Seascape genomics reveals population structure and local adaptation in a widespread coral reef snail, *Coralliophila violacea* (Kiener, 1836)

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ABSTRACT

Local adaptation to different environments may reinforce neutral evolutionary divergence, especially in populations in the periphery of a species' geographic range. Seascape genomics (high-throughput genomics coupled with ocean climate databases) facilitates the exploration of neutral and adaptive variation in concert, developing a clearer picture of processes driving local adaptation in marine populations. This study used a seascape genomics approach to test the relative roles of neutral and adaptive processes shaping population divergence of a widespread coral reef snail, *Coralliophila violacea*. We collected *C. violacea* from colonies of their coral host (*Porites* spp.) at ten locations spanning a large portion of their geographic range. We used RAD-seq to investigate possible local adaptation via genetic-environmental associations with five ocean climate variables. Four genetic partitions were concordant with regions previously observed in mtDNA (Indian Ocean, Coral Triangle, and Hawaiian Islands), with the addition of Vietnam and varying isolation and admixture levels. We identified outlier loci (FDR = 0.10, $N = 72$) among individual localities and between regions (FDR = 0.10, $N =$

34), suggesting that some loci are putatively under divergent selection. Association analyses showed that the two strongest drivers of local adaptation were the annual range and mean of sea surface temperature. Populations that experience lower sea surface temperatures at the periphery of *C. violacea*'s geographic range drive these associations. Our results show that local adaptation to different environments likely reinforces neutral divergence, especially in peripheral populations.

Keywords: Coral reef, *Coralliophila violacea*, local adaptation, peripheral populations, seascape genomics, RAD-seq

INTRODUCTION

Incomplete or ephemeral dispersal barriers, combined with potentially large population sizes and extensive geographic ranges of marine taxa, suggest that natural selection plays a crucial role in generating biodiversity in the ocean (Crandall et al., 2012). Authors are increasingly advocating for speciation models that combine both neutral and adaptive processes (Bowen et al., 2013; Horne, 2014) because natural selection limits realized connectivity between populations (Burgess et al., 2012). Local adaptation to climate and habitat can reinforce neutral divergence patterns driven by gene flow, genetic drift, or mutations (Gavrilets, 2003). In these models, even species with high fecundity and long dispersal distances can have low effective gene flow if selection favors local progeny over those recruiting from different environments.

Empirical evidence indicates that selection plays a more significant role in the diversification of marine taxa than previously thought. For example, several studies show environmental and habitat heterogeneity (Longo & Bernardi, 2015; Rocha & Bowen, 2008;

Teske et al., 2019), or competition among species (Bowen et al., 2013; Briggs, 1992), drive diversification or reinforce nascent allopatric divergence. Similarly, recent work also suggests that divergent selection between habitats or hosts contributes to adaptive variation and speciation (Cheng, 2015; Meyer et al., 2005; Reid et al., 2006; Simmonds et al., 2020; Tornabene et al., 2015).

Natural selection can be particularly influential in peripheral areas of species' ranges where environmental conditions may be at or near the limits of a species' physiological threshold (J. D. DiBattista et al., 2016; Gaither et al., 2010; Johannesson & André, 2006; Kawecki, 2008), creating different selective pressures.

Studies using high-throughput genomic tools have found signals of natural selection driving genetic divergence in peripheral marine populations (Ackiss et al., 2018; Gaither et al., 2015; Saenz-Agudelo et al., 2015). Authors hypothesized various mechanisms driving divergence, including different ecological (e.g., habitats) and environmental factors (e.g., sea surface temperature, salinity, turbidity), but also seasonal fluctuations or environmental heterogeneity (e.g., oceanography) at smaller scales that indicate selective processes at play. As such genomic sequencing, combined with global, high-resolution marine environmental databases (Sbrocco & Barber, 2013), meaning we can now directly assess the role of natural selection driving or reinforcing diversification in the ocean.

In recent decades, geneticists have documented phylogeographic structure in a wide diversity of marine taxa (Bowen et al., 2014; Kelly & Palumbi, 2010), advancing allopatry as a primary speciation model in marine ecosystems, particularly among studies of Indo-Pacific biodiversity (Barber et al., 2011; Bowen et al., 2013; Carpenter et al., 2010; Gaither et al., 2010). Authors often report one or more of the following phylogeographic patterns: 1) population divergence

between Indian and Pacific Ocean basins; 2) population differentiation within the Coral Triangle, the global epicenter of marine biodiversity; or 3) population differentiation on the periphery of the Pacific (e.g., Hawai'i, Marquesas) or Indian Oceans (e.g., Red Sea).

The divergence between the Indian and Pacific Oceans is typically ascribed to the Sunda and Sahul continental shelves' exposure when Plio-Pleistocene glaciations lowered sea levels by 115–130m (Voris, 2000). These intermittent landmass barriers constricted waterways of the Indonesian and Philippine Archipelago, reducing gene flow between the Indian and Pacific Oceans in a wide diversity of marine taxa, including seahorses (Lourie et al., 2005); soldierfish (Craig et al., 2007); anemonefish (Dohna et al., 2015; Timm & Kochzius, 2008); damselfish (Drew & Barber, 2009; Liu et al., 2014; Raynal et al., 2014; Liu et al. 2019); groupers (Gaither et al., 2011); fusiliers (Ackiss et al., 2013); limpets (Kirkendale & Meyer, 2004); snails (Crandall et al., 2008a; Reid et al., 2006; Simmonds et al., 2018); giant clams (DeBoer et al., 2014; Nuryanto & Kochzius, 2009) and seastars (Kochzius et al., 2009). Further studies suggest that modern oceanographic features such as the Halmahera Eddy can limit gene flow within the Coral Triangle by constraining larval exchange, a hypothesis supported by both phylogeographic studies (Ackiss et al., 2013; Barber et al., 2011; DeBoer et al., 2008) and biophysical connectivity models (Kool et al., 2011; Treml et al., 2015).

Although multiple processes contribute to isolation and divergence in the sea (Barber & Meyer 2014), purely allopatric models of marine speciation still face challenges. For example, while Plio-Pleistocene sea levels did constrict Indonesian and Philippine waterways (Voris, 2000), the major pathways for the Indonesian Throughflow, the Makassar Strait, Maluku, and Banda Seas, are deeper than 3,000 m. Thus, even at the lowest sea levels, these dispersal pathways between Pacific and Indian Oceans remained open, requiring authors to invoke

auxiliary processes such as increased upwelling of cold water as a mechanism to limit dispersal (Fleminger, 1986). Moreover, periods of isolation were punctuated by tens of thousands of years of oceanic conditions similar to today's, during which gene flow and population expansion would have occurred, weakening the signal of historical isolation (Crandall et al., 2008b). Similarly, ocean currents in the region vary, both seasonally (Shinoda et al., 2012) and across epochs (Kuhnt et al., 2004), such that oceanographic features may promote isolation during some periods only to be reversed in others.

The coral-eating snail *Coralliophila violacea* (Kiener, 1836) is widespread on coral reefs across the tropical Indo-Pacific, from the Red Sea to the Eastern Pacific (Demond, 1957), and may be subject to both neutral and selective processes for divergence. Previous work on *C. violacea* demonstrated genetic differentiation of snails living on different coral host lineages, despite ongoing gene flow (Simmonds et al., 2020; Simmonds et al., 2018). One lineage was broadly distributed but exhibited phylogeographic structure in the core of its geographic range (i.e., across the Sunda Shelf) and peripheral populations (i.e., Hawai'i). This structure could be attributed to allopatric processes, such as isolation during low sea-level stands or isolation by distance. However, given that environmental conditions vary greatly, it is possible that variation in ocean climate across the range of *C. violacea* could reinforce population divergence via natural selection.

This study combined genome-wide surveys of genetic variation in *C. violacea* with data from marine environmental databases to test the relative roles of neutral and adaptive processes shaping population divergence. Specifically, we test for divergence across known phylogeographic provinces within the Coral Triangle and divergence among peripheral populations in the Indian and Pacific Oceans and the South China Sea.

We then compare neutral and non-neutral variation patterns to geography and environmental variables to assess their relative roles in shaping population divergence.

MATERIALS AND METHODS

Sample collection

To test for patterns of divergence related to geography and local adaptation in *C. violacea*, we collected snails on snorkel or scuba at ten locations spanning a large portion of the snail's geographic range (Table 1, Fig. 1). Sampled populations included both sides of the Sunda Shelf, an area where phylogeographic structure is commonly observed, as well as Hawai'i, the edge of *C. violacea*'s geographic range and an area known to exhibit peripheral isolation (Gaither et al., 2011b; Hodge et al., 2014). In addition, we only used snails collected from *P. lobata* and *P. compressa* coral colonies (clades 2 and 3, the “green group” from Simmonds et al. 2018) that were previously DNA barcoded using RAD-seq data. This selectivity was necessary because *Porites* corals are notoriously challenging to identify *in situ* due to their morphological plasticity and small corallites (Forsman et al., 2015) and because genetically similar colonies can have vastly different morphologies and *vice versa* (Forsman et al., 2009). In total, we collected snails from 1–3 colonies at each location for a total of 71 snails from 32 coral colonies. A portion of each snail's foot tissue was preserved in 95% ethanol and stored at room temperature for DNA analysis.

Figure 1 Map of *Coralliophila violacea* collection locations across the Indo-Pacific region. Sampled populations include 1. Vavvaru. 2. Pulau Weh 3. Hòn Mun 4. Dumaguete 5. Bunaken 6. Lembeh 7 Pulau Mengyatan 8. Nusa Penida 9. Manokwari 10. Ka'a'awa. Raster map made with Natural Earth.

Table 1 Sampling locations for *Coralliophila violacea* collected from coral hosts *Porites lobata* and *P. compressa* from *Porites* lineage 1, corresponding environmental variables from MARSPEC database for each location used in Bayenv2 analysis. Coordinates are in decimal degrees. Location numbers correspond to those in Figure 1. Regions were used for AMOVA analyses. Sea surface salinity (SSS) in psu (practical salinity units). Sea surface (SST) in °C. *N* = no. of individuals.

LOCATION	COUNTRY	REGION	LATITUDE	LONGITUDE	WARMEST MO.		SST	SST RANGE	SSS	SSS RANGE
					SST					
1. Vavvaru	Maldives	Indian Ocean	5.419	73.358	29.42		28.37	1.76	34.93	1.64
2. Pulau Weh	Indonesia	Indian Ocean	5.887	95.348	29.39		28.59	1.40	33.29	1.43
3. Hòn Mun	Vietnam	Vietnam	12.170	109.308	28.20		26.45	4.22	33.34	0.77
4. Dumaguete	Philippines	Coral Triangle	9.332	123.312	28.54		27.61	3.16	33.96	0.92
5. Bunaken	Indonesia	Coral Triangle	1.612	124.783	28.74		28.34	1.05	33.95	0.79
6. Lembeh	Indonesia	Coral Triangle	1.479	125.251	28.81		27.87	2.94	33.95	0.85
7. Pulau Mengyatan	Indonesia	Coral Triangle	-8.557	119.685	28.47		27.51	1.95	34.09	0.68
8. Nusa Penida	Indonesia	Coral Triangle	-8.675	115.513	28.81		27.60	2.12	33.89	1.19
9. Manokwari	Indonesia	Coral Triangle	-0.888	134.085	29.16		28.90	0.78	34.09	1.03
10. Ka'a'awa	USA	Hawai'i	21.584	-157.887	26.43		24.99	2.81	34.92	0.21

Creation of RAD-seq libraries

We extracted DNA from 20 mg of foot tissue using a Qiagen DNeasy Blood and Tissue Kit (Qiagen). We followed the manufacturer's instructions, except in the last step, when we eluted DNA with 100 µl of molecular grade H₂O rather than AE buffer. We estimated initial DNA concentrations using a NanoDrop and visualized DNA quality on a 1%

agarose gel stained with SYBR Green. We used only high-quality DNA with a bright high molecular weight band and minimal smearing. We dried DNA extractions using a SpeedVac on medium heat and reconstituted using molecular grade H₂O to a final uniform 250 ng/μl DNA concentration.

We prepared 2b-RAD libraries following the protocol of Wang et al. (Wang et al., 2012). Alfl restriction enzymes digest reduced representation (1/16th) libraries labeled with individual barcodes and subjected to 18–20 PCR amplification cycles. We electrophoresed products on a 2% agarose gel in a 1× TBE buffer and run at 150 V for 90 minutes. Target bands (165 bp) were visualized with SYBR SAFE dye and excised from the gel. The excised band was then purified using a QIAquick gel purification kit (Qiagen). For a final cleaning step, we used Ampure XP beads (Beckman-Coulter). The QB3 Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley conducted quality checks (qPCR, BioAnalyzer) and sequencing of the resulting libraries, multiplexing 10–20 snails per lane in 5 lanes of a 50 bp single-end run on the Illumina HiSeq 2000 platform.

RAD-seq data processing

To prepare raw sequence data for SNP identification, we truncated all raw reads to the insert size (36 bp), filtered for quality (PHRED scores >20), and discarded empty constructs. We used custom scripts to process the data, available on GitHub https://github.com/z0on/2bRAD_denovo. First, unique tag sequences (minimum sequencing depth 5×) were counted, the number in reverse-complement orientation recorded and merged into one table. All sequences were then clustered in CD-HIT using

a 91% similarity threshold. Next, we defined the reference as the most abundant sequence in the cluster. We then filtered a locus-annotated table from the previous two steps, excluding reads below $5\times$ depth or exhibiting strand bias. The resulting clustered sequences' orientation was then flipped to match the most abundant tag in a cluster. We applied mild allele filters ($10\times$ total depth, allele bias, and strand bias), with the requirement that alleles appear in at least two individuals. We then applied locus filters, allowing a maximum of 50% heterozygotes at a locus, no more than two alleles, genotyped in 30% of samples, and polymorphic. The final set of SNPs was then thinned to one per tag (the one with the highest minor allele frequency) for STRUCTURE analysis and gene-environment association tests to remove linked loci that might be in linkage disequilibrium.

Individual sample filtering steps

From the first 71 individuals, we filtered out those with low genotyping rates ($N = 5$), indicating poor DNA quality by taking the $\log_{10}$ of the number of sites genotyped per individual and removing individuals ≥ 2 standard deviations (SD) of the mean. We also removed individuals ($N = 4$) with high homozygosity (± 2 SD of the mean F inbreeding coefficient), indicating potential contamination. We used the remaining 63 individuals in all analyses. The final data file was in VCF format, and we converted it to other data formats using PGDSpider v2.1.0.1 (Lischer & Excoffier, 2012).

Genetic diversity

We calculated basic population genetic summary statistics using all filtered loci for each location

with ARLEQUIN v3.5 (Excoffier & Lischer, 2010). We estimated the number and frequency of polymorphic loci for each site, observed and expected heterozygosity, and genetic diversity (averaged across all loci).

F_{ST} outlier loci test

We tested loci potentially under natural selection by identifying outlier F_{ST} values using BayeScan v2.1 (Foll & Gaggiotti, 2008). We ran BayeScan with the data structured in two ways to look for outliers at different spatial scales, 1) comparing individual localities and 2) comparing localities grouped into regions (Table 1). We ran the data with a burn-in of 50,000, a thinning interval 10, a sample size 5,000, 100,000 iterations, and 20 pilot runs of 5,000, each examining two different false discovery rates (FDR = 0.10, 0.05).

Population genetic structure

We used the Bayesian model-based clustering method STRUCTURE (Pritchard et al., 2000) to infer the population genetic structure and individual admixture proportions. We examined three datasets consisting of 63 individuals: 1) full RAD-seq dataset using all loci; 2) using only neutral loci as determined by BayeScan; and 3) outlier loci with a false discovery rate of 10% (FDR = 0.10, BayeScan). We ran STRUCTURE with a burn-in period of 20,000 followed by 50,000 MCMC replicates for $K = 1-10$, and ten runs for each K . We used the admixture model, with allele frequencies correlated among populations. We selected the optimal value of K using the ΔK statistic (Evanno et al., 2005) and summarized the results graphically using the program CLUMPAK v1.1 (Kopelman et al., 2015). To test for the significance of genetic partitions identified by STRUCTURE, we ran analyses of molecular variance (AMOVA), both with and

without regional groupings (Table 1). We determined significance by 100,000 random replicates in ARLEQUIN.

Genetic-environment association tests

Because ocean climate variables that impact species distributions (Briggs, 2006) may also structure population genetics (Sanford & Kelly, 2011), we examined differences in allele frequencies associated with environmental variables using a Bayesian framework (Bayenv 2.0; (Coop et al., 2010; Günther & Coop, 2013) that accounts for demographic history. First, we obtained ocean environmental variables from the MARSPEC database (Sbrocco & Barber, 2013). Because of strong correlations among environmental variables, we selected only five in the MARSPEC database for analysis: 1) temperature of the warmest ice-free month (biogeo15), 2) mean annual sea surface temperature (annual sea surface temperature; biogeo13), 3) annual range in sea surface temperature (biogeo16), 4) mean annual sea surface salinity (biogeo08), and 5) annual range in sea surface salinity (biogeo11). Temperature variables are particularly important to this system because coral reefs in locations with high sea surface temperatures are most likely to be impacted by coral bleaching events (Hoegh-Guldberg, 1999). Thus, coral mortality from bleaching could directly impact *C. violacea* living on coral hosts and indirectly by limiting the number and species of hosts available to *C. violacea* in affected reefs.

To create maps of each of the five environmental variables across the study region, we projected the annual mean and range of sea surface salinity (SSS) and temperature (SST), plus the mean warmest monthly temperature at ~5 km resolution onto an equidistant cylindrical world using the ‘*raster*’ package in R. Then, we extracted the five climate variable data to each point location and divided it by a scaling factor of 100 using custom R scripts. Next, we estimated a

covariance matrix with standardized environmental variables for each sampling location, as suggested by the authors of Bayenv2 (Coop et al., 2010; Günther & Coop, 2013), and 3,186 loci that were polymorphic among all sites, for 100,000 iterations, outputting the results every 500 iterations. Finally, we used the last printed covariance matrix for all further analyses. To estimate the Bayes Factor (BF) for each SNP with each ocean climate variable, we ran Bayenv2 for 100,000 Markov chain Monte Carlo (MCMC) iterations. For each ocean climate variable, SNPs with $\log_{10} \text{BF} > 1$ were considered to give substantial-to-strong support for environmentally-associated loci, based on criteria from (Litton & Jefferys, 1984).

Candidate gene annotation

To annotate the function of genes linked to outlier loci and environmentally-associated loci, we aligned sequences containing outlier loci to nucleotides (nr/nt) on the NCBI website using the BLASTN algorithm at two different taxonomic levels, 1) Mollusca (taxid:6447) and 2) Lophotrochozoa (taxid:1206795). We adjusted parameters (word size 7, expected threshold 10, no mask for lookup table, no low complexity filter) to accommodate short-read sequences. We only examined hits with a high percent query coverage (>85%) and used NCBI to identify and annotate any associated genes.

RESULTS

The average number of unique reads per individual was 6.6 million at minimum $5\times$ depth after filtering for quality and removing empty constructs. We sequenced and genotyped 46,148 high-quality RAD-seq loci with $\geq 25\times$ coverage in 71 individuals at ten locations. First, we filtered the dataset for 30% maximum missing data per locus, leaving 7,862 loci, and then thinned to one

SNP per loci to remove any physically linked SNPs for STRUCTURE and F_{ST} analyses, leaving 3,188 SNPs. Next, we removed nine individuals with potential contamination issues (inbreeding coefficient $\geq +2SD$ from the mean) or low DNA quality (missing data $\geq +1SD$ from the mean), leaving 63 individuals.

Genetic diversity

Across all locations, a mean of 47% of loci were polymorphic. Hòn Mun in Vietnam had the highest frequency of polymorphic loci (81%) and the most sequenced individuals (Table 2). Observed heterozygosity ($H_O = 0.196 - 0.570$) and expected heterozygosity ($H_E = 0.225 - 0.586$) varied across locations, but H_O was consistently lower than the H_E at every location (Table 2). Mean genetic diversity over all loci across sites was 0.122, and genetic diversity varied across locations (0.089 – 0.162; Table 2). Genetic diversity was highest in Dumaguete (0.162) and lowest in Bunaken (0.095) (Table 2).

Table 2 Standard diversity indices and summary statistics for all 3,188 loci obtained from *Coralliophila violacea*. N = no. of individuals, no. of polymorphic loci per location, frequency of polymorphic loci, observed heterozygosity (H_O), expected heterozygosity (H_E), and gene diversity over all loci.

Location	N	No. poly. loci	Freq. poly. loci	H_O	H_E	Gene diversity
1. Vavvaru	4	1293	41%	0.345	0.449	0.104
2. Pulau Weh	3	1051	33%	0.459	0.544	0.103
3. Hòn Mun	19	2598	81%	0.196	0.225	0.159
4. Dumaguete	2	1022	32%	0.570	0.586	0.162
5. Bunaken	7	1577	49%	0.281	0.342	0.095
6. Lembeh	6	1515	48%	0.301	0.341	0.114
7. Pulau Mengyatan	4	1143	36%	0.360	0.463	0.089
8. Nusa Penida	9	1881	59%	0.271	0.296	0.115
9. Manokwari	5	1680	53%	0.274	0.357	0.149
10. Ka'a'awa	4	1229	39%	0.353	0.397	0.132

297

298 **Gene flow and genetic structure**

299 Results from STRUCTURE using the full dataset of 3,188 loci or just 3,116 putatively neutral
300 loci confirmed that $K = 4$ was the best-supported number of population partitions (Evanno et al.
301 2005). The four genetic partitions corresponded to recognized distinct biogeographic regions: a)
302 Indian Ocean (1. Vavvaru, 2. Pulau Weh); b) Vietnam (3. Hòn Mun); c) the Coral Triangle (4.
303 Dumaguete, 5. Bunaken, 6. Lembeh, 7. Pulau Mengyatan, 8. Nusa Penida and 9. Manokwari);
304 and d) Hawai'i (10. Ka'a'awa) (Fig. 2). Levels of admixture among these regions were variable.
305 Hawai'i and Vietnam showed minimal admixture from other locations. In contrast, sites in the
306 Indian Ocean had the highest levels of admixture, with numerous individuals exhibiting a mix of
307 alleles from the Indian Ocean and populations to the east. Specifically, Pulau Weh exhibited
308 substantial admixture with the Coral Triangle, and Vavvaru in the Maldives had admixture from
309 both Vietnam and the Coral Triangle. Levels of admixture varied across sites in the Coral
310 Triangle. Pulau Mengyatan and Manokwari had the highest levels, while Dumaguete, Bunaken,
311 Lembeh, and Nusa Penida had the lowest; admixture in these populations was mainly from the
312 Indian Ocean and Vietnam. Results were virtually identical whether we used all loci or neutral
313 loci.

Figure 2 Patterns of genetic structure in *Coralliophila violacea* in the Indo-Pacific region. CLUMPAK-averaged STRUCTURE plots for ten independent runs, clustered and average using CLUMPAK. Each bar represents an individual, and the color is the proportion of assignment to each genetic partition. Locations plotted from left to right and numbered above, and black bars indicate regions as in Table 3.1 **a)** all 3,188 loci at $K = 4$, **b)** 3,116 neutral loci at $K = 4$, **c)** 72 outlier loci at $K = 2$.

Consistent with STRUCTURE results, AMOVA analyses showed significant population structure across all loci (overall $F_{ST} = 0.071$, $p > 0.001$). Hòn Mun in Vietnam was isolated from all other locations (pairwise $F_{ST} = 0.063 - 0.147$; Table 3) except Pulau Mengyatan in southern Indonesia. Ka'a'awa in Hawai'i was also strongly divergent from four other locations (pairwise $F_{ST} = 0.142 - 0.235$; Table 3). Similarly, Pulau Weh in the Indian Ocean was different from all locations (pairwise $F_{ST} = 0.021 - 0.168$; Table 3) except for Vavvaru in the Maldives, Dumaguete in the Philippines, and Ka'a'awa in Hawai'i. AMOVA analyses grouping sites into four biogeographic regions (Indian Ocean, South China Sea, Coral Triangle, Hawai'i; Table 1, Fig. 3) increased the overall F_{ST} to 0.098 ($p > 0.001$), and the F_{CT} was 0.109 ($p > 0.001$). However, regional variation only accounted for 10% of the overall variation, and the majority (90%) was within populations (Table 4).

333 **Table 3** Genetic distances of pairwise populations of *Coralliophila violacea* for all 3,188 SNPs.
 334 Regions follow labeling in Table 1.1. Significant pairwise F_{ST} values at $p > 0.01$ ($\alpha = 0.05$, corrected
 335 for multiple tests using the B-Y method, (Narum, 2006) are shaded.

Location	Indian Ocean		Vietnam	Coral Triangle						Hawai'i
	1	2	3	4	5	6	7	8	9	10
1. Vavvaru	0									
2. Pulau Weh	-0.026	0								
3. Hòn Mun	0.014	0.034	0							
4. Dumaguete	0.063	0.021	0.122	0						
5. Bunaken	0.068	0.072	0.067	0.011	0					
6. Lembah	0.085	0.077	0.109	0.008	0.007	0				
7. Pulau Mengyatan	0.022	0.033	-0.004	-0.025	-0.005	0.002	0			
8. Nusa Penida	0.059	0.052	0.098	0.013	0.000	0.011	-0.020	0		
9. Manokwari	-0.005	-0.022	0.063	0.020	-0.011	0.024	-0.050	0.024	0	
10. Ka'a'awa	0.157	0.168	0.147	0.217	0.221	0.235	0.142	0.223	0.164	0

336 **Table 4** Hierarchical AMOVA of *Coralliophila violacea* populations sampled across all 3,188 loci
 337 with locations grouped by region as indicated in Table 1. Significant F -statistic values at $p > 0.001$
 338 are in boldface.

Source of variation	F -statistics	P-values	% var.
Among regions	$F_{CT} = 0.109$	<0.001	10.93
Among populations	$F_{SC} = -0.012$	0.389	1.09
Within populations	$F_{ST} = 0.098$	<0.001	90.16

Figure 3 Outlier loci among sampled populations of *Coralliophila violacea* as identified by BayeScan. Points to the right of the blue line are outliers.

Outlier tests

Using a false discovery rate (FDR) of 10%, BayeScan revealed 72 outlier loci putatively under directional selection among all locations (FDR = 0.10, 72; FDR = 0.05, 57), and 34 outlier loci among regions (Indian Ocean, Vietnam, Coral Triangle, Hawai'i; FDR = 0.10, 34; FDR = 0.05, 25) (Fig. 4). Considering only these 72 outlier loci, STRUCTURE indicated that $K = 2$ was the best-supported number of genetic partitions (Fig. 2). In contrast to results from all loci or just neutral loci above, the two genetic partitions identified using only outlier loci do not correspond to individual sites. Instead, results recover two groups: 1) Hòn Mun, Vietnam and Ka'a'awa, Hawai'i, two locations separated by ~9,800 km of open ocean, and 2) all other sites (Fig. 2).

Figure 4 Ocean climate variables **a)** temperature of the warmest ice-free month, **b)** annual mean, and **c)** range of sea surface temperature (SST) in °C, **d)** annual mean, and **e)** range of sea surface salinity (SSS) in psu. Data source MARSPEC (Sbrocco & Barber, 2013).

Ocean climate variables and associations with allele frequency differences

Results from MARSPEC show significant variation in the five environmental variables across the sampled region. The temperature of the warmest ice-free month varied by more than 3 °C

across the sampled sites, with the highest temperature in the Indian Ocean (~29.4 °C) and lowest in Hawai‘i (26.4 °C) and Vietnam (28.2 °C) (Table 1, Fig. 4a). The mean annual sea surface temperature (SST) also varied substantially across sampling locations by ~4 °C (Table 1, Fig. 4a). However, in this case, Manokwari (28.90 °C) and Pulau Weh (28.59 °C) at the edges of Indonesia were the warmest, while again Hawai‘i (24.99 °C) and Vietnam (26.45 °C) were the coldest locations (Table 1, Fig. 4a). SST at some sites was very stable, with only small annual ranges: Manokwari (0.78 °C) and Bunaken (1.05 °C). At other locations, SST varied more widely (e.g., Dumaguete (3.16 °C) and Vietnam (4.22 °C); Table 1, Fig. 4b).

Sea surface salinity (SSS) also showed variation across sampling locations (Table 1, Fig. 2.5c). Sites in the two oceanic archipelagos, Vavvaru, Maldives (34.93 psu), and Ka’a’awa, Hawai‘i (34.92 psu), had the highest values. In contrast, salinity at sites in the Coral Triangle was consistently lower (33.89 – 34.09 psu) (Table 1, Fig. 4c). Locations also experienced annual variation in salinity with inputs from freshwater runoff and precipitation (Fig. 4d). Sites in the Indian Ocean, Vavvaru (SSS range = 1.64 psu), and Pulau Weh (SSS range = 1.43 psu) had the most variable salinity while Ka’a’awa (SSS range = 0.21 psu) in Hawai‘i was the most stable (Table 1, Fig. 4d).

We identified a total of 88 SNPs using Bayenv2, for which allele frequency differences correlated with one or more of the five ocean climate variables (Fig. 5). The greatest proportion of these 88 SNPs were associated with annual temperature range ($N = 38$), followed by mean annual temperature ($N = 31$), temperature of the warmest ice-free month ($N = 21$), annual range of salinity ($N = 12$) and mean annual salinity ($N = 10$) (Fig. 5). SNPs associated with temperature had the highest Bayes Factors (Fig. 5). Of these, 22 SNPs were associated with more than one variable ($N = 20$ with two variables, $N = 2$ with three variables). The warmest month's mean SST

and temperature were the most highly correlated, with 15 SNPs shared between them, followed by mean and range of SST ($N = 5$ SNPs). There was no overlap between the 88 loci associated with environmental variables and the 72 outlier loci.

Figure 5 Bayenv2 association of allele frequencies for all polymorphic loci (3,186) with each ocean climate variable. SNPs are ordered by tagID. Blue line indicates significance level above which loci are considered to associate with climate variables (Jeffreys, 1998) strongly.

Mapping and annotation of outliers and environmentally-associated loci

For loci significantly associated with environmental variables, the majority (49/87; 56%) did not map to any mollusc or lophotrochozoan sequences available in the NCBI database

(Table 5). However, we were able to align the other 43% (38/88) of candidate loci to DNA sequences from a variety of molluscs, including an octopus (*Octopus vulgaris*), oysters (*Crassostrea gigas*, *C. virginica*), scallops (*Pecten maximus*, *Mizuhopecten yessoensis*, *Azumapecten farrei*), a clam (*Scapharca broughtonii*), and four marine snails (*Conus episcopatus*, *C. ximenes*, *Thais clavigera*, *Nucella lapillus*) and two freshwater snails (*Pomacea canaliculata*, *Biomphalaria glabrata*). Of these loci, 19 loci were mapped to protein-coding regions. Six of these loci coded for uncharacterized proteins, two microsatellites, and the other 11 were characterized with annotated functions (Table 5).

For outlier loci identified by BayeScan, nearly half (47%; 34/72) mapped to molluscs in the NCBI database, including an octopus (*Octopus pallidus*), oysters (*Crassostrea gigas*, *C. virginica*), a scallop (*Pecten maximus*), a sea hare (*Aplysia californica*), a limpet (*Lottia gigantea*), a freshwater snail (*Pomacea canaliculata*) and marine snail (*Conus episcopatus*) (Table 5). Of these loci, ten had associated predicted protein functions. The top two outlier loci, in terms of percent identity of sequences and the lowest E-value, aligned with a conotoxin from a cone snail (*Conus episcopatus*) and a gene (*CAPLA*) coding for a protein involved in behavioral plasticity in the California seahare (*Aplysia californica*). Interestingly, despite no overlap between the 72 outlier loci identified by BayeScan and the 88 environmentally-associated loci identified by Bayenv2, both sets contained loci mapped to genes coding for conotoxins (Table 5).

419 **Table 5** Annotations for BayeScan outlier loci among sites and Bayenv2 environmentally-
420 associated loci mapped to DNA sequences in the NCBI database.

BAYESCAN								
Tag	Sequence	Organism	Description	Score	E-value	Identity	Gene	GO functions
27523	CGTATGCCGTCTTCTGCTTGA AAAAAAAAAAAAATAC	<i>Conus episcopatus</i>	conotoxin gene	55.4	2.00E-07	97%		conotoxin
29969	AGGACCTGCTACGCAAGCTG CTGCAGGTGGACCTCA	<i>Aplysia californica</i>	CAPL-A	53.6	9.00E-07	94%	<i>CAPL-A</i>	ATP binding, protein serine/threonine kinase activity, behavior plasticity, learning and memory
19005	GGAGAGTGTGATGCAGCCAT CTGCAGTGTCTTTGCA	<i>Coralliophila abbreviata</i>	microsatellite	37.4	0.019	97%		
18735	TGAGATGATCTTGCAACCAC ATGCCTCCATCCCTCT	<i>Aplysia californica</i>	striatin-interacting protein 1-like	35.6	0.066	94%	<i>STRIP1</i>	protein binding, regulation of cell morphogenesis, cortical actin cytoskeleton organization
19841	TGCTTCTGTGGTGCAGGCCA GTGCAAGACCTCAGTC	<i>Biomphalaria glabrata</i>	zinc finger ZZ-type and EF-hand domain- containing protein 1- like	31.9	0.8	91%	<i>ZZEF1</i>	calcium and zinc ion binding
30344	CACTGTGCCAAGGCATGGTA TTGCAAGGGGGAGCCC	<i>Aplysia californica</i>	E3 ubiquitin-protein ligase DTX4-like	30.1	2.8	86%	<i>DTX4 E3</i>	ligase activity, zinc ion binding
36204	AGATCAACAGGGGCATCTCT TTGCTCGGTAAAGTCC	<i>Aplysia californica</i>	protein FAM193A-like	30.1	2.8	86%	<i>FAM193A</i>	uncharacterized protein
32125	AGTAATATGAGTGCATTTAG TTGCAGGAGTCGTGTG	<i>Aplysia californica</i>	epidermal retinol dehydrogenase 2-like	30.1	2.8	86%	<i>SDR16C5</i>	detection of light stimulus involved in visual perception, retinol dehydrogenase activity, keratinocyte proliferation, retinal and retinol metabolism
26718	AGCAGGACAATGCCATACCT TGAAGCAGGACAATGC	<i>Biomphalaria glabrata</i>	chitin biosynthesis protein CHS5-like	28.3	9.7	94%	<i>CHS5</i>	chitin biosynthesis
36113	GACAGCCATCTCGCATAGTT CTGCTGTTCCAGAGCA	<i>Crassostrea gigas</i>	complete sequence	33.7	0.39	86%		
53661	AGTCAAAGCATTGCAGATGT CTGCTATCAAACCTTCT	<i>Lottia gigantea</i>	hypothetical protein	33.7	0.23	91%		
40104	TATATAGAACAAGCAGCAAG ATGCACAAGAGAAGCT	<i>Lottia gigantea</i>	hypothetical protein	31.9	0.8	88%		
36681	TAGTCATCAACAGCATTACA CTGCCATCATTACATG	<i>Biomphalaria glabrata</i>	uncharacterized	30.1	2.8	86%		
48982	GGCTGATGGCTTGCATTATTC TGCTTTATACTTCCT	<i>Bellamyia aeruginosa</i>	microsatellite	30.1	2.8	86%		
30094	CACACACCAGAGGCAGGCAC GTGCACGAGCATACCT	<i>Biomphalaria glabrata</i>	uncharacterized	30.1	2.8	94%		
BAYENV2								
Tag	Sequence	Organism	Description	Score	E-value	Identity	Gene	GO functions
32340	TGTGTTTTTCTTGCATCGCAA TGCATTGTCAATGAG	<i>Aplysia californica</i>	rho GTPase-activating protein 17-like	37.4	0.032	91%	<i>ARHGAP17</i>	GTPase activator activity, signal transduction
13973	TGTTCACTGACGGCAAAAGT ATGCAAAATGAGGTCA	<i>Biomphalaria glabrata</i>	DNA mismatch repair protein Msh2-like	30.1	4.8	94%	<i>MSH2</i>	embryonic development, meiotic mismatch repair, DNA

14160	TGCTCTGAAGGCGCATTGTTT TGCATTTAATTGACC	<i>Biomphalaria glabrata</i>	ubiquitin carboxyl- terminal hydrolase 44- like	33.7	0.39	86%	<i>USP44</i>	repair, oxidative phosphorylation, immune response
26516	GGCGTTCTGTATGCACTTCT TGCTTCAGTTCTCCT	<i>Biomphalaria glabrata</i>	coiled-coil domain- containing protein 94- like	31.9	1.4	88%	<i>Ccdc94</i>	cell division, protein deubiquitination, mitotic nuclear division
31366	AGATCGGAAGAGCACATGTC TGAAGTCCAGTCACGA	<i>Conus episcopatus</i>	conotoxin	53.6	4.00E-07	88%		protein binding
23643	TGTTTGGGGGAGCATGTCCC TGCACACATACCCCT	<i>Crassostrea gigas</i>	calcium-transporting ATPase type 2C member 1-like	30.1	4.8	86%	<i>ATP2C1</i>	conotoxin
33972	TGGGACAGGATTGCAACTTC TTGCCTCAACAGATCT	<i>Crassostrea gigas</i>	ankyrin repeat domain- containing protein 24- like	30.1	4.8	94%	<i>ANKRD24</i>	calcium and manganese ion transport, metabolic process, signal transduction
33093	TAAGGAATGACAGCAAACCA ATGCTACCTCTCCTCT	<i>Octopus bimaculoides</i>	exonuclease 1-like	30.1	4.8	100%	<i>EXO1</i>	protein binding
31520	CGCACGAACTACGCACACTC ATGCATACAAATCATC	<i>Octopus bimaculoides</i>	little elongation complex subunit 2-like	33.7	0.39	88%	<i>ICE2</i>	immune response, DNA repair, mismatch repair, double-strand break repair
30745	GGGCTCTGTCTCGCAATACC GTGCTCAGATAAAGTG	<i>Octopus bimaculoides</i>	A-kinase anchor protein 10, mitochondrial-like	31.9	1.4	88%	<i>AKAP10</i>	snRNA transcription regulation
19751	AGGTCTTCCTGGCACTGATA TGCCAAGCATCCACA	<i>Octopus bimaculoides</i>	glutamate receptor 2- like	31.9	1.4	88%	<i>GRIA2</i>	blood coagulation, signal transduction, protein localization
31348	TGGGAATGTAAAGCAAGATA CTGCTTTTTGTGCGCT	<i>Biomphalaria glabrata</i>	uncharacterized	30.1	4.8	86%		glutamate receptor activity, learning, memory, and behavior
27006	GGTGGGGTCGCTGCAATTCTT TGCGGATCAGGTCTG	<i>Crassostrea gigas</i>	uncharacterized	31.9	1.4	88%		
11426	CGTTTAGTTTTAGCAGGAGC ATGCTTGTCAAAGATA	<i>Halotis discus hannai</i>	microsatellite	33.7	0.39	86%		
17278	AGGTCTCTTTGTGCATAGAC CTGCCAGTGCCTTACT	<i>Rapana venosa</i>	microsatellite	31.9	1.4	94%		
33978	AGTTTGAATGGGGCAGTCAC GTGCTAAGGTGGTGTC	<i>Octopus bimaculoides</i>	uncharacterized	30.1	4.8	91%		
63145	CAAGTTCCAATAGCATTTTAC TGCTTGATGATGCCC	<i>Octopus bimaculoides</i>	uncharacterized	33.7	0.39	91%		
35977	TGTGGATGCTTGGAACATCG GTGCGACAGAGAATCT	<i>Aplysia californica</i>	uncharacterized	33.7	0.39	94%		
15186	CACCCTCTCTATGCAATGACA TGCAAGCCCCCTCT	<i>Aplysia californica</i>	uncharacterized	30.1	4.8	86%		

DISCUSSION

Our results reveal a complex mixture of population genetic patterns in *Coralliophila violacea* that appear to be shaped by both geography and environment. Genome-wide data from >3,000 SNPs identified four genetic partitions concordant with traditionally recognized biogeographic regions (Indian Ocean, South China Sea, Coral Triangle, and Hawai‘i; (Spalding et al., 2007). These findings align with a previous mitochondrial DNA study (Simmonds et al., 2018) and other marine phylogeographic studies from this region (Barber et al., 2011; Carpenter et al., 2010; Crandall et al., 2019). Furthermore, repeated patterns across multiple taxa suggest a common origin (Avice 2000), the most likely broad-scale physical isolation of populations—allopatry.

However, an examination of outlier loci and the correlation between allelic variation and environmental variables also highlights the importance of natural selection in shaping population genetic patterns. More than 2% of loci were outliers, similar to Gaither et al. (2015), showing that divergence is greater than expected under neutrality (Foll & Gaggiotti, 2008). Similarly, nearly 3% of loci had strong correlations with environmental variables. Both Hawai‘i and Vietnam, peripheral populations with similar climate profiles, were differentiated at neutral and outlier loci. In contrast, populations more centrally located in the range of *C. violacea* were only differentiated at neutral loci but not outliers. These results suggest that environmental differences, particularly sea surface temperature, may reinforce the neutral geographic structure, leading to peripheral populations' differentiation. Combined with the ecological differentiation of *C. violacea* on different coral hosts (Simmonds et al., 2020), these results indicate that diversification in marine environments is more complex and varied than currently understood and includes both neutral and selective processes.

Potential role of natural selection in shaping genetic patterns

While neutral loci showed a clear signal of physical limits to gene flow (below), there were also significant associations between frequencies of multiple loci (3% of total) and environmental variables. The strongest genetic associations linked these loci to the SST range and SST mean. Elevated SST can affect gastropods' growth and survival rates, including marine snails (Sanford & Kelly, 2011), so the link between environmentally-associated loci and temperature is not unexpected. However, results also showed an interesting genetic association pattern driven by the temperature at the coldest sites in the peripheral populations Hawai'i and Vietnam. Thus, temperature differences between the Coral Triangle and peripheral populations could enable selection to drive population divergence. For example, Reid et al. (2006) found that environmental and ecological factors (i.e., continental vs. oceanic habitat and primary productivity) shaped intertidal snails' genetics in the Indo-Pacific. Given that peripheral populations like Hawai'i have environmental conditions considered marginal for *Porites*' growth and survival (Polato et al., 2010), such selection could act directly on *C. violacea* or indirectly by selection pressure on their coral hosts. Interestingly, while all four regions sampled show unique environmental signatures when examining the loci under the environmental influence, STRUCTURE only differentiates between two clusters: Vietnam and Hawai'i to the exclusion of all others.

If environmental variation drives natural selection, it is reasonable to expect some overlap between loci with significant associations with environmental variables and the outlier loci that are putatively under selection. However, there was no overlap between these two groups. This seemingly incongruous result may be a function of only examining five

environmental variables or potentially due to uneven sample sizes. We focused on temperature and salinity because of previous studies demonstrating their selection pressure on marine populations (e.g., Berg et al., 2015; DeFaveri et al., 2013; Limborg et al., 2012; Teske et al., 2019). However, other variables can have an impact. For example, Gaither et al. (2015) found that high turbidity and low light conditions explained divergent selection at a visual pigment gene locus in a coral reef fish.

As expected for a non-model organism, few candidate loci aligned to sequences in the NCBI database, highlighting the lack of current genomic resources for gastropods. However, two groups of genes are of interest for future research. First, conotoxin genes showed up as both outlier and environmentally-associated loci. While neurotoxins have not been studied in *Coralliophila*, venoms have been identified in other Neogastropoda (Fänge, 1984) and could be important to deactivate nematocysts to facilitate feeding and living on corals. Second, another gene of interest, *CHS5*, is predicted to be involved in chitin biosynthesis, a substance found in mollusc shells and radulae. Chitin is also used in the epidermis and stomach lining of nudibranchs that feed on Cnidarians as protection from nematocysts (Martin et al., 2007), and *Coralliophila* could similarly deploy chitin.

Populations at the periphery

Hawaiian populations of *C. violacea* were the most geographically isolated, ~7000 km removed from the Coral Triangle, and showed the strongest genetic differentiation. Studies reporting genetic differentiation of Hawaiian populations in Indo-Pacific taxa are common and frequently invoke geographic isolation (Ahti et al., 2016; J. DiBattista et al., 2012; Fernandez-Silva et al., 2015; Leray et al., 2010; Waldrop et al., 2016), and population bottlenecks (Szabó et al., 2014)

as primary drivers. Not only are Hawaiian populations of *C. violacea* differentiated, so too is their host, *Porites lobata* (Baums et al., 2012; Polato et al., 2010), suggesting a common diversification process. Whether by pure allopatry or isolation by distance, there are physical limits to gene flow in *C. violacea* across this vast span of the ocean. In contrast, Vietnam populations are much less geographically isolated from the core of the geographic range of *C. violacea*. Our sampling location was only 10s to 100s of km from other reef systems in the South China Sea with high connectivity to the Coral Triangle (Trembl et al., 2012). Such extreme geographic isolation cannot explain the differentiation of Vietnam.

Allopatric processes may contribute to the strong differentiation of *C. violacea* populations from Vietnam. The South China Sea was partially enclosed during Plio-Pleistocene low sea levels (Ludt & Rocha, 2015; Voris, 2000), resulting in isolation and genetic differentiation of marine taxa (Barber et al., 2000). Ocean circulation patterns also limit water movement from the Coral Triangle into the South China Sea (Kool et al., 2011), creating a barrier dividing populations north and south across the middle of the South China Sea (Trembl et al., 2015). Ocean circulation patterns have been invoked to explain *C. violacea* populations' genetic structure from Taiwan and Taiping, a small island in the South China Sea (Lin & Liu, 2008). These allopatric processes may fully explain differentiation at neutral loci. However, they can't explain the grouping of Hawai'i and Vietnam populations when examining loci exhibiting signals of selection. The similarity of these populations at non-neutral loci, combined with their environmental similarities, strongly argues that environmental variation drives or reinforces differentiation of these peripheral populations.

Interestingly, while peripheral populations in the Indian Ocean (Vavvaru and Pulau Weh) showed a distinct Indian Ocean signature, these populations also had a substantial admixture

generating from the Coral Triangle and the South China Sea. This is not surprising given the volume of sea surface transport from the Pacific into the Indian Ocean through this region (Gordon et al., 2012), a pattern that is observed in a wide variety of taxa (e.g., Drew & Barber, 2009; Williams & Benzie, 1998). However, this region's environmental conditions are also different from other areas of the sampled range, suggesting that selection might act as it does in other parts of the range of *C. violacea*. Thus, gene flow from the Coral Triangle and the South China Sea or other un-sampled areas may limit the signal of environmental selection in the Maldives, despite the relative isolation of these reef ecosystems.

Populations at the center

In contrast to peripheral populations, populations within the Coral Triangle at the core of *C. violacea*'s geographic range showed no evidence of divergence. Populations in the Philippines (Dumaguete), eastern (Manokwari), and central Indonesia (Bunaken, Lembeh, Pulau Mengyatan, Nusa Penida) all had high connectivity, echoing findings for other molluscs in the region (S. H. Cheng et al., 2014; DeBoer et al., 2014; Kirkendale & Meyer, 2004; Nuryanto & Kochzius, 2009) and as predicted by coupled biophysical models of larval dispersal (Kool et al., 2011; Trembl et al., 2015). The only isolation observed by Coral Triangle populations was from populations on the periphery in the South China Sea, the Indian Ocean, and the Central Pacific.

High connectivity, however, is not expected among all populations in the Coral Triangle. Specifically, studies of multiple taxa including giant clams (DeBoer et al., 2008, 2014; Kochzius & Nuryanto, 2008); mantis shrimp (Barber et al., 2006, 2011); echinoderms (Crandall et al., 2008b); and fish (Timm & Kochzius, 2008; Ackiss et al., 2013; Jackson et al., 2014) all show strong isolation of populations spanning the Maluku Sea. This pattern has typically been

attributed to the effects of the Halmahera Eddy (Barber et al., 2006) and is predicted by coupled biophysical models (Kool et al., 2011; Trembl et al., 2015). However, Trembl et al. (2015) found that the Halmahera Eddy filtered only 8.3 – 10.6% of simulated taxa. The strength of this filter was determined by life-history traits such as reproductive output, the timing of spawning, and length of larval dispersal phase. For example, *C. violacea* has high fecundity (Lin & Liu, 1995), spawn larvae most of the year (Soong & Chen, 1991), and likely have a long larval duration (Taylor, 1975). These traits should facilitate crossing seasonally variable dispersal barriers. In contrast, giant clams (*Tridacna*) have relatively short-lived planktonic larvae (9–10 days; Crawford et al., 1986), constraining their dispersal potential, making them more inclined to diverge across filters to gene flow. As such, life history difference likely explains why connectivity in the Coral Triangle isn't limited in *C. violacea*, as it is in other molluscs.

While allopatric divergence is an important process shaping marine taxa's evolutionary history, physical limits to dispersal and gene flow do not operate in isolation. Our results show that local adaptation to different environments may reinforce neutral divergence, especially in peripheral populations. These results add to the growing recognition that various factors impact diversification in coral reef species (Bowen et al., 2013; Fernandez-Silva et al., 2015; Floeter et al., 2007; Hoeksema, 2007; Ludt & Rocha, 2015; Rocha & Bowen, 2008). Advances in high throughput genome-wide sequencing will facilitate the exploration of neutral and adaptive variation in concert. Combined with the improved availability of marine environmental databases, we are at the leading edge of developing a more expansive view of processes driving genetic divergence among populations, leading to clearer approaches to understanding evolution in marine habitats.

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Conflict of Interest Statement

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

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Author Contributions

SES and PHB secured funding, conceptualized the study, worked out the methodology, and led the original manuscript's writing. SES, AFP, SHC, and PHB obtained permits and collected samples. SES, AFP, and SHC conducted lab work. SES performed data analyses and visualization. GM provided resources and project administration. All co-authors contributed to editing and revisions.