

**Table S1.** Wild individuals of Siamese fighting fish (*Betta splendens* Regan, 1910) captured from five different geographical regions in Thailand.

Species	Sample	Sex	Locality
<i>Betta splendens</i>	SP1m1	Male	Bangkok
<i>Betta splendens</i>	SP1m2	Male	Bangkok
<i>Betta splendens</i>	SP1m3	Male	Bangkok
<i>Betta splendens</i>	SP1m4	Male	Bangkok
<i>Betta splendens</i>	SP1m5	Male	Bangkok
<i>Betta splendens</i>	SP1m6	Male	Bangkok
<i>Betta splendens</i>	SP1m7	Male	Bangkok
<i>Betta splendens</i>	SP1m8	Male	Bangkok
<i>Betta splendens</i>	SP1m9	Male	Bangkok
<i>Betta splendens</i>	SP1f1	Female	Bangkok
<i>Betta splendens</i>	SP1f2	Female	Bangkok
<i>Betta splendens</i>	SP1f3	Female	Bangkok
<i>Betta splendens</i>	SP1f4	Female	Bangkok
<i>Betta splendens</i>	SP1f5	Female	Bangkok
<i>Betta splendens</i>	SP1f6	Female	Bangkok
<i>Betta splendens</i>	SP1f7	Female	Bangkok
<i>Betta splendens</i>	SP1f8	Female	Bangkok
<i>Betta splendens</i>	SP1f9	Female	Bangkok
<i>Betta splendens</i>	SP1f10	Female	Bangkok
<i>Betta splendens</i>	SP1f11	Female	Bangkok
<i>Betta splendens</i>	SP1f12	Female	Bangkok
<i>Betta splendens</i>	SP5m1	Male	Chumphon
<i>Betta splendens</i>	SP5m2	Male	Chumphon
<i>Betta splendens</i>	SP5m3	Male	Chumphon
<i>Betta splendens</i>	SP5m4	Male	Chumphon
<i>Betta splendens</i>	SP5m5	Male	Chumphon
Species	Sample	Sex	Locality
<i>Betta splendens</i>	SP5f1	Female	Chumphon
<i>Betta splendens</i>	SP5f2	Female	Chumphon
<i>Betta splendens</i>	SP5f3	Female	Chumphon
<i>Betta splendens</i>	SP5f4	Female	Chumphon
<i>Betta splendens</i>	SP5f5	Female	Chumphon
<i>Betta splendens</i>	SP6m2	Male	Surat Thani
<i>Betta splendens</i>	SP6m3	Male	Surat Thani
<i>Betta splendens</i>	SP6m5	Male	Surat Thani
<i>Betta splendens</i>	SP6m6	Male	Surat Thani
<i>Betta splendens</i>	SP6m7	Male	Surat Thani
<i>Betta splendens</i>	SP6f1	Female	Surat Thani
<i>Betta splendens</i>	SP6f2	Female	Surat Thani
<i>Betta splendens</i>	SP6f3	Female	Surat Thani
<i>Betta splendens</i>	SP6f5	Female	Surat Thani
<i>Betta splendens</i>	SP6f6	Female	Surat Thani
<i>Betta splendens</i>	SP6f9	Female	Surat Thani
<i>Betta splendens</i>	SP6f10	Female	Surat Thani
<i>Betta splendens</i>	SPm1	Male	Lamphun
<i>Betta splendens</i>	SPm2	Male	Lamphun

<i>Betta splendens</i>	SPm3	Male	Lamphun
<i>Betta splendens</i>	SPm4	Male	Lamphun
<i>Betta splendens</i>	SPm5	Male	Lamphun
<i>Betta splendens</i>	SPm6	Male	Lamphun
<i>Betta splendens</i>	SPm7	Male	Lamphun
<i>Betta splendens</i>	SPm8	Male	Lamphun
<i>Betta splendens</i>	SPm9	Male	Lamphun
Species	Sample	Sex	Locality
<i>Betta splendens</i>	SPf1	Male	Lamphun
<i>Betta splendens</i>	SPf2	Male	Lamphun

**Table S2.** Eight microsatellite primer sets developed from Siamese fighting fish (*Betta splendens* Regan, 1910) sourced from Chailertit et al. (2014).

Locus	Primer sequence (5'–3')	Repeat	Ta (°C)	Accession	Size (bp)
BettaMS4	F: GTTTCATCAGGAGCAGCAGCATAA R: CTGTTTGATGGCCGACTTTT	(GA) <sub>n</sub>	59	AB777403	259–315
BettaMS5	F: GTTTCGTCACCTTCTGAGCAAACA R: AAATGCGCTGGGTAGACTTG	(GA) <sub>n</sub>	59	AB777406	198–218
BettaMS8	F: CGTGAGCTGCAAAGAAAACA R: CTGTTTGATGGCCGACTTTT	(GA) <sub>n</sub>	57	AB777407	223
BettaMS15	F: ACTGTAACCGGGCTGTTCTG R: AACGCACCCAGAAACAAATC	(GA) <sub>n</sub>	57	AB777411	216–225
BettaMS17	F: AAGCAGGTCTTTCACCTCCA R: TCACCTGCGTCTAAGTCAA	(GA) <sub>n</sub>	61	AB777413	194–221
BettaMS23	F: GTTTGAGAGAAATGGGTTCTTCG R: TCACTACGCTGCCAAATCAG	(CT) <sub>n</sub> (CA) <sub>n</sub>	55	AB777415	259–315
BettaMS25	F: GTTTGGGTAAAACCCAACTCTGG R: AACGTCACGTGGAACAGATG	(GT) <sub>n</sub>	55	AB777416	194–224
BettaMS40	F: CAGTACATTTGACTGATCGCAGA R: CAGGATGCTTCCTTGGGTAA	(GA) <sub>n</sub>	57	AB777421	136–165

Chailertit V, Swatdipong A, Peyachoknagul S, Salaenoi J, Srikulnath K. Isolation and characterization of novel microsatellite markers from Siamese fighting fish (*Betta splendens*, Osphronemidae, Anabantidae) and their transferability to related species, *B. smaragdina* and *B. imbellis*. Genet Mol Res. 2014; 13: 7157–7162. <https://doi.org/10.4238/2014.September.5.1>. Regan CT. The Asiatic fishes of the family Anabantidae. Proc Zool Soc Lond. 1910; B1909: 767–787.

**Table S3.** Mean number of alleles per locus based on single-nucleotide polymorphism (SNP) and presence-absence (PA) loci from 75 Siamese fighting fish (*Betta splendens* Regan, 1910).

Mean	N	Na	Ne	I	Ho	He	uHe	F
	3.693	0.466	0.466	0.000	0.000	0.000	0.000	
SE	0.025	0.002	0.002	0.000	0.000	0.000	0.000	0.000

Regan CT. The Asiatic fishes of the family Anabantidae. Proc Zool Soc Lond. 1910; B1909: 767–787.

**Table S4.**  $F_{ST}$  showed significant differences between commercial populations of Siamese fighting fish (*Betta splendens* Regan, 1910).

Pop1	Pop2	Fst	p-value
BSP_G	BSP_GIA	0.465	0.001
BSP_G	BSP_GL	0.658	0.001
BSP_GIA	BSP_GL	0.343	0.001
BSP_G	BSP_O	0.726	0.001
BSP_GIA	BSP_O	0.353	0.001
BSP_GL	BSP_O	0.583	0.001
BSP_G	BSP_W	0.625	0.001
BSP_GIA	BSP_W	0.294	0.001
BSP_GL	BSP_W	0.500	0.001
BSP_O	BSP_W	0.522	0.001

Regan CT. The Asiatic fishes of the family Anabantidae. Proc Zool Soc Lond. 1910; B1909: 767–787.

**Table S5.** Genetic divergence among (net nucleotide distance) and within (expected heterozygosity) Siamese fighting fish (*Betta splendens* Regan, 1910) populations, and the proportion of membership of the population samples.

Clusters	Net nucleotide distance				Expected heterozygosity	Proportion of membership
	Cluster A	Cluster B	Cluster C	Cluster D		
Cluster A	-	0.1980	0.1380	0.1586	0.21	0.29
Cluster B	0.1980	-	0.2426	0.2387	0.08	0.23
Cluster C	0.1380	0.2426	-	0.1904	0.11	0.26
Cluster D	0.1586	0.2387	0.1904	-	0.13	0.22

Regan CT. The Asiatic fishes of the family Anabantidae. Proc Zool Soc Lond. 1910; B1909: 767–787.

**Table S6.** Primers used for the development of sex-specific markers for the genotyping assay.

Primer	Sequence (5'–3')	Size (bp)
PA10004900-F	GGTCATCCTCACACTCCT	302
PA10004900-R	CGTTTTATTGTTCCGGCATT	

**Table S7.** Genetic divergence among (net nucleotide distance) and within (expected heterozygosity) populations of Siamese fighting fish (*Betta splendens* Regan, 1910), and proportion of membership of wild population samples.

Clusters	Net nucleotide distance			Expected heterozygosity	Proportion of membership
	Cluster A	Cluster B	Cluster C		
Cluster A	-	0.1964	0.1607	0.55	0.28
Cluster B	0.1964	-	0.0466	0.81	0.43
Cluster C	0.1607	0.0466	-	0.86	0.29

Regan CT. The Asiatic fishes of the family Anabantidae. Proc Zool Soc Lond. 1910; B1909: 767–787.

**Table S8.** *In silico* chromosome mapping aligned to chromosome-level assembly of Siamese fighting fish (*Betta splendens* Regan, 1910) (accession: GCF\_900634795.3).

Locus id	Chromosome	Region	%Query Coverage (60%)	E-Value (0.005)	Percent Identity (55%)
PA100003792	7	9381270–9381338	100%	4.00E-28	100.00%
PA100004846	9	27847987–27848032	66%	3.00E-11	93.48%
PA100004464	9	27782844–27782902	92%	3.00E-17	90.62%
PA100029208	9	28233484–28233525	60%	9.00E-11	95.24%
PA100004900	9	30855785–30855834	72%	3.00E-16	98.00%

PA100031354	9	28616136–28616182	68%	2.00E-13	95.74%
PA100004510	9	28543911–28543979	100%	2.00E-26	98.55%
PA100028166	9	27952615–27952678	92%	1.00E-22	96.88%
PA100004858	9	28667903–28667952	72%	3.00E-16	98.00%
PA100004899	9	30159035–30159077	62%	2.00E-12	97.67%
PA100029201	9	29543275–29543327	76%	1.00E-16	96.23%
PA100029211	9	28750932–28750985	78%	3.00E-17	96.30%
PA100004782	9	31334950–31335019	100%	7.00E-25	97.14%
PA100011835	9	28469956–28470024	100%	2.00E-25	97.10%
PA100004843	9	29420491–29420535	68%	1.00E-09	91.11%
PA100027514	9	28423221–28423289	100%	2.00E-25	97.10%
PA100017083	9	29736793–29736861	100%	2.00E-25	97.10%
PA100021008	9	27255876–27255943	98%	7.00E-25	97.06%
PA100012407	9	28875037–28875093	100%	2.00E-13	82.61%
PA100004465	9	27804560–27804625	97%	3.00E-23	97.01%
PA100025925	9	31673853–31673921	100%	2.00E-25	97.10%
PA100004721	9	27067733–27067790	84%	8.00E-18	94.83%
PA100022272	9	27045850–27045901	75%	3.00E-16	96.15%
PA100004670	9	31930035–31930103	100%	8.00E-24	95.65%
PA100004897	9	27316841–27316886	66%	6.00E-13	95.65%
PA100004903	9	31072802–31072845	63%	1.00E-14	100.00%
Locus id	Chromosome	Region	%Query Coverage (60%)	E-Value (0.005)	Percent Identity (55%)
PA100004567	9	27255731–27255795	94%	2.00E-24	98.46%
PA100020889	9	29921025–29921093	100%	5.00E-20	91.30%
PA100004779	9	26334675–26334743	100%	2.00E-26	98.55%
PA100029590	9	29546367–29546416	72%	4.00E-15	96.00%
PA100004412	9	28450542–28450581	89%	2.00E-12	100.00%
PA100021089	9	26285178–26285246	100%	1.00E-22	94.20%
PA100004556	9	30653569–30653635	98%	3.00E-16	90.00%
PA100004680	9	3410145–3410216	100%	1.00E-08	77.78%
PA100030209	9	27689584–27689652	100%	2.00E-25	97.10%
PA100004773	9	29259455–29259523	100%	2.00E-26	98.55%
PA100004645	9	29259455–29259523	98%	1.00E-27	100.00%
PA100004594	9	29740306–29740366	88%	4.00E-21	96.72%
PA100014942	9	28979422–28979490	100%	2.00E-25	97.10%
PA100004619	9	28238728–28238797	100%	3.00E-23	95.71%
PA100004404	9	27876374–27876450	100%	4.00E-22	89.61%
PA100004762	9	30003040–30003107	100%	2.00E-24	97.10%
PA100004801	9	30620699–30620763	100%	6.00E-13	85.51%
PA100004689	9	28266822–28266891	100%	7.00E-25	97.14%
PA100019317	9	30706839–30706907	100%	2.00E-25	97.10%
PA100010245	9	29244547–29244615	100%	2.00E-25	97.10%
PA100004696	9	26797741–26797810	100%	7.00E-25	97.14%
PA100004408	9	28019594–28019662	100%	1.00E-22	94.20%
PA100004563	9	29100891–29100959	100%	1.00E-22	94.20%
PA100004684	9	31129329–31129397	100%	4.00E-28	100.00%
PA100004381	9	27572488–27572556	100%	4.00E-28	100.00%
PA100013910	9	27498305–27498373	100%	2.00E-25	97.10%
PA100020461	9	30600574–30600642	100%	2.00E-25	97.10%
PA100007736	9	31666984–31667052	100%	2.00E-25	97.10%

PA100017244	9	28247164–28247232	100%	2.00E-25	97.10%
PA100024280	9	26618215–26618278	92%	2.00E-25	100.00%
PA100002311	9	29585295–29585363	100%	4.00E-28	100.00%
PA100004745	9	30712428–30712496	100%	4.00E-28	100.00%
Locus id	Chromosome	Region	%Query Coverage (60%)	E-Value (0.005)	Percent Identity (55%)
PA100004685	9	29878539–29878606	98%	7.00E-25	97.06%
PA100004705	9	27696047–27696107	76%	9.00E-11	85.25%
PA100004431	9	27563896–27563965	100%	3.00E-23	95.71%
PA100004615	9	27648339–27648407	100%	4.00E-28	100.00%
PA100004550	9	27231100–27231168	100%	2.00E-25	97.10%
PA100004551	9	30132934–30133001	100%	1.00E-21	94.20%
PA100005701	9	27704093–27704164	100%	2.00E-19	91.67%
PA100015466	9	30427554–30427622	100%	4.00E-28	100.00%
PA100004628	19	15184077–15184147	100%	2.00E-25	97.18%
PA100002103	9	12148504–12148572	100%	4.00E-28	100.00%
SNP100017094	9	28012619–28012551	100%	4.00E-28	100.00%
SNP100017206	9	27467354–27467422	100%	4.00E-28	100.00%
SNP100029210	9	28750985–28750932	78%	5.00E-20	100.00%
SNP100029235	9	29250216–29250263	69%	4.00E-15	97.92%
SNP100007965	9	28686518–28686586	100%	4.00E-28	100.00%

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**Table S9.** Gene pathways in the putative male-determining region (pMDR) of chromosome 9.

Accession	Start	Stop	Gene symbol
NC_040889.1	26947758	26970809	<i>aacs</i>
NC_040889.1	30847795	30861426	<i>adam9</i>
NC_040889.1	26390420	26404142	<i>adamts12</i>
NC_040889.1	28962819	29033180	<i>adamts3</i>
NC_040889.1	30211382	30223171	<i>adamtsl2</i>
NC_040889.1	26385900	26390295	<i>agpat9l</i>
NC_040889.1	29570659	29619317	<i>antxr2a</i>
NC_040889.1	26661903	26665407	<i>areg</i>
NC_040889.1	26467158	26537032	<i>arl15a</i>
NC_040889.1	29189724	29196923	<i>ascc2</i>
NC_040889.1	29474061	29478641	<i>bmp3</i>
NC_040889.1	30928254	31034308	<i>c5</i>
NC_040889.1	28716487	28734760	<i>camsap1b</i>
NC_040889.1	28281143	28293533	<i>castor1</i>
NC_040889.1	29388118	29402305	<i>cds1</i>
NC_040889.1	26877368	26880870	<i>cenatac</i>
NC_040889.1	28844892	28848124	<i>cfap157</i>
NC_040889.1	29480720	29530595	<i>cfap299</i>
NC_040889.1	31073113	31077836	<i>ciz1b</i>
NC_040889.1	27676311	27686895	<i>ckap2l</i>
NC_040889.1	29915395	29925731	<i>cnot6l</i>
NC_040889.1	27354336	27357287	<i>coq2</i>
NC_040889.1	27923912	27934545	<i>coq5</i>
NC_040889.1	26553159	26574479	<i>cspg4ba</i>
NC_040889.1	28802387	28805356	<i>ctsla</i>
NC_040889.1	28755219	28798749	<i>dapk1</i>

NC_040889.1	30609989	30628460	<i>ddr2l</i>
NC_040889.1	28849529	28864701	<i>dmrt1</i>
NC_040889.1	28876321	28880568	<i>dmrt2a</i>
NC_040889.1	28868330	28872251	<i>dmrt3a</i>
NC_040889.1	27399395	27402291	<i>emb</i>
NC_040889.1	31860028	31863675	<i>endog</i>
NC_040889.1	26654042	26655286	<i>epgn</i>
NC_040889.1	26907697	26913495	<i>erap1b</i>
NC_040889.1	30832192	30841733	<i>ercc6l</i>
NC_040889.1	29931209	29943567	<i>fam102aa</i>
NC_040889.1	28805986	28812813	<i>fbp2</i>
NC_040889.1	28324830	28329078	<i>fbxo21</i>
NC_040889.1	28309958	28321007	<i>fbxw8</i>
NC_040889.1	29532866	29539785	<i>fgf5</i>
NC_040889.1	31798841	31808193	<i>fibcd1a</i>
NC_040889.1	26439603	26445742	<i>fsta</i>
NC_040889.1	26929452	26944939	<i>gak</i>
NC_040889.1	26730830	26733022	<i>gas1a</i>
NC_040889.1	26376315	26382808	<i>gck</i>
NC_040889.1	27334953	27345579	<i>gldc</i>
NC_040889.1	29987080	29993754	<i>gle1</i>
NC_040889.1	26675986	26677766	<i>gpx8</i>
NC_040889.1	30057754	30059936	<i>gstt1a</i>
NC_040889.1	26298921	26308788	<i>hic2</i>
NC_040889.1	27405370	27407451	<i>hint2</i>
NC_040889.1	27704015	27720899	<i>hs3st1l2</i>
NC_040889.1	28598582	28602520	<i>hscb</i>
NC_040889.1	28018525	28024391	<i>hspb8</i>
NC_040889.1	30632390	30633453	<i>ier3</i>
NC_040889.1	27583895	27588971	<i>inpp5e</i>
NC_040889.1	26696158	26699205	<i>isca1</i>
NC_040889.1	26426635	26436919	<i>itga2.2</i>
NC_040889.1	27271678	27285028	<i>jak2b</i>
NC_040889.1	28817338	28844280	<i>kank1a</i>
NC_040889.1	28671809	28712932	<i>kcnt1b</i>
NC_040889.1	27881094	27922642	<i>kremen1</i>
NC_040889.1	27539101	27545163	<i>krt1-c5</i>
NC_040889.1	28177205	28234486	<i>ksr2</i>
NC_040889.1	27548017	27554766	<i>lhx6b</i>
NC_040889.1	26593906	26598594	<i>lipg</i>
NC_040889.1	29925783	29928056	LOC114861258
NC_040889.1	27452464	27463255	LOC114861276
NC_040889.1	27466983	27472812	LOC114861278
NC_040889.1	27463373	27466849	LOC114861280
NC_040889.1	27473105	27475509	LOC114861281
NC_040889.1	27442440	27447386	LOC114861282
NC_040889.1	26600843	26608843	LOC114861288
NC_040889.1	30666683	30676915	LOC114861410
NC_040889.1	30653013	30656027	LOC114861411
NC_040889.1	30646410	30648567	LOC114861412
NC_040889.1	31385686	31400274	LOC114861426

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NC_040889.1	31370489	31385568	LOC114861427
NC_040889.1	31230657	31250824	LOC114861429
NC_040889.1	31260556	31266183	LOC114861430
NC_040889.1	31302095	31319545	LOC114861431
NC_040889.1	31275601	31282969	LOC114861432
NC_040889.1	31291908	31300200	LOC114861434
NC_040889.1	31224149	31230556	LOC114861435
NC_040889.1	31358314	31362765	LOC114861437
NC_040889.1	31251062	31260188	LOC114861439
NC_040889.1	31335656	31339880	LOC114861440
NC_040889.1	31331035	31335905	LOC114861441
NC_040889.1	31362807	31371539	LOC114861442
NC_040889.1	31356086	31358532	LOC114861443
NC_040889.1	31266666	31270865	LOC114861445
NC_040889.1	31440505	31447663	LOC114861446
NC_040889.1	31319463	31324329	LOC114861447
NC_040889.1	31275849	31278060	LOC114861449
NC_040889.1	30804291	30806963	LOC114861464
NC_040889.1	29997913	30022057	LOC114861525
NC_040889.1	31580753	31595422	LOC114861556
NC_040889.1	31565052	31580614	LOC114861557
NC_040889.1	31552732	31557198	LOC114861561
NC_040889.1	31557240	31566102	LOC114861562
NC_040889.1	31550514	31552950	LOC114861563
NC_040889.1	28812996	28815962	LOC114861570
NC_040889.1	31867809	31903593	LOC114861577
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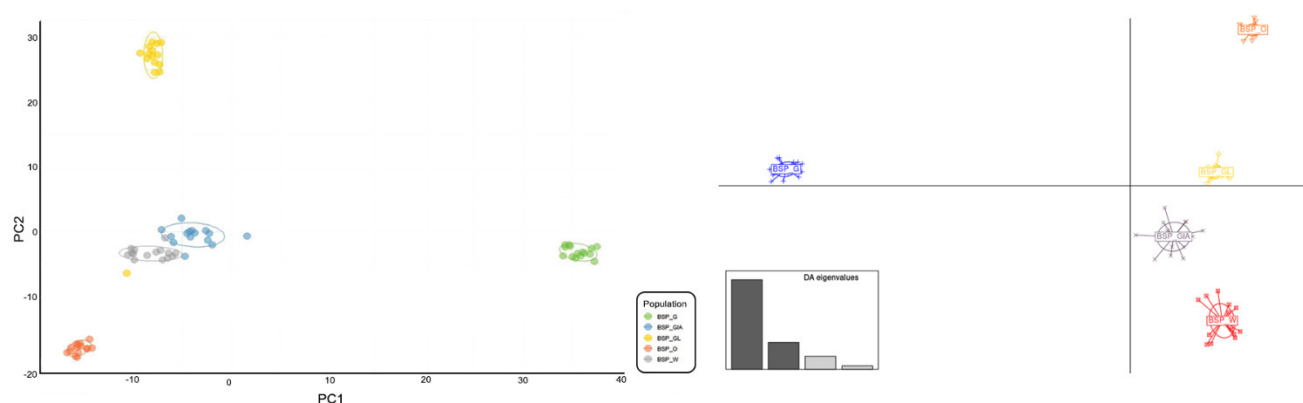
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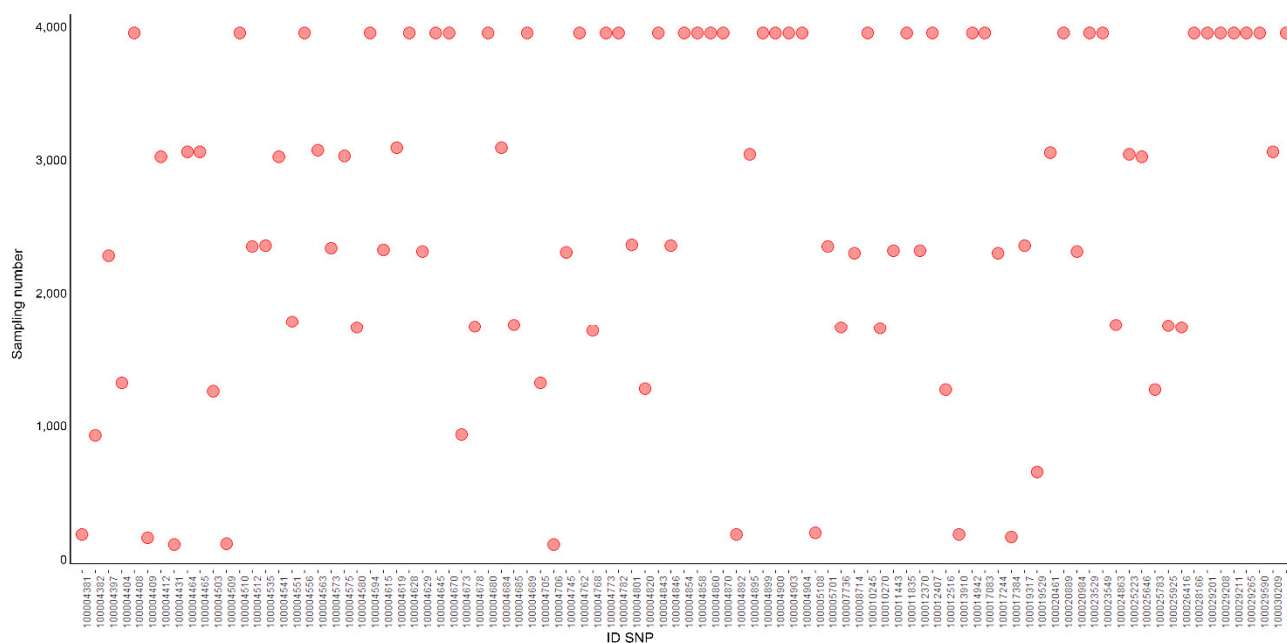
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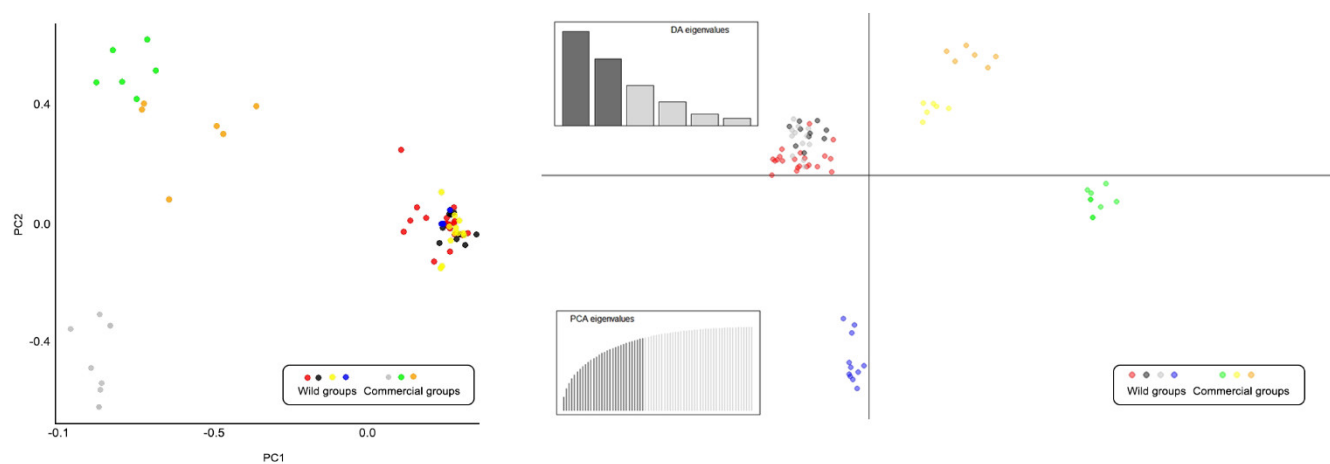
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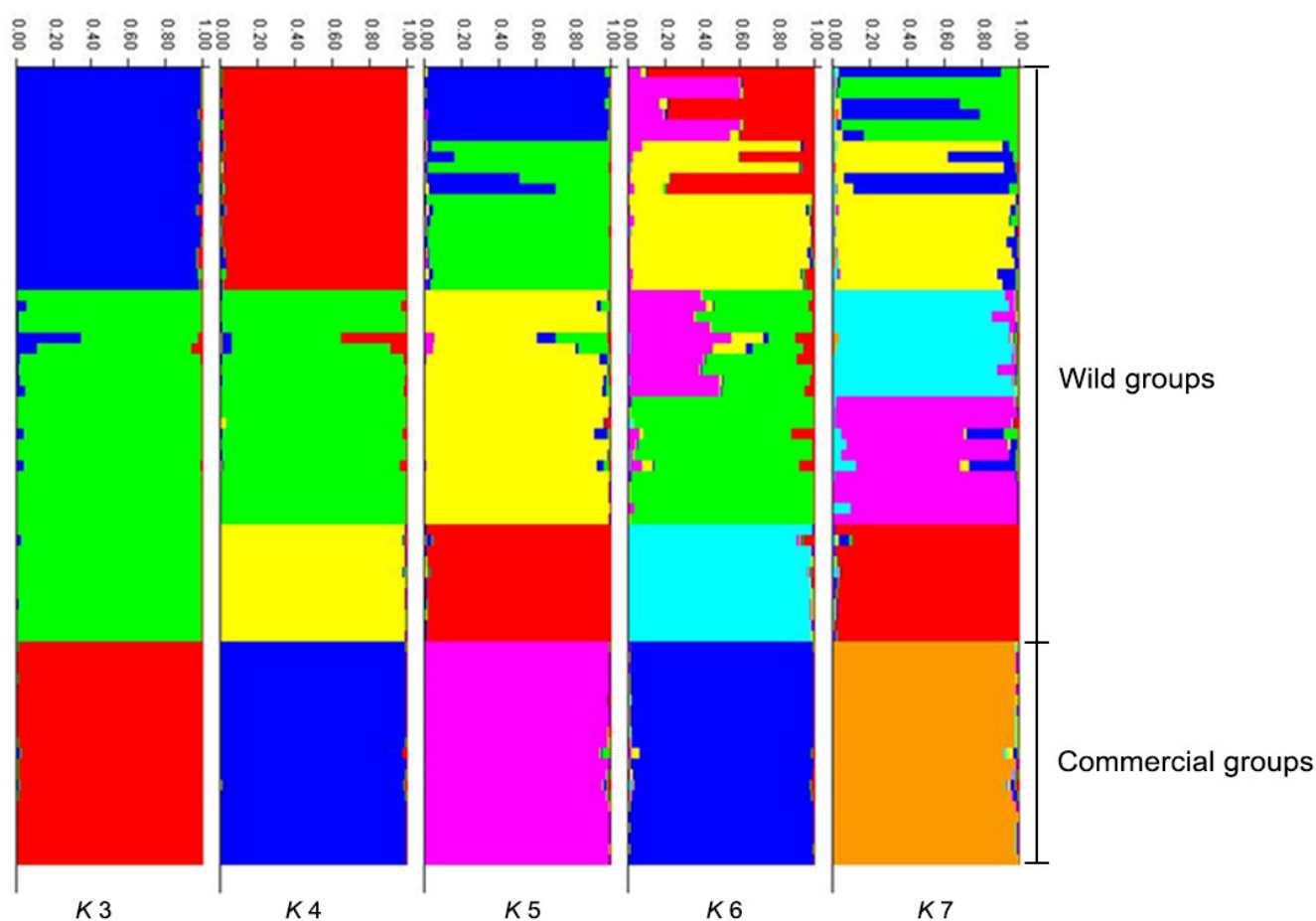
**Figure S1.** Principal component analysis of Siamese fighting fish (*Betta splendens* Regan, 1910) from local farms and discriminant analysis of principal component (DAPC) scatter plots of individuals.



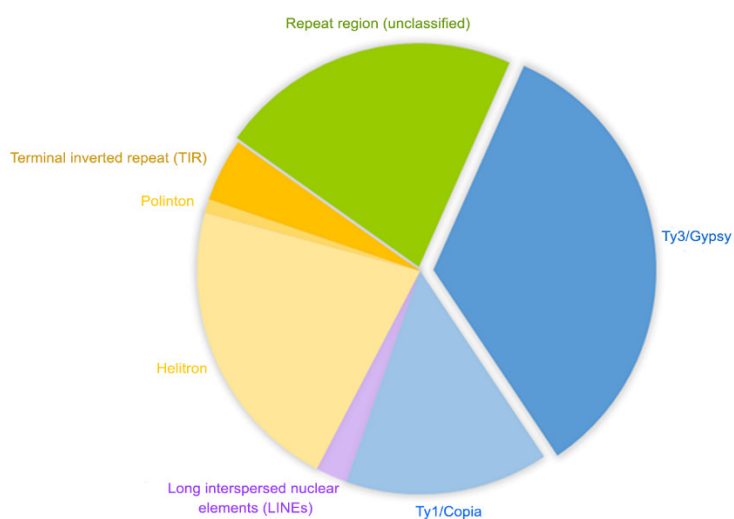
**Figure S2.** Monte Carlo randomization in Siamese fighting fish (*Betta splendens* Regan, 1910).



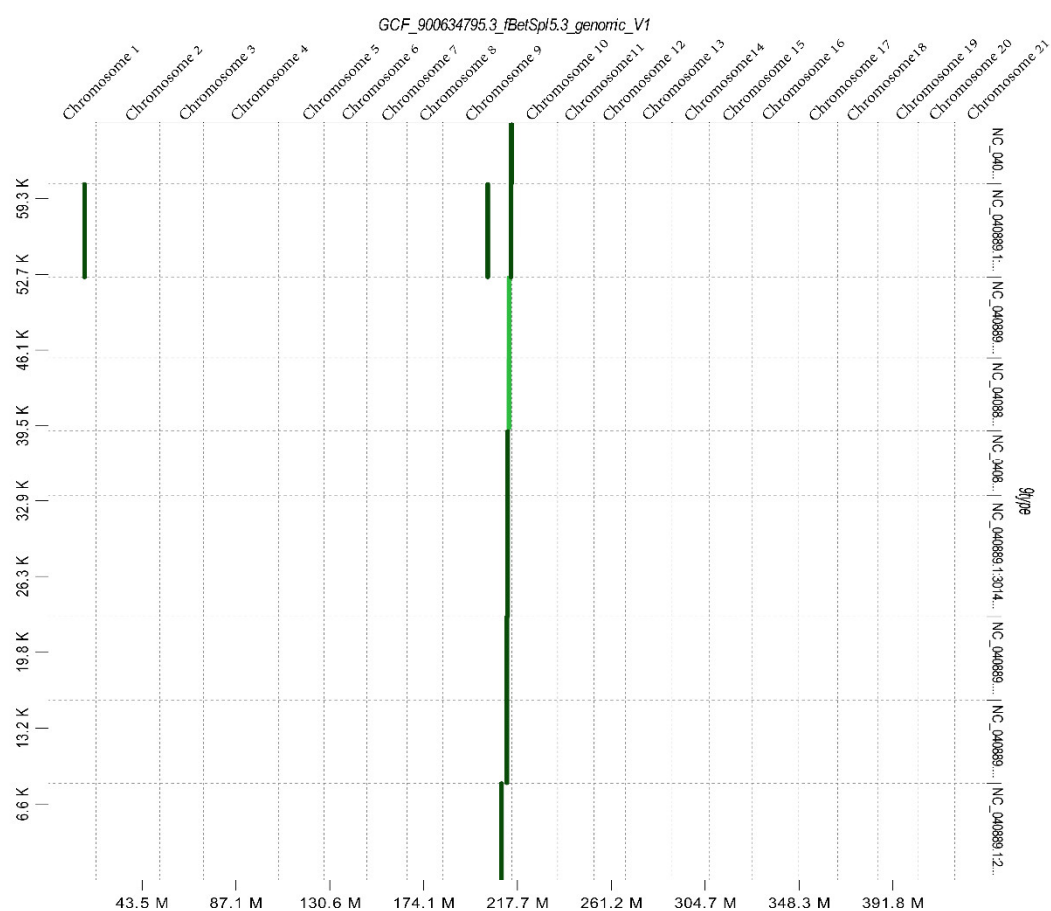
**Figure S3.** Principal component analysis of wild and commercial groups of Siamese fighting fish (*Betta splendens* Regan, 1910) and discriminant analysis of principal component (DAPC) scatter plots of individuals.



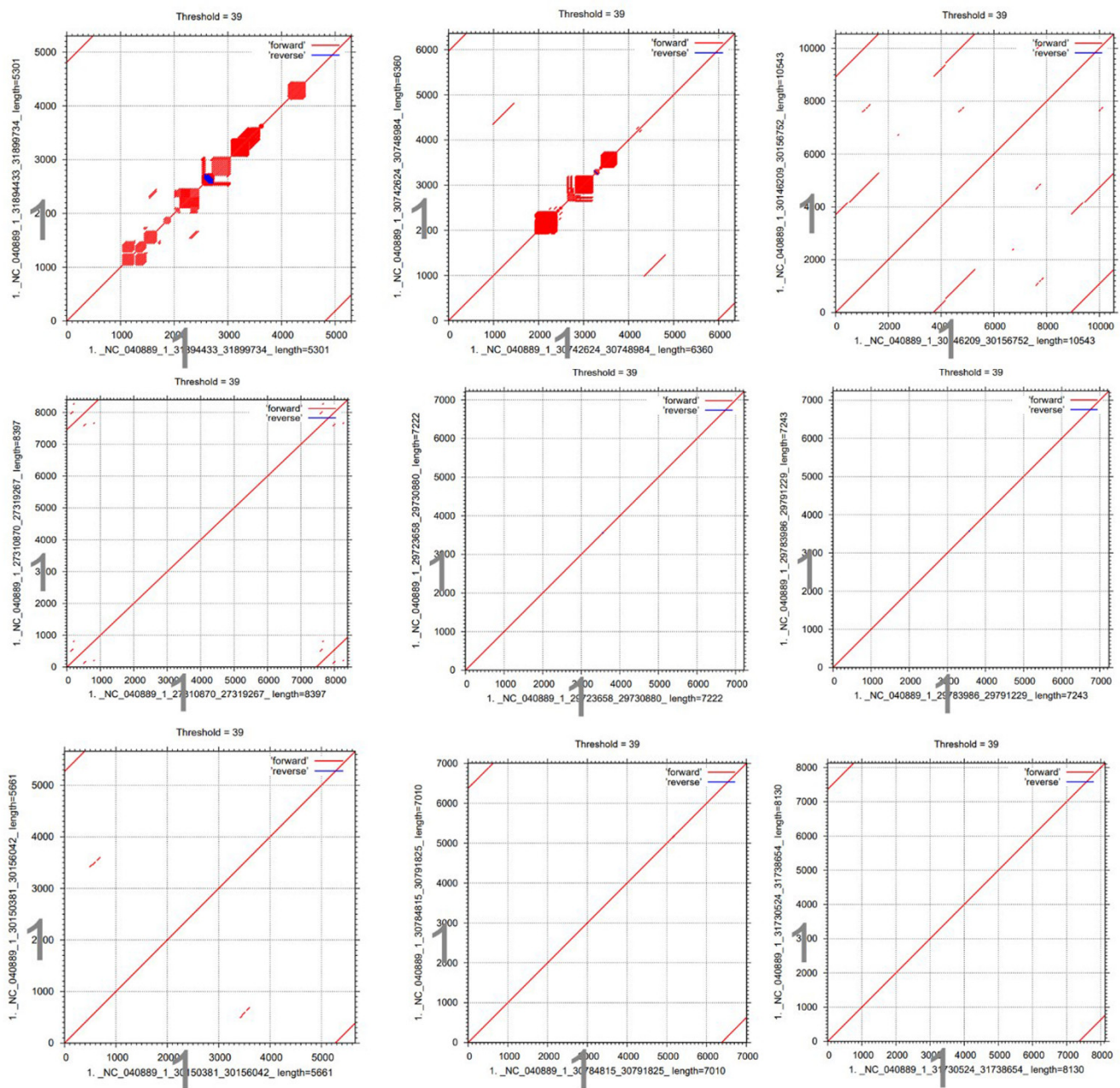
**Figure S4.** Population structure of 75 samples (54 wild and 21 commercial individuals) of Siamese fighting fish (*Betta splendens* Regan, 1910). Plot of Evanno's  $\Delta K$ . Structure bar plots depicting the results of model-based clustering inferred for  $K=3$ . Inferred genetic clusters are indicated by different colors. Each vertical bar on the x-axis represents an individual, and the y-axis represents the proportion of membership (posterior probability) in each genetic cluster,.



**Figure S5.** Repeat searches for single-nucleotide polymorphisms and restriction fragments of male-linked loci in Siamese fighting fish (*Betta splendens* Regan, 1910).

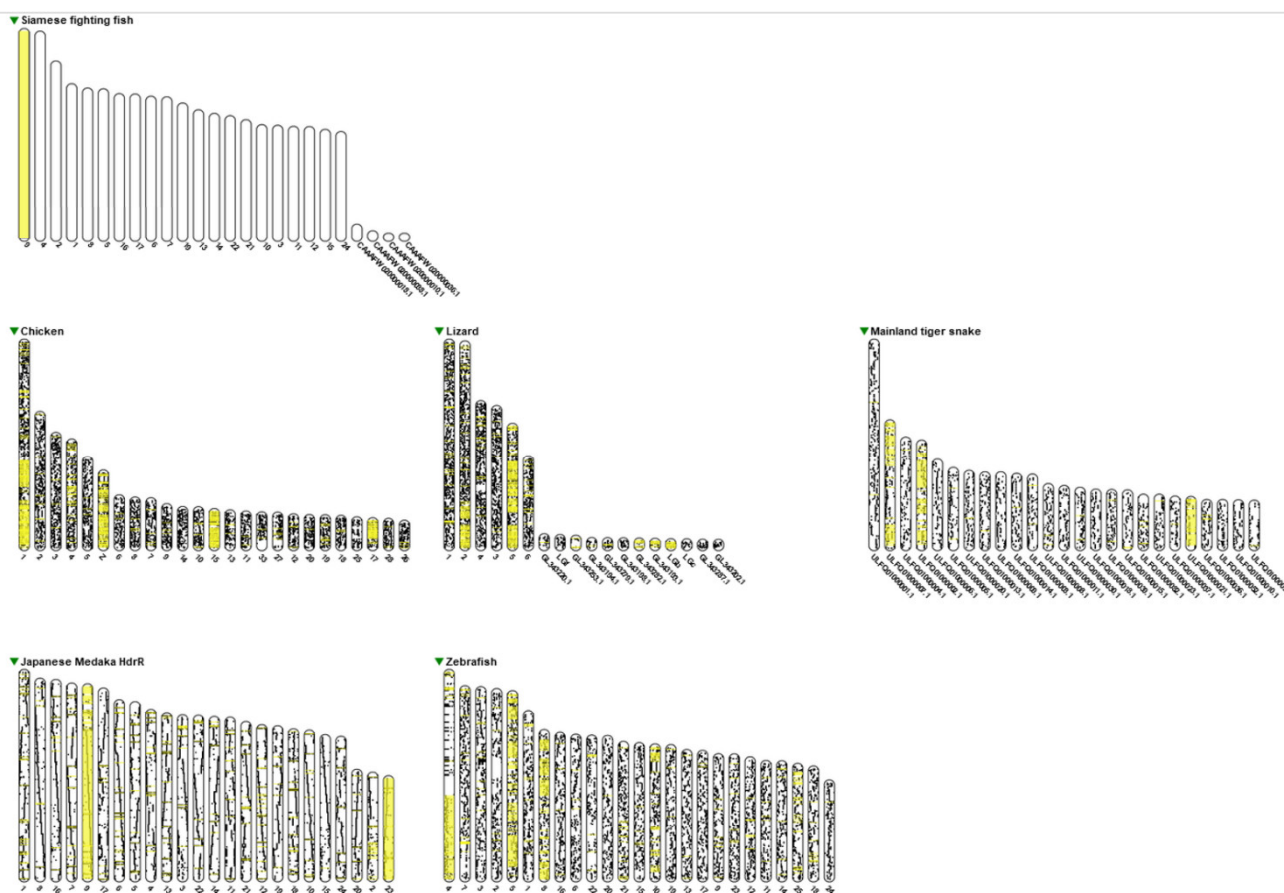


**Figure S6.** All copy numbers were intensively located on chromosome 9 of Siamese fighting fish (*Betta splendens* Regan, 1910), with a few observed on chromosome 1.



**Figure S7.** Dot matrix analysis showing that two of nine types had tandem-arrayed repetitive sequences.





**Figure S8.** Multi-karyotypes of Siamese fighting fish (*Betta splendens* Regan, 1910) with whole-genome sequences compared to those of other vertebrates.