

Supplementary Tables and Figures

Supplementary Table S1. Sequencing data generated for *D. mawsoni* genome assembly and annotation.

	Platform	Total reads No.	Total read base
Genome	Sequel	7,541,877	75,510,543,787
	Novaseq	874,915,446	132,112,232,346
Transcriptome	Sequel	993,300	72,377,817,589

Supplementary Table S2. Statistics for genome assembly of *D. mawsoni*.

	Falcon-unzip	3d-DNA
Number of contigs (scaffolds)	2,440	1,062
Total size of contigs (scaffolds)	924,749,474	926,329,754
Longest contig (scaffold)	24,490,688	46,819,977
N50 contig (scaffold) length	3,228,001	36,983,948
L50 contig (scaffold) count	65	12

Supplementary Table S3. Lengths of 24 chromosome scale scaffolds in *D. mawsoni* genome.

Scaffolds	Length (bp)
HiC_scaffold_5	46,819,977
HiC_scaffold_25	42,898,023
HiC_scaffold_10	42,290,140
HiC_scaffold_38	41,797,624
HiC_scaffold_22	41,781,106
HiC_scaffold_35	41,196,903
HiC_scaffold_26	40,017,477
HiC_scaffold_31	39,955,282
HiC_scaffold_1	39,888,709
HiC_scaffold_6	39,811,704
HiC_scaffold_21	38,643,262
HiC_scaffold_30	36,984,448
HiC_scaffold_4	36,385,023
HiC_scaffold_8	35,318,215
HiC_scaffold_41	35,000,612
HiC_scaffold_11	33,398,360
HiC_scaffold_3	32,861,413
HiC_scaffold_34	30,779,419
HiC_scaffold_32	29,174,432
HiC_scaffold_28	29,051,562
HiC_scaffold_13	26,820,266
HiC_scaffold_33	26,316,149
HiC_scaffold_24	24,955,770
HiC_scaffold_23	13,864,730
Sum	846,010,606

Supplementary Table S4. Benchmarking Universal Single-Copy Orthologs (BUSCO) evaluated for completeness of *D. mawsoni* genome assembly.

actinopterygii_odb9	No	%
Complete BUSCOs (C)	4,197	91.5
Complete and single-copy BUSCOs (S)	3,962	86.4
Complete and duplicated BUSCOs (D)	235	5.1
Fragmented BUSCOs (F)	194	4.3
Missing BUSCOs (M)	193	4.2
Total BUSCO groups searched	4,584	

Supplementary Table S5. Statistics of annotated transposable elements of *D. mawsoni*.

Class	Count	Length occupied (bp)	Percentage of sequences
DNA	62,227	17,248,270	1.86
Academ-1	2,496	641,403	0.07
CMC-Chapaev	177	42,051	0.00
CMC-EnSpm	37,730	15,509,198	1.67
Crypton-A	5,427	892,138	0.10
Crypton-C	56	5,995	0.00
Crypton-H	396	741,879	0.08
Crypton-V	2,079	706,938	0.08
Dada	779	375,943	0.04
Ginger-1	4,223	752,390	0.08
IS3EU	8,841	2,103,489	0.23
Kolobok-T2	63,460	14,974,973	1.62
MULE-MuDR	932	297,762	0.03
Merlin	2,218	785,005	0.08
Novosib	6,734	692,647	0.07
P	9,864	2,212,850	0.24
PIF	163	72,029	0.01
PIF-Harbinger	20,079	6,994,636	0.76
PIF-ISL2EU	2,632	1,314,889	0.14
PiggyBac	5,272	1,659,800	0.18
Sola-1	2,537	234,328	0.03
Sola-2	302	116,271	0.01
TcMar-Fot1	625	229,359	0.02
TcMar-ISRm11	1,361	433,465	0.05
TcMar-Tc1	13,921	3,162,543	0.34
TcMar-Tc2	633	152,091	0.02
Zator	1,009	216,560	0.02
Zisupton	8,959	3,053,901	0.33
hAT	4,660	989,755	0.11
hAT-Ac	114,317	31,354,354	3.38
hAT-Blackjack	5,550	2,062,972	0.22
hAT-Charlie	52,808	15,859,065	1.71
hAT-Pegasus	3,123	581,016	0.06
hAT-Tag1	2,040	325,427	0.04
hAT-Tip100	47,528	11,133,775	1.20
hAT-hAT19	122	73,829	0.01
hAT-hAT5	4,189	1,323,182	0.14
hAT-hAT6	421	98,292	0.01

	hAT-hATm	685	180,033	0.02
	hAT-hobo	2,730	716,015	0.08
LINE		353	251,342	0.03
	CR1	375	86,049	0.01
	I	8,065	3,323,406	0.36
	I-Jockey	1,584	307,115	0.03
	L1	6,680	3,081,651	0.33
	L1-Tx1	2,884	1,249,498	0.13
	L2	74,102	25,380,324	2.74
	Penelope	609	759,171	0.08
	Proto2	353	164,506	0.02
	R2-Hero	706	312,401	0.03
	R2-NeSL	136	121,874	0.01
	RTE-BovB	4,019	3,595,177	0.39
	RTE-X	1,399	565,362	0.06
	Rex-Babar	50,677	10,388,128	1.12
LTR		108	89,952	0.01
	Caulimovirus	136	50,747	0.01
	Copia	2,788	1,354,829	0.15
	DIRS	12,989	18,948,235	2.05
	ERV1	5,658	5,844,206	0.63
	ERVK	1,710	184,858	0.02
	Gypsy	21,970	9,951,831	1.07
	Ngaro	1,264	659,928	0.07
	Pao	2,942	1,873,823	0.20
RC	Helitron	7,371	4,182,969	0.45
SINE		7,211	1,182,027	0.13
	5S	564	121,010	0.01
	Alu	61	56,296	0.01
	ID	437	104,956	0.01
	MIR	19,474	2,654,456	0.29
	tRNA	696	116,016	0.01
	tRNA-Core	1,036	127,774	0.01
	tRNA-L1	1,317	252,943	0.03
Unknown		452,844	98,352,995	10.62
Total transposable elements		1,195,823	336,016,373	36.27

Supplementary Table S6. Genome assemblies used in this study.

Common name	Species	Assembly
Antarctic toothfish	<i>Dissostichus mawsoni</i>	This study
Antarctic icefish	<i>Chaenocephalus aceratus</i>	Kim et al., 2019*
Antarctic dragonfish	<i>Parachaenichthys charcoti</i>	Ahn et al., 2017**
Antarctic bullhead notothen	<i>Notothenia coriiceps</i>	GCA_000735185
Stickleback	<i>Gasterosteus aculeatus</i>	BROAD S1
Spiny chromis	<i>Acanthochromis polyacanthus</i>	ASM210954v1
Midas cichlid	<i>Amphilophus citrinellus</i>	Midas_v5
Orange clownfish	<i>Amphiprion percula</i>	Nemo_v1
Climbing perch	<i>Anabas testudineus</i>	fAnaTes1.1
Pachon cavefish	<i>Astyanax mexicanus</i>	Astyanax_mexicanus-1.0.2
Atlantic herring	<i>Clupea harengus</i>	Ch_v2.0.2
Zebrafish	<i>Danio rerio</i>	GRCz11
Cod	<i>Gadus morhua</i>	gadMor1
Coelacanth	<i>Latimeria chalumnae</i>	LatCha1
Nile tilapia	<i>Oreochromis niloticus</i>	O_niloticus_UMD_NMBU
Japanese medaka HNI	<i>Oryzias latipes</i>	ASM223471v1
Amazon molly	<i>Poecilia formosa</i>	Poecilia_formosa-5.1.2
Yellowtail amberjack	<i>Seriola lalandi dorsalis</i>	Sedor1
Fugu	<i>Takifugu rubripes</i>	fTakRub1.2
Platyfish	<i>Xiphophorus maculatus</i>	X_maculatus-5.0-male

* Kim et al., 2019, Nature Ecology & Evolution, 3, 469–478.

** Ahn et al., 2017, GigaScience, 6, gix060.

Supplementary Table S7. Statistics for genome annotation

Annotation database	Annotated number	Percentage (%)
No. Genes	32,914	-
nr Annotation	30,048	91.3
GO Annotation	24,920	75.7
KEGG Annotation	14,474	44.0
KOG Annotation	19,205	58.3
Pfam Annotation	20,937	63.6
Swissprot Annotation	24,263	73.7
TrEMBL Annotation	29,559	89.8
Interpro Annotation	29,994	91.1

	Count	Length Sum (bp)
Exon	319,003	55,190,635
CDS	316,045	51,218,172

Supplementary Table S8. Genome families of *D. mawsoni* and other fish species.

Species	No. genes	No. genes in orthogroups	No. f unassigned genes	Percentage of genes in orthogroups	Percentage of unassigned genes	No. orthogroups containing species	Percentage of orthogroups containing species	No. species-specific orthogroups	No. genes in species-specific orthogroups	Percentage of genes in species-specific orthogroups
<i>D. mawsoni</i>	32,914	29,718	3,196	90.3	9.7	16,896	67.3	434	1,431	4.3
<i>N. coriiceps</i>	32,661	29,195	3,466	89.4	10.6	18,378	73.2	101	238	0.7
<i>P. charcoti</i>	32,713	31,541	1,172	96.4	3.6	18,544	73.8	74	357	1.1
<i>C. aceratus</i>	30,773	28,579	2,194	92.9	7.1	17,133	68.2	187	611	2.0
<i>A. polyacanthus</i>	24,027	23,624	403	98.3	1.7	15,781	62.8	65	156	0.6
<i>A. citrinellus</i>	23,696	23,353	343	98.6	1.4	15,327	61.0	18	44	0.2
<i>A. percula</i>	23,926	23,385	541	97.7	2.3	15,467	61.6	22	102	0.4
<i>A. testudineus</i>	24,643	24,274	369	98.5	1.5	15,407	61.3	41	154	0.6
<i>A. mexicanus</i>	26,698	25,399	1,299	95.1	4.9	15,579	62.0	135	643	2.4
<i>C. harengus</i>	24,095	23,356	739	96.9	3.1	14,453	57.5	113	489	2.0
<i>D. rerio</i>	30,313	29,438	875	97.1	2.9	15,227	60.6	211	1,663	5.5
<i>G. morhua</i>	20,095	19,504	591	97.1	2.9	13,918	55.4	17	70	0.3
<i>G. aculeatus</i>	20,787	20,223	564	97.3	2.7	14,161	56.4	16	46	0.2
<i>L. chalumnae</i>	19,569	18,915	654	96.7	3.3	13,319	53.0	130	980	5.0
<i>O. niloticus</i>	28,189	27,449	740	97.4	2.6	15,359	61.2	98	629	2.2
<i>O. latipes</i>	22,127	21,519	608	97.3	2.7	14,544	57.9	33	224	1.0
<i>P. formosa</i>	23,615	23,447	168	99.3	0.7	15,191	60.5	26	82	0.3
<i>S. lalandi dorsalis</i>	24,755	24,224	531	97.9	2.1	15,811	63.0	66	141	0.6
<i>T. rubripes</i>	21,411	20,804	607	97.2	2.8	14,158	56.4	50	204	1.0
<i>X. maculatus</i>	23,774	23,285	489	97.9	2.1	15,304	60.9	36	137	0.6

Supplementary Table S9. Gene Ontology of expanded gene families in *D. mawsoni* genome among 20 fish species.

Category	GO ID	GO Name	No. genes	P-Value
Biological	GO:0048468	cell development	116	1.80E-15
Process	GO:0030154	cell differentiation	153	3.30E-13
	GO:0000904	cell morphogenesis involved in differentiation	49	9.70E-12
	GO:0010951	negative regulation of endopeptidase activity	35	1.50E-11
	GO:0006427	histidyl-tRNA aminoacylation	9	1.80E-11
	GO:0052548	regulation of endopeptidase activity	36	3.60E-11
	GO:0022008	neurogenesis	82	5.40E-11
	GO:0010466	negative regulation of peptidase activity	35	1.80E-10
	GO:0048667	cell morphogenesis involved in neuron differentiation	42	5.90E-10
	GO:0030030	cell projection organization	68	7.80E-10
	GO:0052547	regulation of peptidase activity	36	9.70E-10
	GO:0120036	plasma membrane bounded cell projection organization	67	1.00E-09
	GO:0048699	generation of neurons	73	1.30E-09
	GO:0035803	egg coat formation	13	2.60E-09
	GO:0030182	neuron differentiation	69	2.80E-09
	GO:0048812	neuron projection morphogenesis	42	3.00E-09
	GO:0048858	cell projection morphogenesis	42	3.40E-09
	GO:0120039	plasma membrane bounded cell projection morphogenesis	42	3.40E-09
	GO:0051346	negative regulation of hydrolase activity	37	4.70E-09
	GO:0007409	axonogenesis	38	4.90E-09
	GO:0032990	cell part morphogenesis	42	5.60E-09
	GO:0007339	binding of sperm to zona pellucida	13	7.90E-09
	GO:0031175	neuron projection development	47	9.00E-09
	GO:0048666	neuron development	58	9.10E-09
	GO:0061564	axon development	39	9.60E-09
	GO:0009988	cell-cell recognition	13	1.30E-08
	GO:0000902	cell morphogenesis	51	1.40E-08
	GO:0051336	regulation of hydrolase activity	65	2.10E-08
	GO:0032989	cellular component morphogenesis	47	2.60E-08
	GO:0007399	nervous system development	106	3.50E-08
	GO:0007292	female gamete generation	15	4.90E-08
GO:0016043	cellular component organization	215	8.90E-08	
GO:0030162	regulation of proteolysis	36	1.20E-07	
GO:0006418	tRNA aminoacylation for protein	17	1.40E-07	

		translation		
	GO:0048731	system development	209	2.50E-07
	GO:0043038	amino acid activation	17	3.00E-07
	GO:0043039	tRNA aminoacylation	17	3.00E-07
	GO:0048477	oogenesis	13	3.60E-07
	GO:0007411	axon guidance	25	4.60E-07
	GO:0097485	neuron projection guidance	25	5.40E-07
	GO:0015914	phospholipid transport	17	7.80E-07
	GO:0031290	retinal ganglion cell axon guidance	12	1.00E-06
	GO:0090156	cellular sphingolipid homeostasis	5	1.10E-06
	GO:0097742	de novo centriole assembly	5	1.10E-06
	GO:0098535	de novo centriole assembly involved in multi-ciliated epithelial cell differentiation	5	1.10E-06
	GO:0051639	actin filament network formation	5	1.10E-06
	GO:0051248	negative regulation of protein metabolic process	40	1.30E-06
	GO:0006820	anion transport	44	2.70E-06
	GO:0006378	mRNA polyadenylation	9	2.70E-06
	GO:0015748	organophosphate ester transport	17	2.80E-06
	GO:0007338	single fertilization	13	5.50E-06
	GO:0043086	negative regulation of catalytic activity	37	5.70E-06
	GO:1903251	multi-ciliated epithelial cell differentiation	5	6.20E-06
	GO:0006379	mRNA cleavage	8	7.00E-06
	GO:0051960	regulation of nervous system development	27	8.60E-06
	GO:0015698	inorganic anion transport	22	9.80E-06
	GO:0002062	chondrocyte differentiation	9	9.90E-06
Molecular	GO:0004821	histidine-tRNA ligase activity	9	1.80E-11
Function	GO:0004866	endopeptidase inhibitor activity	35	2.30E-11
	GO:0061135	endopeptidase regulator activity	35	4.10E-11
	GO:0030414	peptidase inhibitor activity	35	1.50E-10
	GO:0061134	peptidase regulator activity	35	8.70E-10
	GO:0140326	ATPase-coupled intramembrane lipid transporter activity	12	5.50E-08
	GO:0004857	enzyme inhibitor activity	35	2.10E-07
	GO:0016875	ligase activity, forming carbon-oxygen bonds	17	1.20E-06
	GO:0004812	aminoacyl-tRNA ligase activity	17	1.20E-06
	GO:0004396	hexokinase activity	6	1.70E-06
	GO:0004127	cytidylate kinase activity	5	6.20E-06
	GO:0051427	hormone receptor binding	10	7.60E-06

	GO:0035257	nuclear hormone receptor binding	9	9.90E-06
Cellular	GO:0002178	palmitoyltransferase complex	7	3.40E-08
Component	GO:0035339	SPOTS complex	5	1.10E-06
	GO:0031211	endoplasmic reticulum palmitoyltransferase complex	5	6.20E-06
	GO:0017059	serine C-palmitoyltransferase complex	5	6.20E-06
	GO:1905961	protein-cysteine S-palmitoyltransferase complex	5	6.20E-06

Supplementary Table S10. Gene Ontology of contracted gene families in *D. mawsoni* genome among 20 fish species.

Category	GO ID	GO Name	No. genes	P-Value
Biological	GO:0032482	Rab protein signal transduction	5	3.40E-06
Process	GO:1990778	protein localization to cell periphery	6	1.10E-07
	GO:0006904	vesicle docking involved in exocytosis	4	2.00E-06
	GO:0017157	regulation of exocytosis	5	1.10E-06
	GO:0030011	maintenance of cell polarity	3	3.90E-06
	GO:0048731	system development	25	9.50E-06
	GO:1903361	protein localization to basolateral plasma membrane	3	2.80E-07
	GO:0007399	nervous system development	16	2.60E-06
	GO:0035090	maintenance of apical/basal cell polarity	3	2.40E-06
	GO:0045199	maintenance of epithelial cell apical/basal polarity	3	2.40E-06
	Molecular Function	GO:0004652	polynucleotide adenylyltransferase activity	3
GO:1990817		RNA adenylyltransferase activity	3	7.00E-07
Cellular	GO:0098793	presynapse	7	2.90E-06
Component	GO:0032593	insulin-responsive compartment	3	7.10E-08

Supplementary Table S11. Gene Ontology of specific gene families in *D. mawsoni* genome among four Antarctic fish.

Category	GO ID	GO Name	No. genes	P-Value
Biological Process	GO:1903432	regulation of TORC1 signaling	7	3.50E-05
	GO:0032008	positive regulation of TOR signaling	8	4.30E-05
	GO:0038202	TORC1 signaling	7	5.20E-05
	GO:0090114	COPII-coated vesicle budding	7	5.20E-05
	GO:1904263	positive regulation of TORC1 signaling	7	5.40E-06
	GO:0048194	Golgi vesicle budding	7	1.90E-04
	GO:0006259	DNA metabolic process	107	5.20E-20
	GO:0070278	extracellular matrix constituent secretion	7	3.00E-06
	GO:0007029	endoplasmic reticulum organization	7	1.90E-04
	GO:0071897	DNA biosynthetic process	53	1.60E-29
	GO:0015074	DNA integration	24	1.20E-04
	GO:0036372	opsin transport	7	7.70E-07
	GO:0006278	RNA-dependent DNA biosynthetic process	53	1.10E-34
	GO:0098529	neuromuscular junction development, skeletal muscle fiber	3	1.50E-04
	Molecular Function	GO:0038024	cargo receptor activity	19
GO:0140097		catalytic activity, acting on DNA	62	3.50E-22
GO:0030246		carbohydrate binding	43	5.00E-09
GO:0005044		scavenger receptor activity	19	1.30E-07
GO:0008061		chitin binding	8	1.30E-04
GO:0034061		DNA polymerase activity	51	3.30E-31
GO:0003964		RNA-directed DNA polymerase activity	51	2.30E-35
Cellular Component	GO:0016779	nucleotidyltransferase activity	58	2.90E-20
	GO:0031080	nuclear pore outer ring	7	5.40E-06
	GO:0030127	COPII vesicle coat	8	1.00E-04
	GO:0070971	endoplasmic reticulum exit site	7	1.60E-06
	GO:0012507	ER to Golgi transport vesicle membrane	9	4.20E-05
GO:0030134	COPII-coated ER to Golgi transport vesicle	9	1.70E-04	

Supplementary Table S12. Characterization of transcripts based on splice junctions.

Category	No. Isoforms	No. Genes
Full Splice Match (FSM)	1,189	1,007
Incomplete Splice Match (ISM)	8,166	3,679
Novel In Catalog (NIC)	1,288	902
Novel Not in Catalog (NNC)	9,165	3,966
Genic Genomic	1,981	1,757
Antisense	1,911	257
Fusion	280	160
Intergenic	7,500	7,500

Supplementary Table S13. Summary of transcripts based on structural categories and subcategories.

Categories/Subcategories	anti-sense	FSM	fusion	genic	ISM	intergenic	NIC	NNC	Total
Annotated Transcripts									
3prime_fragment					200				200
5prime_fragment					4,009				4,009
internal_fragment					409				409
intron_retention					257				257
mono-exon		39			3,291				3,330
multi-exon		1,150							1,150
		1,189			8,166				9,355
Novel Transcripts									
at_least_one_novel_splicesite								7,579	7,579
combination_of_known_junctions							1		1
combination_of_known_splicesites							87		87
intron_retention			108				538	1586	2,232
mono-exon	1,185			1918		7032	479		10,614
mono-exon_by_intron_retention							183		183
multi-exon	726		172	63		468			1,429
	1,911		280	1,981		7,500	1,288	9,165	22,125
Total	1,911	1,189	280	1,981	8,166	7,500	1,288	9,165	31,480

Supplementary Table S14. Genes with more than 10 splicing variants.

Cluster ID	No. Isoform	Description	Scaffold	Gene start	Gene End	Transcript length	No. exons
Cluster00001	94	protein FAM133-like	HiC_scaffold_35	26365291	26385663	2731	26
Cluster00002	80	collagen alpha-2(I) chain isoform X1	HiC_scaffold_3	13801711	13810315	3489	27
Cluster00003	74	collagen alpha-1(I) chain-like	HiC_scaffold_41	34480271	34501552	5600	50
Cluster00004	68	collagen alpha-1(I) chain-like	HiC_scaffold_28	21631468	21649356	5597	51
Cluster00005	35	keratin, type I cytoskeletal 13-like	HiC_scaffold_41	6557802	6661239	3348	21
Cluster00006	33	intermediate filament protein ON3-like isoform X1	HiC_scaffold_10	7466753	7478205	3205	17
Cluster00007	31	nucleolar protein 56	HiC_scaffold_25	16890203	16896544	2200	12
Cluster00008	29	nucleolin isoform X1	HiC_scaffold_38	19807945	19816808	3292	18
Cluster00009	27	collagen alpha-1(XVII) chain-like isoform X1	HiC_scaffold_8	15681495	15693908	3839	36
Cluster00010	25	desmoplakin-like isoform X2	HiC_scaffold_38	29900296	29918571	5578	25
Cluster00011	24	leukocyte elastase inhibitor-like isoform X1	HiC_scaffold_33	9263919	9289857	4143	32
Cluster00012	24	polyadenylate-binding protein 1	HiC_scaffold_3	17579339	17585539	2813	14
Cluster00013	22	major histocompatibility complex class I-related gene protein-like	HiC_scaffold_3	2729636	3134308	3374	21
Cluster00014	22	gelsolin-like	HiC_scaffold_38	37524652	37537161	2532	16
Cluster00015	22	keratin, type I cytoskeletal 13-like	HiC_scaffold_31	2677155	2691323	1498	8
Cluster00016	21	myosin-9 isoform X1	HiC_scaffold_41	28581753	28630836	5892	36
Cluster00017	21	actin, cytoplasmic 1	HiC_scaffold_41	26070013	26074393	1855	7
Cluster00018	20	arachidonate 15-lipoxygenase B-like	HiC_scaffold_30	25625705	25657011	5087	27
Cluster00019	19	cadherin-like protein 26 von Willebrand factor A	HiC_scaffold_10	24885335	24895171	3425	17
Cluster00020	18	domain-containing protein 5A-like	HiC_scaffold_35	26670764	26683684	2959	18
Cluster00021	17	elongation factor 2-like	HiC_scaffold_38	13441256	13448740	3253	13
Cluster00022	17	calpastatin isoform X3	HiC_scaffold_25	17073302	17087875	2895	34
Cluster00023	16	heat shock protein HSP 90-beta	HiC_scaffold_32	16767846	16787170	2595	13
Cluster00024	16	heat shock cognate 71 kDa protein	HiC_scaffold_11	31918180	31946563	3271	19
Cluster00025	16	serine/arginine repetitive matrix protein 1 isoform X1	HiC_scaffold_4	12547785	12558681	3248	16
Cluster00026	16	gelsolin-like isoform X1	HiC_scaffold_21	2434770	2511589	2916	25
Cluster00027	15	keratin, type I cytoskeletal 13-like	HiC_scaffold_58	3393820	3413886	2818	15
Cluster00028	15	14-3-3 protein beta/alpha	HiC_scaffold_4	17676840	17683696	2101	6
Cluster00029	15	Tubulin beta chain	HiC_scaffold_4	8929775	8940692	2661	14

Cluster00030	15	eukaryotic translation initiation factor 4B isoform X2	HiC_scaffold_25	17730695	17742324	2912	15
Cluster00031	14	poly(U)-binding-splicing factor PUF60 isoform X2	HiC_scaffold_33	17506101	17514878	2114	12
Cluster00032	14	B-cadherin-like	HiC_scaffold_8	32179861	32193991	2508	17
Cluster00033	14	vigilin isoform X2 plasminogen activator inhibitor 1	HiC_scaffold_38	39094368	39124447	3780	28
Cluster00034	14	RNA-binding protein-like isoform X1	HiC_scaffold_38	15108054	15114110	2636	9
Cluster00035	14	integrin alpha-6-like isoform X1	HiC_scaffold_21	23521889	23548589	3736	25
Cluster00036	13	T-complex protein 1 subunit beta	HiC_scaffold_13	24962254	24972478	1654	15
Cluster00037	13	T-complex protein 1 subunit alpha	HiC_scaffold_32	14758680	14764325	2345	12
Cluster00038	13	14-3-3 protein beta/alpha-B-like	HiC_scaffold_3	17568819	17577116	2304	6
Cluster00039	13	SPARC	HiC_scaffold_22	37884453	37917685	1031	11
Cluster00040	13	casein kinase I isoform X4	HiC_scaffold_22	5822369	5846780	1802	12
Cluster00041	13	T-complex protein 1 subunit zeta	HiC_scaffold_11	13756192	13763360	1839	14
Cluster00042	13	periostin-like isoform X1	HiC_scaffold_35	13775685	13788301	3207	21
Cluster00043	13	nucleolar RNA helicase 2	HiC_scaffold_5	17425608	17434827	3184	16
Cluster00044	13	probable ATP-dependent RNA helicase DDX5 isoform X1	HiC_scaffold_41	30357857	30376133	2603	16
Cluster00045	13	26S proteasome non-ATPase regulatory subunit 1	HiC_scaffold_38	18843247	18879448	3660	27
Cluster00046	13	little elongation complex subunit 2	HiC_scaffold_26	22168996	22181557	4049	24
Cluster00047	13	collagen alpha-2(VI) chain isoform X1	HiC_scaffold_21	27611019	27626632	3223	32
Cluster00048	12	actin-related protein 2-like	HiC_scaffold_24	14963435	15009575	2856	18
Cluster00049	12	eukaryotic peptide chain release factor subunit 1	HiC_scaffold_22	20296399	20304537	2143	11
Cluster00050	12	signal peptide peptidase-like 2A isoform X2	HiC_scaffold_5	14080868	14099489	2101	15
Cluster00051	12	probable global transcription activator SNF2L2	HiC_scaffold_31	29394350	29460810	4719	34
Cluster00052	12	ATP synthase subunit alpha, mitochondrial-like	HiC_scaffold_31	24443187	24449589	2035	11
Cluster00053	12	clustered mitochondria protein homolog	HiC_scaffold_31	14133807	14146588	4553	29
Cluster00054	12	coatamer subunit beta'	HiC_scaffold_21	23637773	23646440	3093	21
Cluster00055	12	deoxycytidylate deaminase	HiC_scaffold_10	21871921	21884100	1596	8
Cluster00056	11	elongation factor 1-gamma	HiC_scaffold_22	22132085	22139863	1524	10
Cluster00057	11	Eukaryotic translation initiation factor 3 subunit C	HiC_scaffold_28	14233086	14244750	3116	21
Cluster00058	11	eukaryotic translation initiation	HiC_scaffold_30	21370913	21378972	2743	19

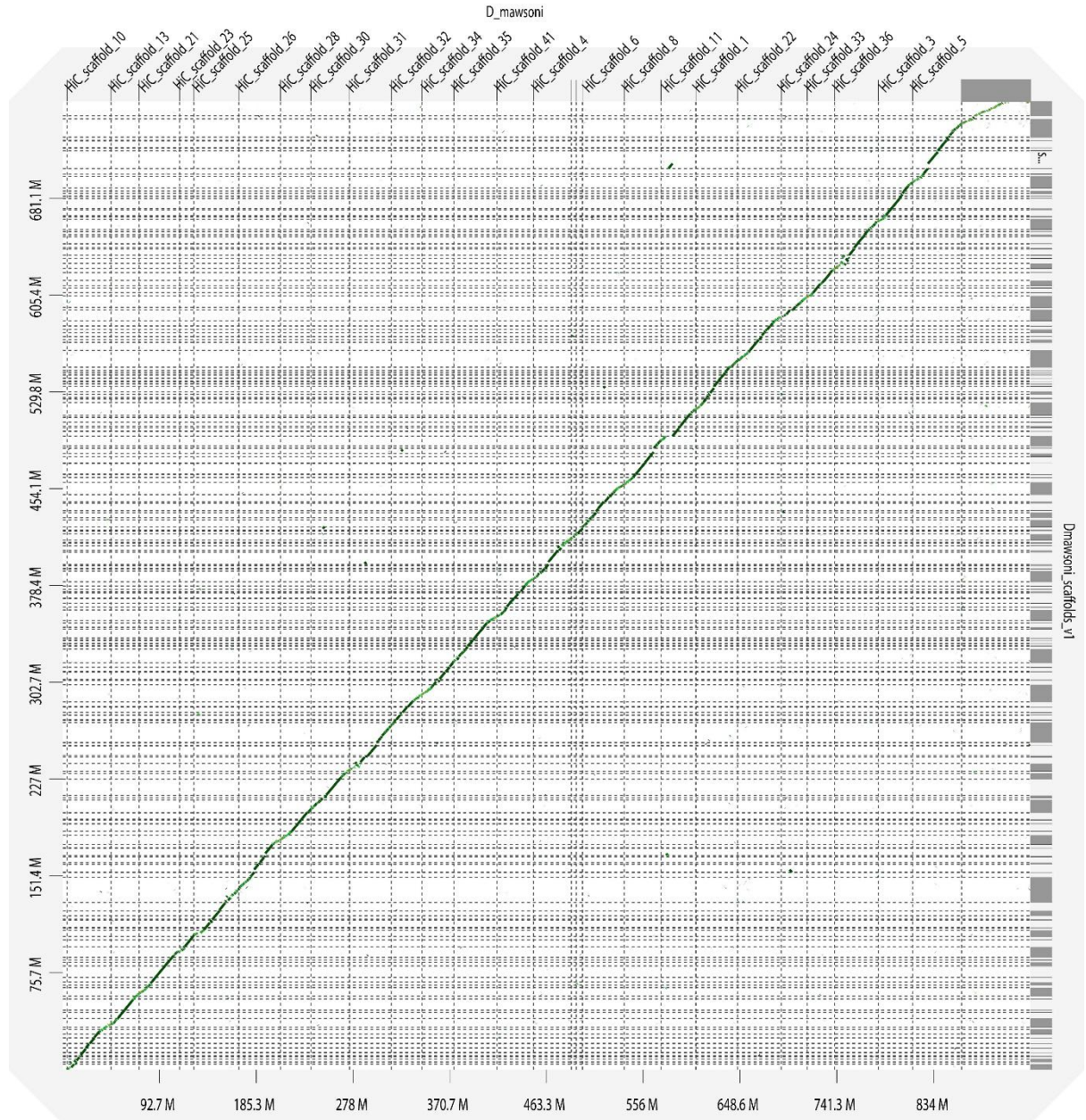
		factor 3 subunit B					
Cluster00059	11	sciellin isoform X1	HiC_scaffold_6	24416009	24428016	1669	16
Cluster00060	11	collagen alpha-2(V) chain-like	HiC_scaffold_6	7838977	7850871	2400	24
Cluster00061	11	eukaryotic translation initiation factor 5	HiC_scaffold_4	23203238	23213907	1687	10
Cluster00062	11	trichohyalin-like isoform X1	HiC_scaffold_1	16522098	16529634	2877	15
Cluster00063	11	plakophilin-3 isoform X2	HiC_scaffold_26	23672015	23689174	3769	14
Cluster00064	11	deoxyribonuclease-1-like	HiC_scaffold_10	21273262	21356630	2227	14
Cluster00065	10	ATP synthase subunit beta, mitochondrial	HiC_scaffold_23	9541936	9545604	1943	10
Cluster00066	10	nucleolar protein 58	HiC_scaffold_23	8965292	8970790	2095	15
Cluster00067	10	T-complex protein 1 subunit eta	HiC_scaffold_24	6520313	6525351	2224	12
Cluster00068	10	protein max isoform X4	HiC_scaffold_32	18428157	18440808	656	4
Cluster00069	10	H-2 class I histocompatibility antigen, Q10 alpha chain-like isoform X2	HiC_scaffold_3	28771169	28780450	2418	9
Cluster00070	10	alcohol dehydrogenase class-3	HiC_scaffold_22	29815705	29848625	2472	21
Cluster00071	10	heat shock 70 kDa protein 4-like	HiC_scaffold_22	18421659	18430960	2968	19
Cluster00072	10	histidine--tRNA ligase, cytoplasmic isoform X2	HiC_scaffold_22	5176609	5190002	2000	12
Cluster00073	10	heterogeneous nuclear ribonucleoprotein A/B-like	HiC_scaffold_11	2874581	2886698	1937	9
Cluster00074	10	N-acetylgalactosaminyltransferase 7 isoform X1	HiC_scaffold_30	1393678	1422330	3272	18
Cluster00075	10	26S proteasome non-ATPase regulatory subunit 2	HiC_scaffold_35	34459933	34477617	2898	21
Cluster00076	10	Actin-related protein 3	HiC_scaffold_6	3244610	3277669	2716	15
Cluster00077	10	ras GTPase-activating-like protein IQGAP1	HiC_scaffold_5	34372247	34419569	4674	37
Cluster00078	10	volume-regulated anion channel subunit LRRC8D-like	HiC_scaffold_41	28488992	28512796	3628	15
Cluster00079	10	calpain-9	HiC_scaffold_30	30296192	30299552	537	4
Cluster00080	10	oxysterol-binding protein-related protein 7-like isoform X2	HiC_scaffold_41	4378065	4404346	3508	22
Cluster00081	10	alpha-actinin-1 isoform X3	HiC_scaffold_4	27863264	27909704	3708	26
Cluster00082	10	kinectin isoform X1	HiC_scaffold_4	16399032	16417531	4583	33
Cluster00083	10	putative aminopeptidase W07G4.4	HiC_scaffold_31	25246354	25252805	1633	16
Cluster00084	10	coronin-1C isoform X2	HiC_scaffold_31	13463564	13471002	990	9
Cluster00085	10	E3 ubiquitin-protein ligase UBR5 isoform X3	HiC_scaffold_1	31203150	31233999	8845	59
Cluster00086	10	desmocollin-2-like	HiC_scaffold_21	19071982	19078911	3294	17

Cluster00087	10	nucleolar GTP-binding protein 1	HiC_scaffold_21	6882345	6896824	2519	17
Cluster00088	10	WD repeat-containing protein 1	HiC_scaffold_25	17170593	17179958	1843	14
Cluster00089	10	mucin-5AC-like	HiC_scaffold_25	6481679	6585019	12786	87
Cluster00090	10	cellular nucleic acid-binding protein isoform X1	HiC_scaffold_10	24689606	24693643	1564	5
Cluster00091	10	14-3-3 protein beta/alpha-1	HiC_scaffold_10	23663526	23667072	1749	7
Cluster00092	10	deoxynucleoside triphosphate triphosphohydrolase SAMHD1	HiC_scaffold_10	21733242	21745122	2541	15

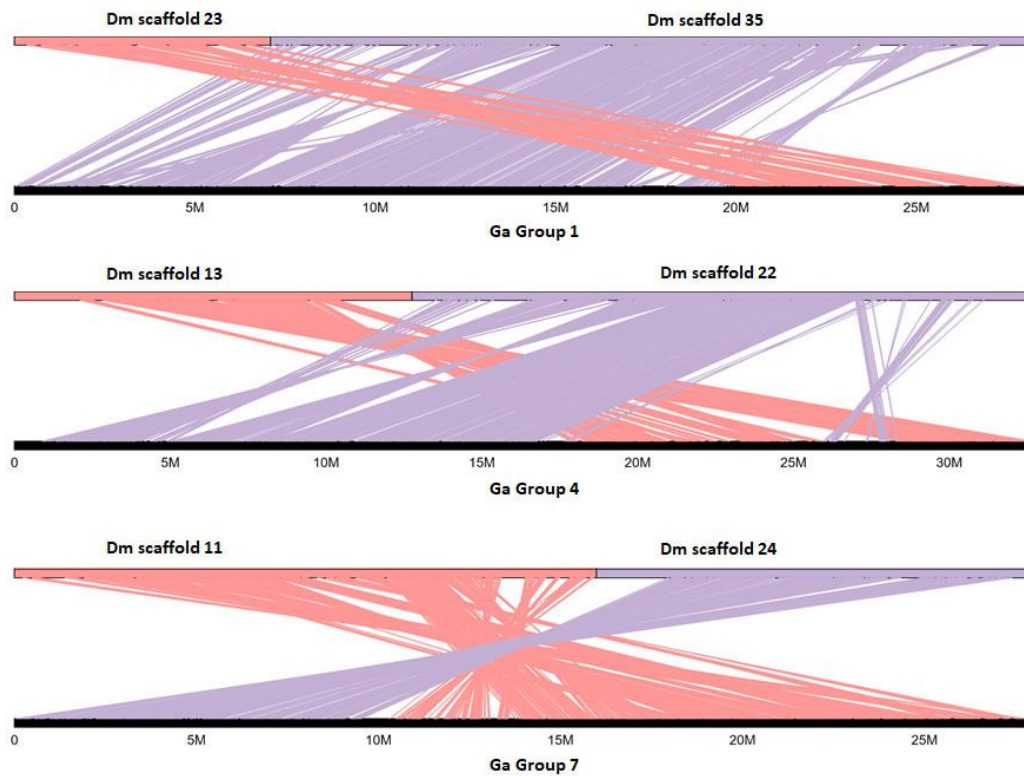
Supplementary Table S15. Gene Ontology of genes with more than 10 splicing variants.

GO Type	GO ID	GO Name	P-Value
Biological Process	GO:0007010	cytoskeleton organization	2.0E-05
	GO:0009894	regulation of catabolic process	2.4E-05
	GO:0043933	protein-containing complex subunit organization	3.7E-05
	GO:0022613	ribonucleoprotein complex biogenesis	4.4E-05
	GO:0030036	actin cytoskeleton organization	7.1E-05
	GO:0034622	cellular protein-containing complex assembly	1.2E-04
	GO:0097435	supramolecular fiber organization	1.6E-04
	GO:0032989	cellular component morphogenesis	2.4E-04
	GO:0006412	translation	2.9E-04
	GO:0043043	peptide biosynthetic process	3.3E-04
	GO:0022607	cellular component assembly	4.4E-04
	GO:0006518	peptide metabolic process	4.8E-04
	GO:0043604	amide biosynthetic process	5.3E-04
	GO:1901566	organonitrogen compound biosynthetic process	6.4E-04
	GO:0048731	system development	7.2E-04
	GO:0065003	protein-containing complex assembly	7.2E-04
	GO:0061061	muscle structure development	9.2E-04
	GO:0043603	cellular amide metabolic process	1.3E-03
	GO:0006996	organelle organization	1.8E-03
	GO:0009888	tissue development	2.0E-03
	GO:0050790	regulation of catalytic activity	6.1E-03
	GO:0044265	cellular macromolecule catabolic process	6.5E-03
	GO:0048513	animal organ development	6.7E-03
	GO:0009057	macromolecule catabolic process	8.8E-03
	GO:0048468	cell development	9.1E-03
	GO:0030154	cell differentiation	1.2E-02
	GO:0006508	proteolysis	2.0E-02
	GO:0009790	embryo development	2.1E-02
	GO:0060429	epithelium development	2.3E-02
	GO:0019538	protein metabolic process	3.3E-02
	GO:1901564	organonitrogen compound metabolic process	3.5E-02
	GO:0072359	circulatory system development	3.8E-02
	GO:0051246	regulation of protein metabolic process	4.2E-02
Molecular Function	GO:0003723	RNA binding	1.2E-11
	GO:0008135	translation factor activity, RNA binding	4.3E-06
	GO:0035639	purine ribonucleoside triphosphate binding	4.1E-04
	GO:0032555	purine ribonucleotide binding	4.8E-04
	GO:0097367	carbohydrate derivative binding	8.1E-04
	GO:0000166	nucleotide binding	1.0E-03
	GO:1901265	nucleoside phosphate binding	1.0E-03

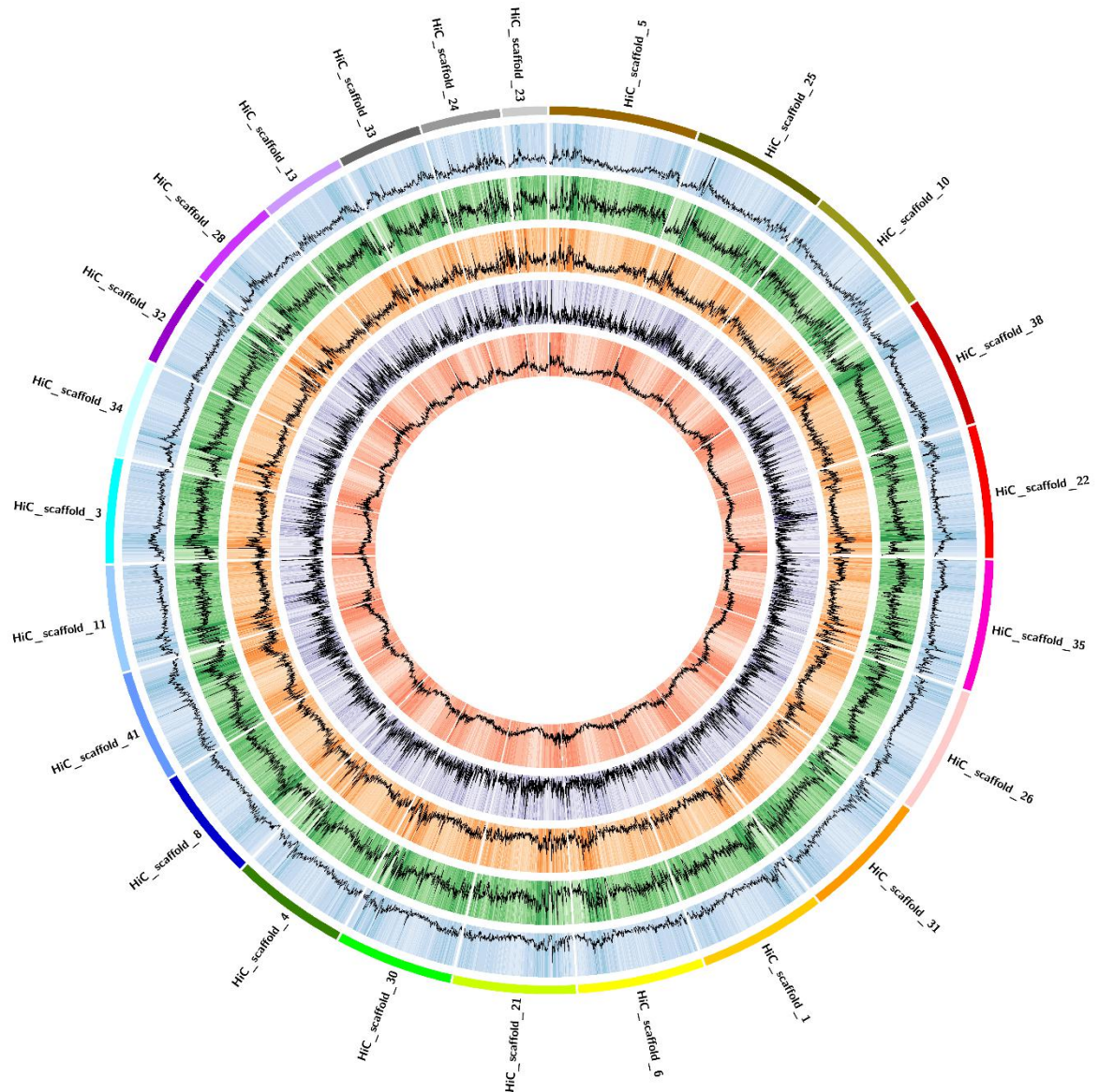
	GO:0043168	anion binding	1.1E-03
	GO:0005524	ATP binding	1.4E-03
	GO:0032559	adenyl ribonucleotide binding	1.6E-03
	GO:0030554	adenyl nucleotide binding	1.6E-03
	GO:0036094	small molecule binding	2.3E-03
	GO:0003676	nucleic acid binding	4.6E-03
	GO:0005509	calcium ion binding	4.7E-03
	GO:0016887	ATPase activity	4.8E-03
	GO:0008092	cytoskeletal protein binding	6.0E-03
	GO:0004175	endopeptidase activity	9.4E-03
	GO:0017111	nucleoside-triphosphatase activity	9.5E-03
	GO:0003779	actin binding	9.8E-03
	GO:0016462	pyrophosphatase activity	1.1E-02
	GO:0016818	hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	1.1E-02
	GO:0016817	hydrolase activity, acting on acid anhydrides	1.2E-02
	GO:0016787	hydrolase activity	2.7E-02
	GO:0070011	peptidase activity, acting on L-amino acid peptides	3.0E-02
Cellular	GO:0099081	supramolecular polymer	1.2E-08
component	GO:0099512	supramolecular fiber	1.2E-08
	GO:0099513	polymeric cytoskeletal fiber	2.9E-06
	GO:0005882	intermediate filament	9.8E-06
	GO:0045111	intermediate filament cytoskeleton	1.1E-05
	GO:0005856	cytoskeleton	1.3E-05
	GO:0043232	intracellular non-membrane-bounded organelle	1.4E-05
	GO:0043228	non-membrane-bounded organelle	1.5E-05
	GO:0031981	nuclear lumen	7.2E-04
	GO:0070013	intracellular organelle lumen	1.7E-03
	GO:0043233	organelle lumen	1.7E-03
	GO:0005634	nucleus	2.0E-03
	GO:0043231	intracellular membrane-bounded organelle	4.1E-03
	GO:0043227	membrane-bounded organelle	4.5E-03



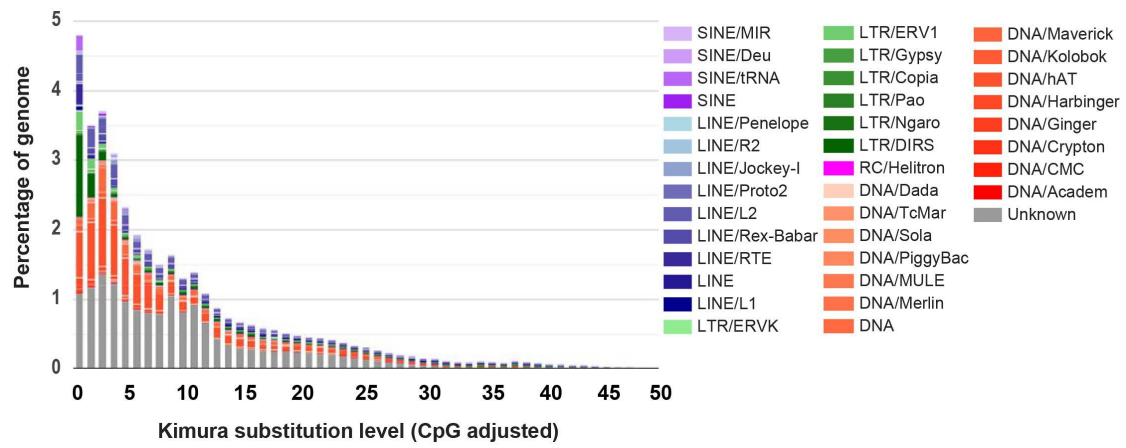
Supplementary Figure S1. Whole-genome alignment between *D. mawsoni* chromosome assembly and short-read assembly. X-axis is newly assembled in this study and Y-axis is Dmawsoni_scaffolds_v1 from GigaScience. Analysis at genomic scale using D-GENIES (<http://dgenies.toulouse.inra.fr/>).



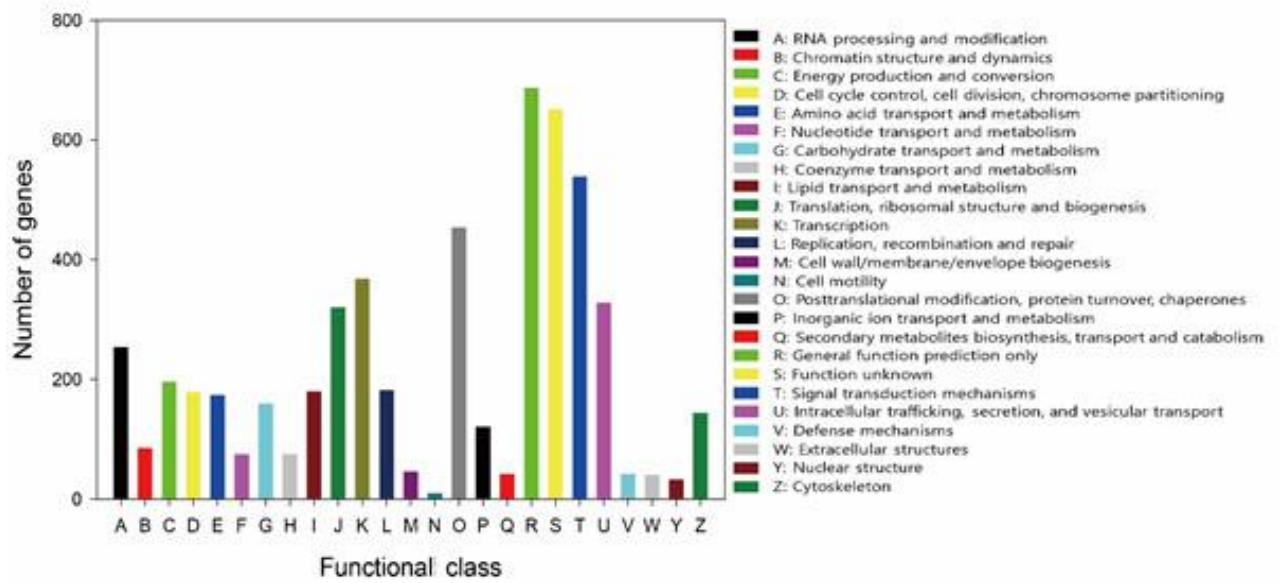
Supplementary Figure S2. *Gasterosteus aculeatus* chromosomes Group 1, Group 4, and Group 7 showing mapped each scaffolds of *D. mawsoni*. Scaffold23 and scaffold35 of *D. mawsoni* aligned to Group 1 of *G. aculeatus* chromosome, scaffold13 and scaffold22 to Group 4, and scaffold11 and scaffold24 to Group 7, respectively. Syntenic gene pairs are connected by lines.



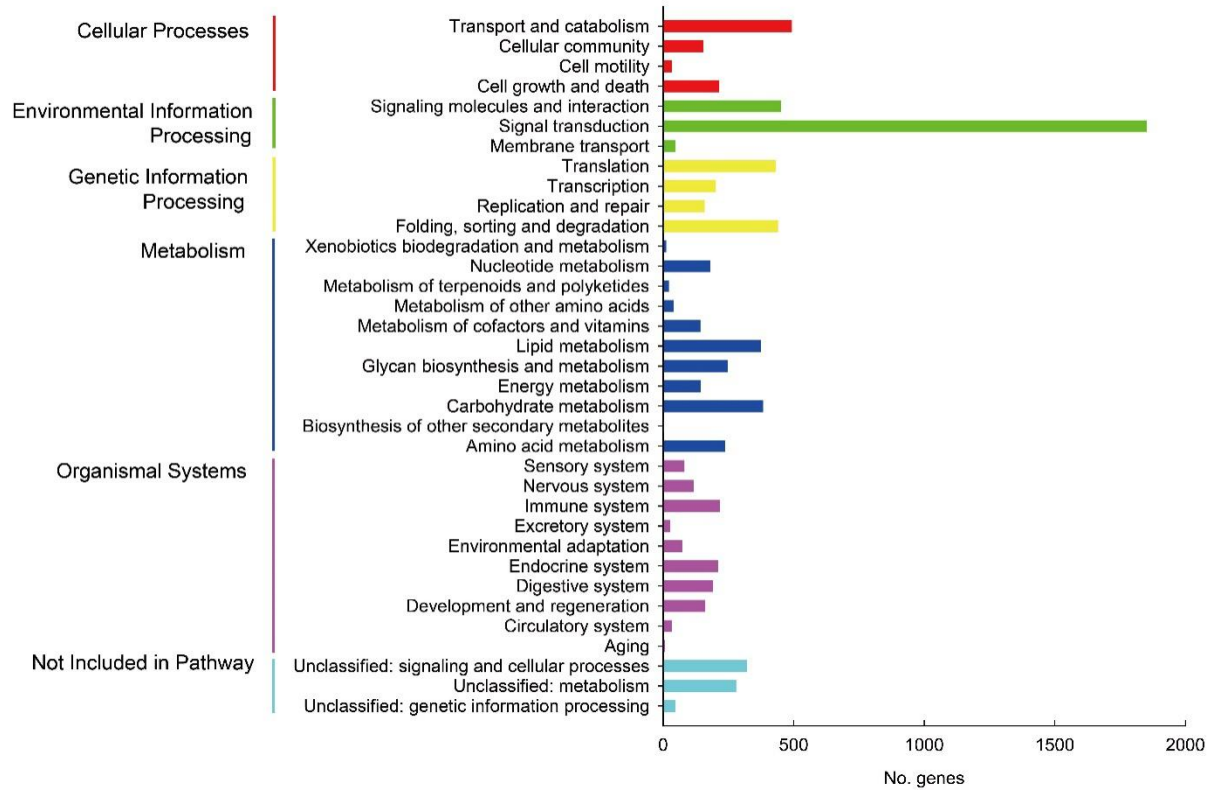
Supplementary Figure S3. Overview of *D. mawsoni* genome and mutations. Features are arranged as SNP (single nucleotide polymorphism), insertion, deletion, gene density, and repeat density from outside to inside in 100 Kb intervals across 24 chromosomes. Density of each feature is plotted as a heatmap with different colors and a line plot.



Supplementary Figure S4. Kimura distance-based copy divergence analysis of transposable elements in *D. mawsoni* genome.



Supplementary Figure S6. Eukaryotic Orthologous Groups (KOG) classification of predicted genes. Results are summarized into 24 functional classes. Horizontal axis indicates each class and vertical axis indicates number of genes in each class.



Supplementary Figure S7. Kyoto Encyclopedia of Genes and Genomes (KEGG) classifications.