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A chromosome-scale reference genome reveals the genetic architecture of sex in a North Atlantic *Sebastes* species, *S. fasciatus*

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Highlights

- This study provides the first chromosome-level genome and first genomic analysis of sex determination in an Atlantic *Sebastes*.
- Sex in *S. fasciatus* is associated with multiple genomic regions, including *amh*, rather than a single strongly differentiated sex chromosome.
- Although *Sebastes* species share broadly conserved genome structure, the study shows that sex-associated architecture can still differ and evolve rapidly.
- The findings show that sex determination in redfish is evolutionarily flexible and relevant to both speciation research and fisheries management.

Abstract

Sex determination systems in teleost fishes are highly labile, subject to frequent turnover, and can play a role in driving divergence and speciation. Here, we present a chromosome-level genome assembly for Acadian redfish (*Sebastes fasciatus*), a commercially and ecologically important Northwest Atlantic species. The genome assembly spans 833.8 Mb across 24 chromosomes with 97% BUSCO completeness. The mitochondrial genome assembled from long reads is 20.1 kb and contains a large tandem repeat region.

Whole-genome sequencing analysis of 141 *S. fasciatus* individuals (N female = 81; N male = 60) from the Northwest Atlantic were then used together with the new genome assembly to identify sex-associated genomic regions on multiple chromosomes. Despite minimal genome-wide differentiation between sexes, we detected discrete sex-associated regions on multiple chromosomes, including a conserved region encompassing the *Anti-Müllerian Hormone* gene (*amh*) (Chr 5), with additional associations near *17 β -hydroxysteroid dehydrogenase* (*hsd17b1*) (Chr 13) and a region on Chr 3 containing genes implicated in hormone signalling and germline development. Together, these results suggest that sex-associated genomic signals in *S. fasciatus* are distributed across multiple chromosomes and loci, including candidates linked to sex determination and differentiation. This work offers the first genomic insights into sex determination of an Atlantic *Sebastes* species, bridging a major gap in our understanding of sex chromosome diversity in rockfishes and providing novel resources for conservation management.

Introduction

Across sexual eukaryotes, the mechanisms that govern sex determination varies widely. In many species, sex is determined genetically. These systems may be chromosomal, such as XY in mammals (male heterogamety) or ZW in birds (female heterogamety), or involve single major loci or multiple genomic regions, as is common in teleost fishes. In addition to determining sex, sex-linked chromosomes or regions are implicated in a range of evolutionary processes (Bachtrog et al., 2014). These include adaptation through the maintenance of alleles that confer advantages under sex-specific or environmental selection pressures (Charlesworth, 2018); genomic conflict arising from antagonistic interactions between sex-linked and autosomal loci (Rice, 1987); and the evolution of suppressed recombination, which can facilitate reproductive isolation (Coyne & Allen Orr, 1998; Kitano & Peichel, 2012). Identifying the specific chromosomes or genomic regions underlying sex determination is therefore key to understanding how sex-linked variation contributes to adaptation, genomic architecture, and speciation.

In mammals and birds, conserved male- or female-heterogametic sex determination systems have been maintained over long evolutionary timescales following the recruitment of sex-determining genes (SDGs) and subsequent degeneration of the heterogametic sex chromosome due to suppressed recombination and the accumulation of deleterious mutations (Graves, 2008). These systems are therefore highly stable and show limited turnover. They include the Y-linked *Sry* gene in mammals (Kashimada & Koopman, 2010; Scharl & Lamatsch, 2023; Sinclair et al., 1990) and the duplicated Z-linked *dmrt1* gene in birds (Ioannidis et al., 2021). In teleost fishes, however, SDGs and their chromosomal locations are often not conserved. Recurrent turnover, whereby new SDGs or genomic regions independently acquire sex-determining roles, drives rapid shifts in the location, structure, and function of sex chromosomes (Kitano et al., 2023). This evolutionary lability is thought to be promoted by processes such as gene duplication, transposable elements and chromosomal rearrangements (Han et al., 2022; Sember et al., 2021). As a result, sex chromosomes in many teleost fish remain homomorphic and difficult to differentiate by karyotyping, even when genetic sex-determining regions are present (Kitano et al., 2024; Sember et al., 2021).

In teleost fish, SDGs commonly belong to one of three protein families and their signalling pathways; doublesex/mab-3-related transcription factors (DMRT), SRY-related HMG-box genes (SOX), and transforming growth factor-beta (TGF- β) (Bertho et al., 2021). The TGF- β pathway is the most common pathway for sex determination across teleost fishes (Drummond, 2005; Fan et al., 2012), with several genes within the pathway co-opted as SDGs including *anti-Müllerian hormone (amh)*, *anti-Müllerian hormone receptor type 2 (amhr2)*, *bone morphogenetic protein receptor type 1b (bmpr1b)*, *gonadal soma-derived factor (gsdf)* and *growth differentiation factor 6 (gdf6)* (Pan et al., 2021). Rockfishes (*Sebastes* spp.) are a species-rich and commercially important marine radiation in which the genomic basis of sex determination remains incompletely characterised, particularly in Atlantic species. In the genus *Sebastes*, rapid speciation (Olivares-Zambrano et al., 2022; Hyde & Vetter, 2007; Kolora et al., 2021), has led to more than 120 species distributed throughout the Northeast and Northwest Pacific (Rabosky et al., 2018) and four in the North Atlantic (Cadigan et al., 2022; Planque et al., 2013), where they are known as redfish. *Sebastes* have an XY sex-determination system (Fowler & Buonaccorsi, 2016), in which a duplicated copy of *amh (amhy)* has been co-opted as the master sex-determining gene, with the functional isoform showing male-limited expression in Northwest Pacific species (Song et al., 2021; Sykes et al., 2023). However, comparative analyses indicate that *amh* copies show low sequence conservation and substantial variation in copy number, chromosomal location, and degree of sex linkage across the genus (Sykes et al., 2023). This heterogeneity complicates identification of a universal master SDG and highlights the rapid and lineage-specific evolution of sex determination mechanisms within this recently diverged genus (Vaux et al., 2020). *Sebastes* therefore provides an excellent system to study SDG turnover.

In this study, we examined the genomic architecture of sex in Acadian Redfish (*Sebastes fasciatus*), from the north Atlantic. Using a novel chromosome-level genome assembly from a male individual combined with population-level whole-genome data using both male and female fish, we characterize sex-associated genomic regions and assess the complexity of sex determination in this species. Using hundreds of *S. fasciatus* individuals collected from Northwest Atlantic Canada, we performed a genome-wide association study (GWAS) and measured genetic diversity statistics to identify sex-associated genomic regions. We extend previous genomic

and genetic studies in this group offering the first genomic insights into sex determination and differentiation in an Atlantic *Sebastes* species.

Methods

Genome assembly and annotation

Tissue from a single male Acadian redfish from the Gulf of Saint Lawrence, Canada (48.183022 N 61.151193 W) was selected for sequencing to assemble a reference genome for the Vertebrate Genomes Project (VGP). Sequencing and genome assembly were conducted at the Vertebrate Genome Lab (VGL; The Rockefeller University, New York, USA). Genomic DNA was sequenced using PacBio Revio HiFi long-read sequencing and Arima Hi-C v2 proximity ligation technology. Assembly was performed using the VGP standard assembly pipeline version 2.0 (Larivière et al., 2024) which implements Hifiasm v0.18.8 (Cheng et al., 2021) and *yahs v1.2a.2* (Zhou et al., 2023). The assembly was then manually curated VGL manual curation team to correct structural errors and assign chromosome numbers. To assess genome assembly completeness we used Benchmarking Universal Single-Copy Orthologs (BUSCO, Manni et al., 2021) v5.8.2 with the Actinopterygii (ray-finned fishes) lineage (actinopterygii_odb12) and the MetaEuk toolkit option (--metaeuk).

We compared genomic structural variation to the Pacific honeycomb rockfish (*S. umbrosus*, GCA_015220745.1). The *S. umbrosus* genome was one of only two annotated chromosome-level *Sebastes* assemblies available at the time and was selected because it is geographically closer (distribution California, USA) to our focal species than *Sebastes schlegelii* (distribution Korea), the only other chromosome-level assembly then available. After removing non-chromosome scaffolds, we used D-GENIES (Cabanettes & Klopp, 2018) to examine dotplot alignments for mismatched and reversed chromosomes between the genomes. We used *S. umbrosus* as the reference and rearranged and reoriented *S. fasciatus* chromosomes based on sequence similarity. Following this, minimap2 (-ax asm5; Li, 2018) was used to align the two FASTA files, and the resulting SAM file was used as input for SyRI v1.7.1 (Goel et al., 2019). SyRI outputs were passed to plotsr v1.1.5 (Goel & Schneeberger, 2022) for visualization. Gene models for the genome assembly were generated with RefSeq protocols (REF?)

Mitochondrial genome assembly

We used PacBio HiFi long reads from the genome assembly raw reads to create a *de novo* mitochondrial genome. The raw HiFi reads were used as input for the MitoHiFi v3.2.2 workflow (Allio et al., 2020; Uliano-Silva et al., 2023) with a *Sebastes fasciatus* (KX897946.1) mitochondrion sequence and annotation as the reference input. In short, the MitoHiFi workflow maps long reads to the selected reference, filters longer than expected reads to prevent nuclear-inserted mitochondrial fragments, and creates contigs with hifiasm. NUMTs, which represent nuclear copies of mitochondrial sequences, can complicate analyses by introducing spurious mitochondrial signals. The resulting contigs are checked for circularity and annotated, and a final representative mitogenome selected. We used the default parameters for the pipeline with the exception of the percent match for contigs, which we increased to 80 percent. We reran MitoHiFi with a 90 percent match parameter to determine if NUMTs had made it through the filtering step. We also used Tandem Repeats Finder v4.09 (Benson, 1999) to examine the extra bases, since extended mitogenome sequences are often the result of repeated regions previously unrecognized by short read assemblers (Formenti et al., 2021).

Sample collection, DNA extraction and genotyping

To investigate the genomic architecture of sex in *S. fasciatus*, a total of N = 274 *Sebastes* were collected from the northwest Atlantic across various Northwest Atlantic Fisheries (NAFO) zones, including from the Gulf of St. Lawrence, Laurentian Channel, and adjacent shelf and slope regions (latitudes 42–49°N, longitudes 55–68°W) ([Figure 2a](#)). Sampling was conducted using research trawl surveys (including Albatros IV, Delaware II, KMKA Voyageur, and Lery Charles) using a variety of trawl gears (e.g., 380 Hurricane Trawl, Campelen 900) between the years 2001 and 2021. All samples were flash frozen (-20 °C) at sea, tissues then excised and preserved in ethanol. Sex for all fish was determined by visual inspection of gonadal morphology. DNA for all samples was extracted using the DNeasy 96 Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. DNA quality was assessed on a 1% agarose gel, and concentration was quantified using the Qubit™ High Sensitivity dsDNA Assay Kit (Thermo Fisher Scientific). Following normalisation, sequencing-ready libraries were prepared using the Illumina DNA Prep Kit (Illumina) with a modified manufacturer's

Sequencing reads from all fish (~10× mean coverage per fish) were aligned to our Acadian redfish (*S. fasciatus*) reference genome (Accession GCA_043250625) using BWA-MEM (v2; Li, 2013) with default parameters, Genome Analysis Toolkit (GATK) v4 MarkDuplicates to remove PCR duplicates, and the GATK v3 RealignerTargetCreator and IndelRealigner for local realignment around indels (McKenna et al., 2010). We used ANGSD (v0.940; Korneliussen et al., 2014) to perform genotype likelihood-based SNP calling using the SAMtools model (-GL 1) (Danecek et al., 2021) and restricting the analysis to unique, properly paired reads with high mapping quality (-minMapQ 30) and base quality (-minQ 20). To reduce the impact of low-complexity regions and alignment artifacts, we excluded triallelic sites, kept only uniquely mapped reads to reduce false negative associated with heteromorphic chromosomes (-uniqueOnly 1 -remove_bads 1 -only_proper_pairs 1 -baq 1 -C 50 -minInd 80% of total samples -SNP_pval 1e-6). Initial filtering and species validation using PCA confirmed two distinct species clusters (see [Appendix A](#) and Figure S1). Following genetic verification, only confirmed *S. fasciatus* individuals (Total N = 141, N female = 81, N male = 60) were retained. The final filtered dataset comprised 139 *S. fasciatus* individuals and 7.2 million SNPs after filtering with PLINK v.1.9 using parameters --geno 0.05, --mind 0.05, --mac 2, and for PCA we added --indep-pairwise 50 5 0.2. This dataset was used for all downstream sex-determination analyses.

We explored the capacity of our new reference genome to enable detection of sex-linked regions using a GWAS, which identifies associations between a phenotype and a genotype (Klein, 2007). Prior to GWAS, we assessed population structure within *Sebastes fasciatus* to evaluate potential geographic genetic structure that could confound association results. PCA was conducted in PLINK v1.9 (Chang et al., 2015) using the filtered SNP dataset. To locate regions of the genome involved in sex determination, we performed GWAS to test for associations between SNP variants

and phenotypic sex, using PLINK v2 (www.cog-genomics.org/plink/2.0/; Chang et al., 2015). Binary sex phenotypes were used as the dependent variable in the association model, and the first two eigenvectors from the above PCA analysis were used as covariates to account for the effect of population structure. Association statistics were adjusted for multiple testing using --adjust. Genes located within 500 bp of SNPs significantly associated with sex were intersected with the genome annotation (GTF) for our assembly (GCA_043250625) using bedtools (v. 2.31.1; Quinlan & Hall, 2010) to identify significant genes.

Assessment of Genomic Divergence

We next assessed the likely mode of sex determination in *S. fasciatus* using criteria from population genetic and sequence alignment summary statistics. To assess genome-wide read depth, we used Mosdepth v0.3.10 (Pedersen & Quinlan, 2018). To identify sex-biased depth signals, normalized read depth was summarized in non-overlapping 500 bp windows and $\log_2$ fold change ($\log_2FC$ male/female). An empirical null distribution was constructed from autosomal windows, and outliers were defined using the upper and lower threshold of the interquartile range (IQR) ($Q3 \pm 3 \times IQR$). Male and female biased regions were defined as windows with $\log_2FC >$ outlier upper threshold and $\log_2FC <$ outlier lower threshold, respectively (Palmer et al., 2019). We used PLINK v2 to calculate pairwise fixation-index (F_{ST}) between males and females across all SNPs. To identify genes with high differentiation between sexes, we calculated mean F_{ST} for each gene from SNP-level F_{ST} values. Genome-wide 10-kb windowed F_{ST} distributions were used to define outliers as values exceeding $Q3 + 3 \times IQR$. Minor allele frequency (MAF) per SNP and linkage disequilibrium (LD) in 10-kb windows were calculated in PLINK v1.9 and estimated separately for males and females using sex-stratified datasets. In addition, LD was estimated using all individuals combined to provide a genome-wide baseline for comparison. Mean and median r^2 values were summarised within candidate genes.

amh Gene

To identify *amh*-like genes in the assembled genome, we compiled reference *amh* sequences from the closest available teleost ortholog group (Perciformes) available in OrthoDB (OrthoDB; Kriventseva et al., 2007; Zdobnov et al., 2017),

together with additional *amh* sequences from *Sebastes koreanus*, *S. pachycephalus*, and *S. schlegelii* (Song et al., 2021) (see Table S1). These sequences were mapped to our chromosome-level assembly using minimap2 v2.24 (H. Li, 2018), with the splice-aware preset (-ax splice). To assess the evolutionary relationships among Perciformes *amh* genes, all reference and putative *amh* sequences identified in this study were aligned using MAFFT v7 (Kato & Standley, 2013). A maximum likelihood phylogenetic tree was inferred using IQ-TREE (v2, Minh et al., 2020), using ModelFinder Plus (MFP) to select the best-fitting substitution model, and node support was evaluated using 1000 ultrafast bootstrap replicates.

All plots were made using ggplot2 (Wickham, 2016).

Results

Genome assembly and annotation

The assembled genome spans 833.8 Mb with a guanine/cytosine (GC) content of 41% and 48.4× coverage, organised into 24 chromosomes and 203 scaffolds (scaffold N50: 36.3 Mb, L50: 11), with a contig N50 of 7.8 Mb (L50: 32) across 674 contigs (GenBank assembly accession GCA_009769545.1, coverage per sequencing technology available at: genomeark.org/genomeark-curated-assembly/Sebastes_fasciatus). A total of 152 scaffolds were unplaced, of which 27 were tentatively assigned to one of the 24 chromosomes. BUSCO results using all 203 scaffolds (Complete:97.0% [Single:96.6%, Double:0.4%], Fragmented:0.8%, Missing:2.2%, n:7207) were similar to results using only the 24 chromosome scaffolds (Complete:96.6% [Single:96.3%, Double:0.3%], Fragmented:0.7%, Missing:2.7%, n:7207).

Mitogenome assembly using MitoHiFi with the -p80 flag resulted in a 20,103 bp circular, gene-complete assembly containing all 37 expected mitochondrial genes. This assembly is approximately 3,700 bp longer than the NCBI reference, *Sebastes fasciatus* (KX897946.1), and 2,000 bp longer than any other *S. fasciatus* mitogenome on NCBI. Tandem Repeats Finder analysis of this assembly detected a 3.6 kb tandem repeat region between positions 16,455 and 20,099, consisting of a 277 bp repeat unit (alignment score = 7,290; Supplementary Table 1) repeated approximately 13.2 times

[Figure S2](#)). To test whether this additional length represented residual NUMT contamination, we reran MitoHiFi using the more stringent -p90 flag, however this produced a 15,373 bp assembly missing two expected genes and lacked circularity.

Synteny and structural rearrangements

SyRI identified the majority of aligned regions as syntenic for both the Honeycomb Rockfish, *S. umbrosus*, and Acadian Redfish, *S. fasciatus*, genomes ([Figure 1](#); 762.58 Mb and 779.26 Mb, respectively). As the *S. fasciatus* genome is larger than the reference *S. umbrosus*, it contained a greater number of unaligned bases (37.11 Mb versus 23.09 Mb for *S. umbrosus*). We detected approximately twice as many duplications in *S. fasciatus* (993 events, 1.59 Mb) compared to *S. umbrosus* (358 events, 1.28 Mb), along with 437 translocations and 109 inversions between the assemblies. Within this broader landscape of structural variation, the genomic regions containing *amh* differ between *S. fasciatus* and *S. umbrosus*, with conserved synteny interrupted by numerous SNPs on chr 4, and lineage-specific insertions, deletions and SNPs on chr 5. This suggests localised structural divergence within otherwise collinear *amh*-associated region.

Whole genome resequencing and genomic variation between sexes

Using the *S. fasciatus*-only dataset, PCA revealed minimal population structure, with PC1 and PC2 explaining 1.82% and 1.34% of the variance respectively ([Figure 2b](#)). Modest spatial clustering by collection location was evident, indicating weak population structure among samples however this structure did not align with phenotypic sex. This structure was accounted for in downstream analyses to minimize potential confounding of sex-associated signals.

Genome-wide association analysis identified SNPs significantly associated with sex after multiple testing correction, distributed across three chromosomes (chr 3, chr 5 and chr 13, see [Table 1](#)). The genomic inflation factor (λ) was 1.034, indicating minimal inflation in our model. Significant SNPs on chr 5 (N SNPs = 9) overlapped annotations for *amh*, an SDG previously identified in *Sebastes*. On chr 13 (N SNPs = 6) significant SNPs overlapped with gene *moto* (NC_133807.1: 21349264-21466878). All significant SNPs in this gene are located approximately 4.7–5.0 kb upstream of the *hydroxysteroid (17-beta) dehydrogenase 1 (hsd17b1)* (FDR $p < 1e-7$) (NC_133807.1:

21406409-21410726) transcription start site, both encoded on the minus strand. Both *moto* and *hsd17b1* have previously been associated with sex differentiation in Teleost fishes. Significant SNPs on chr 3 (N SNPs = 250) formed a peak between 36.2 - 37.01 Mb ([Figure 2c](#) and overlapped annotations for seven genes, all uncharacterised except for *sgcz* ([Table 1](#); Table S2). BLAST searches of uncharacterized genes in this region identified homology to genes with known roles in sex hormone signalling and gonadal differentiation (see [Table S2](#) & [Appendix B](#)). These include members of the *TGF- β* pathway (*tgfb2*, *tgfb3*), repeatedly implicated in fish sex-determination and gonadal differentiation. Together, multiple genomic regions were identified that may modulate sex determination and development via regulatory effects on hormone signalling and germline differentiation, alongside the primary SDG. Additional examination of these putative sex-linked regions on chr 3, 5, and 13 show sex-specific genomic signatures. Consistent with the GWAS results, these regions showed elevated genetic differentiation between sexes relative to the genome-wide background GWAS-(mean F_{ST} 0.012–0.187). Several genes exceeded the genome-wide outlier threshold ($Q3 + 3 \times IQR$ of windowed F_{ST}), including *amh* on chr 5 (mean $F_{ST} = 0.186$) and LOC141764957 on chr 3 (mean $F_{ST} = 0.187$) (see Table S3). Genes overlapping GWAS-significant regions also showed distinct sex-specific patterns of MAF. On chromosome 3, mean MAF was higher in females (0.152) than in males (0.091), whereas on chromosome 13, MAF was elevated in both sexes (mean = 0.120 in males; mean = 0.136 in females). These differences were significant for both sexes relative to their respective genome-wide averages ($p < 0.05$). On 5, males had a lower mean MAF (0.065) than females (0.118), which was significantly different from the autosomal average for females ($p = 3.79 \times 10^{-3}$) but not for males ($p = 0.30$) ([Figure 3a](#)). Genes overlapping GWAS-significant regions show non-uniform patterns of sex-biased read depth, with discrete windows exhibiting outlier depth differences (Table S3). On chr 3, *sgcz* exhibits pronounced sex-specific depth heterogeneity across its length, while LOC141764959 exhibited male-biased depth ($\log_2FC \sim +0.6$ to $+1.1$, see Table S3) and bi-modal heterozygosity consistent with a segregating sex-linked haplotype or copy-number polymorphism (see Figure S3). Several genes on chr 3 show modest male-biased LD (e.g., LOC141764959–957, mean $r^2 \sim 0.02$ –0.04 in males vs 0.013–0.02 in females), consistent with localised recombination suppression. In contrast, we observed female-biased depth ($\log_2FC \approx -0.5$) coverage at *amh* on chr 5 ([Figure 3b](#)) and *moto* restricted to short regions (<1 kb) rather than spanning the

full gene (Figure S4). These regions exhibit high LD in both sexes with minimal sex differences (mean r^2 0.04–0.05 vs. autosomal mean r^2 = 0.01), suggesting structural variation rather than sex-linked recombination suppression (Table S4).

Examination of genome annotations and mapping of *amh* homologues to our assembled genome matched two regions (Table S5). The first matched the *amh* copy on chr 5, and the second on chr 4. Examination of genome annotations confirmed the presence of *amh* paralogues. In our genome, the *amh* gene located on chr 5 is located on the reverse strand and comprises 7 exons. In contrast, LOC141767045 (Muellerian-inhibiting factor-like) is found on chr 4 on the forward strand, with 2 exons. The maximum likelihood phylogeny of *amh* sequences in *Sebastes* recovered two distinct clades of *amh* genes in *Sebastes* corresponding to the *amhy* and *amha* paralogues. Our *amh* sequences on chr 4 and chr 5 both form part of the *amha* clade (Figure 3c).

Discussion

Sex determination systems in teleost fishes are highly variable, frequently turnover, and can be central to intraspecific divergence and speciation. Here we present a chromosome-level genome assembly for Acadian redfish (*Sebastes fasciatus*) that reveals a largely conserved genomic architecture relative to other *Sebastes* species, despite ecological and phenotypic diversification across the genus. Integrating this assembly with population genomic data identified multiple sex-associated regions on chromosomes 3, 5, and 13, including the conserved *anti-Müllerian hormone* (*amh*) gene, which has been repeatedly implicated in teleost sex determination. The presence of additional loci linked to steroidogenesis and gonadal differentiation suggests that sex determination and development in *S. fasciatus* likely involves genes acting alongside *amh*. Together, these results highlight the complexity and evolutionary lability of sex determination pathways in teleosts and demonstrate the value of chromosome-level genomic resources for resolving the genetic architecture underlying sex determination and diversification in *Sebastes*.

Here, we presented a chromosome-level genome assembly for Acadian redfish (*Sebastes fasciatus*) spanning 833.3 Mb, comparable in size to other *Sebastes* including *Sebastes umbrosus* (honeycomb rockfish, 800.9 Mb), *Sebastes aleutinaus*

(rougeye rockfish, 889.5 Mb). Synteny analysis reveals 93–95% conservation between *S. umbrosus* and *S. fasciatus*, indicating a largely conserved genomic architecture across the genus despite ecological and phenotypic diversity. This high-quality assembly provides a valuable resource for studying the genetic basis of adaptation, speciation, and sex determination in Atlantic redfish and for comparative analyses across the genus. We also assembled the mitochondrial genome of *S. fasciatus* using long reads. The mitochondrial control region of *S. fasciatus* contained a 3.6 kb tandem repeat array (positions 16,455–20,099), consisting of a 277 bp repeat unit repeated ~13.2 times. Although mitochondrial genomes are generally conserved in gene content across vertebrates, the non-coding control region (D-loop) in teleosts often exhibits high structural variability (Lee et al., 1995). This variability is often driven by tandem repeat arrays and length polymorphisms that are difficult to resolve without long-read data (The Vertebrate Genomes Project Consortium et al., 2021). Repeat expansions and copy number variation in the control region has been documented in multiple *Sebastes* species; early work showed that *S. fasciatus*, *S. mentella*, and *S. marinus* (now known as *S. norvegicus*) share a tandem repeat array of ~275 bp with 9–17 copies per individual and frequent heteroplasmy, indicating dynamic repeat polymorphism in this locus (Bentzen, 1998). Analyses of other rockfishes show that tandem repetitive sequences in the control region contribute to differences in overall mitogenome length between species and between conspecifics (Sandel et al., 2018). Relatively long tandem repeats occur in the mitochondrial genomes of many teleosts (Ludwig et al., 2000; Mjelle et al., 2008) and vertebrates (Hoelzel, 1993). Repeats contribute substantially to mitochondrial genome size variation and, through their potential to form secondary structures, may influence replication dynamics and the generation of length polymorphism (Nardi et al., 2012). Our assembly confirms this pattern, suggesting that control region repeats are a major source of structural variation in *Sebastes* mitogenomes.

Integration of our new genome assembly with population genomic data enabled the identification of multiple regions and genes associated with sex determination (chr 3, chr 5, and chr 13) in *S. fasciatus*. Among these, we recovered the *anti-Müllerian hormone* (*amh*) gene on chr 5, a gene repeatedly recruited as a SDG in *Sebastes* lineages (Song et al., 2021; Sykes et al., 2023). We also identified *moto* and *hydroxysteroid (17-beta) dehydrogenase 1* (*hsd17b1*) on chr 13, also implicated in sex

determination in teleost fish (Kawasaki et al., 2015, 2025; Pan et al., 2022). A sex-biased region on chr 3 between 36.2 - 37.01 Mb containing multiple genes was also identified. The multi-chromosomal distribution of sex-associated regions (chr 3, chr 5, and chr 13) suggest multiple loci that likely act alongside *amh*, previously determined as the primary sex-determining gene in *Sebastes*, to modulate sexual development or sex determination in *S. fasciatus*.

The GWAS identified *amh* on chr 5 as strongly associated with sex in *S. fasciatus*. Anti-Müllerian hormone belongs to the conserved TGF- β gene family and often acts as a sex-determining locus in teleosts through duplication or allelic divergence (Zhu et al., 2025). In Northwest Pacific rockfishes, an *amh* duplication (*amhy*) is the sex-determining locus, with evidence that the duplicated copy originated from an autosomal *amh* and became sex-linked via translocation (Song et al., 2021). The copy of *amh* on chr 5 in *S. fasciatus* is sex-associated, yet phylogenetic analysis shows it clusters with canonical *amha*, rather than with *amhy* copies from other *Sebastes* species. This indicates that sex association in *amh* can evolve independently from the male-specific *amhy* documented in other rockfishes, providing a potential example of repeated lineage-specific recruitment of the same gene family for sex determination within *Sebastes*. The conservation of both *amh* paralogs across deeply diverged Atlantic (*S. fasciatus*) and Pacific (*S. umbrosus*) *Sebastes* species in our study supports the early origin of the *amh* duplication, thought to predate the divergence of *Hozuki* and *Sebastes* (Sykes et al., 2023). Although both sexes carry this copy, we identified a localised region with female-biased coverage and reduced heterozygosity, consistent with structural variation affecting sex association. Female-specific intronic insertions in related *Sebastes amh* paralogs have been shown to disrupt transcription and the TGF- β domain (Song et al., 2021), highlighting the potential functional consequences of structural variants. These observations extend previous findings in tilapia (Curzon et al., 2020), pufferfish (Duan et al., 2021), and pike (Pan et al., 2021b), where findings show structural variation can drive sex association even in the absence of sex-limited copies. Overall, our results underscore that teleosts can involve independent, lineage-specific modifications of canonical genes, and demonstrate the utility of chromosome-level assemblies for detecting subtle structural mechanisms underlying sex-associated loci.

On chr 13, the sex-association signal localised to a region where two genes with known roles in teleost reproduction overlap. Here, we identified a nested gene architecture wherein the *hsd17b1* gene resides within the *moto* locus. The GWAS signal was localised ~5 kb upstream of *hsd17b1*, and suggests sex-associated variation may affect both genes. *Moto* (ENSEMBL:ENSDARG00000090664) exhibits differential expression between male and female Zebrafish (*Danio rerio*) (Pan et al., 2022), and mutations in *moto* affect spermatogenesis (Kawasaki et al., 2015). *Hydroxysteroid (17-beta) dehydrogenase 1* catalyzes the interconversion between estrogens and androgens, a key step in ovarian differentiation, and acts as the sex-determining gene in several teleosts including greater amberjack (*Seriola dumerili*) (Koyama et al., 2019a), olive flounder (Zou et al., 2020) and sturgeon (*Acipenser baerii*) (Lasalle et al., 2024). The role of *hsd17b1* in *Sebastes* remains unknown, but it may function downstream of *amh*, potentially influencing steroid production and male gonad development, consistent with steroidogenic pathways linked to *amh* in European sea bass (Mascoli et al., 2025). This locus, along with the lineage-specific recruitment of *amh*, suggests a modular system in which multiple SDGs have been independently co-opted into the sex determination and differentiation pathway in Acadian redfish.

On chromosome 3, patterns of sex-specific variation were complex. Although mapping depth was sometimes male biased, females showed lower heterozygosity relative to males in regions surrounding candidate genes. Linkage disequilibrium in this region was elevated in males relative to females in this region, suggesting reduced effective recombination in males. Significant GWAS SNPs overlapped multiple genes with established or putative roles in sex differentiation and gonadal development. These include *sgcz*, associated with musculature development (Pan et al., 2021); *gpat2*, which shows male-biased expression in olive flounder and is involved in glycerolipid biosynthesis linked to spermatogenesis (Zhang et al., 2016); *igf2bp3*, a conserved RNA-binding protein linked to embryonic and germline development in zebrafish (Vong et al., 2021); and *mitfa*, a pigmentation gene that has been linked to sex expression and sexual dimorphism in teleost's (Kottler & Scharl, 2018). Additionally, we identified several members of the TGF- β signalling pathway, including *tgfb2* which plays a role in ovarian differentiation and development in Chinese tongue sole (*Cynoglossus semilaevis*) (Li et al., 2026). The co-localization of these

functionally relevant genes within a sex-differentiated genomic region suggests this chr 3 locus may play a modulatory or complementary role in the sex determination and differentiation network of *S. fasciatus*. Finally, we also observed a region on chr 1 showing a weaker, near-significant association with sex. Although this signal did not reach genome-wide significance, it may reflect population-specific variation in sex-linked loci or structural differences between populations and suggest that additional, less pronounced sex-associated regions may exist and warrant further investigation in future studies. Future studies using population genomic datasets in other Atlantic redfish, such as *S. mentella*, and across multiple populations of *S. fasciatus*, will help determine whether distributed sex-associated loci are a general feature of the genus or lineage-specific.

Recent genomic studies have revealed that even in species with well-characterised sex chromosomes, sex development often involves complex regulatory regions distributed across the genome, with many sex-associated regions potentially contributing to sex differentiation and initial sex determination. In addition to the primary *amh* locus on chr 5, we identified a putative modifier locus on chromosome 3, *hsd17b1* on chr 13 as sex-associated in *S. fasciatus*. This pattern aligns with observations in other teleosts, where secondary or minor sex-associated loci have been identified, such as in zebrafish (*Danio rerio*) (Luzio et al., 2015), coral trout (*Plectropomus leopardus*) (Gao et al., 2023), and salangid fishes (Li et al., 2020). While genuinely polygenic sex determination, in which many independently segregating loci collectively determine sex, is rare in vertebrates (Schartl & Lamatsch, 2023) our findings suggest that multiple loci may modulate aspects of sexual phenotype or differentiation. Functional studies will be required to confirm whether these secondary loci on chr 1 and chr 13 directly influence gonadal fate or act downstream in sexual differentiation pathways.

Applications

This chromosome-level reference genome for *Sebastes fasciatus* (Acadian redfish) represents a critical resource for both applied fisheries science and evolutionary biology. It will enable detailed studies of evolutionary relationships among *Sebastes* species and the genetic basis of traits underlying adaptation to diverse marine environments. With the genus *Sebastes* being highly speciose and divergence

in some redfish linked to factors such as depth gradients and habitat specialization (Bourret et al., 2025; Olivares-Zambrano et al., 2022; Johansen et al., 2025), our assembly offers a foundation for future research into the genomic mechanisms underlying speciation and ecological diversification in this group. The genome may also enable the development of sex identification tools. These are essential for managing sex-specific harvest strategies in long-lived, late-maturing species such as redfish, particularly where sex ratios influence spawning success, recruitment, and long-term population viability. Genetic sex-identification tools have been developed in a variety of fish species and have proven useful for exploited fish including short-snout icefish (*Neosalanx brevirostris*) (Yang et al., 2024), Greenland halibut (*Reinhardtius hippoglossoides*) (Estévez-Barcia et al., 2025) and various tuna species (Nielsen et al., 2024). Integrating sex-linked genomic information into fisheries management has the potential to refine stock assessments and guide sex-specific conservation measures, contributing to the long-term sustainability and resilience of exploited fish populations.

Conclusion

In the present study, we assembled the genome and mitogenome of the Acadian redfish (*S. fasciatus*) and leveraged whole-genome sequencing to genotype millions of SNPs across both male and female individuals to identify sex associated regions in the genome. Genome-wide association analysis identified multiple sex-associated loci on chromosomes 3, 5, and 13. In *S. fasciatus*, the identification of previously identified SGD *amh* on chr 5, along with multiple sex-associated loci on other chromosomes, suggests that a conserved primary signal may act alongside loci involved in hormone signalling and gonadal differentiation. These findings offer the first genomic perspective on sex chromosome evolution in redfish in the North Atlantic, and create new tools for redfish management.

Data Availability Statement

The Acadian redfish (*S. fasciatus*) chromosome-level genome is publicly available through GenBank, ENA and UCSC Genome Browser under accession number GCA_043250625 BioProject PRJNA1137994 and at https://www.genomeark.org/genomeark-all/Sebastes_fasciatus.html. SNP genotypes

527 and phenotypic sexes of the samples and code to perform all analysis are available
528 on Dryad (xxx).
529
530

Figures

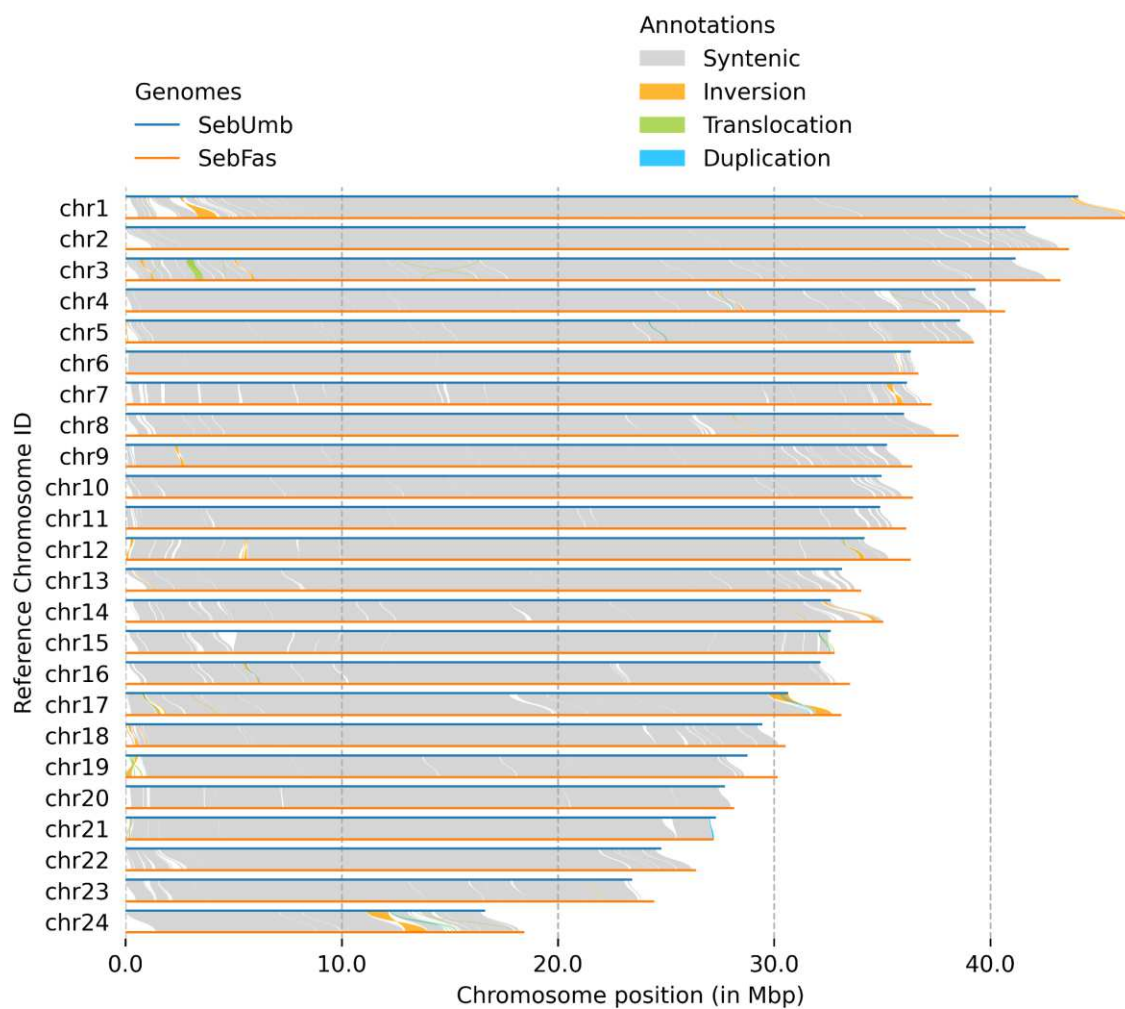


Figure 1 Structural variation between *Sebastes umbrosus* (blue chromosomes) and *Sebastes fasciatus* (orange chromosomes) genomes. White areas between chromosomes indicate unaligned regions.

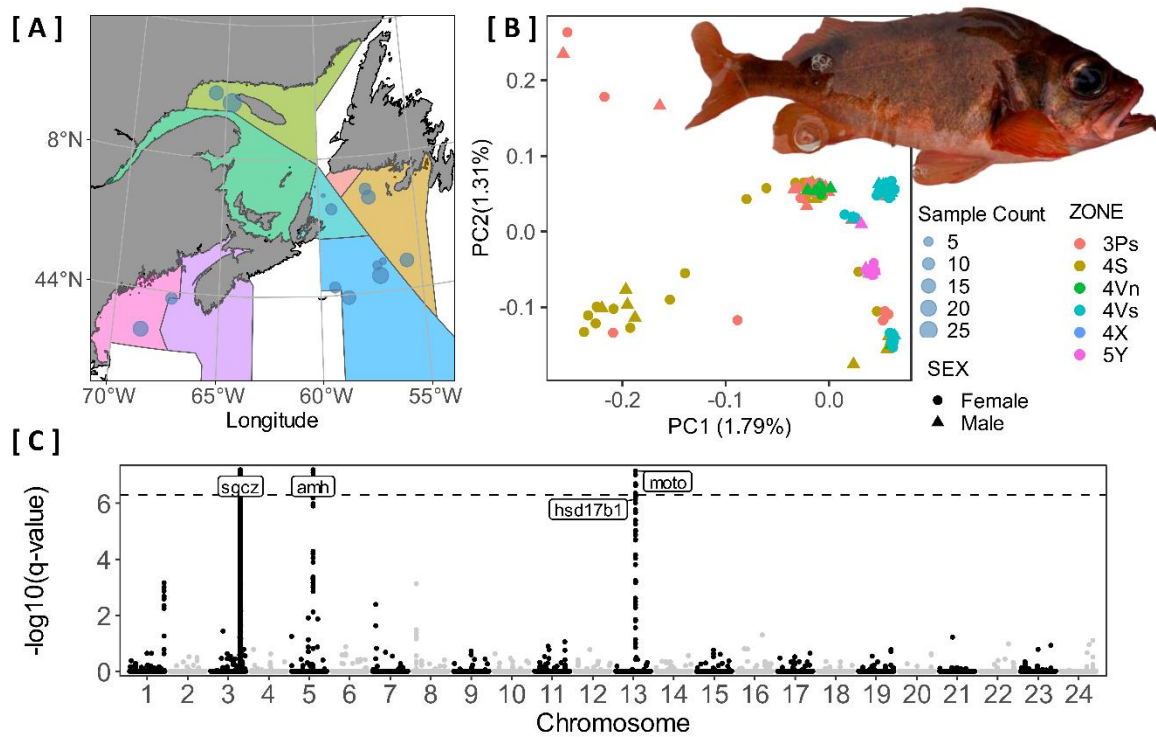


Figure 2. (A) Map of Northwest Atlantic Fisheries Organization (NAFO) zones showing the distribution of *S. fasciatus* sampling locations used for genome-wide association study (GWAS). (B) Principal component analysis (PCA) of genetic variation; male (triangle) and female (circle) *S. fasciatus* are indicated and coloured by Northwest Atlantic Fisheries Organisation (NAFO) zone (ZONE). PCA axes were used as covariates in the GWAS to control for population stratification. (C) Manhattan plot of GWAS for sex determination. The y-axis shows adjusted $-\log_{10}(q\text{-values})$ of genetic variants across chromosomes after Bonferroni correction. Selected SNPs are labelled with overlapping gene annotations. Dotted horizontal line indicates the genome-wide significance threshold ($p = 5 \times 10^{-7}$). Unplaced scaffolds not shown. Inset shows *S. fasciatus* collected from Bonne Bay, Newfoundland. Photo by Tyler Breech.

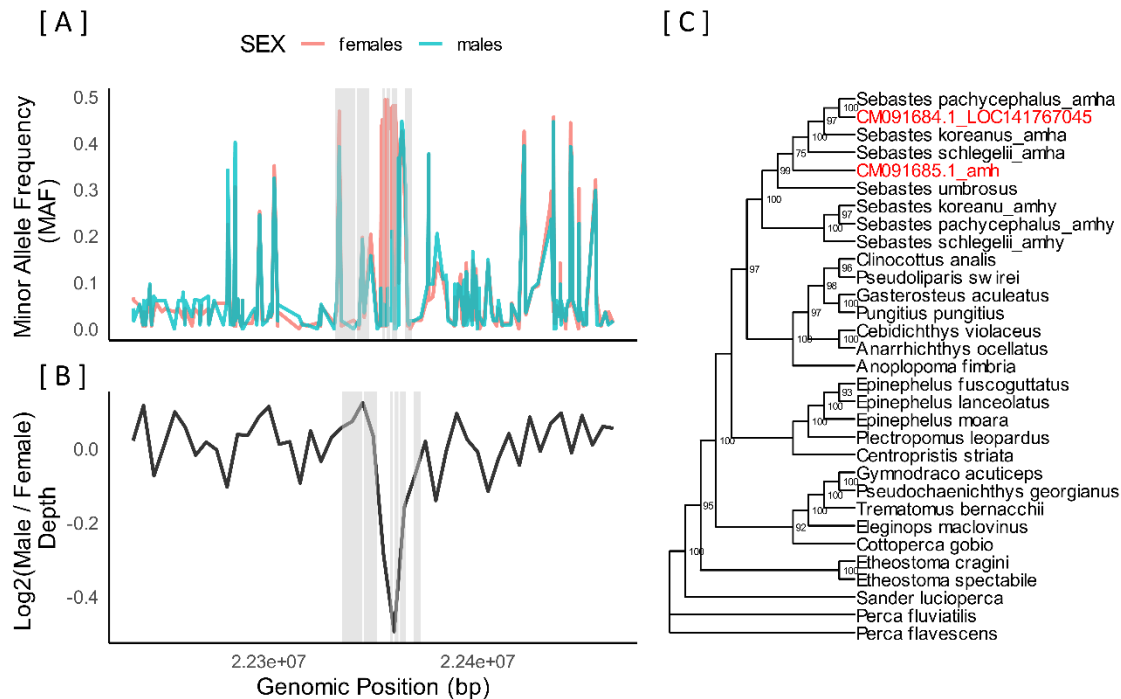


Figure 3. (A) Minor allele frequency (MAF) in male and female *Sebastes fasciatus* across the *amh* region on chr 5, *amh* exon highlighted in grey. Elevated female MAF suggests female-specific polymorphism at this locus. **(B)** Log₂ fold-change in sequencing depth (male/female) across *amh*; grey shading indicates exons. Reduced male depth and MAF may reflect male-specific deletions or copy number variation. **(C)** Maximum likelihood phylogeny of *amh* sequences from *S. fasciatus* chr 4 (CM091684.1) and chr 5 (CM091685.1, highlighted in red), including *amha* and *amhy* from Perciformes (OrthoDB; Kriventseva et al., 2019) and *Sebastes amh* homologous (Song et al., 2021).

Tables

Table 1. Summary of significant genome-wide association (GWAS) SNPs (+/- 250 bp) by chromosome, including top associated genes determined from genome annotations or BLAST homology for uncharacterized loci (homologs in rockfish indicated with *), putative roles in sex determination or reproductive processes.

chr #	Number of significant SNPs	Number of Genes	Annotation	Description
chr 3	250	7	<i>sgcz</i> , <i>igf2bp3</i> *, <i>tgfb2</i> *, <i>tgfb3</i> *, <i>mitfa</i> *	Genes on this chromosome show homology to genes involved in hormone signalling and germline development (Pan et al., 2021)
chr 5	9	1	<i>amh</i>	Master SDG in <i>Sebastes</i> and other teleosts (Song et al., 2021).
chr 13	6	2	<i>moto</i> , <i>hsd17b1</i>	The gene <i>moto</i> is linked to sex of Zebrafish (Kawasaki et al., 2025) and <i>hsd17b1</i> plays a role in the metabolism of sex hormones and is the SDG in various teleosts (Fan et al., 2021; Koyama et al., 2019; Lasalle et al., 2024).

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- 903

Supplementary Tables

Table S1. Consensus repeat unit identified in the mitochondrial genome of *S. fasciatus*. The repeat unit, detected using Tandem Repeats Finder, is 277 bp in length and occurs approximately 13 times in tandem between positions 16,455 and 20,099. The sequence shown represents the consensus pattern across all repeat copies.

Consensus pattern (277 bp)

TTTTTTTATGGTTTAAAAATGTTTTTATTTACATTATTATAAGTTTTTTTTTGATTAA
TCTGATAAAATTTAAAAAAGTGTTAGGCGGGGCCCAACAAAGCCCCGCCTGC
ATAAGAGATTGTCCTAGCTTAACTATTAATTCACCCATAAAAAACATAAGATATAT
ACCCATCAACCCCCGGGCCGCAAGTAGAGTATTAAATCACAAGGGGCATTAAAT
CCCCTGAGATGACTAACATTTACATACGCAAATTCAAAAAACAGGAAAGTCCC
GAGCCAT

913 Table S2. BLAST annotation of selected top GWAS SNPs on chr 3, highlighting gene
 914 hits with putative roles in sex determination or differentiation. Only a subset of
 915 candidate genes is shown, see Appendix B for full list.

Gene	Chr	Description	Link to function in Teleosts	References
<i>amh</i>	5	Key male sex-determining gene in many teleost fish. Involved in testis development.	<i>Amh</i> and its duplication <i>amhy</i> has been previously described as the master SDG gene in Sebastes.	(Song et al., 2021)
<i>hsd17b1</i>	13	Estrogen activator, androgen inactivator enzyme.	Sex determining gene in some teleosts such as golden pompano (<i>Trachinotus ovatus</i>) and Siberian sturgeon (<i>Acipenser baerii</i> , Brandt 1869).	(Fan et al., 2021; Lasalle et al., 2024)
<i>sgcz</i>	3	Transmembrane proteins stabilizing cell membranes	NA	(Crosbie et al., 1999)
<i>gpat2*</i>	3	Receptor, involved in germline stem cell maintenance and spermatogenesis	Male-biased expressed gene in olive flounder	(Zhang et al., 2016)
TGFB pathway genes including	3	TGF- β pathway is broadly implicated in sex differentiation	<i>Tgfb2</i> shows sex-biased in expression and potentially involved in ovarian	(Li et al., 2026)

<i>tgfb2,</i> <i>tgfbr3</i> and <i>tab1*</i>			differentiation/s exual development	
<i>igf2bp3</i>	3	Conserved RNA-binding protein	Linked to embryonic and germline development in zebrafish	(Vong et al., 2021)
<i>mitfa</i>	3	Key transcription factor in melanocyte and possibly pigment-linked sexual traits	Pigment genes can be linked to sex expression and sexual dimorphism in Telesost	(Kottler & Schartl, 2018)
<i>crebbpa</i>	3	Coactivator involved in transcription regulation, potentially affecting gonad-specific gene expression	CREB-related factors are among the regulators that modulate critical genes like <i>cyp19a1a</i> , which controls estrogen synthesis.	(Sharma et al., 2022)

917

918 Table S3. Summary of candidate sex-associated genes showing male/female differences in normalised read depth (mean log2 fold
919 change, standard deviation, and statistical significance) alongside population differentiation (FST) metrics.

Gene ID	Mean Log2FC	SD (Log2FC)	P (depth)	FDR (depth)	Mean FST	FST P-value	FST FDR
LOC141764948	-0.0203	0.0582	0.000518	0.00259	0.019	0	0
LOC141764955	-0.0168	0.0827	0.00224	0.0065	0.078	0	0
LOC141764957	-0.0716	0.163	0.0737	0.0921	0.187	0	0
LOC141764958	0.115	0.387	0.122	0.136	0.165	0	0
LOC141764959	0.809	0.182	0.0313	0.0446	0.054	0	0
LOC141764963	0.0358	0.0672	2.62E-07	2.62E-06	0.039	0	0
<i>amh</i>	-0.0771	0.204	0.57	0.57	0.186	0	0
<i>hsd17b1</i>	-0.16	0.225	0.00586	0.00977	0.113	0.001	0.001
<i>moto</i>	-0.0213	0.084	0.00281	0.0065	0.012	0	0
<i>sgcz</i>	-0.0152	0.187	0.00325	0.0065	0.077	0	0

920

921 Table S4. Mean linkage disequilibrium (r^2) across chromosomes with candidate sex-
 922 linked genes.

Chromosome	Sex	Mean r^2	Median r^2	SD r^2	Number of pairs
CM091683.1 (3)	Female	0.018	0.00387	0.0521	20020095
	Male	0.0311	0.00554	0.0976	18016758
CM091685.1 (5)	Female	0.0625	0.00491	0.161	29877
	Male	0.0785	0.00509	0.192	26536
CM091693.1(13)	Female	0.0198	0.00484	0.0657	775115
	Male	0.0284	0.00679	0.0845	7.03E+05

923

924

925 Table S5. Mapping of NCBI *AMH* ortholog sequences to the reference genome using
 926 Minimap2, showing the mapped chromosomes, as well as the start and end positions
 927 of each alignment.

Chr Number	Chr Name	Number of mapped reads (Total 29)	Total alignment bases	Start pos	End pos
4	CM091684.1	29	25782	4365694	4369496
5	CM091685.1	29	52525	22343308	22346111

928

929

Appendix A

Because *S. fasciatus* is often misidentified with the closely related *S. mentella*, we additionally included *S. mentella* individuals to validate species assignments and to ensure that inferred sex-associated signals were not driven by cryptic species structure. Genotypes of the full dataset of N = 274 fish was filtered using PLINK v1.9 (Chang et al., 2015), for missingness in both SNPs and fish, minor allele frequency and linkage disequilibrium (LD) (--geno 0.05, --mind 0.05, --maf 0.05 and -indep-pairwise 50 5 0.2). We performed principal component analysis (PCA) on this multispecies dataset to corroborate assigned species identification. The PCA revealed two clearly separated genetic clusters corresponding to *S. mentella* and *S. fasciatus* (Fig S1), with no intermediate individuals observed. Following genetic confirmation, only sampled genetically verified in this study as *S. fasciatus* were retained for focal analyses.

946 **Supplementary Figures**

947

948

949

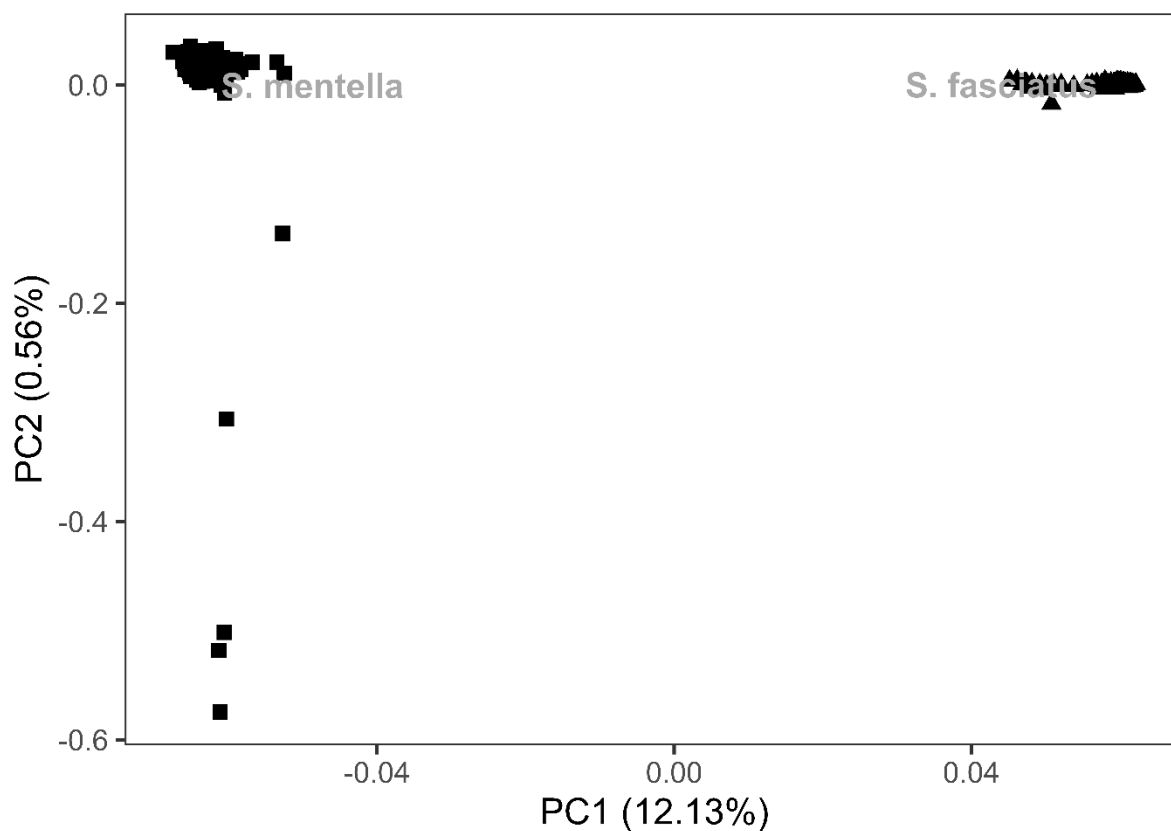
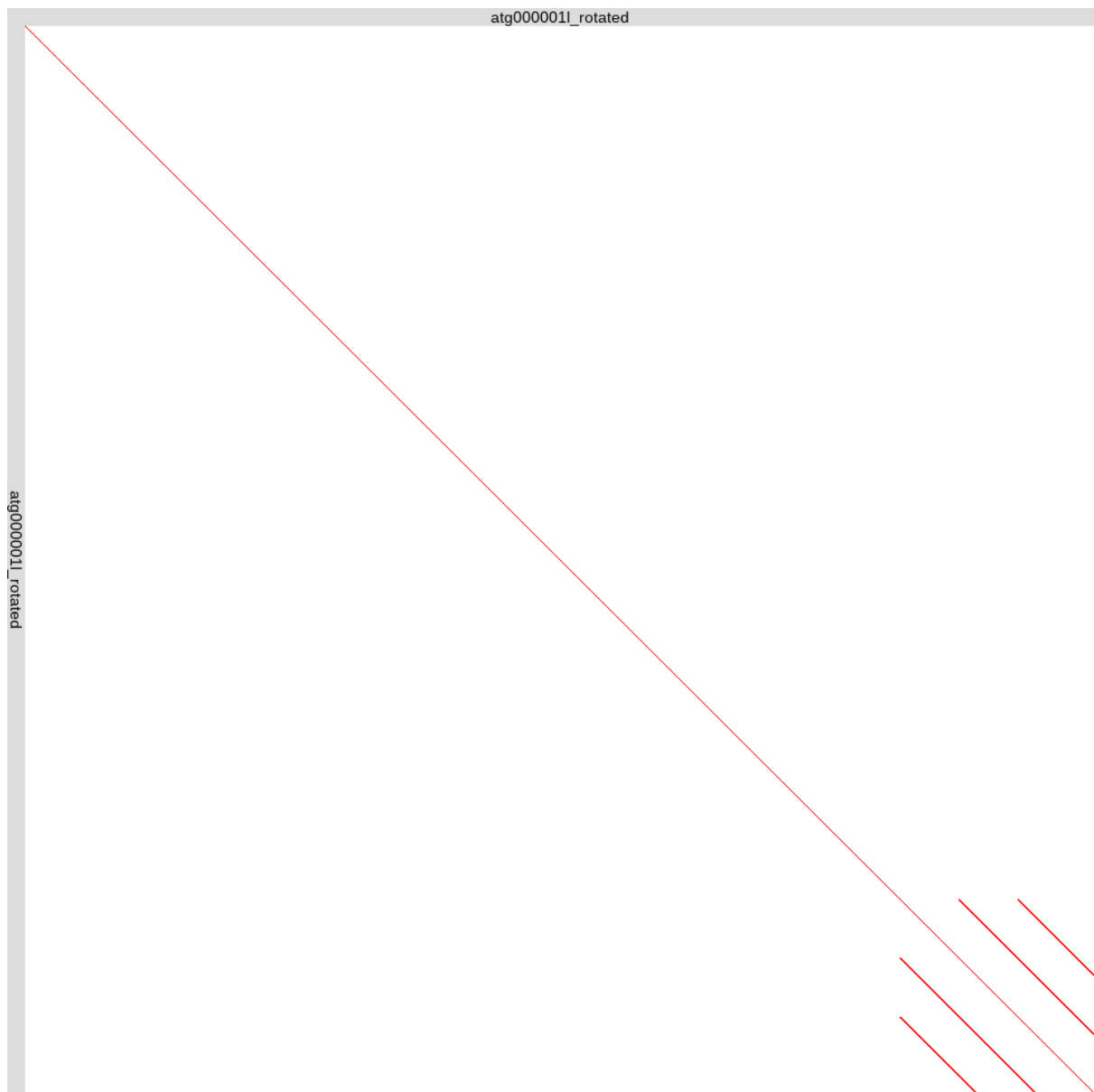


Fig S1. Principal component analysis (PCA) of genetic variation (PC1 vs. PC2) including *S. fasciatus* and *S. mentella* samples to identify potential hybrids. Shapes indicate species, and groups are labelled.



955

956 Fig S2. Dotplot of the self-alignment of the *S. fasciatus* mitochondrial genome. The
957 strong diagonal offset from the main axis indicates a tandem repeat region,
958 corresponding to a ~3.6 kb segment composed of approximately 13 copies of a 277
959 bp repeat, located between positions 16,455 and 20,099.

960

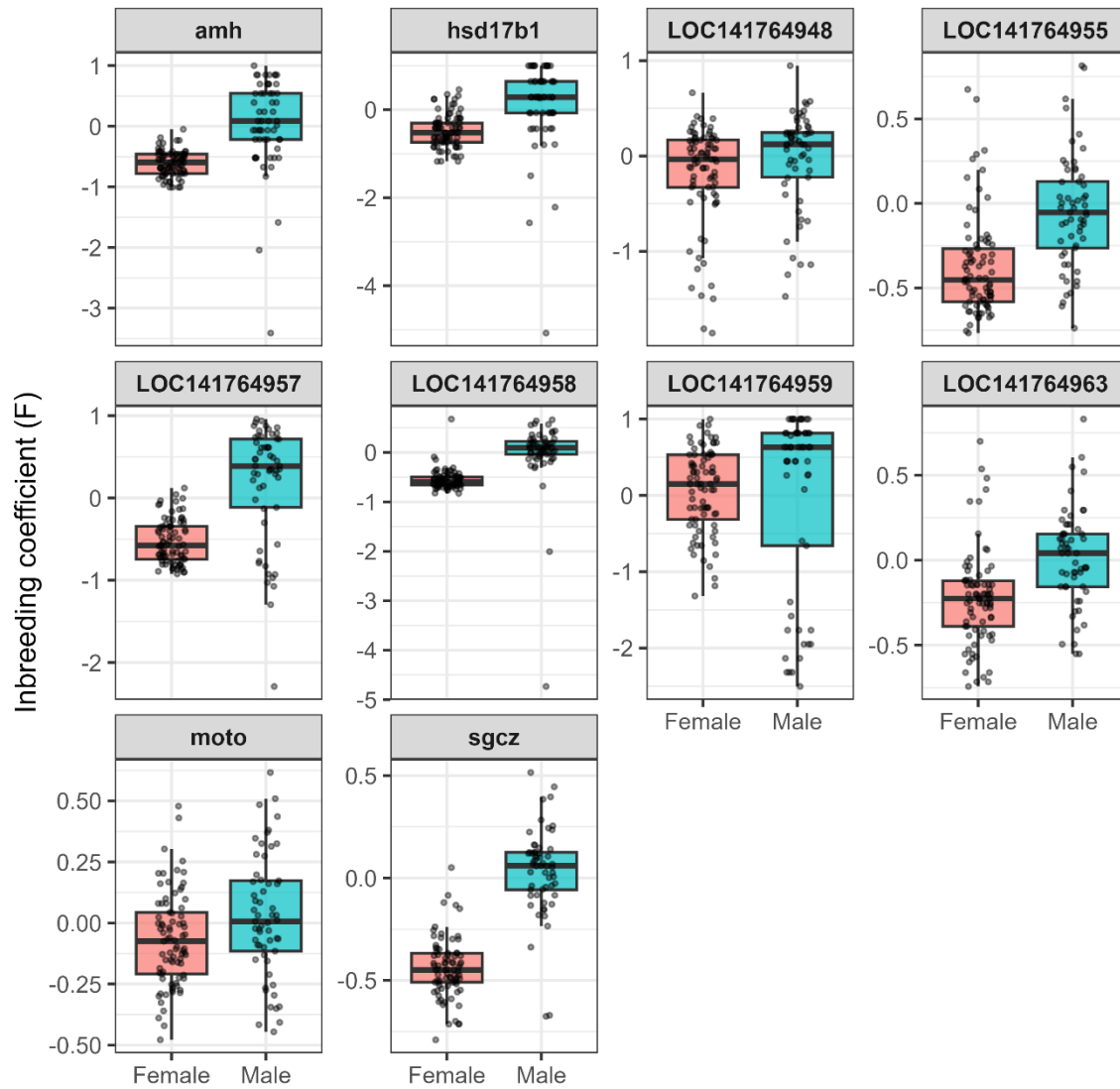


Fig S3. Inbreeding coefficient (F) at sex-associated genes in males and females.

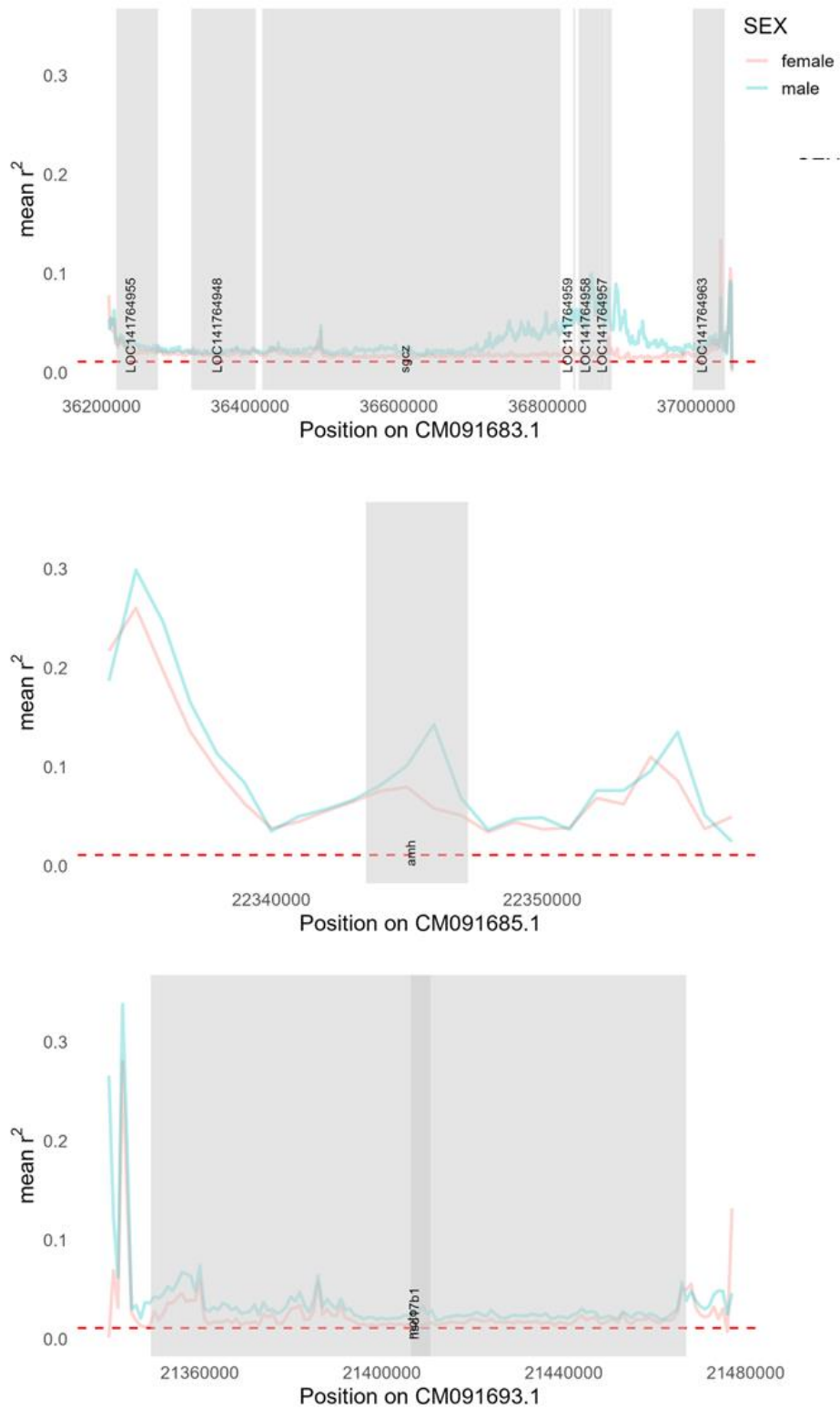


Fig S4. Linkage disequilibrium (LD) across chromosomes. Mean LD (r^2) of each site against all other sites in a 10kb window, separated by sex (male vs. female).

969 **Appendix B. Full list of BLAST hits for top**
970 **GWAS SNPs (± 250 bp) on chr 3.**

971 atad2b
972 camta1a
973 cdh13
974 cdh16
975 cds2
976 clcn1a
977 cntn3a.1
978 cntn5
979 cops4
980 crebbpa
981 cyfip2
982 dclk1a
983 ecm2
984 fam20cb
985 fat2
986 fbln2
987 fgf13a
988 furinb
989 gfra1b
990 gpat2
991 grin2ab

992	hecw2a
993	helz
994	igf2bp3
995	igsf5b
996	itfg1
997	itprid2
998	kcnc2
999	kcnma1a
1000	ksr2
1001	lama4
1002	ldb2a
1003	map4l
1004	matn4
1005	mcf2l2
1006	mier2
1007	mitfa
1008	mmp11a
1009	myom2b
1010	naif1
1011	ncf4
1012	nfascb
1013	nlgn1

1014	olfm2a
1015	otud7a
1016	oxr1a
1017	pam
1018	pard3bb
1019	patj
1020	pdcb
1021	pde4ca
1022	phactr2
1023	phf14
1024	pin1
1025	ppfibp2b
1026	prodh2
1027	rabep1
1028	rally
1029	sgpp2
1030	si:ch211-201h21.5
1031	si:dkey-112m2.1
1032	si:dkey-92j12.5
1033	sirt6
1034	slc24a3
1035	slc5a11

1036	slc66a3
1037	smyd1b
1038	snrka
1039	spred2a
1040	srp68
1041	srpx2
1042	stx8
1043	tab1
1044	tcaim
1045	tgfb2
1046	tgfbr3
1047	tmtc2b
1048	tusc3
1049	ube2d1b
1050	ube2ql1
1051	uckl1b
1052	unc5db
1053	wdr75
1054	wipf1b