Received: 14 June 2022 | Revised: 22 August 2022 | Accepted: 30 August 2022

DOI: 10.1002/ece3.9341

Ecology and Evolution Open Access WILEY

RESEARCH ARTICLE

Speciation genomics and the role of depth in the divergence of rockfishes (*Sebastes*) revealed through Pool-seq analysis of enriched sequences

Daniel Olivares-Zambrano[1] | **Jacob Daane**[2] | **John Hyde**[3] | **Michael W. Sandel**[4,5] | **Andres Aguilar**[1]

[1]Department of Biological Sciences, California State University Los Angeles, Los Angeles, California, USA

[2]Department of Biology and Biochemistry, University of Houston, Houston, Texas, USA

[3]National Oceanic and Atmospheric Administration, National Marine Fisheries ServiceNational Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla, California, USA

[4]Biological and Environmental Sciences, University of West Alabama, Livingston, Alabama, USA

[5]Department of WIldlife, Fisheries, and Aquaculture, Mississippi State University, Mississippi State, Mississippi, USA

Correspondence
Andres Aguilar, Department of Biological Sciences, California State University Los Angeles, Los Angeles, CA, USA.
Email: aaguil67@calstatela.edu

Present address
Daniel Olivares-Zambrano, Department of Marine and Environmental Biology, University of Southern California, Los Angeles, California, USA

Funding information
Council on Ocean Affairs Science and Technology, California State University, Grant/Award Number: COAST-GDP-2019-001 and CSUCOAST-OLIDAN-CSULA-AY1920; National Institutes of Health, Grant/Award Number: R25GM061331; National Science Foundation, Grant/Award Number: DEB-2121145

Abstract

Speciation in the marine environment is challenged by the wide geographic distribution of many taxa and potential for high rates of gene flow through larval dispersal mechanisms. Depth has recently been proposed as a potential driver of ecological divergence in fishes, and yet it is unclear how adaptation along these gradients' shapes genomic divergence. The genus *Sebastes* contains numerous species pairs that are depth-segregated and can provide a better understanding of the mode and tempo of genomic diversification. Here, we present exome data on two species pairs of rockfishes that are depth-segregated and have different degrees of divergence: *S. chlorostictus–S. rosenblatti* and *S. crocotulus–S. miniatus*. We were able to reliably identify "islands of divergence" in the species pair with more recent divergence (*S. chlorostictus–S. rosenblatti*) and discovered a number of genes associated with neurosensory function, suggesting a role for this pathway in the early speciation process. We also reconstructed demographic histories of divergence and found the best supported model was isolation followed by asymmetric secondary contact for both species pairs. These results suggest past ecological/geographic isolation followed by asymmetric secondary contact of deep to shallow species. Our results provide another example of using rockfish as a model for studying speciation and support the role of depth as an important mechanism for diversification in the marine environment.

KEYWORDS
demography, depth, marine, Pacific Ocean, rockfish, selection

TAXONOMY CLASSIFICATION
Genetics, Genomics

1 | INTRODUCTION

Understanding mechanisms of speciation in the marine environment remains difficult due to the lack of apparent geographical barriers and high rates of gene flow among populations (Dennenmoser et al., 2017). Most marine species demonstrate high dispersal capabilities and connectivity among populations, which can impede local adaptive processes and differentiation (Bierne et al., 2003; Carreras et al., 2017). However, even with the homogenizing effects of gene flow, there is potential for local adaptation that may be the driving force for genomic differentiation in the marine environment (Whitney et al., 2018).

Early work on speciation in marine fishes was thought to be a consequence of geographic isolating mechanisms. The formation of land barriers (Bermingham et al., 1997; Bernardi et al., 2004), islands (Leray et al., 2010), and physical boundaries generated from oceanographic processes (Gaither & Rocha, 2013; Hubert et al., 2012) were used from a biogeographical perspective to describe speciation patterns in marine fishes. However, the role of pelagic larval duration in contributing to gene flow among populations suggested that allopatric divergence may be rarer in marine fish (reviewed in Bindea et al., 2013). Although large-scale allopatric events can drive marine speciation, there is evidence that other isolating mechanisms occur in the marine environment (Faria et al., 2021; Rocha et al., 2005). Studies have demonstrated the possibility for closely related species to be sympatrically distributed, indicating ecology may be important (Burford, 2009; Crow et al., 2010; Rocha et al., 2005). Although Rocha et al. (2005) found evidence for sympatrically distributed reef fishes, there remains a lack of knowledge for how ecological speciation applies to temperate marine environments. Furthermore, sympatric or parapatric distribution of species contradicts existing knowledge that gene flow can be a barrier to speciation. This creates an apparent "marine speciation paradox," or how can marine speciation occur in the face of high apparent gene flow (Faria et al., 2021; Johannesson, 2009)?

The model of ecological speciation is important in understanding the speciation process in the marine environment (Bernardi, 2013; Puebla, 2009). Numerous studies have now documented the role that ecological specialization, especially in fishes, plays in speciation. A number of environmental factors have been documented that lead to ecological divergence in the marine environment; these include temperature (Teske et al., 2019) and salinity (Momigliano et al., 2017), which are often correlated with other habitat characteristics (e.g., depth and latitude). Depth has already been identified as a potential factor in the diversification of rockfishes (Behrens et al., 2021; Heras & Aguilar, 2019; Hyde et al., 2008; Ingram, 2010; Sivasundar & Palumbi, 2010), and depth may be important in driving speciation for other marine organisms (Carlon & Budd, 2002; Gaither et al., 2018; Hirase et al., 2021; Prada & Hellberg, 2013). Thus, adaptation to these environmental differences can lead to divergence in life history traits, such as spawning behavior, which subsequently drives reproductive isolation between incipient species.

Rockfishes (genus *Sebastes*) inhabit temperate waters across the Atlantic and Pacific Ocean, with 60 different species found in the North Pacific that have radiated over the past 5 million years (Johns & Avise, 1998). Species are found from rocky intertidal habitats to depths greater than 1500 m (Love et al., 2002). Given the ecological partitioning and habitat similarity between recently diverged forms of rockfish, ecological speciation may have contributed to their divergence (Behrens et al., 2021; Burford, 2009; Pavoine et al., 2009). Depth has been proposed as an important component in the diversification of rockfishes (Behrens et al., 2021; Heras & Aguilar, 2019; Hyde et al., 2008; Ingram, 2010; Sivasundar & Palumbi, 2010).

This study aims to identify genomic regions that have contributed to differentiation among recently diverged Northern Pacific species pairs of rockfish: (*S. chlorostictus–S. rosenblatti* and *S. crocotulus–S. miniatus).* The two species pairs occur along a continuum of divergence, with *S. chlorostictus–S. rosenblatti* diverging approximately 0.21 Mya and *S. crocotulus–S. miniatus* diverging approximately 2.3 Mya (Hyde & Vetter, 2007). Both species pairs are found at different depths. *S. chlorostictus* occurs between 60 and 240 m, while *S. rosenblatti* occurs between 100 and 490 m. *S. miniatus* occurs at 30–100 m, while *S. crocotulus* occurs between 100 and 200 m (Hyde & Vetter, 2007). Our goals are to determine whether depth-segregated speciation is a result of selective sweeps that generates islands of genomic differentiation or "divergence islands" (Via, 2012; Wolf & Ellegren, 2017). We will also examine islands of genomic differentiation to see whether they are shared across species pairs. Sharing of these divergence islands may indicate parallel evolutionary pressures in depth adaptation. Finally, we also investigated the demographic history of speciation in these species pairs, to see whether similar patterns exist and whether these patterns are consistent with ecological speciation.

2 | METHODS AND ANALYSIS

2.1 | Sample collection

Ethanol-preserved fin clips for the following depth-separated species pairs were obtained from *S chlorostictus–S. rosenblatti* and *S. crocotulus–S. miniatus* (Table 1). High molecular weight DNA was obtained using standard phenol–chloroform methods followed by ethanol precipitation (Sambrook & Russell, 2006). DNA integrity was checked on a 2% agarose gel and quantified on a Qubit fluorometer before preparing the samples for Illumina library preparation.

2.2 | Targeted sequence capture design

We designed a series of oligonucleotide capture baits that could efficiently enrich DNA sequencing libraries across Perciformes, a large order of more than 2200 species that includes *Sebastes* (Daane et al., 2016, 2019; Nelson et al., 2016). We targeted protein-coding exons, conserved non-coding elements (CNEs),

TABLE 1 Rockfish samples used for this study

Scientific name	Common name	Location	*n*	Year	Latitude	Longitude
S. rosenblatti	Greenblotched Rockfish	Palos Verdes, CA USA	7	1996	33.81	−118.44
		Guadalupe Island, MX	4	1996	29.16	−118.27
		La Jolla, CA USA		1994	32.87	−117.31
		60 Mile Bank, CA USA		1994	32.11	−118.24
		San Nicholas Island, CA USA		1994	33.20	−119.51
S. chlorostictus	Greenspotted Rockfish	Point Reyes, CA USA	5	1998	38.07	−123.53
		Osborne Bank, CA USA	3	2005	33.36	−119.03
		Tanner Bank, CA USA	3	2007	32.70	−119.06
		San Clemente Island, CA USA	3	2007	32.78	−118.36
		San Nicholas Island, CA USA	3	2007	33.28	−119.51
		Palos Verdes, CA USA	3	2018	33.69	−118.33
S. crocotulus	Sunset Rockfish	Tanner Bank, CA USA	10	2004	32.69	−119.07
		San Quintin, MX	10	2005	30.67	−116.13
S. miniatus	Vermilion Rockfish	La Jolla Canyon, CA USA	5	2000	32.83	−117.25
		Punta Baja, MX	5	1994	29.89	−115.82
		Shelter Cove, CA USA	3	2008	40.25	−124.4
		Depoe Bay, OR USA	4	2008	44.8	−124.07
		Halfmoon Bay, CA USA	3	2003	37.46	−122.43

miRNAs and ultra-conservative elements (UCNEs) for enrichment. Protein-coding exons were extracted from Ensembl BioMart for the three-spined stickleback (*Gasterosteus aculeatus*, BROAD S1), the Japanese medaka (*Oryzias latipes*, MEDAKA1), and green-spotted puffer (*Tetraodon nigroviridis*, TETRAODON 8.0; Kinsella et al., 2011). CNEs were defined from the constrained regions >50 bp within the Ensembl compara 11-way teleost alignment that did not overlap with coding sequences (Ensembl release-91) (Herrero et al., 2016). miRNA hairpins were identified from miRbase and UCNEs from UCNEbase (Dimitrieva & Bucher, 2013; Kozomara & Griffiths-Jones, 2010).

We used BLASTN (ncbi-blast-2.2.30+; parameters "-max_target_seqs 1 -outfmt 6") to identify each targeted element within multiple perciform genome assemblies. The majority of capture baits were designed against the genome of the Chabot de Rhénanie *Cottus rhenanus* (ASM145555v1). Importantly, certain genetic regions may be absent or highly divergent within the genome of this sculpin but remain conserved in other Perciformes. To account for these regions and ensure their capture, we iteratively designed capture baits from the genomes of the shorthorn sculpin *Myoxocephalus scorpius* (ASM90031295v1) (Malmstrøm et al., 2016), the sablefish *Anoplopoma fimbria* (AnoFim1.0) (7), the golden redfish *S. norvegicus* (ASM90030265v1) (Malmstrøm et al., 2016), the flag rockfish *S. rubrivinctus* (SRub1.0), the rougheye rockfish *S. aleutianus* (ASM191080v2), the European perch *Perca fluviatilis* (ASM90030264v1) (Rondeau et al., 2013), and the three-spined stickleback *G. aculeatus* (BROAD S1). For each species, we included new capture baits if the targeted elements were either not identified (coverage <70% or a BLASTN E-value >0.001), or had <85% identity to an existing capture bait. As a result of this iterative addition of sequence information from new species, there should be oligonucleotide capture baits of at least 85% identity to each clade included in the capture design. This multi-species design enables efficient enrichment across distantly related perciform fishes. The final species composition of the capture baits: *C. rheanus* (62.0%), *M scorpius* (6.7%), *A. fimbria* (6.4%), *S. norvegicus* (5.9%), *S. rubrivinctus* (2.0%), *S. aleutianus* (1.8%), *P. fluviatilis* (5.3%), and *G. aculeatus* (9.8%).

SeqCap EZ Developer (cat #06471684001) capture oligos were designed in collaboration with the Nimblegen design team to standardize oligo annealing temperature, reduce probe redundancy, and remove low complexity DNA regions. The capture design contained sequence from 492,506 regions (81,493,221 total bp) across all eight perciform reference genomes. Accounting for probe redundancy between the perciform reference genomes, the final capture design comprised 407,084 distinct elements, including 285,872 protein-coding exons, 118,406 conserved non-coding elements, 2508 UCNEs, and 298 miRNAs (see Daane et al., 2016).

2.3 | Exome sequencing

Exome sequencing of 20 individuals from four different species was done using a pool-seq approach (Table 1) (Daane et al., 2016; Schlötterer et al., 2014). DNA from 20 individuals was pooled in equimolar amounts, and libraries were constructed using the Kapa HyperPlus kit (Table S1). Enrichment of exome sequences was done following the approach of Daane et al. (2019). Individually barcoded libraries were quantified using qPCR, pooled, and sequenced on an Illumina HiSeq4000 at the UC Berkeley Vincent Coates Genomics facility with 150 PE sequencing.

2.4 | Read mapping

Following sequencing, reads were demultiplexed, then trimmed, and quality filtered with Trimmomatic (Bolger et al., 2014) using the parameters: ILLUMINACLIP:TruSeq3-PE-2:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36" ILLUMINACLIP:TruSeq3-PE-2:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36. The resulting high-quality trimmed reads were mapped to the *S. umbrosus* genome (Kolora et al., 2021; assembly fSebUmb1.pri) with bwa-mem (Li & Durbin, 2010). Resulting BAM files were converted to mpileup format with samtools (Table S1) (Daane et al., 2019), and regions surrounding indels were masked with the identify-indel-regions.pl script for subsequent analysis. Allele frequencies were estimated with Popoolation2 (Kofler et al., 2011), and loci with a minor allele frequency (MAF) less than 0.05 were removed for subsequent analyses. We use this MAF cutoff to avoid any bias from fixed or nearly fixed variants between species.

We identified divergence islands across species pairs using outlier approaches. We estimated F_{ST} for species pairs using the parameters in Popoolation2: –suppress-noninformative –min-count 6 –min-coverage 80 –max-coverage 500 –min-covered-fraction 1 –window-size 1 –step-size 1 –pool-size 20 (Kofler et al., 2011; Li et al., 2009). Results from the F_{ST} analysis were then plotted utilizing qqman to generate Manhattan Plots in R (Turner, 2018). Our comparisons include two species pairs that span a range of divergence across the speciation continuum (Behrens et al., 2021; Hyde et al., 2008). Identification of clear regions of divergence in the more diverged species pair possesses additional challenges, as drift and recombination may erode any signals associated with speciation-related divergence (Quilodrán et al., 2020).

2.5 | Sliding window analysis

To identify divergence islands, we applied an approach similar to Holliday et al. (2016) and Renaut et al. (2013). We performed a sliding window analysis of F_{ST} across each chromosome, using a window of 10 adjacent SNPs and sliding the window every two SNPs, to identify the number of regions that contained SNPs greater than the top one percentile for F_{ST} of the genome-wide analysis. To assess significance, we randomly sampled 10 SNPs from across the genome with replacement for their F_{ST} values 100,000 times. For each of these subsamples, we estimated the proportion of top one percentile F_{ST} SNPs present. The proportion of top one percentile SNPs in each window, over the resampled dataset, was used to determine significance for the original dataset ($p < .001$) to reduce the signal from false positives.

2.6 | Gene ontology enrichment analysis

We identified genes found in outlier windows from our sliding window analysis. These served as our candidate list of genes that was then compared with the background list of all annotated genes found from our exome dataset. To test for enrichment of molecular pathways and/or function between the background and candidate lists and to allow for the visualization of the gene ontology networks, we used Cytoscape-CLUEGO and its plug-in CLUEPedia (Bindea et al., 2009, 2013). Cytoscape-CLUEGO utilizes hypergeometric testing followed by Bonferroni multiple testing corrections between a candidate gene list and a custom background list (Bindea et al., 2009). For Cytoscape-CLUEGO, all annotations were made using the *D. rerio* genome from the Gene Ontology Consortium (Ashburner et al., 2000), provided as it was the closest related species to genus *Sebastes* in this analysis package. Finally, Cytoscape-CLUEGO groups genes by GOterm to avoid redundancy in the results.

2.7 | Demography of speciation

We utilized folded site frequency spectra in δaδi (Gutenkunst et al., 2009) to determine the demographic history of speciation in each of the species pairs. To generate datasets for this analysis, we used all identified SNPs from Popoolation2 (before filtering for MAF as above). To assure independence (linkage equilibrium) of each SNP, we randomly sampled one SNP every 1,000,000 bp across the genome. Raw SNP frequencies were converted into δaδi SNP format using genomalicious (https://rdrr.io/github/j-a-thia/genomalicious/). We explored seven simple two-population models in δaδi: no migration, symmetric migration, asymmetric migration, symmetric migration followed by isolation, asymmetric migration followed by no migration, secondary contact with symmetric migration, and secondary contact with asymmetric migration. We hypothesize that if ecological speciation has occurred in these species pairs, due to adaptation to different depths, we would expect to observe a demographic history with at least some gene flow. We utilized the δaδi optimization procedure from Portik et al. (2017) (https://github.com/dportik/dadi_pipeline). We ran four iterations of optimizations for each model with 10, 20, 30, and 40 replications, respectively. For each species pair, we compared models using AIC and Δ AIC. We did not convert parameters from the best fit model into biologically meaningful values, as our goal was simply to reconstruct a reasonable demographic scenario for each species pair.

3 | RESULTS

3.1 | Genome assembly and coverage

We obtained sequences for pooled sequenced from each species that contained 20 individuals. The average read depth was 10.36 across species and >99% of the reads mapped back to the *S. umbrinus* genome (Table S1).

3.2 | SNP calling and F_{ST}

We used F_{ST} to identify islands of divergence between each species pair and found F_{ST} values for 48,106 SNPs in *S. crocotulus–S. miniatus* and 52,626 SNPs in *S. chlorostictus–S. rosenblatti*. Mean F_{ST} for *S. crocotulus–S. miniatus* was 0.10 with a standard error of 0.0014; for *S. chlorostictus–S. rosenblatti*, mean F_{ST} was 0.03 with a standard error of 0.0003. We found a total of 10 non-overlapping windows in the *S. crocotulus–S. miniatus* comparison that passed our significance threshold ($p < 0.0001$) and 33 windows for *S. chlorostictus–S. rosenblatti* (Figure 1; Tables S2 and S3). There were two windows that were shared in both comparisons, one on chromosome 6 and the other on chromosome 12 (Figure 1, Tables S2–S4).

3.3 | Enrichment analysis of significant F_{ST} windows and candidate genes

Genes found within significant F_{ST} windows for *S.chlorostictus–S. rosenblatti* were enriched for pathways related to neuropeptide signaling, monovalent inorganic cation inorganic homeostasis, galanin receptor activity, proton-transporting two-sector ATPase complex—catalytic domain, active transmembrane transporter activity, P-P-bond-hydrolysis driven transmembrane transporter activity, and proton transmembrane transporter activity (Bonferroni <0.05). Eighteen of the 200 candidate genes in this species pair were enriched within these GO terms. Of the 18 genes: five are related to ATP binding, ATP synthase or ATPase activity (*abcb8*, *atp5f1e*, *atp6v1h*, *atp1b2b*, *and atp6ap1a*), four are solute carriers (*slc4a2b*, *slc12a9*, *slc2a10*, *and slc9a7*), and two are integrin beta subunits (*itb4r and itb4r2a*). The remaining seven genes have functions related to melanophore production stimulation, germ cell migration, behavioral, ectodermal placode development, cell proliferation inhibition, and MHC class I binding activity (*adcyap1b*, *ca15b*, *galr1b*, *oprk1*, *pnocb*, *pth2*, *and tap2t*) (Table 2, Figure 2a, Table S5).

Genes found within significant F_{ST} windows for *S.crocotulus–S. miniatus* were enriched for pathways related to bicellular tight junction, positive regulation of actin filament polymerization, histone lysine methylation and SWI/SNF superfamily-type complex (Bonferroni <0.05). Alone, 12 candidate genes from a list of 126 candidate genes were enriched for these GO terms and provided functional and cellular pathway insight. Of the 12, three are involved in chromatin remodeling (*arid1ab*, *arid1b*, and *tfpt*), three are involved in methylation (*dnmt1*, *setd1a*, and *setdb1b*), four are related to cytoskeletal organization (*zgc:158689*, *tbcd*, *cldnk*, and *baiap2a*), one to GTP binding (*cdc42ep4a*), and one to intracellular membrane organization (*rab13*) (Table 3, Figure 2b, Table S5).

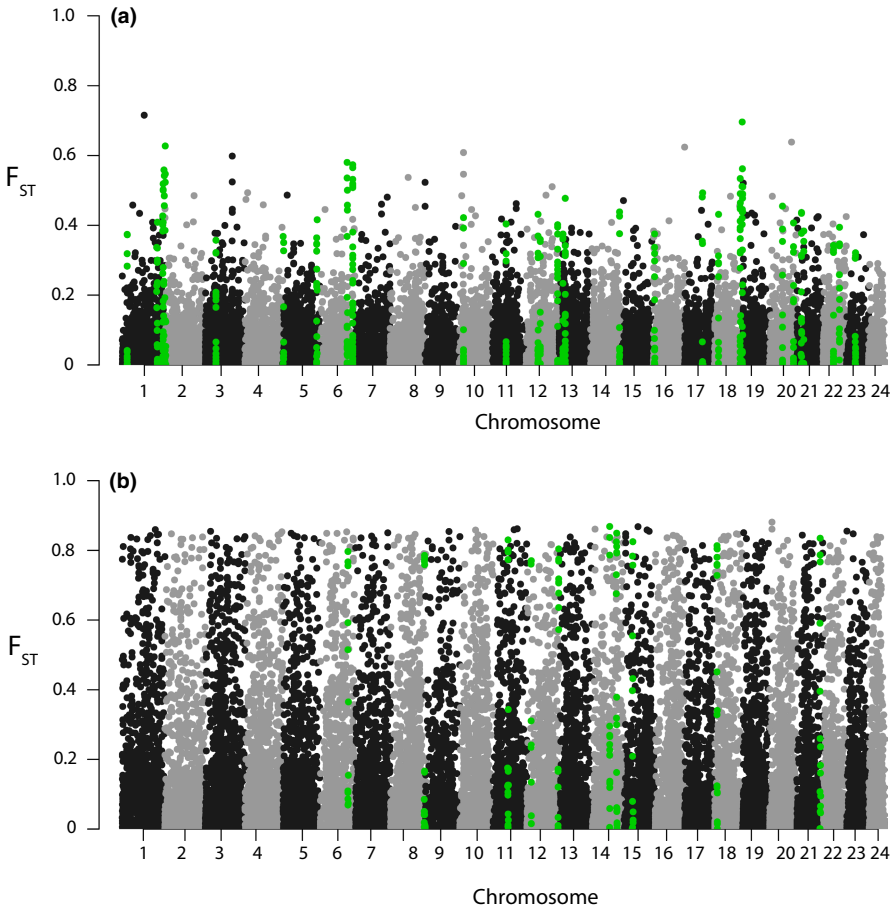

FIGURE 1 Manhattan plot of individual F_{ST} values based on pooled exome sequencing between *Sebastes chlorostictus–Sebastes rosenblatti* (a) and *S. crocotulus–S. miniatus* (b) species pairs displaying individual SNP F_{ST} values by chromosome number (aligned to the *S. umbrosus* genome). Highlighted green values are of SNPs that reside in windows that contain a higher-than-expected proportion of F_{ST} values above the top 1% of genome-wide estimated differentiation ($p < .001$).

TABLE 2 Enriched GO terms in *Sebastes chlorostictus–Sebastes rosenblatti* candidate genes found near genomic islands of divergence utilizing Cytoscape-CLUEGO (Bonferroni <0.05)

GOID	GOTerm	Ontology Source	Term *p* value	Term *p* value Corrected with Bonferroni	Group *p* value	Group *p* value Corrected with Bonferroni	GOLevels	GOGroups	% Associated Genes	Nr. Genes	Associated Genes Found
GO:0055067	monovalent inorganic cation homeostasis	GO_BiologicalProcess-EBI-UniProt-GOA_27.03.2019_00h00	.00	.00	.00	.00	[7]	Group0	13.64	6.00	[atp1b2b, atp6ap1a, ca15b, slc12a9, slc4a2b, slc9a7]
GO:0007218	neuropeptide signaling pathway	GO_BiologicalProcess-EBI-UniProt-GOA_27.03.2019_00h00	.00	.00	.00	.00	[4, 5, 6]	Group1	10.94	7.00	[adcyap1b, galr1b, ltb4r, ltb4r2a, oprk1, pnocb, pth2]
GO:0004966	galanin receptor activity	GO_BiologicalProcess-EBI-UniProt-GOA_27.03.2019_00h00	.00	.00	.00	.00	[6, 7, 8, 9]	Group1	33.33	3.00	[galr1b, ltb4r, ltb4r2a]
GO:0033178	proton-transporting two-sector ATPase complex, catalytic domain	GO_CellularComponent-EBI-UniProt-GOA_27.03.2019_00h00	.00	.02	.00	.01	[3, 4, 5]	Group2	18.75	3.00	[atp5f1e, atp6ap1a, atp6v1h]
GO:0022804	active transmembrane transporter activity	GO_BiologicalProcess-EBI-UniProt-GOA_27.03.2019_00h00	.00	.03	.00	.01	[6]	Group2	4.29	10.00	[abcb8, atp1b2b, atp5f1e, atp6ap1a, atp6v1h, slc12a9, slc2a10, slc4a2b, slc9a7, tap2t]
GO:0015405	P–P-bond-hydrolysis-driven transmembrane transporter activity	GO_BiologicalProcess-EBI-UniProt-GOA_27.03.2019_00h00	.00	.02	.00	.01	[8]	Group2	7.06	6.00	[abcb8, atp1b2b, atp5f1e, atp6ap1a, atp6v1h, tap2t]
GO:0015078	proton transmembrane transporter activity	GO_BiologicalProcess-EBI-UniProt-GOA_27.03.2019_00h00	.00	.04	0.00	.01	[8, 9, 10]	Group2	7.58	5.00	[atp5f1e, atp6ap1a, atp6v1h, slc2a10, slc9a7]

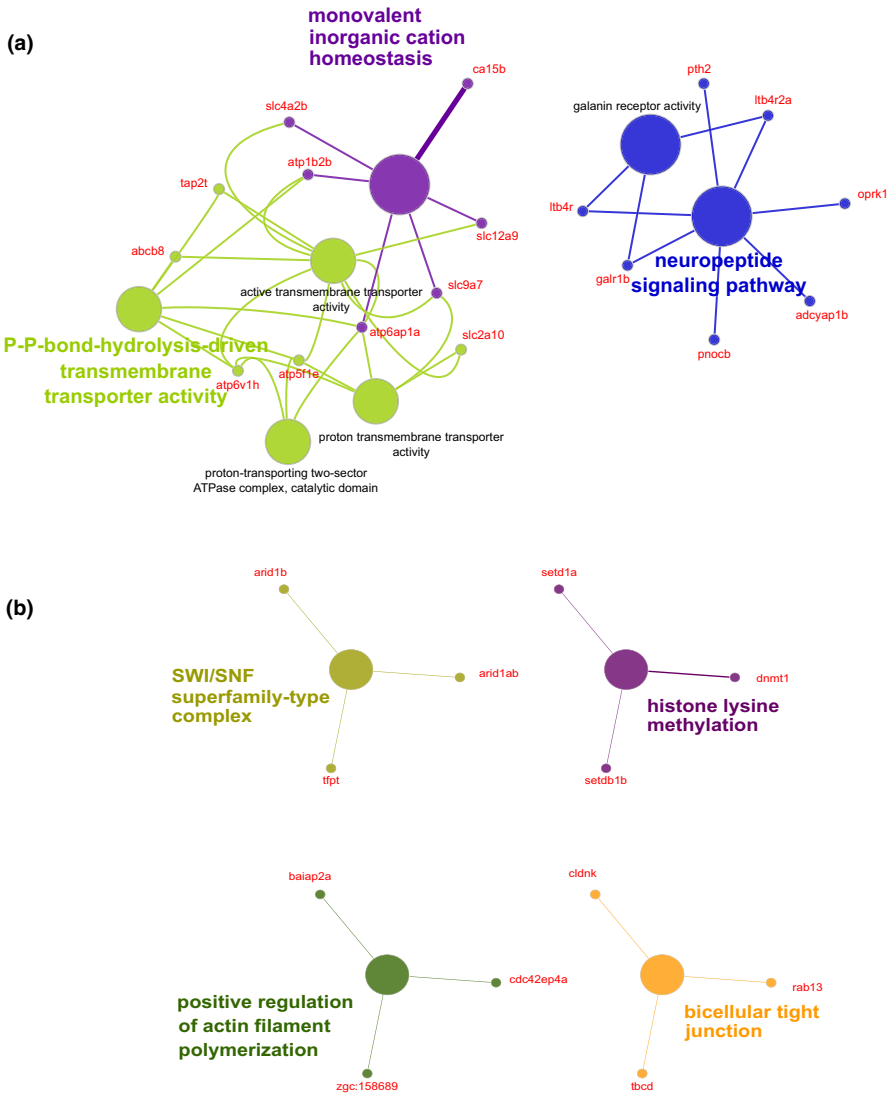

FIGURE 2 Significantly enriched GO terms (Bonferroni <0.05) for genes found in genomic islands of divergence identified via sliding window analysis for the *Sebastes chlorostictus–Sebastes rosenblatti* (a) and *S. crocotulus–S. miniatus* (b) comparisons using Cytoscape-CLUEGO. The different color circles represent unique functional GO terms. Linked functional GO terms illustrate a functional pathway. The candidate genes that connect each significant GO term are labeled red. Finally, highlighted terms are known as a leading term as they are the most significant GO term from the analysis.

3.4 | Demography of speciation

In order to assess the demographic history of speciation within our two species pairs, we tested seven models of population divergence using δaδi. We used pruned datasets that consisted of 5368 and 5310 SNPS for the *S. rosenbaltti–S. chlorostictus* and *S. miniatus–S. crocotulus* species pairs, respectively. For both species pairs, we found the secondary contact with asymmetric gene flow from the deep to shallow species to be the best model (Tables 4 and 5).

4 | DISCUSSION

The presumed lack of geographic barriers and propensity for high levels of gene flow poses challenges for studying speciation in the marine environment. Our results build on a growing body of work that indicate the genus *Sebastes* is a good model for better understanding of the mechanisms that promote speciation in the marine environment (Behrens et al., 2021; Burford & Bernardi, 2008; Heras & Aguilar, 2019; Ingram, 2010; Kolora et al., 2021). Using pooled exome sequences, we were able to uncover islands of genomic differentiation in two different *Sebastes* species pairs that exhibit depth segregation. We were able to identify a greater number of islands in the *S. chlorostictus–S. rosenblatti* than in the *S. crocotulus–S. miniatus* pair, which is expected given the disparity in divergence time observed in these taxa (Hyde & Vetter, 2007). Recently diverged species pairs are likely to retain signals of divergent selection associated with speciation as these signals erode over the amount of time species pairs are isolated (Quilodrán et al., 2020). Enriched GO terms for genes found within the *S. chlorostictus–S. rosenblatti* species pair divergence islands suggest that genes involved in neuropeptide signaling and cellular homeostasis are important in the divergence of this species pair. A less clear pattern was observed for *S. crocotulus–S. miniatus*, but we were able to identify shared islands between the two species pairs. This work builds upon previous findings in the genus *Sebastes* and suggests a more complex pattern of genomic divergence as it relates to speciation in this group.

Exome-wide analysis revealed a number of enriched GO terms found within significant outlier regions for the *S. chlorostictus–S.*

TABLE 3 Enriched GO terms in *Sebastes crocotulus–Sebastes miniatus* candidate genes found near genomic islands of divergence utilizing Cytoscape-CLUEGO (Bonferroni <0.05)

GOID	GOTerm	Ontology Source	Term *p* value	Term *p* value Corrected with Bonferroni	Group *p* value	Group *p* value Corrected with Bonferroni	GOLevels	GOGroups	% Associated Genes	Nr. Genes	Associated Genes Found
O:0005923	bicellular tight junction	GO_CellularComponent-EBI-UniProt-GOA_27.03.2019_00h00	.01	.04	.01	.03	[4]	Group0	6.52	3.00	[cldnk, rab13, tbcd]
GO:0030838	positive regulation of actin filament polymerization	GO_BiologicalProcess-EBI-UniProt-GOA_27.03.2019_00h00	.01	.03	.01	.03	[5, 6, 7, 8, 9, 10, 11]	Group1	6.98	3.00	[baiap2a, cdc42ep4a, zgc:158689]
GO:0034968	histone lysine methylation	GO_BiologicalProcess-EBI-UniProt-GOA_27.03.2019_00h00	.01	.04	.01	.04	[6, 7, 8, 9, 10]	Group2	6.25	3.00	[dnmt1, setd1a, setdb1b]
GO:0070603	SWI/SNF superfamily-type complex	GO_CellularComponent-EBI-UniProt-GOA_27.03.2019_00h00	.01	.05	.01	.04	[4, 5, 6, 7, 8, 9, 10, 11, 12, 13]	Group3	6.12	3.00	[arid1ab, arid1b, tfpt]

TABLE 4 Results of the demographic model analysis (δaδi) for the *Sebastes chlorostictus–Sebastes rosenblatti* species pair

Model abbreviation	Log-l	AIC	delta AIC	theta	nu1	nu2	m	m1→2[a]	m2→1[a]	T	T1	T2
sec_contact_asym_mig	−2558.41	5128.82	0	997.87	0.6277	0.1753		0.1814	9.157		7.5564	0.3685
anc_asym_mig	−2654.33	5320.66	191.84	538.23	1.3647	0.7972		0.1427	1.7962		3.1908	0.0143
no_mig	−2673.41	5352.82	224	1366.47	0.1264	0.1427				0.0275		
asym_mig	−2676.33	5362.66	233.84	782.12	0.9455	0.5535		0.1723	2.3527	0.9105		
sym_mig	−2780.87	5569.74	440.92	215.43	2.6643	3.0234	0.261			5.7288		
sec_contact_sym_mig	−2780.65	5571.3	442.48	173.31	3.3009	3.7447	0.2146			6.4535		
anc_sym_mig	−2845.23	5700.46	571.64	335.37	1.7422	1.7978	2.296				3.7806	0.2338

Note: The log likelihood (Log-l), Akaike Information Criteria (AIC), difference in AIC values compared with the best model (delta AIC), scaled ancestral population sizes (theta, nu1, and nu2), migration rates (m – symmetrical; m1→2 and m2→1 – asymmetrical), and scaled divergence times (T, T1, and T2) are reported for each model.

[a]1: *S. chlorostictus*; 2: *S. rosenblatti*.

TABLE 5 Results of the demographic model analysis (δaδi) for the *Sebastes crocotulus–Sebastes minatus* species pair

Model abbreviation	Log-l	AIC	delta AIC	theta	nu1	nu2	m	m1→2[a]	m2→1[a]	T	T1	T2
sec_contact_asym_mig	−1799.93	3611.86	0	151.38	0.6573	2.5635		9.0238	1.0233		28.6354	0.4318
no_mig	−1895.39	3796.78	184.92	1246.52	0.0311	0.0544				0.0022		
sym_mig	−2083.7	4175.4	563.54	114.23	4.5392	5.5264	0.8282			20.2339		
asym_mig	−2082.8	4175.6	563.74	260.87	1.6168	2.8042		2.5446	1.4564	8.119		
sec_contact_sym_mig	−2087.1	4184.2	572.34	325.39	1.6042	1.8633		2.5214			2.9266	2.7467
anc_asym_mig	−2112	4236	624.14	145.73	2.2964	6.0197		2.5924	0.6727		17.1097	0.0377
anc_sym_mig	−2230.2	4470.4	858.54	1071.12	0.3464	0.8302	0.6722				0.0132	0.0119

Note: The log likelihood (Log-l), Akaike Information Criteria (AIC), difference in AIC values compared with the best model (delta AIC), scaled ancestral population sizes (theta, nu1, and nu2), migration rates (m – symmetrical; m1→2 and m2→1 – asymmetrical), and scaled divergence times (T, T1, and T2) are reported for each model.

[a]1: *S. crocotulus*; 2: *S. miniatus*.

rosenblatti comparison including cation homeostasis and the neuropeptide signaling pathway. The identification of genes in the neuropeptide signaling pathways in the *S. chlorostictus–S. rosenblatti* species pair is more directly related to ecological divergence and speciation in this group. Wang et al. (2021) point out the interplay between chemosensory divergence and ecological speciation. They focus on the importance of chemosensory drive in this process with a particular focus on diet adaptations. While dietary differences may exist in the *S. chlorostictus–S. rosenblatti* pair, it is likely adaptation to depth-related features is more important. Hyde and Vetter (2007) proposed a mechanism by which depth segregation could lead to divergence in *Sebastes*. In *Sebastes*, juveniles are attracted to species-specific habitat types during settlement, and homing to a different depth-related habitat could contribute to sensory drive that would eventually lead to reproductive isolation via habitat differences (Heras et al., 2015).

We can only speculate on the relative importance of these pathways to the ecological divergence of this species pair. Genes involved in homeostatic functioning are likely crucial in maintaining cellular stability in the face of environmental differences. While there has not been adequate characterization of depth-related habitat differences for any of the species we studied or the physiological demands of these environments, we can hypothesize that differences in temperature, pH, and salinity exist along the depth gradient that may drive local adaptation for these and other species. Overall, the enrichment of candidate genes near significant F_{ST} windows of genomic islands of differentiation for the *S. chlorostictus–S. rosenblatti* pair provides functional descriptions of genes and gene networks related to behavior, development, homeostasis, and immunity, supporting previous work on rockfish that has found similar evolutionary evidence corresponding to depth driving their speciation (Behrens et al., 2021; Hyde & Vetter, 2007; Ingram, 2010).

We found fewer enriched gene ontology groups for the *S. crocotulus–S. miniatus* species pair, concordant with the finding of fewer significant outlier windows. This finding is likely due to the increased divergence of this species pair compared with *S. chlorostictus* and *S. rosenblatti*. It is possible that our approach is not applicable to more diverged species pairs and would benefit from methods that could account for levels of intraspecific variation (Cruickshank & Hahn, 2014). The amount of divergence between *S. crocotulus* and *S. miniatus* has likely eroded most of the signal associated with speciation due to the increased effects of drift and recombination (Quilodrán et al., 2020). The significant gene ontology terms that we did identify for this pair were associated with basic housekeeping functions (actin regulation of polymerization, the SWI/SNF pathways, histone lysine methylation pathways, tight junction, and regulation of actin filament polymerization) and cannot be associated with differences in depth for this species pair. The SWI/SNF pathways act as ATP-dependent chromatin remodelers that repress and activate genes and are associated with cardiovascular development (Table S4). The finding that histone lysine methylation pathways are enriched in this species pair could be related to hybrid sterility, as this pathway has been found to be related to hybrid sterility in mice

(Mihola et al., 2009). Histone lysine methylation could indicate a postzygotic barrier in this species pair (Sha et al., 2020). It may be that the enriched genes found in this species pair are indicative of postzygotic barriers as chromatin remodeling genes are known to cause hybrid sterility and these molecular functions may be more present in longer diverged species of rockfish; however, additional work needs to be done to assess the validity of these findings.

We found a limited number of shared outlier windows between the two species pairs, a window on chromosome 6 and one on chromosome 12. These overlapping windows contained only four annotated genes (Table S4), some of which are involved in the basic metabolism. The lack of clear shared divergence islands across species pairs is likely due to the difference in estimated divergence times. This pattern was also observed by Behrens et al. (2021) in a comparison of genome-wide divergence in three *Sebastes* species pairs and demonstrates the limitations of using F_{ST} in these types of studies (Quilodrán et al., 2020).

4.1 | Comparison to previous work

A recent study of the genomic architecture of speciation in *Sebastes* found evidence for two "islands" across two different chromosomes (Behrens et al., 2021). As in our study, Behrens et al. (2021) also utilized the *S. crocotulus–S. miniatus* pair and they found evidence for six regions of elevated genomic differentiation; however, this study also utilized an additional species pair (*S. carnatus–S. chrysomelas*) that has a recent divergence (similar to *S. chlorostictus–S. rosenblatti)*. A more direct comparison of divergence islands with our study is not entirely possible, as Behrens et al. (2021) used different approaches and reference genome. They used SNPs derived from reduced representation sequencing (ddRAD-Seq) to identify regions of high divergence between species pairs and whole genome resequencing of a single individual from *S. carnatus* and *S. chrysomelas* to identify "functionally" divergent SNPs (Behrens et al., 2021). The approaches to identifying the function of outliers were also different between the two studies; Behrens et al. (2021) focused on identifying genes that contained outlier SNPs, while we looked at genes found within outlier windows and tested for the enrichment of GO terms for these genes. Regardless, we did not find similar gene sets in our analyses, with Behrens et al. (2021) finding a set of genes related to vision and immune function. Clearly, future work should focus on employing whole genome approaches and standardized genomic resources for *Sebastes* species.

4.2 | Demography of speciation

Examination of a limited set of demographic models indicated that the same model, secondary contact with asymmetric gene flow, was most likely for both species pairs. This suggests that the invasion of a novel habitat (deeper water in this case) is followed by a period of isolation in these species pairs. Models of ecological speciation predict that gene flow should persist upon invasion of the new ecological space. However, a recent study on depth-segregated ecomorphs in *S. mentella* found support for demographic models that were similar to those found in this study (Benestan et al., 2021). Benestan et al. (2021) suggested that divergence in *S. mentella* was relatively recent (0.5 MYA) and driven by changes in sea level during the Pleistocene. Support for the secondary contact model found in this study is also supported by other studies of speciation history in marine organisms (Fairweather et al., 2018; Filatov et al., 2021; Leder et al., 2021). Another aspect of our analysis is that the directionality of asymmetric gene flow, following isolation, went consistently from the deeper species to the shallower species. It is unclear whether this pattern will hold across other depth-segregated species pairs in *Sebastes*, but could be indicative of climatic shifts impacting the depth distribution of these species. Future work on Northeastern Pacific *Sebastes* will determine whether the overall pattern of isolation followed by secondary contact holds across depth-segregated species pairs.

4.3 | Limitations

Our work is limited in that we utilized a pool-seq approach and only focused on enriched exome, CNE and UCE sequences. The main advantage of the pool-seq approach is that it reduces the overall cost of sequencing (Schlötterer et al., 2014). It does have the disadvantage that allele frequency estimates can be biased, but this is overcome with increased sequencing coverage (Schlötterer et al., 2015). We intentionally utilized high coverage regions in our SNP discovery steps (40–500× coverage) to reduce any error. On top of this, we utilized enriched sequences in our analysis, which has the advantage of reducing sequencing efforts to protein-coding regions of the genome but would potentially be missing signals from extragenic regions. We were also limited in any inference from the comparison between the more divergent species pair *(S. crocotulus–S. minatus*), and future work in this area should focus on more recently derived species.

5 | CONCLUSIONS

Our exome scan of two *Sebastes* species pairs revealed a handful of genes and pathways associated with depth-related divergence. There were a small number of shared islands of divergence between the pairs, but islands of divergence were more readily detected in the pair with more recent divergence. In the *S. chlorostictus–S. rosenblatti* pair, we found enrichment for the neuropeptide synthesis pathway in outlier loci, which suggests that chemosensory drive may be involved in depth-related speciation for this pair. Our analysis of demography of speciation revealed support for a similar model of divergence for the two pairs (isolation followed by secondary contact), which has been observed in other marine taxa. These results

build on the growing knowledge of speciation history in the genus *Sebastes* and suggest *Sebastes* will continue to be a valuable model in understanding mechanisms of speciation in temperate marine fishes.

AUTHOR CONTRIBUTIONS

Daniel Olivares-Zambrano: Data curation (lead); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). **Jacob Daane:** Methodology (equal); writing – review and editing (equal). **John Hyde:** Data curation (equal); resources (equal); writing – review and editing (equal). **Michael Sandel:** Resources (equal); writing – review and editing (equal). **Andres Aguilar:** Conceptualization (equal); formal analysis (equal); funding acquisition (lead); project administration (lead); supervision (lead); writing – original draft (equal); writing – review and editing (equal).

ACKNOWLEDGMENTS

This project was supported by grants from the CSU Council on Ocean Affairs, Science & Technology (COAST-GDP-2019-001) and the National Science Foundation (DEB-2121145) to A. Aguilar. D. Olivares-Zambrano was supported by a CSU Council on Ocean Affairs, Science & Technology Graduate Student Research Award (CSUCOAST-OLIDAN-CSULA-AY1920) and by a grant from the National Institute of General Medical Sciences of the National Institute of Health (R25GM061331) (to K. Foster). Earlier drafts of this manuscript were improved with comments from K. Fisher and P. Krug (CSULA). We thank M. P. Harris at Harvard Medical School for technical laboratory support.

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

All Illumina sequence data have been uploaded to the NCBI-SRA (project # PRJNA839756). All code for pool-seq analysis is located on: https://github.com/aaguil67/aacode/blob/main/Sebastes_Olivares_1/Filtering_Mapping_Popoolation.

ORCID

Andres Aguilar https://orcid.org/0000-0002-4409-9597

REFERENCES

Ashburner, M., Ball, C., Blake, J., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., & Ringwald, M. (2000). Gene Ontology: Tool for the unification of biology. *Nature Genetics*, *25*, 25–29. https://doi.org/10.1038/75556

Behrens, K. A., Girasek, Q. L., Sickler, A., Hyde, J., & Buonaccorsi, V. P. (2021). Regions of genetic divergence in depth-separated *Sebastes* rockfish species pairs: Depth as a potential driver of speciation. *Molecular Ecology*, *30*(17), 4259–4275. https://doi.org/10.1111/mec.16046

Benestan, L. M., Rougemont, Q., Senay, C., Normandeau, E., Parent, E., Rideout, R., Bernatchez, L., Lambert, Y., Audet, C., & Parent, G. J. (2021). Population genomics and history of speciation reveal fishery management gaps in two related redfish species (*Sebastes mentella* and *Sebastes fasciatus*). *Evolutionary Applications*, *14*(2), 588–606.

Bermingham, E., McCafferty, S. S., & Martin, A. P. (1997). Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. In T. D. Kocher & C. A. Stepien (Eds.), *Molecular systematics of fishes*. Academic Press.

Bernardi, G., Bucciarelli, G., Costagliola, D., Robertson, D. R., & Heiser, J. B. (2004). Evolution of coral reef fish *Thalassoma* spp.(Labridae). 1. Molecular phylogeny and biogeography. *Marine Biology*, *144*(2), 369–375.

Bernardi, G. (2013). Speciation in fishes. *Molecular Ecology*, *22*(22), 5487–5502.

Bierne, N., Bonhomme, F., & David, P. (2003). Habitat preference and the marine-speciation paradox. *Proceedings of the Royal Society of London B: Biological Sciences*, *270*, 1399–1406.

Bindea, G., Galon, J., & Mlecnik, B. (2013). CluePedia Cytoscape plugin: Pathway insights using integrated experimental and *in silico* data. *Bioinformatics*, *29*(5), 661–663.

Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A., Fridman, W. H., Pages, F., Trajanoski, Z., & Galon, J. (2009). ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics*, *25*, 1091–1093.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*, 2114–2120.

Burford, M. O. (2009). Demographic history, geographical distribution and reproductive isolation of distinct lineages of blue rockfish (*Sebastes mystinus*), a marine fish with a high dispersal potential. *Journal of Evolutionary Biology*, *22*(7), 1471–1486.

Burford, M. O., & Bernardi, G. (2008). Incipient speciation within a subgenus of rockfish (*Sebastosomus*) provides evidence of recent radiations within an ancient species flock. *Marine Biology*, *154*, 701–717.

Carlon, D. B., & Budd, A. F. (2002). Incipient speciation across a depth gradient in a scleractinian coral? *Evolution*, *56*, 2227–2242. https://doi.org/10.1111/j.0014-3820.2002.tb00147.x

Carreras, C., Ordóñez, V., Zane, L., Kruschel, C., Nasto, I., Macpherson, E., & Pascual, M. (2017). Population genomics of an endemic Mediterranean fish: differentiation by fine scale dispersal and adaptation. *Scientific Reports*, *7*(1), 1–12.

Crow, K. D., Munehara, H., & Bernardi, G. (2010). Sympatric speciation in a genus of marine reef fishes. *Molecular Ecology*, *19*(10), 2089–2105.

Cruickshank, T. E., & Hahn, M. W. (2014). Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. *Molecular ecology*, *23*(13), 3133–3157.

Daane, J. M., Dornburg, A., Smits, P., MacGuigan, D. J., Hawkins, M. B., Near, T. J., Detrich, H. W., III, & Harris, M. P. (2019). Historical contingency shapes adaptive radiation in Antarctic fishes. *Nature Ecology & Evolution*, *3*, 1102–1109.

Daane, J. M., Rohner, N., Konstantinidis, P., Harris, D. S., & M., P. (2016). Parallelism and epistasis in skeletal evolution identified through use of phylogenomic mapping strategies. *Molecular Biology and Evolution*, *33*(1), 162–173.

Dennenmoser, S., Vamosi, S. M., Nolte, A. W., & Rogers, S. M. (2017). Adaptive genomic divergence under high gene flow between freshwater and brackish-water ecotypes of prickly sculpin (*Cottus asper*) revealed by Pool-Seq. *Molecular Ecology*, *26*, 25–42.

Dimitrieva, S., & Bucher, P. (2013). UCNEbase—a database of ultraconserved non-coding elements and genomic regulatory blocks. *Nucleic Acids Research*, *41*, D101–D109.

Fairweather, R., Bradbury, I. R., Helyar, S. J., de Bruyn, M., Therkildsen, N. O., Bentzen, P., Hemmer-Hansen, J., & Carvalho, G. R. (2018). Range-wide genomic data synthesis reveals transatlantic vicariance and secondary contact in Atlantic cod. *Ecology and Evolution*, *8*(23), 12140–12152.

Faria, R., Johannesson, K., & Stankowski, S. (2021). Speciation in marine environments: Diving under the surface. *Journal of Evolutionary Biology.*, *34*, 4–15.

Filatov, D. A., Bendif, E. M., Archontikis, O. A., Hagino, K., & Rickaby, R. E. (2021). The mode of speciation during a recent radiation in open-ocean phytoplankton. *Current Biology*, *31*(24), 5439–5449.

Gaither, M. R., Gkafas, G. A., de Jong, M., Sarigol, F., Neat, F., Regnier, T., Moore, D., Grocke, D. R., Hall, N., Liu, X., Kenny, J., Lucaci, A., Hughes, M., Haldenby, S., & Rus Hoelzel, A. (2018). Genomics of habitat choice and adaptive evolution in a deep-sea fish. *Nature Ecology & Evolution*, *2*, 680–687.

Gaither, M. R., & Rocha, L. A. (2013). Origins of species richness in the Indo-Malay-Philippine biodiversity hotspot: Evidence for the centre of overlap hypothesis. *Journal of Biogeography*, *40*(9), 1638–1648.

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genetics*, *5*(10), e1000695.

Heras, J., & Aguilar, A. (2019). Comparative transcriptomics reveals patterns of adaptive evolution associated with depth and age within marine rockfishes (*Sebastes*). *Journal of Heredity*, *110*, 340–350.

Heras, J., McClintock, K., Sunagawa, S., & Aguilar, A. (2015). Gonadal transcriptomics elucidate patterns of adaptive evolution within marine rockfishes (*Sebastes*). *BMC Genomics*, *16*, 656–672.

Herrero, J., Muffato, M., Beal, K., Fitzgerald, S., Gordon, L., Pignatelli, M., Vilella, A. J., Searle, S. M. J., Amode, R., Brent, S., Spooner, W., Kulesha, E., Yates, A., & Flicek, P. (2016). Ensembl comparative genomics resources. *Database*, *2016*, bav096. https://doi.org/10.1093/database/bav096

Hirase, S., Yamasaki, Y. Y., Sekino, M., Nishisako, M., Ikeda, M., Hara, M., Merilä, J., & Kikuchi, K. (2021). Genomic evidence for speciation with gene flow in broadcast spawning marine invertebrates. *Molecular Biology and Evolution*, *38*(11), 4683–4699.

Holliday, J. A., Zhou, L., Bawa, R., Zhang, M., & Oubida, R. W. (2016). Evidence for extensive parallelism but divergent genomic architecture of adaptation along altitudinal and latitudinal gradients in populus trichocarpa. *New Phytologist*, *209*(3), 1240–1251.

Hubert, N., Meyer, C. P., Bruggemann, H. J., Guerin, F., Komeno, R. J., Espiau, B., Causse, R., Williams, J. T., & Planes, S. (2012). Cryptic diversity in Indo-Pacific coral-reef fishes revealed by DNA-barcoding provides new support to the centre-of-overlap hypothesis. *PLoS One*, *7*(3), e28987.

Hyde, J. R., Kimbrell, C. A., Budrick, J. E., Lynn, E. A., & Vetter, R. D. (2008). Cryptic speciation in the vermillion rockfish (*Sebates miniatus*) and the role of bathymetry in the speciation process. *Molecular Ecology*, *17*, 1122–1136.

Hyde, J. R., & Vetter, R. D. (2007). The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). *Molecular Phylogenetics Evolution*, *44*, 790–811.

Ingram, T. (2010). Speciation along a depth gradient in a marine adaptive radiation. *Proceedings of the Royal Society B: Biological Sciences*, *278*, 613–618.

Johannesson, K. (2009). Inverting the null-hypothesis of speciation: a marine snail perspective. *Evolutionary Ecology*, *23*(1), 5–16.

Johns, G. C., & Avise, J. C. (1998). Tests for ancient species flocks based on molecular phylogenetic appraisals of *Sebastes* rockfishes and other marine fishes. *Evolution*, *52*, 1135–1146.

Kinsella, R. J., Kähäri, A., Haider, S., Zamora, J., Proctor, G., Spudich, G., Almeida-King, J., Staines, D., Derwent, P., Kerhornou, A., Kersey, P., & Flicek, P. (2011). Ensembl BioMarts: a hub for data retrieval across taxonomic space. *Database*, *2011*, bar030. https://doi.org/10.1093/database/bar030

Kofler, R., Pandey, R. V., & Schlötterer, C. (2011). PoPoolation2: Identifying differentiation between populations using sequencing of pooled DNA samples (Pool-Seq). *Bioinformatics*, *27*, 3435–3436.

Kolora, S. R. R., Gregory, O. L., Vazquez, J. M., Stubbs, A., Kamalakar, C., Jainese, C., Seeto, K., McCrea, M., Sandel, M. W., Vianna, J. A., Maslenikov, K., Bachtrog, D., Orr, J. W., Love, M., & Sudmant, P. H. (2021). Origins and evolution of extreme life span in Pacific Ocean rockfishes. *Science*, *374*(6569), 842–847.

Kozomara, A., & Griffiths-Jones, S. (2010). miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Research*, *39*(suppl_1), D152–D157.

Leder, E. H., André, C., Le Moan, A., Töpel, M., Blomberg, A., Havenhand, J. N., Lindström, K., Volckaert, F. A. M., Kvarnemo, C., Johannesson, K., & Svensson, O. (2021). Post-glacial establishment of locally adapted fish populations over a steep salinity gradient. *Journal of Evolutionary Biology*, *34*(1), 138–156.

Leray, M., Beldade, R., Holbrook, S. J., Schmitt, R. J., Planes, S., & Bernardi, G. (2010). Allopatric divergence and speciation in coral reef fish: The three-spot Dascyllus, Dascyllus trimaculatus, species complex. *Evolution: International Journal of Organic Evolution*, *64*(5), 1218–1230.

Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics*, *26*, 589–595.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*, 2078–2079.

Love, M. S., Yoklavich, M., & Thorsteinson, L. (2002). *The rockfishes of the Northeast Pacific*. University of California Press.

Malmstrøm, M., Matschiner, M., Tørresen, O., Star, B., Snipen, L. G., Hansen, T. F., Baalsrud, H. T., Nederbragt, A. J., Hanel, R., Salzburger, W., Stenseth, N. C., Jakobsen, K. S., & Jentoft, S. (2016). Evolution of the immune system influences speciation rates in teleost fishes. *Nature Genetics*, *48*, 1204–1210. https://doi.org/10.1038/ng.3645

Mihola, O., Trachtulec, Z., Vlcek, C., Schimenti, J. C., & Forejt, J. (2009). A mouse speciation gene encodes meiotic histone H3 methyltransferase. *Science*, *323*(5912), 373–375.

Momigliano, P., Jokinen, H., Fraimout, A., Florin, A. B., Norkko, A., & Merila, J. (2017). Extraordinarily rapid speciation in a marine fish. *Proceedings of the National Academy of Sciences, USA*, *114*, 6074–6079.

Nelson, J. S., Grande, T. C., & Wilson, M. V. H. (2016). *Fishes of the World* (5th ed.). John Wiley & Sons, Inc.

Pavoine, S., Love, M., & Bonsall, M. (2009). Hierarchical partitioning of evolutionary and ecological patterns in the organization of phylogenetically-structured species assemblages: application to rockfish (genus: *Sebastes*) in the Southern California bight. *Ecology Letters*, *12*, 898–908.

Portik, D. M., Leaché, A. D., Rivera, D., Barej, M. F., Burger, M., Hirschfeld, M., Rödel, M. O., Blackburn, D. C., & Fujita, M. K. (2017). Evaluating mechanisms of diversification in a Guineo-Congolian tropical forest frog using demographic model selection. *Molecular Ecology*, *26*, 5245–5263. https://doi.org/10.1111/mec.14266

Prada, C., & Hellberg, M. E. (2013). Long prer-eproductive selection and divergence by depth in a Caribbean candelabrum coral. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 3961–3966. https://doi.org/10.1073/pnas.1208931110

Puebla, O. (2009). Ecological speciation in marine v. freshwater fishes. *Journal of Fish Biology*, *75*(5), 960–996.

Quilodrán, C. S., Ruegg, K., Sendell-Price, A. T., Anderson, E. C., Coulson, T., & Clegg, S. M. (2020). The multiple population genetic and demographic routes to islands of genomic divergence. *Methods in Ecology and Evolution*, *11*(1), 6–21. https://doi.org/10.1111/2041-210X.13324

Renaut, S., Grassa, C. J., Yeaman, S., Moyers, B. T., Lai, Z., Kane, N. C., Bowers, J. E., Burke, J. M., & Rieseberg, L. H. (2013). Genomic

islands of divergence are not affected by geography of speciation in sunflowers. *Nature Communications*, *4*(1), 1–8.

Rocha, L. A., Robertson, D. R., Roman, J., & Bowen, B. W. (2005). Ecological speciation in tropical reef fishes. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*, 573–579.

Rondeau, E. B., Messmer, A. M., Sanderson, D. S., Jantzen, S. G., von Schalburg, K. R., Minkley, D. R., Leong, J. S., Macdonald, G. M., Davidsen, A. E., Parker, W. A., Mazzola, R. S. A., Campbell, B., & Koop, B. F. (2013). Genomics of sablefish (*Anoplopoma fimbria*): expressed genes, mitochondrial phylogeny, linkage map and identification of a putative sex gene. *BMC Genomics*, *14*(1), 1–19.

Sambrook, J., & Russell, D. W. (2006). Standard ethanol precipitation of DNA in microcentrifuge tubes. *Cold Spring Harbor Protocols*, *1*, pdb-prot4456.

Schlötterer, C., Kofler, R., Versace, E., Tobler, R., & Franssen, S. U. (2015). Combining experimental evolution with next-generation sequencing: A powerful tool to study adaptation from standing genetic variation. *Heredity*, *114*, 431–440.

Schlötterer, C., Tobler, R., Kofler, R., & Nolte, V. (2014). Sequencing pools of individuals— mining genome-wide polymorphism data without big funding. *Nature Reviews Genetics*, *15*, 749–763.

Sha, Q. Q., Zhang, J., & Fan, H. Y. (2020). Function and regulation of histone H3 lysine-4 methylation during oocyte meiosis and maternal-to-zygotic transition. *Frontiers in Cell and Development Biology*, *8*, 597498. https://doi.org/10.3389/fcell.2020.597498

Sivasundar, A., & Palumbi, S. R. (2010). Life history, ecology and the biogeography of strong genetic breaks among 15 species of Pacific rockfish, Sebastes. *Marine Biology*, *157*(7), 1433–1452.

Teske, P. R., Sandoval-Castillo, J., Golla, T. R., Emami-Khoyi, A., Tine, M., Von Der Heyden, S., & Beheregaray, L. B. (2019). Thermal selection as a driver of marine ecological speciation. *Proceedings of the Royal Society B*, *286*(1896), 20182023.

Turner, S. D. (2018). Qqman: An R package for visualizing GWAS results using Q-Q and manhattan plots. *Journal of Open Source Software*, *3*, 1.

Via, S. (2012). Divergence hitchhiking and the spread of genomic isolation during ecological speciation-with-gene-flow. *Philosophical Transactions of the Royal Society B*, *367*, 451–460.

Wang, X., Verschut, T. A., Billeter, J. C., & Maan, M. E. (2021). Seven questions on the chemical ecology and neurogenetics of resource-mediated speciation. *Frontiers in Ecology and Evolution*, *9*, 71.

Whitney, J. L., Bowen, B. W., & Karl, S. A. (2018). Flickers of speciation: Sympatric colour morphs of the arc-eye hawkfish, *Paracirrhites arcatus*, reveal key elements of divergence with gene flow. *Molecular Ecology*, *27*, 1479–1493.

Wolf, J., & Ellegren, H. (2017). Making sense of genomic islands of differentiation in light of speciation. *Nature Review Genetics*, *18*, 87100.

SUPPORTING INFORMATION

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

How to cite this article: Olivares-Zambrano, D., Daane, J., Hyde, J., Sandel, M. W., & Aguilar, A. (2022). Speciation genomics and the role of depth in the divergence of rockfishes (*Sebastes*) revealed through Pool-seq analysis of enriched sequences. *Ecology and Evolution*, *12*, e9341. https://doi.org/10.1002/ece3.9341