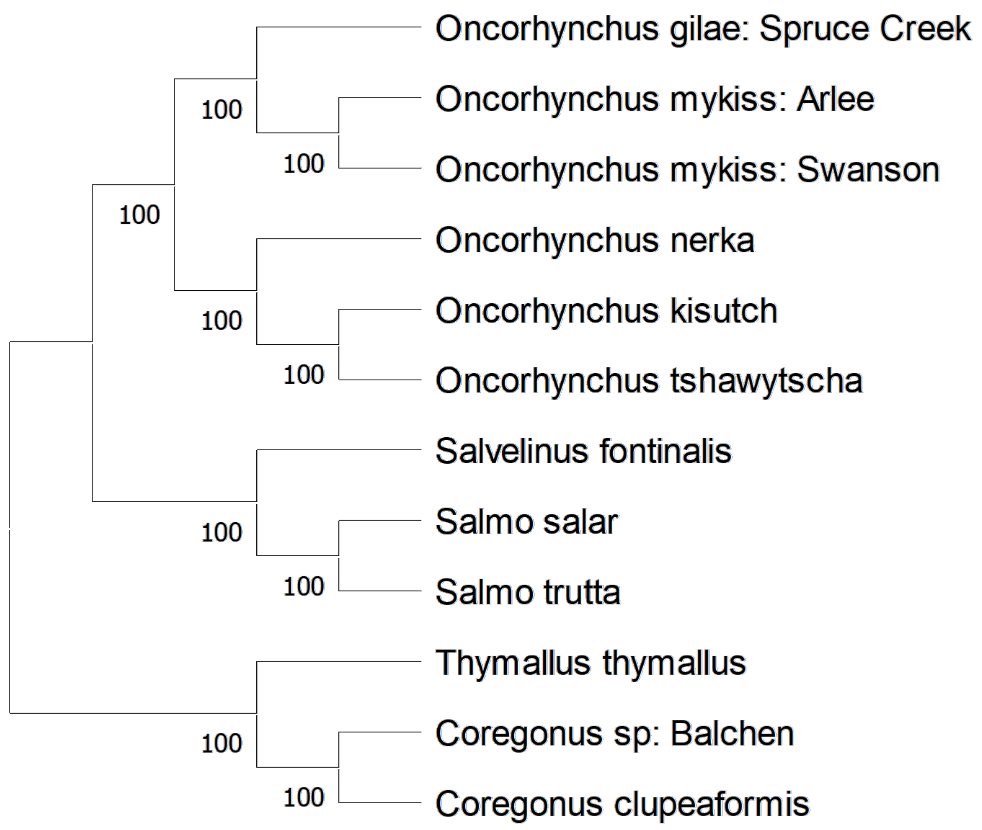
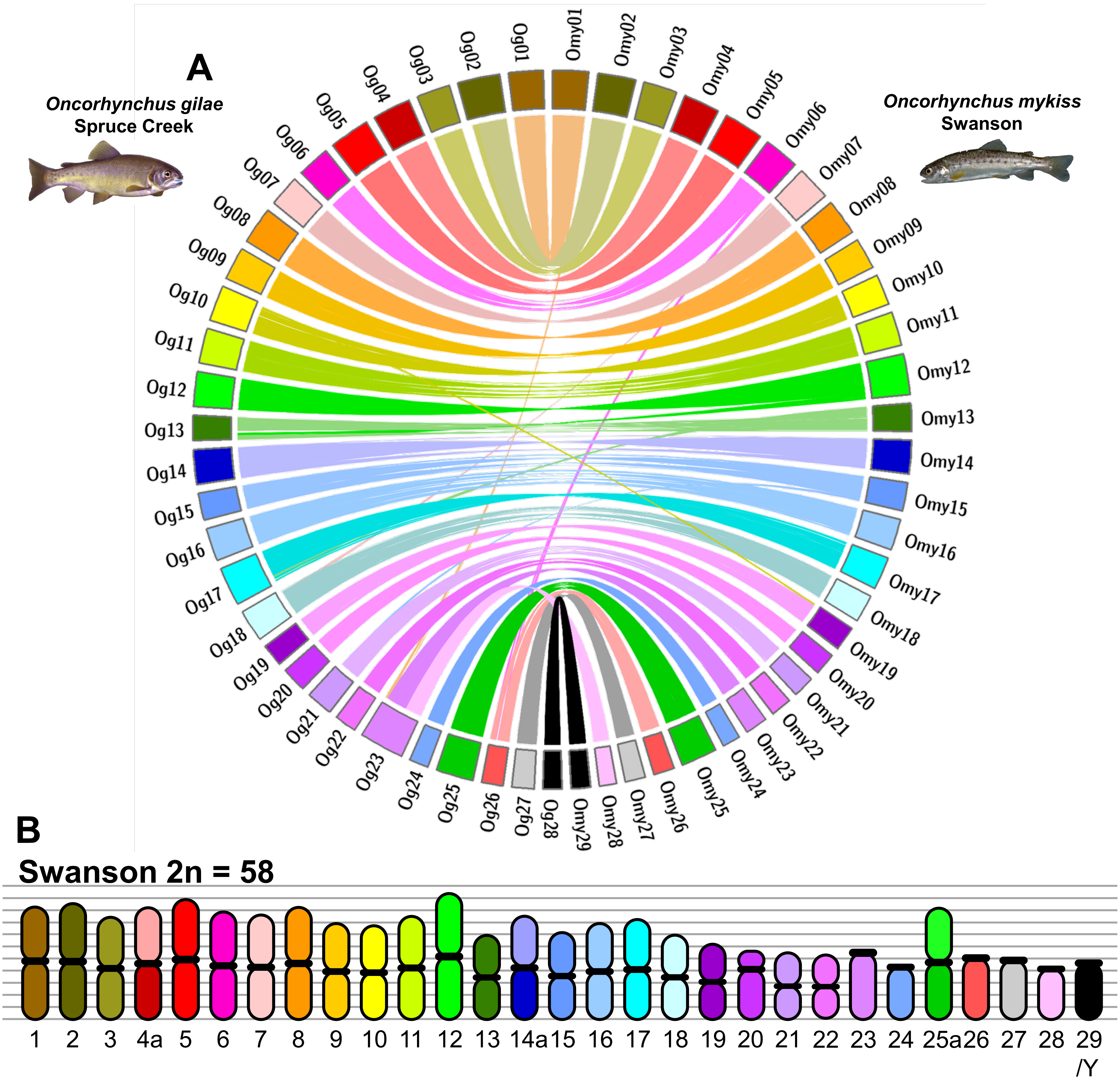
Supplemental Figure 1



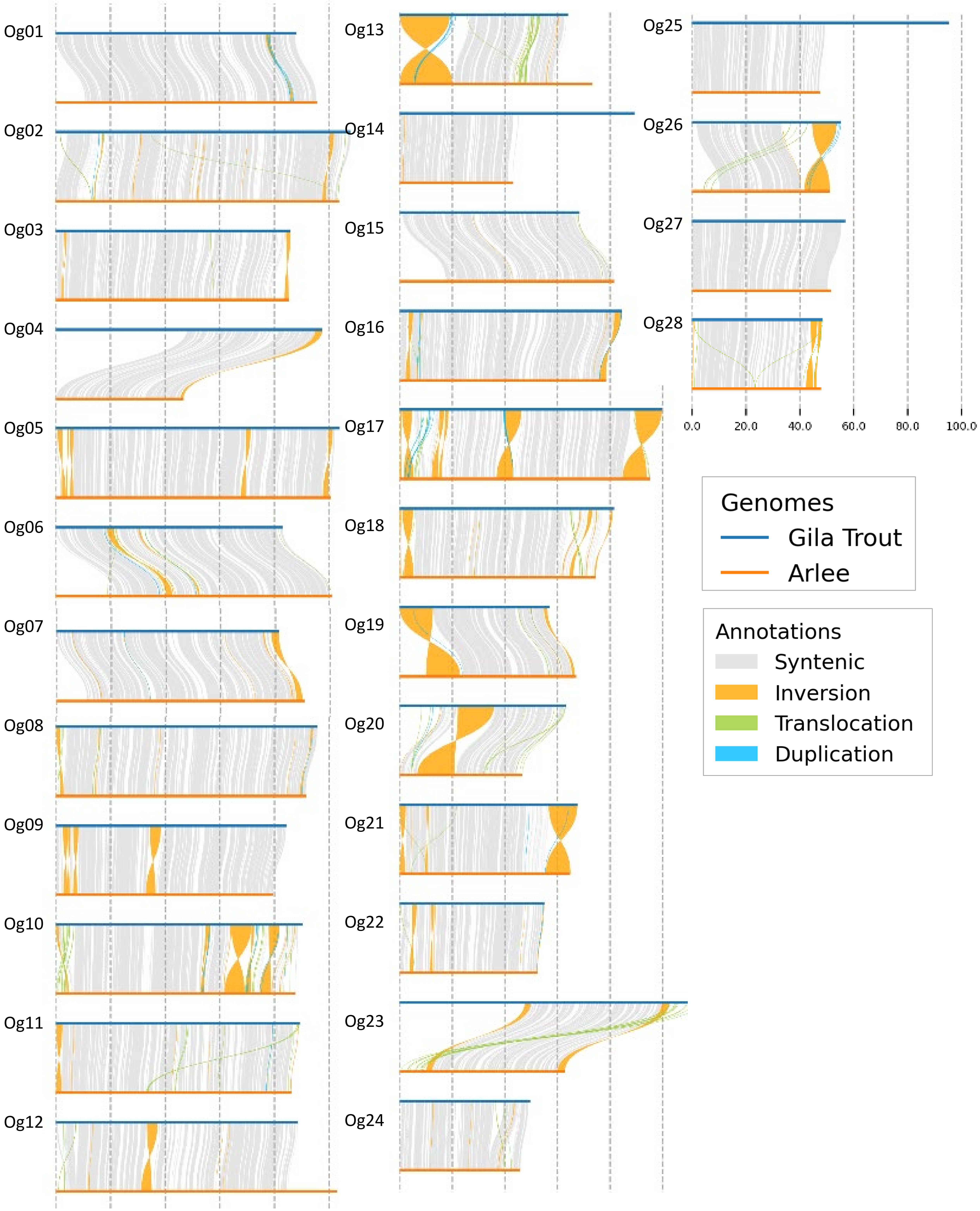
Supplemental Figure 1 | Phylogenetic tree of select salmonid species used in full genome analysis. Phylogenetic tree was generated from BUSCO genes using methods outlined in https://github.com/mcmurtrs/Making-a-Phylogenetic-Tree-with-BUSCO-Genes . The tree was constructed using CIPRES science gateway [79] using RAxML-HPC2 on ACCESS v. 8.2.12 [80]. The two *Coregonus* species and *T. thymallus* were set as outgroups. Bootstrap values were set for 1000 iterations with a bootstrap seed. Bootstrap values are indicated to the left of the branch points and the species names are indicated to the right.

Supplemental Figure 2



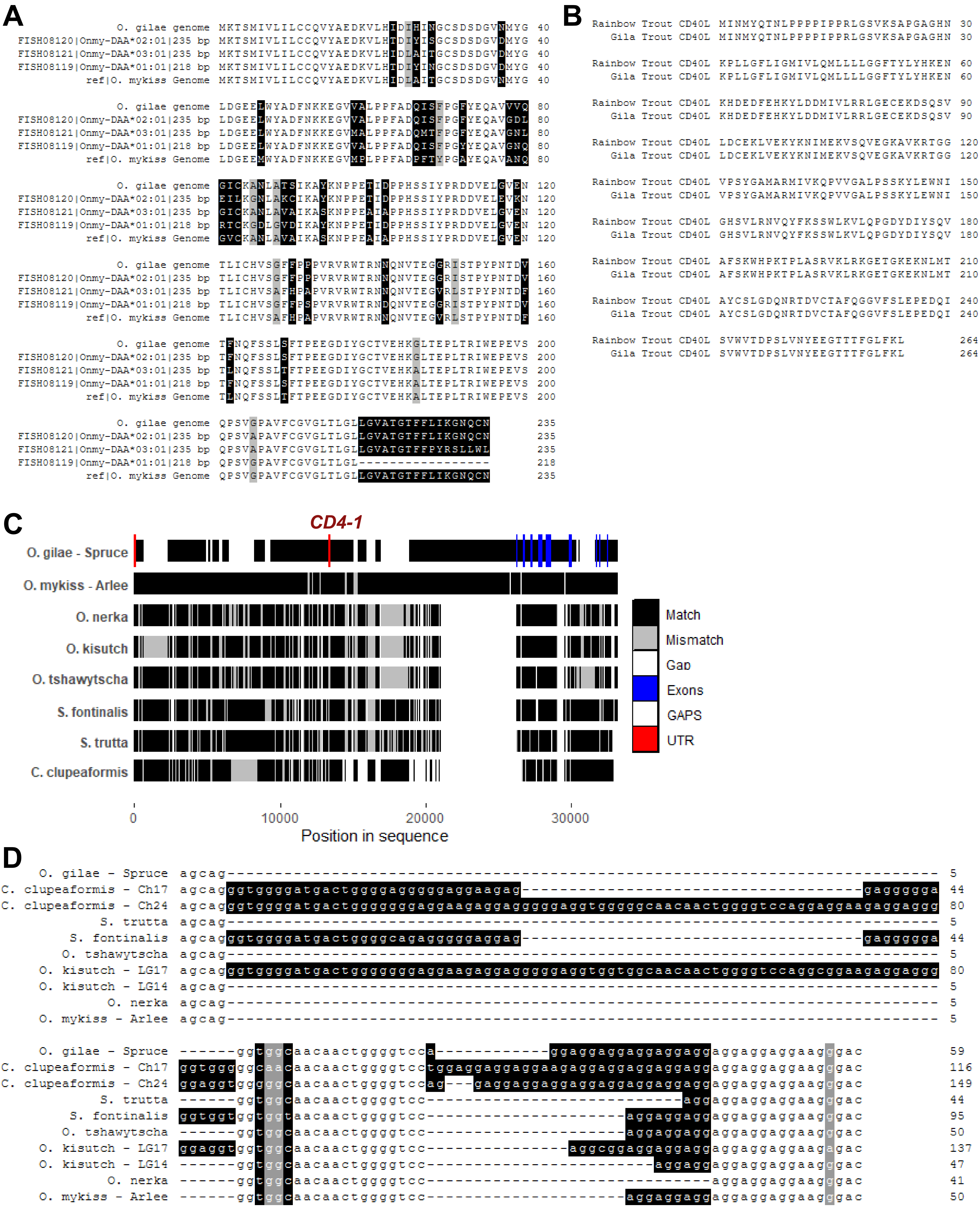
Supplemental Figure 2 | Circos plot of final Gila Trout chromosomes against the Swanson Rainbow Trout chromosomes. Chromosomes are represented by solid-colored blocks around the outside of the circle and are labeled Og for Gila Trout Chromosomes and Omy for Rainbow Trout. Colored ribbons within the circle represent regions of matching sequence greater than 500 kbp in length. A) Comparison of Gila Trout chromosomes with Swanson Rainbow Trout chromosomes. B) hypothetical ideograms of Swanson Rainbow Trout chromosomes based on chromosomes size from NCBI. Gray bars range from 0 to 110 Mb increasing at 10 Mb increments.

Supplemental Figure 3



Supplemental Figure 3 | Plotsr of chromosomes from Gila Trout vs Rainbow Trout Arlee chromosomes. Large rearrangements identified between Gila Trout and Arlee Rainbow Trout chromosomes using Plotsr. Because of program limitations, only 28 of 32 Arlee chromosomes were successfully mapped against the Gila Trout chromosomes. Species name is indicated to the left and chromosomes are indicated by colored lines. Chromosome size is indicated on the x-axis in Mb. Syntenic sequences are represented by light-gray ribbons between the chromosome lines, Inversions are represented in orange ribbons, translocations in green, and duplications in blue.

Supplemental Figure 4



A

```
           O. gilae genome MKTSMIVLILCCQVYAEDKVLHIDIHINGCSDSDGVNMYG  40
FISH08120|Onmy-DAA*02:01|235 bp MKTSMIVLILCCQVYAEDKVLHTDIYISGCSDSDGVDMYG  40
FISH08121|Onmy-DAA*03:01|235 bp MKTSMIVLILCCQVYAEDKVLHIDLAITGCSDSDGVDMYG  40
FISH08119|Onmy-DAA*01:01|218 bp MKTSMIVLILCCQVYAEDKVLHTDIYINGCSDSDGVDMYG  40
        ref|O. mykiss Genome MKTSMIVLILCCQVYAEDKVLHIDLAITGCSDSDGVNMYG  40

           O. gilae genome LDGEELWYADFNKKEGVVALPPFADQISFPGFYEQAVVVQ  80
FISH08120|Onmy-DAA*02:01|235 bp LDGEELWYADFNKKEGVVALPPFADQISFPGFYEQAVGDL  80
FISH08121|Onmy-DAA*03:01|235 bp LDGEELWYADFNKKEGVMALPPFADQMTFPGFYEQAVGNL  80
FISH08119|Onmy-DAA*01:01|218 bp LDGEELWYADFNKKEGVVALPPFADQISFPGYYEQAVGNQ  80
        ref|O. mykiss Genome LDGEEMWYADFNKKEGVMPLPPFADPFTYPGAYEQAVANQ  80

           O. gilae genome GICKANLATSIKAYKNPPETIDPPHSSIYPRDDVELGVEN 120
FISH08120|Onmy-DAA*02:01|235 bp EILKGNLAKCIKAYKNPPETIDPPHSSIYPRDDVELEVKN 120
FISH08121|Onmy-DAA*03:01|235 bp GICKANLAVAIKASKNPPEAIAPPHSSIYPRDDVELGVEN 120
FISH08119|Onmy-DAA*01:01|218 bp RTCKGDLGVDIKAYKNPPETIDPPHSSIYPRDDVELGVEN 120
        ref|O. mykiss Genome GVCKANLAVAIKASKNPPEAIAPPHSSIYPRDDVELGVEN 120

           O. gilae genome TLICHVSGFFPPPVRVRWTRNNQNVTEGGRISTPYPNTDV 160
FISH08120|Onmy-DAA*02:01|235 bp TLICHVSGFFPPPVRVRWTRNNQNVTEGGRISTPYPNTDV 160
FISH08121|Onmy-DAA*03:01|235 bp TLICHVSAFHPAPVRVRWTRNNQNVTEGVRLSTPYPNTDF 160
FISH08119|Onmy-DAA*01:01|218 bp TLICHVSGFFPSPVRVRWTRNDQNVTEGGRISTPYPNTDV 160
        ref|O. mykiss Genome TLICHVSAFHPAPVRVRWTRNNQNVTEGVRLSTPYPNTDF 160

           O. gilae genome TFNQFSSLSFTPEEGDIYGCTVEHKGLTEPLTRIWEPEVS 200
FISH08120|Onmy-DAA*02:01|235 bp TFNQFSSLSFTPEEGDIYGCTVEHKGLTEPLTRIWEPEVS 200
FISH08121|Onmy-DAA*03:01|235 bp TLNQFSSLTFTPEEGDIYGCTVEHKALTEPLTRIWEPEVS 200
FISH08119|Onmy-DAA*01:01|218 bp TFNQFSSLSFTPEEGDIYGCTVEHKALTEPLTRIWEPEVS 200
        ref|O. mykiss Genome TLNQFSSLTFTPEEGDIYGCTVEHKALTEPLTRIWEPEVS 200

           O. gilae genome QPSVGPAVFCGVGLTLGLLGVATGTFFLIKGNQCN      235
FISH08120|Onmy-DAA*02:01|235 bp QPSVAPAVFCGVGLTLGLLGVATGTFFLIKGNQCN      235
FISH08121|Onmy-DAA*03:01|235 bp QPSVAPAVFCGVGLTLGLLGVATGTFFPYRSLLWL      235
FISH08119|Onmy-DAA*01:01|218 bp QPSVGPAVFCGVGLTLGL-----------------      218
        ref|O. mykiss Genome QPSVGPAVFCGVGLTLGLLGVATGTFFLIKGNQCN      235
```

B

```
Rainbow Trout CD40L  MINMYQTNLPPPPIPPRLGSVKSAPGAGHN  30
   Gila Trout CD40L  MINMYQTNLPPPPIPPRLGSVKSAPGAGHN  30

Rainbow Trout CD40L  KPLLGFLIGMIVLQMLLLLGGFTYLYHKEN  60
   Gila Trout CD40L  KPLLGFLIGMIVLQMLLLLGGFTYLYHKEN  60

Rainbow Trout CD40L  KHDEDFEHKYLDDMIVLRRLGECEKDSQSV  90
   Gila Trout CD40L  KHDEDFEHKYLDDMIVLRRLGECEKDSQSV  90

Rainbow Trout CD40L  LDCEKLVEKYKNIMEKVSQVEGKAVKRTGG 120
   Gila Trout CD40L  LDCEKLVEKYKNIMEKVSQVEGKAVKRTGG 120

Rainbow Trout CD40L  VPSYGAMARMIVKQPVVGALPSSKYLEWNI 150
   Gila Trout CD40L  VPSYGAMARMIVKQPVVGALPSSKYLEWNI 150

Rainbow Trout CD40L  GHSVLRNVQYFKSSWLKVLQPGDYDIYSQV 180
   Gila Trout CD40L  GHSVLRNVQYFKSSWLKVLQPGDYDIYSQV 180

Rainbow Trout CD40L  AFSKWHPKTPLASRVKLRKGETGKEKNLMT 210
   Gila Trout CD40L  AFSKWHPKTPLASRVKLRKGETGKEKNLMT 210

Rainbow Trout CD40L  AYCSLGDQNRTDVCTAFQGGVFSLEPEDQI 240
   Gila Trout CD40L  AYCSLGDQNRTDVCTAFQGGVFSLEPEDQI 240

Rainbow Trout CD40L  SVWVTDPSLVNYEEGTTTFGLFKL       264
   Gila Trout CD40L  SVWVTDPSLVNYEEGTTTFGLFKL       264
```

C

D

```
   O. gilae - Spruce  agcag---------------------------------------------------------------------------  5
C. clupeaformis - Ch17  agcagggtggggatgactggggagggggaggaagag------------------------------------gaggggga  44
C. clupeaformis - Ch24  agcagggtggggatgactgggggggaggaagaggaggggaggtgggggcaacaactggggtccaggaggaagaggaggg  80
          S. trutta  agcag---------------------------------------------------------------------------  5
      S. fontinalis  agcagggtggggatgactggggcagagggggaggag------------------------------------gaggggga  44
     O. tshawytscha  agcag---------------------------------------------------------------------------  5
  O. kisutch - LG17  agcagggtggggatgactgggggggaggaagaggaggggaggtggtggcaacaactggggtccaggcggaagaggaggg  80
  O. kisutch - LG14  agcag---------------------------------------------------------------------------  5
           O. nerka  agcag---------------------------------------------------------------------------  5
  O. mykiss - Arlee  agcag---------------------------------------------------------------------------  5

   O. gilae - Spruce  ------ggtggcaacaactggggtcca------------ggaggaggaggaggaggaggaggaggaagggac  59
C. clupeaformis - Ch17  ggtggg ggcaaccaacaactggggtcctggaggaggaggaagaggaggaggaggaggaggaggaggaagggac  116
C. clupeaformis - Ch24  ggaggtgggggcaacaactggggtccag---gaggaggaggaggaggaggaggaggaggaggaggaagggac  149
          S. trutta  ------ggtggcaacaactggggtcc---------------------------aggaggaggaggaagggac  44
      S. fontinalis  ggtggtggtggtaacaactggggtcc---------------------aggaggaggaggaggaggaagggac  95
     O. tshawytscha  ------ggtggcaacaactggggtcc---------------------aggaggaggaggaggaggaagggac  50
  O. kisutch - LG17  ggaggtggtggcaacaactggggtcc---------------aggcggaggaggaggaggaggaggaagagac  137
  O. kisutch - LG14  ------ggtggcaacaactggggtcc------------------------aggaggaggaggaggaagggac  47
           O. nerka  ------ggtggcaacaactggggtcc------------------------------aggaggaggaagggac  41
  O. mykiss - Arlee  ------ggtggcaacaactggggtcc---------------------aggaggaggaggaggaggaagggac  50
```

Supplemental Figure 4 | Additional sequence alignments for genes of interest. A) Protein alignment of Gila Trout DAA against Rainbow Trout DAA sequences obtained from IGMT and the DAA sequence found in the Arlee Rainbow Trout genome. Sequence labels are located to the left of the alignment and the amino acid position is listed to the left. Amino acids with no identity or similarity are colored in black, amino acids with some similarity are colored in gray, and '-' represent sequence gaps. B) Protein alignment of Gila Trout CD40L and Rainbow Trout CD40L. C) Full alignment of Gila Trout CD4.1 against other salmonids showing the 5'UTR (red). D) Nucleotide alignment of the variable region of Nup42 exon 2. nucleotides with no identity or similarity are colored in black, nucleotides with some similarity are colored in gray, and '-' represent sequence gaps.

Supplementary Table 1 | Repeat Masker analysis of Gila Trout, Rainbow Trout, and Atlantic salmon genomes

			Gila Trout Spruce Creek			*O. mykiss* Arlee			*Salmo salar*		
Type	Order	Superfamily	# of Elements	Length (bp)	%	# of Elements	Length (bp)	%	# of Elements	Length (bp)	%
Retroelements			3294873	881684104	37.94	3443977	846101003	36.13	4179559	1001198504	36.32
	SINE		113888	13367469	0.58	119338	13569901	0.58	156289	17145351	0.62
	LINE		2824477	708947476	30.51	2959835	688465854	29.4	3558982	815838012	29.6
		L2/CR1/Rex	1625419	367598787	15.82	1700289	377927329	16.14	2080395	460659087	16.71
		R1/LOA/Jockey	10919	3782952	0.16	11717	4000370	0.17	14118	5382634	0.2
		R2/R4/NeSL	33292	14083251	0.61	37729	13632826	0.58	35911	9779365	0.35
		RTE/Bov-B	155019	64834843	2.79	167500	57983015	2.48	175353	60809928	2.21
		L1/CIN4	238714	82172608	3.54	243880	58315323	2.49	266324	53349717	1.94
	LTR		356508	159369159	6.86	364804	144065248	6.15	464288	168215141	6.1
		BEL/Pao	1323	508270	0.02	1365	528055	0.02	1481	560413	0.02
		Ty1/Copia	11451	2643358	0.11	9682	2523576	0.11	26681	5836047	0.21
		Gypsy/DIRS1	53948	38160021	1.64	52868	26730025	1.14	62608	27551795	1
		Retroviral	18884	7281500	0.31	20143	7943215	0.34	28563	12812379	0.46
DNA			1142443	377257080	16.23	1190726	388989551	16.61	1410478	466512667	16.92
		hobo-Activator	268219	69775129	3	280046	69761867	2.98	319867	77911547	2.83
		Tc1-IS630-Pogo	520612	237583339	10.22	540213	245869889	10.5	559795	253597294	9.2
		MULE-MuDR	671	164231	0.01	691	168029	0.01	848	201252	0.01
		PiggyBac	13183	4557409	0.2	13684	4722036	0.2	13474	5076264	0.18
		Tourist/Harbinger	27560	5875814	0.25	29069	5840920	0.25	32980	6743357	0.24
Rolling-circles			2048	399708	0.02	2146	456952	0.02	2584	633120	0.02
Unclassified			67850	9521455	0.41	73302	10064196	0.43	80575	11094116	0.4
Total interspersed repeats				**1268462639**	**54.58**		**1245154750**	**53.17**		**1478805287**	**53.65**
Other Elements	Small RNA:		1064	122305	0.01	1853	225950	0.01	689	94197	0
	Satellites:		2	1979	0	1	1199	0	0	0	0
	Simple repeats:		560140	48604361	2.09	612186	51626021	2.2	669367	72012520	2.61
	Low complexity:		65631	6890442	0.3	70167	7741822	0.33	77941	9541855	0.35

RepeatMasker was Run using a custom repeat library generated from 2126 entries on the Rainbow Trout obtained from FishTEDB.

RepeatMasker version 4.1.6 was used in default mode and run with rmblastn version 2.14.1+