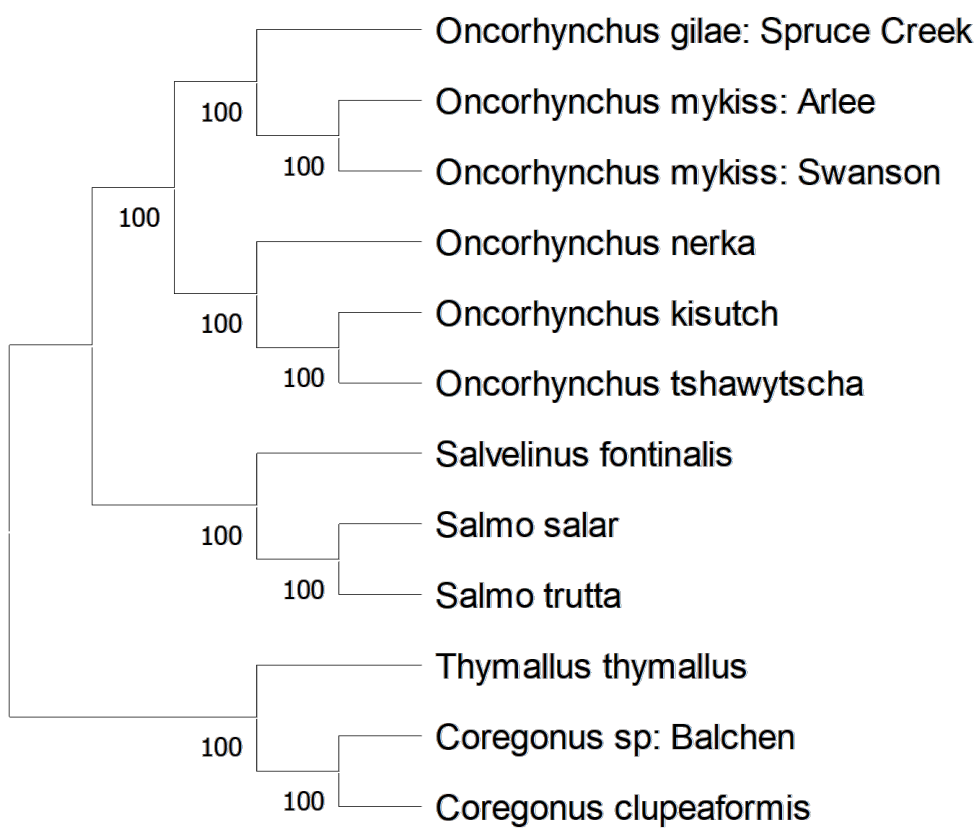
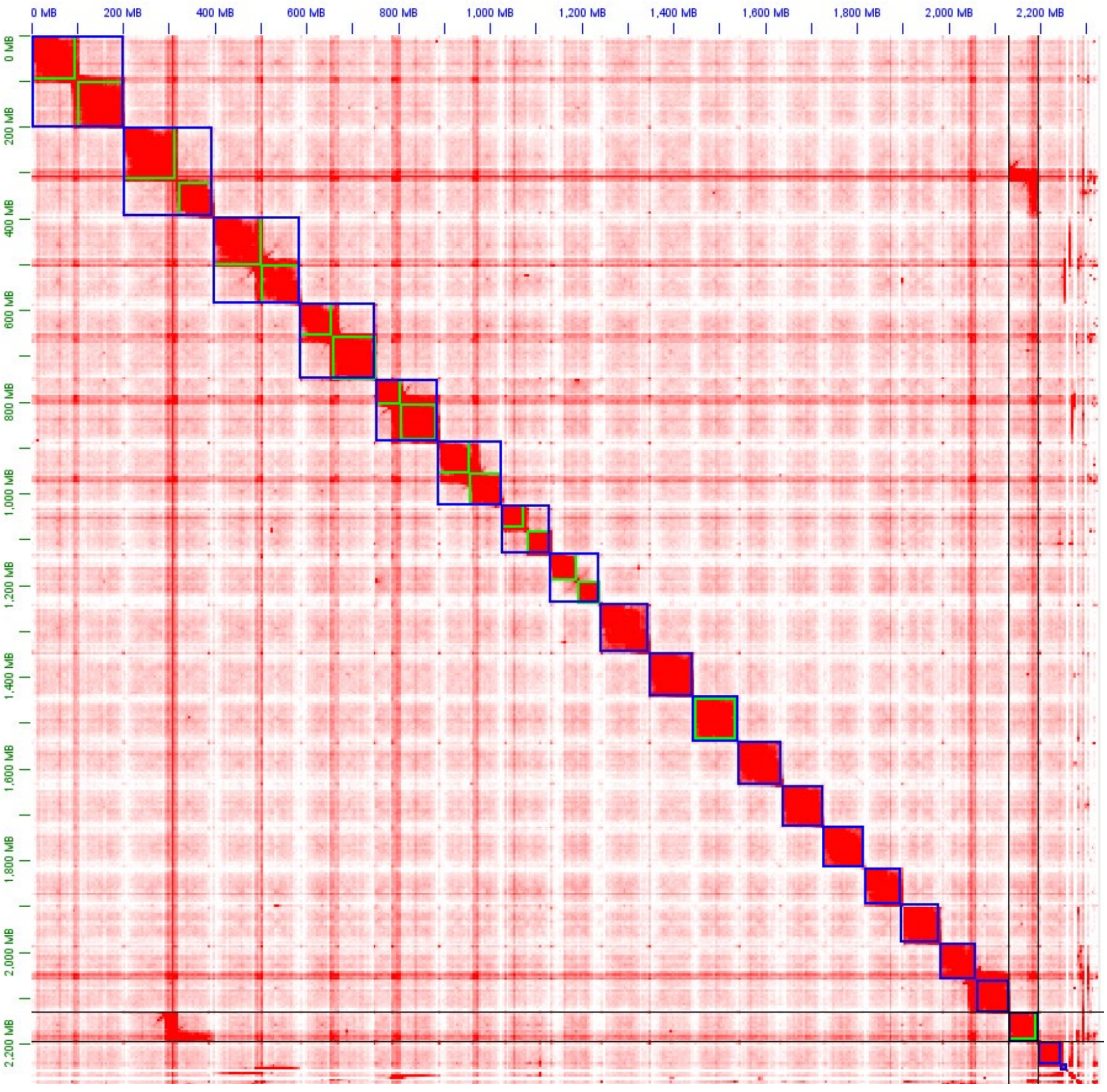
Supplemental Figure 1



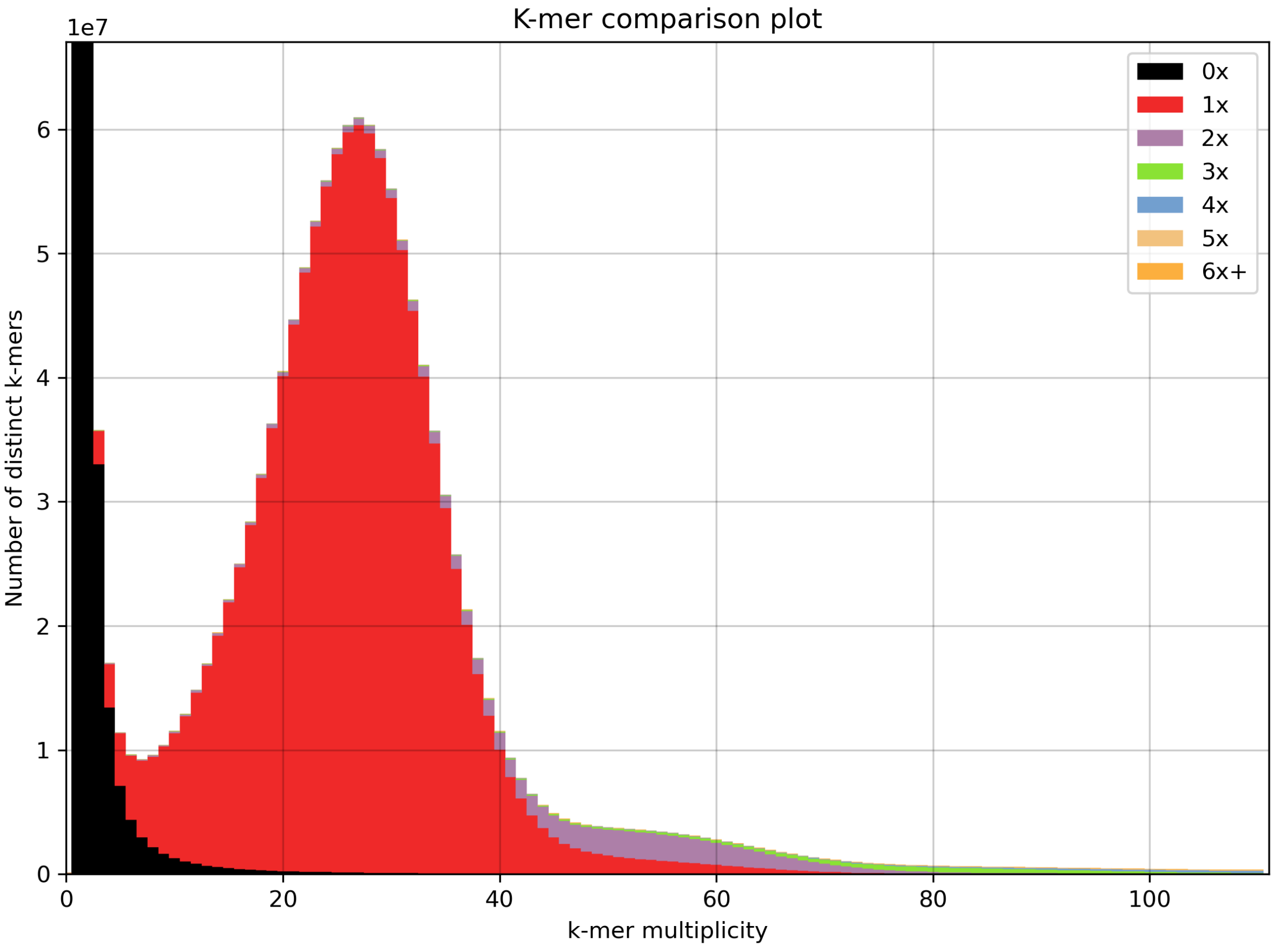
Supplemental Figure 1 | Phylogenetic tree of select salmonid species used in full genome analysis. Phylogenetic tree was generated from BUSCO genes using methods outlined in https://github.com/mcmurtrs/Making-a-Phylogenetic-Tree-with-BUSCO-Genes . The tree was constructed using CIPRES science gateway [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



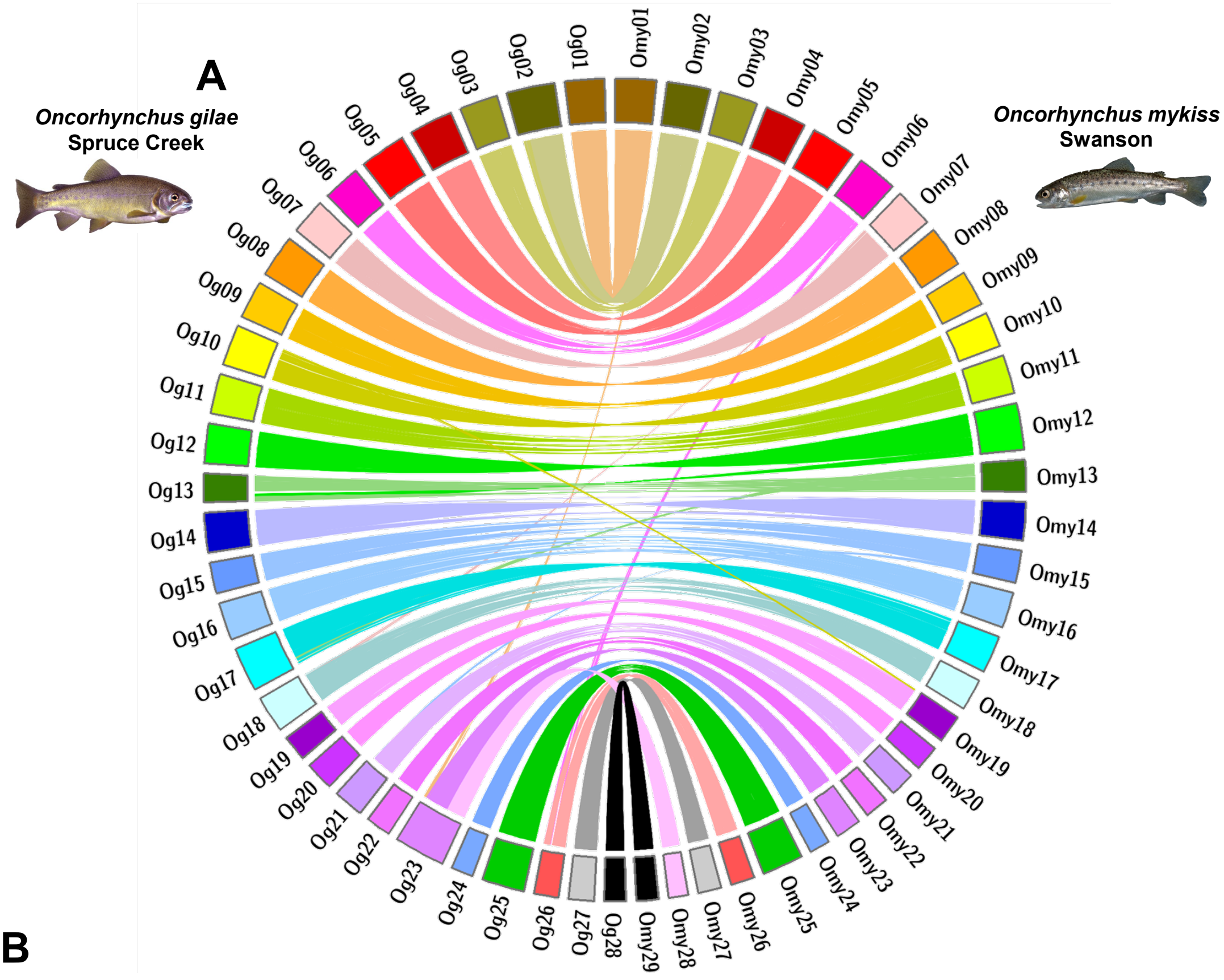
Supplemental Figure 2 | Juicebox heatmap of secondary HiC analysis post-secondary processing through Juicebox highlighting chimeric chromosomes. Red boxes indicate contact points of sequences and their proximity to one another. Blue boxes indicate contact domains while green boxes indicate identified chromosomes that could not be easily separated using Juicebox. See methods (Identification and numbering of Chromosome edges) for process of chimeric chromosome separation.

Supplemental Figure 3



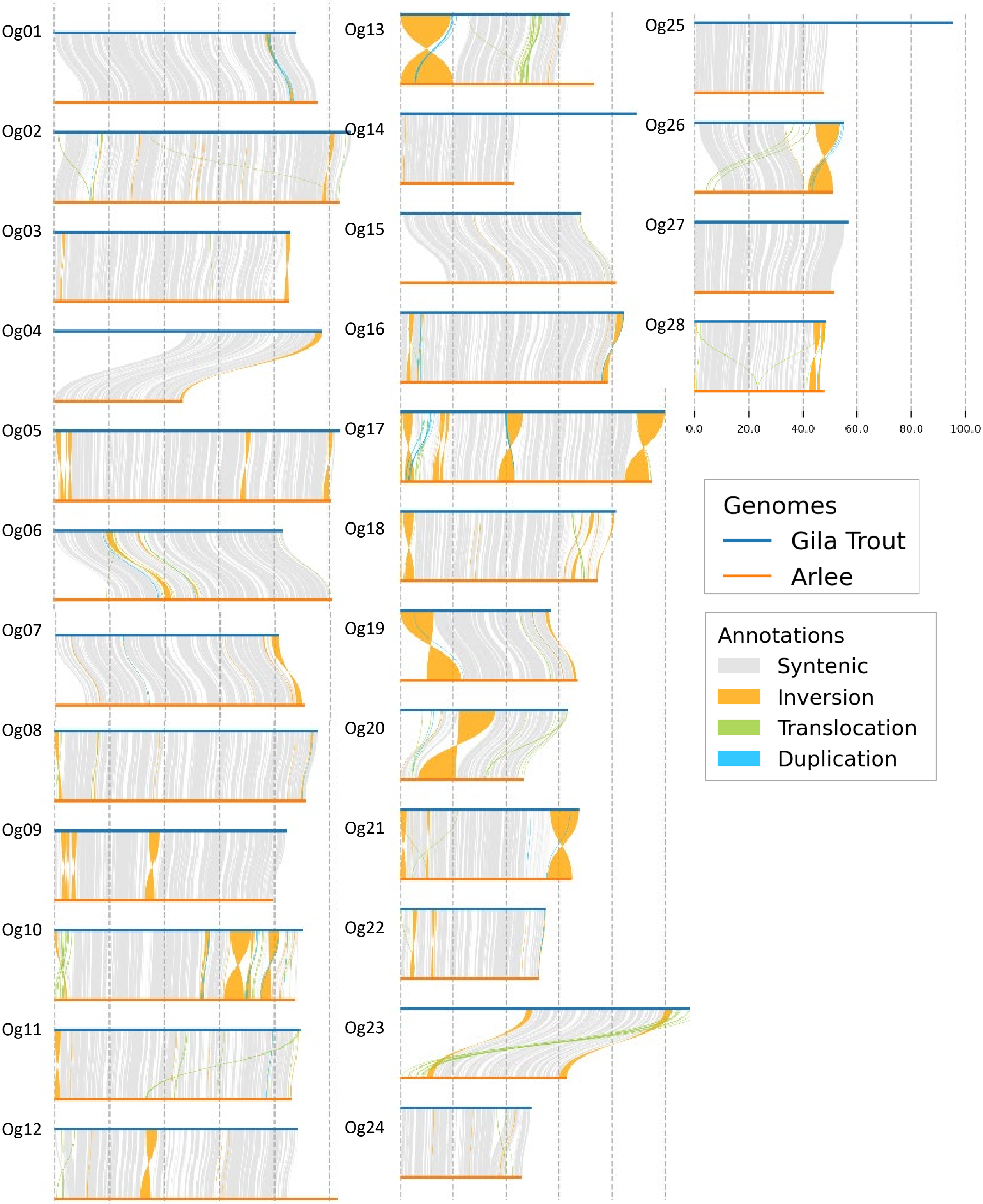
Supplemental Figure 3 | k-mer comparison plot generated using KAT comp command to compare k-mers between initial read data and final Gila Trout genome. Stacked histograms indicating k-mer frequency vs. assembly copy number against the final Gila Trout genome. The black peak indicates absent k-mers (those absent from the assembly which often represent sequencing errors [66]) while the red peek indicates k-mers that appear once, purple twice and so on (see key in left corner). There is distinct separation between the 0x and 1x peeks indicating that absent k-mers are not collapsing into the mapped k-mers. The small peak at 2x also indicates that the genome represents a mostly homozygous individual.

Supplemental Figure 4



Supplemental Figure 4 | 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 5



Supplemental Figure 5 | 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 6

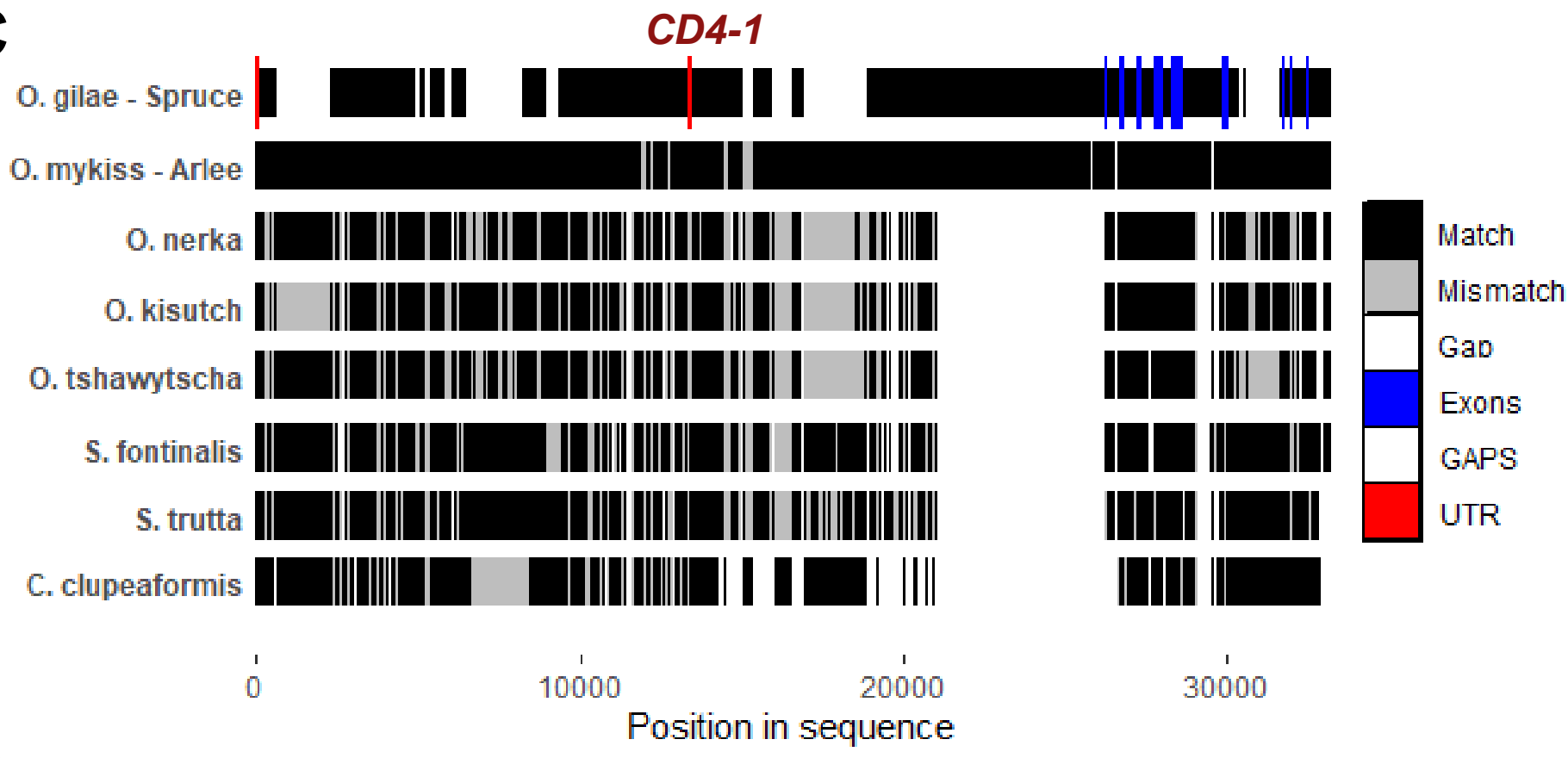
A

```
                    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   agcaggtggggatgactggggaggggaggaagag-----------------------------------------gaggggga  44
C. clupeaformis - Ch24   agcaggtggggatgactgggggggaggaagaggaggggggaggtgggggcaacaactggggtccaggaggaagaggaggg  80
            S. trutta    agcag----------------------------------------------------------------------------------------  5
         S. fontinalis   agcaggtggggatgactggggcagaggggggaggag-----------------------------------------gaggggga  44
        O. tshawytscha   agcag----------------------------------------------------------------------------------------  5
      O. kisutch - LG17  agcaggtggggatgactgggggggaggaagaggaggggggaggtggtggcaacaactggggtccaggcggaagaggaggg  80
      O. kisutch - LG14  agcag----------------------------------------------------------------------------------------  5
            O. nerka     agcag----------------------------------------------------------------------------------------  5
      O. mykiss - Arlee  agcag----------------------------------------------------------------------------------------  5

      O. gilae - Spruce  ------ggtggcaacaactggggtcca----------ggaggaggaggaggaggaggaggaggaaggac  59
C. clupeaformis - Ch17   ggtgggggcaacaacaactggggtcctggaggaggaggaagaggaggaggaggaggaggaggaaggac  116
C. clupeaformis - Ch24   ggaggtgggggcaacaactggggtccag---gaggaggaggaggaggaggaggaggaggaggaaggac  149
            S. trutta    ------ggtggcaacaactggggtcc----------------------------aggaggaggaggaaggac  44
         S. fontinalis   ggtggtggtggtaacaactggggtcc----------------------aggaggaggaggaggaggaaggac  95
        O. tshawytscha   ------ggtggcaacaactggggtcc----------------------aggaggaggaggaggaggaaggac  50
      O. kisutch - LG17  ggaggtggtggcaacaactggggtcc----------------aggcggaggaggaggaggaggaggaagagac  137
      O. kisutch - LG14  ------ggtggcaacaactggggtcc----------------------aggaggaggaggaggaggaaggac  47
            O. nerka     ------ggtggcaacaactggggtcc----------------------------aggaggaggaaggac  41
      O. mykiss - Arlee  ------ggtggcaacaactggggtcc----------------------aggaggaggaggaggaggaaggac  50
```

Supplemental Figure 6 | 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.