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Natural frequencies of hybridization in Caribbean reef-building corals

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Research Article

Keywords: Coral reef restoration, Caribbean, hybridization, adaptive introgression

Posted Date: September 1st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10683541/v1

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Additional Declarations: No competing interests reported.

Abstract

While hybridization and introgression are widespread across the tree of life, the frequencies by which they occur remain relatively poorly documented, even among those foundation species that generate and maintain ecosystems. Corals in the genera *Acropora* and *Orbicella* have produced the vast majority of shallow-water coral biomass in the Caribbean basin since the onset of the Pleistocene and are currently threatened by multiple global and local stressors. The natural frequencies of within-genera hybridization are unknown, which limits our capacity to guide basin-wide efforts to manage remaining stands and restore coral diversity. Of 744 unique *Acropora* genets collected at 42 reefs within the eastern and northern Caribbean prior to a massive die-off in 2023 and 2024, 81% were pure species lineages, while the remainder were mostly F1/F2 hybrids and fewer backcrossed hybrids. Of 1,049 unique *Orbicella* genets collected at 47 reefs, 86% were pure *Orbicella* species, while F1/F2 hybrids dominated the remainder. Thus, the presence of pure species lineages and locally-produced, cryptic hybrids across the Caribbean basin indicate that species boundaries in these genera are maintained despite long-standing hybridization, and provide a baseline for efforts to restore the Caribbean reef-builders and their genetic diversity.

Significance: Because of the collapse of Caribbean coral reef populations in recent decades, millions of dollars are being invested in restoring populations of the reef-building corals *Acropora* and *Orbicella* across massive spatial scales. Our results have two important implications for these efforts. First, at a practical level, our study reveals cryptic hybrids that are unfortunately difficult to identify morphologically either within coral nurseries or the field. Second, by showing that naturally occurring hybrids are common and geographically widespread, our study challenges the assumption that restoration efforts should focus exclusively on pure species lineages and provides an empirical target for pure and hybrid diversity that can be used in conservation planning.

Introduction

Coral reefs are centers of marine biodiversity, provide crucial ecosystem services and functioning, and are impacted by an expanding array of local and global threats, including overfishing, pollution, habitat destruction, disease, and climate change (Mumby and Steneck 2008; Bellwood et al. 2019; Vega Thurber et al. 2025). These threats are especially evident within the Caribbean. In particular, the two coral genera *Acropora* and *Orbicella* that produced more coral biomass than any other coral genera since the onset of the Pleistocene (Pandolfi et al. 2002; Pandolfi and Jackson 2006; Jackson and O'Dea 2023) have suffered severe basin-wide declines (Gardner et al. 2003) and functional extinction in some areas (Manzello et al. 2025). In response, millions of USD are now being poured into the restoration of coral genetic diversity and ecological functioning of these two reef-building genera across the Caribbean (Hughes et al. 2023; Muller et al. 2025).

Most reef-building corals, including *Acropora* and *Orbicella*, have the opportunity to hybridize and thereby alter local ecological and evolutionary dynamics (Willis et al. 2006; Arnold and Fogarty 2009). In a

conservation setting, coral hybridization has yielded strong debate over the degree to which resources should explicitly prioritize hybrid lineages (Richards and Hobbs 2015; Chan et al. 2019; Grupstra et al. 2024; Riginos et al. 2024). However, despite the evolutionary and conservation interest, the frequency and dynamics of their hybridization on natural reefs remain surprisingly poorly described.

There are two extant Caribbean *Acropora* (Oken, 1815; F. Acroporidae Verrill, 1901), both of which are critically endangered. *Acropora palmata* (Lamarck, 1816; Elkhorn coral) and *cervicornis* (Lamarck, 1816; Staghorn coral) have fossil records that date between approximately 6.6 and 3.6 million years ago (Fig. 1A; (McNeill et al. 1997; Budd and Johnson 1999; Vollmer and Palumbi 2007; Precht et al. 2019). A morphologically - intermediate coral *A. prolifera* (Fused Staghorn coral) was initially described as a separate species, has a fossil record that dates back 125,000 years at a minimum (Precht et al. 2019), and contains genetic signatures of backcrosses in a relatively limited sampling of field colonies (Oppen et al. 2000; Vollmer and Palumbi 2002, 2007; Kitchen et al. 2019, 2022; Palumbi et al. 2012). While it has been argued that F2 *Acropora* corals have not been observed in the field (Locatelli et al. 2024), the evidence is largely based on studies with relatively few samples (Kitchen et al. 2019), a sampling protocol that targeted pure species lineages apriori (García-Urueña et al. 2022; Kitchen et al. 2022; Bright et al. 2024; Baums et al. 2026), or low-resolution genetic markers that may not robustly identify the full spectrum of hybrid status (Oppen et al. 2000; Vollmer and Palumbi 2002; Palumbi et al. 2012; Japaud et al. 2019; see Discussion in Oppen et al. 2000; Fogarty 2012; Hughes et al. 2023).

The Caribbean *Orbicella* (F. Merulinidae Milne Edwards & Haime, 1857; Budd et al. 2012) include *annularis* (Lobed Star coral; Ellis & Solander, 1786), *faveolata* (Mountainous Star coral; Ellis & Solander, 1786) and *franksi* (Boulder Star coral; Gregory, 1895). Fossil records indicate that species complex dates to at least 6.5 million years ago and that 3–5 species co-existed at any given timepoint. Modern-day *faveolata* diverged from other lineages approximately 3–4 million years ago, while *annularis* and *franksi* diverged much more recently (0.5 mya; (Pandolfi et al. 2002; Fig. 1B). There is mixed evidence on whether these species potentially hybridize (Fukami et al. 2004; Fukami and Knowlton 2005; Levitan et al. 2004, 2011; Szmant et al. 1997; Fogarty et al. 2012a). Importantly, we lack evidence for rates of hybridization and backcrossing from natural field samples in *Orbicella*, in part because few species-specific genetic markers are available (Levitan et al. 2011).

Here, we collected and genotyped single nucleotide polymorphisms (SNPs) using restriction-site associated DNA sequencing (RADseq) of 2,060 colonies from the Dry Tortugas, Florida and United States Virgin Islands (Figure S1; Table S1) in *Acropora* (n = 42 reefs) and *Orbicella* (n = 47 reefs). We then assessed clonal diversity, lineage diversification, and the hybridization frequencies to help fill guide debate over maintenance and restoration of coral diversity.

Results

Clonal diversity

Out of the 910 *Acropora* individuals successfully sequenced, we detected 744 unique genotypes; the remaining 166 individuals were clonal replicates. We found a range of 1 to 18 clonal replicates per unique genotype for morphospecies *A. palmata* and 1 to 13 for *A. cervicornis* (Figure S5) and genotypic richness per population of 87.4 ± 3.6% and 83.4 ± 4.3%, respectively (mean ± S.E.; Figure S6). Of the 14 *prolifera* colonies sequenced from a single location in the Dry Tortugas (Florida), 12 were unique (85.6%). Rarefaction curve analysis of all *Acropora* combined indicated that we rarely sampled the full genotypic diversity at these sites, with the possible exception of a single population in Florida (Figure S6). Out of 1070 *Orbicella* individuals sequenced, we detected 1051 unique genotypes; the remaining 19 individuals were clonal replicates. We found a range of 1 to 7 clonal replicates per unique genotype for the morphospecies *annularis* and 1 to 2 for *franksi* (Figure S5). All *faveolata* were unique. The genotypic richness per population of 97.1 ± 1.7% for *annularis*, and 99.7 ± 0.3% for *franksi*, and rarefaction curve analysis indicated we did not sample the full genotypic diversity per site (Figure S7).

Species divergence and hybridization - Acropora

A maximum-likelihood phylogeny reveals that field-identified *A. prolifera* morphospecies occurred at an intermediate position between the pure *cervicornis* and *palmata*, suggesting an F1 hybridization event for these samples (Fig. 1A). Pairwise Euclidean genetic distances between pure individuals within each species was 3.7% for *palmata*, 6.0% for *cervicornis*, 4.5% for *prolifera* while between-species divergence was at least five-fold greater (29.8%; Figure S8; Table S2).

In total, 81% of *Acropora* genets were pure *cervicornis* or *palmata*, and approximately 19% (143) of the 744 unique *Acropora* genotypes were F1/F2 hybrids or backcrosses (Table 1; Fig. 2C). A large fraction of hybrids had an intermediate hybrid index, consistent with F1/F2 hybrids (69, or 48%). Hybridization is geographically-widespread, as they were collected from 28 of the 35 reefs with at least 10 unique *Acropora* genets sequenced (Figure S11A). There were significantly more hybrids per unique genet in the Dry Tortugas (mean ± SE = 57.6% ± 17.8; n = 4) than in the USVIs (16.0% ± 3.1; n = 31; $F_{1,33} = 16.13$; $p < 0.001$).

Rates of field-misidentification differed by morphospecies (Table 1). Of 462 *palmata* morphospecies, 96% were genetically confirmed, while < 1% were F1/F2 hybrids and 3% were *palmata* backcrosses. Of 270 *cervicornis* morphospecies, 56% were genetically confirmed, 3% were *palmata*, 20% were F1/F2 hybrids, 13% were *cervicornis* backcrosses and 8% were *palmata* backcrosses. Hybrids tend to look *cervicornis*-like even when substantial amounts of *palmata* nuclear background are present (Fig. 1A).

The 12 field-identified *prolifera* collected in Florida are an approximately 50:50 mix of both genetic lineages, as indicated by PC (Fig. 2A) and admixture analysis (Figure S9). Using a hybrid index, 11 of these 12 were confirmed as F1/F2 hybrids and 1 was a *palmata* backcross. Interpopulation ancestry (Q10; Figure S12B) also suggests that F1 hybrids are reproducing with themselves (creating F2 crosses) and several generations of backcrosses with both parental lineages.

Mitochondrial lineages (sensu Kitchen et al. 2022) are unevenly distributed across *cervicornis* and *palmata* nuclear backgrounds (Fig. 3). Pure and backcrossed *cervicornis* were dominated by the *cervicornis* mitotype (97 and 100%, respectively, or 33 of 34 total). In contrast, pure *palmata* lineages had 61% (89/145) *palmata* mitotypes and 61% (11/18) of *palmata*-backcrossed genets had *palmata* mitotypes. F1 hybrids were evenly split between *cervicornis* and *palmata* mitotypes (7 and 5, respectively, or 58% *cervicornis*).

A genomic cline analysis (sensu Gompert et al. 2017; Pulido-Santacruz et al. 2018) provides an indication of how ancestry-informative markers, or AIMs, are introgressed across individuals with varying hybrid indices. The centers and gradients of genomic clines at 113 AIMs have similar centers and linear slopes in allele frequency (Figure S12A), indicating introgression consistent with neutral processes rather than selection at these SNPs (Gompert et al 2017).

Species divergence and hybridization - Orbicella

A maximum-likelihood phylogeny reveals a separation among all three *Orbicella*, with *O. faveolata* as the ancestral lineage with a split from two descendant sister taxa *O. franksi* and *O. annularis* (Fig. 1B). Pure species had within-species pairwise Euclidean genetic distance of 6.8%, 7.5% and 8.6%, respectively (Figure S8). Genetic distance between pure *faveolata* versus *annularis* and *franksi* was 14.8% and 14.6%, respectively, which is approximately 2-fold greater than within faveolata (Table S2). Genetic distance between the *annularis* and *franksi* was 10.4%, which is comparable to distances within these species.

The first axis of the principal components analysis (PC1) explained 17% of the divergence among genotypes and splits *faveolata* from the other two *Orbicella* species (*annularis* and *franksi*), which were split at the 2nd PC axis (4.0%; Fig. 3A). An admixture of k = 2 also splits *faveolata* from the other species (Figure S10) and admixture analysis of k = 3 shows the split between the three species (Fig. 3B). We note that PC1 and PC2 correlated strongly with admixture lineages (k = 3; see Methods).

Of the 1051 *Orbicella* samples collected, 13% (133) were hybrids between *faveolata* and the other two species (*franksi* and *annularis*) and most genets had a hybrid index consistent with F1/F2 hybrids (63, or 47%; Table 1, Fig. 3C). In contrast, we detected very few hybrids of the sister taxa *franksi* and *annularis* (10 of 1051, or < 1% of all samples); note the bimodal distribution along PC2 between pure *franksi* and *annularis* in the PC analysis with few individuals between these extremes (Fig. 3A). Cryptic hybrids were geographically widespread, as they were sequenced within 34 of the 40 reefs with at least 10 unique *Orbicella* genets (Figure S11B). There was no difference in the hybrids per unique genet between the Dry Tortugas (mean ± SE = 7.4% ± 2.2; n = 11) than in the USVIs (11.3% ± 1.4; n = 29; $F_{1,38} = 2.28$; $p = 0.140$).

The misidentification of pure *Orbicella* lineages was common across all three morphospecies (Table 1). Only 66% of field-identified *annularis* was confirmed genetically, while 71% of *franksi* and 80% of *faveolata* morphospecies were confirmed genetically. Cryptic hybrids were slightly more frequent among *annularis* morphospecies (25%) relative to the hybrid frequency among *franksi* (13%) and *faveolata* morphospecies (7%). Eight of the 10 *franksi-annularis* hybrids we detected were field-identified as

annularis. The centers and gradients of the genomic clines at 230 AIMs that define *faveolata* versus *annularis/franksi* (Figure S12B) are relatively similar.

Symbiont communities

We detected differences between coral genera in the proportion of *Symbiodiniaceae* clades (Fig. 5). *Acropora* species are dominated by *Brevicolum* (mean and S.E. across all individuals = 55.3% ± 0.8%) and *Symbiodinium* (38.6% ± 0.8%). *Orbicella* species tended to be dominated by *Cladocopium* (66.9% ± 1.1%), with some *Brevicolum* (25.8% ± 1.0%), although a large proportion of *annularis* colonies were dominated by *Brevicolum. Orbicella* individuals had proportionately more heat-tolerant *Durusdinium* (6.7% ± 0.6%) than did *Acropora* individuals (0.6% ± 0.1%). In general, visual inspection indicates that pure and hybrid lineages within genera did not consistently differ in their symbiont frequencies.

Discussion

Most *Acropora* colonies (80.8%) were pure *cervicornis* or *palmata* while most *Orbicella* were pure *faveolata, annularis* or *franski* (86.4%). The strongly bimodal distributions in the hybrid indices in both genera indicate strong post-zygotic barriers to reproduction with or without pre-zygotic barriers (Gompert et al. 2017; Pulido-Santacruz et al. 2018). Simultaneously, *Acropora* and *Orbicella* hybrids and backcrosses are geographically widespread and relatively frequent (i.e., 19% and 14%, respectively) on northern and eastern Caribbean coral reefs. These estimates represent the most comprehensive survey of hybridization using high-resolution genetic markers in either reef-building genera. We address the implications of these patterns for our understanding of the ecology and evolution of the two genera, as well as for the basin-wide resource-intensive effort to manage and restore Caribbean reefs.

Acropora

The mechanisms maintaining species boundaries and minimizing even more rampant hybridization in *Acropora* remain unclear. *Acropora cervicornis* and *palmata* have broadly sympatric distribution, few barriers to fertilization, and no clear temporal differentiation in spawning times, nor hybrid inviability. It is possible (Fogarty 2012; Fogarty et al. 2012b) hybridization is inversely density dependent; when parental species were at high densities, as they were throughout the Pleistocene and until the last several decades (Cramer et al. 2020), conspecific fertilization may have been more frequent than heterospecific fertilization events. If true, then modern reefs may be witnessing greater hybridization frequencies with declines in density.

Our findings are consistent with laboratory studies that crossed F1 hybrids and *cervicornis* (Fogarty et al. 2012b; Fogarty 2012) outplanted the F2 larvae after settlement and survirval was comparable to or higher than survivorship of conspecific *palmata* or *cervicornis* (Fogarty 2012), suggesting F2s are viable and maybe even favored in some habitats. The evolutionary mechanism driving post-zygotic barriers

among these two lineages remains unknown. Our finding that female-derived *cervicornis* mitotype are more common than *palmata* mitotypes in hybrids with *palmata* nuclear background, is also consistent with laboratory evidence of more promiscuous *cervicornis* than *palmata eggs* (Fogarty et al. 2012b), generating greater *cervicornis* mitochondrial introgression into *palmata* nuclear backgrounds than the reverse. We note that an alternative is that *cervicornis* mitotype might have been the ancestral condition, in which case the patterns reflect incomplete lineage sorting.

Our results indicate that field identification of pure and hybrid lineages in *Acropora* is potentially misleading. While field-identification of *palmata* was accurate (i.e., 96% was genetically confirmed), field-identified *cervicornis* was confirmed at a relatively low rate (56%). This is largely because hybrids and backcrosses have are cryptic and have *cervicornis*-like morphologies. Similarly, Kitchen et al. 2024 also report that genetically confirmed hybrids were more likely to be identified as *cervicornis* than *palmata*. It is tempting to interpret this morphological asymmetry as the consequence of the maternally inherited genome, given the strong asymmetry in mitochondrial introgression. This hypothesis awaits testing however.

Our analysis of genomic clines of 113 ancestral-informative markers (AIMs) suggest that introgression of these loci reflects neutral evolutionary processes. It is possible or even likely that our survey did not detect AIMs that were strongly divergent and also under selection, as has been tentatively suggested by other studies with either fewer loci (Palumbi et al. 2012) or samples (Kitchen et al. 2019). Evidence for such “speciation” genes will require a more comprehensive survey of the genome and exhaustive natural sampling regime than is currently available.

Orbicella

The mechanisms maintaining species boundaries and minimizing rampant hybridization are similarly unclear in *Orbicella* as in *Acropora*, though our results are broadly consistent with some laboratory and field data. Levitan et al. (2004) suggested that *faveolata* gametes are largely incompatible with *franksi* and *annularis*, while (Szmant et al. 1997) concluded no strong pre-zygotic barrier to cross-fertilization among these crosses. The very low frequency of *franksi* and *annularis* hybrids in the field (< 1%) may be largely maintained by differences in spawning time (i.e., *franksi* spawns approximately two hours earlier; Levitan et al. 2011; Fogarty et al. 2012a; Gómez-Corrales et al. 2025) since *franksi* and *annularis* gametes are compatible. The evolutionary mechanism driving post-zygotic barriers among these three lineages remains unknown.

An alternative explanation for the frequency of hybrids that we detected is that we may have mis-identified hybrids between *annularis* and *franski* because the parents have a relatively high frequency of ancestral polymorphisms that did not sort since the separation from *faveolata*. Indeed, the magnitude of the divergence among all three species is relatively small, relative to the divergence among individuals within these species (Table S2) and the AIMs that we analyzed had a relatively modest allele frequency difference (i.e, > 0.6). The presence of a binomial distribution of hybrid index as well as a seeming

enrichment of F1/F2 hybrids (i.e., HI at approximately 0.5; Fig. 4c) is consistent with recent hybridization and is unlikely to be explained by (a lack of) lineage sorting.

As with *Acropora*, our analysis of genomic clines of ancestral-informative markers (AIMs) suggest that these have a center and slopes that are similar, and thus largely reflect neutral rates of introgression. It is possible or even likely that our survey did not detect AIMs that were more strongly divergent and also under selection. Recent genomic evidence indicates (Gómez-Corrales et al. 2025) molecular signals of positive selection in proteins that are associated with “chemo/photo/thermo-reception” a genomic region that we likely did not recover with our SNP survey.

Symbiont community

Different genera host different Symbiodiniaceae communities, as has been seen previously (Thornhill et al. 2006; Cunning et al. 2024), and our data were broadly consistent with those previous studies. *Acropora* was dominated by *Breviolum* and *Symbiodinium*. This is somewhat surprising, given *cervicornis* (Thornhill et al. 2006; Baums et al. 2010; Lirman et al. 2014; Cunning et al. 2021) and *palmata* (Thornhill et al. 2006) tend to be dominated by *Symbiodinium. Orbicella* was dominated by *Cladocopium* and *Brevicolum*; previous studies sometimes show high variation in symbiont communities (Garren et al. 2006; Thornhill et al. 2006; Cunning et al. 2024), but these two symbionts are consistently recorded among *annularis* (Thornhill et al. 2006; Edmunds et al. 2014; Kennedy et al. 2015), *faveolata* (Thornhill et al. 2006; Aguilar et al. 2024; Cunning et al. 2024; Klein et al. 2024) and *franksi* (Thornhill et al. 2006; Cunning et al. 2024). With few exceptions, hybrids and pure lineages held largely similar communities within genera. *Orbicella* was more likely to have greater frequency of the heat-tolerant *Durusdinium* than was *Acropora* but neither genus was dominated by the genus (6.7 vs 0.6%, respectively). This suggests that our sampling occurred after recovery from heat-stress events or sampling within benign habitats, as heat and bleaching tends to increase *Durusdinium* frequencies (Baker 2003).

Implications for coral restoration

While pure species lineages dominated Caribbean reefs, locally-produced, cryptic hybrids were present across the Caribbean basin. These results indicate that species boundaries are maintained despite long-standing hybridization, and provide an important baseline for basin-wide efforts to restore reef-builders and their genetic diversity. The central dogma of conservation genetics is that ideal sources for local reef restoration are local populations of pure species lineages, as this minimizes the potential for outbreeding depression and accidental co-invasion of parasites. Alternatively, these risks may be overstated, or at least, less influential on genetic diversity than is inbreeding depression in those cases in which genetic diversity is already exceedingly low (Edmands 2007; Frankham 2015). Indeed, given the magnitude of the coral extinction crisis, recent coral restoration practioners have suggested transplantation of Caribbean conspecific populations 1000s of kilometers away (Baker et al. 2025; Muller et al. 2025; Humanes et al. 2026) or different coral species from the Pacific Ocean (Camacho et al. 2026).

Our results expand this debate to include hybrids. Specifically, we argue that the relatively high frequency and geographically widespread nature of hybrids means that restoration should explicitly include hybrids, or at the least, not use precious resources to minimize hybridization. While there are worthy reservations about scenarios in which hybrid genomes swamp pure species lineages (Willis et al. 2006; Chan et al. 2019; VanWynen et al. 2021), hybrids of these two genera are commonplace and have not already swamped pure lineages. Moreover, hybridization may be already providing an adaptive role, introgressing genes into pure lineages for the past several million years (Grupstra et al. 2024).

Methods

Collection

In June and July 2021, and May thru August 2022, we collected coral tissue from across the United States Virgin Islands and Dry Tortugas, Florida USA (Figure S1; Table S1). At each location, we targeted 10–20 individuals at less than 10m depth from five morphospecies (*Acropora cervicornis*, *A. palmata*, *O. faveolata*, *O. annularis*, and O. *franksi*). A small number of the hybrid *A. prolifera* morphotype were also collected when available. Individual corals of the same species were collected at least 1m apart. Each coral was geolocated (using a floating GPS unit) and photographed. We hammered a plastic tag on the reef substrata near each coral colony if it was not already tagged by other researchers. Samples were preserved in 90–95% ethanol or a DMSO-EDTA preservative (Gaither et al. 2011), shipped to the College of Charleston, and stored at 4º or -20ºC. We extracted *Acropora* samples with Machery-Nagel Nucleospin Blood and Tissue kit using manufacturer's instructions. We extracted *Orbicella* samples with a dispersion buffer/phenol-chloroform-isoamyl alcohol extraction protocol, followed by a Zymo DNA Clean and Concentrator Kit following manufacturer's protocols (Sturm 2020). DNA quality and quantity were evaluated visually with a 2% agarose gel electrophoresis.

Genotyping and filtering SNPs

Acropora and *Orbicella* RADseq libraries were created following (Parchman et al. 2012). We digested gDNA with two restriction enzymes, EcoRI and MseI, and ligated adaptors containing unique 8 to 10 bp barcodes to the digested DNA of each individual. The products were then PCR amplified in two independent reactions with standard Illumina primers. All amplicons were pooled and shipped to the University of Texas Genomic Sequencing and Analysis Facility or the Tufts University Core Facility, which used Pippin Prep® to isolate the 300–450 bp fraction. This fraction was then single-read sequenced (SE150) with Illumina Novaseq. We used custom scripts to demultiplex into sample-specific FASTQ-formatted files. When individuals were run within multiple libraries or sequencing runs, we combined all data per individual into a single FASTQ file per individual. Demultiplexed reads were aligned to the *Acropora palmata* genome (Locatelli et al. 2024) or *Orbicella faveolata* genome (Young et al. 2024) with bwa mem (Li and Durbin 2010) using default settings. Samples with at least 250K aligned reads were used to identify SNPs with MAF > 0.01 and default settings with samtools/bcftools 1.0 (Danecek et al. 2021), and SNPs with less than one read across 50% of individuals were removed. We analyzed

genotype likelihoods at 121,388 SNPs across 910 *Acropora* individuals and at 146,311 SNPs across 1070 *Orbicella* individuals.

We identified clonal ramets using ngsRelate (Korneliussen and Moltke 2015), as defined by a threshold Hedrick's Rab (0.85 for *Acropora*; 0.90 for *Orbicella*) individuals. This threshold was based on the bimodal distribution of values (Figure S2) following (Arnaud-Haond et al. 2007). We calculated Rab for *Acropora* with SNPs that had greater read depth than in the initial SNPset (ie., at least one read across 75% of individuals, yielding 92277 SNPs) and used the initial SNPset (146,311) to calculate Rab for *Orbicella*. We calculated genotypic richness as the number of unique genets divided by the number of individuals sequenced. We also made rarefaction-based sampling estimates of richness using vegan (Oksanen et al. 2024) and calculated eMLG, or the expected number of MLG at the lowest common sample size per population. This per-population eMLG metric and genotypic richness were highly correlated in *Acropora* (Pearson's Correlation coefficient = 0.960; $p < 0.0001$; df = 37) and *Orbicella* (Pearson's Correlation coefficient = 0.995; $p < 0.0001$; df = 39). We then haphazardly chose one ramet of each genotype for subsequent analyses. After re-filtering SNPs with clonal repeats removed, we generated genotype likelihoods at 105,518 SNPs for *Acropora* for 744 individuals and 170,680 SNPs for *Orbicella* for 1051 individuals.

Defining pure lineages and putative hybrids

We generated principle components and admixture analyses on genotype likelihoods using PCangsd (Meisner and Albrechtsen 2018) and ngsAdmix (k = 2–10; (Skotte et al. 2013) using default settings. We used these to identify pure and putatively hybrid individuals in each genus using thresholds that were created after visual inspection of the 1st PC axis and admixture lineage. For *Acropora*, the 1st PC axis strongly correlated with a k = 2 admixture lineage (Pearson's correlation coefficient = 0.997; df = 742; $p < 0.001$; Figure S3C) and thresholds are visualized in Figure S3A-B. *Orbicella* is a three-species complex, with one ancestral species (*faveolata*) and two sister descendent taxa (*annularis* and *franksi*) (Fig. 1). The 1st PC axis, which separates *faveolata* from the others, correlated with the one of the three admixture lineages (Pearson's correlation coefficient = 0.997, df = 1049; $p < 0.001$, Figure S4C). We created thresholds to separate *faveolata* from *annularis/franksi* pure lineages after visual inspection of the distribution of each parameter (Figure S4B-C). We then removed these pure *faveolata* and putative *faveolata*-(*annularis*/*franksi*) hybrids, and used PC2 axis and a 2nd admixture lineage to assess pure *annularis*, pure *franksi* and *annularis-franksi* hybrids. PC2 and the two admixture lineages were strongly correlated (Pearson's correlation coefficient > 0.99; df = 487, $p < 0.001$ for both; regression not shown).

Maximum likelihood phylogenies

To create maximum likelihood phylogenies for pure *Acropora* genets (*cervicornis, palmata*, and field-identified *prolifera*), we used the SNPs that had at least one read in 80% of individuals, and removed samples with fewer than 15 reads per locus per individual. We were left with 480 individuals, and used vcftools (Danecek et al. 2011) to identify those SNPs with < 5% missing genotype calls (25,842). A fasta-formatted file was generated using a custom Python code (Ortiz 2019). We generated a ML tree with

iQtree, a GTR_80 model of evolution, and 1000 fast bootstrap replicates ((Trifinopoulos et al. 2016). There were 16767 parsimony-informative and 2722 singleton sites used for this analysis. ML estimates of pairwise Euclidean genetic distances among individuals were used to assess within and between species divergence.

We repeated the protocol for pure *Orbicella* genets (*faveolata*, *annularis*, and *franksi*) with few exceptions. We used the SNPs that had at least one read in 75% of individuals, and removed samples with fewer than 75 reads per locus per individual, leaving 359 individuals. We then filtered SNPs with < 15% missing genotype calls (69,773), leaving 51016 parsimony-informative and 8963 singleton sites used for this analysis.

Hybrid index and genomic cline analysis

To generate per-individual hybrid index (or HI, the proportion of the genome inherited from one lineage), genomic clines (i.e., introgression of alleles across the hybrid index), and interpopulation ancestry (or Q10), we ran *bgchm* (Gompert et al. 2024) on genotype likelihoods of putative hybrids. We first identified ancestry-informative markers (or AIMs), which we defined as SNPs with an allele frequency difference (or δ) greater than 90% between pure *Acropora* genets and 60% between pure *faveolata* and the *annularis/franksi* lineage. We explored a range of δ used to define AIMs (δ = 0.7–1.0 for *Acropora* and δ = 0.6–0.75 for *Orbicella*). In general, the hybrid index was less affected by changes in δ than was interpopulation ancestry (analyses not shown), as predicted by (Wiens et al. 2025). This yielded 113 AIMs for *Acropora* and 230 AIMs for *Orbicella* to calculate a per-individual HI, Q10, and genomic clines. The modest number of AIMs are comparable to those used in other studies (Pulido-Santacruz et al. 2018; Gompert et al. 2024; Wiens et al. 2025). We did not pursue an analysis for *annularis-franksi* hybrids because the low number (n = 10) precluded robust analysis.

For *Acropora*, the per-individual hybrid index was closely correlated to an admixture Q proportion (df = 181, Pearson's correlation coefficient = 0.899; $p < 0.001$), and with PC1 (df = 181, Pearson's correlation coefficient = 0.912, $p < 0.001$). For *Orbicella*, the per-individual hybrid index was closely correlated to one of the admixture Q lineage (k = 3; Pearson's correlation coefficient = 0.824, df = 131, $p < 0.001$), and with PC1 (Pearson's correlation coefficient = 0.876, df = 131, $p < 0.001$). We used HI to define pure lineages (i.e,. HI > 0.9 and HI < 0.1), F1/F2 hybrids (i.e,. HI between 0.4 and 0.6), and backcrosses (i.e, the remainder) in subsequent analyses.

Acropora Mitochondrial lineages

To assess whether mitochondrial lineages in our samples were from *A. cervicornis* or *palmata*, we aligned fastq files that had at least 250k reads to a mitochondrial *A. cervicornis* genome (MW246490; Kitchen et al. 2019) using bwa, made genotype calls, and used *bcftools consensus* on the individual vcf-formatted files to create mitochondrial sequences per sample. We excluded samples that had less than 1000 bp aligned to the mitochondria. Individual samples were then aligned to each of 15 mitochondrial genomes (Kitchen et al. 2022); three mitochondrial genomes haphazardly chosen from three

haplogroups of *A. palmata* and from two haplogroups of *A. cervicornis*. After filtering out sites with missing data, we calculated pairwise genetic distances using default parameters in ape::dist.dna (Paradis and Schliep 2019). We inferred a mitotype if the sample was least divergent from one species and most divergent from a different species; if the species was identical, then we excluded that sample from final analysis. We confirmed results for a subset of samples with parsimony trees generated with phangorn::pratchet (Schliep 2011) using default parameters. We fit a binomial regression to assess whether mitotype (*cervicornis* or *palmata*) depended on the hybrid index.

Symbiont community assemblage

We assessed the symbiont community using our RADseq dataset following (Manzello et al. 2019). We aligned all reads using default parameters in bowtie2 (Langmead and Salzberg 2012) to the four major Caribbean lineages (LaJeunesse et al. 2018) concatenated into a single FASTA-formatted file: *Symbiodinium microadriacticum*, formerly Clade A (Aranda et al. 2016); *Breviolum minutum* (Shoguchi et al. 2013), formerly Clade B; *Cladocopium goreaui* (Chen et al. 2022), formerly Clade C1; and *Durusdinium trenchii* (Dougan et al. 2024), formerly Clade D. We used samtools idxstat to summarize results and kept samples if the number of aligned reads was greater than 1000 (*Acropora*, n = 617; *Orbicella*, n = 1037). These RADseq-based assessments of symbiont communities strongly correlate with those generated by a traditional metabarcoding approach (Eckert et al. 2024).

Declarations

Data sharing plans

All raw genomic data will be uploaded to GenBank. Datasets and code at GitHub will be archived at Zenodo.

Funding information

Funding was provided by the National Park Service (BUIS/DRTO/SARI/VICR/VIIS PMIS # 264595) and the National Science Foundation (OCE-1924599; DEB-2344085).

Author Contribution

E.S. prepared figures and wrote the main manuscript text. The results were collected by all authors. All authors reviewed the manuscript.

Acknowledgements

Scientific research and collection and export permits were provided by the Government of the Virgin Islands Endangered species (DFW21045U) and US Department of Interior (VICR-2021-SCI-0002, VIIS-

2021-SCI-0012, SARI-2021-SCI-0004 and BUIS-2020-SC1-0001). We thank Jenna Charydczak, Eric Wehmeyer and Rebecca Hauser for help with laboratory work, and Ryan Eckert and Joshua Voss for help with DNA extraction of *Orbicella*. The author(s) would like to acknowledge the 1S10OD032203-01 (Tufts University Core Facility Genomics Core) for their support of DNA sequencing.

Data Availability

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Tables

Tables are available in the Supplementary Files section.

Figures

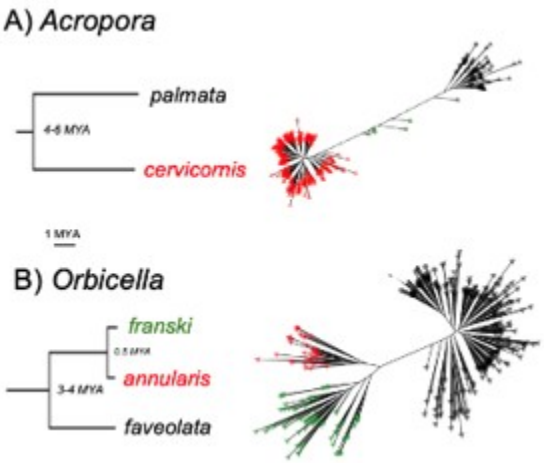


Figure 1

Phylogeny of Caribbean (A) *Acropora* and (B) *Orbicella* species. Topology and estimated divergence times are based on mitochondrial sequence and fossil evidence (Palumbi et al. 2012, Fukami and Knowlton 2005). A maximum likelihood topology (100% consensus) of SNPs of pure species is shown. Hybrids are removed except for five individuals that were field-identified (and confirmed using SNPs) as the F1 *Acropora prolifera* (green). Triangles and circles indicate samples collected in US Virgin Islands and Florida, respectively.

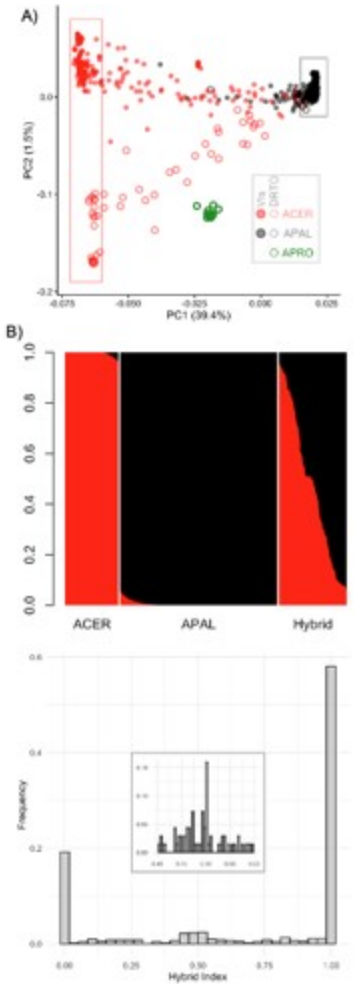


Figure 2

Acropora hybridization patterns. A) Principal components analysis. Symbols indicate collection at Dry Tortugas Florida (DRTO) or the US Virgin Islands (VIs). Colors are morphospecies*cervicornis* (ACER), *palmata* (APAL) and *prolifera* (APRO). B) admixture analysis, where colors are based on k=2 genomic clusters. C) Distribution of hybrid index calculated from ancestry-informative markers (AIMs; 113 loci) for all samples (0 = ACER; 1 = APAL), and putative hybrids (inset). Boxes in A indicate criteria for identifying pure individuals of each species; the samples outside of boxes were putative hybrids (n = 143).

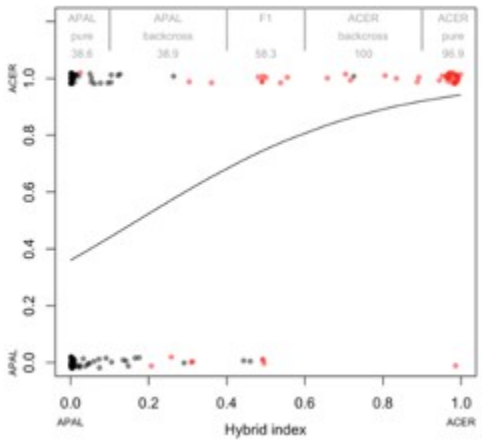


Figure 3

Acropora introgression plotted against their hybrid index. The black line is a binomial regression fit ($p<0.001$). Proportion of individuals that have an ACER (*A. cervicornis*) mitotype is indicated for pure, backcross and F1 genomic backgrounds. Colors indicate the morphospecies *A. cervicornis* (ACER; red) or *A. palmata* (APAL; black)

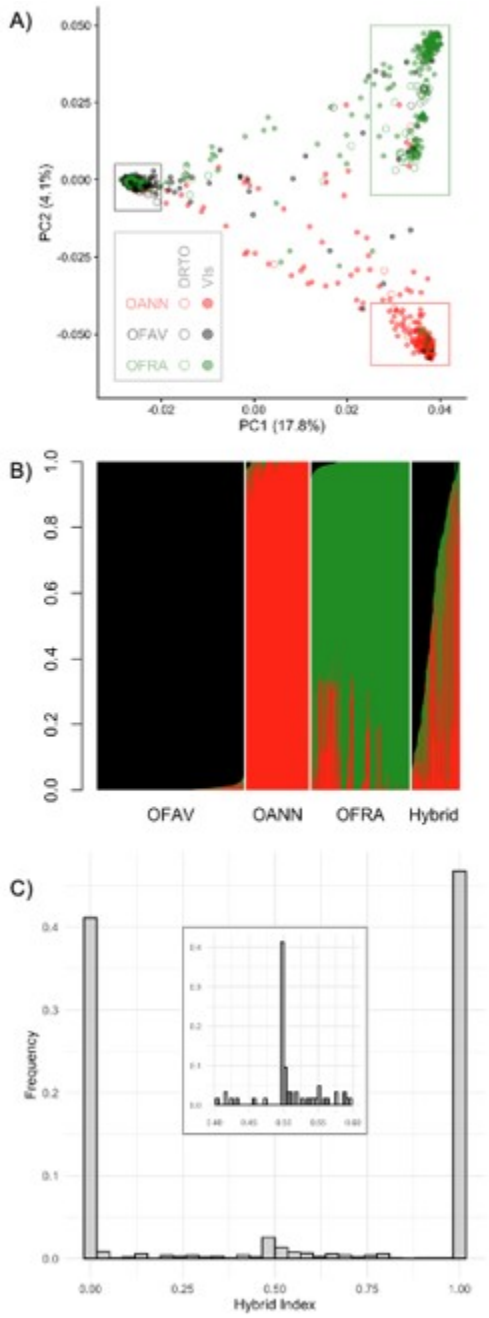


Figure 4

Orbicella hybridization patterns. A) Principal components analysis. Symbols indicate collection at Dry Tortugas Florida (DRTO) or the US Virgin Islands (VIs). Colors indicate the morphospecies *annularis* (OANN), *faveolata* (OFAV), and *franksi* (OFRA). B) admixture analysis, where colors are based on k=3 genomic clusters. C) Distribution of hybrid index calculated from ancestry-informative markers (AIMs; 230 loci) for all samples (0 = ACER; 1 = APAL), and putative hybrids (inset). Boxes in A indicate criteria for identifying pure individuals of each species; the samples outside of boxes were putative hybrids (n = 143).

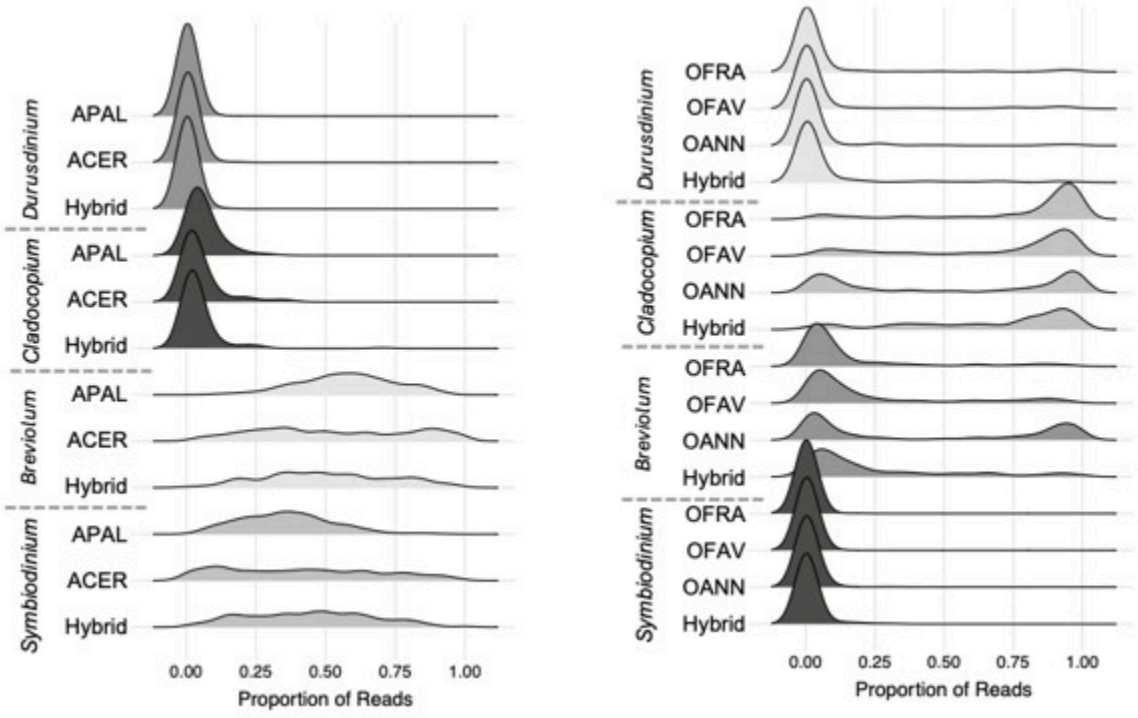


Figure 5

Proportion of Symbiodineaceae genera across *Acropora* (left) and *Orbicella* (right) individuals. The pure vs hybrid assignments were made using PC and NGSadmix. ACER, APAL and APRO refer to *A. cervicornis*, *A. palmata* and *A. prolifera,* and *OANN, OFAV and OFRA refer to O. annularis, O. faveolata, and O. franksi.*

Supplementary Files

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- SupplementalNEE.pdf
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