

Cophylogeny and specificity between cryptic coral species (*Pocillopora* spp.) at Mo'orea and their symbionts (Symbiodiniaceae)

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Abstract

The congruence between phylogenies of tightly associated groups of organisms (cophylogeny) reflects evolutionary links between ecologically important interactions. However, despite being a classic example of an obligate symbiosis, tests of cophylogeny between scleractinian corals and their photosynthetic algal symbionts have been hampered in the past because both corals and algae contain genetically unresolved and morphologically cryptic species. Here, we studied co-occurring, cryptic *Pocillopora* species from Mo'orea, French Polynesia, that differ in their relative abundance across depth. We constructed new phylogenies of the host *Pocillopora* (using complete mitochondrial genomes, genomic loci, and thousands of single nucleotide polymorphisms) and their Symbiodiniaceae symbionts (using ITS2 and *psbA*^{ncr} markers) and tested for cophylogeny. The analysis supported the presence of five *Pocillopora* species on the fore reef at Mo'orea that mostly hosted either *Cladocopium latusorum* or *C. pacificum*. Only *Pocillopora* species hosting *C. latusorum* also hosted taxa from *Symbiodinium* and *Durusdinium*. In general, the *Cladocopium* phylogeny mirrored the *Pocillopora* phylogeny. Within *Cladocopium* species, lineages also differed in their associations with *Pocillopora* haplotypes, except those showing evidence of nuclear introgression, and with depth in the two most common *Pocillopora* species. We also found evidence for a new *Pocillopora* species (haplotype 10), that has so far only been sampled from French Polynesia, that warrants formal identification. The linked phylogenies of these *Pocillopora* and *Cladocopium* species and lineages suggest that symbiont speciation is driven by niche diversification in the host, but there is still evidence for symbiont flexibility in some cases.

KEYWORDS

coevolution, cospeciation, holobiont, mutualism, niche differences

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1 | INTRODUCTION

For organisms that form symbiotic partnerships with microbes, the identity and composition of symbionts is often critical to the performance and function of the holobiont (the host and its symbionts) (Rohwer et al., 2002; Roughgarden et al., 2018; van Oppen & Medina, 2020). Especially when host-symbiont partnerships are obligate, variation among host individuals and species in their symbiont identity may explain some of the variation in their response to environmental gradients or disturbances (Abbott et al., 2021; del Campo et al., 2020; Innis et al., 2018). However, the extent to which the interaction between host and symbiont itself evolves remains unclear in many cases. Therefore, identifying host-symbiont specificity, and the extent to which host and symbiont lineages codiversify through shared evolutionary histories, is necessary for understanding the ecological and evolutionary dynamics of the holobiont, and the degree to which symbioses promote or restrict adaptation to climate change (Compant et al., 2010; Kaltenpoth et al., 2014; Seah et al., 2017; Takiya et al., 2006).

Most scleractinian corals form obligate symbiotic relationships with photosynthetic dinoflagellate algae in the family Symbiodiniaceae. In some corals, symbiont flexibility and the environment often play a substantial role in the composition of Symbiodiniaceae hosted (Boulotte et al., 2016; Cunning et al., 2015; Putnam et al., 2012; Quigley et al., 2019). However, there is also evidence for some level of host-symbiont specificity (Forsman et al., 2020; LaJeunesse & Thornhill, 2011; Parkinson & Baums, 2014), though direct evidence for concordant evolutionary histories is often considered lacking (Rowan & Powers, 1991; van Oppen & Medina, 2020).

The degree of host-symbiont specificity, and the potential for coevolution, should relate to the mode of symbiont transmission (Baird et al., 2009, 2021; Goodnight, 2000; Hartmann et al., 2017; Zeng et al., 2017). Most (~71%) scleractinian coral species obtain their symbionts horizontally (i.e., acquired from the environment), which is common in species that spawn gametes with external fertilization (broadcast spawning) (Baird et al., 2009). These coral species are expected to host greater symbiont diversity, and necessarily host algal species that are able to live outside the coral (Fujise et al., 2021; Quigley et al., 2017), reducing specificity and decoupling their evolutionary histories. In contrast, other coral species obtain their symbionts vertically (i.e., from parent to offspring; Hirose et al., 2000; Hirose & Hidaka, 2006). Vertical transmission is more common in coral species with internal fertilization, where the embryo develops within the polyp before release as a motile planula larva (brooders) (Baird et al., 2009). Coral species with vertical transmission are expected to host lower algal diversity (Bongaerts et al., 2015), and algal species that are highly host specialized and often unable to live and propagate outside the coral host (Fujise et al., 2021). As a result, partnerships with hosts that vertically transmit algal symbionts are expected to be more stable over time, facilitating coadaptation of partners as a result of their shared reproductive fate (Fisher et al., 2017). Therefore,

especially in species with vertical transmission, the main cause of symbiont speciation is thought to be niche diversification provided by speciation of the host, since the host provides the habitat that modulates natural selection on the symbiont (Thornhill et al., 2014). Nevertheless, there are few direct tests of this hypothesis (Lewis et al., 2019; Turnham et al., 2021).

Estimates of cophylogeny, or the concordance between the phylogenies of hosts and symbionts, provide a means to identify host-symbiont specificity and shared evolutionary histories. Congruence between the phylogenies of coral hosts and algal symbionts may be the product of coevolution or cospeciation. Strictly speaking, coevolution involves reciprocity; that is, evolutionary change in one species in response to the traits of a second species, followed by evolutionary response by the second species to the change in the first species (Janzen, 1980; Thompson, 1994). Cospeciation is concordant patterns of speciation that do not necessarily involve reciprocity, such as speciation of the coral host and subsequent tracking and diversification of the algal symbionts (Lewis et al., 2019), or shared biogeography and similar responses to common environments (Althoff et al., 2012; Nuismer et al., 2010). However, cophylogeny between corals and their photosynthetic algal symbionts has not yet been formally tested or quantified (LaJeunesse et al., 2010; Pinzon & LaJeunesse, 2011), though there is recent evidence that the age at which algal symbiont species diverged from each other corresponds to when their hosts diversified (Turnham et al., 2021), evolutionary associations between modes of symbiont transmission and reproduction (Hartmann et al., 2017), evolutionary stability and maintenance of photosynthetic algae in corals (Gault et al., 2021), and cophylogeny between corals and bacterial symbionts (O'Brien et al., 2019; Pollock et al., 2018).

Estimates of cophylogeny are important because several factors could limit the concordance between host and symbiont phylogeny when it is expected to be high (i.e., when there is vertical transmission). Some coral species with vertical transmission appear to exhibit a mixed mode of symbiont acquisition, allowing larvae to associate with symbionts that are not detected in the maternal colony (Quigley et al., 2018). A mixed mode of symbiont transmission may allow juveniles to have greater flexibility in different environments than strictly vertical transmission. Even if symbionts are transmitted vertically, an individual can host a diverse community of 'background' symbionts, even when there is often only one taxa detected, that are transitory and may depend on environmental conditions (Kriefall et al., 2022; Lee et al., 2016; Rouzé et al., 2019; Strader et al., 2022). As a result, even in species with vertical transmission, the relationships between scleractinian host and symbiont can be dynamic over ecological timescales (Quigley et al., 2019; Reich et al., 2017). Certain species of algal symbionts may share evolutionary histories with the coral host, while other species within the same host clade may not. Identifying associations between host and symbiont that are maintained in different habitats and in response to environmental stressors is therefore necessary for confidently detecting

coevolutionary relationships, as well as generating new hypotheses for what drives these associations.

Tests of cophylogeny between corals and their algal symbionts have been hampered in the past by unresolved taxonomy and phylogenetic relationships within both the coral host and the algal symbiont. Both the coral host and the symbiont contain morphologically-cryptic species, making it difficult to identify associations without rigorous genomic information. In particular, the coral genus *Pocillopora* is notorious for containing multiple cryptic species because of morphological plasticity and overlapping morphological phenotypes (Marti-Puig et al., 2014; Paz-García et al., 2015; Pinzón et al., 2013). Despite being a well-studied coral genus (Flot et al., 2008; Gélin et al., 2017; Johnston et al., 2017; Oury et al., 2021; Schmidt-Roach et al., 2014), with a monophyletic radiation approximately 3 million years old (Johnston et al., 2017), the identification and placement of some mitochondrial lineages is not yet fully resolved. Similarly, Symbiodiniaceae, the dinoflagellates that form the symbiotic partnership with scleractinians, have recently received taxonomic revision at the family and genus level (LaJeunesse et al., 2018), and the extent to which variation in genetic sequences reflects species is only just beginning to be clarified (Lewis et al., 2019; Thornhill et al., 2014; Turnham et al., 2021).

Here, we investigate *Pocillopora*–Symbiodiniaceae associations in multiple co-occurring cryptic *Pocillopora* species that differ in their responses to thermal stress and in their niche space across depths at Mo'orea, French Polynesia (Burgess et al., 2021; Johnston et al., 2022). Pocilloporid corals are a particularly interesting group because colonies that broadcast spawn actually transmit their symbionts vertically in their eggs (Hirose et al., 2000; Johnston et al., 2020; Massé et al., 2013; Schmidt-Roach et al., 2012), unlike many other broadcast spawning corals that do not transmit their symbionts to offspring (Baird et al., 2009). Eggs are relatively small (~100 µm diameter) and negatively buoyant upon release (Schmidt-Roach et al., 2012), in contrast to other broadcast spawning species that release eggs without algal symbionts, which possibly reflects selection to facilitate fertilization while avoiding symbiont stress from radiation at the sea surface that causes harm to hosts (Hartmann et al., 2017). Previous studies on *Pocillopora* have found both symbiont specificity and flexibility (Cunning et al., 2013; Noreen et al., 2013; Pinzon & LaJeunesse, 2011; Rouzé et al., 2019; Schmidt-Roach et al., 2013). We used multiple genomic data sets to reconstruct phylogenetic relationships and determine the placement of mitochondrial haplotypes that were not included in previous *Pocillopora* phylogenomic analyses (Johnston et al., 2017). We also assessed Symbiodiniaceae relationships using sequence variation in both ITS2 and *psbA*^{ncf} markers in order to determine whether there is support for their cophylogeny, or multiple colonization events of distantly related Symbiodiniaceae into *Pocillopora*. Given the recent evidence of codiversification described for some *Pocillopora* lineages with *Cladocopium* (Turnham et al., 2021), we hypothesize that there will be specific *Pocillopora*–*Cladocopium* associations that are not likely to differ across depths, reflecting cospeciation.

2 | MATERIALS AND METHODS

2.1 | *Pocillopora* phylogenetic analyses

2.1.1 | Taxon sampling

Tissue samples from 44 *Pocillopora* colonies were collected using SCUBA in August 2019 from six sites (LTER 1–6) and three depths (5, 10, and 20m) around Mo'orea, French Polynesia (Table S1). The samples contained nine mitochondrial open reading frame (mtORF) haplotypes including haplotype 1a, 2, 3a, 3b, 3f, 3h, 8a, 10, 11. Haplotype 1a contains two species: *P. meandrina* and *P. grandis* (Johnston et al., 2017). While colonies of the same genotype are well known in *P. acuta* (haplotype 5) (e.g., Strader et al., 2022), we did not sample clones. mtORF identification was carried out as described in Johnston et al. (2022) with haplotype numbering following Forsman et al. (2013) and Pinzón et al. (2013). The species names associated with certain haplotypes are only nominal names based on the morphospecies type (based on Veron & Stafford-Smith, 2000) originally assigned to the first description of the haplotype in Johnston et al. (2022), Forsman et al. (2013) and Pinzón et al. (2013). Tissue samples were stored in salt-saturated DMSO (dimethyl sulphoxide) buffer (Gaither et al., 2011) until DNA was extracted. Specimens are stored at Florida State University.

2.1.2 | DNA extraction and quantification

Genomic DNA was extracted from tissue using the OMEGA (Bio-Tek) E.Z.N.A. Tissue DNA Kit. Three elutions (50, 100, 100 µl) were collected in HPLC grade H₂O. Extractions were inspected on a 1% agarose gel. All three elutions were combined and DNA was speedvac concentrated. Extractions were quantified using the Qubit dsDNA HS Assay kit with the Qubit Fluorometer (ThermoFisher Scientific).

2.1.3 | Library preparation and sequencing

ezRAD libraries (Toonen et al., 2013) were generated following Knapp et al. (2016) as in Johnston et al. (2017). Briefly, samples were digested with the isoschizomer restriction enzymes *Mbo*I and *Sau*3AI (New England BioLab), which cleave at GATC cut sites, and libraries were generated with the KAPA HyperPrep Kit (Roche) using TruSeq DNA indexes (Illumina). Libraries were size selected at 350–700 bp and sequenced on the MiSeq platform as paired-end 300 bp runs at Florida State University.

2.1.4 | Reference assemblies and phylogenetic analyses

Four different data sets were generated for *Pocillopora* phylogenetic analyses: a concatenated genomic loci data set, complete

mitochondrial genomes, and two single nucleotide polymorphism (SNP) data sets; one in which SNPs were removed based on proximity (unlinked SNP data set) and one in which SNPs were not removed based on proximity (linked SNP data set). All four data sets were generated from the same RAD libraries and subject to different filtering criteria and downstream analyses. Our concatenated genomic loci data set comprised 57 individuals, which included the 44 *Pocillopora* sampled from the fore reef of Mo'orea, plus 11 additional *Pocillopora* individuals from Johnston et al. (2017) that were collected from Eastern Australia (*P. acuta*, *P. damicornis*, *P. verrucosa*), Hawai'i (*P. acuta*, *P. damicornis*, *P. verrucosa*, *P. grandis*, *P. meandrina*, *P. ligulata*), Clipperton Atoll (haplotype 2), and Mexico (*P. grandis*), and two outgroup individuals collected from Eastern Australia (*Seriatopora hystrix* and *Stylophora pistillata*). IPYRAD version 0.9.60 (Eaton & Overcast, 2020) was used to generate the concatenated genomic loci data set by mapping to the *P. verrucosa* genome (Buitrago-López et al., 2020) with a clustering threshold of 85%, a minimum depth of eight reads to call majority rule base calls, a minimum of eight samples per locus, a maximum allowed SNP threshold of 20% per locus, and a maximum of 50% heterozygous sites per locus. The number of raw reads obtained per library ranged from 326,778–7,780,151. After trimming, the number of reads that mapped to the *P. verrucosa* genome (Buitrago-López et al., 2020) using IPYRAD version 0.9.60 (Eaton & Overcast, 2020) ranged from 101,804–6,146,286 per library, with the number of loci in the concatenated genomic data set ranging from 176–17,689 among samples, resulting in a sequence matrix consisting of 92.5% missing sites. EXABAYES version 1.4.1 (Aberer et al., 2014) with default parameters was used to generate a Bayesian phylogeny of this concatenated genomic loci data set.

To generate the data set of complete mitochondrial genomes, all 57 libraries were mapped to the *P. grandis* mitochondrial genome, accession number EF526303 (Flot & Tillier, 2007), using BWA MEM version 0.7.17 (Li & Durbin, 2009). The use of a reference genome from a closely related species is not expected to affect inferences and follows current best practices (Shafer et al., 2017). Consensus sequences were generated using BCFTOOLS MPILEUP and BCFTOOLS CALL (Li, 2011) and converted to fasta format using Seqtk (<https://github.com/lh3/seqtk>). A single individual with 100% coverage of the reference was chosen per mtORF haplotype, with the exception of *P. acuta* and *P. damicornis*, for which previously sequenced complete mitochondrial genomes were used, accession numbers NC_009797 and EU_400213, respectively (Chen et al., 2008; Flot & Tillier, 2007). Two outgroups, *Seriatopora caliendrum* (EF633601) and *S. hystrix* (EF633600) (Chen et al., 2008), were also included. Mitochondrial genomes were aligned in GENEIOUS version 9.1.8. A Bayesian phylogeny of this data set was generated using BEAST version 2.6.2 with the GTR model of evolution, a random local clock to allow for variation in the evolutionary rate across the tree, and the birth-death model as the tree prior to allow for differences in speciation and extinction rates across the tree, which is important when outgroup taxa are included in analyses (Bouckaert et al., 2019). The MCMC was run for 10,000,000 generations with sampling every 1000 steps and the first 10% was removed as burnin. A maximum likelihood phylogeny

was generated using RAXML-NG (Kozlov et al., 2019) with the GTR+G model and 10,000 bootstrap replicates.

To generate an unlinked SNP data set, trimmed reads of all libraries from step two of IPYRAD version 0.9.60 (Eaton & Overcast, 2020) were aligned to the *P. verrucosa* genome (Buitrago-López et al., 2020) using BWA MEM version 0.7.17 (Li & Durbin, 2009). The unlinked SNP data set included *P. ligulata*, mtORF haplotypes 2, 3a, 3b, 3f, 3h, 8a, 10, and 11, *P. meandrina*, and *P. grandis*. SAMtools (Li et al., 2009) was used to convert sam files to sorted bam files. PCR duplicates were removed with MarkDuplicates and then variants were called with HaplotypeCaller using GATK version 4.2 (McKenna et al., 2010; van der Auwera et al., 2013). The variant call file (VCF) was subsequently filtered using VCFTOOLS version 0.1.6 (Danecek et al., 2011). For mtORFs that had more than three individuals sequenced per haplotype, only the three individuals with the least missing data were retained. *Pocillopora acuta*, *P. damicornis*, and outgroup taxa were removed from this data set entirely due to large amounts of missing data (>80%) relative to the other libraries. The VCF was filtered to remove sites with missing data and indels, and then sites were thinned by 5000 bp to extract unlinked SNPs. The unlinked SNP data set for SNAPP contained 2770 SNPs. The VCF file was converted to binary nexus format using the python script VCF2PHYLIP version 2.0 (Ortiz, 2019). To generate a species tree, we used the SNAPP plugin in BEAST2 version 2.6.2, which implements the multispecies coalescent model (Bouckaert et al., 2014). All taxa were treated as distinct species, u and v were calculated from the data, and a gamma distributed prior with $\alpha = 2$ and $\beta = 200$ was used. The analysis ran for 4,000,000 Markov chain Monte Carlo (MCMC) generations, sampling every 1000 steps. Convergence was assessed with TRACER version 1.6 and the first 10% were removed as burnin.

2.1.5 | Discriminant analysis of principal components

We also generated a data set that retained linked SNPs, that is, SNPs that were not thinned by proximity, for discriminant analysis of principal components (DAPC) to distinguish clusters of *Pocillopora*. We used a linked SNP file for DAPC analysis because DAPC does not use a model of evolution and therefore makes no assumptions about the underlying population genetics model, for example, those concerning linkage equilibrium or Hardy-Weinberg equilibrium. To generate the linked SNP data set, we filtered the above VCF file (before filtering for SNAPP) using VCFTOOLS version 0.1.6 (Danecek et al., 2011) by first removing individuals from (Johnston et al., 2017) that were previously sequenced on the Illumina GAIIx platform in 2013 (SS1, SD6, SD2, Pacu01, Pacu02, R17, SD1, SD4, J001, and R16). SNPs were retained if they were biallelic, had no more than 25% missing data, and had a mean sequencing depth of greater than or equal to six. In total, 7887 SNPs were used for the DAPC analysis. Using the package ADEGENET version 2.1.3 in R (Jombart & Collins, 2015), we used the function find. Clusters to find the best number of K , which was chosen based on the lowest Bayesian information criterion (BIC).

The number of PC axes was determined using the `optim.a.score` function. Seven PCA axes and four discriminant analysis axes were retained.

Additionally, we used nQuire (Weib et al., 2018) to investigate ploidy with the linked SNP data set and found that a diploid model was the best fit for all *Pocillopora* libraries.

2.2 | Symbiodiniaceae analyses

2.2.1 | Taxon sampling

Tissue samples from 217 *Pocillopora* colonies were collected from the fore reef of Mo'orea at LTER sites 1–6 in 2019. These samples included nine mtORF haplotypes (haplotype 1a, which includes *P. meandrina* and *P. grandis*, as well as 2, 3a, 3b, 3f, 3h, 8a, 10, and 11). *Pocillopora acuta* can be found on the fringing reef at Mo'orea (Rouzé et al., 2019) but was not included in our collection because we only sampled the fore reefs. The 44 *Pocillopora* samples used for phylogenetic analyses are a subset of these 217 samples. For four *Pocillopora* lineages (haplotype 10, the lineage containing *P. meandrina* and haplotype 8a, *P. grandis*, and the lineage containing haplotypes 11/2), samples were collected at 5, 10, and 20m depths (Table S2 and Johnston et al., 2022).

2.2.2 | DNA extraction, ITS2 library preparation, and sequencing

Genomic DNA was extracted from tissues using Chelex 100 (Bio-Rad). Samples were incubated in 150 µl of 10% Chelex 100 for 60 min at 55°C followed by 15 min at 95°C. The supernatant was then used for PCR amplification of the ribosomal internal transcribed spacer 2 (ITS2) region using the SYM_VAR primer pairs (Hume et al., 2018). Initial PCR was performed using Phusion High-Fidelity MasterMix (ThermoFisher Scientific) with an initial denaturation step at 98°C for 2 min, followed by 35 cycles of 98°C for 10 s, 56°C for 30 s, and 72°C for 30 s, and a final extension step at 72°C for 5 min. Amplified DNA was cleaned using AMPure XP beads and used as template for an index PCR in which unique combinations of Nextera XT index primers were used for each sample. Eight cycles of PCR were performed as described above with an annealing temperature of 55°C. Libraries were then cleaned, normalized, and pooled for sequencing on an Illumina MiSeq platform with 2×300 paired end reads.

Demultiplexed forward and reverse fastq files were passed to SymPortal (Hume et al., 2019) remotely, which removed non-Symbiodiniaceae sequences and then grouped Symbiodiniaceae sequences by genera. Being a ribosomal gene, there are usually multiple ITS2 copies within a single Symbiodiniaceae cell. ITS2 sequence variation can therefore come from intragenomic variation among gene copies, in addition to intergenomic variation among Symbiodiniaceae genotypes. To parse ITS2 sequence variation into

intra- and intergenomic variation, SymPortal identifies repeatedly co-occurring sequences with similar relative abundances as “defining intragenomic variants” (DIVs), which are then collapsed into ITS2 type profiles that may represent distinct taxonomic units above, at, or below the species level (Hume et al., 2019). The full (post-quality-filtering) diversity of ITS2 sequence variation was used for downstream statistical analysis, while type profiles aided in visualization. However, since ITS2 is not a species-level marker, the *psbA^{ncf}* marker was also used to better resolve Symbiodiniaceae species-level diversity.

2.2.3 | *Cladocopium* species identification

To complement information provided by ITS2 type profiles and increase certainty in species identification, we also used the noncoding plastid minicircle (*psbA^{ncf}*) to distinguish between *Cladocopium* species (Moore et al., 2003) for 66 *Pocillopora* colonies in our collection. These 66 *Pocillopora* colonies encompassed the diversity of ITS2 type profiles obtained from SymPortal for which *Cladocopium* was dominant. The *psbA^{ncf}* region was amplified using the primers and protocol of (Moore et al., 2003), and the region was sequenced in both the forward and reverse directions. Sequences were aligned and manually checked in GENEIOUS version 9.1.8 (Biomatters Ltd). To add further confidence in *Cladocopium* species identification, we added 41 *psbA^{ncf}* sequences from (Turnham et al., 2021), which encompass a wide geographic range across the Pacific, including two *C. goreau* sequences used as an outgroup, and three sequences from Pinzon and LaJeunesse (2011) collected from *Pocillopora* haplotype 2 colonies from Clipperton Atoll. A Bayesian phylogeny of this larger data set was generated using BEAST version 2.6.2 (Bouckaert et al., 2019). We used the GTR model of evolution, a random local clock, and the birth-death model as the tree prior, for the same reasons outlined for the *Pocillopora* mitogenome tree. The MCMC was run for 266,000,000 generations with sampling every 1000 steps and the first 20% was removed as burnin. Clades within *C. latusorum* and *C. pacificum* were identified by high posterior support (>95%) at deep monophyletic nodes, though divergence between these clades was greater in *C. latusorum* than in *C. pacificum*.

2.2.4 | Analyses of Symbiodiniaceae among *Pocillopora* species

We used principal coordinates analysis (PCoA; `cmdscale` function in the R package `vegan` [Oksanen et al., 2019]) with a Bray–Curtis dissimilarity measure to visualize differences in Symbiodiniaceae ITS2 sequence diversity between *Pocillopora* species or haplotype. Differences between groups were tested using permutational multivariate analysis of variance (PERMANOVA) with 99,999 permutations (`adonis` function in the R package `vegan`). These analyses were run to only include colonies dominated by either *C. latusorum* or *C. pacificum*.

2.2.5 | Analyses of Symbiodiniaceae within *Pocillopora* species across depth

We also investigated whether *Pocillopora* species maintain specificity in their Symbiodiniaceae composition across depth (5, 10, and 20m) for four *Pocillopora* species at Mo'orea. These included two species with similar relative abundances across depth (*P. grandis*, and haplotypes 11/2), and two species with different relative abundances across depth (haplotype 10, which is most prevalent at 20m depth, and *P. meandrina* and haplotype 8a, which is most prevalent at 5 m depth; Johnston et al., 2022). We used PCoA with a Bray–Curtis dissimilarity measure to visualize differences in Symbiodiniaceae ITS2 sequence diversity within *Pocillopora* species across depth. The `vegan pairwise.adonis2` function with 99,999 permutations was used to investigate pairwise differences across depth. As above, these analyses were run to only include colonies dominated by either *C. latusorum* or *C. pacificum*, depending on the *Pocillopora* species. Within each *Pocillopora* species, differences in ITS2 type profiles were also investigated across depth for those colonies found to host only *C. latusorum* or *C. pacificum* using Pearson's chi-squared test for count data (function `chisq.test` function in R).

2.3 | Cophylogenetic analysis of *Cladocopium*–*Pocillopora*

To test for phylogenetic congruence, *Pocillopora* host and *Cladocopium* symbiont phylogenetic trees, based on *psbA^{ncr}*, were assessed using the Procrustean Approach to Cophylogeny, PACo (R package `paco` Balbuena et al., 2013). PACo is a distance-based global-fit method that quantifies the topological congruence between two phylogenetic trees and identifies the particular associations contributing to the cophylogenetic structure. The method allows for multiple host-symbiont associations and does not require fully resolved phylogenies. Instead of testing for random association between the host and symbiont taxa, PACo explicitly tests the dependence of the symbiont phylogeny on the host phylogeny, which assumes that symbiont speciation is driven by host speciation. PACo does this by carrying out a PCoA on the host and symbiont phylogenetic distances separately, then using Procrustes superimposition to rotate and scale the symbiont tree to fit that of the host, tests for the congruence of host-symbiont associations. The null hypothesis tested is that the algal symbiont branches are randomly associated to the coral host branches. The alternative hypothesis is that at least some part of the symbiont ordination is constrained by that of the corals and, thus, the coral-algae associations are mirrored in phylogenetic congruence. The analysis of the residual sums of squares of each topographical link between algal and host trees provides a direct measure of the importance of each specific link to the global fit and is useful for quantifying evidence for which symbiont species or genetic clades track corals more than others. Smaller residual sums of squares mean greater importance. Jackknife 95% confidence intervals also are provided. Including *psbA^{ncr}* sequences from multiple

colonies, rather than from just one representative colony for each *Pocillopora* species, allowed us to investigate lineage diversity both within *Cladocopium* species as well as *Pocillopora* species specificity with a *Cladocopium* species or lineage.

For *Cladocopium*, we used the *psbA^{ncr}* Bayesian tree of 110 taxa described above, but only included symbiont–host links for the 66 taxa we sequenced the *psbA^{ncr}* region for in the PACo analysis. For *Pocillopora*, a Bayesian phylogenetic tree was generated using EXABAYES version 1.4.1 (Aberer et al., 2014) with default parameters by selecting a single representative for *P. verrucosa*, *P. meandrina*, *P. grandis*, and haplotypes 2, 8a, and 11 with the highest coverage from the concatenated genomic loci data set generated by `ipyrad`. Phylogenetic trees were imported into R using the “APE PACKAGE” (Paradis et al., 2004).

3 | RESULTS

3.1 | *Pocillopora* phylogenetic analyses

Analyses using two approaches to delineate *Pocillopora* species (DAPC analysis using 7887 linked SNPs and SNAPP analysis using 2770 unlinked SNPs) supported five species on the fore reef at Mo'orea. Furthermore, *P. ligulata* (haplotype 6a) from Hawai'i was distinct from any species at Mo'orea and does not occur there, but is most closely related to haplotypes 11/2 (Figures 1 and 2). The five species of *Pocillopora* recovered from the fore reef at Mo'orea were: (1) haplotypes 11/2, (2) *P. meandrina* (haplotype 1a) and haplotype 8a, (3) *P. verrucosa* (haplotypes 3a, 3b, 3f, and 3h), (4) haplotype 10, and (5) *P. grandis* (haplotype 1a). Note that *P. acuta* also occurs at Mo'orea, but only on the fringing reef (Rouzé et al., 2019). In total, there are six species of *Pocillopora* at Mo'orea.

Haplotypes 11/2, *P. meandrina*/haplotype 8a, and *P. verrucosa* each formed distinct clusters indicating ongoing or recent gene flow among the mtORF haplotype lineages within each cluster. In the mitochondrial phylogeny, haplotype 2 was more ancestral to the clade containing *P. ligulata* and haplotype 11 (Figure 3b), but in the nuclear genomic (Figure 3a) and SNAPP phylogenies (Figure 1), *P. ligulata* recovered as ancestral to the clade containing haplotypes 11 and 2. Both SNP data sets indicated that haplotypes 11 and 2 are not reciprocally monophyletic, indicating some level of recent or ongoing gene flow between them (Figures 1 and 2). Within *P. verrucosa*, there was no consistent clustering of haplotypes 3a, 3b, 3f, and 3h (Figures 1, 2 and 3a,b). Haplotype 8a was not differentiated from *P. meandrina* in the genomic, SNAPP, or DAPC data sets.

Haplotype 10 and *P. grandis*, in contrast, showed no evidence of introgression with other mtORF haplotypes. In all data sets (genomic, complete mitochondrial genomes, DAPC and SNAPP), haplotype 10 was consistently recovered as distinct from *P. verrucosa* (Figures 1, 2 and 3a,b), indicating that it is a genetically distinct sister species to *P. verrucosa*. In both the genomic and SNAPP data sets, *P. grandis* was recovered as distinct from the clade that contains *P. meandrina* and haplotype 8a. In the mitochondrial data set, however, *P. meandrina*

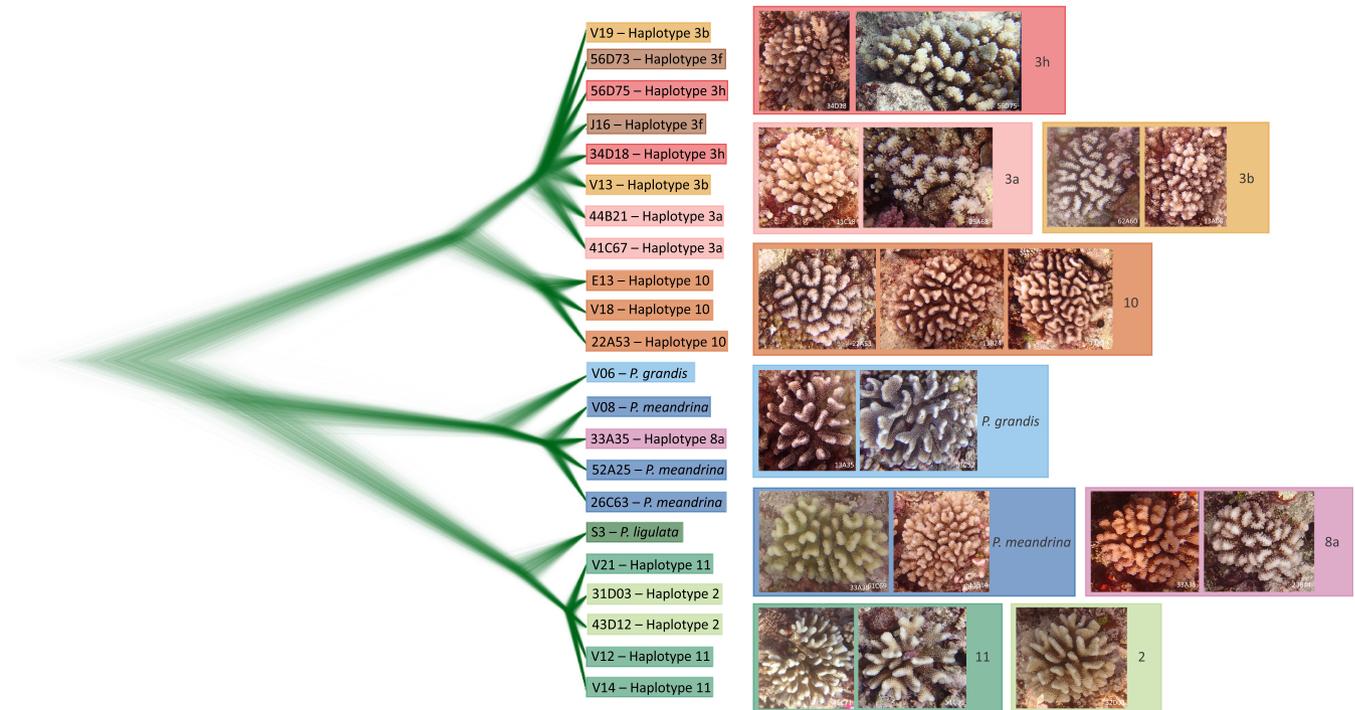


FIGURE 1 *Pocillopora* species tree. The cloudogram distribution from SNAPP showing the Bayesian analysis of 2770 nuclear, unlinked, biallelic SNPs.

and *P. grandis* formed a monophyletic clade distinct from haplotype 8a (Figure 3b).

3.2 | *Pocillopora*—Symbiodiniaceae specificity

The majority (215 of 217) of *Pocillopora* colonies sampled from Mo'orea hosted Symbiodiniaceae from the genus *Cladocopium* (Figure 4). Four out of 41 (10%) *P. grandis* colonies hosted *Symbiodinium* spp. and one hosted *Durusdinium* spp. (2%). Five out of 26 (19%) haplotype 11 and haplotype 2 colonies hosted *Durusdinium glynnii*. The undescribed *Cladocopium* species identified with the C116 DIV was hosted by one *P. verrucosa* colony, two *P. meandrina* colonies, one *P. grandis* colony, and one haplotype 11 colony (Figure 4).

Phylogenetic analysis of the *Cladocopium* *psbA^{ncr}* region indicated that the majority of symbionts hosted by *Pocillopora* were either *C. latusorum* or *C. pacificum* (Figure 5a). Sequences from colonies hosting C116 DIVs could not be aligned with those from *C. latusorum* and *C. pacificum*, and therefore were not included in the phylogenetic analysis. *Cladocopium latusorum* was hosted by *P. meandrina* and haplotype 8a, *P. grandis*, and haplotypes 11/2, and one haplotype 10 colony. *Cladocopium pacificum* was hosted exclusively by haplotype 10 and *P. verrucosa*. Largely, and following (Turnham et al., 2021), we found that *C. latusorum* could be identified by the C42a DIV in their ITS2 type profile, and that *C. pacificum* could be identified by the C1d DIV in their profile (Figures 4 and 5a). However, there were also some ITS2 type profiles from haplotype 10, *P. verrucosa*, and haplotypes 11/2

colonies that did not contain C42a or C1d. These ambiguous ITS2 type profiles were C1.C42.2 (one colony), C1.C3.C42.2.C1b.C3cg (two colonies), C42.2.C1.C42i.C3.C1b.C1au (one colony), C1.C42.2.C3cg.C1b.C115k.C45c (two colonies), C1.C42.2.C3.C1b.C1au.C115n (two colonies), C1.C42.2.C1b.C1au.C3.C3cg.C1j (15 colonies), C1.C1ag.C1ah.C42.2.C3cg.C1b.C3 (three colonies), and C1.C42.2.C1b.C3cg.C3.C1au.C41p (7 colonies). Of these colonies, only three, with type profiles C1.C42.2 and C1.C3.C42.2.C1b.C3cg, had low sequencing depth. All of these samples, however, could be assigned to either *C. latusorum* or *C. pacificum* using the *psbA^{ncr}* marker. Analysis of full ITS2 sequence diversity revealed additional unique sequence variants that were associated with *Pocillopora* species (Figure S1) and ITS2 type profiles (Figure S2), but which were not included as DIVs in the ITS2 type profiles predicted by SymPortal.

3.2.1 | Symbiodiniaceae composition among *Pocillopora* species

ITS2 sequence diversity differed significantly between most *Pocillopora* species and haplotypes (Figure 6a, Table 1). The greatest difference in ITS2 sequence diversity was between the *Pocillopora* lineage that contains *P. meandrina*, haplotype 8a, and *P. grandis*, and the other *Pocillopora* species and haplotypes. Symbiodiniaceae ITS2 diversity did not differ between *P. meandrina* and haplotype 8a ($p = .371$), or between haplotypes 11 and 2 ($p = .888$), providing further support for hybridization or introgression between these *Pocillopora* groups.

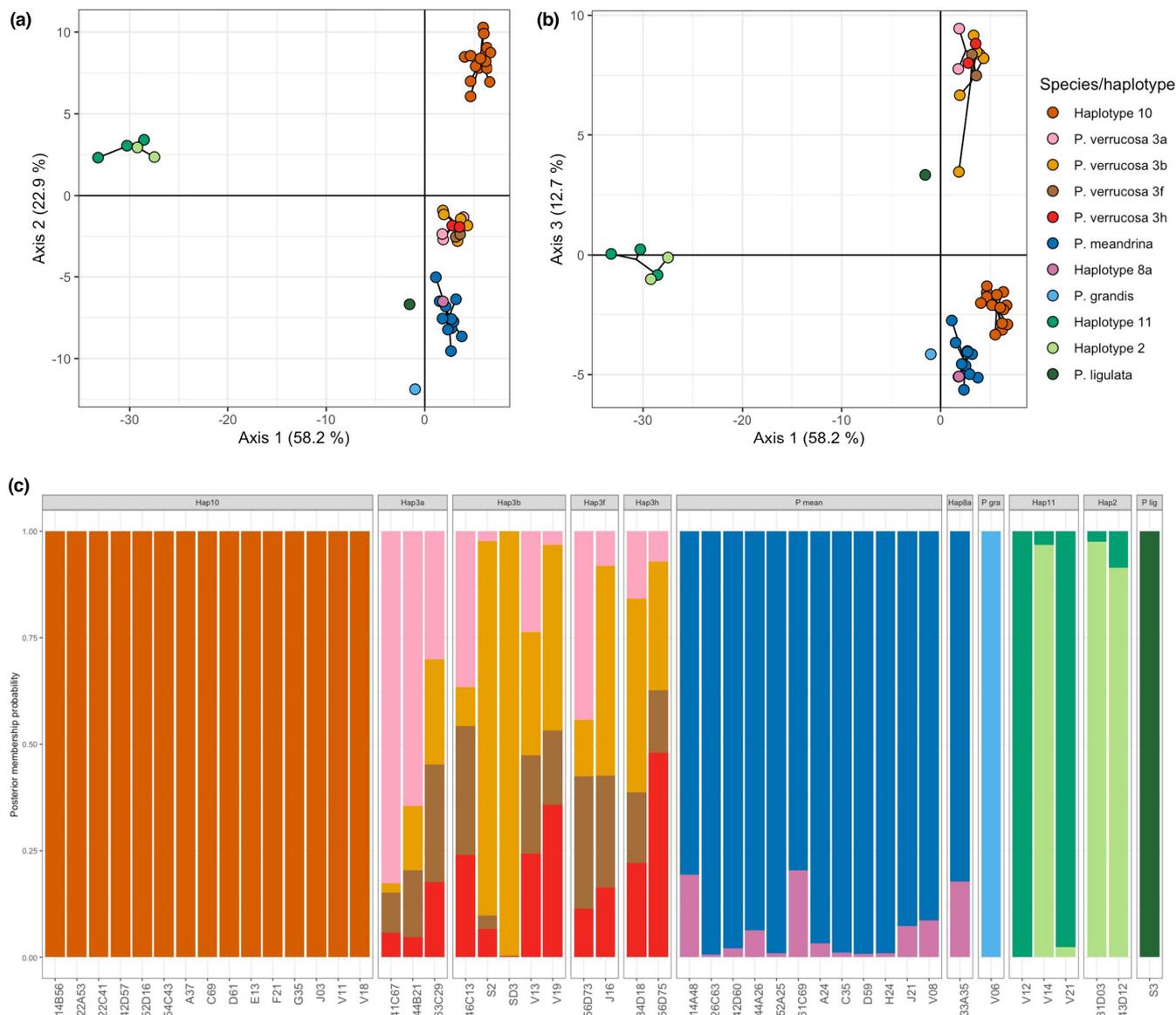


FIGURE 2 Discriminant analysis of principal components (DAPC) plots for *Pocillopora* using 7887 nuclear biallelic SNPs that were not filtered for linkage showing genetic clusters in (a) and (b), and the posterior membership probability of individuals to these DAPC clusters in (c).

3.2.2 | Symbiodiniaceae composition within *Pocillopora* species across depth

Within *Pocillopora* species, ITS2 sequence diversity differed across depth for some *Pocillopora* species but not others (Figure 6b–e, Tables 1 and 2). Pairwise comparisons of ITS2 sequence diversity were significant within *C. pacificum* hosted by haplotype 10 between 5–10 and 5–20m depths (Figure 6b, Table 2). Additionally, Pearson's chi-squared test indicated that ITS2 type profiles within *C. pacificum* also differed significantly across depth ($\chi^2 [20] = 36.917; p = .012$). Within *P. meandrina* and haplotype 8a, pairwise comparisons of ITS2 sequence diversity within *C. latusorum* were significant between 5–20 and 10–20m depths (Figure 6b, Table 2). However, ITS2 type profiles did not differ significantly across depth. ITS2 sequence diversity did not differ across depth in *P. grandis* or haplotypes 11/2 (Table 2).

3.3 | Cophylogenetic analysis of *Cladocopium*–*Pocillopora*

We found strong dependence of the *Cladocopium* phylogeny on the *Pocillopora* phylogeny (Figure 7). The test for the extent to which the coral–algal associations are mirrored in phylogenetic congruence yielded a global residual sum of squares (m^2) of 0.0009 with a permutational p -value of $p < .0001$, indicating that the *Cladocopium* phylogeny is not randomly associated with the *Pocillopora* phylogeny. We also identified three clades within *C. latusorum* and two clades within *C. pacificum*.

The specific coral–algal associations that contributed the most (i.e., smallest squared residuals) to the global phylogenetic congruence were the dependence of *C. latusorum* clade II on *P. grandis*, and the dependence of *C. pacificum* clades IV and V on *Pocillopora*

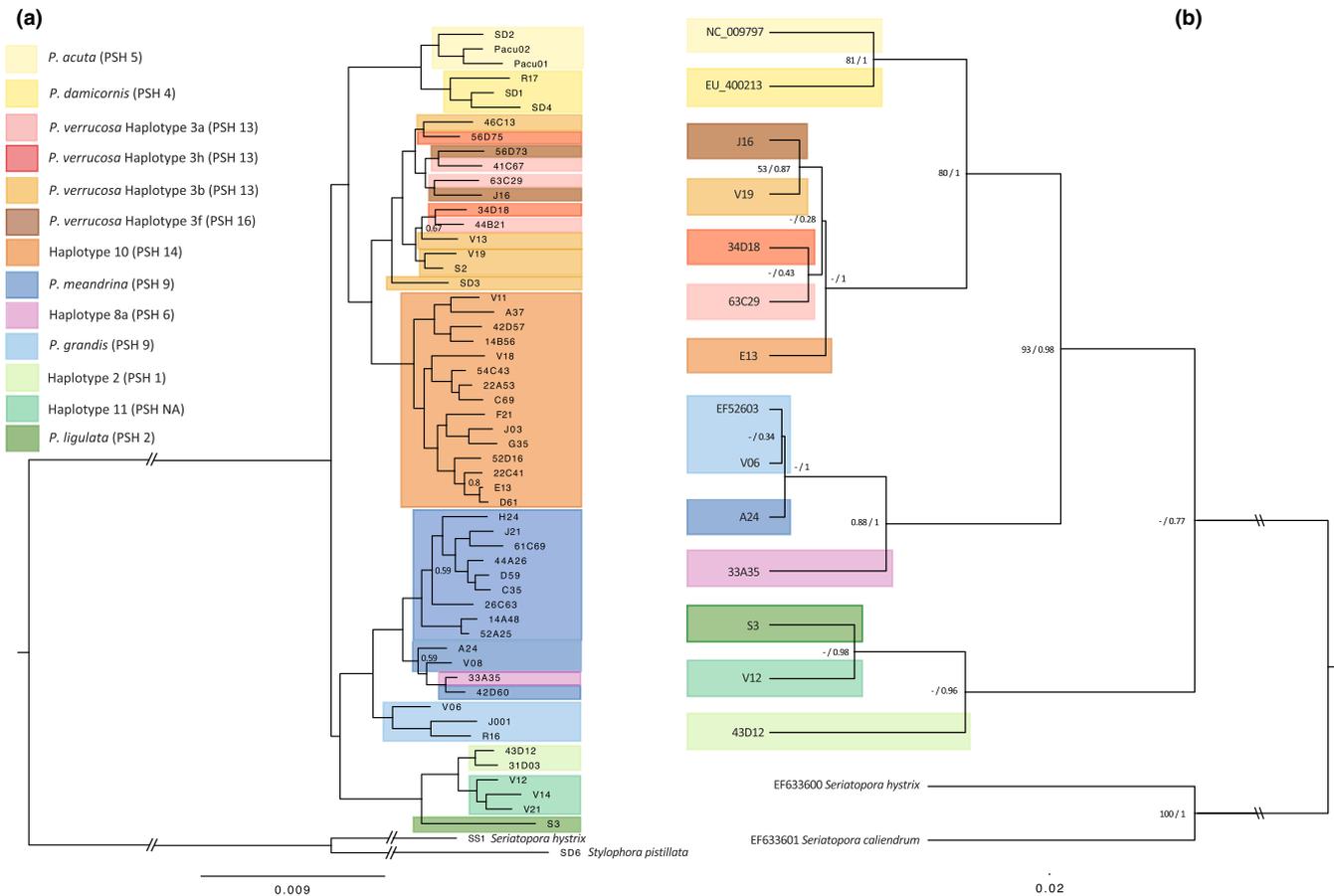


FIGURE 3 *Pocillopora* genomic and mitochondrial phylogenies. (a) Nuclear genomic Bayesian phylogeny of *Pocillopora* with outgroups *Seriatopora* and *Stylophora*. All nodes are >95% CI unless otherwise indicated. (b) Maximum and Bayesian phylogenetic analysis of complete mitochondrial genomes. Mitochondrial haplotype identification follows Pinzón et al. (2013) and Forsman et al. (2013). For ease of comparison among studies using different naming, the primary species hypothesis (PSH) presented in Gélín et al. (2017) is added in parentheses.

sister taxa haplotype 10 and *P. verrucosa*. The weakest associations contributing to the overall phylogeny were the dependence of *C. latusorum* clade III and its dependence on haplotype 11 (Figure 5b). Although there was high specificity between haplotypes 11/2 and *C. latusorum* clade III, the contribution of these links was low because of the low branch support within this clade.

4 | DISCUSSION

Identifying the dependence of symbiont lineages on their host lineages is important for understanding the evolutionary history and ecological responses of the holobiont (Corbin et al., 2017). Despite the obligate symbiotic relationships between photosynthetic algae (Symbiodiniaceae) and scleractinian coral hosts being a classic example of a holobiont, explicit tests of phylogenetic congruence have been absent. Furthermore, formal, quantitative tests of cophylogeny between corals and their algal symbionts have been hampered in the past because both corals and algae contain genetically unresolved and morphologically-cryptic species. Here, we used multiple genomic data sets from samples collected across multiple depths and sites at

Mo'orea, French Polynesia, to resolve previously well-studied mitochondrial lineages into species and found strong evidence of cophylogeny between *Pocillopora* species and their algal symbionts. Our analyses supported the presence of five co-occurring *Pocillopora* species on the fore reef of Mo'orea that largely hosted two species of host-specialized algae (*Cladocopium latusorum* and *C. pacificum*). *Pocillopora acuta* can also be found at Mo'orea but is only found on the fringing reef, not on the fore reef, and largely hosts *Durusdinium* in this environment (Rouzé et al., 2019; Strader et al., 2022) and was therefore not included in our analyses. In general, the *Cladocopium* phylogeny, including multiple clades within species, mirrored the *Pocillopora* phylogeny (Figure 7). Certain *Cladocopium* species and lineages within species showed stronger dependence with specific *Pocillopora* species than others. Such cophylogeny is probably a consequence of the life history of broadcast-spawning *Pocillopora*, which, unlike many broadcast spawning corals from other genera, transmit algal symbionts to the next generation in the eggs (Hirose et al., 2000; Schmidt-Roach et al., 2012). However, we also found that some *Pocillopora* colonies were dominated by symbiont taxa from *Symbiodinium* and *Durusdinium*. Taxa from *Symbiodinium* and *Durusdinium* also occur in other host taxa ranging from jellyfish

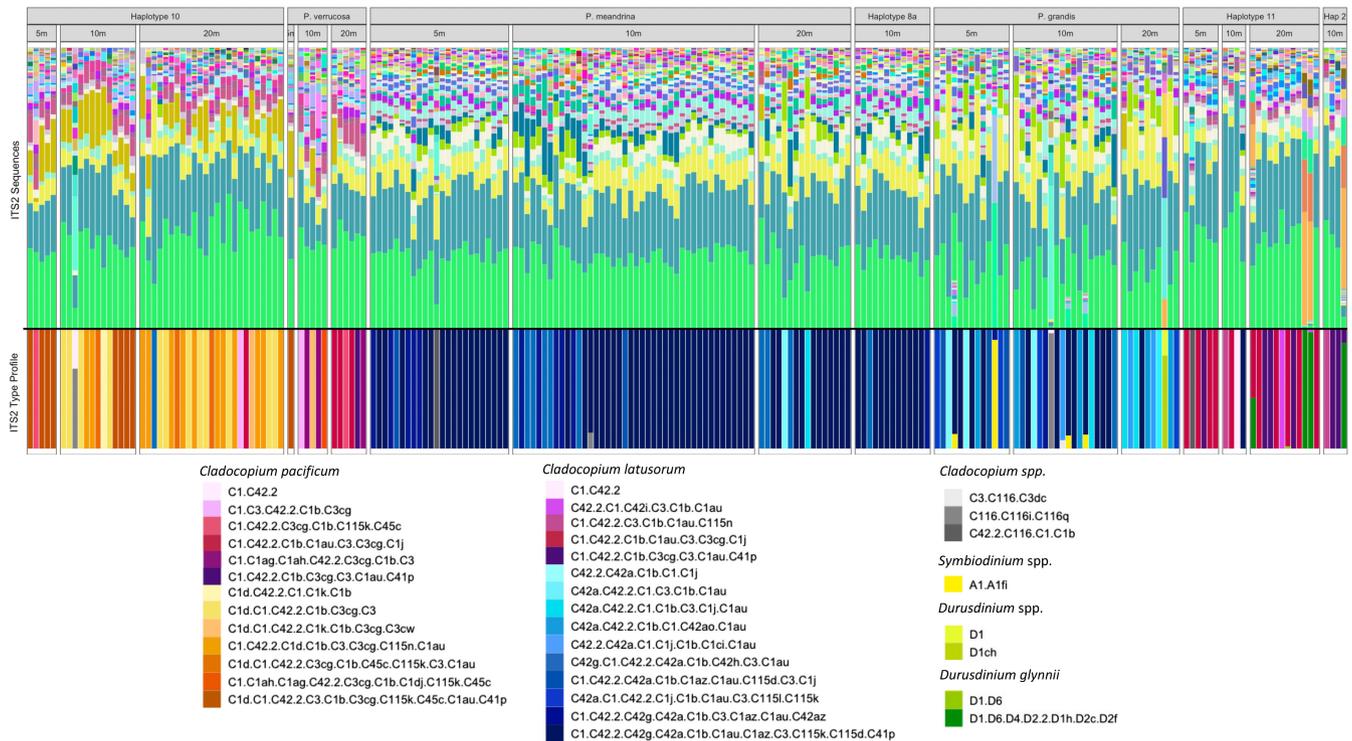


FIGURE 4 Diversity of ITS2 sequences (based on the post_med output from SymPortal) and ITS2 type profiles within each colony of *Pocillopora* species or haplotype. Each column represents a colony and colonies are binned by depth sampled within each species or haplotype. For *C. pacificum* and *C. latorum*, species identity was based on psbA sequence data. *D. glynnii* was identified from the ITS2 type profile. ITS2 sequence colour key found in Figure S3.

(Mammone et al., 2021; Sachs & Wilcox, 2006) to clams (DeBoer et al., 2012; Poo et al., 2021), and may have a free-living stage (Fujise et al., 2021; Pochon et al., 2014). The presence of symbiont taxa from *Symbiodinium* and *Durusdinium* suggests that there may also be some degree of horizontal transmission in broadcast-spawning *Pocillopora*, as has been observed in the brooding *P. damicornis* (Epstein et al., 2019). While speciation of *C. latorum* and *C. pacificum* appears to be driven by niche diversification provided by speciation in *Pocillopora* (Turnham et al., 2021), we are now in a position to understand more about certain associations of symbiont clades within more *Pocillopora* species. Furthermore, *Pocillopora* still harbours generalist symbionts that may also be transmitted vertically but could be acquired horizontally as well.

Recently, Turnham et al. (2021) described two new species of Symbiodiniaceae in the genus *Cladocopium* and found that they codiversified with different *Pocillopora* lineages, probably as a result of maternal vertical transmission of symbionts. They found that *C. latorum* codiversified with the lineage that contains *P. meandrina*, haplotype 8a, and *P. grandis*, and that *C. pacificum* codiversified with *P. verrucosa*, and that this speciation event in *Cladocopium* coincided with the diversification of these extant *Pocillopora* lineages approximately 3MYA. Here, we provide additional symbiont associations for several *Pocillopora* species and provide a formal test of cophylogeny. Specifically, we found that, in addition to *P. meandrina*, haplotype 8a, and *P. grandis*, haplotypes 11/2 also host *C. latorum*. In addition to *P. verrucosa*, haplotype 10 also hosts *C. pacificum*. Furthermore, we

show that there are unique associations between *Pocillopora* species and different lineages within *C. latorum* and *C. pacificum*, providing further evidence for the cospeciation hypothesis.

In the past, cophylogeny between corals and their algal symbionts was expected to be rare or absent (van Oppen & Medina, 2020) because many coral species exhibit horizontal transmission (Baird et al., 2009), many algal symbiont species exhibit flexibility to associate with different hosts (LaJeunesse et al., 2018), and even coral species with vertical transmission can have some degree of horizontal acquisition of symbionts (Quigley et al., 2018, 2019). Other coral species, including members from *Porites* and *Montipora*, also exhibit vertical transmission and harbour host-specialist species of *Cladocopium* (Forsman et al., 2020; Hoadley et al., 2021; LaJeunesse et al., 2004; LaJeunesse & Thornhill, 2011), but the extent of cophylogeny within these groups remains to be tested. Members of the genus *Cladocopium* associate with a broad diversity of hosts in addition to corals, including other cnidarians, clams, ciliates, flatworms, foraminifera, and sponges, but can be highly host specialized (LaJeunesse et al., 2018). Therefore, an important next step to test the links between vertical transmission and cospeciation is to compare estimates of cophylogeny among more groups of corals and compare groups with vertical versus horizontal transmission.

The functional differences, if any, between *C. latorum* and *C. pacificum* in their effects on the coral host are not clear. However, the high specificity and evidence for shared evolutionary history still generates hypotheses for the degree to which symbioses in this

group promote or restrict adaptation to climate change (Compant et al., 2010). For example, the ability to switch or shuffle symbionts in response to environmental change may be lower in coral species with high symbiont specificity. To test this, it will be informative to know the extent to which *Pocillopora* species hosting *C. latusorum* or *C. pacificum* differ in their ability to switch or shuffle symbionts. In our data set, only *Pocillopora* species that typically host *C. latusorum* showed the ability to host taxa from *Symbiodinium* and *Durusdinium*. In fact, only those with similar relative abundances between 5 and 20 m (*P. grandis*, and haplotype 11 and 2: Johnston et al., 2022) hosted symbionts from genera other than *Cladocopium*. For example, *Durusdinium glynnii*, a heat-tolerant host generalist (LaJeunesse et al., 2010), was detected in five haplotype 11/2 colonies. *D. glynnii* forms stable, long-term associations with hosts that are often found in highly variable environments (Innis et al., 2018; LaJeunesse

et al., 2010; McGinley et al., 2012; Rouzé et al., 2019; Sawall et al., 2014). Interestingly, haplotypes 11/2 experienced the greatest bleaching mortality following the bleaching event at Mo'orea in 2019 (Burgess et al., 2021), thus the association with *D. glynnii* in the survivors sampled here could reflect the outcome of selective mortality or symbiont shuffling following this event. Furthermore, the decreased thermal tolerance of haplotypes 11/2 may relate to their unique association with *C. latusorum* Clade III (Figure 5), reduced fitness from introgression among hosts (Kim et al., 2018; Muhlfeld et al., 2009), or the interplay between both (Miller et al., 2010). Similarly, four *P. grandis* colonies contained 12%–91% *Symbiodinium* spp. This genus contains both free-living and obligate symbionts, and generalist species found in many tropical environments and in a diversity of hosts such as jellyfish, giant clams, and other coral genera (LaJeunesse, 2017). Some taxa are highly infectious and

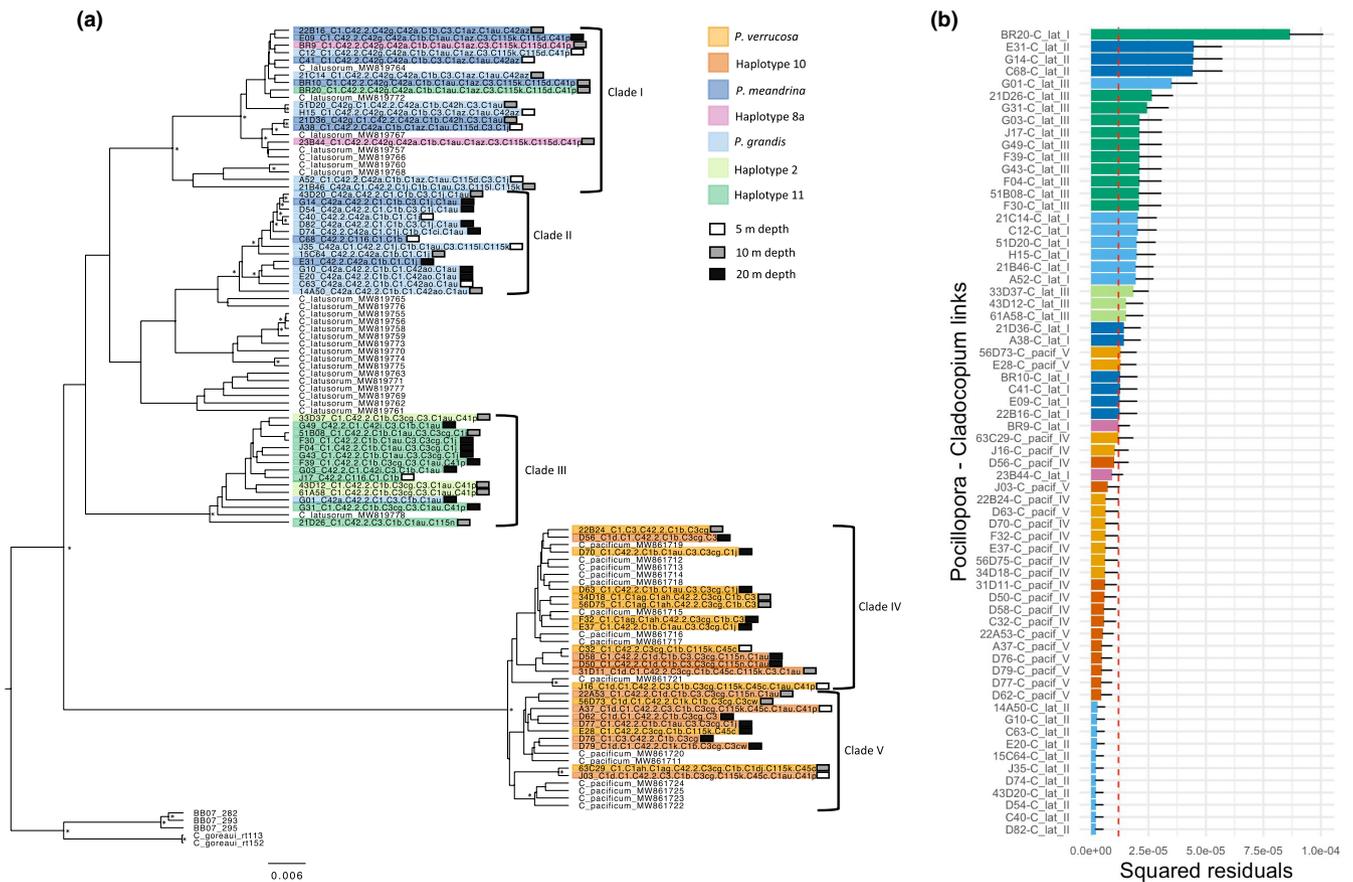
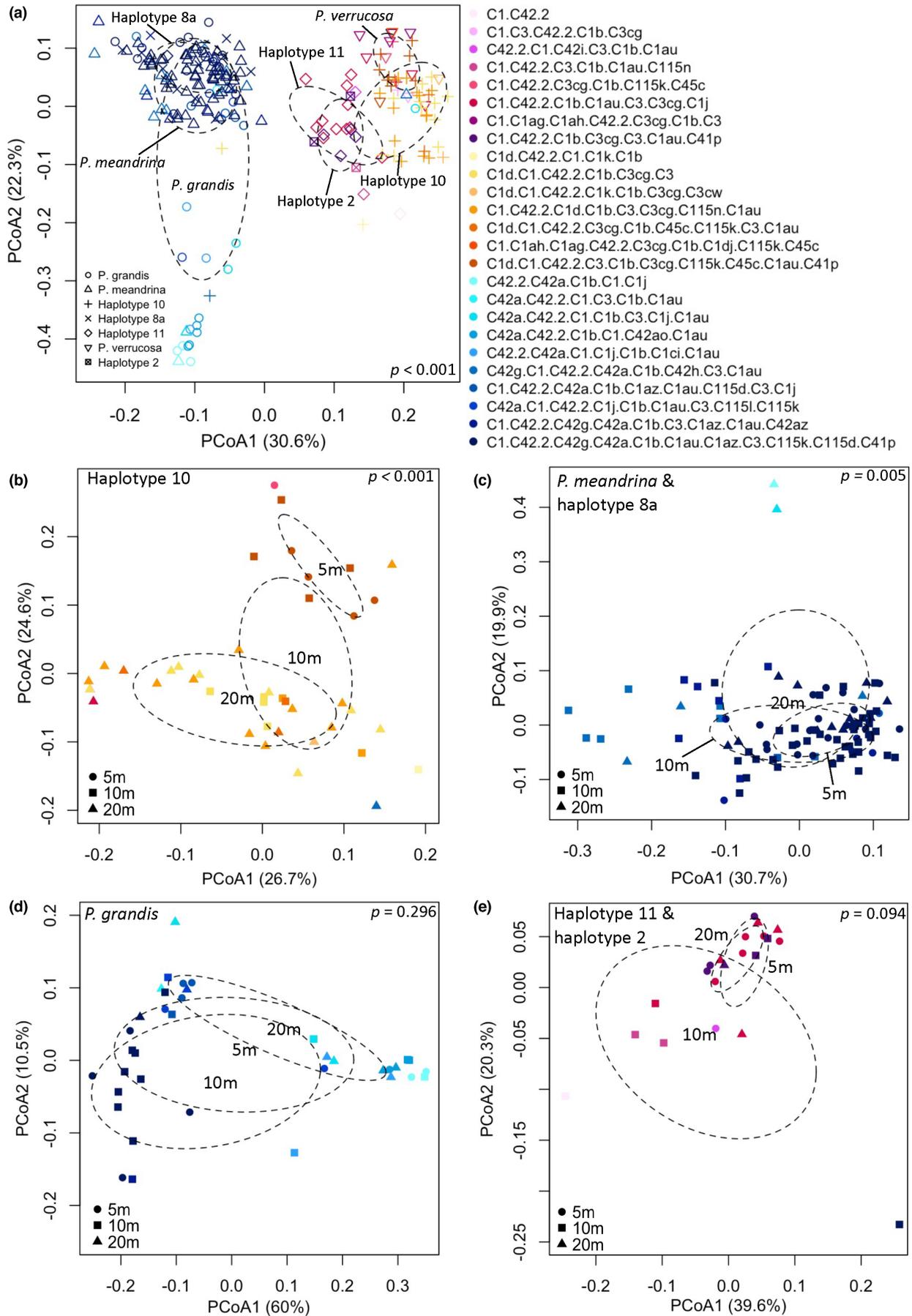


FIGURE 5 *Cladocopium* psbA Bayesian tree and PACo procrustean analysis of host symbiont links. (a) *Cladocopium* psbA Bayesian tree that includes 66 samples from this study (those identified in colour by *Pocillopora* species/haplotype). Depth of collection is indicated to the right of taxa labels. Taxa labelled as BB07 are from Pinzon and LaJeunesse (2011) and all other taxa are from Turnham et al. (2021). (b) Contributions of individual *Pocillopora* host–*Cladocopium* symbiont links to the procrustean fit. Jackknifed squared residuals (bars) and upper 95% confidence intervals (error bars) resulting from applying PACo to symbiont distances. The *Pocillopora* species/haplotype each psbA sequence was extracted from are indicated by the bar colour. The median squared residual value is shown (red dashed line).

FIGURE 6 Principal coordinate analyses of Symbiodiniaceae ITS2 sequence diversity for (a) *Pocillopora* species and mtORF haplotypes sampled from the fore reefs of Mo'orea, and then across depth for (b) haplotype 10, (c) *P. meandrina* and haplotype 8a, (d) *P. grandis*, and (e) haplotypes 11/2. Symbols are coloured by ITS2 type profile and PERMANOVA results are presented in the top or bottom right corner of each plot.



	df	Sum Sq	Pseudo-F	R ²	p-value
<i>Pocillopora</i> species/haplotype	6	1.294	18.469	.356	<.001
Residuals	201	2.346		.644	
Haplotype 10 by depth	2	0.068	3.247	.143	<.001
Residuals	39	0.408		.857	
<i>P. meandrina</i> and haplotype 8a by depth	2	0.039	2.426	.051	.005
Residuals	91	0.732		.949	
<i>P. grandis</i> by depth	2	0.045	1.160	.062	.296
Residuals	35	0.676		.938	
Haplotype 11 and haplotype 2 by depth	2	0.018	1.535	.146	.095
Residuals	18	0.106		.854	

Note: Boldface indicates statistical significance with $p < .05$, p -values based on 99,999 permutations.

Abbreviations: df, degrees of freedom; Pseudo-F, F value by permutation; Sum Sq, sum of squares.

TABLE 1 PERMANOVA results for ITS2 sequence diversity for *Pocillopora* species and haplotypes, and for *Pocillopora* species and haplotype across depth, when only colonies hosting *C. latusorum* and *C. pacificum* were included. *Pocillopora verrucosa* was not included in depth analyses because of low sample size

when attracted to certain hosts, can swarm in high densities next to these available hosts (Yamashita et al., 2014). In the sea anemone, *Exaiptasia pallida*, colonization by *S. microadriaticum* was found to be akin to a form of parasitism in which there is no apparent benefit to the host (Gabay et al., 2018). It is interesting that *Symbiodinium* was only detected in *P. grandis*. In the Red Sea, *P. verrucosa*, increasingly hosts *Symbiodinium* with decreasing latitude, indicating that there may be some benefit to this symbiosis with increasing sea water temperature (Sawall et al., 2014). But whether the *Symbiodinium* taxa hosted in *P. grandis* at Mo'orea is the same as that hosted in the Red Sea is not known. Additionally, whether this symbiosis with *P. grandis* is long-lasting or brief is not known, nor whether this *Symbiodinium* spp. is more attracted to *P. grandis* than other *Pocillopora* species at Mo'orea. Alternatively, the *Cladocopium* lineages hosted by *P. grandis* may be poorer competitors with this *Symbiodinium* spp. than those hosted by other *Pocillopora*, or the immune system of *P. grandis* may be less able to detect this *Symbiodinium* spp.

A higher rate of sexual reproduction within a *Cladocopium* species or clade could lead to greater potential for adaptation to different environments. Recently, cytological evidence of reproduction of *C. latusorum* in *P. meandrina* was found to be of mixed mode; mainly asexual with sexual reproduction occurring in <1%–5% of cells *in hospite* (Figueroa et al., 2021). While there is some evidence that sexual reproduction in free-living Symbiodiniaceae may be triggered by stress, such as nutrient deficiency (Pfiester, 1989), the triggers of sexual reproduction in symbiotic Symbiodiniaceae, such as *C. latusorum* (Fujise et al., 2021), are not known. These triggers may be both biotic and abiotic, differ by *Cladocopium* species or lineage, and/or be controlled by the host. The depth differences we detected in the symbiont ITS2 sequence diversity and type profiles within *Pocillopora* haplotype 10 may indicate the potential for adaptive divergence in *C. pacificum*, as hypothesized in other coral species (Bongaerts et al., 2011, 2013).

By genetically identifying species of both *Pocillopora* and Symbiodiniaceae, our analysis overcomes a major hurdle that has

prevented tests of coevolution and cospeciation in corals and their symbiotic algae. For example, we can also confirm that haplotype 10 (Forsman et al., 2013) is a distinct *Pocillopora* species that warrants formal identification. Although haplotype 10 hosted the same algal species (*C. pacificum*) as its sister species *P. verrucosa* (haplotypes 3a, 3b, 3f, and 3h), it tended to host a different psbA^{ncr} clade and ITS2 type profile. Haplotype 10 and *P. verrucosa* are both more common at 20m than 5m, though haplotype 10 is far more abundant than *P. verrucosa* at all depths at Mo'orea (Johnston et al., 2022). Furthermore, geographic sampling of *Pocillopora* to date indicates that haplotype 10 may be endemic to French Polynesia while *P. verrucosa* is widely distributed from the Tropical Eastern Pacific to the Red Sea and Arabian Gulf (Forsman et al., 2013; Gélín et al., 2017; Mayfield et al., 2015). As a result, we hypothesize that haplotype 10 diverged in sympatry from *P. verrucosa*, and that this is reflected in some of the genetic divergence in *C. pacificum* lineages among these two *Pocillopora* species.

Pocillopora haplotypes showing evidence for introgression and similar ecology also exhibited similar *Cladocopium* composition. In contrast to Johnston et al. (2017), in which colonies were sampled from across the Pacific and where evidence of hybridization was found only between the youngest *Pocillopora* species (the brooders *P. acuta* and *P. damicornis*), here we found evidence of introgression in multiple branches of the phylogeny in co-occurring *Pocillopora* corals from Mo'orea, French Polynesia. Genomic data indicated fewer species than mitochondrial data. Because mitochondrial evolution proceeds more slowly than nuclear evolution in anthozoans (Shearer et al., 2002), our genomic analysis suggests relatively recent introgression between *P. meandrina* (mitochondrial haplotype 1a) and haplotype 8a, and between haplotypes 11 and 2, even though these haplotypes resolved as reciprocally monophyletic in our genomic analysis. Our finding of introgression between *P. meandrina* (mitochondrial haplotype 1a) and haplotype 8a also contrasts with Gélín et al. (2017) who designated haplotype 1a and haplotype 8a as distinct primary species hypotheses (PSH) using mostly

TABLE 2 Pairwise comparisons of ITS2 sequence diversity for *Pocillopora* species and haplotypes across depth. ITS2 sequence diversity analyses include sequences when only colonies hosting *C. latusorum* and *C. pacificum* were included

	5–10m				5–20m				10–20m						
	df	Sum Sq	Pseudo-F	R ²	p-value	df	Sum Sq	Pseudo-F	R ²	p-value	df	Sum Sq	Pseudo-F	R ²	p-value
Haplotype 10	1	0.025	2.872	.161	.032	1	0.056	5.061	.153	.001	1	0.020	1.888	.051	.069
Residuals	15	0.131	.839	.839		28	0.309		.847		35	0.376		.949	
<i>P. meandrina</i> and haplotype 8a	1	0.015	2.078	.027	.054	1	0.018	2.310	.059	.028	1	0.026	2.771	.039	.015
Residuals	76	0.533	.973	.973		37	0.295		.941		69	0.636		.961	
<i>P. grandis</i>	1	0.012	0.592	.021	.63	1	0.016	0.934	.047	.357	1	0.039	1.975	.076	.106
Residuals	27	0.548	.979	.979		19	0.331		.953		24	0.474		.924	
Haplotype 11 and haplotype 2	1	0.012	1.380	.121	.192	1	0.003	1.090	.083	.347	1	0.012	1.841	.116	.082
Residuals	10	0.085	.879	.879		12	0.034		.917		14	0.093		.884	

Note: Boldface indicates statistical significance with $p < .05$, p -values based on 99,999 permutations. Abbreviations: df, degrees of freedom; Pseudo-F, F value by permutation; Sum Sq, sum of squares.

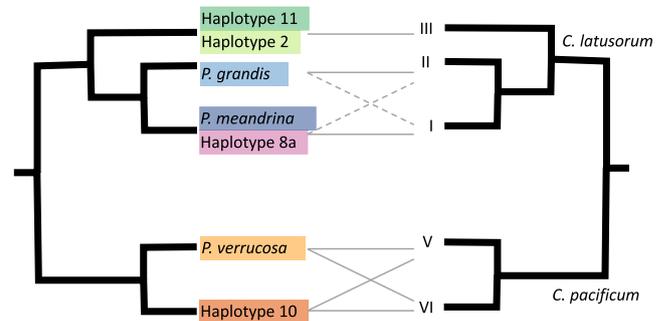


FIGURE 7 Schematic summary of *Pocillopora* host–*Cladocopium* symbiont cophylogeny, based off analyses shown in Figure 6. Dashed lines indicate minor links between host and symbiont clades reflecting greater flexibility.

mitochondrial markers (Figure 3). Similarly, we found no evidence that haplotype 3f (PSH 16) is a distinct species from haplotypes 3a, 3b, 3h (PSH 13) using nuclear and mitochondrial genomes (compare with Gélin et al., 2017). Furthermore, despite a lack of divergence in the mitochondrial genome in *P. meandrina* and *P. grandis*, these species are distinct, confirming previous findings (Johnston et al., 2017). Similarly, mitochondrial data suggests that haplotype 2 is ancestral to haplotype 11 and *P. ligulata*. Haplotype 2 is geographically widespread, found from Clipperton Atoll in the Tropical Eastern Pacific to Madagascar in the western Indian Ocean, but is relatively rare throughout this range (Gélin et al., 2017; Marti-Puig et al., 2014; Pinzón et al., 2013) and at Mo'orea (Burgess et al., 2021). In contrast, haplotype 11 has, to date, only been sampled from French Polynesia (Burgess et al., 2021; Forsman et al., 2013; Johnston et al., 2022). Given the basal placement of haplotype 2 in the mitochondrial phylogeny, and their respective geographic distributions, we hypothesize that haplotype 11 and *P. ligulata* diverged from haplotype 2 in isolation. However, the nuclear introgression observed between haplotypes 11 and 2, and the lack of differentiation in symbionts hosted, suggest that there is some gene flow between these haplotypes at Mo'orea. At Clipperton Atoll, haplotype 2 hosts a yet to be described species that is different from that hosted in Mo'orea, which is neither *C. latusorum* nor *C. pacificum*, that is closely related to *C. goreauii* (Figure 5a; taxa beginning with BB07) (Pinzon & LaJeunesse, 2011). Whether there are fitness impacts when interspecific crosses occur between *Pocillopora* that host different algal species, such as the failure to transmit the algal symbiont or reduced symbiont growth if transmitted, is not known. Given the emerging evidence, the mosaic of *Pocillopora* species distributions, and thus respective interactions, is probably evolutionarily complex across time and space, giving rise to lineages and species following a pattern of reticulate evolution, as has been hypothesized for many other coral species (Arrigoni et al., 2016; Diekmann et al., 2001; Veron & Stafford-Smith, 2000).

AUTHOR CONTRIBUTIONS

ECJ and SCB designed the study; ECJ and SCB collected samples from the field; ECJ did the laboratory work; RC did the ITS2

sequencing; RC contributed analytical tools and data interpretation; ECJ and SCB analyzed the data; ECJ and SCB wrote the first draft of the manuscript. All authors contributed to the final version of the manuscript and approved it for publication.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

OPEN RESEARCH BADGE



This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at <https://doi.org/10.5281/zenodo.6710608>.

DATA AVAILABILITY STATEMENT

All data and R code used to create the plots can be found at <https://doi.org/10.5281/zenodo.6710608>. All raw sequence data can be accessed under NCBI BioProject PRJNA852278: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA852278>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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