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REPORT

# Population genetic structure of the broadcast spawning coral, *Montastraea cavernosa*, demonstrates refugia potential of upper mesophotic populations in the Florida Keys

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**Abstract** In the Florida Keys, coral cover on shallow reef systems (0–30 m) has declined over the past several decades, punctuated by severe losses during coral disease outbreaks and bleaching events. However, certain areas within the Florida Keys, especially the Dry Tortugas and many upper mesophotic habitats (30–60 m), have maintained relatively healthy coral communities, even in the face of recent severe and widespread coral disease outbreaks. Relatively little is known about the genetic connectivity of corals among these sites or the potential for mesophotic sites to act as refugia by contributing to metapopulation recovery and persistence. Using a paired shallow and upper mesophotic sampling design, we assessed the genetic connectivity of a dominant, broadcast spawning coral species, *Montastraea cavernosa*, across the Northern and Southern Dry Tortugas, Lower Florida Keys, and Upper Florida Keys. A genetic dataset based on a suite of > 9000 single-nucleotide polymorphism loci indicated that the level of vertical genetic connectivity between paired shallow and upper mesophotic populations varied significantly based on location. Shallow and upper mesophotic *M. cavernosa* populations in the Northern Dry Tortugas and the Upper Keys were genetically similar. In contrast, populations were significantly differentiated across depth in the Lower Keys and Southern Dry Tortugas. While upper mesophotic populations in the Lower Keys and Southern Dry Tortugas were distinct from their shallow counterparts, there was evidence of relatively high levels of genetic connectivity to both the shallow and upper mesophotic populations downstream in the Upper Keys. These results suggest that while vertical connectivity between paired shallow and mesophotic populations can vary, certain upper mesophotic populations may fill an important role in maintaining coral metapopulations throughout the Florida Keys and should be considered in future management strategies.

**Keywords** Population genetics · Genetic connectivity · Mesophotic coral ecosystems · *Montastraea cavernosa* · Marine protected areas

## Introduction

Coral reef ecosystems in the Florida Keys are some of the most well-studied reefs in the tropical western Atlantic and were among the earliest to receive formalized conservation management (Jaap et al. 2008). Despite this long history of study and management, Florida Keys' reefs have become severely degraded over the past several decades, including substantial losses of important reef-building species and coral cover declines greater than 50% between 1998 and 2011 (Ruzicka et al. 2013). The Florida Keys follow the general trend in accelerated coral decline and phase shifts to macroalgal dominance that occurred across many Caribbean reefs since the 1980s (Gardner et al. 2003). These declines can be attributed to a combination of stressors

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including warming ocean temperatures and thermally induced coral bleaching events (Manzello 2015), hurricanes (Gardner et al. 2005), overfishing and disease events resulting in a loss of key herbivorous taxa (e.g., *Diadema antillarum*, Lessios et al. 1984; Kramer and Heck 2007), nutrient enrichment of coastal waters (Lapointe et al. 2019), and multiple coral disease outbreaks including persistent outbreaks of white diseases that drove the widespread loss of *Acropora* corals (Aronson and Precht 2001; Porter et al. 2001; Joyner et al. 2015). More recently, stony coral tissue loss disease (SCTLD) has rapidly spread throughout the majority of Florida's coral reef and to other reefs across the Caribbean (Muller et al. 2020). However, as of fall 2020, SCTLD has not been reported within the Dry Tortugas and preliminary surveys conducted during summer 2019 found relatively low disease prevalence across upper mesophotic habitat (30–60 m) throughout the Florida Keys (Roth et al. 2020).

Coral reefs in the Dry Tortugas and upper mesophotic coral reef ecosystems throughout the Florida Keys are relatively isolated from localized anthropogenic impacts and support comparatively healthy coral communities and higher coral cover (Haskell et al. 2000; Gil-Agudelo et al. 2020). The combination of these attributes suggests that these areas may function as coral population refugia (Riegl and Piller 2003; Bongaerts et al. 2010). To evaluate the potential for hypothesized refugia to contribute to coral metapopulation recovery, the levels of population connectivity among potential refugia and more highly impacted locations must be quantified, thereby informing prioritized management efforts (Mcleod et al. 2019). Additionally, quantification of genetic diversity across natural coral populations is critical to informing novel coral management tools currently being developed and employed in the Florida Keys, including coral restoration and reef-wide efforts to bank species and genetic diversity ex situ (Schopmeyer et al. 2012; Hagedorn et al. 2018; Baums et al. 2019; Precht 2019).

Population genetic approaches have been widely implemented to quantify genetic diversity, measure gene flow, and infer patterns of connectivity among coral populations throughout the tropical western Atlantic, including sites within the Florida Keys (Serrano et al. 2014, 2016; Drury et al. 2017; Rippe et al. 2017; Studivan and Voss 2018). Studies of coral genetic connectivity across the Florida Reef Tract have assessed a variety of coral species with different reproductive mechanisms and life histories, examined vertical connectivity across depth gradients, and employed a variety of molecular markers (Baums et al. 2010; Serrano et al. 2014, 2016; Manzello et al. 2019). The coral *Montastraea cavernosa* is a cosmopolitan Atlantic species, extreme depth-generalist, broadcast spawner, important reef builder, and a dominant species in the Florida Keys coral community (Reed 1985; Budd et al. 2012; Burman et al. 2012). These ecological and life history characteristics contribute to its widespread use in the investigation of coral population genetic structure and diversity (Nunes et al. 2009; Budd et al. 2012; Goodbody-Gringley et al. 2012; Serrano et al. 2014; Studivan and Voss 2018; Eckert et al. 2019; Dodge et al. 2020; Drury et al. 2020; Sturm et al. 2020).

Previous research on *M. cavernosa* population genetic structure throughout the Florida Keys has identified high levels of horizontal connectivity within the same depth zone across the Upper Keys, Lower Keys, and the Dry Tortugas (Serrano et al. 2014). However, there are varying patterns in the level of vertical connectivity across depth zones from 0 to 30 m within sampling regions. Genetic connectivity of *M. cavernosa* also appears to be limited among shallow populations in the northern Florida Keys ($\sim$ 5 m), upper mesophotic populations in the Dry Tortugas ($\sim$ 30 m), and lower mesophotic populations at Pulley Ridge ($>$ 60 m), a reef $\sim$ 160 km west of the Dry Tortugas (Studivan and Voss 2018; Drury et al. 2020). No previous studies have characterized or compared *M. cavernosa* population genetic structure between paired shallow (0–30 m) and upper mesophotic (30–60 m) depth zones across reef habitats throughout the Florida Keys.

The Deep Reef Refugia Hypothesis posits that mesophotic reefs, by nature of their depth and distance from land, are inherently buffered from anthropogenic stressors that more severely impact shallow coral communities (Glynn 1996; Bongaerts et al. 2010). Ecosystem health, community structure, and population dynamics of upper mesophotic coral ecosystems in the Florida Keys are still understudied relative to their shallow counterparts. However, recent assessments have characterized upper mesophotic areas with relatively higher coral cover and lower prevalence of bleaching and disease as compared to shallow reef habitats (Reed et al. 2015). An assessment of the population genetic structure of these upper mesophotic populations and quantification of connectivity to shallow reef populations is needed to evaluate the refugia potential of these populations and to determine effective management strategies.

This study addresses these research gaps by employing a high-resolution 2bRAD single-nucleotide polymorphism (SNP) genotyping approach to generate a suite of thousands of SNP markers dispersed throughout the genome (Wang et al. 2012). Using these SNPs, *M. cavernosa* population genetic structure and genomic diversity were quantified and compared across paired shallow and upper mesophotic sites (hereafter referred to as "mesophotic") in the Northern and Southern Dry Tortugas, Lower Keys, and Upper Keys.

## Materials and methods

*Montastraea cavernosa* corals were sampled during August and September 2019 from four reef locations along the Florida Keys (Table 1, Fig. 1). The distances between coinciding shallow and upper mesophotic sampling locations within a sample population were minimized, not exceeding ~ 10 km. Shallow and mesophotic samples were collected by SCUBA and technical divers, respectively, using hammers and masonry chisels to collect ~ 5 $cm^2$ of coral tissue which was placed in an individually labeled sampling bag. Scaled photographs of each colony were taken pre- and post-sampling, and colony depth was recorded. Once samples were surfaced they were immediately preserved in 100% molecular-grade ethanol and maintained at − 20 °C until transported back to FAU-HBOI. Once back at FAU-HBOI the ethanol was replaced with fresh 100% molecular-grade ethanol, and the samples were stored at − 80 °C until DNA extraction.

Prior to DNA extraction, tissue was scraped from 1 to 2 coral polyps using a sterile scalpel for each sample. The tissue was then placed in a 2-mL tube with 500 µL of TRIzol reagent. The samples were stored in TRIzol at 4 °C for a minimum of 1 h up to multiple days. Tubes were centrifuged at 20,000 × g for 2 min to pellet the tissue, which was transferred to a new 2-mL safe-lock tube with ~ 0.075 g of 0.5-mm glass beads for maceration. Genomic DNA was extracted using a modified dispersion buffer/phenol–chloroform-isoamyl alcohol extraction (Sturm 2020). The extracted DNA was cleaned using a Zymo DNA Clean and Concentrator Kit following manufacturer's protocols and then eluted in 15 µL of nuclease-free water. DNA quality was checked on a NanoDrop 2000 (Thermo Fisher), and dsDNA quantity was measured using a broad-range assay kit on a Qubit 4.0 fluorometer (Thermo Fisher).

Samples were diluted to 25 ng $\mu L^{-1}$ concentrations and 100 ng of DNA was added to each digestion reaction with

**Table 1** *Montastraea cavernosa* samples from shallow and mesophotic sites across four locations in the Florida Keys

| Location | Sample population | Average depth (m) | Site | $n_c$ | $n_a$ | $n_g$ | Latitude | Longitude |
|---|---|---|---|---|---|---|---|---|
| Southern Dry Tortugas (DTS) | DTS-Shallow | 25.7 | | **30** | **28** | **28** | | |
| | | | Riley's Hump | 26 | 24 | 24 | 24.5178 | − 83.0979 |
| | | | Riley's Hump | 4 | 4 | 4 | 24.5114 | − 83.0952 |
| | DTS-Mesophotic | 36.2 | | **37** | **37** | **37** | | |
| | | | Riley's Hump | 20 | 20 | 20 | 24.4940 | − 83.0961 |
| | | | Riley's Hump | 11 | 11 | 11 | 24.4893 | − 83.1227 |
| | | | Riley's Hump | 6 | 6 | 6 | 24.4847 | − 83.1107 |
| Northern Dry Tortugas (DTN) | DTN-Shallow | 16.6 | | **30** | **27** | **27** | | |
| | | | Sherwood Forest | 23 | 21 | 21 | 24.6602 | − 83.0789 |
| | | | Sherwood Frest | 7 | 6 | 6 | 24.6404 | − 83.1029 |
| | DTN-Mesophotic | 32.2 | | **30** | **23** | **23** | | |
| | | | Sherwood Forest | 13 | 10 | 10 | 24.6521 | − 83.1031 |
| | | | Sherwood Forest | 17 | 13 | 13 | 24.6404 | − 83.1029 |
| Lower Keys (LK) | LK-Shallow | 17.8 | | **30** | **30** | **30** | | |
| | | | Big Coppitt | 29 | 29 | 29 | 24.4930 | − 81.5989 |
| | | | Big Coppitt | 1 | 1 | 1 | 24.4911 | − 81.6060 |
| | LK-Mesophotic | 31.4 | Big Coppitt | **13** | **13** | **13** | 24.4940 | − 81.5879 |
| Upper Keys (UK) | UK-Shallow | 23.6 | | **35** | **34** | **32** | | |
| | | | Carysfort | 20 | 20 | 18 | 25.2204 | − 80.2009 |
| | | | Elbow | 7 | 6 | 6 | 25.1440 | − 80.2526 |
| | | | Elbow | 8 | 8 | 8 | 25.1442 | − 80.2521 |
| | UK-Mesophotic | 40.6 | | **32** | **25** | **25** | | |
| | | | Carysfort | 17 | 10 | 10 | 25.2175 | − 80.1934 |
| | | | Elbow | 8 | 8 | 8 | 25.1522 | − 80.2316 |
| | | | Elbow | 7 | 7 | 7 | 25.1582 | − 80.2206 |
| | | | **Grand Total** | **237** | **217** | **215** | | |

Sample totals include the number of *M. cavernosa* collected in the field ($n_c$ = 237), the number of samples used in downstream analyses ($n_a$ = 217), and the number of unique individuals identified after the removal of clones ($n_g$ = 215). Bolded sample numbers are sample population totals. GPS coordinates are in decimal degrees (WGS84)

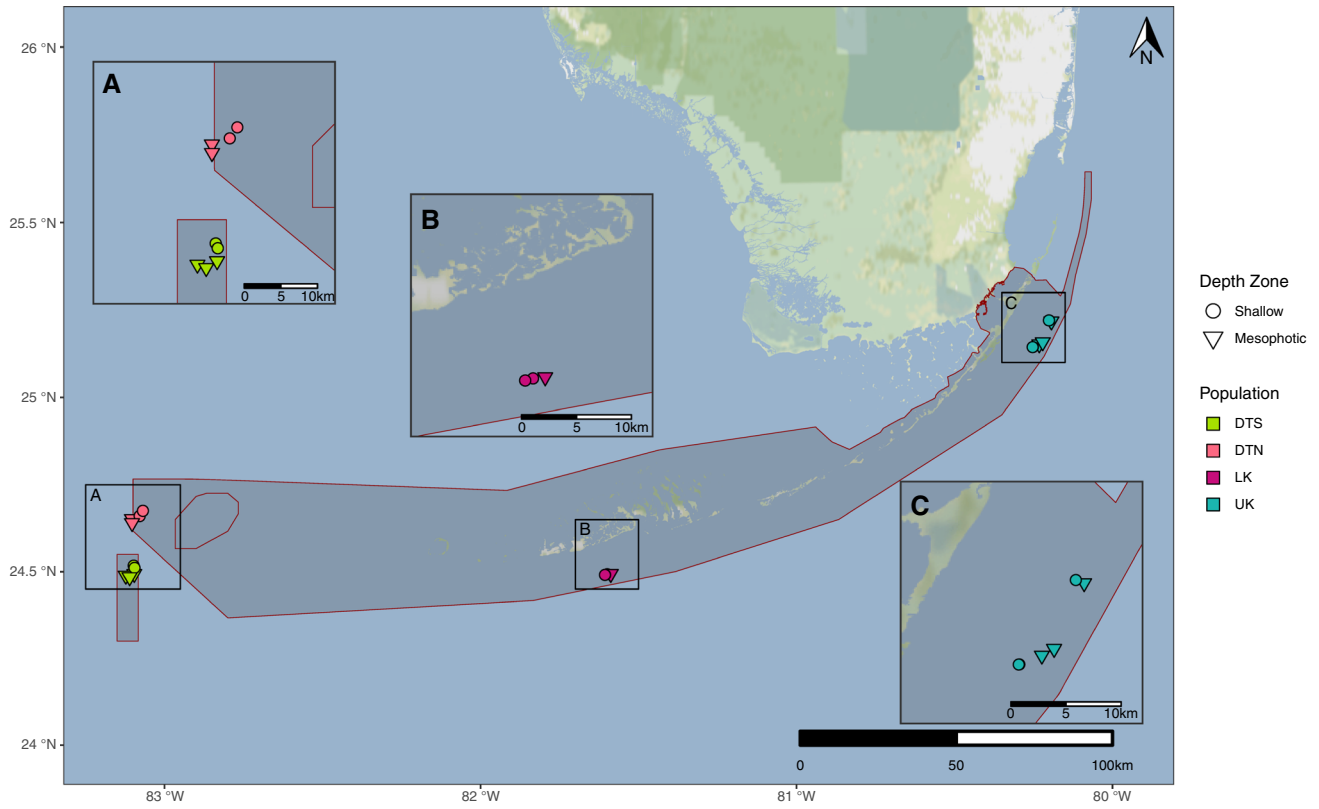


**Fig. 1** *Montastraea cavernosa* sampling sites throughout four reef locations across the Florida Keys National Marine Sanctuary, Southern Dry Tortugas (DTS, A), Northern Dry Tortugas (DTN, A), Lower Keys (LK, B), and Upper Keys (UK, C). Sanctuary boundaries are outlined in red. All sampling sites within each reef location are indicated by their shallow or mesophotic depth zonation

BcgI enzyme. 2bRAD SNP libraries were prepared following Wang et al. (2012) including modifications described in the protocol's GitHub repository (https://github.com/z0on/2bRAD_denovo). Notably, 12 uniquely indexed 3' adaptors were incorporated, allowing 12 sample ligations to be pooled prior to amplification. Fully degenerate 5' adapters were also included, allowing PCR duplicate removal from downstream analyses. Additionally, triplicate libraries were prepared for three samples and used as a sequencing quality check and to identify natural clones (Manzello et al. 2019). Pooled libraries were further combined in equimolar amounts based on qPCR relative quantification. The final pooled library was size selected with Pippin Prep (Sage Science) before conducting 100-bp single-end sequencing on an Illumina NovaSeq S1 flow cell at the University of Texas at Austin's Genome Sequencing and Analysis Facility.

Reads were initially demultiplexed by the sequencing facility using PCR and TruSeq indices and were further demultiplexed by the in-line 3' adaptor indices, deduplicated, and trimmed using custom Perl scripts (https://github.com/z0on/2bRAD_denovo). The sequences were further quality-filtered using the fastq_quality_filter in the FASTX-Toolkit v0.0.14 ($\geq$ 90% of bases with Phred quality scores $\geq$ 20; Gordon and Hannon 2010). Trimmed and quality-filtered reads were first aligned to a meta-reference of concatenated genomes from the four Symbiodiniaceae genera known to associate with coral hosts (formerly clades A–D), *Symbiodinium microadriacticum* (Aranda et al. 2016), *Breviolum minutum* (Shoguchi et al. 2013), *Cladocopium goreaui* (Liu et al. 2018), and *Durusdinium trenchii* (Shoguchi et al. 2021) using the sequence aligner *Bowtie2* (Langmead and Salzberg 2012), separating unaligned reads from those that aligned to the Symbiodiniaceae reference. Unaligned reads were then mapped to the *M. cavernosa* genome (Rippe et al. 2021). Reads that aligned to the Symbiodiniaceae reference were re-aligned to the *M. cavernosa* genome, filtering out reads which mapped to both algal and coral references, resulting in reads that aligned exclusively to either the *M. cavernosa* genome or the Symbiodiniaceae meta-genome for downstream analyses. Counts of alignments to each of the four Symbiodiniaceae genomes were used as a proxy for the relative abundance of these four algal symbiont genera associated with each sample.

The program ANGSD v0.933 (Korneliussen et al. 2014) was used to generate genotype likelihoods for the *M. cavernosa* dataset, which incorporates probabilistic

uncertainty based on sequencing and mapping quality scores for a particular locus. Conducting population genetic analyses on the raw genotype likelihoods is more statistically robust than working with hard-called genotypes, especially when sequencing coverage is variable across samples. ANGSD was run with the following filters: minimum mapping quality scores of 20, minimum base quality scores of 25, $p$-value of $10^{-5}$ that a locus is variable, at least 75% of non-missing genotypes across samples, minimum $p$-value for deviation from Hardy–Weinberg equilibrium of $10^{-5}$, minimum $p$-value for strand bias of $10^{-5}$, minimum allele frequency of 0.05, and a filter that removed any tri-allelic SNPs. An identity-by-state (IBS) matrix was generated for the full dataset and used to create a cluster dendrogram using the function *hclust* in *R* (R Core Team 2019). Pairs of samples that exhibited levels of genetic similarity equivalent to the level of the technical triplicate groups were identified as naturally occurring genetic clones and the clone/replicate that had the highest number of reads was retained for further analyses.

ANGSD was re-run on the clones-removed dataset with the same filter parameters as described above to generate an IBS genetic distance matrix. The IBS matrix was used to conduct a principal coordinates analysis (PCoA) using the *cmdscale* function in the package *vegan* v2.5.6 in *R* (Oksanen et al. 2019). *poppr* was used to conduct an analysis of molecular variance (AMOVA, 99 permutations) on the converted BCF file from ANGSD, and the package *StAMPP* v1.5.1 was used to calculate pairwise fixation indices ($F_{ST}$) between the populations and corresponding $p$-values (99 permutations, Pembleton et al. 2013; Kamvar et al. 2014). ANGSD was also run on the clones-removed dataset including both variant and invariant sites and without filters that distort allelic frequencies. Heterozygosity was calculated from ANGSD-generated genotype likelihoods using a custom *R* script (https://github.com/z0on/2bRAD_denovo).

Population structure models for clusters $K = 1$–11 (the number of sample populations + 3 to evaluate if there are cryptic genetic clusters within a sample population) were generated using the program NGSAdmix, which conducts admixture analysis directly on genotype likelihoods (Skotte et al. 2013). The most likely number of genetic clusters (the best value of $K$) was estimated from 10 NGSAdmix iterations run for each value of $K$. These runs were imported into the web-based program CLUMPAK, which employs the Evanno method and the web-based program StructureSelector which uses the Puechmaille method to evaluate the optimal values of $K$ (Evanno et al. 2005; Kopelman et al. 2015; Puechmaille 2016; Li and Liu 2018).

Two programs were employed to identify outlier SNP loci, BAYESCAN v2.1 (50,000 burn-in, 5000 iterations, Foll and Gaggiotti 2008) and BayeScEnv v1.1 (50,000 burn-in, 5000 iterations, Villemereuil and Gaggiotti 2015). Both programs use similar Bayesian models to conduct genome scans and identify loci putatively under natural selection pressures using differences in allele frequencies among populations. However, BayeScEnv additionally incorporates environmental variables as covariates to detect local adaptation linked to each variable, in this case average depth of each sample population. Outlier SNP loci were compared against annotated gene regions (within ± 2 kb) of the *M. cavernosa* genome to identify putative functional effects (Rippe et al. 2021). A rank-based gene ontology (GO) analysis was conducted using the GO_MWU method to identify GO categories significantly over-represented among gene regions with outlier SNP loci (https://github.com/z0on/GO_MWU; Wright et al. 2015).

## Results

Sequencing generated a total of 1.62 billion reads, averaging 7.3 million reads per sample library. Following PCR de-duplication and quality filtering steps a total of 611 million reads were retained, or an average of 2.7 million reads per sample library. On average, only 2.5% of quality-filtered reads per sample aligned to the Symbiodiniaceae genomes. A small proportion of the quality-filtered reads, on average 0.16% per sample, were excluded from downstream analyses due to dual alignment to both symbiont and host references. An overwhelming majority (on average 98.4%) of the reads that aligned exclusively to the Symbiodinaceae reference aligned to the *Cladocopium* genome (Supp. Figure 1).

Two pairs of natural clones were identified in the cluster dendrogram (Supp. Figure 2). Both pairs of clones were sampled from the shallow Upper Keys, and in situ colony photographs verified that these colonies were not accidental re-samples of the same colony. With natural clones and technical triplicate libraries removed, ANGSD generated a total of 9,891 SNP loci that passed the previously described filters. Heterozygosity calculated across all sites (invariant and variant) and averaged across individuals within a sampling population did not vary widely, ranging from 0.00238 to 0.00265 (Table 2).

In the PCoA, shallow and mesophotic Upper Keys samples clustered tightly together and with samples from the mesophotic Lower Keys and Southern Dry Tortugas populations (Fig. 2). Shallow Lower Keys and Southern Dry Tortugas samples clustered together and were relatively distanced from the previously described cluster. Shallow and mesophotic samples from Northern Dry Tortugas appear to be genetic intermediaries between the two clusters.

**Table 2** Mean heterozygosity and standard deviation calculated for each sample population across all sites (variant and invariant). $n_g$ indicates the number of unique genotypic individuals within each sample population

| Location | Depth zone | $n_g$ | Mean heterozygosity | Standard deviation |
|---|---|---|---|---|
| DTS | Shallow | 28 | 0.00253 | 0.00039 |
| | Mesophotic | 37 | 0.00250 | 0.00041 |
| DTN | Shallow | 27 | 0.00265 | 0.00062 |
| | Mesophotic | 23 | 0.00241 | 0.00035 |
| LK | Shallow | 30 | 0.00246 | 0.00033 |
| | Mesophotic | 13 | 0.00239 | 0.00034 |
| UK | Shallow | 32 | 0.00238 | 0.00024 |
| | Mesophotic | 25 | 0.00265 | 0.00059 |

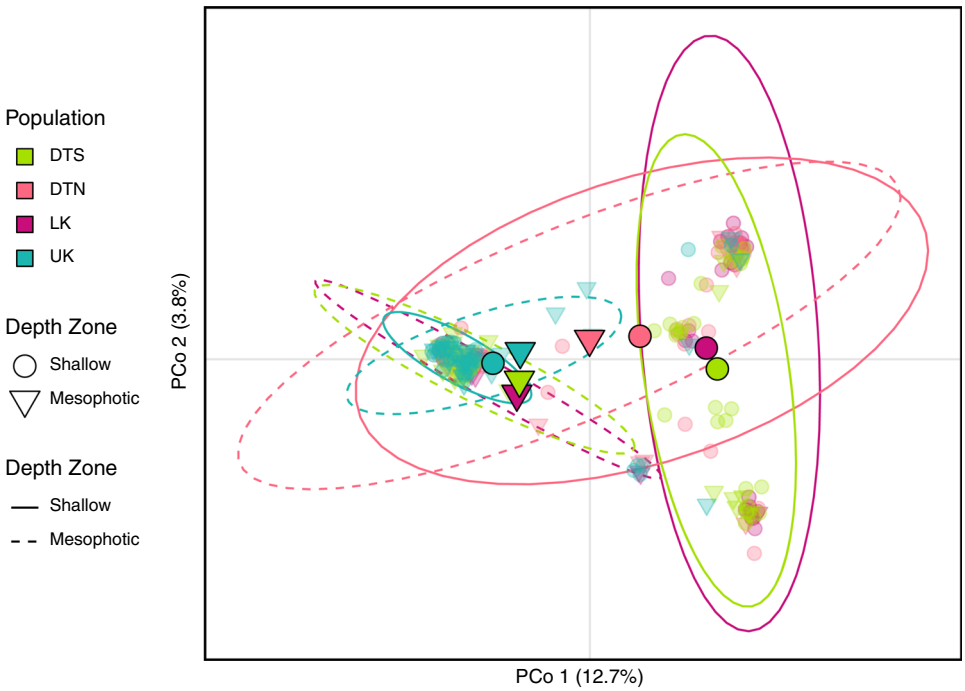


**Fig. 2** Principal coordinates analysis based on Identity-By-State (IBS) distance matrix visualizing clustering of individual samples indicated by small, transparent circles (shallow) and triangles (mesophotic) and population centroids indicated by large circles and triangles. Prediction ellipses assume a multivariate *t*-distribution. Percent variation explained by each axis is indicated

AMOVA attributed a significant amount of genetic variation (2.96%, SS = 21,815.81, $p < 0.01$) to differences among sample populations. Pairwise $F_{ST}$ values identified significant pairwise population differentiation (FDR-corrected $p < 0.05$) for all comparisons with the exception of mesophotic Southern Dry Tortugas and mesophotic Upper Keys (Fig. 3). Pairwise $F_{ST}$ values between shallow and mesophotic Upper Keys and shallow and mesophotic Northern Dry Tortugas were both relatively low ($F_{ST}$ = 0.004 for both pairwise comparisons). In contrast, pairwise $F_{ST}$ values between shallow and mesophotic Lower Keys and shallow and mesophotic Southern Dry Tortugas were much higher ($F_{ST}$ = 0.065 and 0.062, respectively). Pairwise $F_{ST}$ values for between site comparisons mirrored the clustering patterns in the PCoA with low $F_{ST}$ values among shallow/mesophotic Upper Keys, mesophotic Lower Keys, and mesophotic Southern Dry Tortugas ($F_{ST}$ = 0–0.008), and low $F_{ST}$ values between shallow Lower Keys and shallow Southern Dry Tortugas ($F_{ST}$ = 0.003).

The optimal number of genetic clusters ($K$) varied across evaluation estimators between $K$ = 2 and $K$ = 3; therefore, admixture plots for both models were produced and analyzed (Fig. 4). Patterns in genetic structure among sample populations reflected patterns of genetic similarity revealed in the PCoA and pairwise $F_{ST}$ heat map. In the $K$ = 2 plot, the majority of the shallow populations including Southern Dry Tortugas, Northern Dry Tortugas, and Lower Keys populations are dominated by the genetic cluster indicated in blue, while the majority of the mesophotic populations including Southern Dry Tortugas, Lower Keys, and Upper Keys are dominated by the genetic cluster shown in yellow. In contrast, mesophotic Northern Dry Tortugas samples had a high likelihood of membership to each of the two proposed genetic clusters, and the shallow Upper Keys is dominated by the yellow genetic cluster that mostly dominates the mesophotic populations. In the $K$ = 3 model,

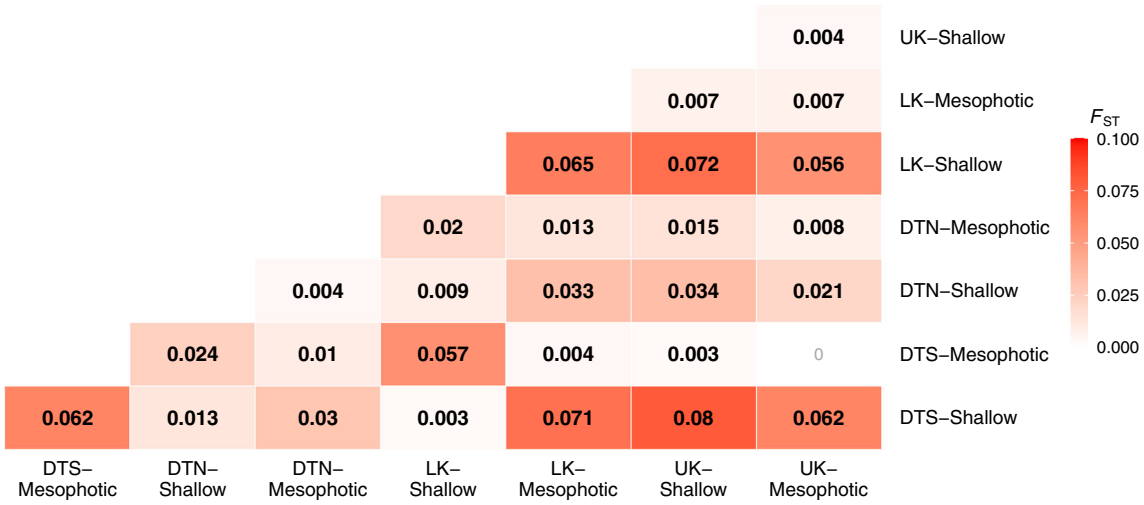


**Fig. 3** Heat map representations of pairwise population differentiation as estimated by fixation index ($F_{ST}$). Values within cells are estimated $F_{ST}$ with increasing intensity of the color red corresponding to increasing $F_{ST}$ values. Bolded $F_{ST}$ values denote significant differentiation between populations (post FDR-correction, $p < 0.05$)

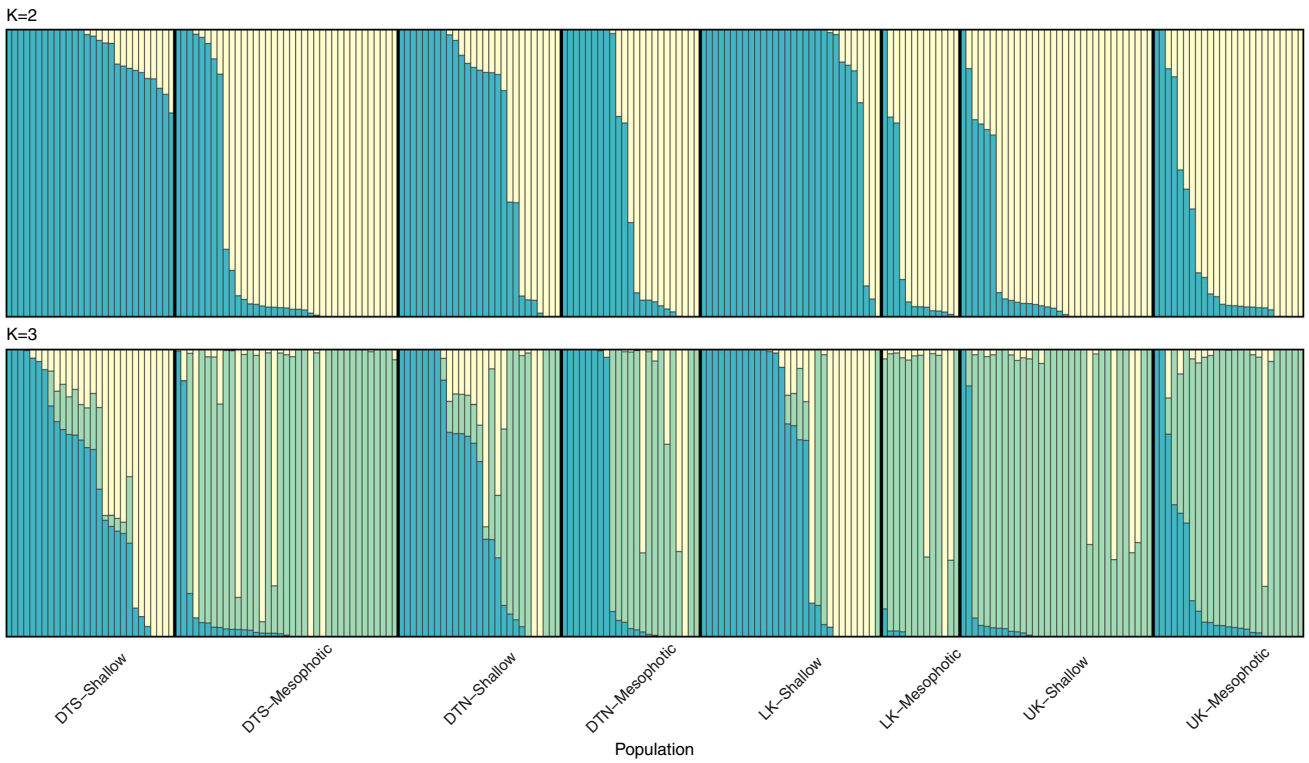


**Fig. 4** Population structure models generated via admixture analysis conducted on genotype likelihoods with the program NGSAdmix. The Evanno and Puechmaille methods were used to estimate the optimal values of $K$, and the models $K = 2$ and $K = 3$ were selected as the most likely number of genetic clusters, represented by the colors yellow, blue, and green

samples mainly from the shallow Southern Dry Tortugas, and Lower Keys populations tended to be dominated by either a blue or yellow genetic cluster, while mesophotic Southern Dry Tortugas, Lower Keys, and Upper Keys, as well as shallow Upper Keys, were dominated by the green genetic cluster. The shallow and mesophotic Northern Dry Tortugas populations were more variable, with multiple samples dominated by each of the three genetic clusters as well as samples that were relatively admixed.

The program BAYESCAN identified a total of 353 outlier SNP loci (FDR-corrected $p$-value $< 0.1$) potentially under diversifying selection. Of these 353 putative outlier

SNP loci, 116 of them were identified within ± 2 kb of 119 M. *cavernosa* annotated gene regions (Supplementary Table S1). Many of these genes were of unknown function, but a number also clustered into a variety of orthologous group categories, including those that function in post-translational modification, protein turnover, and chaperone functions as well as signal transduction (Tatusov et al. 2000). However, GO enrichment analysis with GO_MWU failed to identify any GO categories that were significantly over-represented among annotated gene regions with outlier SNP loci (FDR = 0.1). Additionally, BayeScEnv failed to identify any depth-associated outlier SNP loci.

## Discussion

Population genetic structure analyses including PCoA, AMOVA, pairwise $F_{ST}$ comparisons, and admixture analysis based on 9,891 SNP loci demonstrate significant genetic structure among *M. cavernosa* populations in the Florida Keys. The level of vertical genetic connectivity between paired shallow and mesophotic populations was location specific. Shallow and mesophotic *M. cavernosa* populations from the Lower Keys and Southern Dry Tortugas exhibited relatively high levels of vertical pairwise genetic differentiation despite less than 2 km and 4 km maximum horizontal distance between pairwise shallow and mesophotic sampling sites within a reef location (Fig. 3). In contrast, shallow and mesophotic *M. cavernosa* populations in Northern Dry Tortugas and Upper Keys populations exhibited comparatively low levels of genetic differentiation, even with maximum horizontal distances of 10 km between some shallow and mesophotic sampling sites within the Upper Keys (Fig. 3).

A similar microsatellite-based study that assessed *M. cavernosa* genetic structure among depth zones from 0 to 30 m across the Upper Keys, Lower Keys, and the Northern Dry Tortugas (Serrano et al. 2014) also found high levels of genetic differentiation between 0–10 m and 25–30 m populations in the Lower Keys, but much lower levels of genetic differentiation between populations at these two depths in the Northern Dry Tortugas and Upper Keys. The present study suggests that these location-specific patterns in genetic differentiation persist in populations beyond 30 m, into the upper mesophotic zone. Future sampling of this species across finer-scale depth gradients in both the shallow and upper mesophotic zones within the Florida Keys may help to characterize location-specific genetic "break-points," which may not align with traditional definitions of shallow and mesophotic depth zonation (Eckert et al. 2019).

Location-specific patterns in vertical genetic connectivity of *M. cavernosa* populations across a depth gradient are not unique to the Florida Keys. Microsatellite-based analyses also indicate there is no significant genetic differentiation between shallow and mesophotic populations of *M. cavernosa* in the northwest Gulf of Mexico (Studivan and Voss 2018). In contrast, *M. cavernosa* populations along the Belize Barrier Reef exhibit strong genetic structuring across a depth gradient, especially between relatively shallow (10 and 16 m) and relatively deep (25 and 35 m) populations (Eckert et al. 2019). Similarly, a SNP-based analysis of *M. cavernosa* in Cuba found significant levels of genetic differentiation between the mesophotic Banco de San Antonio population and all other shallow populations (Sturm et al. 2020).

While a single mesophotic coral population may exhibit rather limited connectivity to its nearby shallow counterpart, that same reef may demonstrate high levels of genetic similarity to shallow populations downstream over much greater horizontal distances. The results of this study suggest that mesophotic Southern Dry Tortugas and Lower Keys populations, while highly differentiated from their local shallow counterparts, are highly connected to both mesophotic and shallow populations downstream in the Upper Keys. Similar patterns have been observed in *M. cavernosa* population genetic structure on a regional scale. While nearby shallow and upper mesophotic populations in Belize are highly differentiated, mesophotic populations in Belize exhibit relatively high levels of genetic similarity to the Dry Tortugas, despite > 1000 km of separation (Studivan and Voss 2018).

*Montastraea cavernosa*'s reproductive characteristics may partially contribute to the observed patterns of genetic differentiation among shallow and mesophotic populations in the Florida Keys. *Montastraea cavernosa* is a gonochoric broadcast spawner; male and female colonies simultaneously release buoyant gametes approximately 1 week following the full moon during July–September (Szmant 1991). Gametes are thought to mix and fertilize in the water column near the surface. Very little is known about the temporal dynamics of spawning across shallow and mesophotic depth zones or across different locations within the Florida Keys. However, in Flower Garden Banks National Marine Sanctuary, spawning of *M. cavernosa* occurs synchronously across shallow and mesophotic depth zones where nightly spawning windows for the species can be as long as 4 h (Vize 2006). Even with synchronous spawning, the greater depths mesophotic gametes must traverse to reach the surface may cause barriers to fertilization between shallow and mesophotic gametes, especially in hydrodynamic environments, where surface waters are quickly advected away (Levitan et al. 2004). Possible temporal variation in spawning and/or fertilization between shallow and mesophotic *M. cavernosa* populations, in combination with local hydrodynamic regimes,

may contribute to the location specific patterns in vertical connectivity observed across the Florida Keys.

Biophysical models generated to characterize potential *M. cavernosa* larval connectivity across the Florida Keys in some cases mirror the assessments of population connectivity based on the genetic dataset generated in this study. One of the most relevant biophysical studies employed a high-resolution hydrodynamic model to simulate a 2010 *M. cavernosa* spawning event to characterize potential patterns in larval connectivity among reefs in the Florida Keys (Frys et al. 2020). Reefs across the Lower Keys, especially deeper, outer-shelf locations are dominated by the northward moving Florida Current which can transport larvae upwards of $\sim$ 100 km prior to settlement. Shallower, near-shore sites in the Lower Keys are also influenced by slightly weaker, but continuous, southward flow from the inner-shelf through breaks in the Lower Keys toward the outer-shelf. This may be a possible driver of differentiation between shallow and mesophotic populations in the Lower Keys (Figs. 2, 3). More nearshore, shallow Lower Keys sites may have higher self-recruitment or receive incoming larvae from inner-shelf reefs while mesophotic sites further offshore on the outer-shelf are more likely to receive larvae from upstream sources or have their larvae advected away by the Florida Current. Modeled connectivity pathway links are shorter on the outer-shelf of the Upper Keys, likely due to strong tidal influences that generally keep larvae close to the reef, contributing to self-recruitment and retention of larvae sourced from upstream reefs (Frys et al. 2020). These biophysical dynamics may contribute to the close genetic clustering of the majority of shallow and mesophotic individuals in the Upper Keys (Fig. 2).

However, in contrast with the genetic results presented here, which suggest that there are relatively high levels of genetic connectivity, especially between populations in the Southern Dry Tortugas and the Lower Keys and Upper Keys, the biophysical model employed by Frys et al. (2020) simulated extremely limited larval connectivity between the Dry Tortugas and the Lower Keys and Upper Keys. The dispersal model reproduced a single spawning event based on hydrodynamic conditions from August to September, 2010. Other hydrodynamic and physical connectivity studies focused on the southwest Florida shelf and Florida Keys have validated their models over multi-year scales (Kourafalou et al. 2018; Olascoaga et al. 2018; Valle-Levinson et al. 2020). These studies found that hydrodynamic conditions, especially near the shelf break, exhibit high intra- and inter-annual variability stemming from a combination of wind forcing, the meandering Loop Current/Florida Current system, and the related shedding and propagation of eddies. Mesophotic Southern Dry Tortugas sites are closest to the shelf break and therefore are more likely to be subjected to an oceanic current influence when compared to shallow Southern Dry Tortugas sites and the Northern Dry Tortugas which are more interior on the shelf (Valle-Levinson et al. 2020). This may contribute to the depth-dependent genetic differentiation observed in the Southern Dry Tortugas that is not identified in the Northern Dry Tortugas. In addition, the flow across both Northern and Southern Dry Tortugas sites is oftentimes parallel in directionality which suggests that there is limited physical connectivity between them (Valle-Levinson et al. 2020). This may explain why *M. cavernosa* populations in the Dry Tortugas exhibit higher levels of genetic connectivity to more physically distant downstream sites than to one another. Hydrodynamic models applied to simulations of larval connectivity of the bicolor damselfish, *Stegastes partitus*, which exhibit similar spawning periods and pelagic larval duration to *M. cavernosa*, suggest that Loop Current/Florida Current-eddy interactions can drive sporadic but significant pulses of larvae from the Dry Tortugas (and from further upstream sites at Pulley Ridge) that settle in the Lower Keys and Upper Keys, possibly leaving the genetic signature observed in our dataset (Vaz et al. 2016).

We identified 353 outlier SNPs, 3.5% of all genotyped SNP loci, as putatively undergoing diversifying selection. The prevalence of outlier SNP loci is consistent with other coral population genetic studies based on RADseq SNP approaches (Devlin-Durante and Baums 2017; Drury et al. 2020). Thirty-two percent of these identified outlier SNPs occurred within a $\pm$ 2 kb range of annotated gene regions which may potentially have functional implications for this species, especially as it relates to protein modification or signal transduction (Supplementary Table S1). In contrast, BayeScEnv did not identify any outlier SNP loci when depth was incorporated as an environmental co-variate, suggesting that patterns in genetic differentiation between shallow and upper mesophotic populations may not be driven by local adaptation to a shallow or upper mesophotic environment. These results contrast with another study investigating the population genetic structure of *M. cavernosa* across a shallow to lower mesophotic depth gradient in Florida (Drury et al. 2020). Drury et al. (2020) found a significant correlation between depth and the minor allele frequency of outlier SNP loci, a pattern which was mainly driven by unique allelic patterns in the lower mesophotic Pulley Ridge population. However, *M. cavernosa* populations at Pulley Ridge are highly genetically isolated from other nearby populations in the region and exhibit high rates of clonality, so it is unknown if these unique allelic patterns are signatures of adaptive selection to the lower mesophotic environment or driven by high rates of asexual reproduction, inbreeding, or genetic bottlenecks (Studivan and Voss 2018; Drury et al. 2020). Still,

it is possible that the environmental conditions in the upper mesophotic zone where this study's samples were collected were not divergent enough from the conditions in the nearby shallow environments to generate genomic signatures of local adaptation. This, in combination with the high levels of genetic similarity between some shallow and mesophotic populations, suggests that local hydrodynamic conditions, rather than local adaptation to depth-associated environmental conditions, may be the main driver of population genetic differentiation of *M. cavernosa* across the Florida Keys.

The samples for this study were collected in 2019, years after SCTLD was identified in the Upper Keys (Sharp et al. 2020). The disease has since spread through the Lower Keys down to Key West, although it has yet to be observed within the Dry Tortugas as of late 2020 (Roth et al. 2020). The potential impact that SCTLD-driven mortality events may have on genetic diversity, and therefore, patterns of population genetic structure and connectivity are not yet understood. During this sampling expedition in August–September, 2019, prevalence of SCTLD was minimal on shallow reefs of the Lower and Upper Keys and not observed across the Dry Tortugas or at mesophotic sites. In later surveys conducted from October 2019 to February 2020 between 10 and 25 m depths, SCTLD prevalence on *M. cavernosa* in the Upper Keys remained low (average 0.2%) but was much higher across sites within the Lower Keys, averaging 7.5% (Sharp et al. 2020). Overall, the majority of active disease and recently dead colonies observed during that time period were in the Lower Keys.

The timing and progression of the disease throughout the Keys suggest that our sampling period may have occurred after peak SCTLD prevalence and mortality in the Upper Keys but before outbreaks reached the Lower Keys. Since *M. cavernosa* is an intermediately susceptible species to SCTLD and no sampling was completed before the outbreak reached the Upper Keys, it is difficult to determine if the patterns in population genetic structure and variation was influenced by SCTLD (FKNMS and FDEP 2018). For example, if more susceptible genets across the shallow Upper Keys were members of a distinct lineage, their loss may have decreased the population genetic structure and genetic diversity, resembling that of the mesophotic Upper Keys, Lower Keys, and Southern Dry Tortugas in our dataset. However, Serrano et al. (2014) found similarly low levels of genetic differentiation between 0–10 m and 25–30 m populations in the Upper Keys before SCTLD was first identified and described. This suggests that the lack of major genetic structuring across depth observed in our study in the Upper Keys is not driven solely by SCTLD related mortality of susceptible genets.

Ultimately, this genetic characterization of a dominant species across the Florida Keys can inform management efforts within Florida Keys National Marine Sanctuary. While diversity parameters like heterozygosity did not vary widely among populations (Table 2), these data do provide a genomic diversity benchmark that should be considered in coral restoration efforts. In addition, some upper mesophotic communities which are, as of yet, relatively unimpacted by SCTLD outbreaks may serve as important genetic refugia and possible sources of larvae for connected shallow coral populations. Further characterization and continued monitoring of these upper mesophotic reefs are needed to better understand the ecosystem services they provide and risks they may face. Overall, the genetic results of this study highlight the need to conserve these important mesophotic reef communities and incorporate them into our understanding of coral metapopulation dynamics in the Florida Keys.

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**Declarations**

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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