more influenced by spatiotemporal factors. While host genetic structure remained the most important predictor of microbial community assemblage, environmental factors and age class emerged as two to three times more influential in shaping the microbial community than the Symbiodiniaceae community (Fig. 3c). Spatiotemporal patterns may be driven by neutral ecological processes, such as priority effects during colonization or the environmental abundance of taxa. Conversely, age- and site-specific

communities may indicate a functional role of certain taxa in particular environments and life stages.

Supporting this, we found some microbial community differences to be driven by known functionally important taxa. One such case is the differential abundance of *Vibrio* species between adults and juveniles. *Vibrio spp*. is an opportunistic pathogen (Munn, 2015) and has been associated with coral bleaching (Ben-Haim et al., 2003). This suggests that particular *Vibrio* strains may be age-class specific pathogens, or, more likely to invade age-specific communities. Another case is between-site differences in *Endozoicomonas* spp. abundance. *Endozoicomonas* has been suggested to be a beneficial microbial symbiont in corals which can modulate environmental stress (Peixoto et al., 2017; Tandon et al., 2022). These site-specific differences may support a functional role of *Endozoicomonas* in certain environments.

Within the largest cryptic genetic group, we observed significant genetic separation between adult and juvenile corals, which suggests that the local selection regime has changed over the few hundred years that separate the times of recruitment of adults and juveniles. However, we could not identify any SNPs under diverging selection between these subgroups, suggesting that genetic drift over time may be the actual explanation of genetic divergence between age cohorts. While our failure to find SNP outliers may be a result of insufficient power due to small sample size, the lack of association between age class and holobiont community suggests that despite host evolution (whether adaptive or neutral) the holobiont community still can change independently in response to the environment.

Conclusion

We demonstrated cryptic genetic structure is the most important factor in determining both coral-associated Symbiodiniaceae and microbial communities. Local environment and age of the host have much less effect, although the microbial community is more responsive to these factors than Symbiodiniaceae. This strong fidelity to host genetic clusters suggests the holobiont components cannot easily evolve independently from each other and underlying host genetic structure must be weighed in determining the acclimatization potential of the holobiont to future conditions. Still, this fidelity is not complete, and host evolution over a relatively short time scale (few hundred years) did not induce divergence of other holobiont components, which suggests that they may be in fact sufficiently independent from the host genetic background to help speed up coral adaptation.

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Data Availability

All sequencing (2bRAD and 16S/1TS2 amplicon) data generated can be found at NCBI BioProject PRJNA1048506. All scripts and intermediate products (including metadata) used to complete analysis can be found at https://github.com/cb-scott/PoritesHolobiont_Final or archived at doi: 10.5281/zenodo.10384279.

Author Contributions

Conceptualization: CBS and MVM; Data curation: CBS and RS; Formal analysis: CBS and RS; Funding acquisition: CBS and MVM; Investigation: CBS and RS; Methodology: MVM; Project administration: MVM; Resources: MVM; Software: all; Supervision: MVM; Validation: MVM; Visualization: CBS; Writing – original draft: CBS; Writing - review & editing: CBS and MVM.

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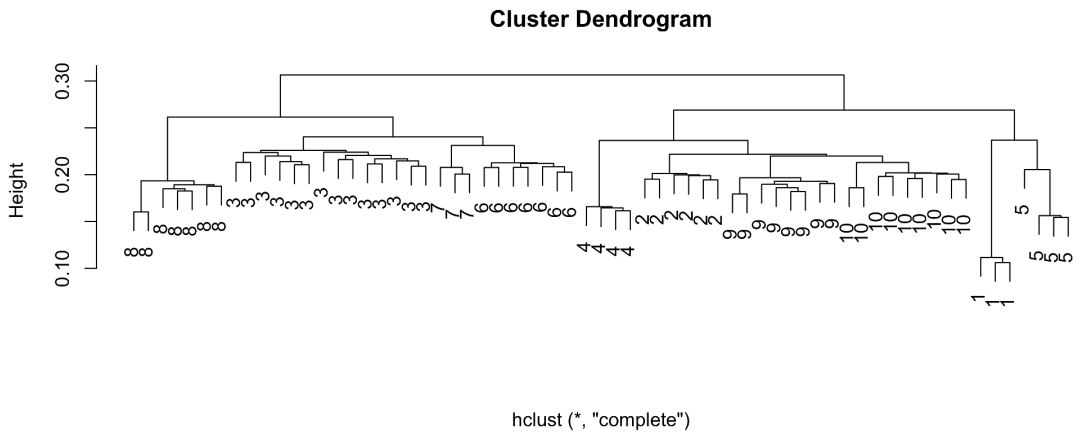
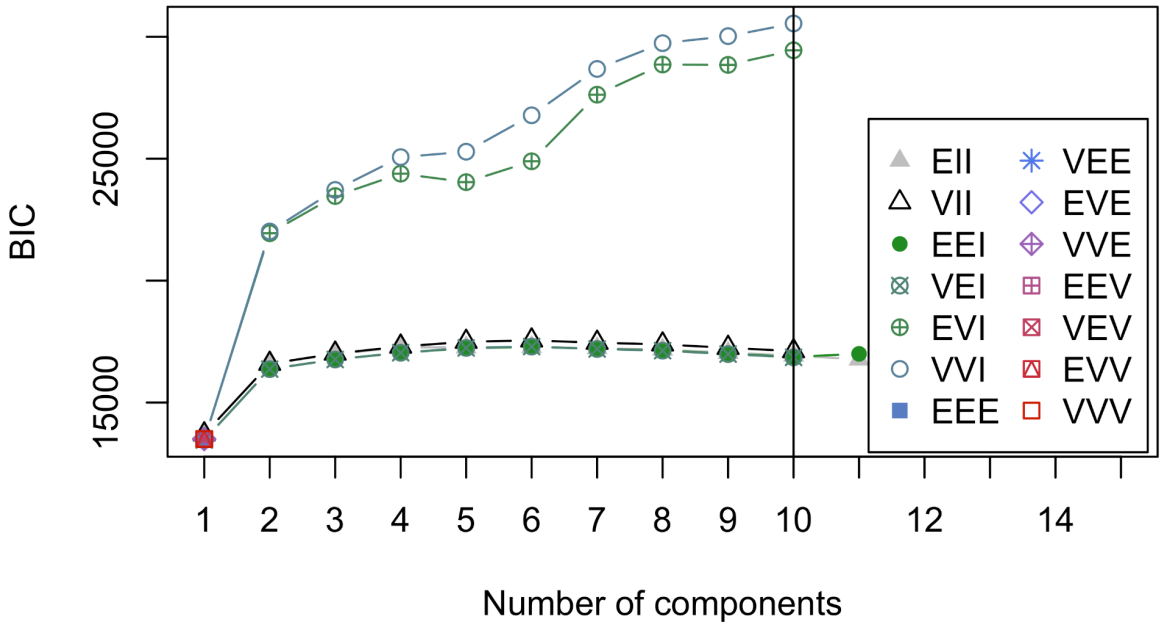
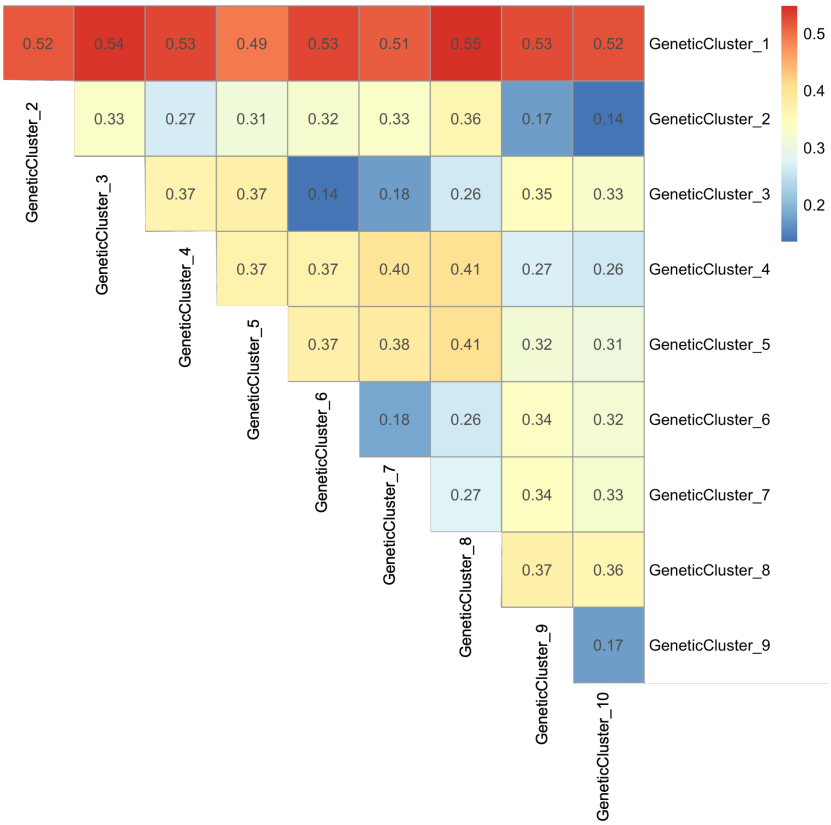
Supplemental Figures



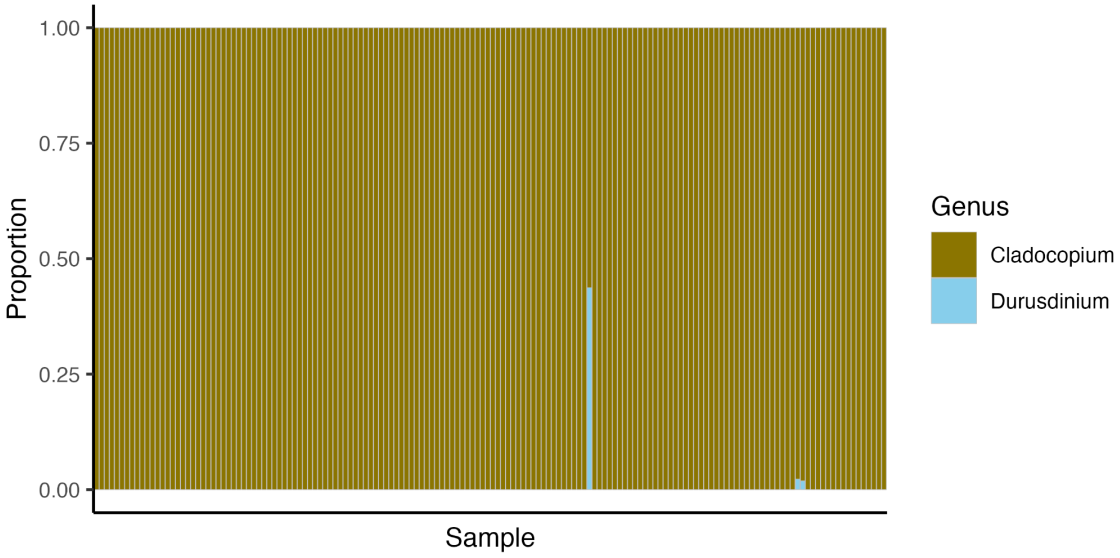
Supplemental Figure 1. Hierarchical clustering results of genetic data. Hierarchical clustering of ANGSD identity by state matrix aligns well with Bayesian-assigned genetic clusters (leaf labels).



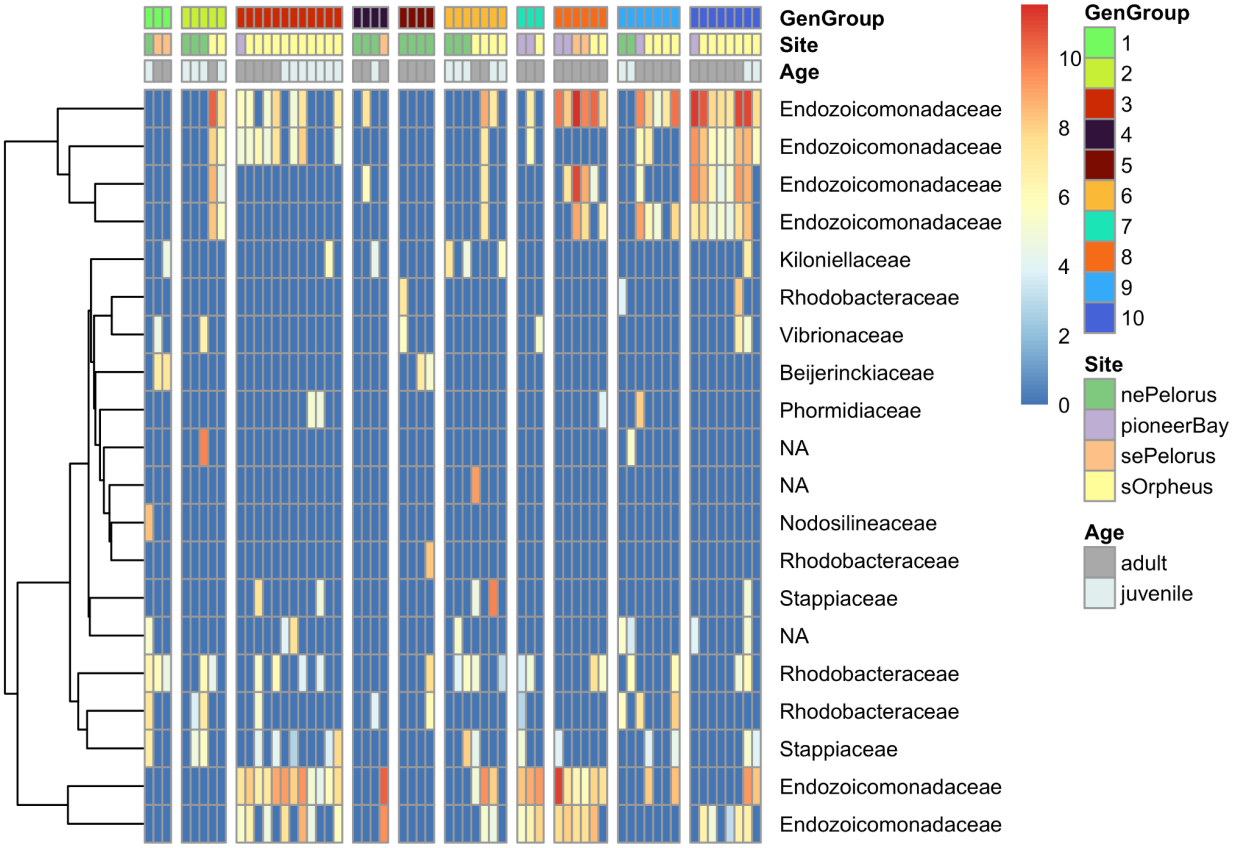
Supplemental Figure 2. Bayesian Information Criterion used for choosing optimum number of clusters. BIC is maximized at 10 clusters, the value chosen for subsequent analyses.



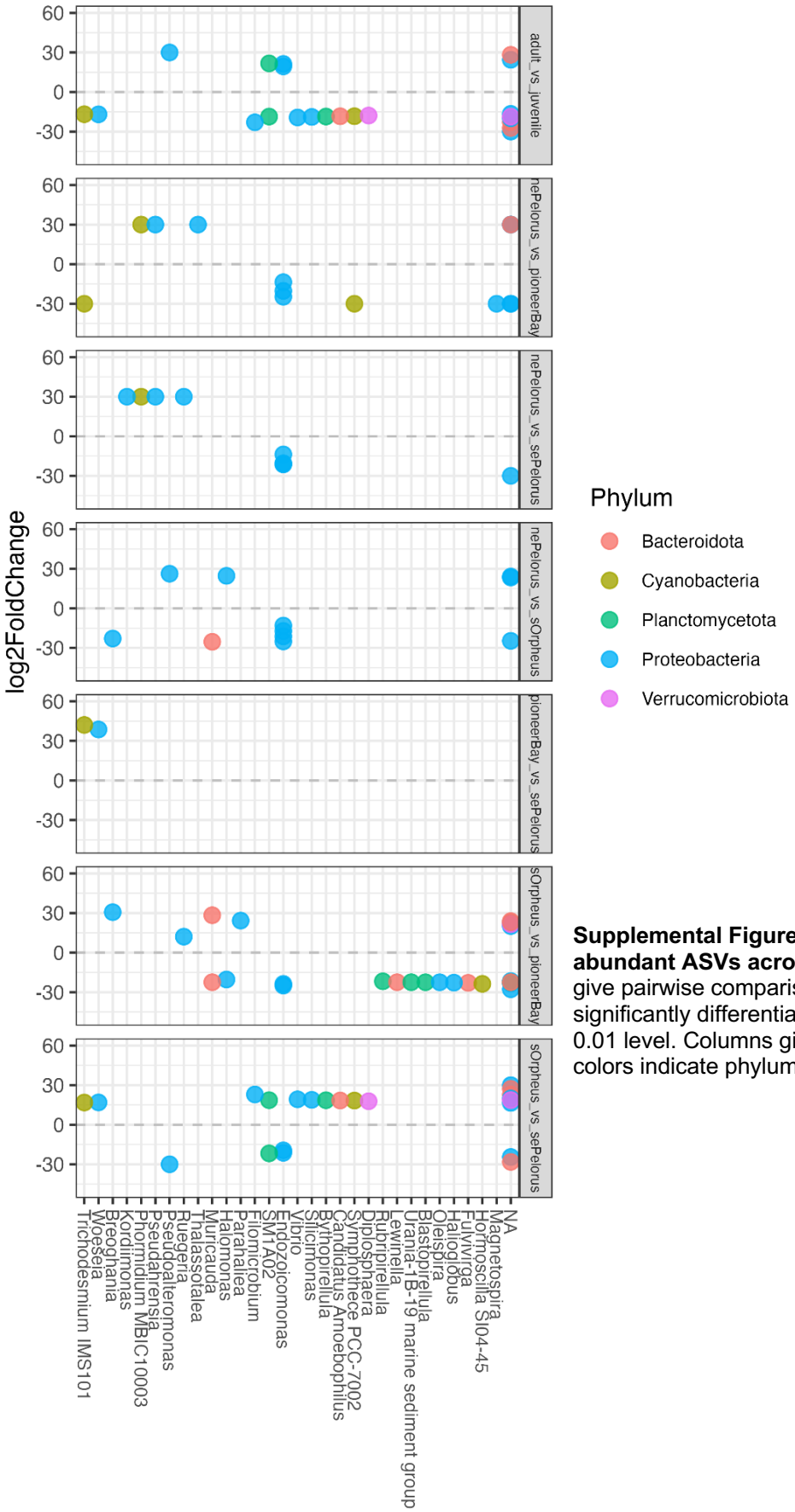
Supplemental Figure 3. Pairwise F_{ST} between Bayesian-identified genetic clusters. Calculations of pairwise F_{ST} between clusters indicate a high degree of genetic divergence between cryptic lineages.



Supplemental Figure 4. ASV assignments for ITS2 sequences reveal all samples are dominated by Cladocopium sp. All Symbiodiniaceae ASVs were assigned predominantly to *Cladocopium* sp., with few hosting background levels of *Durusdinium* sp.



Supplemental Figure 5. Top 20 most abundant ASVs across samples. Top average 20 most abundant bacterial ASVs occurring in samples. Fill shows abundance by log2 normalized counts. Rows show ASV abundance across samples, with assigned family indicated on the right. NA values indicate no assignment to the family level for a particular ASV. Columns reflect individual samples, separated by genetic group. Site and age for each sample are indicated at the top of the plot. Genetic group is colored the same as in Figure 2.



Supplemental Figure 6. Significantly differentially abundant ASVs across all comparisons. Panels give pairwise comparisons, by age class and site, of significantly differentially abundant ASVs at the α < 0.01 level. Columns give genus-level assignments, colors indicate phylum-level assignment.

Supplemental Tables

Table 1. PERMANOVA results for drivers of host genetic structure show site is the most important factor. Site has the strongest (and only significant) effect on *Porites* spp. genetic structure.

Predictor	R^2	F	p
Site	0.084	1.87	0.005
Age	0.021	1.44	0.105
Residual	0.895		

Table 2. PERMANOVA results for drivers of Symbiodiniaceae community structure support gradient forest results. Host genetics have the strongest (and only significant) effect on symbiont community, followed by sampling site, then age.

Predictor	R^2	F	p
GeneticGroup	0.409	6.62	0.001
Site	0.022	1.09	0.331
Age	0.0067	0.98	0.441
Residual	0.562		

Table 3. PERMANOVA results for drivers of microbial community structure show a significant role of genetics, site, and age. Host genetics have the strongest effect on symbiont community, followed by sampling site, then age. However, counter to the results of the Symbiodiniaceae analysis, all correlations are significant.

Predictor	R^2	F	p
GeneticGroup	0.158	2.45	0.001
Site	0.0368	1.72	0.001
Age	0.013	1.835	0.003
Residual	0.792		