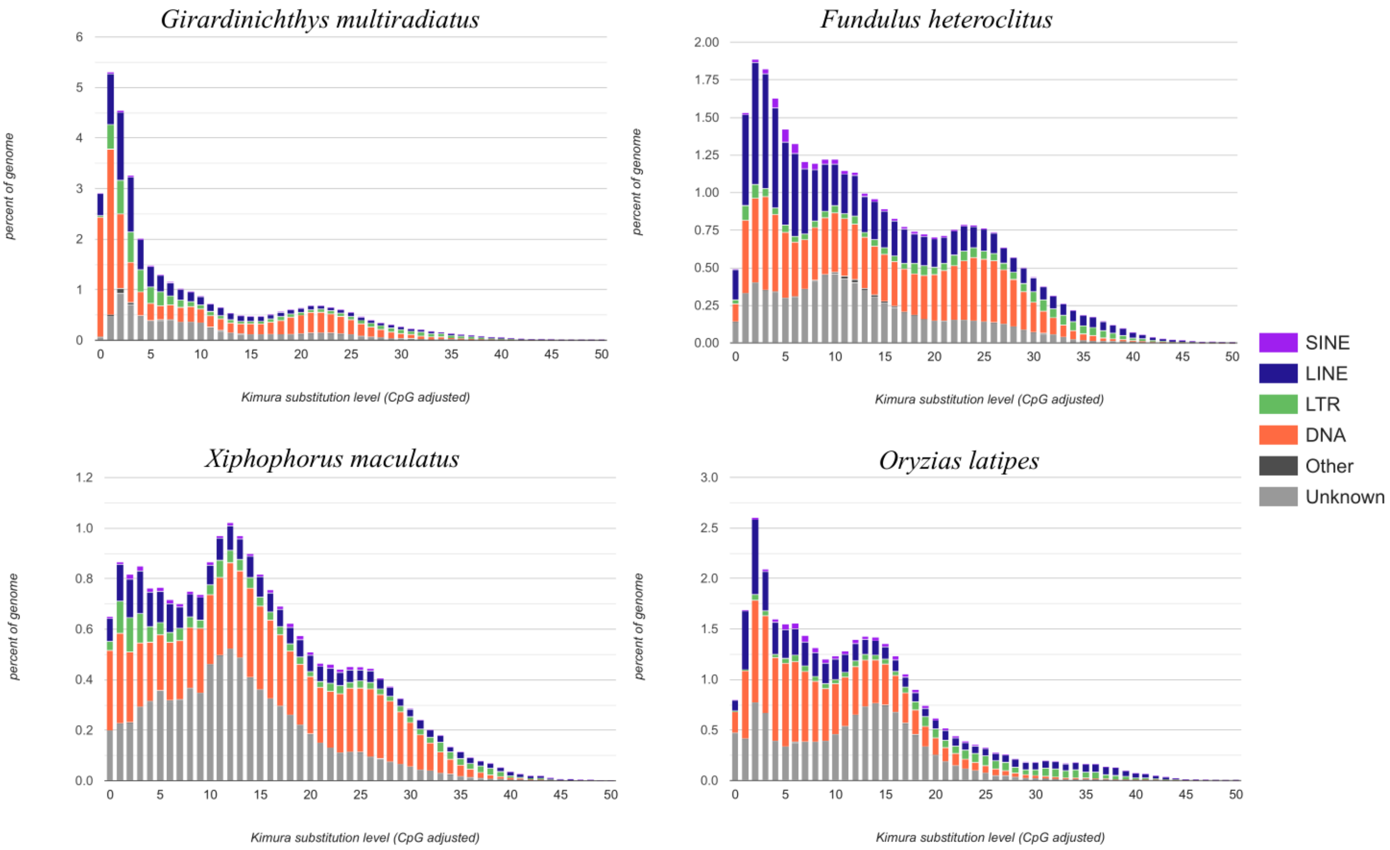
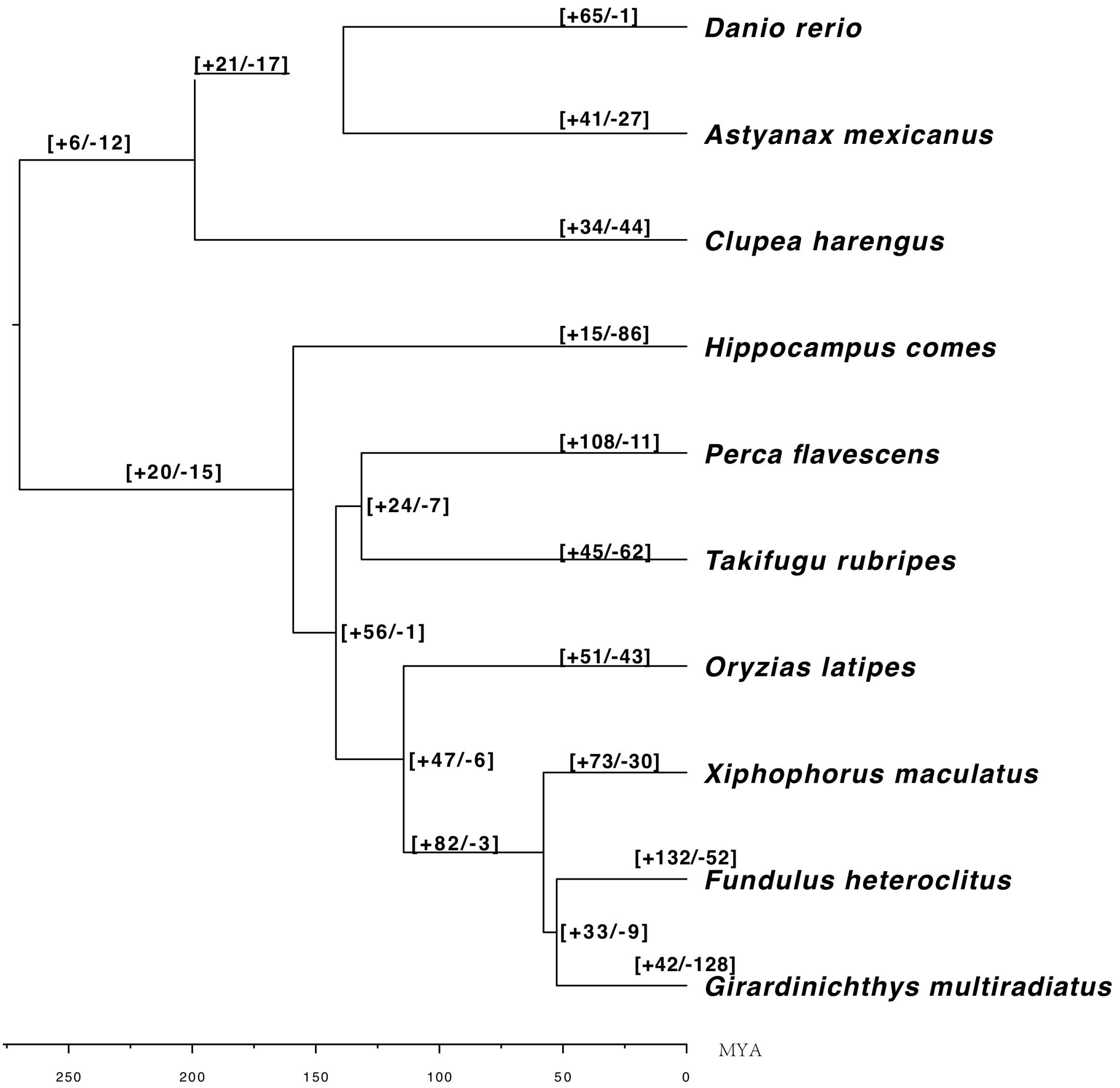
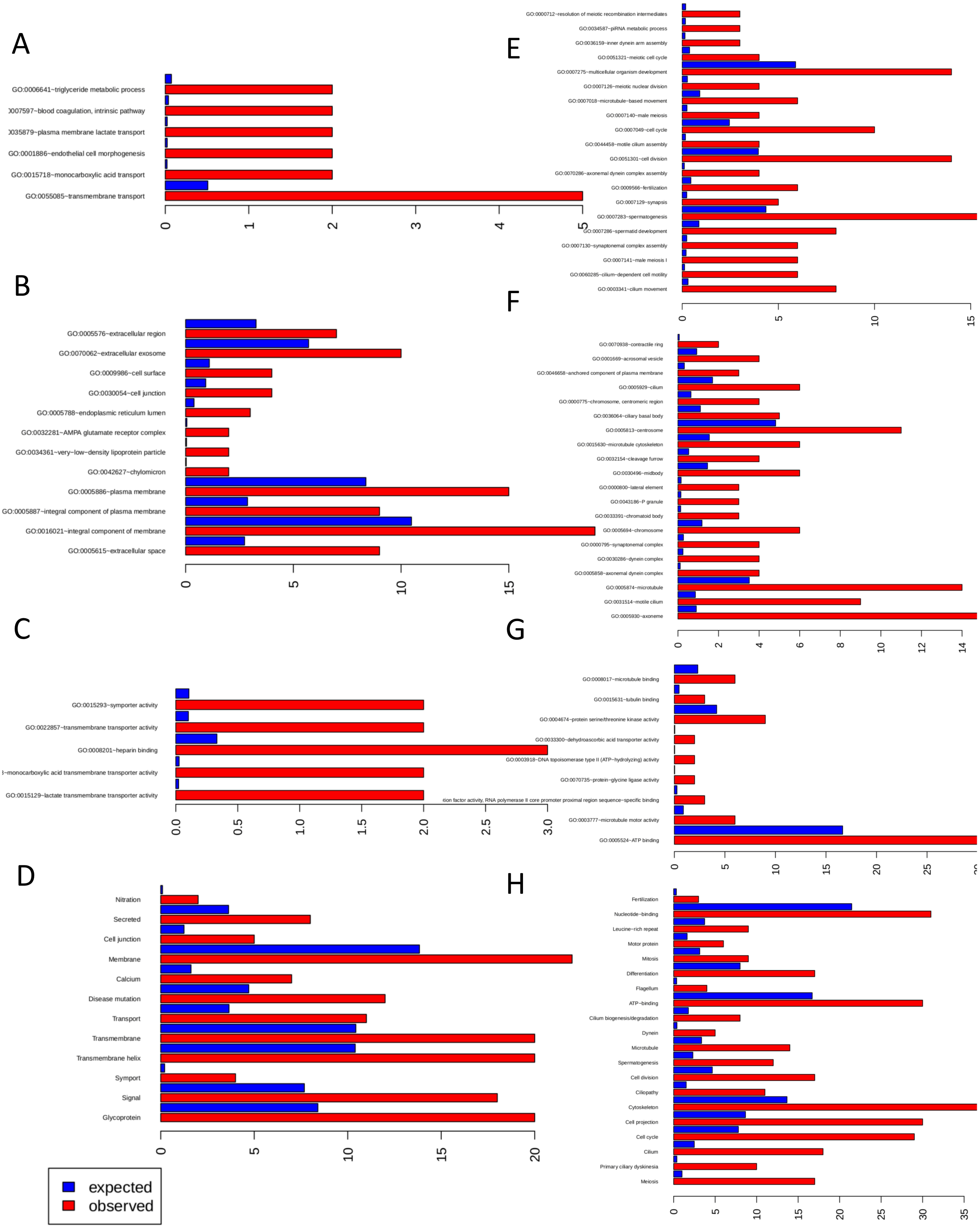


Supplemental Fig. S1. Kimura distance-based copy divergence analyses of transposable elements in *Girardinichthys multiradiatus*, *Fundulus heteroclitus*, *Xiphophorus maculatus* and *Oryzias latipes*.

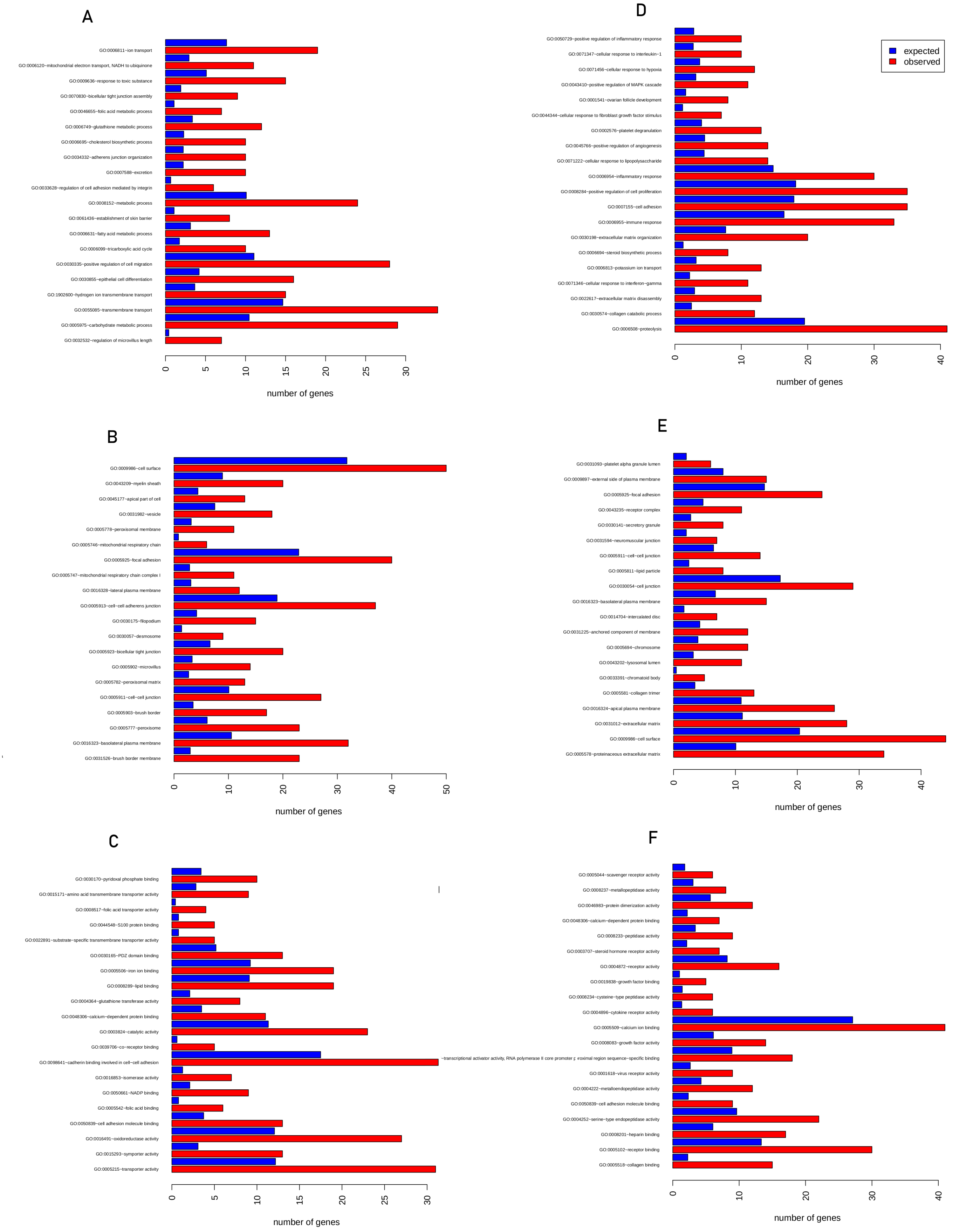


Supplemental Fig. S2. Phylogenetic tree displaying species divergence time and number of gene families expanded or contracted on each branch. Numbers of expand gene family are shown in square brackets next to plus mark; number of contracted gene family are shown in square brackets next to minus mark.

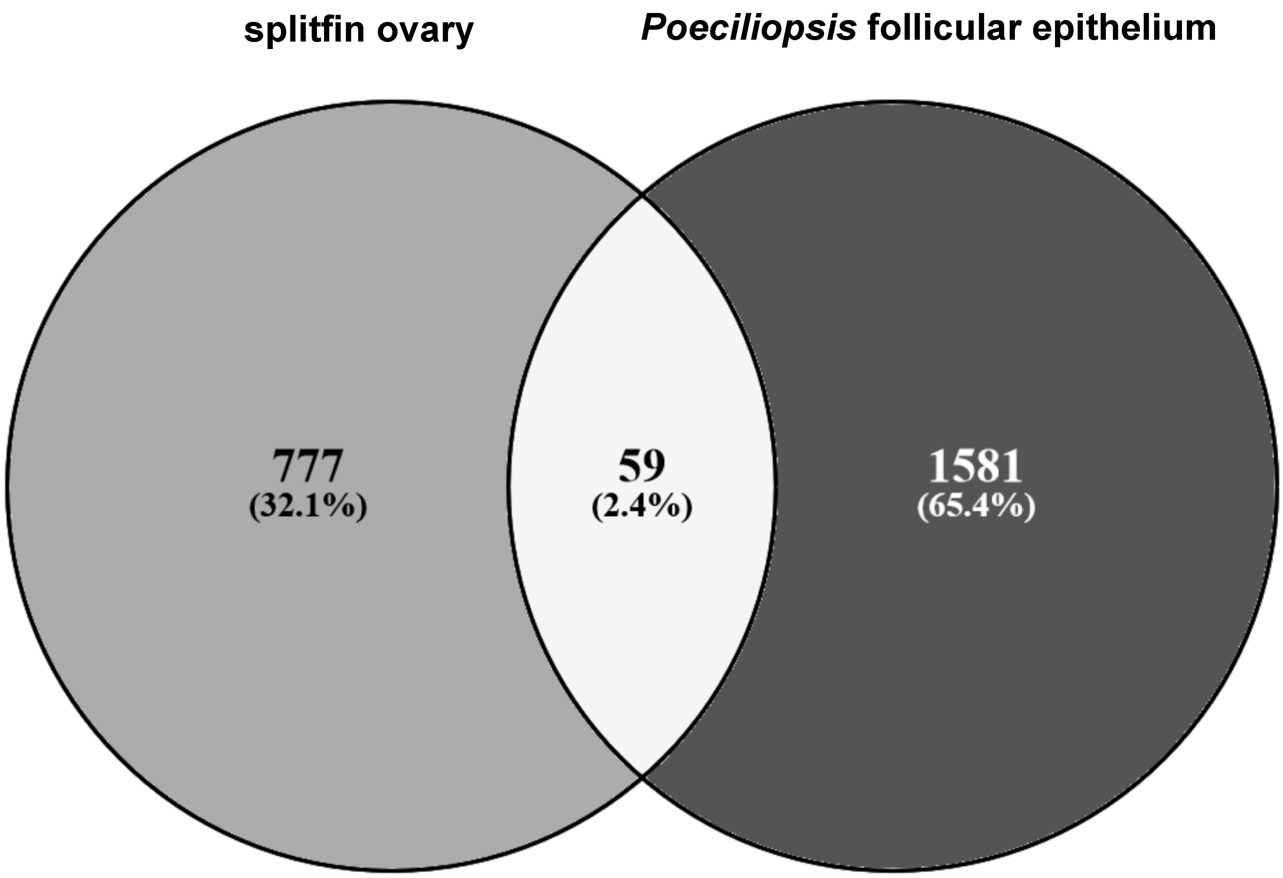


Supplemental Fig. S3. Barplots of enriched GO terms for genes upregulated in female brain (A-D) and upregulated in male brain (E-H). Upregulated is defined as DESeq2 baseMean > 5 and fold change > 2 and adjusted P-value < 0.05. Categories are GO Biological Process (A,E), GO Cellular Component (B,F) and GO

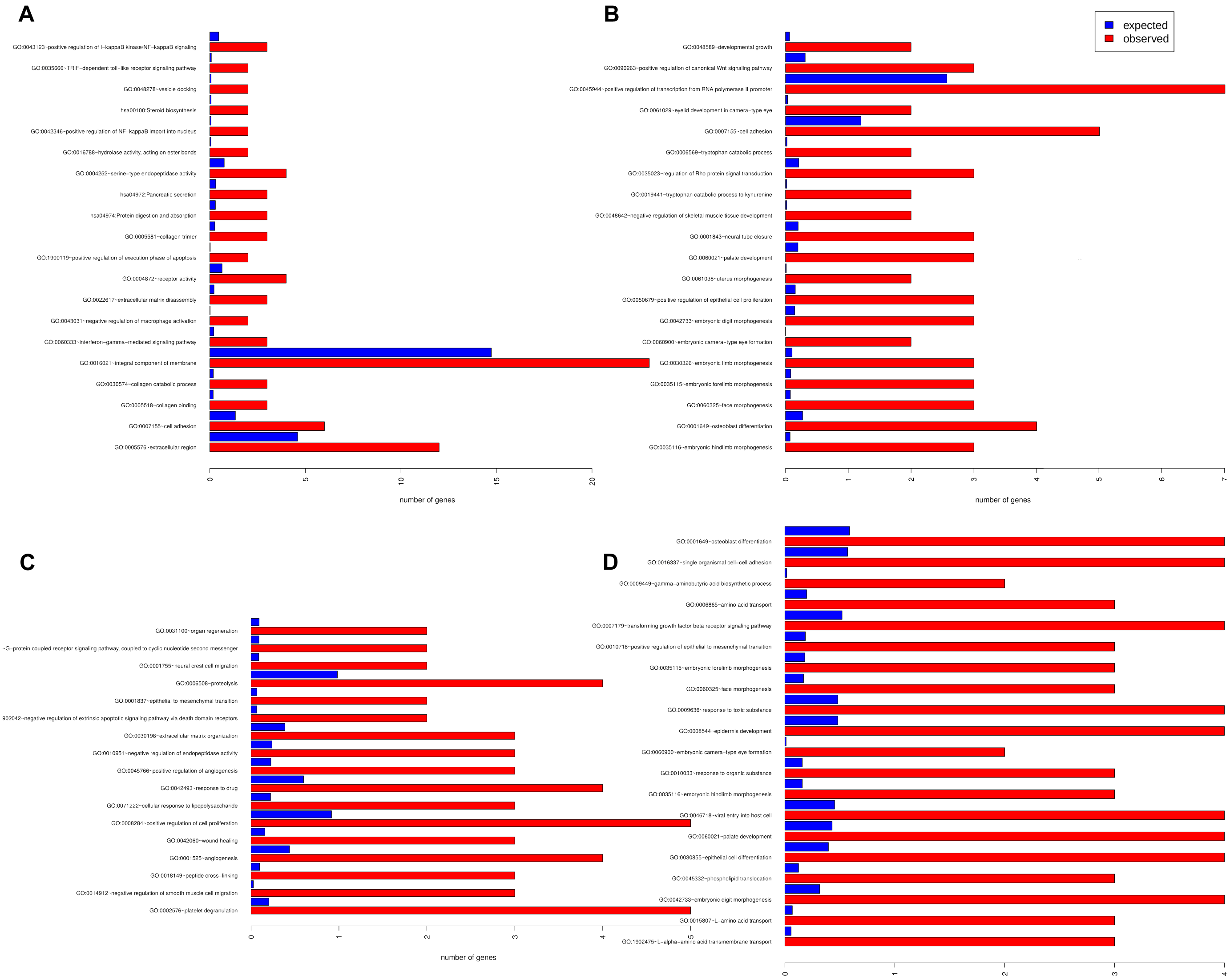
Molecular Function (C,G) and Uniprot keywords (D,H) . Blue indicating the number of expected genes given the gene selection would be random, red indicating the number of observed genes. Significance of GO-terms is decreasing from bottom to top. A maximum of 20 categories is shown.



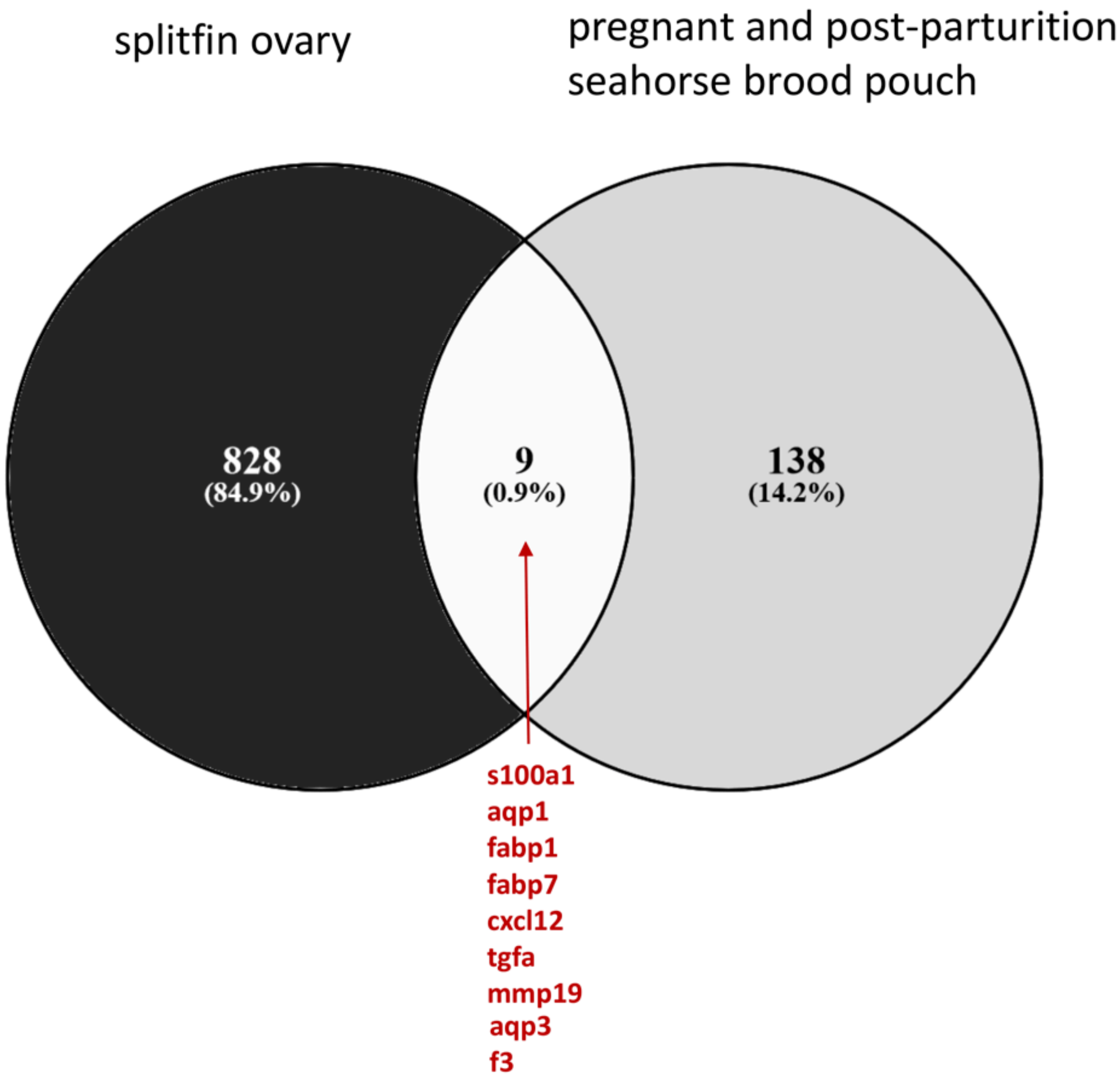
Supplemental Fig. S4. Barplots of enriched GO terms for genes enhanced in trophotaenia (A-C) and enhanced in ovary (D-F). Mainly expressed in a tissue is defined as DESeq2 baseMean > 5 and log2 fold change > 2 compared to all other tissue types in the study. GO categories are Biological Process (A,D), Cellular Component (B,E) and Molecular Function (C,F). Blue indicating the number of expected genes given the gene selection would be random, red indicating the number of observed genes. Significance of GO-terms is decreasing from bottom to top. A maximum of 20 categories is shown.



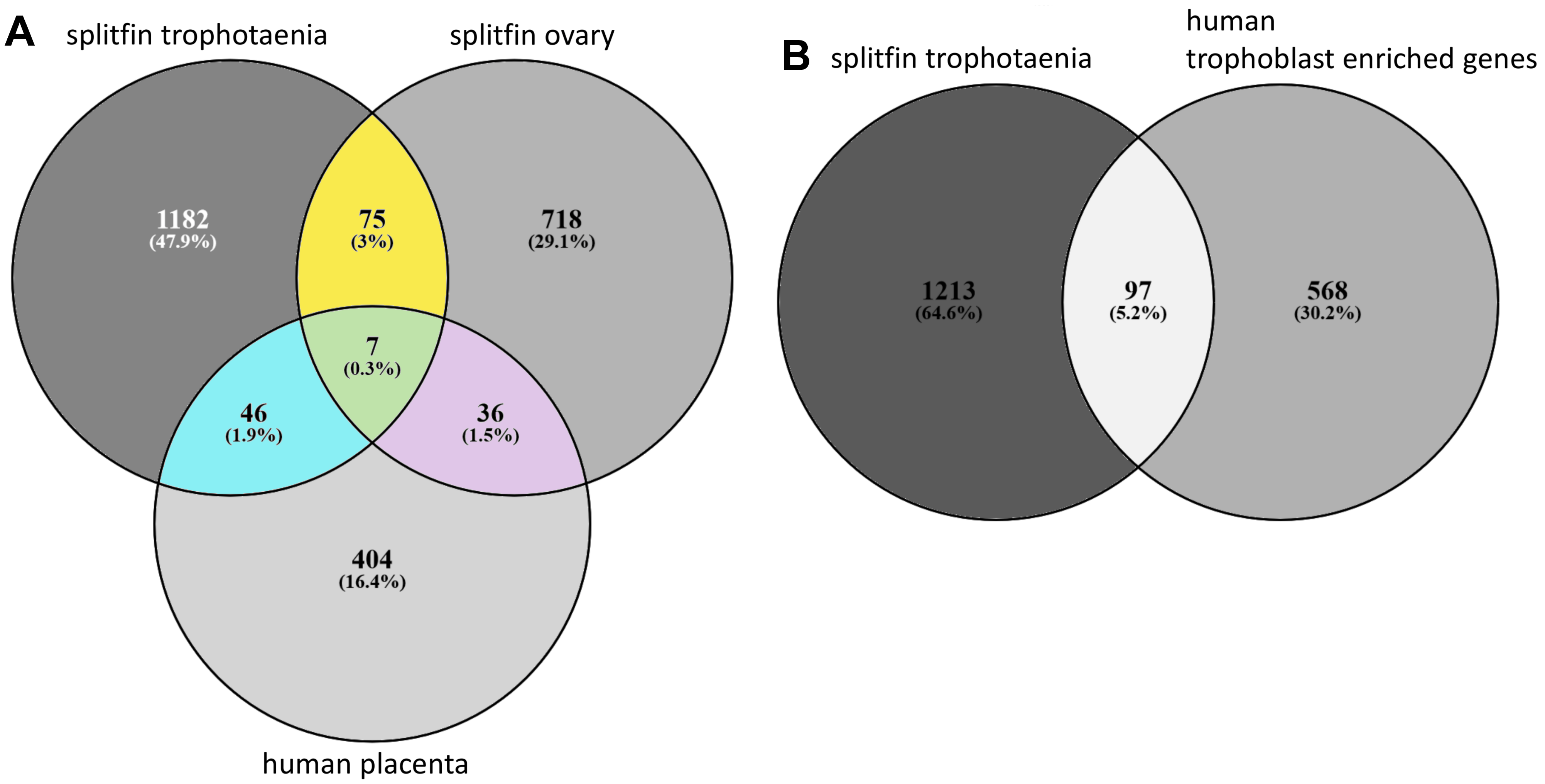
Supplemental Fig. S5. Venn diagram representing the relation between genes with enhanced expression in ovary of *G. mulitradiatus* and genes uniquely expressed in the follicular epithelium of *P. retropinna* as compared to *P.turrubarensis*.



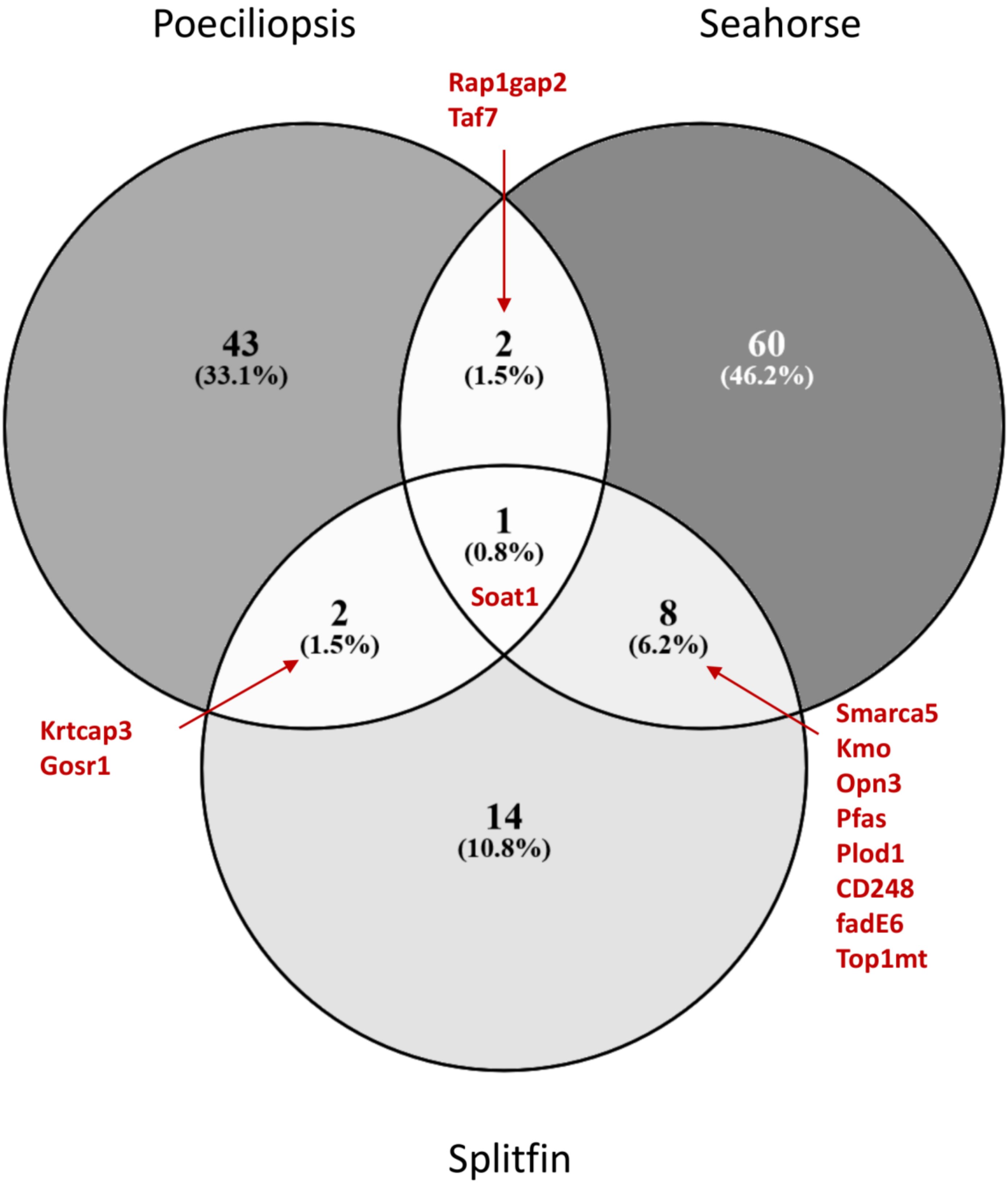
Supplemental Fig. S6. Enrichment of specific gene categories of overlapping genes between species. (A) Barplots of different enriched functional categories for overlapping genes between splitfin ovary and genes significantly up-regulated in P.retropinna as compared to P.turrubarensis (Guernsey et al.2020). (B) Barplots of enriched GO terms for 'Biological Process' for overlapping genes between splitfin trophotaenia and human placenta, (C) between splitfin ovary and human placenta (D) and between splitfin trophotaenia and human trophoblasts. Blue bars indicate the number of expected genes and red bars the number of observed genes given the gene selection would be random. Significance of GO-terms is decreasing from bottom to top. A maximum of 20 categories is shown.



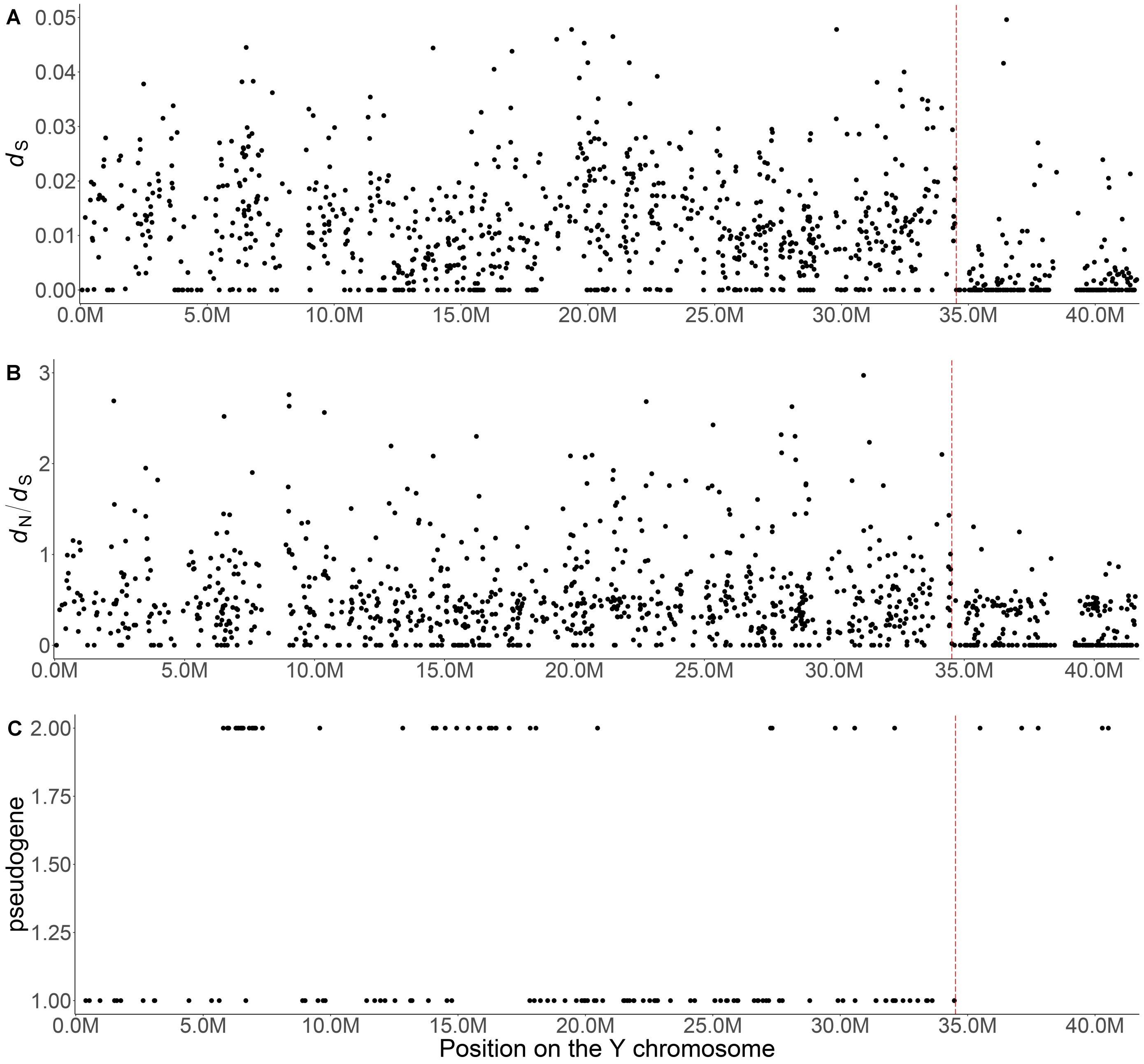
Supplemental Fig. S7. Venn diagram representing the relation between genes with enhanced expression in ovary of *G. mulitradiatus* and genes significantly upregulated in the pregnant (compared with the nonpregnant) and post-parturition (compared with the late-pregnant) seahorse brood pouch.



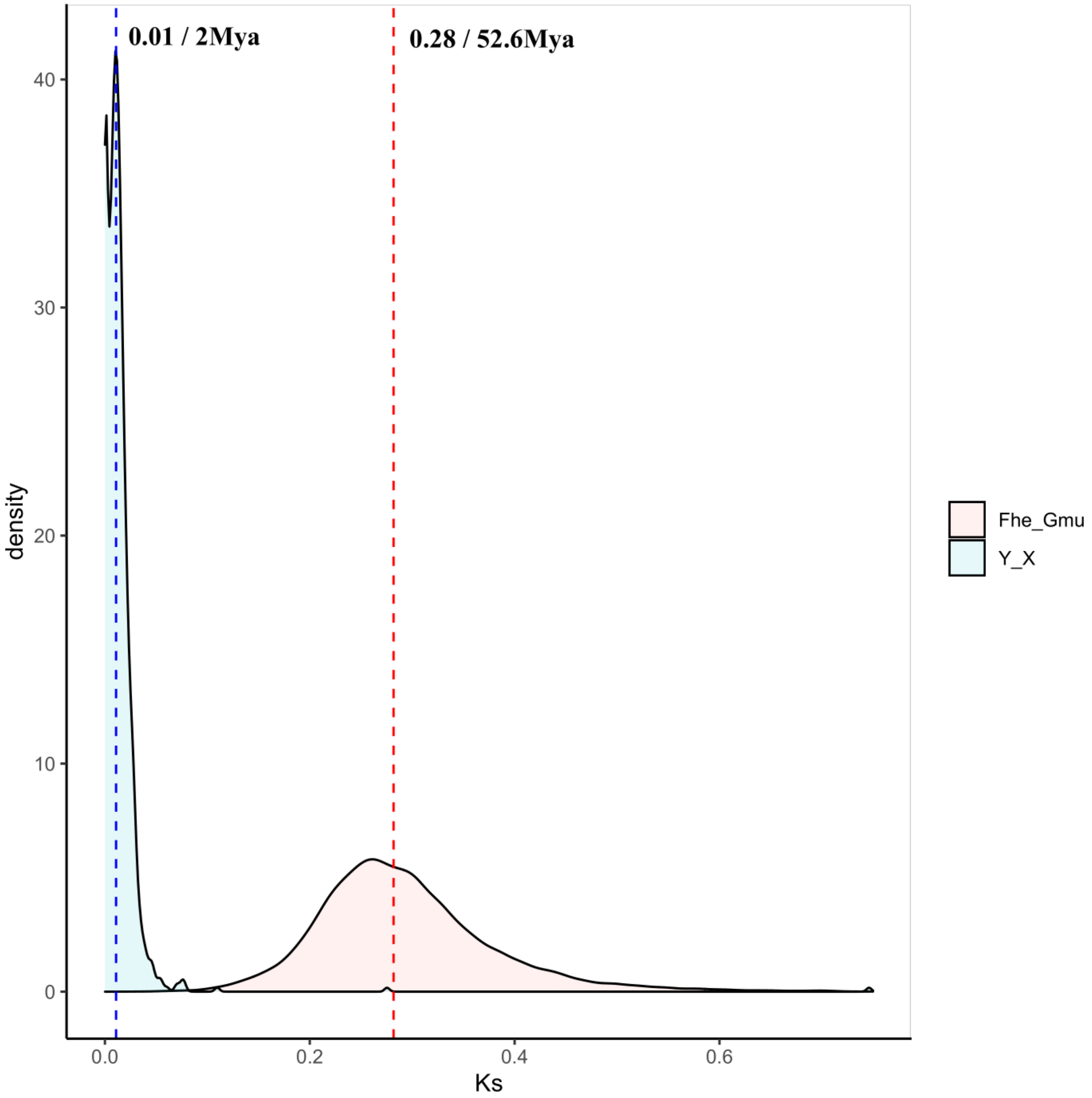
Supplemental Fig. S8. Venn diagrams representing the relation between splitfin and human placental expression. (A) genes with enhanced expression in ovary and trophotaenia of *G. mulitradiatus* and human placenta (dataset combining the embryonal and maternal part). (B) genes with enhanced expression in splitfin trophotaenia and human trophoblast, which give rise to the embryonal part of the placenta.



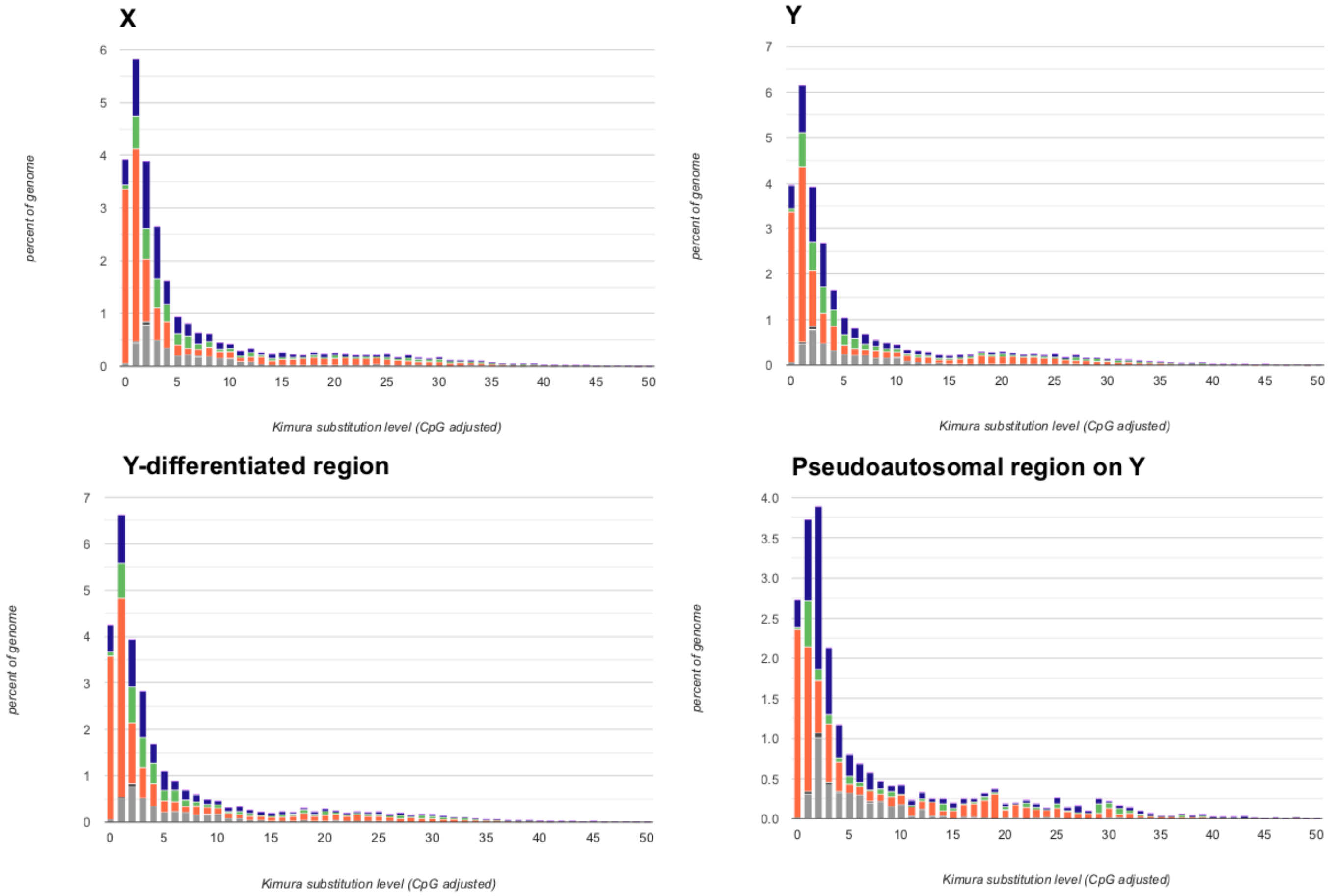
Supplemental Fig. S9. Venn diagram representing the relation between genes positively selected in *G. mulitradiatus*, seahorse and *P. retropinna*.



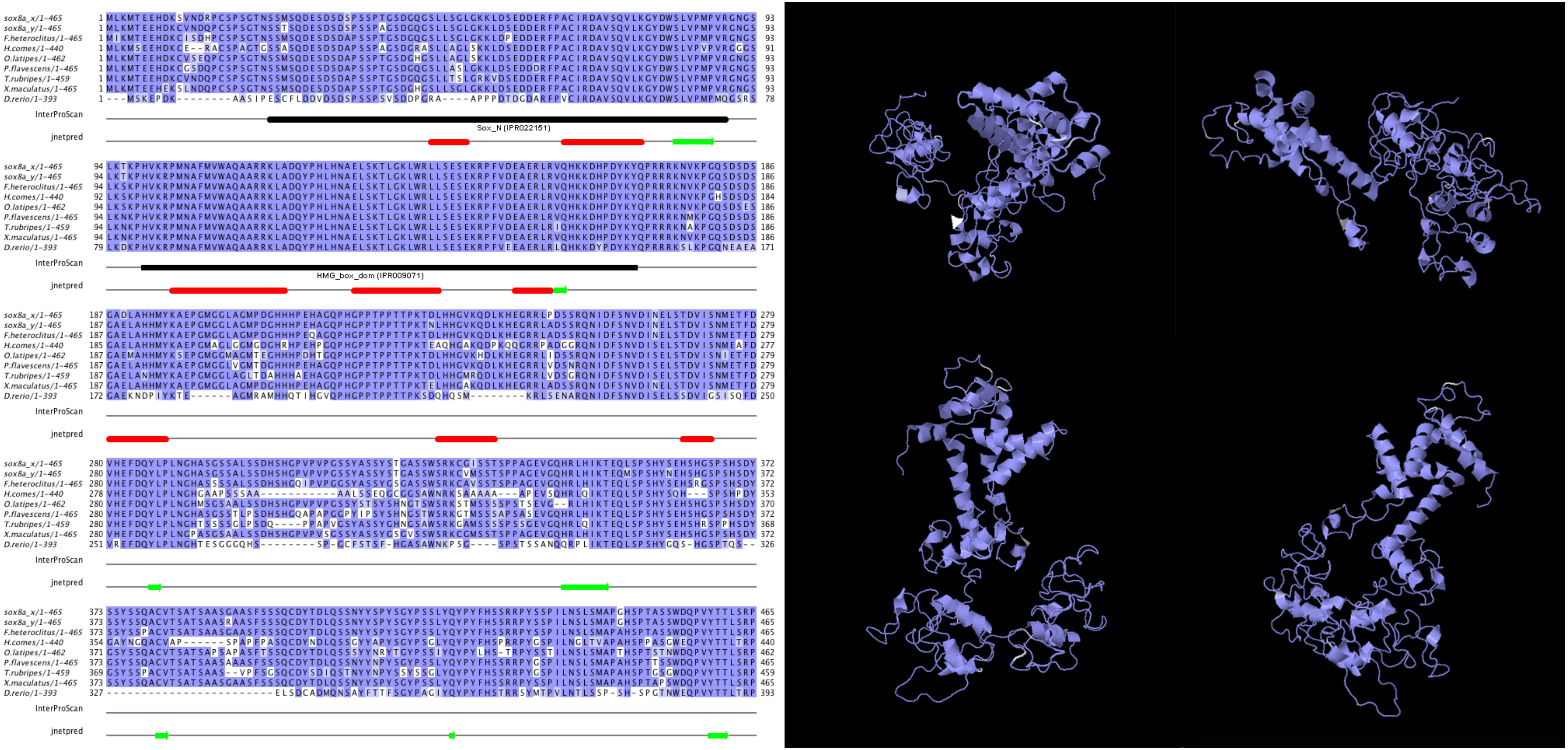
Supplemental Fig. S10. Comparison between chromosome X and Y. (A) Pairwise dS values of X and Y gene pairs/alleles. (B) Pairwise dN/dS values of X and Y gene pairs/alleles. (C) Pseudogenes identified on X (y=2.00) and on Y (y=1.00).



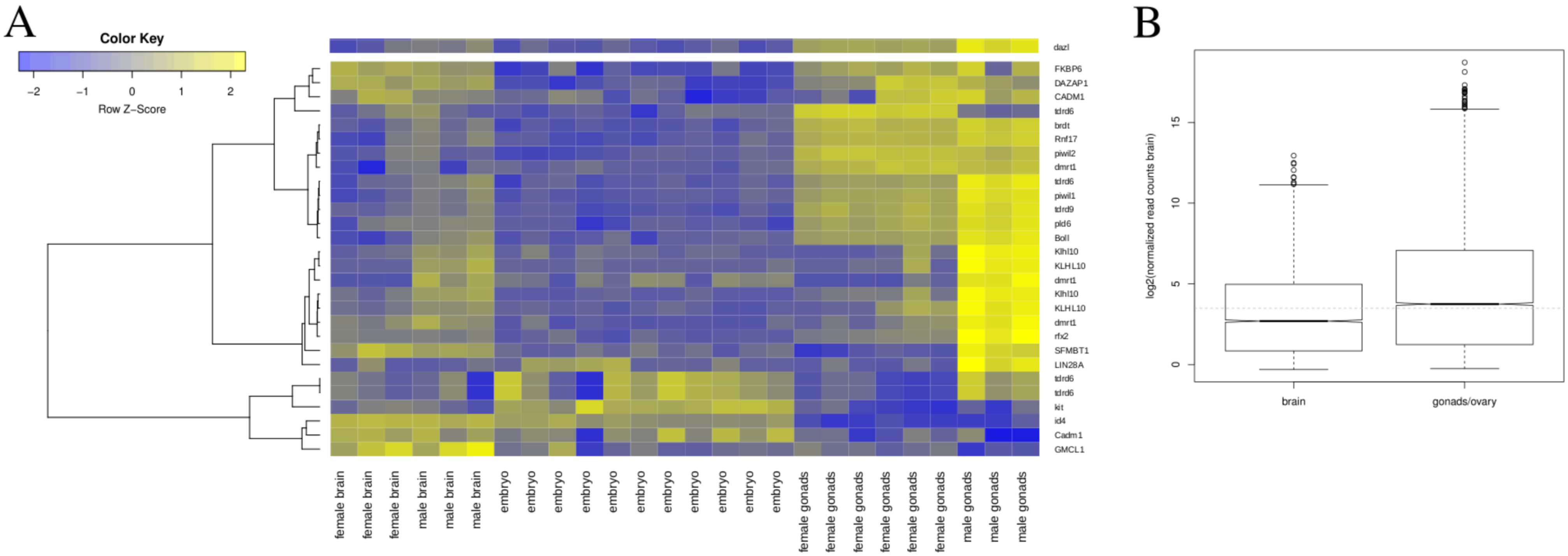
Supplemental Fig. S11. Estimation of the time when the Y-chromomsome originated. The distribution of pairwise dS values of X and Y gene pairs/alleles was compared to that of pairwise dS between *Girardinichthys multiradiatus* and *Fundulus heteroclitus* orthologs, the time of origin of the Y-chromomsome was calculated linearly.



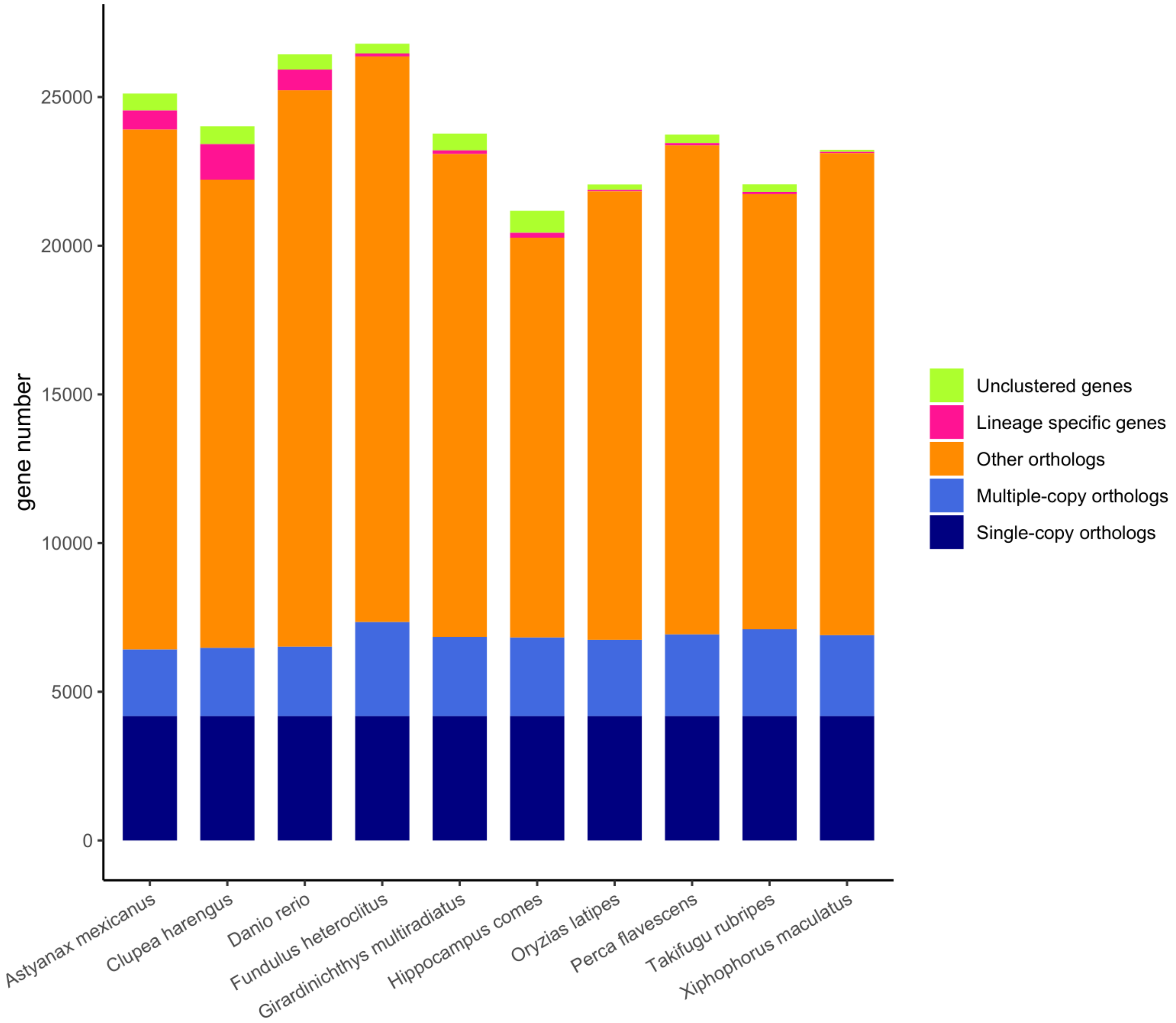
Supplemental Fig. S12. Kimura distance-based copy divergence analyses of transposable elements on the X chromosome, the Y chromosome, the Y-differentated region and the pseudoautosomal region on the Y.



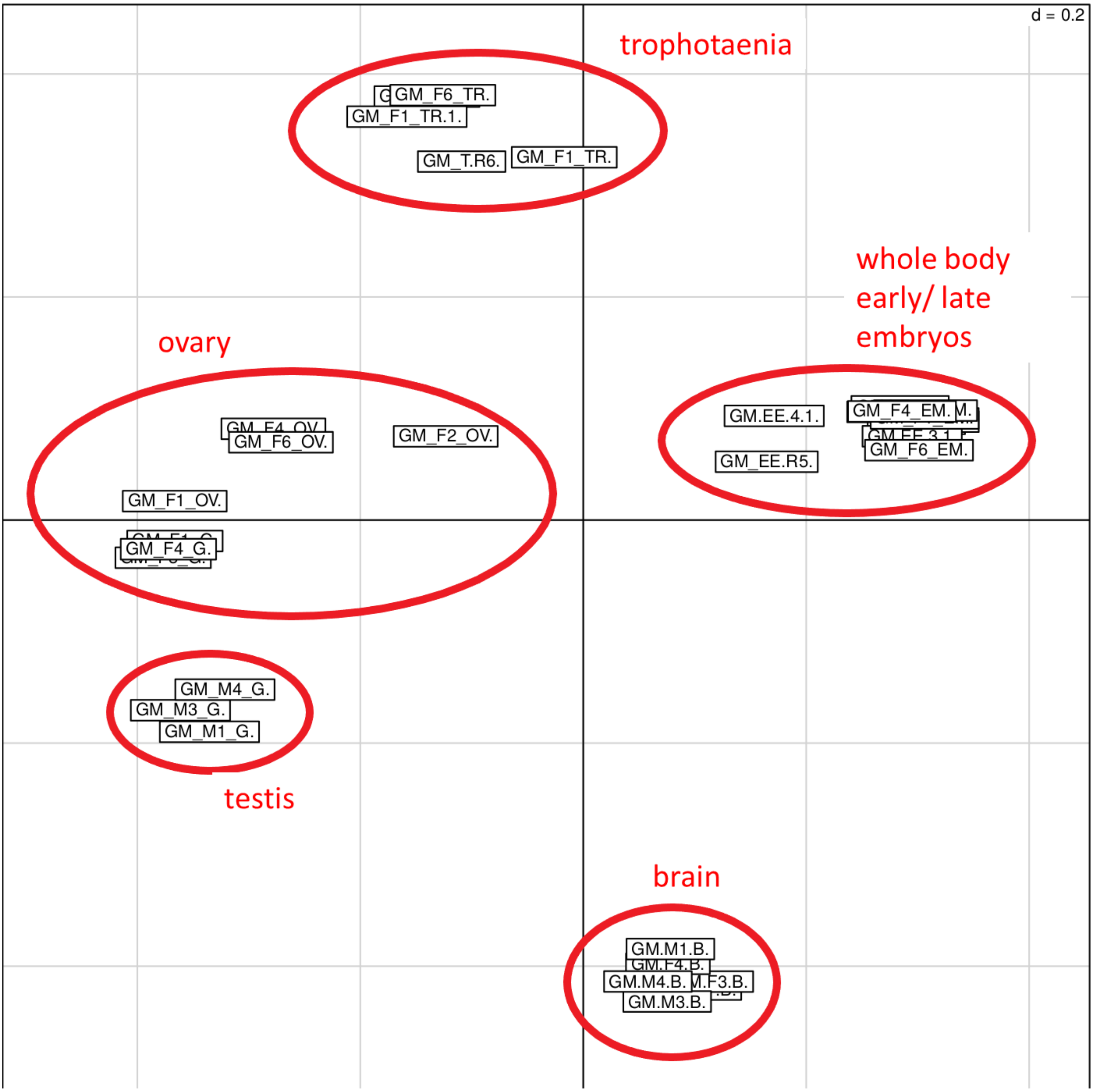
Supplemental Fig. S13. Alignment and 3D structure of Sox8a. Left: an alignment of Sox8a sequences from different species. Right: the predicted 3D structure of Sox8a Y allele shown in four perspectives.



Supplemental Fig. S14. Expression of Dazl regulated genes. A) Heatmap showing RNAseq-based expression levels of genes in brain, embryos and gonads regulated by Dazl. B) Boxplot of gene expression in brain and gonads of all genes differentially regulated (baseMean>10, logFC>1, adj.P<0.05) in brain showing that these genes are differentially regulated in brain, but mostly expressed at a lower level than in the gonads. The dotted line shows the median expression of all genes in brain.



Supplemental Fig. S15. Gene categories in ten teleost species. Single-copy orthologs: orthologs revealing single copy in each of the ten speices; Multiple-copy orthologs: orthologs that are in present in every species and revealing multiple copies in some species; Other orthologs: orthologs that are found in some species but not in all ten; Lineage specific genes: genes with no ortholog found in the other nine species.



Supplemental Fig. S16. Correspondence Analysis of the splitfin samples based on RNA-seq read counts of all genes.