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Genetic structuring and species boundaries in the Atlantic stony coral *Favia* (Scleractinia, Faviidae)

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Abstract

Scleractinian corals are the main modern builders of coral reefs, which are major hot spots of marine biodiversity. Southern Atlantic reef corals are understudied compared to their Caribbean and Indo-Pacific counterparts and many hypotheses about their population dynamics demand further testing. We employed thousands of single nucleotide polymorphisms (SNPs) recovered via ezRAD to characterize genetic population structuring and species boundaries in the amphi-Atlantic hard coral genus *Favia*. Coalescent-based species delimitation (BFD* – Bayes Factor Delimitation) recovered *F. fragum* and *F. gravida* as separate species. Although our results agree with depth-related genetic structuring in *F. fragum*, they did not support incipient speciation of the 'tall' and 'short' morphotypes. The preferred scenario also revealed a split between two main lineages of *F. gravida*, one from Ascension Island and the other from Brazil. The Brazilian lineage is further divided into a species that occurs throughout the Northeastern coast and another that ranges from the Abrolhos Archipelago to the state of Espírito Santo. BFD* scenarios were corroborated by analyses of SNP matrices with varying levels of missing data and by a speciation-based delimitation approach (DELINEATE). Our results challenge current notions about Atlantic reef corals because they uncovered surprising genetic diversity in *Favia* and rejected the long-standing hypothesis that Abrolhos Archipelago may have served as a Pleistocenic refuge during the last glaciations.

KEYWORDS

Bahia, cryptic species, DAPC, incipient speciation, mid-Atlantic ridge, SNAPP

1 | INTRODUCTION

Shallow-water coral populations span more than 3000 km of the Brazilian coast, forming the only true coral reefs of the South Atlantic, harbouring 21 species of symbiotic scleractinian corals, four of which are endemic (Leão et al., 2016). Unlike Caribbean and Indo-Pacific reefs, which are more diverse and dominated by corals with branching morphologies, species with massive morphologies dominate Brazilian reefs (Leão et al., 2003). The unique coral assemblages of the Southwestern Atlantic inhabit an environment that could be considered challenging for other scleractinians (Castro et al., 2012; Leão et al., 2003), such as the inshore shallow reefs of the Eastern coast of Brazil, which are heavily affected by riverine sediment input (Bastos et al., 2015; Dominguez, 2009; Vieira et al., 2019). These river deltas may limit the dispersion of reef organisms.

For instance, the São Francisco, Jequitinhonha and Doce rivers possibly restrict the geographic distribution of *Millepora* spp. (de Souza et al., 2017) and different clades of Symbiodiniaceae associated with *Mussismillia hispida* are distributed according to distinct reef habitats limited by river mouths, which might be acting as ecological barriers (Picciani et al., 2016).

Favia gravida Verril, 1986 is one of these tolerant species, frequently found in shallow intertidal pools, but occasionally in deeper reefs up to 15 m (Leão et al., 2003; Teschima et al., 2019). While this species has high morphological plasticity both within and between populations (Amaral & Ramos, 2007; Laborel, 1970), *F. gravida* and its sister species, the Caribbean endemic *Favia fragum* (Esper, 1793), differ with respect to the completeness of septal cycles (*F. gravida* always presents four complete septal cycles, whereas in *F. fragum* the fourth cycle is usually incomplete—Figure S1). However, many of the morphological characters used to differentiate these two species are highly variable, and some qualitative traits are subjective, for example, '[...] somewhat longer series [...], more widely spaced septal teeth' (Budd et al., 2012), which further complicates species identification. At the other end of the population–species continuum, some scleractinian coral experts do not even recognize them as separate species, despite their disjoint geographic distributions (e.g. Veron, 2000).

Information on the dispersal potential and the connectivity patterns within and between these species is fragmentary. Although presenting multiple events of larval release per year, the (brooded) larvae of *Favia* remain in the water column only for a short period and are ready to settle within 24–48 h after release (Calderon et al., 2000; Carlon & Olson, 1993; Lewis, 1974). Because these larvae have a philopatric dispersal behaviour, both species are presumably unable to commonly exchange recruits with distant populations, reducing gene flow and potentially leading to isolation (Jones et al., 2009; Warner et al., 2016). Nunes et al. (2011), using the intron and exon of β-*tubulin* and the intron of *PaxC*, and Teschima et al. (2022), employing ITS and *masc* nDNA, recovered significant levels of genetic differentiation among *F. gravida* populations in the South Atlantic and Goodbody-Gringley et al. (2010), also based on ITS and β-*tubulin*, showed strong genetic structuring among populations of *F. fragum* in the Caribbean Sea. Furthermore, evidence of habitat partitioning and incipient speciation between two morphotypes of *F. fragum* across a depth gradient in the Bocas del Toro region of Panama (Carlon & Budd, 2002), coupled with a mating system characterized by high rates of self-fertilization (Brazeau et al., 1998; Carlon & Lippe, 2011) indicates that genetic structuring in the species may occur even on small geographic scales, suggesting a high potential for isolation-by-distance and perhaps cryptic speciation.

The taxonomy of Scleractinia is traditionally rooted in skeletal traits, which present a high degree of inter- and intraspecific variation (Todd, 2008). In closely related taxa with overlapping quantitative traits, the selection of diagnostic characters may have a subjective component (Budd & Klaus, 2001; Fujita et al., 2012) and environmentally mediated plasticity of those traits complicates the matter even further (Dubé et al., 2017; Johnston et al., 2017; Ow & Todd, 2010; Paz-García et al., 2015). Changes in morphological characters do not always coincide with the process of lineage divergence, creating a 'grey zone' in species delimitation (de Queiroz, 2007). For example, molecular and morphological approaches to species delimitation come into conflict when genetically distinct lineages have no identifiable phenotypes (i.e. cryptic species) or high morphological disparity but no significant genetic structure (Bickford et al., 2007; Fišer et al., 2018). Both scenarios are rife among hard corals, responsible for building one of the most diverse ecosystems on the planet, coral reefs (Moberg & Folke, 1999). As an attempt to produce more reliable estimates of species limits, model-based species delimitation using genomic data has become increasingly popular (Leaché et al., 2014; Smith & Carstens, 2020; Yang & Rannala, 2010). Coalescent-based approaches, for example, are not dependent on diagnostic characters and reciprocal monophyly to access species status, bypassing the problems highlighted previously (see Fujita et al., 2012). Furthermore, advances in sequencing technology and analytical improvements enabled biologists to work with high-density genomic markers and thus obtain reconstructions with higher phylogenetic resolution. One of these sequencing methods, restricted-associated DNA sequencing (RAD-Seq), consists of a set of protocols that uses restriction enzymes to provide a reduced representation of a given genome (see Andrews et al., 2016 for a review). In reef-building corals, the discovery of cryptic diversity using high-density genomic markers has shown that cryptic speciation might be pervasive in the group (Arrigoni et al., 2020; Gijsbers et al., 2022; Gómez-Corrales & Prada, 2020; Wepfer et al., 2020), a trend that studies based on traditional morphological characters were not able to identify. These results illustrate the complexity of coral taxonomy and species boundaries and highlight the need for a more rigorous evaluation of the species limits in the Atlantic *Favia* using genomic data. These molecular approaches also have practical implications for conservation efforts: accurate estimation of species richness and distribution is essential to the management and protection of these pivotal ecosystems (van Oppen & Coleman, 2022).

In this study, we examine patterns of population differentiation and species boundaries in Atlantic *Favia* spp.

according to single nucleotide polymorphism (SNP) data obtained through ezRAD, a variation of RADseq (Toonen et al., 2013). We assessed the genetic population structuring of Caribbean *F. fragum* and Southeastern Atlantic *F. gravida*, including a population from Ascension Island, located in the mid-Atlantic Province, which is likely a divergent lineage based on a recent molecular study (Teschima et al., 2022). Considering the restricted larval dispersal of *Favia* spp. and their philopatric settlement behaviour, we expect to recover high genetic differentiation among these regions. Finally, considering previous morphological and genetic evidence (Carlon & Budd, 2002), we use these data to test a potential species boundary between two Panamanian *F. fragum* ecotypes.

2 | MATERIALS AND METHODS

2.1 | Sampling

We selected individuals from eight populations of *Favia gravida*, seven from the Brazilian coast and one from Ascension Island, and two populations of the Caribbean *Favia fragum*, from the Bocas del Toro region of Panama, corresponding to the two different ecotypes identified by Carlon and Budd (2002) (Figure 1, Table 1). Samples of these two ecotypes, named Tall and Short based on their distinctive corallite morphologies, were the same as those analyzed at microsatellite loci by Carlon and Lippe (2011). At this site, the two ecotypes were completely isolated by habitat: the Tall ecotype occurred in shallow seagrass habitat (<1.5 m depth), while the Short ecotype occurred in a slightly deeper reef habitat (3–5.0 m depth) (Table 1). Colony fragments were collected by snorkeling and/or SCUBA diving. Coral tissue samples were preserved in DMSO solution (Gaither et al., 2011), in chaos solution (Fukami et al., 2004) or 90% alcohol and stored at −20°C until extraction.

2.2 | Molecular analysis

We extracted DNA from tissue samples of *F. fragum* using the Omega E.Z.N.A Tissue DNA kit and Invitrogen PureLink Genomic DNA kit in the case of *F. gravida*, following the manufacturers' instructions. Extractions were purified using 1.8× AmPureXP magnetic beads. DNA quality was assessed via electrophoresis in 1.5% agarose gel, ensuring that only high molecular weight DNA was carried over to the digestion step. Quantification was performed using a Qubit 2 Fluorometer and the dsDNA High Sensitivity Assay kit.

Libraries were prepared following the ezRAD protocol (Toonen et al., 2013). Briefly, samples were digested using the enzyme DpnII (New England Biolabs), in 50 μL reactions containing 5 μL DpnII NEB 10× Buffer, 2 units of DpnII and 200–1000 ng of DNA. Digestions were incubated at 37°C for 3 h, then heat-inactivated for 20 min at 65°C, purified using 1.8× AMPureXP beads, run in 1.5% agarose gels and considered successful if producing a smeared band. Library preparation began with the KAPA HyperPrep Library kit (Roche Sequencing Store) following Knapp et al. (2016) with minor modifications. DNA samples were end-repaired and a-tailed, and then adapter ligation was performed using IDT xGen Stubby Adapters and Unique Dual Index (UDI) primer pairs. These index-ligated products were size selected with Mag-Bind Magnetic Beads (Omega Bio-Tek) targeting fragments in

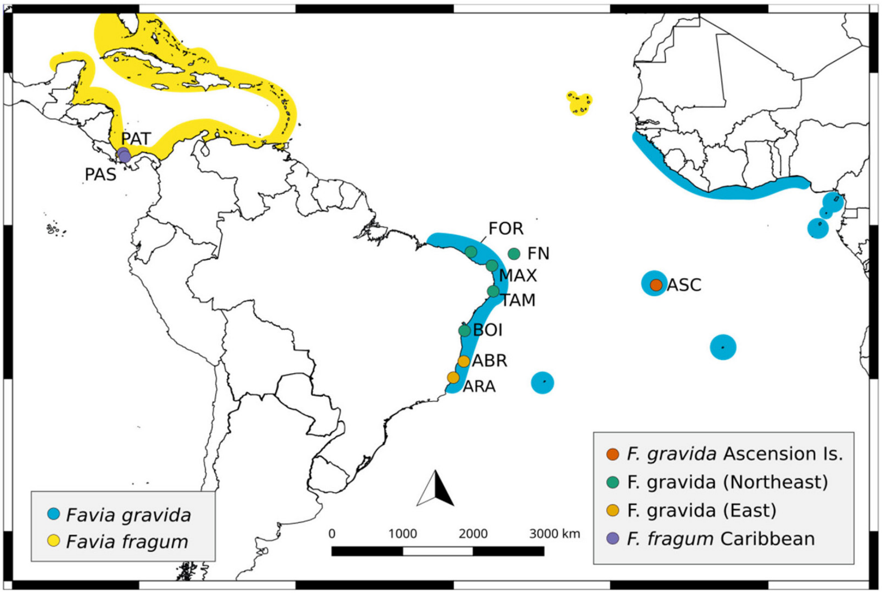


FIGURE 1 Geographic ranges of *Favia fragum* and *F. gravida* modified from Aronson et al. (2008). Circles corresponding to sampling sites are colour-coded according to clusters obtained in the DAPC and sNMF analyses. Sampling sites on the map are coded according to Table 1.

TABLE 1 Geographic distribution and number of samples (*N*) of *Favia* sequenced in the present study. Sampling site codes are used in figures and text.

Species	Region	Sampling site	N	Depth (m)	Code	Lat, long
F. fragum	Caribbean	Hospital Bight, Panama, reef, (Short morphotype)	10	3.0–5.0	PAS	9.30 N, 82.14 W
		Hospital Bight, Panama, seagrass, (Tall morphotype)	8	1.0	PAT	9.30 N, 82.14 W
F. gravida	Brazil	Fortaleza, Ceará	8	3.0–4.0	FOR	3.71 S, 38.82 W
		Fernando de Noronha Archipelago, Pernambuco	10	3.0–5.0	FN	3.85 S, 32.44 W
		Maxaramguape, Rio Grande do Norte	8	0.5–2.0	MAX	5.39 S, 35.25 W
		Tamandaré, Pernambuco	10	0.5–2.0	TAM	8.73 S, 35.08 W
		Boipeba, Bahia	11	0.5–4.0	BOI	13.58 S, 38.82 W
		Abrolhos Archipelago, Bahia	9	8.0–15.0	ABR	18.20 S, 38.78 W
		Aracruz, Espirito Santo	2	0.5–1.5	ARA	20.01 S, 40.15 W
	Mid-Atlantic Ridge	Ascension Island	9	–	ASC	7.93 S, 14.36 W

a 350–700 bp range in two steps, with DNA:bead ratios of 1:0.6 and 1:0.2, respectively. Samples were amplified using 6–10 PCR cycles with KAPA HiFi Hotstart Ready-mix (Omega Bio-Tek) and purified using 1:1 DNA:AMPure XP beads. Libraries were validated using Qubit dsDNA HS kit, Agilent 2100 Bioanalyzer and qPCR, and then pooled and sequenced as paired-end (2×150 bp) reads on the Illumina HiSeq 4000 sequencer at the Research Technology Support Facility (RTSF) Genomics Core, at Michigan State University.

2.3 | Bioinformatics

We assessed raw read quality with FASTQC (Andrews, 2010) and subsequent processing was performed with the bioinformatic pipeline dDocent (Puritz et al., 2014). This pipeline is faster, has a smaller memory footprint and yields loci with better sample coverage than other widely used RADseq pipelines (Jungwirth, 2017). First, universal adapters and reads with Phred <30 were excluded from further analyses. We randomly chose three individuals from each population to create the de novo assembly reference onto which all samples would be mapped. We used dDocent scripts ReferenceOpt.sh and RefMapOpt.sh to select the optimal combination of parameters for the de novo assembly, aiming at maximizing the number of paired mapping reads while minimizing the number of mismatched reads. We set the minimum level of similarity among sequences in the same cluster, or clustering threshold (*c*), to 90%, the minimum within individual coverage level (*k*1) to 3 and the minimum number of individuals sharing a read (*k*2) to 4. Within dDocent, sequences from all individuals were mapped against the reduced representation reference and SNPs were called with FreeBayes (Garrison & Marth, 2012). We performed a local BLAST against complete genomes of *Breviolum*, *Cladocopium*, *Fugacium*, and *Symbiodinium* species in order to detect and remove endosymbiont contamination. A coral host dataset was created by *BLASTing* the assembled contigs against a scleractinian database assembled from 29 nuclear and 12 complete mitochondrial coral genomes, downloaded from GenBank (Table S1). We employed the program HS-BLASTN (Chen et al., 2015) with a threshold e-value set to 1e^{-05} in both analyses.

The resulting raw variant call files (VCF) were filtered using VCFTools (Danecek et al., 2011). We applied a minimum quality filter of 30 and a minimum mean depth of 3, then removed individuals with more than 55% missing loci. To assess the influence of missing data on the results we created three subsets with 0%, 10% and 20% missing loci. Finally, we removed indels, filtered out loci with minor allele frequencies lower than 0.01 and kept a single SNP per locus in downstream analyses.

2.4 | Genetic diversity

We estimated the number of private alleles, rarefied allelic richness and Nei's gene diversity (Nei, 1973) for all sampling sites with the R packages *hierfstat* (Goudet, 2005) and *poppr* (Kamvar et al., 2014). We checked for the presence of clones through identical multilocus genotypes (MLGs) using the R package *adegenet*. We also estimated Hamming distances to flag genotypes with exceptionally low divergence, indicating the same clonal lineage, using the R package *poppr*. Given the lack of sample replicates in our sequencing strategy, we applied a threshold of $d = 0.025$ to identify potential repeated genotypes (Reynes et al., 2021). To avoid bias due to variable sampling sizes, private alleles were estimated using random subsamples based on the smaller sample size, with ($n = 2$) and without ($n = 5$) the ARA sampling site (Table 2).

2.5 | Population structure

Population structure was assessed with two multivariate methods: (linear) discriminant analysis of principal components (DAPC) and non-negative matrix factorization (sNMF) using the R package LEA (Frichot & François, 2015). The implementation of DAPC in *adegenet* (Jombart & Collins, 2015) subjects the SNP matrix to a PCA and performs a discriminant analysis on a number of first principal components, chosen via cross-validation. We used two approaches to define the groupings for DAPC: supervised discrimination treating each sampling site as a population and a non-supervised approach in which the optimal number of populations was estimated by *k*-means clustering analysis. The sNMF approach estimates the genome fraction from each sample that originated from a certain number of gene pools, or putative ancestral populations (*k*). The ancestry coefficients were estimated for 1–10 ancestral populations and the optimal value of *k* was selected based on the cross-entropy criterion (CEC) after 100 replicates for each *k*-value. To detect fine-scale genetic substructure we also performed a separate analysis using only the Caribbean *F. fragum* samples, applying the same filtering parameters previously described.

We calculated the pairwise F_{ST} values according to Weir and Cockerham (1984) with the R package *hierfstat* (Goudet, 2005), and assessed the significance by estimating the 95% confidence intervals for each pairwise comparison. The existence of isolation by distance (IBD) was assessed with a Mantel test performed with the R package *vegan* (Dixon, 2003), comparing matrices of pairwise genetic ($F_{ST}/(1-F_{ST})$) and geographic distances based on 1000 permutations. The geographic distances in kilometers were approximated as the shortest distances connecting sampling sites by sea using Google Earth (Gorelick et al., 2017).

2.6 | Species delimitation

Alternative species boundaries were evaluated using the Bayes Factors Delimitation (BFD*) method of Leaché et al. (2014), a coalescent-based approach implemented in the software SNAPP (Bryant et al., 2012). This method uses path sampling to approximate the marginal likelihoods of competing species delimitation scenarios. Since

TABLE 2 Number of private alleles, mean allelic richness, and Nei's unbiased gene diversity values for Atlantic *Favia* spp. sampling sites.

Sampling sites	*N*	MLG	Priv. alleles ($N = 5$)	Priv. alleles ($N = 2$)	AL, 95% CI [LL; UL]	Gene diversity
PAS	8	8	69	77	1.223 [1.205; 1.241]	0.121
PAT	8	8	81	87	1.243 [1.224; 1.260]	0.118
ASC	6	6	178	134	1.220 [1.200; 1.238]	0.100
FOR	5	5	25	28	1.209 [1.191; 1.227]	0.101
FN	9	9	24	37	1.201 [1.184; 1.217]	0.100
MAX	7	7	15	25	1.202 [1.185; 1.218]	0.097
TAM	7	7	20	15	1.180 [1.163; 1.197]	0.084
BOI	9	9	10	10	1.212 [1.196; 1.228]	0.086
ABR	7	7	98	47	1.254 [1.236; 1.271]	0.126
ARA	2	2	–	78	1.263 [1.237; 1.288]	0.131

Note: Sampling sites are coded according to Table 1. Private alleles were estimated based on a random subsample corresponding to the smaller sample size with ($n = 2$) and without ($n = 5$) the ARA sampling site.

Abbreviations: AL, allelic richness; CI, confidence interval; LL, lower limit; MLG, number of multilocus genotypes; *N*, number of samples; UL, upper limit.

SNAPP is computationally intensive and sensitive to missing data, we used the dataset with 0% missing data from a subset of 21 individuals. We tested for the existence of species boundaries in the following scenarios: (a) Brazil, Ascension Island and Caribbean; (b) Brazilian samples were split between Northeastern and Eastern populations (see Section 3); (c) Caribbean samples were split between Tall and Short morphotypes; (d) Short morphotype population further subdivided according to the clusters recovered from sNMF and DAPC analyses (see Section 3); and (e) current taxonomy, that is, samples from Brazil and Ascension Island lumped as a single species (Table 3). SNAPP analyses were performed in BEAST v.2.4.5 (Bouckaert et al., 2014) with a path sampling run of 48 steps (MCMC length = 1000,000, pre-burnin = 10,000). We ranked alternative models based on their marginal likelihood estimate (MLE) and used these values to calculate corresponding Bayes factors (BF). Bayesian preference was scored according to the canonical scale of Kass and Raftery (1995).

We also conducted a speciation-based delimitation approach using the software DELINEATE (Sukumaran et al., 2021). The guide tree was obtained from two independent SNAPP runs performed on the *g coral* dataset and considering all individuals (MCMC length = 3000,000) using BEAST v.2.4.5 (Bouckaert et al., 2014). Results from both runs were combined and convergence was assessed with Tracer v.1.7 (Rambaut et al., 2018). Trees were summarized into a Maximum Clade Credibility Tree (MCCT) with a tree-annotator, with a 20% burn-in. We constrained Caribbean *F. fragum* and Ascension *Favia* as known separate species a priori, while allowing DELINEATE to infer species assignments for the Brazilian sub-populations, which were left unconstrained, with a cumulative probability threshold of 0.99.

3 | RESULTS

3.1 | Bioinformatics

A total of 85 individuals were successfully sequenced. FASTQ files had an average of 3.6 million 151 bp reads per library. After trimming and removal of low-quality reads, the dataset consisted of 1,654,067 raw SNPs, with an average sequencing depth of 18×. Unique contigs that blasted against the Symbiodiniaceae database were removed from further analyses, resulting in the *holobiont* dataset. The *coral host* dataset consisted of 862,411 unique contigs that successfully blasted against our local Scleractinian database. Seventeen samples with >55% missing loci were removed from further analyses. After the remaining filtering steps, we created subsets of the data with different amounts of missing data (Table 4), ranging from 998 to 21,691 SNPs.

Only 157 of our unique contigs BLASTed against endosymbiont genomes, the most common biological contaminant in coral samples. Regardless, there are no available reference genomes for Atlantic *Favia* or any other Atlantic Faviidae, and although some of the remaining contigs in the *holobiont* dataset might belong to coral DNA that was not represented in our local database, some might belong to other microorganisms that make up the holobiont. In order to provide a more reliable description of Atlantic *Favia* biology and population patterns, we will present the results from the *coral host* datasets in the following sections, consisting exclusively of reads that matched the BLASTed coral database. Results obtained from the *holobiont* dataset are shown in Appendix S1.

3.2 | Genetic diversity

We did not recover any duplicated multilocus genotypes (MLG). Furthermore, the distribution of pairwise distances among samples was continuous from 0.04 to 0.18 (Figure S2), above the threshold value ($d = 0.02$) chosen according to Reynes et al. (2021), suggesting the absence of clonality in our samples. Nei's unbiased gene diversity (expected heterozygosity) within sampling sites ranged from 0.08 in TAM to 0.13 in ARA. Mean allelic richness ranged from 1.18 in TAM to 1.26 in ARA. Ascension Island had the highest count of private alleles, while the sampling sites from the Northeast Brazilian population had the lowest counts.

TABLE 3 BFD* results for the alternative species delimitation hypothesis. MLEs were obtained via thermodynamic integration, using 48 steps. The model in boldface represents the preferred species delimitation hypothesis.

Species delimitation model	# of species	MLE	BF
A: Brazil, Ascension, Caribbean	3	−18,996.74	−102.74
B: BR Northeast, BR East, Ascension, Caribbean	**4**	**−18,945.37**	**0.00**
C: BR Northeast, BR East, Ascension, Short, Tall	5	−18,951.66	−12.58
D: BR Northeast, BR East, Ascension, Short1, Short2, Tall	6	−18,957.52	−24.3
E: Brazil+Ascension, Caribbean (current taxonomy)	2	−19,365.07	−839.4

Abbreviations: BF, Bayes factor; MLE, marginal likelihood estimate.

3.3 | Population structure

The sNMF analysis of the coral host dataset with no missing data (g) yielded the lowest resolution and recovered four distinct ancestral gene pools ($k=4$): *F. fragum* from Panamá, *F. gravida* from Ascension Island in addition to East (BR East) and Northeast (BR Northeast) Brazilian *F. gravida* populations (Figure 2a). The alignment with 10% missing data (g) provided enhanced resolution within the Brazilian coast, recovering three populations: BR East, three northeastern subpopulations, plus FOR and the FN grouped in a third population (Figure 2b). The least filtered alignment, with 20% missing data (g) resulted in equally low CEC values when k ranged from 4 to 7. This dataset recovered three individuals from the Short morphotype of the Caribbean *F. fragum* as a separate population. BOI samples were recovered as a separate population, however, it is noteworthy that three individuals exhibit significant signs of admixture with other populations. MAX and TAM samples were grouped together, and ABR and ARA were again recovered as a single subpopulation ($k=7$, Figure 2c).

TABLE 4 SNP count for each dataset containing different percentages of missing data.

		SNP count	
Dataset	**Missing data (%)**	**Holobiont**	**Coral host**
G8	20	21,691	11,820
G9	10	8617	3612
G10	0	1429	998

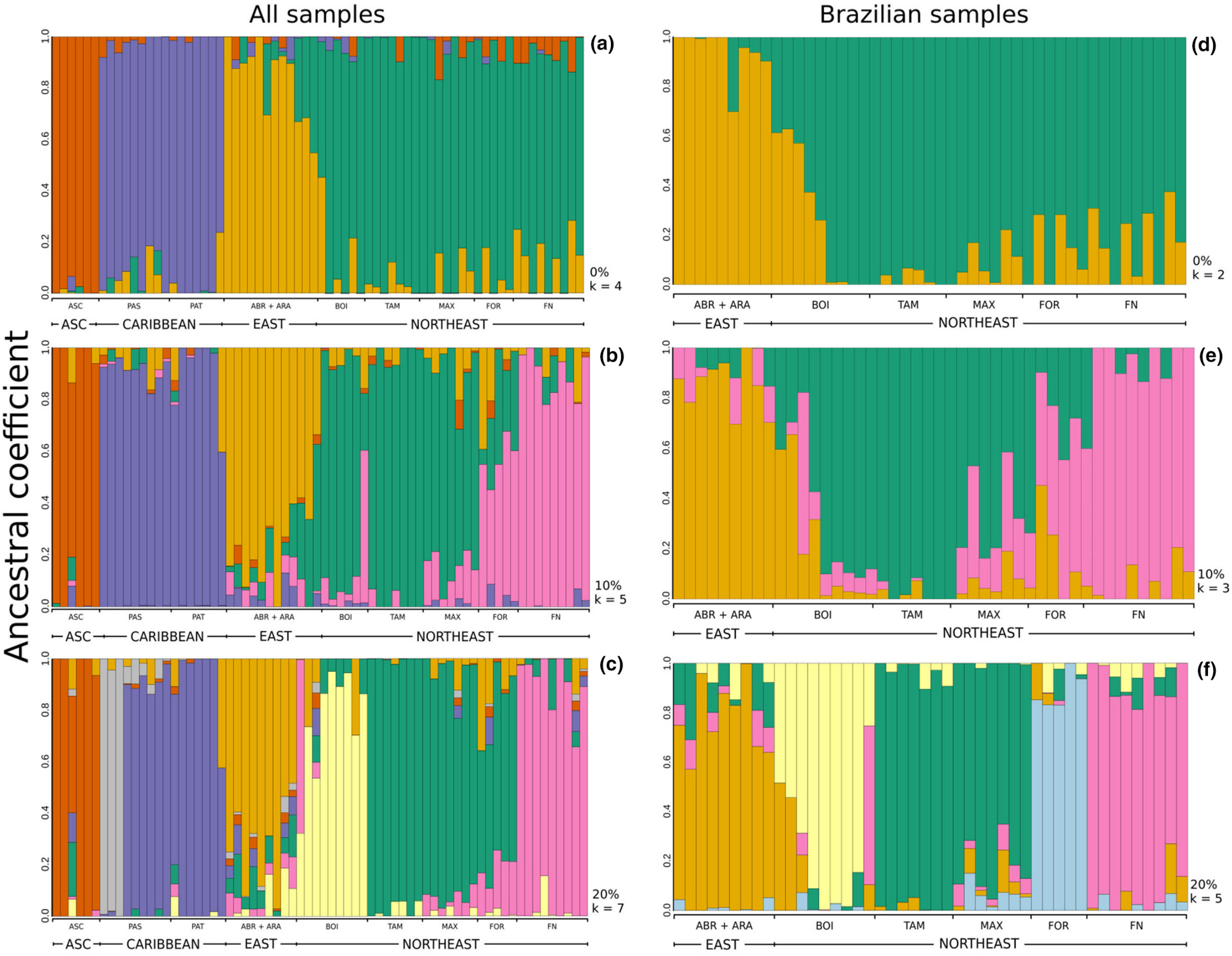


FIGURE 2 sNMF barplots for (a–c) Atlantic *Favia* and (d–f) Brazilian *F. gravida* based on the *coral host* datasets. K represents the optimal value obtained from cross-entropy analysis. Percentage values represent the missing data percentage in each dataset. ABR, Abrolhos; ARA, Aracruz; ASC, Ascension Island; BOI, Boipeba; FN, Fernando de Noronha; FOR, Fortaleza; MAX, Maxaranguape; PAS, *F. fragum* short morphotype (Panama); PAT, *F. fragum* tall morphotype (Panama); TAM, Tamandaré.

In order to investigate the fine-scale genetic structure of *F. gravida* along the Brazilian coast, we performed separate analyses for those samples. The dataset with no missing data again yielded the lowest resolution. The optimal number of ancestral populations was *k* = 2 according to the *g* dataset, dividing the Brazilian coast into BR East and BR Northeast populations, with the exception of three individuals from BOI that had a larger percentage of their ancestral gene pool assigned to BR East (Figure 2d). The *g* dataset recovered three subpopulations within the Brazilian coast (Figure 2e), and the *g dataset* further resolved BOI and FOR each in a separate subpopulation (*k* = 5, Figure 2f). Samples from TAM showed the lowest levels of admixture across all datasets.

The *holobiont* dataset recovered similar results but with higher resolution within the Brazilian samples (Figure S2). Regardless, the pattern was the same across all datasets, with higher *k*-values recovering phylogeographically coherent subpopulations nested within the main clusters, except for the subpopulation within the Short morphotype of *F. fragum* (Figure 2c and Figure S2).

The same general pattern was observed in the DAPC analysis (Figure 3). When sampling sites were used as a priori information, the first axis separated *F. gravida* from *F. fragum*, while the second axis mostly isolated the Ascension Island samples (Figure 3a). With no prior information, the *k*-means analysis recovered *k* = 4 as the optimal number of clusters (Figure 3b,c), corresponding to the four main ancestral gene pools observed in the sNMF analysis (Figure 2a). The samples from BOI that had higher ancestral proportions corresponding to BR East in the sNMF analysis were also assigned to that cluster in the DAPC (Figure 3d).

To assess fine-scale spatial distribution within the Brazilian *F. gravida* we ran a second DAPC using only samples from the Brazilian coast (Figure 4). The first axis explained ~47% of the genetic variation, separating samples from TAM and MAX from the remaining populations. TAM was the most differentiated site, which agrees with the low levels of admixture revealed by sNMF. With no prior information on sampling sites, the k-means recovered *k* = 2 as the optimal number of clusters and samples were separated into BR East and BR Northeast (Figure 4b).

We also created a subset of the data including only *F. fragum* samples (Table 5). sNMF analysis using *g* revealed *k* = 2 as the lowest CEC value, with three individuals of the Short morphotype (PAS) assigned to a distinct subpopulation (Figure 5a). The lowest CEC value for both *g* and *g* was *k* = 3 and the remaining PAS samples were recovered in a third cluster, while all the samples corresponding to the Tall morphotype (PAT) had high proportions of a distinct ancestral gene pool (Figure 5b). We performed a DAPC on the *g* alignment with no prior information and *k* = 3 was returned as the optimal number of clusters (Figure 5c). All PAT individuals clustered together while the same 3 aforementioned PAS samples

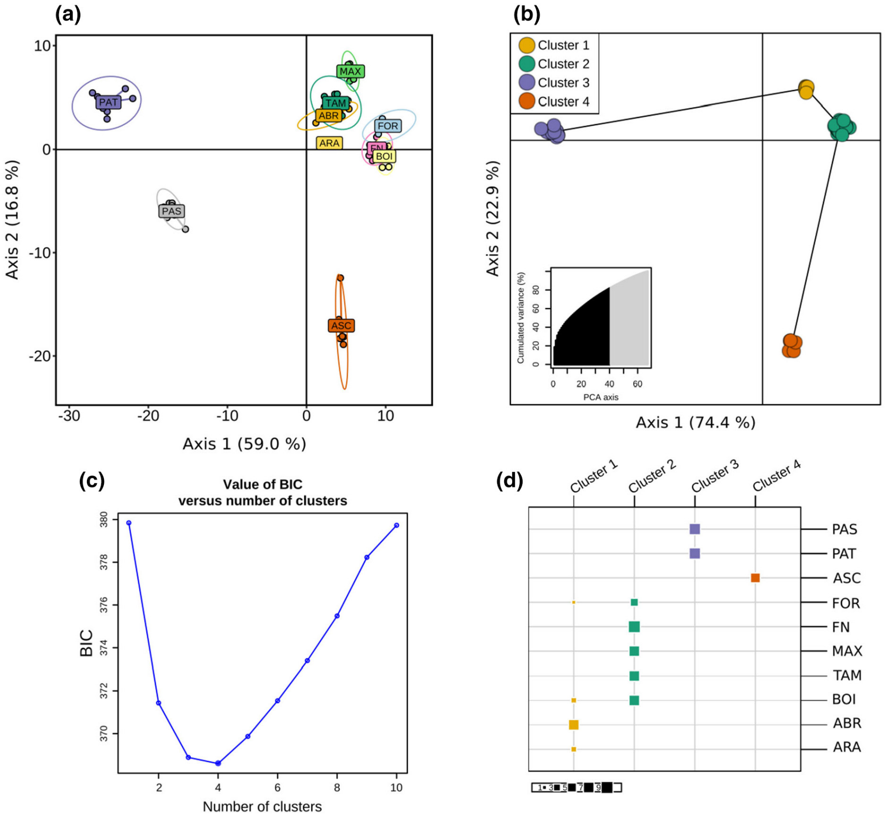


FIGURE 3 (a) Discriminant analysis of principal components (DAPC) plot of Atlantic *Favia* samples (*g* dataset) using sampling sites as *priors* (b) DAPC plot with no prior information. Colours represent distinct clusters (*k* = 4). The number of retained PCs is indicated by the black bars in the inset bar graph. (c) Optimal numbers of clusters recovered by the Bayesian Information Criterion (BIC). (d) Number of individuals from each sampling site that were assigned to each DAPC cluster in (b). Sampling site acronyms are in Table 1.

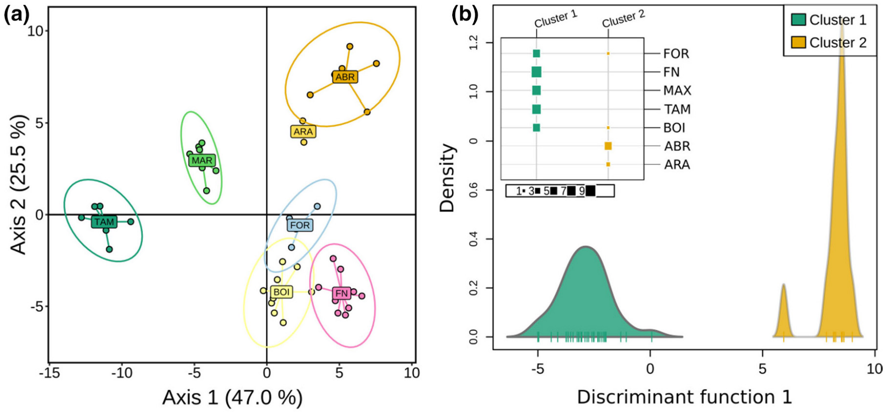


FIGURE 4 (a) Discriminant analysis of principal components (DAPC) plot of Brazilian *Favia gravida* samples (*g* dataset) using sampling sites as a priori grouping information; and (b) DAPC showing the density plot. Colours represent distinct clusters ($k=2$) recovered by the Bayesian Information Criterion (BIC). Sampling site acronyms are in Table 1.

TABLE 5 SNP count for each *coral host Favia fragum* dataset containing different percentages of missing data.

Dataset	Missing data (%)	SNP count
G8	20	22,064
G9	10	12,813
G10	0	5544

were placed in a separate cluster. The first axis explained >70% of the variation, separating these three PAS individuals from the remaining Caribbean samples. The second axis explained ~30% of the variation and separated samples from the Tall morphotype from the other two clusters comprised of the Short morphotype.

The pairwise F_{ST} values, computed according to Weir and Cockerham (1984), were consistent with the clustering recovered from DAPC and sNMF analyses (Figure 6). F_{ST} values ranged from 0.02 (between ABR and ARA, i.e. the two sampling sites that make up the BR East population) to 0.20 (between ARA and ASC, in the mid-Atlantic Ridge). The largest F_{ST} values were consistently between Ascension Island and all other populations, followed by the Caribbean populations and Coastal Brazil (Figure 6, all $F_{ST}>0.15$, red cells in heatmap). Bootstrapping across loci showed that all F_{ST} values are significantly different from zero. Isolation by distance results revealed a moderate correlation between genetic and geographic distances ($r=.40$, $p=<.01$). Smoothed local regression indicates that the significant correlation is quasi-linear (Figure 6).

3.4 | Species delimitation

We compared five different species delimitation models under a coalescent-based framework (Table 3). Model B had the largest marginal likelihood (ML = −18,945.37) and Bayes factors were calculated relative to this model. Bayes factors were >10 for all pairwise comparisons, giving decisive support for model B as the preferred species delimitation model. Model B is represented by four putative species: Ascension Island, Caribbean *F. fragum* and the two clusters of Brazilian samples, BR East and BR Northeast.

The SNAPP results considering all samples were plotted into a 'cloudogram', representing the posterior distribution of species tree topologies (Figure 7). The MCCT was used by DELINEATE as a guide tree, resulting in a maximum likelihood estimate of the species delimitation giving high support for two separate species along the Brazilian coast (Figure 8), corroborating the BDF* results. We conducted a second analysis constraining only Caribbean *F. fragum* with fixed species assignment, which recovered a single amphi-Atlantic *Favia* species (Figure S3). Given the convincing combined results of sNMF, DAPC and BFD* analyses and the support from additional species tree analysis from nuclear markers (Teshima et al., 2021) that the Ascension Island is a separate evolving lineage, we argue that unconstraining its species assignment leads to bias in the DELINEATE analysis.

4 | DISCUSSION

4.1 | Atlantic Favia

Our results showed significant genetic variation within the Atlantic *Favia* among surveyed regions. Both the admixture and principal components approaches divided the samples into four main clusters: Caribbean *F. fragum* and three clusters within *F. gravida*: mid-Atlantic Ascension Island, Northeastern and Eastern Brazil. Although presenting some levels of admixture, these four groups originated from different ancestral gene pools in all sNMF analyses (Figure 2, Figure S2) and as distinct, non-overlapping clusters in the DAPC (Figure 3). Furthermore, Bayes factor delimitation (BFD*) gave decisive support (BF > 10 for all comparisons between alternative models) for the hypothesis that these four genetic

FIGURE 5 Short (PAS—3–4 m depth) and Tall (PAT—1 m depth) morphotypes of the Caribbean *Favia fragum*. Assignment plots represent sNMF analysis of datasets with (a) no missing data (*g*) and (b) 20% missing data (*g*). (c) Discriminant Analysis of Principal Components (DAPC) plot based on the dataset with 10% missing data (*g*) and no prior information.

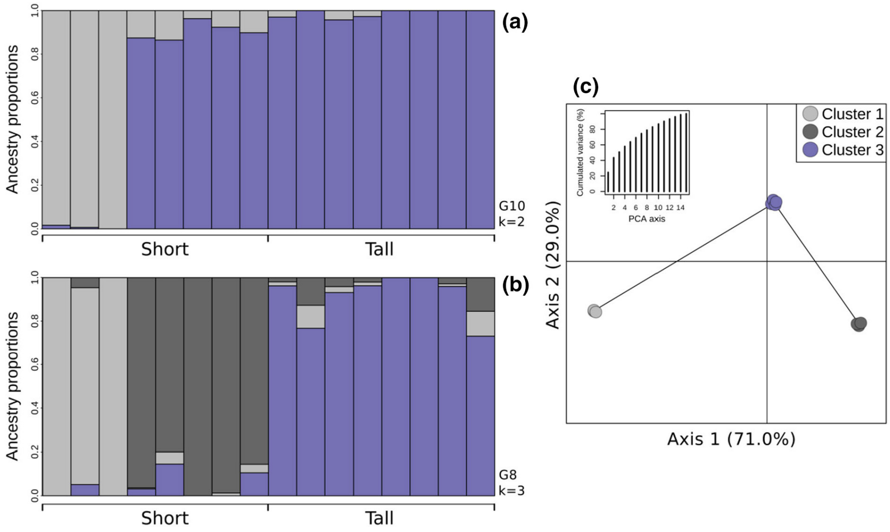
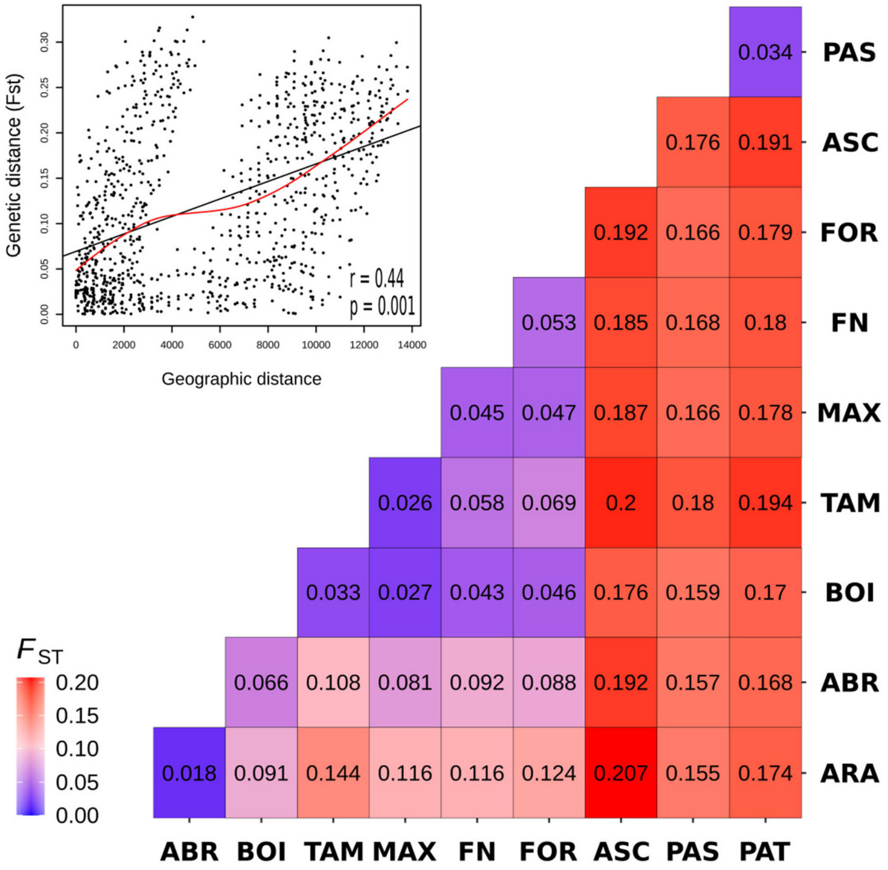


FIGURE 6 Heatmap of pairwise F_{ST} values (according to Weir & Cockerham, 1984) among Atlantic *Favia* sampling sites based on the *g* dataset. Inset graph shows the scatter plot of the Mantel test showing the relationship between genetic (estimated as $F_{ST}(1–F_{ST})^{-1}$) and geographic distances. Smoothed local regression line is represented in red.



populations are, in fact, separate species. This result was corroborated by DELINEATE, which resolved the Eastern and Northeastern Brazilian split as separate species (Figure 8).

Strong genetic differentiation between species that span the Brazilian and Caribbean biogeographic provinces has been widely recorded for marine organisms based on different genetic markers, for instance, allozymes in sea anemones (Vianna et al., 2003), mtDNA of the intertidal fiddler crab *Uca rapax* (Laurenzano et al., 2013), wrasses (Rocha et al., 2005) and bridled gobies (Volk et al., 2021, which also analysed SNPs), and microsatellites of reef sharks (Bernard et al., 2017). The same pattern was also recovered in reef-building corals (Nunes et al., 2009), including *Favia* spp. (Nunes et al., 2011; Teshima et al., 2021).

The main barrier between these regions is the Amazon River delta, the world's largest drainage system, which may have acted as a geographic barrier to the dispersion of marine organisms since the Early Pliocene (~5 Mya)

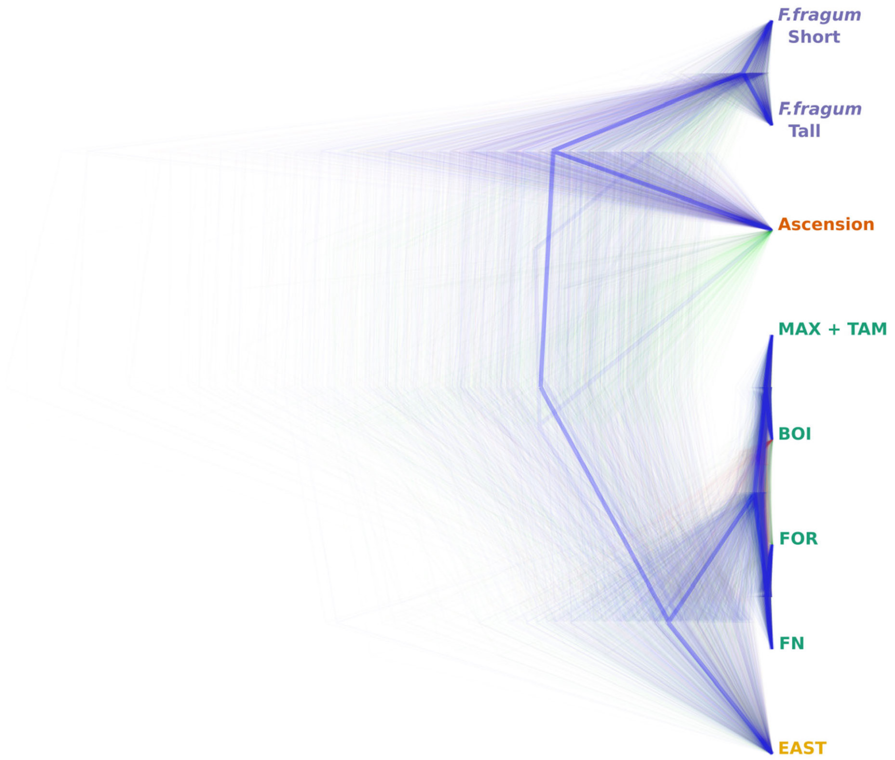


FIGURE 7 Cladogram representing the posterior distribution of species trees inferred with SNAPP. Thinner blue lines indicate greater tree topology credibility, while the thicker blue line represents the maximum clade credibility tree. Green and red thinner lines represent disagreements in the tree topology. Tips are coded according to Table 1.

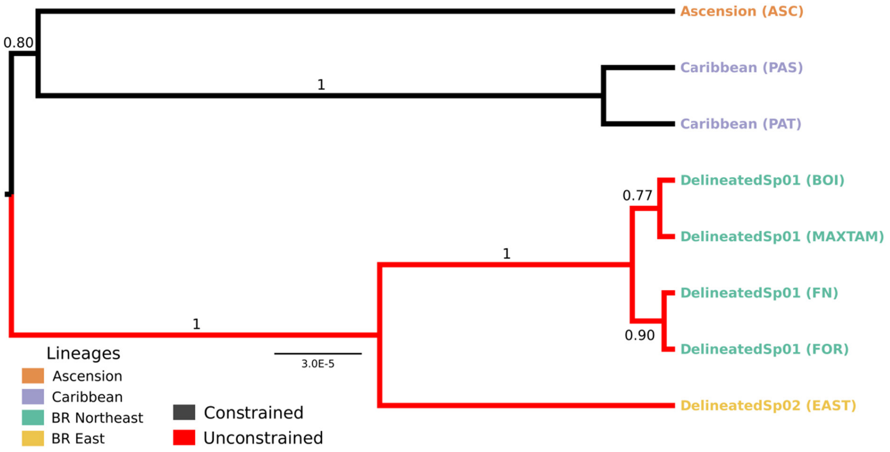


FIGURE 8 Maximum clade credibility tree (MCCT) obtained by SNAPP representing the maximum likelihood estimate of the species delimitation. Black branches represent constrained (fixed species assignment) and red branches represent unconstrained population lineages (lineages with unknown species identity and whose membership was allowed to vary). Tip labels show species assignments as suggested by DELINEATE followed by the population label in parenthesis, and are colour-coded according to the lineages recovered by SNAPP. Branch labels represent posterior probabilities for the MCCT.

when it reached its current geographic configuration (Latrubesse et al., 2010). Overcoming the low salinity and high turbidity of this freshwater discharge is probably a rare event in animals with poor dispersal capacity, such as *Favia* (Tosetto et al., 2022). Considering the age of the Amazon barrier and that of the modern *Favia* (4–6 My—Schwartz et al., 2012), the genetic differentiation recovered in this study is strong evidence that the Caribbean *F. fragum* and the South Atlantic *F. gravida* are indeed separate lineages, despite the low levels of admixture indicated by the sNMF results. Although faster and more accurate than other popular programs for population structure analysis (Frichot et al., 2014), sNMF tends to overestimate admixture even in low differentiation simulated scenarios (Wang, 2022).

F_{ST} values between Ascension Island *F. gravida* and the remaining populations were comparable to, and in some cases higher than, those observed between *F. gravida* and *F. fragum* populations ($F_{ST}=0.207$ between ASC and ARA—Figure 6) and DAPC and sNMF analysis showed marked separation between Ascension and Brazilian genetic clusters. Additionally, SNAPP recovered Ascension Island and the Northeastern and Eastern populations of *F. gravida* as separate species. Significant genetic differentiation between Brazilian, mid-Atlantic and West-African samples of reef-building corals and hydrocorals was previously reported using nuclear and mitochondrial markers (de Souza et al., 2017; Nunes et al., 2009, 2011). In the study conducted by Teshima et al. (2021), *F. gravida* samples from Ascension Island did not share ITS and *masc* haplotypes with Brazilian populations and were at least six mutational steps away from the remaining *Favia* spp. samples. The authors suggested the possibility of a cryptic species of *Favia* in this region.

The stretch of Atlantic Ocean separating Western and Eastern Atlantic fauna is considered a 'soft barrier' that is, it works as a biogeographic filter and not as a hard obstacle (Floeter et al., 2008; Luiz et al., 2012). This leads to moderate isolation among lineages of the mid-Atlantic Ridge Province, which encompasses Ascension and Saint Helena Islands, from populations along the Brazilian coast. Ascension is a volcanic island, which originated ~1–2 Mya (Ashmole & Ashmole, 1997) and it is located approximately 3000 km from Brazil. *Favia gravida* from this island grows as encrusting colonies down to depths of 5 m whereas in shallow-water tidal pools colonies are free-living, a feature never observed in any other site (Hoeksema, 2012; Zibrowius et al., 2017). Genetic isolation of island populations is not an uncommon trait, characterized by high genetic differentiation in various fish species (Hemmer-Hansen et al., 2007; Pinheiro et al., 2017) and reef-building corals (Ayre & Hughes, 2004) when compared to their coastal counterparts. Also, remote islands tend to have high rates of endemic marine organisms (Dawson, 2016; Robertson, 2001) and the mid-Atlantic Ridge Province is recognized as an important center of endemism for marine species (Roberts et al., 2002). Altogether, these results support the presence of a third species of *Favia* endemic to Ascension Island. However, it is important to acknowledge the absence of samples from mid-Atlantic and Western African islands, such as Saint Helena, in our study. This limitation underscores the need for additional sampling efforts to facilitate comprehensive taxonomic revision. Notably, colonies from Ascension Island were first described as *Platygyra ascensionis* Ridley, 1881, which was later considered a junior synonym of *F. gravida* (Hoeksema & Wirtz, 2013), further highlighting the urgency of a taxonomic revision.

4.2 | Brazilian coast

Genetic studies of *F. gravida* show contrasting patterns of population structuring along the Brazilian coast. Nunes et al. (2011) recovered significant differentiation between two sampling sites from the recently discovered Brazilian Northeastern and Eastern populations based on one intron and one exon of *B-tubulin* and a *Pax-C* intron. Using a more comprehensive sampling scheme, Teschima et al. (2022) recovered a single haplotype from both ITS and *masc* along the Brazilian coast, with private haplotypes being observed only in the Rocas Atoll and Fernando de Noronha Archipelago. Nonetheless, the authors acknowledge the possible limitations linked to marker resolution, particularly considering the potential for ITS intragenomic variability, which may lead to phylogenetic inconsistency, especially among closely related lineages (Wörheide et al., 2004). On the contrary, our results using thousands of high-resolution markers and different statistical approaches, indicate that the two main genetic clusters recovered on the Brazilian coast are separately evolving lineages. It is worth noting that two individuals from the BR Northeast Boipeba sampling site exhibited high percentages of their admixture coefficients assigned to BR East (Figure 2). Given the intermediate geographical location of this site (Figure 1), it is plausible that this region is part of a hybridization zone. This would also explain the minor discrepancies in the posterior distribution of species tree topologies (Figure 7). However, delimitation analyses based on the multispecies coalescent model often underestimate the number of species when handling hybrid samples (Wagner et al., 2017; Zhang et al., 2011), a trend previously observed in anthozoans (Quattrini et al., 2019). Because the scenario where BR East and BR Northeast were lumped into a single lineage (table—model A) ranked among the least favoured by BFD*, we are confident in the species delimitation results given the conservative nature of the methods employed.

More resolution for population structure within Brazil was obtained with the addition of SNPs to the alignments as increasing percentages of missing data were allowed. Moderate amounts of missing data add valuable information to phylogenetic and population genetics studies, as previously reported using empirical and simulated data (Hodel et al., 2017; Huang & Knowles, 2016). This might be attributed not only to the larger number of characters but also to the potential incorporation of loci featuring higher mutation rates or subjected to natural selection, which may improve resolution and reveal recent population differentiation (Gagnaire et al., 2015).

During the Holocene, the development of coral reefs in Brazil was linked to sea level changes and the Amazon river basin flow. Because all coral species in Brazil are found in the Abrolhos Bank, it has been hypothesized that its bryozoan-dominated reef structures acted as a refuge during a period of low sea levels and high sedimentation that was initiated ~5 ky ago. This period led to a reduction in coral cover as reefs grew closer to the shore (Bastos et al., 2018; Dechnik et al., 2019; Laborel, 1970; Leão et al., 2003; Leão & Kikuchi, 2005). After this regression period, when connectivity between sites was re-established, Abrolhos would have acted as a source of recruits that re-populated the coast (Kikuchi & Leão, 1998; Leão & Kikuchi, 2001). Under this hypothesis, we would expect to find the highest genetic diversity and number of private alleles in the Abrolhos region (Comps et al., 2001; Hewitt, 2000; Provan & Bennett, 2008), and admixture among populations of a single species along the coast. Instead, maximum allelic richness and gene diversity were found in ARA and not in ABR, and the number of private alleles in the former population was also higher ($n = 78$ vs. $N = 47$, Table 2). The consistent recovery of two divergent lineages on the Brazilian coast suggests that the hypothesis of Abrolhos serving as the unique refugium from where recolonization started is unlikely. Although narrow, the Brazilian continental shelf presents numerous suitable shallow-water habitats near the coast (Alberoni et al., 2020). For instance, according to paleoclimatic modeling, *Mussismilia braziliensis* sustained a limited near-shore distribution along the northern coast of Bahia, north of Abrolhos Bank (Menezes et al., 2020). Furthermore, *Favia* can withstand turbid waters and variations in temperature and salinity (Pereira, Calderon, et al., 2020; Pereira, Fonseca, et al., 2020), making it well adapted to near-shore reefs subjected to high sediment deposition and constant subaerial exposure (Kikuchi & Leão, 1998; Loiola et al., 2019).

Although presenting signs of admixture, there is also marked population structure in the Northeast population (Figure 2), most likely due to philopatric behaviour and low dispersal capacity of *F. gravida* larvae (Calderon et al., 2000; Carlon & Olson, 1993). Larvae of brooders are competent for settlement within hours after release (Harrison & Wallace, 1990), leading to shorter PLDs and thus philopatry and restricted gene flow among distant populations (Carlon, 1999; Thomas et al., 2015; Underwood et al., 2009; Warner et al., 2016). Unlike *Favia*, the aforementioned *Mussismilia hispida* and *Millepora alcicornis* are broadcasters with presumably better dispersal capabilities (Lewis, 2006; Neves & Pires, 2002), although exceptions to this general pattern are not uncommon among broadcasters (reviewed in Harrison, 2011). Additionally, multiple *Millepora* species are capable of successfully dispersing through fragmentation (Edmunds, 1999), which is unlikely for *Favia* due to its massive morphology.

Brazilian reefs harbour 21 species of scleractinian corals, a low number when compared to the richer Caribbean reefs, with which it has faunistic similarities (Leão et al., 2016). Nonetheless, the reef fauna of Brazil presents a high degree of endemism (Leão et al., 2003). With the recognition of these newly discovered cryptic *Favia* lineages as separate species, the number of endemic stony corals on the Brazilian coast will increase to eight, almost 35% of the total number of coral species. Endemic species with restricted distributions, as is the case of BR East and Ascension Island lineages, require special attention in conservation efforts, since they face a higher risk of extinction (Roberts & Hawkins, 1999). The lower gene diversity and allelic richness values suggest that the individual subpopulations in the Northeastern lineages might be at risk, which is especially alarming due to the increasing threat to marine species posed by climate change (Manes et al., 2021).

4.3 | Favia fragum ecotypes

Previous work has provided morphological and genetic evidence for two ecotypes within the Caribbean species *Favia fragum* in the Bocas del Toro of Panama (Carlon et al., 2011; Carlon & Budd, 2002). The Tall ecotype (PAT) preferentially occurs in shallow seagrass habitats while the Short ecotype (PAS) is more common in deeper (>3 m) coral reefs. These papers document significant morphological and genetic divergence across this depth gradient and suggest that ecological differences between ecotypes are reducing gene flow and setting the stage for speciation. A subsequent paper employing 15 microsatellites revealed even more fine-scale clustering within ecotypes, but also evidence for mixed ancestry (Carlon & Lippe, 2011). Our results recovered low levels of genetic variation between Short and Tall ecotypes ($F_{ST} = 0.034$), but clustering analysis (sNMF) showed signs of finer structuring, assigning three individuals of the Short ecotype to a distinct lineage regardless of the degree of missing data. Focusing on only the *F. fragum* samples and allowing for more missing data increased resolution and recovered the remaining Short ecotypes in a third population with distinct ancestral coefficients, which was corroborated by the DAPC. This suggests that the Short ecotype of *F. fragum* may actually encompass more than one lineage, as previously uncovered by the microsatellite data (Carlon & Lippe, 2011). Our Tall ecotype samples, on the contrary, show no finer structuring and some individuals show signs of admixture with both Tall ecotype subpopulations. Differentiation between *F. fragum* ecotypes found in shallow and deep habitats is present in our data, a trend that was documented for other scleractinians (Bongaerts et al., 2010; De Palmas et al., 2018). Furthermore, the subdivision within the Short morphotype indicates the existence of genetic lineages within microhabitats. Nevertheless, the species delimitation models that considered a split within the Caribbean *F. fragum* (models C and D) were not more likely than the model that lumped the two ecotypes into one species, suggesting that the observed variation is most likely due to intra-specific rather than inter-specific differentiation and that gene flow remains between ecotypes.

5 | CONCLUSION AND FUTURE DIRECTIONS

Population structure and formal species delimitation approaches recovered decisive support for four candidate species: *F. fragum* from the Caribbean, a lineage from Ascension Island, and two lineages from the Brazilian coast. Within these lineages, we also recovered a fine-scale population structure. Our genomic approach contributes to building a more comprehensive seascape of the population dynamics of reef-building organisms, especially along the Brazilian coast, which hosts unique reef coral endemism and the South Atlantic's sole true coral reefs.

Although *F. fragum* and *F. gravida* are recognized as separate species by specialists (Budd et al., 2012; Nunes et al., 2008), only in 2022 the International Union for the Conservation of Nature (IUCN) Red List included *F. gravida* in its assessment. Both species are currently categorized as 'least concern', with their population trend listed as 'unknown'. (Aronson et al., 2008; Kitahara et al., 2021). This underscores the scarcity of population data required for reliable assessments. The implementation of conservation plans typically requires taxonomically accepted species (Agapow et al., 2004), and since the IUCN Red List is largely used by government agencies as an indicator of the global biodiversity and species extinction risks, our results indicate the need for a reassessment of the Atlantic *Favia* in this database. Given that our data offer support for at least four candidate species, we suggest that each of these regions requires an independent management strategy, given their unique evolutionary history.

AUTHOR CONTRIBUTIONS

C.L.A: writing—original draft (lead); writing—review and editing (equal); formal analysis (lead); conceptualization (supporting); investigation (lead). **M.S.B:** conceptualization (lead); writing—original draft (supporting); writing—review and editing (equal); formal analysis (supporting); funding acquisition (lead); resources. **D.B.C.:** writing—review and editing (equal); funding acquisition; resources. **C.Z.:** writing—review and editing (equal); resources. **R.J.T.:** writing—review and editing (equal); funding acquisition; resources.

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DATA AVAILABILITY STATEMENT

Raw sequence reads are deposited in the SRA (BioProject PRJNA842338). Scripts and VCF files used for the analyses are deposited on the Dryad database (https://doi.org/10.5061/dryad.0vt4b8h4t). Benefits Generated: benefits from this research accrue from the sharing of our data and results on public databases as described above.

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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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