

Figure S2. *Dmrt2a* sequence analysis in spotted scat. **A, I)** At most 14 bp indel in intron 1 of male and female sequence **A, II)** PAGE gel with primers spanning the indel region (Marker-1) did not show distinguishable bands in males and female. **B, I)** At most 10 bp indel in intron 1 of male and female sequence. **B, II)** PAGE gel with primers spanning the indel region (Marker-5) did not show distinguishable bands between males and female. At least 16 TG single sequence repeats (SSR-1 and SSR-2) preceded indel 1 and indel 5. The indel 1 and the indel 5 are positioned first and last on intron 1.

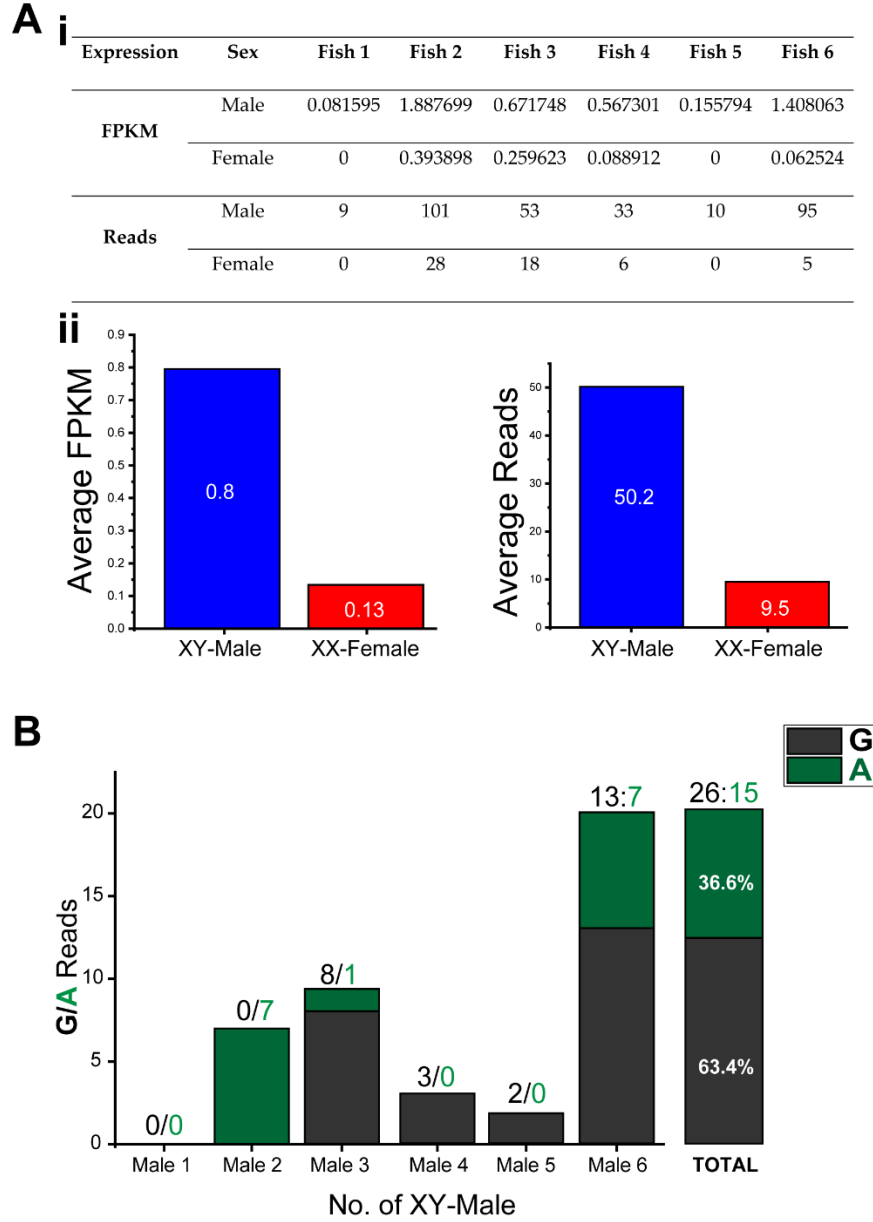


Figure S3. A) *Dmrt2a* transcript expression by transcriptome analysis. i) *Dmrt2a* FPKM and reads values from 6 male and 6 female transcriptome data. ii) Graph of average FPKM values and reads numbers from the 6 males and females in "i" above. **B)** The proportion of G and A sub-types in six XY-males (male 1 - 6). The SNP region (SNP: I) on the Exon 2 of *Dmrt2a* transcript was used as a query sequence to blast the raw RNA-seq data of 6 XY-males using blastX. The proportion of clean reads with either G or A were determined. Male 1 has very low reads, and the SNP site was not found. Male 2 and (4 and 5) did not have G and A alleles, respectively. On average, the proportion of the G allele is more (63.4%) than A (36.6%).

Table S1. Primer sequences used in this study

Primer name	Forward 5' – 3'	Reverse 5' – 3'	Product size bp
Cloning (Open Reading Frame, ORF)			
Dmrt2a	GCTAATTCAATTCTTGTCTG GAGTCTG	GACAAACGTGACATTTATAGTG CAAG	1786
Dmrt2b	GCTCAGTGACAGCAGCAACA TTG	GCACTTCATCACTTTCCATTGTC CTG	1400
Cloning gDNA			
Dmrt2a	GTTGCGAGTTTGAAATCGAC GTG	ACAAACGTGACATTTATAGTGC	4042
Tissue distribution / Gene expression			
Dmrt2a	GACAACGCCGGTTGCGAGTT	TCCACCACCAGCAGGCAGTT	311
Dmrt2b	TGTCCACTCAGCCGGAAGGA	CTCCAGCGGCAGAAGCGTTT	271
<i>β-actin</i>	GAGAGGTTCCGTTGCCCAGA G	CAGACAGCACAGTGTTGGCGT	
Markers			
Marker-1	TGGAGAGACAGCGGGTGATG	TGAAGGTGAGCACATGGGCATA	129
Marker-2	TGTATATGCCCATGTGCTCAC CTTC	GGGAAACAGCACTGACTGACC CGAG	147, 139
Marker-3	AGATCCTGAGCAGCAGATAT TGTAGG	TGAAACTAAAAATAGTACTGGT TTCTTAC	160, 148
Marker-4	GACCATAACGAAGGCCGAAC ACATT	AAAGTGGGACAGTGAGTCAAA TGG	543, 444
Marker-5	ATGGTACTGTATGGATGTCTT TCTG	AACTAAGCCTGACTGCATTACT TCA	158
Marker-6	CGTCCCAGTTTAACAGCGAT A	TGTTGTGACAGTGCTGGACATG	158, 144
Marker-7 (SNP)	AGTTGTGCTAGACCTCCATCT CATG	CTAATGCCACCAAGTCAAGTAT GTT	500

Table S2. NSBI and Ensembl sequence IDs of Dmrt proteins used in this study

Species name	Accession Number
Dmrt2/a	
Zebrafish, <i>Danio rerio</i>	NM_130952.1
Nile tilapia, <i>Oreochromis niloticus</i>	NM_001279767.1
Fugu, <i>Takifugu rubripes</i>	ENSTRUG00000015666.3
Medaka, <i>Oryzias latipes</i>	ENSORLG00000022893.1
Spotted gar, <i>Lepisosteus oculatus</i>	XM_006627124.2
Coelacanth, <i>Latimeria chalumnae</i>	XM_014491555.1
Stickleback, <i>Gasterosteus aculeatus</i>	ENSGACG00000014508.1
Frog, <i>Xenopus laevis</i>	NM_001096256.1
Lizard, <i>Anolis carolinensis</i>	XM_008103322.2
Chicken, <i>Gallus gallus</i>	XR_005843092.1
Mouse, <i>Mus musculus</i>	NM_145831.3
Human, <i>Homo sapiens</i>	NC_000009.12
Spotted scat, <i>Scatophagus argus</i>	MG765301.1
Dmrt2b	
Zebrafish, <i>Danio rerio</i>	NM_001079976.1
Nile tilapia, <i>Oreochromis niloticus</i>	NM_001279473.1
Fugu, <i>Takifugu rubripes</i>	ENSTRUG00000018484.3
Medaka, <i>Oryzias latipes</i>	ENSORLG00000025087.1
Spotted gar, <i>Lepisosteus oculatus</i>	XM_006634912.2
Stickleback, <i>Gasterosteus aculeatus</i>	ENSGACG00000006816.1
Spotted scat, <i>Scatophagus argus</i>	MG765302.1
Dmrt5	
Spotted scat, <i>Scatophagus argus</i>	MG765305.1