Molecular Phylogenetics and Evolution 173 (2022) 107515

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Resolving the phylogenetic relationship among recently diverged members of the rockfish subgenus *Sebastosomus*

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ARTICLE INFO

Keywords:
Phylogeny
Sebastes
ddRADseq
Sebastosomus
Adaptive radiation
Single Nucleotide Polymorphism
Cryptic species

ABSTRACT

Rapid speciation is an important aspect of adaptive radiations, but can obfuscate phylogenetic relationships among taxa. For recent radiations, there are challenges to reconstructing the relationships among the species due to often shorter branch lengths. Resolution of these relationships is further confounded when studies only use a few genetic markers. Double digest restriction-site associated DNA sequencing (ddRADseq) is a method of next generation sequencing that identifies many single nucleotide polymorphisms (SNPs) throughout the genome. This increases statistical power to resolve close phylogenetic relationships like those found within an adaptive radiation. We used this approach to understand the evolutionary history of the rockfishes of the genus *Sebastes*, which experienced an adaptive radiation between 3 and 5 mya. Here, we reconstructed the phylogenetic relationships among six species of rockfish within the subgenus *Sebastosomus* using over 11,600 SNPs. This reconstruction includes the two recently diverged species, *Sebastes mystinus* and *S. diaconus*, that were first described genetically in 2008 using mtDNA control region sequence data and six microsatellite loci. We confirmed the relationship of these cryptic species as sister-taxa and found evidence that *S. melanops* and *S. flavidus* were also sister-taxa. The latter contradicts prior studies but is supported by our reconstruction using nuclear DNA and measures of genetic differentiation tests and a discriminant analysis of principal components. The relationships between the species of *Sebastosomus* are further supported by morphological, biological, and ecological justifications.

1. 1.Introduction

One of the fundamental questions in biology is understanding how quickly the speciation process can occur. Adaptive radiations, rapid speciation within a species group in response to a new ecological context, serve as a natural experiment of this process. The rockfish, genus *Sebastes,* is one of the few examples of an ancient adaptive radiation in marine systems (Johns & Avise 1998). In addition to the initial radiation, there is evidence of more recent speciation events, which may be influenced by *Sebastes* spatial and temporal dispersal patterns (Burford and Bernardi 2008, Burford et al. 2011, Hyde and Vetter 2009;). *Sebastes* diverged into approximately 60 species roughly between 3 and 5 million years ago by some estimates (Wourms 1991, Johns and Avise 1998). Within the genus *Sebastes*, Hyde and Vetter (2007) conducted a comprehensive analysis of the phylogenetic relationships using mtDNA control region. However, recently acknowledged changes in some sister species and even cryptic species (Burford and Bernardi 2008) have not been reevaluated with all species in this monophyletic group using more than a few nuclear DNA loci (see analysis of whole genome sequencing that included a subset of the species in *Sebastosomus*; Kolora et al. 2021).

While the origin of the genus *Sebastes* was a historic adaptive radiation (3 to 5 million years ago), the subgenus *Sebastosomus* within this group has experienced recent speciation events within the cryptic species clade that includes *S. entomelas*'s divergence from the cryptic sister-taxa (*S. mystinus* and *S. diaconus*) and the divergence between these cryptic sister taxa, approximately 1.0 mya and 200 kya, respectively (Burford and Bernardi 2008). Previous studies identified *Sebastosomus* as a monophyletic group comprised of five species, *Sebastes mystinus* (blue rockfish), *Sebastes entomelas* (widow rockfish), *Sebastes flavidus* (yellowtail rockfish), *Sebastes serranoides* (olive rockfish), and *Sebastes melanops* (black rockfish), and one additional species, *Sebastes ciliates* (dusky rockfish; Kendall 2000). However, *S. ciliates* was removed from this group based on several genetic analyses (Johns and Avise 1998, Rocha-Olivares et al. 1999, Hyde and Vetter 2007). Later analysis by

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https://doi.org/10.1016/j.ympev.2022.107515
Received 6 December 2021; Received in revised form 28 April 2022; Accepted 29 April 2022
Available online 13 May 2022

Burford and Bernardi (2008) using mtDNA and microsatellite loci determined that the former species *S. mystinus* was composed of two cryptic species that diverged approximately 200 kya (Burford and Bernardi 2008, Buford 2009).

All species of *Sebastosomus* have overlapping regions and, depending on the species, are found in temperate, rocky reefs, in nearshore reefs on the continental shelf, and on offshore regions of the Northern Pacific Coast of North America (Table 1). Typical of many marine species, rockfish have a pelagic larval phase ranging from three to six months, with members of *Sebastosomus* averaging around four months. The pelagic stage is important for marine species because it is the primary period of dispersal, and the length of time spent as larvae may affect the dispersal capacity of fish species. Dispersal is important to the speciation process because it can influence patterns of both temporal and spatial isolation between populations. The two recently described cryptic species in particular overlap along a 450 km range from central Oregon to northern California (Burford and Bernardi 2008) and are most abundant in nearshore recreational and live fish fisheries in this region relative to other rockfish species. Since the discovery of the cryptic species, the two species were formally named *S. mystinus* (blue rockfish) and *S. diaconus* (deacon rockfish; Frable et al. 2015). However, the phylogenetic relationships among all six *Sebastosomus* species have not been formally addressed with multi-nuclear genomic DNA.

Although mtDNA control region sequence data combined with microsatellite data proves useful for detecting hybridization and characterizing relationships of recently diverged species, there are limitations to these data when constructing phylogenies (Rubinoff et al., 2005). We can overcome issues such as marker bias, heritability, effective population size differences, and low statistical power using high throughput nuclear DNA, especially when combined with mtDNA markers (Zhang and Hewitt 2003). With species that have undergone radiation events over a short period of time like *Sebastosomus*, constructing phylogenies with thousands of single nucleotide polymorphisms (SNPs) can overcome the limitations of other genetic markers. The goal of this study was to establish the phylogenetic relationships within the monophyletic grouping *Sebastosomus* for all five original species and the newly described *S. diaconus* using a double-digest restriction-associated DNA sequence (ddRADseq) approach and compare the results to previous reconstructions using mtDNA and nuclear DNA (RAG1) sequence data. Here, we can better understand the relationships among the six members of an ancient species flock that also encompasses a relatively recent speciation event.

2. Materials and methods

2.1. Samples

To reconstruct the phylogenetic relationships, we used individuals from all six species, some of which were analyzed previously and classified genetically as either Type 1 or Type 2 *S. mystinus,* now described as *S. diaconus* and *S. mystinus,* respectively (Burford and Bernardi 2008, Buford 2009; Frable et al. 2015). We sampled the remaining four species of *Sebastosomus* (*S. melanops*, *S. flavidus*, *S. serranoides*, and *S. entomelas*) within their respective ranges (Table 2). This provided a sampling of all extant members of the subgenus for phylogenetic reconstruction.

2.2. DNA extraction, sequencing, and genomic library building

We extracted genomic DNA from fin clips using the Qiagen DNEasy Blood and Tissue Extraction Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer protocol. We quantified DNA concentrations using a Qubit fluorometer (Qubit 2.0; Invitrogen, Carlsbad, CA USA). We built ddRADseq libraries using the enzyme pairs *SphI* and *MluCI* and

Table 2
The samples of species of the *subgenus Sebastosomus*. This includes the number of individuals, which sequence library they were found in, and the populations they were drawn from.

Species	N	Library	Populations
S. mystinus Type 1 (S. diaconus)	11	Library 1; Library 3	Neah Bay, Washington (N = 6); Cape Argo, Oregon (N = 5)
S. mystinus Type 2 (S. mystinus)	10	Library 1; Library 3	Big Creek, California (N = 2); Fort Ross, California (N = 1); Monterey Bay, California (N = 3); Point Lobos, California (N = 2); Stillwater, California (N = 2)
S. flavidus	15	Library 2	ORYR (N = 15), Newport, Oregon
S. melanops	16	Library 1; Library 2	BLCKOR (N = 5), Newport, Oregon; BlackUnk (N = 1), California; SJBR (N = 10), Strait of San Juan de Fuca, Washington
S. serranoides	14	Library 2	MBOR (N = 1), Monterey Bay, California; PBOR (N = 12), Pismo Beach, California; PtOR (N = 1), Point Lobos, California
S. entomelas	18	Library 1; Library 2	MBWR (N = 2), Monterey Bay, California; SCWR (N = 1) Santa Cruz, California; SEN0 (N = 9), Point Lobos, California

Table 1
Characteristics of species in Sebastosomus* including depth category, offshore versus nearshore, maximum (max) depth (meters), abundance, parturition (larval release), pelagic larval duration (larval phase), and settlement characteristics (initial settlement depth and time, and any subsequent movement).

Species	Coloration	Depth (max)	Abundance	Parturition	Larval Phase	Settlement
Offshore						
S. flavidus (yellowtail rockfish) Clade 2	Brown/brass, lighter ventral area, dusky yellow fins, deep body	90–180 m (549 m)	BC - Central CA, & San Miguel Is.	Nov-Jan	3–4 months	Apr to Aug, first in canopy, then deeper
S. entomelas (widow rockfish) Clade 1	Brown/dusky brown, lighter ventral area, black fins, deep body	140–210 m (549 m)	BC - N. CA	Jan-Feb (CA) Feb-Mar (OR)Apr. (BC)	4–5 months	June-July, initially shallow in dense kelp, then deeper
Nearshore						
S. melanops (black rockfish) Clade 2	Black/gray, black spotting, lighter ventral area, elongate body	0–55 m (366 m)	BC - Central CA	Jan-Mar	4–6 months	May-July, shallow region < 20 m
S. serranoides (olive rockfish) Clade 1	Brown/green–brown, white dorsal spotting, elongate body	0–120 m (172 m)	N. CA - S. CA	Dec-Mar, Peak Jan.	4–6 months	Apr-Sept, initially shallow then deeper
S. mystinus and S. diaconus (blue and deacon rockfish) Clade 1	Black/light gray, two vertical black stripes by eyes, deep body	0–90 m (549 m)	Deacon Queen Char Island to N CA & Blue OR - Central CA	Oct-Mar Peak Dec/Jan	3–5 months	Apr-June, initially schooling, then shallow bottom

*Laroche and Richardson 1981, Echeverria 1987, Moser, et al. 1996, Orr et al. 1998, Love et al. 2002, Bobko and Berkeley 2004, Ammann 2004; BC = British Columbia, CA = California, OR = Oregon, N = northern, S = southern.

following the protocol and method outlined in Burford Reiskind et al. (2016). We constructed three libraries, for a total of 85 individual rockfish from the subgenus *Sebastosomus* (Table 2). We conducted single-end sequencing of 100 bp fragments of the libraries on the Illumina HiSeq 2500 following specifications at North Carolina State University Genomic Sequencing Laboratory (Raleigh, NC, USA).

2.3. Bioinformatic pipeline

We used FASTQC (Babraham Bioinformatics; https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) to check the quality of the reads, using a high phred score criterion, prior to processing the barcodes as outlined in Burford Reiskind et al. (2016). We then ran the *process_radtags* script to filter and de-multiplex our variable length barcodes in STACKS v.1.24 (Catchen et al. 2011). We trimmed the reads to 90 base pairs to make all read lengths identical in length as required by the STACKS platform. For SNP detection, we ran the denovo pipeline (*denovo.pl*) available in STACKS with the following parameters: $m = 3$ (minimum stack depth), $M = 2$ (mismatches allowed between loci within an individual), and $n = 2$ (mismatches allowed between loci when combining them in a catalog; Catchen et al. 2011). These parameters were originally tested in an analysis that included *S. mystinus*, *S. diaconus*, *S. melanops*, *S. serranoides*, and *S. flavidus* and tested a range of values for *m* and *n* (Burford Reiskind et al. 2016). We then used the population pipeline (*populations*) in STACKS with parameters as follows: minimum read depth per individual at a locus ($m = 5$), number of populations loci present in ($p = 6$; for the six species), proportion of individuals within a population (species) that have these loci ($r = 0.75$). We generated PLINK output files for further SNP filtering and downstream analyses (PLINK v1.19 https://pngu.mgh.harvard.edu/purcell/plink/), as it is considered versatile for large NextGen sequenced data. We filtered the data set for minimum allele frequencies (MAF) in PLINK (–maf 0.10), removing all loci with a minimum allele frequency of 0.10 or lower, and we filtered loci missing in more than 10% of individuals for all analyses (–geno 0.10), which retained only loci that were present in 90% or more individuals. We then used the program PGDSpider v.2.1.1.0 (Lischer and Excoffier 2012; https://www.cmpg.unibe.ch/software/PGDSpider/) to transform the PLINK dataset in various input file formats required by the software we utilized: GENEPOP v.4.2 (Rousset 2008), DAPC analysis implemented in ADEGENET (Jombart 2008), and TreeMix v1.13 (Pickrell et al., 2012). We measured genetic diversity (H_E), inbreeding coefficient (F_{IS}), and genetic differentiation (pairwise F_{ST}), and we conducted a pairwise exact G test in GENEPOP (MCMC parameters: 20 000 dememorization, 500 batches, 10 000 iterations per batch). We conducted a Discriminant Analysis of Principal Components (DAPC) in R using the package ADEGENET and used ggplot to graph individual loadings.

2.4. Phylogeny construction

We used TreeMix (ver 1.13; Pickrell et al., 2012) to construct the maximum likelihood (ML) phylogenetic tree using 23,340 SNPs from 14,332 ddRADseq reads for the six species of *Sebastosomus*. Treemix incorporates allele frequency data to determine the most likely tree allowing for admixture. We used a K = 500 (creating 500 blocks of SNPs) to account for linkage among SNPs and produced unrooted trees, and one tree with a root of *S. melanops*. To confirm branching patterns, we conducted a bootstrap analysis using 1000 replicates for the unrooted trees in TreeMix and 500 replicates for the rooted tree. We then constructed phylogenies using three different target trees (three tree topologies with the greatest support), keeping target tree heights, with TreeAnnotator (Drummond and Rambaut 2007 v.2.6.4) and created figures using TreeFig (http://tree.bio.ed.ac.uk/software/figtree/ v.1.4.4). To corroborate our findings with TreeMix, we also used SVD Quartets (Chiffman and Kubatko 2014) implemented in PAUP* (version v4a168; Swofford 2002) to generate a species tree under multispecies coalescence, treating each SNP as an independent locus. We exhaustively searched all possible quartets and used 1,000 bootstrap replicates to assess node support.

3. Results

After filtering first with the *de novo* and population pipeline parameters in STACKS and further filtering *maf* = 0.10 (removing loci with 0.10 or less frequency) and *geno* = 0.10 (retaining loci found in 90% of individuals), we retained 23,340 polymorphic loci (for number of loci filtered at each step see Bioinformatic Pipeline Notes in Dryad account https://doi.org/10.5061/dryad.6t1g1jx1n) across 14,332 ddRADseq reads. After evaluating both individuals and loci for missing data, we found one individual from *S. entomelas* that had over 80% missing data and this individual was removed from all following analyses, resulting in a total of 84 individuals.

With the 23,340 SNPs, we used GENEPOP to measure the genetic diversity, inbreeding coefficient, and genetic differentiation (pairwise F_{ST} and pairwise exact test) of the six species (Table 3). We found significant differences in pairwise F_{ST} values for all comparisons (pairwise exact G test) after correcting for multiple comparisons by enforcing strict Bonferroni corrections (alpha level = 0.05). *Sebastes mystinus* and *S. diaconus* had lower pairwise F_{ST} values ($F_{ST} = 0.313$) than other pairwise comparisons. We found lower F_{ST} values ($F_{ST} = 0.514$) between *S. melanops* and *S. flavidus*. Both *S. mystinus* and *S. diaconus* show similar pairwise F_{ST} values when compared to their closest sister taxa, *S. entomelas*, based on previous phylogenetic reconstructions. *Sebastes diaconus* showed slightly greater genetic differentiation than *S. mystinus* (*S. diaconus* & *S. mystinus* versus *S. entomelas* $F_{ST} = 0.871$ versus 0.783, respectively; Table 3). We found a similar relationship in the pairwise F_{ST} values between *S. serranoides* and either *S. flavidus* or *S. melanops*, with *S. melanops* showing slightly greater genetic differentiation than *S. flavidus* ($F_{ST} = 0.864$ versus 0.850, respectively; Table 3). Finally, we found F_{ST} values ranging from 0.840 or higher comparing any of *S. diaconus*, *S. mystinus*, or *S. entomelas* to any of *S. flavidus*, *S. serranoides*, and S. *melanops* (Table 3).

We completed a DAPC analysis we retained 23,340 SNPs of the six species after cross-validating the data using 95% of samples and found that 10 PCs produced the lowest error and highest success rate (Fig. 1, LD 1 versus 2, other axes compared in Supplemental Fig. 1). We found considerable overlap between *S. diaconus* and *S. mystinus*, and a close relationship between these sister-taxa and *S. entomelas* (Fig. 1). Due to varying degrees of relatedness among all six species and to further resolve the relationship among the cryptic species clade, we see that these three species are more closely related to each other than to the other clade, yet are distinct species matching what we found in the pairwise F_{ST} measures (Fig. 1, Supplemental Fig. 1). In contrast, *S. melanops, S. flavidus*, and *S. serranoides* all showed greater genetic divergence from each other than from the central group.

The phylogenetic tree of *Sebastosomus* showed a similar pattern of relationships to what we found in the pairwise F_{ST} and DAPC analyses. We found similar bootstrap support among two tree topologies both unrooted and with *S. melanops* as the root (Fig. 2A & B). There was no evidence of migration or gene flow among species and genetic drift patterns corroborated the results from pairwise F_{ST} and DAPC (TreeMix, Fig. 3). We identified two consistent clades within this monophyletic group. Clade 1 was composed of *S. mystinus* and *S. diaconus* as sister-taxa and included *S. entomelas* for all reconstructions (Fig. 2). Clade 2 was consistently composed of *S. flavidus* and *S. melanops* as sister-taxa in all trees except for the tree rooted with *S. melanops* (Fig. 2). However, we found that the sixth species, *S. serranoides,* was assigned to either the first clade or second clade, depending on the reconstruction (TreeMix versus SVD Quartets; Fig. 2).

Table 3
Pairwise F_{ST} comparisons among the six species of Sebastasomus. All pairwise differences are significant in the pairwise exact test (G-test in GenePop) at an alpha level of 0.05 after strict Bonferroni corrections for multiple comparisons.

	S. diaconus	S. mystinus	S. entomelas	S. flavidus	S. melanops
S. mystinus	0.313				
S. entomelas	0.871	0.783			
S. flavidus	0.886	0.840	0.897		
S. melanops	0.896	0.850	0.904	0.514	
S. serranoides	0.941	0.902	0.942	0.850	0.864

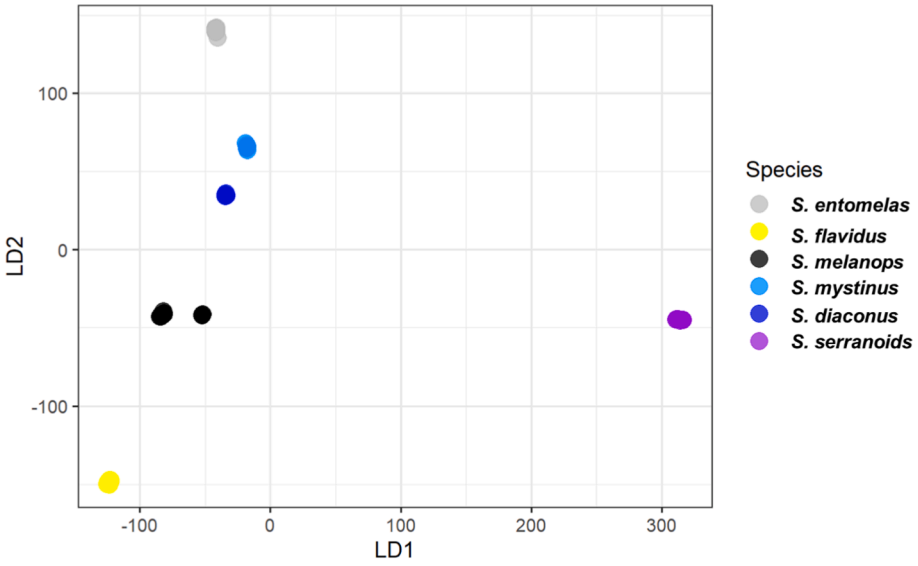

Fig. 1. Results of the DAPC analysis of the six species in the subgenus Sebastosomus with 30 PCs. Displaying the first two axes (LD1 and LD2). We explore other axes in Supplemental Fig. 1. Constructed using 23,340 loci across 84 individuals. Sebastes melanops, S. diaconus, S. mystinus, S. serranoides, S. entomelas, and S. flavidus are all represented.

4. Discussion

While the phylogenetic relationships of *Sebastes* species have been previously analyzed (Hyde and Vetter 2007, Johns and Avise 1998, Rocha-Olivares et al. 1999), including an analysis of the subgenus *Sebastosomus* (Burford and Bernardi 2008), there has only been a few reconstructions that use nuclear DNA (Kolora et al. 2021, subset of *Sebastes* species). There has not been a full reconstruction using multi-nuclear markers of *Sebastosomus* since *S. mystinus* and *S. diaconus* were first described. In comparison to previous phylogenetic analyses, the results of this study showed strong support for the previously identified clade of *S. mystinus*, *S. diaconus*, and *S. entomelas* (Clade 1) and inconsistent support for a clade that contained the other three species, *S. melanops*, *S. serranoides*, and *S. flavidus* (Clade 2; Hyde and Vetter 2007, Johns and Avise 1998, Rocha-Olivares et al. 1999, Burford and Bernardi 2008). This study finds a similar pattern to what was found in Kolora et al. (2021); however, the Kolora et al. (2021) study did not include all six species in the subgenus and included many other *Sebastes* species. Therefore, while it supports our findings, we are limited in making direct comparisons. In comparison with previous studies, including Burford and Bernardi's (2008) analysis using mtDNA and microsatellite loci, we found support for *S. melanops* and *S. flavidus* as sister-taxa within their clade much like *S. mystinus* and *S. diaconus*. In addition, *S. serranoides* either joined Clade 1 or Clade 2 depending on the reconstruction. Finally, we found a closer relationship between *S. entomelas* and *S. mystinus* than between *S. entomelas* and *S. diaconus*.

While Burford and Bernardi (2008) identified two cryptic species of *S. mystinus* (*S. diaconus* and *S. mystinus*), they also showed these two species were closely related to *S. entomelas* and estimated that the divergence occurred approximately 1.0 mya based on mtDNA control region data. However, depending on the molecular marker (mtDNA versus nuclear DNA) and the reconstruction, the degree of differentiation and the assignment of individuals to species-level branch groups in the tree varied. For example, in the mtDNA neighbor-joining tree, individual samples of *S. entomelas* embedded with one or both of the cryptic species (see Burford and Bernardi 2008), while the ML tree based on nuclear DNA markers (RAG1) separated the two species. They concluded that there was likely incomplete lineage sorting between the two species (Burford and Bernardi 2008). Incomplete lineage sorting would explain shared ancestry without contemporary introgression, which is consistent with the cryptic species, *S. diaconus* and *S. mystinus,* and *S. entomelas* being morphologically distinct, having a depth distribution difference, and having different population centers despite having a largely overlapping range (Love et al. 2002). In this study, measures of genetic divergence supported a closer relationship between *S. entomelas* and *S. mystinus* than between the former and *S. diaconus* (Fig. 1, Table 3). The greater divergence between *S. diaconus* and *S. entomelas* could be due to sympatric coexistence during the speciation process leading to character displacement or greater genetic divergence between these two species. *S. diaconus* has acquired more derived characteristics than *S. mystinus*, thus increasing the genetic divergence between *S. entomelas* and *S. diaconus*. These species likely do not interact as currently *S. entomelas* is an offshore species while both *S. mystinus* and *S. diaconus* are nearshore. In addition, the peak timing of settlement for *S. entomelas* is later in the season and typically they move to deeper water sooner than the other two species (Table 1). This and the lack of evidence of introgression among the lineages (Fig. 3) gives little support to contemporary introgression. *Sebastes diaconus* is found across the same range as *S. entomelas*, which supports the two species further differentiated during the speciation process. Overall, we are unable to

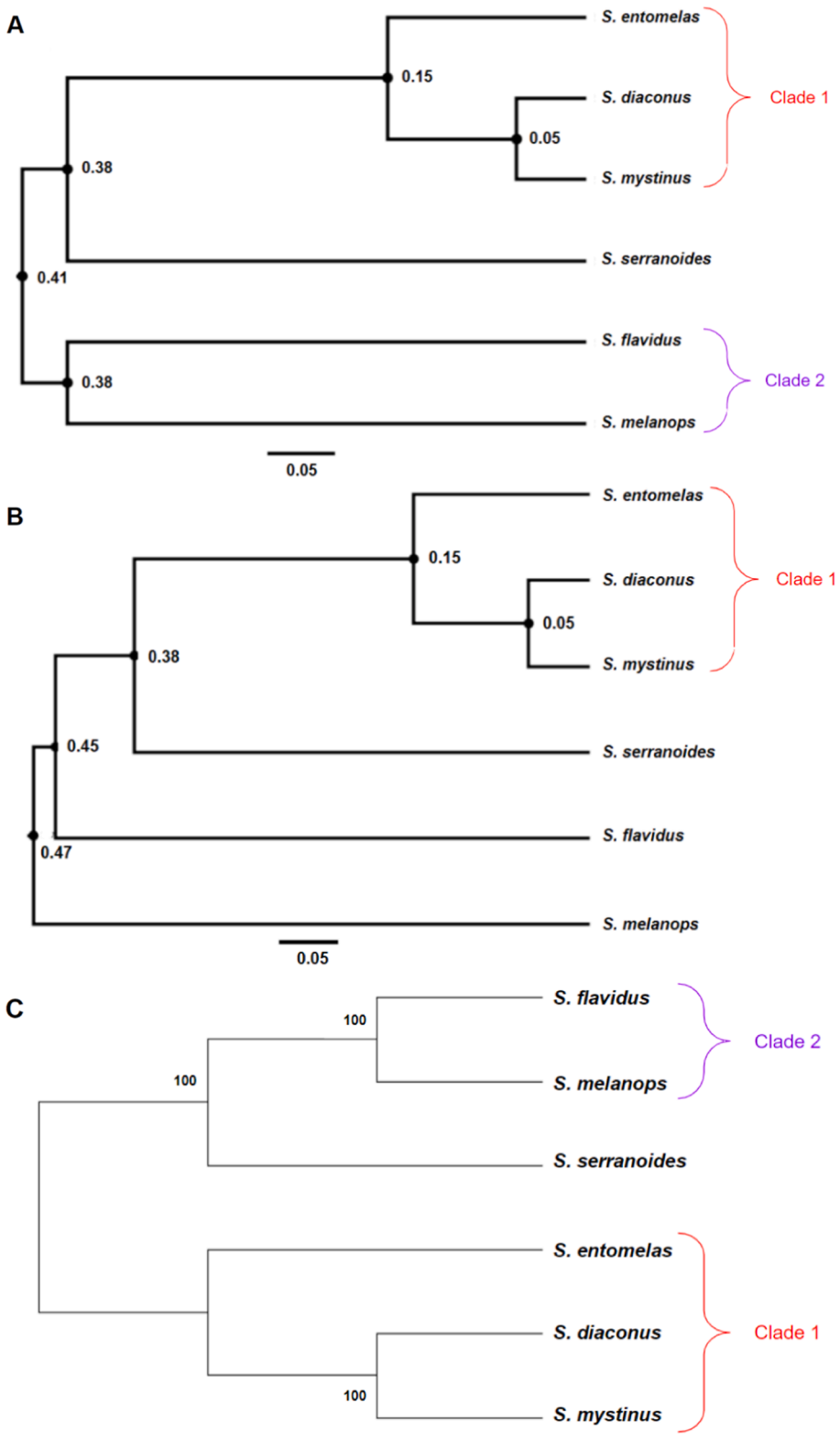

Fig. 2. Maximum likelihood and SVD Quartets phylogenetic trees of subgenus Sebastosomus. Trees A and B are generated in TreeMix (v1.13) built using TreeAnnotator (v.2.6.4) and TreeFig (v.1.4.4) keeping target tree heights with no root.. The A and B trees are with and without a root (*B rooted by S. melanops*) and include a bootstrap analysis using 1000 replicated to confirm branching patterns and a K = 500 to account for SNP linkage. Branch length standard error is as indicated on the graphs. Trees are constructed using 23,340 SNPs across 84 individuals. The C tree constructed using SVD Quartets (Chiffman and Kubatko 2014) implemented in PAUP* (version v4a168; Swofford 2002) to generate a species tree under multispecies coalescence, Each of the 23,340 SNPs are treated as an independent locus. All possible quartets are searched and 1,000 bootstrap replicates are used to assess node support. Trees are constructed using 23,340 loci across 84 individuals.

resolve these alternative hypotheses without an extensive comparative genomic analysis. Sampling along the range of overlap between *S. diaconus* and *S. entomelas* and greater sampling of *S. mystinus*, and a demographic analysis similar to what was done for the two cryptic species in Buford (2009), would provide a better understanding of the speciation process and evolutionary history of these three species in Clade 1.

The previous reconstruction by Burford and Bernardi (2008) had three species in Clade 2, *S. flavidus*, *S. serranoides*, and *S. melanops*, with *S. serranoides* and *S. flavidus* as sister-taxa. Here, we found evidence that supports *S. melanops* and *S. flavidus* as a sister-taxa in all unrooted trees and the evaluation of migration and genetic drift (Fig. 3), and with *S. serranoides* joining Clade 1 in one of the reconstructions and Clade 2 in the second one. This contradicts previous analyses, one using seven

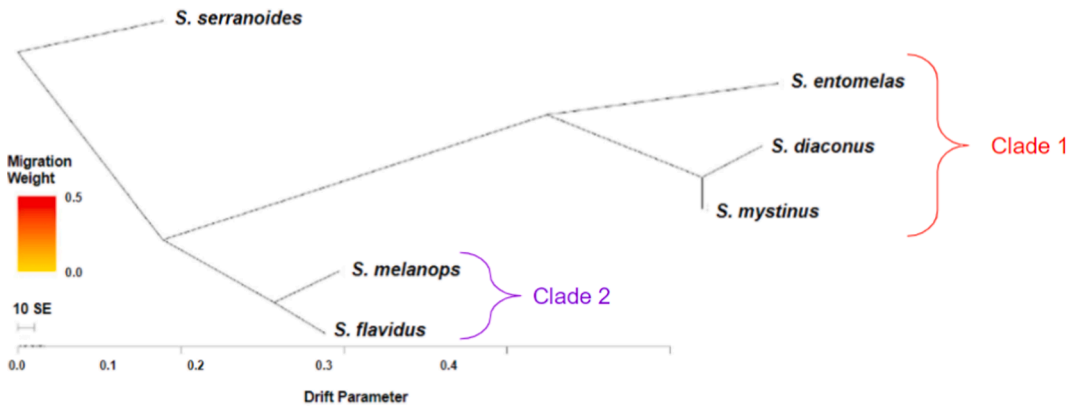

Fig. 3. Results of the ML reconstruction TreeMix tree with *S. serranoides* as the root with K = 500, to test for lineage migration and degree of genetic drift. There is no evidence of migration among the branches (there are no colored lines among species) and the degree of genetic drift is greatest between Clade 1 S. mystinus (blue), *S. diaconus*(deacon), and *S. entomelas* (widow) rockfish, and Clade 2 of *S. melanops* (black) and *S. flavidus* (yellowtail) rockfish. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mtDNA genes and two nuclear genes (Hyde and Vetter 2007) and one using mitochondrial control region (CR) sequence data (Burford and Bernardi 2008). In these previous reconstructions, both analyses using mtDNA control region sequences showed a closer relationship between *S. serranoides* and *S. flavidus* than either to *S. melanops* (Hyde and Vetter 2007, Burford and Bernardi 2008). In contrast, Burford and Bernardi (2008) showed that *S. melanops* and *S. flavidus* were more closely related to each other than to *S. serranoides* in the neighbor-joining tree with 750 bps of the RAG1 nuclear sequence data. Yet, the tree generated with RAG1 data showed a clear distinction between Clade 1 (both cryptic species and *S. entomelas*) and Clade 2 (*S. serranoides*, *S. melanops*, and *S. flavidus*). This suggests that there is a discrepancy between phylogenetic reconstructions that include mtDNA versus nuclear DNA markers. This may be due to introgression that occurred during the speciation process among these three species, similar to what is suggested within Clade 1. In this study, we further supported *S. melanops* and *S. flavidus* as a sister group in both of the analyses of genetic differentiation (Table 3, Figs. 2 and 3), showing the relatively closer relationship between these two species. Notably, while *S. flavidus* and *S. melanops* share the majority of their range, *S. serranoides* only overlaps with the southernmost locations of their range, suggesting an ecological basis for this relationship. In addition, while all three species have overlapping settlement periods, S. *melanops* typically stays shallow after settlement compared to the other two (Table 1). With a more extensive sampling of all three species throughout their ranges we could begin to unravel the evolutionary history within this clade and begin to draw generalizations about speciation within the genus *Sebastes*.

Overall, these phylogenetic reconstructions of the monophyletic subgenus *Sebastosomus* has strong support from several different nuclear DNA analyses as well as strong morphological, biological, and ecological justifications to reinforce these analyses. This suggests that further analysis of the discrepancy between mtDNA and nuclear DNA reconstructions, will likely reveal the mechanism of speciation within this monophyletic group. Further sampling of the subgenus within regions of sympatry and allopatry will provide critical information on how abiotic factors (environmental shifts throughout their ranges), evolutionary history (times of isolation and sympatry), and species interactions (range overlap and character displacement) help shape the speciation process within *Sebastosomus*. Ideally, given the analysis that revealed evidence of cryptic species (Hyde and Vetter 2009), a more thorough sampling and multiple markers of both mtDNA and nuclear DNA might help understand the past and present evolutionary trajectory of the genus *Sebastes* overall. This would also help inform trajectories for other nearshore, rocky reef fishes and invertebrates that co-exist with this group along the eastern Pacific.

5. Conclusion

We constructed a phylogenetic tree of the monophyletic group *Sebastosomus* including six species using ddRADseq techniques and nuclear DNA establishing the relationship of the newly defined *S. diaconus* to the rest of the clade. This revealed the anticipated relationship between *S. mystinus*, *S. diaconus*, and *S. entomelas*. However, the three species in question are closely related that supports the constructed phylogenetic relationships but highlights the closer relationship between *S. mystinus* and *S. entomelas*. Further exploration of this relationship, especially in the context of the difference between a nearshore versus offshore species respectively, would further highlight the evolutionary history between these two cryptic species and *S. entomelas*. Additionally, exploration of the overlap region of the two cryptic species would also aid in the understanding of this relationship and how it fits into the context of *Sebastosomus*. Here, we show that the relationships among the species in clade 1 are likely formed by their evolutionary history and ecology. Notably, our phylogenetic tree did contradict the previously described relationship between *S. melanops*, *S. flavidus*, and *S. serranoides* in other studies using mtDNA. Our trees are supported by the pairwise F_{ST} results showing lower genetic divergence between *S. melanops* and *S. flavidus* as compared to S. *serranoides*. This study provides a deeper understanding of the relationships among the six species of this monophyletic group than previous analyses and establishes the position and relationship of *S. diaconus* within the *Sebastosomus* subgenus.

CRediT authorship contribution statement

E.N. Wallace: Conceptualization, Investigation, Writing – original draft. **E.M.X. Reed:** Methodology, Writing – review & editing. **A. Aguilar:** Methodology, Writing – review & editing. **M.O. Burford Reiskind:** Conceptualization, Investigation, Methodology, Resources, Writing – original draft, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank John Soghigian for assistance with the phylogenetic reconstruction and Michael H. Reiskind for logistical support in the laboratory. We would also like to thank Giacomo

Bernardi, for providing adult samples of olive rockfish.

Funding

This work was supported by the Wynne Innovation Grant from the CAL Dean's Enrichment Grant Programme at NCSU awarded to MO Burford Reiskind.

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