fishes

MDPI

Article

In Silico Chromosome Mapping of the Male-Specific/Linked Loci in the Jade Perch (*Scortum barcoo*) Suggests Chromosome 19 as the Putative Y Sex Chromosome

Thitipong Panthum [1,2,3], Pish Wattanadilokchatkun [1], Kitipong Jaisamut [1], Worapong Singchat [1,3], Syed Farhan Ahmad [1,2,3], Narongrit Muangmai [1,4], Prateep Duengkae [1,3], Agostinho Antunes [5,6] and Kornsorn Srikulnath [1,2,3,7,8,*]

1 Animal Genomics and Bioresource Research Unit (AGB Research Unit), Faculty of Science, Kasetsart University, 50 Ngamwongwan, Bangkok 10900, Thailand; thitipong.pa@ku.th (T.P.); pish.wa@ku.th (P.W.); worapong.si@ku.th (W.S.); syedfarhan.a@ku.th (S.F.A.); ffisnrm@ku.ac.th (N.M.); fforptd@ku.ac.th (P.D.)
2 Interdisciplinary Graduate Program in Bioscience, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
3 Special Research Unit for Wildlife Genomics (SRUWG), Department of Forest Biology, Faculty of Forestry, Kasetsart University, 50 Ngamwongwan, Bangkok 10900, Thailand
4 Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, 50 Ngamwongwan, Bangkok 10900, Thailand
5 CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos, s/n, 4450-208 Porto, Portugal; aantunes@ciimar.up.pt
6 Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal
7 Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand
8 Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart University, Bangkok 10900, Thailand
* Correspondence: kornsorn.s@ku.ac.th; Tel.: +66-25625444

Abstract: Jade perch (*Scortum barcoo*) has an XX/XY sex-determination system (SDS); however, its sex chromosomes and sex-determining region remain unknown. The recent availability of the jade perch chromosome-level genomic data provides a valuable resource for pinpointing the location of functional genes and the whole genomic structure. In this study, we conducted. In silico chromosome mapping of male-specific/linked loci of jade perch and identified a potential 11.18 Mb male-linked region localized on chromosome 19 (SBA19). Repeat annotations of the male-linked region revealed an abundance of transposable elements, particularly Ty3/Gypsy and novel repeats. Sequence analysis of this region identified a remnant of *amh* gene, which is considered a potential candidate for SDS in many teleosts. A duplicate copy of *amh* remnant was located at SBA6. These duplicated *amh* copies were highly similar to those of XX/XY SDS in teleosts, in which one copy of *amh* was identified on the Y sex chromosome. Taken all together, we hypothesize SBA19 as the putative sex chromosome and the 11.18 Mb male-linked region to be a potential male-determining region.

Keywords: jade perch; in silico chromosome mapping; sex determination; sex chromosome; *amh*

Key Contribution: 1. The jade perch exhibits XX/XY sex determination system. 2. These loci were mapped to the recently published reference genome of jade perch. 3. We identified the jade perch chromosome 19 as a putative sex chromosome. 4. An 11.18 Mb male-linked region could be a potential male-determining region.

check for updates

Citation: Panthum, T.; Wattanadilokchatkun, P.; Jaisamut, K.; Singchat, W.; Ahmad, S.F.; Muangmai, N.; Duengkae, P.; Antunes, A.; Srikulnath, K. In Silico Chromosome Mapping of the Male-Specific/Linked Loci in the Jade Perch (*Scortum barcoo*) Suggests Chromosome 19 as the Putative Y Sex Chromosome. *Fishes* **2023**, *8*, 482. https://doi.org/10.3390/fishes8100482

Academic Editors: Yang Wang and Deshou Wang

Received: 29 July 2023
Revised: 21 September 2023
Accepted: 26 September 2023
Published: 27 September 2023

1. Introduction

Jade perch or Barcoo grunter (*Scortum barcoo*, [1]), Terapontidae) originally inhabited the Barcoo River in Lake Eyre Basin, Australia [2]. Recently, jade perch has been cultured by

several industrial growers for future commercial aquaculture production owing to its rapid growth, highly efficient feed conversion, and strong disease resistance in subtropical and tropical regions [3,4], although there is a risk of spread of this species into natural water sources as an alien species [5,6]. With regard to sexual dimorphism, mature jade perch females are larger than mature males and require lower nutrient levels for body size and weight growth during maturation [7,8]. Thus, the production of an all-female population has economic implications for the aquaculture market. Despite its high nutritional value for human consumption and its high market price, current jade perch farming faces seed supply limitations due to inconsistent and difficult production due to a lack of information about sex determination and sex-determining genes [9]. We previously investigated genome-wide single-nucleotide polymorphisms (SNPs) to identify genomic variants associated with sex-linked regions, which suggested that jade perch likely exhibits a male heterogametic XX/XY sex determination system (SDS) [9]. We identified 14 male-specific/linked loci without chromosomal locations. However, sex determining regions (SDRs), which are important to better understand sex determination mechanisms, remain unknown. Teleosts display a large diversity in SDSs, including genetic sex determination (GSD), environmental sex determination, and interactions between the two systems [10–14]. Different systems of GSD have been identified in a particular species with one system (XX/XY or ZZ/ZW), or both XX/XY and ZZ/ZW systems existing within the same genus, species, and even at the population level [15–21]. Because of this variability, several genes and SDRs might initiate SDS in a species, known as a polygenic sex determination (PSD) system [22]. In Siamese fighting fish (*Betta splendens*, [23]), a large number of male-specific/linked loci were identified on chromosome 9 (BSP9), but several additional male-specific/linked loci were identified on BSP7 and BSP19, indicating the existence of a PSD system [20]. A similar PSD system was observed in zebrafish (*Danio rerio* [24]) with chromosomes 4 (DRE4), DRE5, and DRE16 [25,26]. Thus, chromosomal localization of male-specific/linked loci coupled with potential sex chromosomal genomic analysis are required to better understand sex chromosome and SDR in jade perch, which could allow in the future its sex manipulation in aquaculture.

Recently, a chromosome-level genome assembly with 24 pairs of jade perch chromosomes (accession: GCA_023238725.1) facilitated the comparison of genomic structures among teleosts at the molecular level and revealed extensive linkage homology with frequent chromosomal rearrangements in teleosts [27]. Here, we used the reference baseline genome data of the jade perch to generate a chromosome map of male-specific/linked loci from our previous study using In silico mapping of the jade perch genome. We mapped genes known to be involved in sex developmental in teleosts to identify potential sex chromosomes, and performed gene ontology (GO) and repeat annotation to search for homologies between the jade perch and other vertebrates using comparative genomic analyses. Elucidation of the genetic architecture of SDSs is necessary to identify putative sex chromosomes.

2. Materials and Methods

2.1. In Silico Mapping of Male-Specific/Linked Loci to the Reference Genome

The male-specific/linked loci identified in our previous study, which included twenty jade perch individuals (ten males and ten females) [12] were aligned to the chromosome-level assembly of jade perch (accession: GCA_023238725.1) using NCBI-BLASTn using default parameters [27]. The loci that passed the 100% filtering criterion with all males were designated as male-specific loci, whereas those passing in 70%–90% of all males were considered male-linked loci. The output mapped file was filtered with the most significant hits (identity: >95%; alignment length: >65 bp) and then parsed using custom Geneious Prime 2021.1.1 (Biomatters, Auckland, New Zealand; https://www.geneious.com) (accessed on 20 November 2022) to generate a file format for visualization of a chromosome map. An ideogram was also generated from BLASTn alignments of experimentally validated male-specific/linked loci using Geneious Prime 2021.1.1. Sixty-six functional genes

of sex development and the sex determination process in teleosts were also mapped onto chromosome-level genome assembly of jade perch to identify the association of potential sex chromosomes (Sensitivity: Medium-Low sensitivity/fast, -Fine Tuning: Iterate up to 5 times, −51% similarity) (https://www.geneious.com, accessed on 20 November 2022) (Table S1).

2.2. Homology Searching

The male-specific/linked loci that met our criteria were globally BLAST searched against the National Center for Biotechnology Information (NCBI) database. We then investigated the homologies between the male-specific/linked loci and the available reference jade perch (accession: GCA_023238725.1) and other teleosts, including European seabass (*Dicentrarchus labrax* [28]), Asian seabass (*Lates calcarifer* [29]), Chinese seabass (*Lateolabrax maculatus* [30]), American black bass (*Micropterus salmoides* [31]), Nile tilapia (*Oreochromis niloticus* [32]), zebrafish (*Danio rerio* [24]), three-spined stickleback (*Gasterosteus aculeatus* [33]), medaka (*Oryzias latipes* (Temminck and Schlegel) [34]), Japanese pufferfish (*Takifugu rubripes* [35]), green-spotted pufferfish (*Tetraodon nigroviridis*, [36]), Southern platyfish (*Xiphophorus maculatus* [37]), Siam fighting fish (*Betta splendens*, [23]), green anole (*Anolis carolinensis*, [38]), Indian cobra (*Naja naja* [39]), and chicken (*Gallus gallus* [40]). Using the Geneious Prime 2021.1.1, we used all loci to search the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi: accessed on 20 November 2022) and RepBase version 19.11 (Genetic Information Research Institute, http://www.girinst.org/repbase/: accessed on 20 November 2022). RepBase is a specialized database with repeated or other significant sequences, and it only reports results with E-values < 0.005. Query coverage required >60% identity. This process was repeated three times to accommodate the inclusion of a new genome assembly version at various stages in the pipeline.

2.3. Functional Annotation and Gene Ontology of Male-Linked Regions

To understand the biological functions of male-linked regions, we designated the potential region where male-specific/linked loci were frequently mapped with a high density in the jade perch genome. BLASTn was performed with the candidate region against the reference annotation consisting of the gene dataset of jade perch [41]. The reference gene dataset of the three-spined stickleback, which contains a large functional gene dataset, was also retrieved from the Ensembl database using the Biomart package for comparison [42]. BLASTn results were generated as a tabular formatted output file, and only significant hits (identity > 95% and alignment length > 65 bp) were retained. All gene sequences from the reference dataset that corresponded to the region with significant hits were extracted and mapped against the proteome dataset (including total annotated proteins). The proteomic dataset was downloaded from UniProtKB [43]. UniProtKB is a collection of functional information on proteins with accurate, consistent, and rich annotations. Functional annotations and gene ontology enrichment analysis were conducted on the filtered gene hits using ShinyGO (0.76) implemented in R/Bioconductor packages. The analysis utilized the best matching species genome as a reference and standard settings, including a 0.05 FDR p value threshold [44]. The matching transcripts were further processed to detect associated GO terms related to biological processes (BPs), Molecular functions (MFs), and Cellular components (CCs). Three databases, UniProtKB, Gramene Proteins Database (GR protein), and Protein Data Bank (PDB), were also used to identify GO categories. The InterProScan (IPS) function in Blast2GO software (https://www.biobam.com/blast2go/) (accessed on 20 November 2022) was used to retrieve protein domains and motif information. Blast2GO also produced enzyme code (EC) numbers for transcripts with an e-value less than 10^{-5}. Enrichment of ontologies was performed using Fisher's exact test, with p-value of <0.05.

2.4. DNA Marker Validation

The male-linked region of jade perch was verified for the presence of *amh* gene using a PCR-based approach. Nucleotide sequences of *amh* gene were randomly selected to de-

velop PCR-based markers (Chromosome 6: Primer MLR1_F1: AGCCGACATCAACAACTGCC and MLR1_R1: ACACTCCTTCACCACCACATCC and Chromosome 19: Primer MLR2_F2: TGCTGCTCAACTCCTACATC and MLR2_R2: ACTCCTTCACCACCACATCC). The positive control used in the study was the *amh* gene (hAMHRII-F: AAAGGTACCATGCTAGGGTCTTTGGGGCTT and hAMHRII-R: AAATCTAGAACAGGAGAAAGGGTACAGG) as described by Kamiya et al. [45]. The applicability of the *amh* gene on male-linked region PCR assays was further tested and validated using jade perch specimens as mentioned above. PCR amplification was performed using 15 µL of 1X ThermoPol buffer containing 1.5 mM MgCl2, 0.2 mM dNTPs, 5.0 µM primers, 0.5 U Taq polymerase (Apsalagen Co., Ltd., Bangkok, Thailand), and 25 ng genomic DNA. The PCR protocol was as follows: initial denaturation at 98 °C for 5 min; 35 cycles of 98 °C for 20 s, 58 °C for 45 s, and 68 °C for 2 min; and a final extension at 68 °C for 5 min. The PCR products were visualized by electrophoresis in 1% agarose gel to examine the presence or absence as a consequence of allele-specific DNA markers. For the PCR-based sequencing, the PCR products were purified using FavorPrep GEL/PCR Purification Mini Kit (Favorgen Biotech Corp., Ping-Tung, Taiwan). Nucleotide sequences of the DNA fragments were determined by the DNA sequencing service of First Base Laboratories Sdn Bhd (Seri Kembangan, Selangor, Malaysia). Multiple sequence alignment was used to search the nucleotide sequences of the PCR product with the candidate SNP sequence by log-expectation (MUSCLE) (http://www.ebi.ac.uk/Tools/msa/muscle) (accessed on 20 November 2022) using default parameters to confirm the identity of the amplified DNA fragments.

2.5. Determination of Repetitive Elements in Male-Linked Regions

A *de novo* TE (transposable element) library was generated from male-linked regions of jade perch chromosome-level genome where most male-specific/linked loci were frequently located using the Extensive de novo TE Annotator (EDTA), with species parameter set to "others" [46]. The inbuilt RepeatModeler (Institute for Systems Biology, Washington, DC, USA) [47] was used to identify the remaining TEs and other repetitive elements that might have been overlooked by the EDTA algorithm (sensitive 1). TE and other repetitive element identifications were performed using RepeatMasker (RM; version 1.332) as the compatible version of the standard NCBI blastn program (NCBI/RMBLAST) (version 2.6.0+) search engine. Genomic regions spanning high levels of male-linked regions were compared to those of the TE annotation library. The TEs and repetitive elements annotated within the male-linked regions were then extracted using bedtools [48]. All sequences of unclassified elements were subsequently re-mapped onto the whole-genome sequence using D-GENIES (Copyright© INRA 2016) [49] to examine their localization. Multiple hits (short reads of $\geq$100 bp and $\geq$1 kb genomic reads at an error rate of $\sim$15%) of unclassified elements with high abundance were pooled for each genomic segment where they were examined for tandem arrangement within the segment using dot matrix analysis of the nucleotide sequences using MAFFT version 7 (Copyright © 2013 Kazutaka Katoh). The following parameters were used: (1) scoring matrix of 200PAM/k = 2 and (2) gap opening penalty of 1.53. The plots and alignments were executed with a threshold score of 39 (E = 8.4 $\times$ 10 $-$ 11). (http://mafft.cbrc.jp/alignment/server/, accessed 20 November 2022).

2.6. Characterization of Microsatellites in Jade Perch Genome

Microsatellites in the jade perch genome were identified using Krait version 1.3.3 [50] with default scanning parameters for perfect, imperfect, and compound microsatellites to examine the association between high density of microsatellite distribution and potential SDR [51,52]. The imperfect microsatellite selection criteria were as follows: (*i*) minimum sequence length and sequence repeat number were set to 8 bp and 3 times, (*ii*) maximum consecutive edits (including substitutions and indels) were specified as 3 bp, and (*iii*) the penalty cost was set to 1 for mismatch and 2 for indels (gaps), and the minimum required score to identify imperfect microsatellites was set to 10. For compound microsatellites, the maximum distance allowed between adjacent microsatellites was 10 bp. To facilitate

comparisons among different repeat types, relative density (RD) and relative abundance (RA) were used as parameters for microsatellite analysis. RA indicates the number of microsatellites per Mb of the sequence analyzed, and RD is the length (in bp) of microsatellites per Mb of the sequence analyzed. Krait v1.3.3 was applied to estimate the GC content and chromosome sequence size of the jade perch (accession: GCA_023238725.1).

2.7. Comparative Genomics between Jade Perch and Other Vertebrates

Multiple karyotypes in male-linked regions of jade perch were compared to identify linkage homology with whole-genome sequences of other teleosts, namely, European seabass, Asian seabass, Chinese seabass, American black bass, Nile tilapia, zebrafish, three-spined stickleback, medaka, Japanese pufferfish, green-spotted pufferfish, Southern platyfish, and Siam fighting fish, while green anole, Indian cobra, and chicken were used as the outgroup by simulation of the Ensembl database and Genomicus and linkage homology portals [27,52,53]. Genes are represented by small black dots, leading to diagonal lines inside chromosomes, with the order being similar to the reference, and line breaks representing rearrangements. They were overlaid with the color of the chromosome of the reference genome where the homologous gene was located, thus pointing to large-scale homologous chromosome segments.

3. Results

3.1. Chromosomal Localization of Sex-Specific/Linked Loci in Jade Perch

Contig and scaffold N50 values of this chromosome-level genome assembly were 4.5 Mb and 28.6 Mb, respectively, in Geneious prime version 2023.1. A map of twenty-four chromosomes was then constructed with a total length of 642.9 Mb. We sequenced 28,033 loci and compared a number of loci by filtering with a gradually changing set of criteria for sex-specific/linked loci.In silico chromosome mapping of all male-specific/linked loci for chromosome-level assembly (accession: GCA _023238725.1) revealed that male-specific loci (locus id:100021722) were localized to chromosome 6 (position: 28000000–29333791) of jade perch (SBA6), whereas 8 (locus id:100026100, 100026101, 100011760, 100017263, 100033965, 100033966, 100026920, and 100026527) of 13 male-linked loci were mapped onto SBA19 (position: 64700–11252534), and the remaining five loci onto SBA8, SBA12, SBA14, SBA16, and SBA23 (Figure 1 and Table 1). The locus ID was identified based on our previous study [9]. Hereafter, we designated male-linked regions, which were identified on SBA6 within 1.3 Mb (28000000–29333791 bp) as "male-linked region1: MLR1" and SBA19 within 11.18 Mb (locus 64700–11252534 bp, accession: GCA_023238725.1) as "MLR2" as a consequence of high frequency of In silico chromosome mapping by male-specific/linked loci.

3.2. Homology of Putative Male-Specific/Linked Loci and Gene Mapping to Reference Genome

A total of 14 male-specific/linked loci of the jade perch shared sequence homology with the genomes of European seabass, Asian seabass, Chinese seabass, American black bass, Nile tilapia, zebrafish, three-spined stickleback, medaka, Japanese puferfsh, green-spotted pufferfish, Southern platyfish, and Siam fighting fish, as well as green anole, Indian cobra, and chicken (Table 2). Based on global BLAST analyses using the NCBI database, one male-specific locus on SBA6 and eight male-linked loci on SBA19 were homologous with putative functional genes (Table 2). Four male-specific/linked loci of jade perch (SBA19) showed partial homology with *sdk2b* (sidekick cell adhesion molecule 2b), which is related to cell-cell communication, cell junction organization, and SDK interactions, *nrg2b* (pro-neuregulin-2, membrane-bound isoform X3), which enables signaling receptor binding activity, animal organ development, and intracellular signal transduction, *mybpc2b* (myosin binding protein Cb), associated with muscle tissue development, including adaxial cell musculature system), and *kazna* (Kazrin-A), which is related to the formation of the cornified envelope. No loci were included in the sex developmental pathway. Moreover, In silico chromosome mapping of sixty-six functional genes related to sex determination and

sex development in teleosts was mapped onto jade perch chromosomes. However, only *amh* (anti-Mullerian hormone receptor) was successfully localized to both SBA6 (outside MLR1) and SBA19 (inside MLR2) (Figure 1).

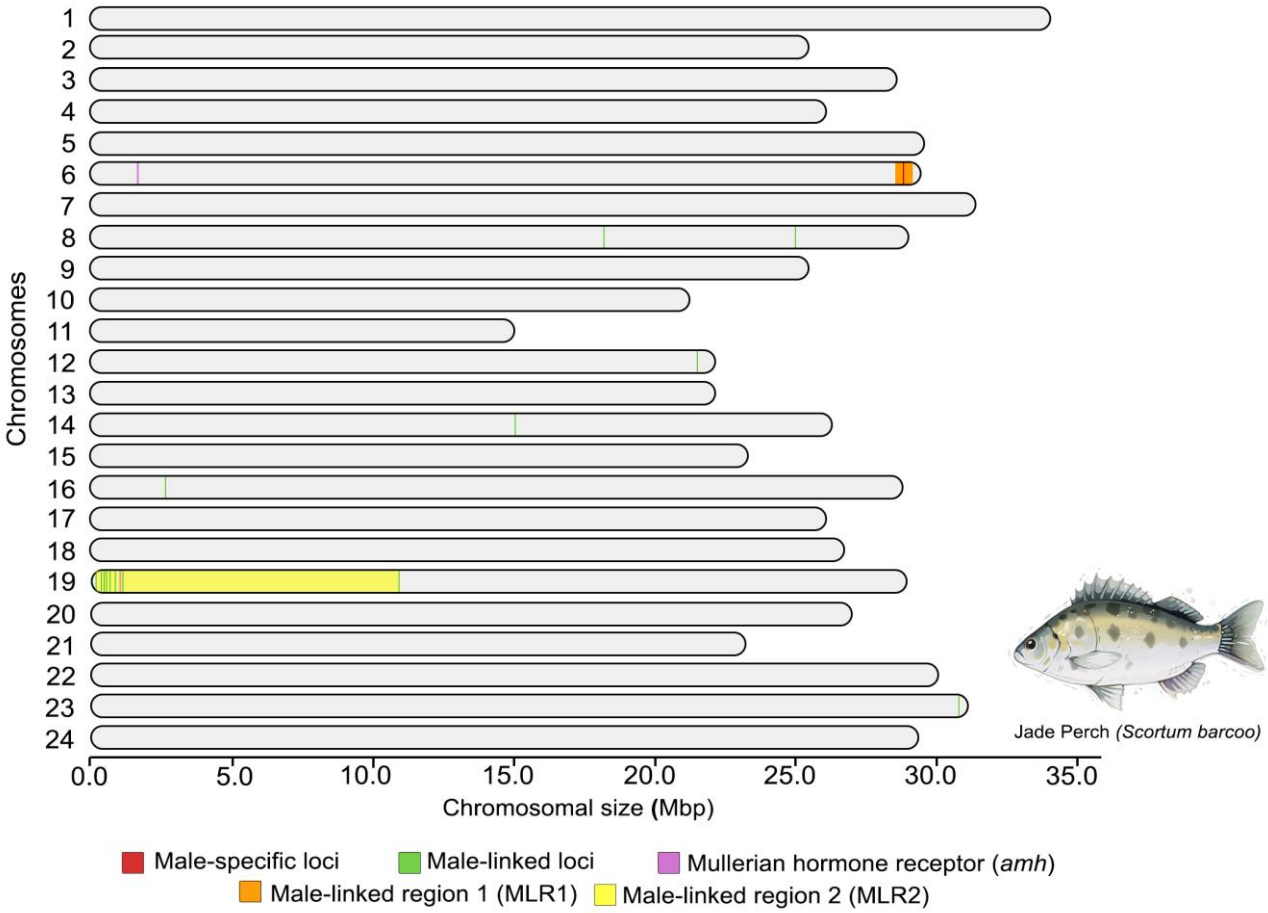

Figure 1. In silico chromosome mapping showing the distribution of male-specific/linked loci and Anti-Müllerian hormone (*amh*) in jade perch (*Scortum barcoo* [1]).

3.3. Functional Classification and Enrichment Analysis of Male-Linked Region

Gene ontology (GO) enrichment analyses were performed using MLRs derived from SBA6 and SBA19 to classify putative functions in the comparisons. The GO-enriched categories of MLR1 on SBA6 in sex-specific regions of biological processes (BPs) were mainly involved in anterograde synaptic vesicle transport, synaptic vesicle cytoskeletal transport, and vesicle cargo loading. Molecular functions (MFs) were mostly related with cyclin binding, CAMP response element binding protein binding, and RNA polymerase II CTD heptapeptide repeat kinase activity. Cellular components (CCs) were largely connected with AP-3 adaptor complex, axon cytoplasm, and endoplasmic reticulum exit site. The GO-enriched categories of MLR2 on SBA19 in BPs revealed involvement mostly in primary sex determination, negative regulation of female gonad development, response to gonadotropin-releasing hormone and positive regulation of male gonad development. MFs were mainly related with retinoic acid-responsive element binding, and ligand-activated transcription factor activity. CCs were primarily involved in the plasma membrane, intercalated disc and RNA polymerase II transcription regulator complex (Figure 2).

Table 1. In silico chromosome mapping of all male-specific/linked loci in chromosome-level assembly genome of jade perch (*Scortum barcoo* (SBA) [1]) (accession: GCA_023238725.1).

Locus ID	SBA6	SBA8	SBA12	SBA14	SBA16	SBA19	SBA23	% Identity	% Query Cover
100011760						397248–397316		98.4	98.1
100017263						554368–554436		98.1	98.4
100018397		25088227–25088295						92.0	92.0
100021722	28916249–28916318							64.3	64.3
100024764					2710511–2710579			98.6	98.6
100026092		18269334–18269402						92.0	92.0
100026100				15105253–15105322		64730–64799		95.7 (SBA14)/ 98.1 (SBA19)	97.1 (SBA14)/ 98.4 (SBA19)
100026101				15105253–15105322		64730–64799		95.7 (SBA14)/ 98.1 (SBA19)	97.1 (SBA14)/ 98.4 (SBA19)
100026527		426602–426670				426602–426670		98.1	98.4
100026834							30995241–30995309	100.0	100
100026920						10882240–10882308		98.1	98.4
100027734			21559150–21559218					98.6	98.6
100033965						509282–509350		98.1	98.4
100033966						509282–509350		98.1	98.4

Table 2. Chromosomal locations of other vertebrates on homologous sequences of jade perch were homologous with several vertebrates. The bracket symbols indicate a gene.

Locus ID	GAC	DLA	LCA	ONI	DRE	OLA	TRU	TNI	XMA	BSP	ACA	LMA	MSA	NNA	GGA
100021722	20	7	15	11	16	16	7	8	3	16	scaffold	-	scaffold	micro chromosome	23
100026100	5 (*nrg2b*)	13	8	2	14	10 (*nrg2b*)	14	1	23	10	4	-	-	3	13
100026101	15	3	19	19	17	22	2	10	19	22	1	5	-	1	3
100011760	7	21	scaffold	10	21	14	15	-	11	14	scaffold	5	-	micro chromosome	4
100017263	21	-	-	-	-	-	-	-	-	-	-	7	-	-	-
100018397	18	8	7	15	-	24	16	14	15	24	-	2	-	1	3
100026920	12 (*kazna*)	15	6	20	8	7	3	9 (*kazna*)	1	7	-	1	-	micro chromosome	21
100027734	5 (*mybpc2b*)	12	23	8	24 (*mybpc2b*)	19	1	2	10	19	6	8	-	Z	31
100026092	8	18	17	4	22	4	20	-	9	4	-	6	scaffold	1	28
100024764	5 (*sdk2b*)	12	23	-	12	19	-	2	10	19	-	-	scaffold	2	18
100033965	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100033966	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100026527	2	19	2	1	7	3	13	5	4	3	scaffold	9	-	3	10
100026834	9	20	5	-	22	-	-	-	-	1	1	3	scaffold	1	3

European seabass (*Dicentrarchus labrax*: DLA) [28], Asian seabass (*Lates calcarifer*: LCA) [29], Chinese seabass (*Lateolabrax maculatus*: LMA) [30], American black bass (*Micropterus salmoides*: MSA) [31], Nile tilapia (*Oreochromis niloticus*: ONI) [32], zebrafish (*Danio rerio*: DRE) [24], threespine stickleback (*Gasterosteus aculeatus*: GAC) [33], medaka (*Oryzias latipes*: OLA) [34], Japanese puferfsh (*Takifugu rubripes*: TRU) [35], green-spotted pufferfish (*Tetraodon nigroviridis*: TNI) [36], Southern platyfish (*Xiphophorus maculatus*: XMA) [37], Siam fighting fish (*Betta splendens*: BSP) [23], green anole (*Anolis carolinensis*: ACA) [38], Indian cobra (*Naja naja*: NNA) [39], and chicken (*Gallus gallus*: GGA) [40].

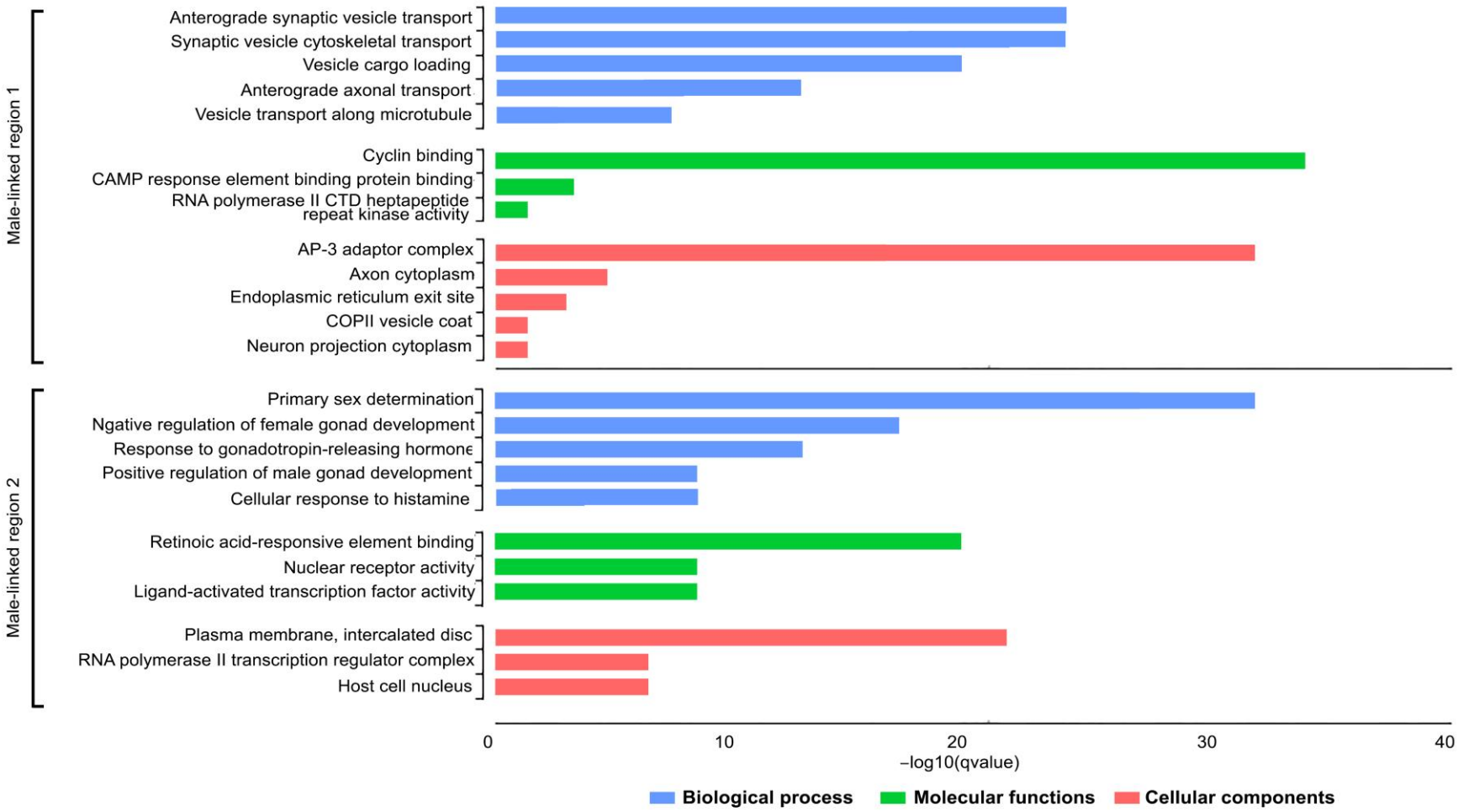

Figure 2. Gene ontology (GO) functional classification of male-linked region, instead of Anti-Müllerian hormone (*amh*), of jade perch (*Scortum barcoo* [1]) using Blast2GO. Histograms of the frequency of transcripts annotated to specific GO categories; biological process, molecular functions, and cellular components are represented by blue, green, and pink bars, respectively.

3.4. Annotation of Repetitive Elements in the Specific Region

MLRs were then screened for TE abundance, and masked areas were recorded using the extensive de novo TE Annotator (EDTA) and RepeatMasker (RM; version 1.332). Most repeats were identified as 42.86% DNA transposons (helitrons, hAT, and terminal inverted repeats (TIR)), 19.04% long terminal repeat (LTR), 19.04% target site duplication (TSD), 9.52% LTR retrotransposons (Ty3/Gypsy and Ty1/Copia), and 9.52% unclassified repeat region. Ty3/Gypsy accounted for 22.22% of the MLR1. Moreover, most repeats were identified as 45.90% DNA transposons (helitrons, hAT, and terminal inverted repeats (TIR)), 18.03% long terminal repeat (LTR), 18.03% target site duplication (TSD), 9.02% LTR retrotransposons (Ty3/Gypsy and Ty1/Copia), and 9.02% unclassified repeat region. Ty3/Gypsy accounted for 52.38% of the MLR2 (Table 3). To investigate unclassified repetitive elements extensively, all unclassified sequences were characterized using D-GENIES. Two novel repetitive element types were identified from MLR1 and 11 novel repetitive element types from the MLR2 were identified. The two novel types from MLR1 were specifically located on SBA6, whereas the 11 novel repetitive element types from MLR2 were predominantly located on SBA19 and a few copy numbers on SBA1, SBA3, SBA14, and SBA24 (Figure S1a,b). A dot matrix analysis showed that only one (CM041549.1:1993427–1996037) of the 13 novel repetitive element types was tentatively composed of tandem repetitive sequences (Figure S2). Moreover, another novel repetitive element type (CM041549.1:7466839–7478173) with low copy numbers was found in the genomes of medaka (Figure S3).

3.5. Validation of Male-Linked Region with DNA Markers

The *amh* gene in the male-linked region of jade perch was validated using a PCR-based method. However, the same DNA band pattern was observed in both male and female jade perch (Figure S4).

3.6. Microsatellite Distribution in Jade Perch Genome

The total length of the jade perch genome contained 812,134 microsatellite loci, consisting of 384,683 (24%), 1,163,799 (74%) and 30,269 (2%) perfect, imperfect and compound microsatellite loci, respectively (Table S2, Figure S5). The RA of perfect microsatellites was 584.88 loci/Mb, and the RD was 12,802.75 bp/Mb, while the RA of imperfect and compound microsatellites were 1769.48 loci/Mb and 46.02 loci/Mb, respectively. The RD of imperfect and compound microsatellites were 57,999.96 bp/Mb and 3160.19 bp/Mb, respectively (Table S2). The most frequent motif of perfect and imperfect microsatellites was $(AC)_n$, whereas the most frequent motif of compound microsatellites was $(CA)_n$–$(CA)_n$. The average length of perfect microsatellite loci (total perfect microsatellite length/total perfect microsatellite counts) was approximately 21.89 bp, that of imperfect microsatellite loci (total imperfect microsatellite length/total imperfect microsatellite counts) was approximately 32.78 bp, and that of compound microsatellite loci (total compound microsatellite length/total compound microsatellite counts) was approximately 68.67 bp (Table S3). The highest proportion of microsatellite repeat motif distribution was observed in SBA19 with perfect, imperfect, and compound microsatellites (19,226 loci, 426,694 bp; 53,724 loci, 1,817,765 bp; and 1,709 loci, 127,655 bp, respectively), and the lowest proportion was observed in SBA11 with perfect, imperfect, and compound microsatellites (9349.00, 196,225, 28,125, 881,647, and 619 loci, 40,968 bp, respectively) (Figure 3 and Table S3).

Table 3. Repeat searches for male-linked region on chromosome 6 and 19 of jade perch (*Scortum barcoo* [1]).

Repeat Class	Chromosome 6		Male-Linked Region 1		Chromosome 19		Male-Linked Region 2	
	Fragments	**Percentage (%)**	**Fragments**	**Percentage (%)**	**Fragments**	**Percentage (%)**	**Fragments**	**Percentage (%)**
LTR retrotransposon	9.00	4.07	2.00	0.90	21.00	7.98	11.00	4.18
Ty3/Gypsy	9.00	4.00	2.00	0.90	20.00	7.60	10.00	3.80
Ty1/Copia	-	-	0.00		1.00	0.38	1.00	0.38
Long terminal repeat (LTR)	18.00	8.14	4.00	1.81	42.00	15.97	22.00	8.37
DNA transposon	167.00	75.57	9.00	4.07	137.00	52.09	56.00	21.29
Helitron	5.00	2.26	0.00	0.00	8.00	3.04	3.00	1.14
hAT	12.00	5.43	2.00	0.90	4.00	1.52	2.00	0.76
Terminal inverted repeat (TIR)								
-CACTA	99.00	44.80	3.00	1.36	78.00	29.66	31.00	11.79
-Tc1/mariner	8.00	3.62	2.00	0.90	5.00	1.90	1.00	0.38
-PIF/Harbinger	4.00	1.81	0.00	0.00	9.00	3.42	4.00	1.52
-Mutator	39.00	17.65	2.00	0.90	33.00	12.55	15.00	5.70
Target site duplication	18.00	8.14	4.00	1.81	42.00	15.97	22.00	8.37
Repeat region (unclassified)	9.00	4.07	2.00	0.90	21.00	7.98	11.00	4.18

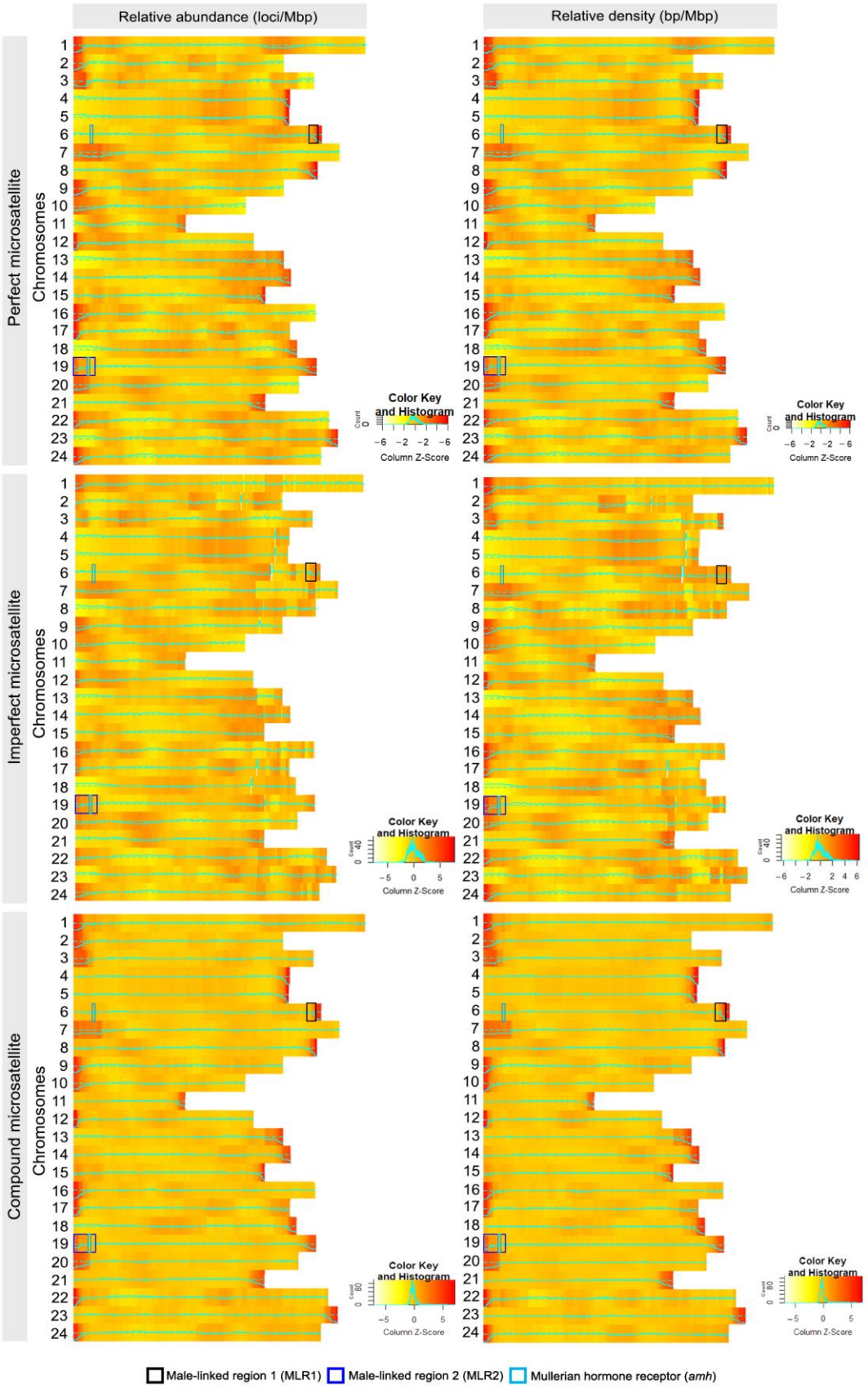

Figure 3. Microsatellite abundance and density in 24 chromosomes of jade perch (*Scortum barcoo* [1]).

3.7. Linkage Homology of Jade Perch and Other Vertebrates

Male-linked regions of the jade perch were compared with whole-genome sequences of other vertebrates to identify linkage homology among species. MLR1 on SBA6 was homologous to three-spined stickleback chromosome 20, European seabass chromosome 7, Asian seabass chromosome 15, scaffold (NW_024040152.1) of American black bass, Nile tilapia chromosome 11, zebrafish chromosome 16, medaka chromosome 16, Japanese puferfish chromosome 7, green-spotted pufferfish chromosome 8, Southern platyfish chromosome 3, Siam fighting fish chromosome 16, scaffold (GL343480.1) of green anole, Indian cobra microchromosomes, and chicken chromosome 23. Moreover, the MLR2 on SBA19 was homologous to three-spined stickleback chromosome 5, European seabass chromosome 12, Asian seabass chromosome 23, Chinese seabass chromosome 8, Nile tilapia chromosome 8, zebrafish chromosome 24, medaka chromosome 19, Japanese puferfish chromosome 1, green-spotted pufferfish chromosome 2, Southern platyfish chromosome 10, Siam fighting fish chromosome 19, green anole chromosome 6, Indian cobra chromosome Z, and chicken chromosome 31.

4. Discussion

The number of genome sequencing projects in teleosts has increased in recent years, with the understanding of sex determination impacting many teleosts [54–57], and being key to better comprehend the mechanisms that shape the diversity of their sex chromosomes and sex determination. Our recent studies have revealed that the jade perch has an XX/XY SDS [9]. Here, we combined male-specific/linked loci information and genome database analysis to identify sex chromosomes and a putative SDR in jade perch. Moreover, we predicted a potential male-specific *amh* gene in the male-linked regions of jade perch.

4.1. Sign of Sex Chromosome in Jade Perch

A male-specific locus derived from our previous genome-wide SNP analysis [9] was determined in SBA6 and the sequence data within 1.3 Mb (position: 28000000–29333791 bp) as male-linked region 1 (MLR1). Moreover, eight male-linked loci were located on SBA19 within 11.18 Mb (position: 64700–11252534 bp), which were assigned to MLR2. Further identification at the physical and molecular levels of sex chromosomes might be characterized by the occurrence of repetitive sequences, linkage homology of sex chromosomes in other species, different base mutations such as substitution or indels between a homologous pair in different sexes, and identification of potential sex determining genes [16,20]. Repetitive elements can drive chromosomal rearrangements that further induce sex chromosome differentiation and heterochromatin propagation [58–63]. Most microsatellite repeat motifs are also distributed in SBA19. Our analysis revealed a high density of microsatellites on SBA19 near MLR2. Similar distribution of microsatellite repeats, identified by molecular cytogenetics, genome informatics, and chromosomics on sex chromosomes, has been reported in many vertebrates, suggesting the possible role of microsatellite repeats in sex chromosome differentiation and evolution [20,59,61,64–66]. A high microsatellite distribution was identified around the SDR and its neighboring regions, which might expand the segment of differentiation on the sex chromosomes [51,67]. Eleven novel repetitive element types were observed in MLR2, of which one type was randomly distributed in other chromosomes with low copy numbers. In the female Siamese cobra (*Naja kaouthia* [68]), PBI-DdeI satellite DNA is distributed in many snake lineages, with high amplification found in the W chromosome of females [62]. Similar findings have been observed for the SDR of medaka and Siamese fighting fish [20,69]. Apart from site-specific repetitive elements, a certain portion of MLR2 also contained an abundance of TEs, compared with the entire SBA19. Especially, multiple copy number of Ty3/Gypsy were also detected, while the Ty3/Gypsy copies are frequently distributed on sex chromosomes in snakeskin gourami (*Trichopodus pectoralis*, [23], North African catfish (*Clarias gariepinus*, [70]), bighead catfish (*Clarias macrocephalus*, [71]) [18,19,72–75]. By contrast, there was no evidence of a large repetitive element distribution on MLR1. It should be considered that frequent amplification of the repeats

has a structural role in heterochromatinization and promotes further sex chromosome differentiation [58,62,76,77]. These results collectively suggest an association of the sex chromosome phenomenon with SBA19. By contrast, the male-specific locus in MLR1 on SBA6 might be an accidental occurrence in the sample population study [9].

MLR2 is partially homologous to the Indian cobra W sex chromosome, which is highly conserved in the snake lineage [64,65,69–82]. This result was similar to the comparative linkage homology of sex-specific/linked loci or the segments of several teleosts, such as bighead catfish, snakeskin gourami, Siamese fighting fish, and other amniotes [19,20,74]. This suggests an expansion of the super sex chromosome from ancestral amniotes to teleosts [20,61,64,65,81,83]. Convergent evolution is assumed to be the driving force causing the divergence of sex chromosomes among phylogenetically distant or closely related taxa [20,61,64,65,81,83,84]. SBA19 may be enriched with genes contributing to the sex determination cascade or genes with sexually antagonistic effects. The involvement of the genomic region with primary sex determination in biological processes was revealed by the GO-enriched categories of MLR2 on SBA19. One interesting issue is that remnants of *amh* gene were observed in MLR2 on SBA19, but not in MLR1 on SBA6. Thus, whether this gene relates to the SDR of the jade perch remains unclear.

4.2. The Existence of Amh Gene on SBA19 Might Associate the Sex Determining Region of Jade Perch

One functional gene, *amh* gene, which is related to SDS in teleosts, was tentatively predicted in two locations of the jade perch genome: outside the MLR1 on SBA6 and in the MLR2 of SBA19 by informatic analysis. The anti-Müllerian hormone, which is a product of the *amh* gene, is responsible for inhibiting the development of the Müllerian ducts in the female reproductive tract during embryogenesis, thus promoting male development in amniotes [85–87]. However, *amh* plays an important role in the proliferation and differentiation of germ cells during male gonadal development in teleosts [87]. Interestingly, duplications of *amh* genes have been identified in many teleosts, such as Patagonian pejerrey (*Odontesthes hatcheri* [88]), blackspotted (*Gasterosteus wheatlandi* [89]), Japan Sea (*Gasterosteus nipponicus* [90]), three-spined stickleback, and brook stickleback (*Culaea inconstans* [91]), all of which exhibit the XX/XY system [92,93]. The *amh* gene might be a male-specific paralog and has been validated as a male sex-determining gene in Nile tilapia, Cobaltcap silverside (*Hypoatherina tsurugae* [94]), northern pike [95], and rockfish [96–100]. One paralog was identified on the autosome, known as *amha*, which may play a role in oocyte maturation, whereas another paralog was located on the Y sex chromosome, known as *amhy*, which has gene expression levels related to suppressing ovary development and stimulating testis differentiation in males [93]. Both *amhy* and *amha* share high homology in nucleotide and amino acid sequences, and they contain seven exons in teleosts [92,98]. It is likely that we identified a remnant of the exon in the putative structure of *amh* gene in both MLR1 and MLR2. In Argentinian silverside (*Odontesthes bonariensis* [101]), Patagonian pejerrey (*O. hatcheri* [88]) *amhy*, a specific insertion of approximately 100–600 bp was observed within the third or fourth introns, which are the most evident structural differences between the two paralogs [92,98]. However, we were unable to directly confirm the structural difference of *amh* in the jade perch with the available sequencing data (GCA_023238725.1_ASM2323872v1). All three approaches rely on reliable sequencing data, and reads of repetitive regions cannot be aligned. Therefore, it is not possible to infer whether these structures were important in the recruitment of *amh* duplicates for sex determination in jade perch, or the possibility of pseudogenes [27,102]. The most likely explanation is that we observed a remnant of *amh* gene in MLR2, which collectively suggests that the putative sex chromosome of jade perch may be chromosome 19. However, no reports have confirmed the causative roles of this gene and its polymorphisms in sex determination in jade perch. The 11.18 Mb MLR2 would be an important target for future functional investigations.

Consideration of linkage homology in the MLR2 on SBA19, which surrounds the remnant of *amh* gene, suggests that MLR2 might be the male-determining region of jade

perch. The highest linkage disequilibrium in SBA19 may reflect recombination suppression between the X and Y chromosomes in MLR2, and elevated sequence divergence between male and female individuals. Sex chromosomes represent a pair of originally homologous chromosomes that diverged by restricting recombination during evolution because of the origin of a sex-determining locus on one member of the pair [83]. MLR2 was evolutionarily conserved with regions in European seabass chromosome 12, Chinese seabass chromosome 8, and three-spined stickleback chromosome 5, which are the bass lineage; however, duplicated *amh* genes were not identified in these bass species [92,93,98]. By contrast, the duplicated *amh* gene might be identified in the jade perch genome, whereas the duplicated *amh* gene was detected in medaka, which is not in close evolutionary lineage with the jade perch [27]. This suggests that duplication of *amh* gene probably occurred after divergence between the jade perch and bass lineages [100,103]. Multiple putative TEs were detected around the remnant of *amh* gene in the MLR2 on SBA19, rather than twice the average number detected across the entire SBA19. Similarly, Y-linked sex-determining gene of yellow catfish was found to have Y-specific accumulation of TEs in a recent study, indicating their potential role in sex determining region [104,105]. Meanwhile, TEs may be involved in sex determination and act as regulatory elements by transcriptional rewiring of the SD regulatory network during the evolution of novel master SD genes [106]. These results raise the hypothesis of *amh* duplication and insertion of the two paralogs in the jade perch genome. TEs around the paralog of *amh* on SBA19 or SBA6 may trigger duplication and insertion. The process of gene duplication insertion has been reported for a sex-determination gene with *amh* gene in many teleosts, such as medaka, Patagonian pejerrey, northern pike, yellow perch, and threespine stickleback [92,99,106–109]. This level of convergence implies that duplication is an important process in the recruitment of *amh* gene as the master sex determination gene on the Y sex chromosome, but not on the X sex chromosome [100]. DNA markers were developed to confirm the presence and distinguish the *amh* gene in MLR1 and MLR2 between male and female specimens. However, no difference was observed in the DNA band pattern between males and females. It is hypothesized that there might be allelic differences, such as base substitutions, in the sex-determining gene such as *amh* gene on the Y chromosome, as observed in Korean rockfish (*Sebastes schlegelii*, [96]) and Japanese flounder (*Paralichthys olivaceus* [109]) [92,96,100,110]. Both homologous chromosomes (the X and Y sex chromosomes) need, therefore, to be sequenced fully along the length of the *amh* gene. Positional cloning and copy number analysis of *amh* gene on X and Y sex chromosomes (SBA19) are required for further elucidation. Construction of a high-quality genome assembly based on both short- and long-read sequencing data from male and female jade perch and molecular cloning are needed to resolve the structure of the *amh* gene on SBA19 and SBA6 and to identify their functional roles. Therefore, knock-in and knockout transgenic experiments are necessary to fully uncover these mechanisms.

5. Conclusions

Based on male-linked SNP loci derived from jade perch in our previous study [9], the jade perch exhibited XX/XY SDS. In this study, we mapped these loci to the recently published reference genome of jade perch. We identified the jade perch chromosome 19 as a putative sex chromosome. Repetitive element identification, GO enrichment analyses, and segmental linkage homology of sex chromosomes in vertebrates also support the potential of SBA19 as a sex chromosome. The remnant of the duplicated *amh* gene was determined in the jade perch genome, in which one copy of the remnant *amh* was located in the male-linked region of SBA19. This is similar to many teleosts with the XX/XY system, including duplicated *amh*, and the Y sex chromosome also contains *amh*.

Positional cloning and high-quality next-generation genome sequencing are required to unravel the complex genetic sex determination system of this species. Preliminary genomic prediction results indicate that the ability to manage genetic improvement in jade perch increases the economic potential of this species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8100482/s1, Figure S1: The 2 novel repetitive element types from the MLR1 were located on chromosome 6 of jade perch (*Scortum barcoo* (McCulloch and Waite, 1917)). (b) The 11 novel repetitive element types from the MLR2 were predominantly located on chromosome 19 of jade perch and a few copy numbers on chromosome 1, 3. 14 and 24; Figure S2: Dot matrix analysis showing that only 1 (ID: CM041549.1:1993427–1996037) of the 13 novel repetitive element types was tentatively composed of tandem-arrayed repetitive sequences.; Figure S3: The novel repetitive element (ID: CM041549.1:7466839–7478173) from the MLR2 on chromosome 19 of jade perch (*Scortum barcoo* McCulloch and Waite, 1917) was distributed in the medaka genome with low copy numbers.; Figure S4: Agarose gel electrophoresis of PCR products in the validation test in male and female individuals of jade perch (*Scortum barcoo* (McCulloch and Waite, 1917)). (a) Primer MLR1 (136 bp) (b) Primer MLR2 (138 bp) and (c) The positive control (141 bp).; Figure S5: Pie diagram showing the portion of each microsatellite type in the jade perch (*Scortum barcoo* (McCulloch and Waite, 1917)).; Table S1: List of gonadal genes implicated in sex determination and differentiation of teleosts.; Table S2: Summary of perfect, imperfect, and compound microsatellites detected in the genome of jade perch (*Scortum barcoo* (McCulloch and Waite, 1917)).; Table S3: Details of perfect microsatellites in each chromosome of jade perch (*Scortum barcoo* (McCulloch and Waite, 1917)). References [111,112] are cited in the Supplementary Materials.

Author Contributions: Conceptualization, T.P. and K.S.; methodology, T.P., P.W., S.F.A. and K.S.; software, T.P. and P.W.; validation, T.P. and K.S.; formal analysis, T.P., P.W., S.F.A. and K.S.; investigation, T.P. and K.S.; data curation, T.P. and K.S.; writing—original draft preparation, T.P. and K.S.; writing—review and editing, T.P., P.W., K.J., W.S., S.F.A., N.M., P.D., A.A. and K.S.; visualization, T.P. and K.S.; supervision, K.S.; project administration, K.S.; funding acquisition, K.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the High-Quality Research Graduate Development Cooperation Project between Kasetsart University and the National Science and Technology Development Agency (NSTDA) (Grant no. 6417400247) awarded to T.P. and K.S.; the Thailand Science Research and Innovation through the Kasetsart University Reinventing University Program 2021 (Grant no. 3/2564) awarded to T.P., and K.S.; the National Research Council of Thailand (NRCT/2565); Fundamental Fund: Kasetsart University Research and Development Institute (KURDI) (FF(S-KU)17.66) awarded to S.F.A., and K.S.; and the Kasetsart University Research and Development Institute (KURDI) awarded funds to N.M. and K.S. No funding source was involved in the study design, collection, analysis, and interpretation of the data, writing of the report, or decision to submit the article for publication. International SciKU Branding (ISB), Faculty of Science, Kasetsart University awarded funding to W.S. and K.S.

Institutional Review Board Statement: All animal care and experimental procedures were approved by the Animal Experiment Committee of Kasetsart University, Thailand (approval no. ACKU64-SCI-010), and conducted in accordance with the Regulations on Animal Experiments at Kasetsart University.

Informed Consent Statement: Not applicable.

Data Availability Statement: The full dataset and metadata of this publication are available from the Dryad Digital Repository. Dataset, https://datadryad.org/stash/share/P6fDtif_Ig3ZLeYfYCc98BUadnpzHBZXkG8wYoNL-w8 (https://doi.org/10.5061/dryad.hhmgqnkhn.) (accessed on 20 November 2022).

Acknowledgments: We thank Sompon Muangthong and Bundit Ruttanasatian (Ayutthaya Farm, Ayutthaya, Thailand) for supplying the jade-perch specimens. This work was financially supported by the Office of the Ministry of Higher Education, Science, Research and Innovation, and Thailand Science Research and Innovation through the Kasetsart University Reinventing University Program 2021. We thank the Center for Agricultural Biotechnology (CAB) at the Kasetsart University Kamphaeng Saen Campus for support with server analysis services. We also thank the Faculty of Science, Kasetsart University, Thailand, for providing the supporting research facilities (6501.0901.1).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jade Perch (*Scortum barcoo*). Available online: https://www.gbif.org/pt/species/2374545 (accessed on 20 December 2022).
2. Ihwan, M.; Syahnon, M.; Fakhrul, I.; Marina, H.; Ambak, M. New report on trichodiniasis (Protozoa: Ciliophora: Peritrichida) in Jade perch; *Scortum barcoo* from Peninsular Malaysia. *J. Fish. Aquat. Sci.* **2016**, *11*, 437–443. [CrossRef]
3. Renaudeau, D.; Collin, A.; Yahav, S.; Basilio, V.D.; Gourdine, J.L.; Collier, R.J. Adaptation to tropical climate and research strategies to alleviate heat stress in livestock production. *Adv. Anim. Vet. Sci.* **2010**, *1*, 378–379. [CrossRef]
4. Araujo, G.S.; Silva, J.W.A.D.; Cotas, J.; Pereira, L. Fish farming techniques: Current situation and trends. *J. Mar. Sci. Eng.* **2022**, *10*, 1598. [CrossRef]
5. De Almeida, M.J.S. *The Paradox of Alien Invasive Species: Negative and Positive Effects on Biodiversity and Ecosystem Services*; Faculty of Sciences, University of Porto: Porto, Portugal, 2013.
6. Essl, F.; Lenzner, B.; Bacher, S.; Bailey, S.; Capinha, C.; Daehler, C.; Dullinger, S.; Genovesi, P.; Hui, C.; Hulme, P.E.; et al. Drivers of future alien species impacts: An expert-based assessment. *Glob. Change Biol.* **2020**, *26*, 4880–4893. [CrossRef] [PubMed]
7. Snelson, F.F. Social and Environmental Control of Life History Traits in Poeciliid Fishes. In *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*; Meffe, G.K., Snelson, F.F., Jr., Eds.; Prentice Hall: Englewood Cliffs, NJ, USA, 1989; pp. 149–162.
8. Ponce de Leon, J.L.; Rodríguez, R.; Leon, G. Life-history patterns of *Cuban poeciliid* fishes (Teleostei: Cyprinodontiformes). *Zoo Biol.* **2013**, *32*, 251–256. [CrossRef] [PubMed]
9. Suntronpong, A.; Panthum, T.; Laopichienpong, N.; Nguyen, D.H.; Kraichak, E.; Singchat, W.; Ariyaraphong, N.; Ahmad, S.F.; Muangmai, N.; Duengkae, P.; et al. Implications of genome-wide single nucleotide polymorphisms in jade perch (*Scortum barcoo*) reveals the putative XX/XY sex-determination system, facilitating a new chapter of sex control in aquaculture. *Aquaculture* **2022**, *48*, 737587. [CrossRef]
10. Devlin, R.H.; Nagahama, Y. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* **2022**, *208*, 191–364. [CrossRef]
11. Mank, J.E.; Promislow, D.E.L.; Avise, J.C. Evolution of alternative sex determining mechanisms in teleost fishes. *Biol. J. Linn. Soc.* **2006**, *87*, 83–93. [CrossRef]
12. Baroiller, J.F.; D'Cotta, H.; Bezault, E.; Wessels, S.; Hoerstgen-Schwark, G. Tilapia sex determination: Where temperature and genetics meet. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2009**, *153*, 30–38. [CrossRef]
13. Wang, H.P.; Piferrer, F.; Chen, S.L.; Shen, Z.G. *Sex Control in Aquaculture*; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2008.
14. Wang, D.; Pan, Z.; Wang, G.; Ye, B.; Wang, Q.; Zuo, Z.; Zou, J.; Xie, S. Gonadal transcriptome analysis and sequence characterization of sex-related genes in *Cranoglanis bouderius*. *Int. J. Mol. Sci.* **2022**, *23*, 15840. [CrossRef]
15. Zhou, Q. A swimy locus on Y chromosome of the platyfish (*Xiphophorus maculatus*) is derived from a novel DNA transposon Zisupton. *Gene* **2012**, *503*, 254–259. [CrossRef] [PubMed]
16. Martínez, P.; Vinas, A.M.; Sanchez, L.; Díaz, N.; Ribas, L.; Piferrer, F. Genetic architecture of sex determination in fish: Applications to sex ratio control in aquaculture. *Front. Genet.* **2014**, *5*, 340. [CrossRef] [PubMed]
17. Wilson Sayres, M.A. Genetic diversity on the sex chromosomes. *Genome Biol. Evol.* **2018**, *10*, 1064–1078. [CrossRef] [PubMed]
18. Nguyen, D.H.M.; Panthum, T.; Ponjarat, J.; Laopichienpong, N.; Kraichak, E.; Singchat, W.; Ahmad, S.F.; Muangmai, N.; Peyachoknagul, S.; Na-Nakorn, U.; et al. An investigation of ZZ/ZW and XX/XY sex determination systems in North African catfish (*Clarias gariepinus*, Burchell, 1822). *Front. Genet.* **2021**, *11*, 562856. [CrossRef] [PubMed]
19. Nguyen, D.H.M.; Ponjarat, J.; Laopichienpong, N.; Panthum, T.; Singchat, W.; Ahmad, S.F.; Kraichak, E.; Muangmai, N.; Duengkae, P.; Peyachoknagul, S.; et al. Genome-wide SNP analysis of hybrid clariid fish reflects the existence of polygenic sex-determination in the lineage. *Front. Genet.* **2022**, *13*, 789573. [CrossRef] [PubMed]
20. Panthum, T.; Jaisamut, K.; Singchat, W.; Ahmad, S.F.; Kongkaew, L.; Wongloet, W.; Dokkaew, S.; Kraichak, E.; Muangmai, N.; Duengkae, P.; et al. Something fishy about Siamese fighting fish (*Betta splendens*) sex: Polygenic sex determination or a newly emerged sex-determining region? *Cells* **2022**, *11*, 1764. [CrossRef]
21. Fedder, J. Sex determination and male differentiation in Southern swordtail fishes: Evaluation from an evolutionary perspective. *Fishes* **2023**, *8*, 407. [CrossRef]
22. Roberts, N.B.; Juntti, S.A.; Coyle, K.P.; Dumont, B.L.; Stanley, M.K.; Ryan, A.Q.; Roberts, R.B. Polygenic sex determination in the cichlid fish *Astatotilapia burtoni*. *BMC Genom.* **2016**, *17*, 835. [CrossRef]
23. Regan, C.T. The Asiatic fishes of the family Anabantidae. *Proc. Zool. Soc. Lond.* **1910**, *B1909*, 767–787.
24. Zebrafish (Danio rerio). Available online: https://www.gbif.org/species/9797255 (accessed on 20 December 2022).
25. Nagabhushana, A.; Mishra, R.K. Finding clues to the riddle of sex determination in zebrafish. *J. Biosci.* **2016**, *41*, 145–155. [CrossRef]
26. Aharon, D.; Marlow, F.L. Sexual determination in zebrafish. *Cell. Mol. Life Sci.* **2022**, *79*, 8. [CrossRef] [PubMed]
27. Lu, Y.; Li, R.; Xia, L.; Cheng, J.; Xia, H.; Zhan, Q.; Yu, D.; You, X.; Gu, R.; Xu, J.; et al. A chromosome-level genome assembly of the jade perch (*Scortum barcoo*). *Sci. Data.* **2022**, *9*, 408. [CrossRef] [PubMed]
28. European Seabass (*Dicentrarchus labrax*). Available online: https://www.fishbase.se/summary/dicentrarchus-labrax.html (accessed on 20 December 2022).
29. Asian Seabass (*Lates calcarifer*). Available online: https://www.fishbase.se/summary/Lates-calcarifer.html (accessed on 20 December 2022).

30. Chinese Seabass (*Lateolabrax maculatus*). Available online: https://www.gbif.org/pt/species/2392026 (accessed on 20 December 2022).
31. Lacépède, B.G.E. *Histoire Naturelle des Poissons: IV*; Chez Plassan: Paris, France, 1802; Volume 4, pp. 1–16.
32. Nile Tilapia (*Oreochromis niloticus*). Available online: https://www.gbif.org/species/4285694 (accessed on 20 December 2022).
33. Gasterosteus aculeatus. Available online: https://www.gbif.org/species/4286327 (accessed on 20 December 2022).
34. Medaka (*Oryzias latipes*). Available online: https://www.gbif.org/species/2368377 (accessed on 20 December 2022).
35. Japanese Pufferfish (*Takifugu rubripes*). Available online: https://www.gbif.org/species/2407604 (accessed on 20 December 2022).
36. Green-spotted Pufferfish (*Tetraodon nigroviridis*). Available online: https://www.gbif.org/species/5213566 (accessed on 20 December 2022).
37. Southern Platyfish (*Xiphophorus maculatus*). Available online: https://www.gbif.org/species/2350164 (accessed on 20 December 2022).
38. Green Anole (*Anolis carolinensis*). Available online: https://www.gbif.org/species/2466939 (accessed on 20 December 2022).
39. Indian Cobra (*Naja naja*). Available online: https://www.gbif.org/species/2470351 (accessed on 20 December 2022).
40. Chicken (*Gallus gallus*). Available online: https://www.gbif.org/species/9326020 (accessed on 20 December 2022).
41. O'Leary, N.A.; Wright, M.W.; Brister, J.R.; Ciufo, S.; Haddad, D.; McVeigh, R.; Rajput, B.; Robbertse, B.; Smith-White, B.; Ako-Adjei, D.; et al. Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic. Acids. Res.* **2016**, *44*, D733–D745. [CrossRef]
42. Kinsella, R.J.; Kähäri, A.; Haider, S.; Zamora, J.; Proctor, G.; Spudich, G.; Almeida-King, J.; Staines, D.; Derwent, P.; Kerhornou, A.; et al. Ensembl BioMarts: A hub for data retrieval across taxonomic space. *Database J. Biol. Databases Curation* **2011**, *2011*, bar030. [CrossRef] [PubMed]
43. Lima, T.; Auchincloss, A.H.; Coudert, E.; Keller, G.; Michoud, K.; Rivoire, C.; Bulliard, V.; de Castro, E.; Lachaize, C.; Baratin, D.; et al. HAMAP: A database of completely sequenced microbial proteome sets and manually curated microbial protein families in UniProtKB/Swiss-Prot. *Nucleic Acids Res.* **2009**, *37*, D471–D478. [CrossRef]
44. Ge, S.X.; Jung, D.; Jung, D.; Yao, R. ShinyGO: A graphical gene-set enrichment tool for animals and plants. *Bioinformatics* **2020**, *36*, 2628–2629. [CrossRef] [PubMed]
45. Kamiya, T.; Kai, W.; Tasumi, S.; Oka, A.; Matsunaga, T.; Mizuno, N.; Fujita, M.; Suetake, H.; Suzuki, S.; Hosoya, S.; et al. A trans-species missense SNP in Amhr2 is associated with sex determination in the tiger pufferfish, *Takifugu rubripes* (fugu). *PLoS Genet.* **2012**, *8*, e1002798. [CrossRef]
46. Ou, S.; Su, W.; Liao, Y.; Chougule, K.; Agda, J.R.A.; Hellinga, A.J.; Lugo, C.S.B.; Elliott, T.A.; Ware, D.; Peterson, T.; et al. Benchmarking transposable element annotation methods for creation of a streamlined, comprehensive pipeline. *Genome Biol.* **2019**, *20*, 275. [CrossRef]
47. RepeatMasker. Available online: http://www.repeatmasker.org (accessed on 20 December 2022).
48. Manee, M.M.; Jackson, J.; Bergman, C.M. Conserved noncoding elements influence the transposable element landscape in drosophila. *Genome Biol. Evol.* **2018**, *10*, 1533–1545. [CrossRef]
49. Cabanettes, F.; Klopp, C. D-GENIES: Dot plot large genomes in an interactive, efficient and simple way. *PeerJ* **2018**, *6*, e4958. [CrossRef]
50. Du, L.; Zhang, C.; Liu, Q.; Zhang, X.; Yue, B.; Hancock, J. Krait: An ultrafast tool for genome-wide survey of microsatellites and primer design. *Bioinformatics* **2018**, *34*, 681–683. [CrossRef]
51. Wattanadilokchatkun, P.; Panthum, T.; Jaisamut, K.; Ahmad, S.F.; Dokkaew, S.; Muangmai, N.; Duengkae, P.; Singchat, W.; Srikulnath, K. Characterization of microsatellite distribution in Siamese fighting fish genome to promote conservation and genetic diversity. *Fishes* **2022**, *7*, 251. [CrossRef]
52. Lee, J.; Hong, W.Y.; Cho, M.; Sim, M.; Lee, D.; Ko, Y.; Kim, J. Synteny portal: A web based application portal for synteny block analysis. *Nucleic Acids. Res.* **2016**, *44*, W35–W40. [CrossRef]
53. Nguyen, N.T.T.; Vincens, P.; Crollius, H.R.; Louis, A. Genomicus 2018: Karyotype evolutionary trees and on-the-fly synteny computing. *Nucleic Acids. Res.* **2018**, *46*, D816–D822. [CrossRef] [PubMed]
54. Volff, J.N. Genome evolution and biodiversity in teleost fish. *Heredity* **2005**, *94*, 280–294. [CrossRef] [PubMed]
55. Purcell, C.M.; Seetharam, A.S.; Snodgrass, O.; Ortega-García, S.; Hyde, J.R.; Severin, A.J. Insights into teleost sex determination from the *Seriola dorsalis* genome assembly. *BMC Genom.* **2018**, *19*, 31. [CrossRef] [PubMed]
56. Sember, A.; Nguyen, P.; Perez, M.F.; Altmanová, M.; Ráb, P.; Cioffi, M.D.B. Multiple sex chromosomes in teleost fishes from a cytogenetic perspective: State of the art and future challenges. *Philos. Trans. R. Soc. B* **2021**, *376*, 20200098. [CrossRef]
57. Ramos, L.; Antunes, A. Decoding sex: Elucidating sex determination and how high-quality genome assemblies are untangling the evolutionary dynamics of sex chromosomes. *Genomics* **2022**, *114*, 110277. [CrossRef]
58. Kejnovsky, E.; Hobza, R.; Cermak, T.; Kubat, Z.; Vyskot, B. The role of repetitive DNA in structure and evolution of sex chromosomes in plants. *Heredity* **2009**, *102*, 533–541. [CrossRef]
59. Matsubara, K.; Uno, Y.; Srikulnath, K.; Matsuda, Y.; Miller, E.; Olsson, M. No interstitial telomeres on autosomes but remarkable amplification of telomeric repeats on the W sex chromosome in the sand lizard (*Lacerta agilis*). *J. Hered.* **2015**, *106*, 753–757. [CrossRef]
60. Carducci, F.; Barucca, M.; Canapa, A.; Biscotti, M.A. Rex retroelements and teleost genomes: An overview. *Int. J. Mol. Sci.* **2018**, *19*, 3653. [CrossRef] [PubMed]

61. Singchat, W.; O'Connor, R.E.; Tawichasri, P.; Suntronpong, A.; Sillapaprayoon, S.; Suntrarachun, S.; Muangmai, N.; Baicharoen, S.; Peyachoknagul, S.; Chanhome, L.; et al. Chromosome map of the Siamese cobra: Did partial synteny of sex chromosomes in the amniote represent "a hypothetical ancestral super-sex chromosome" or random distribution? *BMC Genom.* **2018**, *19*, 939. [CrossRef] [PubMed]
62. Thongchum, R.; Singchat, W.; Laopichienpong, N.; Tawichasri, P.; Kraichak, E.; Prakhongcheep, O.; Sillapaprayoon, S.; Muangmai, N.; Baicharoen, S.; Suntrarachun, S.; et al. Diversity of PBI-DdeI satellite DNA in snakes correlates with rapid independent evolution and different functional roles. *Sci. Rep.* **2019**, *9*, 15459. [CrossRef] [PubMed]
63. Furman, B.L.S.; Metzger, D.C.H.; Darolti, I.; Wright, A.E.; Sandkam, B.A.; Almeida, P.; Shu, J.J.; Mank, J.E. Sex chromosome evolution: So many exceptions to the rules. *Genome Biol. Evol.* **2020**, *12*, 750–763. [CrossRef]
64. Singchat, W.; Sillapaprayoon, S.; Muangmai, N.; Baicharoen, S.; Indananda, C.; Duengkae, P.; Peyachoknagul, S.; O'Connor, R.E.; Griffin, D.K.; Srikulnath, K. Do sex chromosomes of snakes, monitor lizards, and iguanian lizards result from multiple fission of an "ancestral amniote super-sex chromosome"? *Chromosome Res.* **2020**, *28*, 209–228. [CrossRef]
65. Singchat, W.; Ahmad, S.F.; Sillapaprayoon, S.; Muangmai, N.; Duengkae, P.; Peyachoknagul, S.; O'Connor, R.E.; Griffin, D.K.; Srikulnath, K. Partial amniote sex chromosomal linkage homologies shared on snake W sex chromosomes support the ancestral super-sex chromosome evolution in amniotes. *Front. Genet.* **2020**, *11*, 948. [CrossRef]
66. Deakin, J.E.; Ezaz, T. Understanding the evolution of reptile chromosomes through applications of combined cytogenetics and genomics approaches. *Cytogenet. Genome Res.* **2019**, *157*, 7–20. [CrossRef]
67. Tennessen, J.A.; Wei, N.; Straub, S.C.K.; Govindarajulu, R.; Liston, A.; Ashman, T.L. Repeated translocation of a gene cassette drives sex-chromosome turnover in strawberries. *PLoS Biol.* **2018**, *16*, e2006062. [CrossRef]
68. Siamese Cobra (Naja kaouthia). Available online: https://www.gbif.org/es/species/2470368 (accessed on 20 December 2022).
69. Uno, Y.; Asada, Y.; Nishida, C.; Takehana, Y.; Sakaizumi, M.; Matsuda, Y. Divergence of repetitive DNA sequences in the heterochromatin of medaka fishes: Molecular cytogenetic characterization of constitutive heterochromatin in two medaka species: *Oryzias hubbsi* and *O. celebensis* (Adrianichthyidae, Beloniformes). *Cytogenet. Genome Res.* **2013**, *141*, 212–226. [CrossRef]
70. North African Catfish (*Clarias gariepinus*, Burchell, 1822). Available online: https://www.gbif.org/species/5202793 (accessed on 20 December 2022).
71. Bighead Catfish (*Clarias macrocephalus*, Günther, 1864). Available online: https://www.gbif.org/species/5202728 (accessed on 20 December 2022).
72. Na, J.K.; Wang, J.; Ming, R. Accumulation of interspersed and sex-specific repeats in the non-recombining region of papaya sex chromosomes. *BMC Genom.* **2014**, *15*, 335. [CrossRef]
73. Puterova, J.; Kubat, Z.; Kejnovsky, E.; Jesionek, W.; Cizkova, J.; Vyskot, B.; Hobza, R. The slowdown of Y chromosome expansion in dioecious *Silene latifolia* due to DNA loss and male-specific silencing of retrotransposons. *BMC Genom.* **2018**, *19*, 153. [CrossRef] [PubMed]
74. Nguyen, D.H.M.; Panthum, T.; Ponjarat, J.; Laopichienpong, N.; Kraichak, E.; Singchat, W.; Ahmad, S.F.; Muangmai, N.; Peyachoknagul, S.; Na-Nakorn, U.; et al. Genome-wide SNP analysis suggests male heterogamety in bighead catfish (*Clarias macrocephalus*, Günther, 1864). *Aquaculture* **2021**, *543*, 737005. [CrossRef]
75. Srikulnath, K.; Ahmad, S.F.; Singchat, W.; Panthum, T. Do Ty3/Gypsy transposable elements play preferential roles in sex chromosome differentiation? *Life* **2020**, *12*, 522. [CrossRef]
76. Matsubara, K.; O'Meally, D.; Azad, B.; Georges, A.; Sarre, S.D.; Graves, J.A.; Matsuda, Y.; Ezaz, T. Amplification of microsatellite repeat motifs is associated with the evolutionary differentiation and heterochromatinization of sex chromosomes in Sauropsida. *Chromosoma* **2016**, *125*, 111–123. [CrossRef]
77. Ahmad, S.F.; Singchat, W.; Jehangir, M.; Panthum, T.; Srikulnath, K. Consequence of paradigm shift with repeat landscapes in reptiles: Powerful facilitators of chromosomal rearrangements for diversity and evolution. *Genes* **2020**, *11*, 827. [CrossRef] [PubMed]
78. Matsubara, K.; Nishida, C.; Matsuda, Y.; Kumazawa, Y. Sex chromosome evolution in snakes inferred from divergence patterns of two gametologous genes and chromosome distribution of sex chromosome-linked repetitive sequences. *Zool. Lett.* **2016**, *2*, 19. [CrossRef]
79. Laopichienpong, N.; Muangmai, N.; Chanhome, L.; Suntrarachun, S.; Twilprawat, P.; Peyachoknagul, S.; Srikulnath, K. Evolutionary dynamics of the gametologous *CTNNB1* gene on the Z and W chromosomes of snakes. *J. Hered.* **2017**, *108*, 142–151. [CrossRef]
80. Laopichienpong, N.; Tawichasri, P.; Chanhome, L.; Phatcharakullawarawat, R.; Singchat, W.; Kantachumpoo, A.; Muangmai, N.; Suntrarachun, S.; Matsubara, K.; Peyachoknagul, S.; et al. A novel method of caenophidian snake sex identification using molecular markers based on two gametologous genes. *Ecol. Evol.* **2017**, *7*, 4661–4669. [CrossRef]
81. Singchat, W.; Ahmad, S.F.; Laopichienpong, N.; Suntronpong, A.; Panthum, T.; Griffin, D.K.; Srikulnath, K. Snake W sex chromosome: The shadow of ancestral amniote super-sex chromosome. *Cells* **2020**, *9*, 2386. [CrossRef]
82. Suryamohan, K.; Krishnankutty, S.P.; Guillory, J.; Jevit, M.; Schröder, M.S.; Wu, M.; Kuriakose, B.; Mathew, O.K.; Perumal, R.C.; Koludarov, I. The Indian cobra reference genome and transcriptome enables comprehensive identification of venom toxins. *Nat. Genet.* **2020**, *52*, 106–117. [CrossRef]
83. Ezaz, T.; Srikulnath, K.; Graves, J.A.M. Origin of amniote sex chromosomes: An ancestral super-sex chromosome, or common requirements? *J. Hered.* **2017**, *108*, 94–105. [CrossRef] [PubMed]

84. Kratochvíl, L.; Gamble, T.; Rovatsos, M. Sex chromosome evolution among amniotes: Is the origin of sex chromosomes non-random? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2021**, *376*, 20200108. [CrossRef] [PubMed]
85. Capel, B. Vertebrate sex determination: Evolutionary plasticity of a fundamental switch. *Nat. Rev. Genet.* **2017**, *18*, 675–689. [CrossRef] [PubMed]
86. Adolfi, M.C.; Nakajima, R.T.; Nóbrega, R.H.; Schartl, M. Intersex, hermaphroditism, and gonadal plasticity in vertebrates: Evolution of the Müllerian duct and *Amh/Amhr2* signaling. *Annu. Rev. Anim. Biosci.* **2019**, *7*, 149–172. [CrossRef]
87. Yan, Y.L.; Batzel, P.; Titus, T.; Sydes, J.; Desvignes, T.; BreMiller, R.; Draper, B.; Postlethwait, J.H. A hormone that lost its receptor: Anti-Mullerian hormone (*amh*) in zebrafish gonad development and sex determination. *Genetics* **2019**, *213*, 529–553. [CrossRef]
88. Patagonian Pejerrey (*Odontesthes hatcheri*). Available online: https://www.gbif.org/pt/species/172797066/verbatim (accessed on 20 December 2022).
89. Putnam, F.W. Remarks on a supposed nondescript species of Gasterosteus from Massachusetts. *Proc. Essex Inst.* **1867**, *5*, 1866–1867.
90. Higuchi, M.; Sakai, H.; Goto, A. A new threespine stickleback, *Gasterosteus nipponicus* sp. nov. (Teleostei: Gasterosteridae), from the Japan sea region. *Ichthyol. Res.* **2014**, *61*, 341–351. [CrossRef]
91. Brook Stickleback (*Culaea inconstans*). Available online: https://www.gbif.org/species/2356556 (accessed on 20 December 2022).
92. Hattori, R.S.; Murai, Y.; Oura, M.; Masuda, S.; Majhi, S.K.; Sakamoto, T.; Fernandino, J.I.; Somoza, G.M.; Yokota, M.; Strüssmann, C.A. A Y-linked anti-Müllerian hormone duplication takes over a critical role in sex determination. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 2955–2959. [CrossRef]
93. Jeffries, D.L.; Mee, J.A.; Peichel, C.L. Identification of a candidate sex determination gene in Culaea inconstans suggests convergent recruitment of an *Amh* duplicate in two lineages of stickleback. *J. Evol. Biol.* **2022**, *35*, 1683–1695. [CrossRef]
94. Cobaltcap Silverside (*Hypoatherina tsurugae*). Available online: https://www.gbif.org/species/2411930 (accessed on 20 December 2022).
95. Northern Pike (*Esox lucius*). Available online: https://www.gbif.org/species/113226615 (accessed on 20 December 2022).
96. Hilgendorf, F.M. Uebersicht über die japanischen Sebastes-Arten. *Ges. Naturforsch. Freunde Berl.* **1880**, *1880*, 166–172.
97. Li, M.; Sun, Y.; Zhao, J.; Shi, H.; Zeng, S.; Ye, K.; Jiang, D.; Zhou, L.; Sun, L.; Tao, W.; et al. A tandem duplicate of anti-Müllerian hormone with a missense SNP on the Y chromosome is essential for male sex determination in Nile tilapia, *Oreochromis niloticus*. *PLoS Genet.* **2015**, *11*, e1005678. [CrossRef] [PubMed]
98. Bej, D.K.; Miyoshi, K.; Hattori, R.S.; Strüssmann, C.A.; Yamamoto, Y. A duplicated, truncated *amh* gene is involved in male sex determination in an old world silverside. *G3 Genes. Genomes. Genet.* **2017**, *7*, 2489–2495. [CrossRef] [PubMed]
99. Pan, Q.; Feron, R.; Yano, A.; Guyomard, R.; Jouanno, E.; Vigouroux, E.; Wen, M.; Busnel, J.M.; Bobe, J.; Concordet, J.P.; et al. Identification of the master sex determining gene in Northern pike (*Esox lucius*) reveals restricted sex chromosome differentiation. *PLoS Genet.* **2019**, *15*, e1008013. [CrossRef] [PubMed]
100. Song, W.; Xie, Y.; Sun, M.; Li, X.; Fitzpatrick, C.K.; Vaux, F.; O'Malley, K.G.; Zhang, Q.; Qi, J.; He, Y. A duplicated *amh* is the master sex-determining gene for *Sebastes* rockfish in the Northwest Pacific. *Open. Biol.* **2021**, *11*, 210063. [CrossRef]
101. Argentinian Silverside (*Odontesthes bonariensis* Valenciennes, 1835). Available online: https://www.gbif.org/species/2412370 (accessed on 20 December 2022).
102. Rafati, N.; Chen, J.; Herpin, A.; Pettersson, M.E.; Han, F.; Feng, C.; Wallerman, O.; Rubin, C.J.; Péron, S.; Cocco, A.; et al. Reconstruction of the birth of a male sex chromosome present in Atlantic herring. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 24359–24368. [CrossRef]
103. Halm, S.; Rocha, A.; Miura, T.; Prat, F.; Zanuy, S. Anti-Müllerian hormone (*Amh/Amh*) in the European sea bass: Its gene structure, regulatory elements, and the expression of alternatively-spliced isoforms. *Gene* **2007**, *388*, 148–158. [CrossRef]
104. Gong, G.; Xiong, Y.; Xiao, S.; Li, X.Y.; Huang, P.; Liao, Q.; Han, Q.; Lin, Q.; Dan, C.; Zhou, L.; et al. Origin and chromatin remodeling of young X/Y sex chromosomes in catfish with sexual plasticity. *Natl. Sci. Rev.* **2023**, *10*, nwac239. [CrossRef]
105. Schartl, M.; Schories, S.; Wakamatsu, Y.; Nagao, Y.; Hashimoto, H.; Bertin, C.; Mourot, B.; Schmidt, C.; Wilhelm, D.; Centanin, L.; et al. Sox5 is involved in germ-cell regulation and sex determination in medaka following co-option of nested transposable elements. *BMC Biol.* **2018**, *16*, 16. [CrossRef]
106. Matsuda, M.; Nagahama, Y.; Shinomiya, A.; Sato, T.; Matsuda, C.; Kobayashi, T.; Morrey, C.E.; Shibata, N.; Asakawa, S.; Shimizu, N. DMY is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature* **2022**, *17*, 559–563. [CrossRef]
107. Nanda, I.; Kondo, M.; Hornung, U.; Asakawa, S.; Winkler, C.; Shimizu, A.; Shan, Z.; Haaf, T.; Shimizu, N.; Shima, A.; et al. A duplicated copy of *DMRT1* in the sex-determining region of the Y chromosome of the medaka, *Oryzias latipes*. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11778–11783. [CrossRef]
108. Feron, R.; Zahm, M.; Cabau, C.; Klopp, C.; Roques, C.; Bouchez, O.; Eché, C.; Valière, S.; Donnadieu, C.; Haffray, P.; et al. Characterization of a Y-specific duplication/insertion of the anti-Mullerian hormone type II receptor gene based on a chromosome-scale genome assembly of yellow perch, *Perca flavescens*. *Mol. Ecol. Resour.* **2020**, *220*, 531–543. [CrossRef] [PubMed]
109. Peichel, C.L.; McCann, S.R.; Ross, J.A.; Naftaly, A.F.S.; Urton, J.R.; Cech, J.N.; Grimwood, J.; Schmutz, J.; Myers, R.M.; Kingsley, D.M.; et al. Assembly of the threespine stickleback Y chromosome reveals convergent signatures of sex chromosome evolution. *Genome Biol.* **2020**, *21*, 177. [CrossRef] [PubMed]
110. Japanese Flounder (*Paralichthys olivaceus*). Available online: https://www.gbif.org/pt/species/2408877 (accessed on 3 April 2023).

111. Tsakogiannis, A.; Manousaki, T.; Lagnel, J.; Sterioti, A.; Pavlidis, M.; Papandroulakis, N.; Mylonas, C.C.; Tsigenopoulos, C.S. The transcriptomic signature of different sexes in two protogynous hermaphrodites: Insights into the molecular network underlying sex phenotype in fish. *Sci. Rep.* **2018**, *8*, 3564. [CrossRef] [PubMed]
112. McCulloch, A.R.; Waite, E.R. Results of the South Australian Museum expedition to strzelecki and cooper creeks. September and October, 1916. (k) Pisces. *Trans. R. Soc. S Aust.* **1917**, *41*, 472–475.