

SUPPLEMENTAL INFORMATION

Figure S1 Density curves of synonymous mutations to assess false orthologs for the pairwise comparisons of *H. carbonarium* (A), *H. flavolineatum* (B) and *H. macrostomum* (C). Histograms indicate overall distribution of pair-wise dS values for each pairwise comparison, and bars indicate the density of genes assigned to each Gaussian component (Component1= blue; 2= red; 3= green; all genes= black). Genes assigned to the third component (green) were excluded from the analyses, as they had the highest dS values and were assumed to be inaccurate calls.

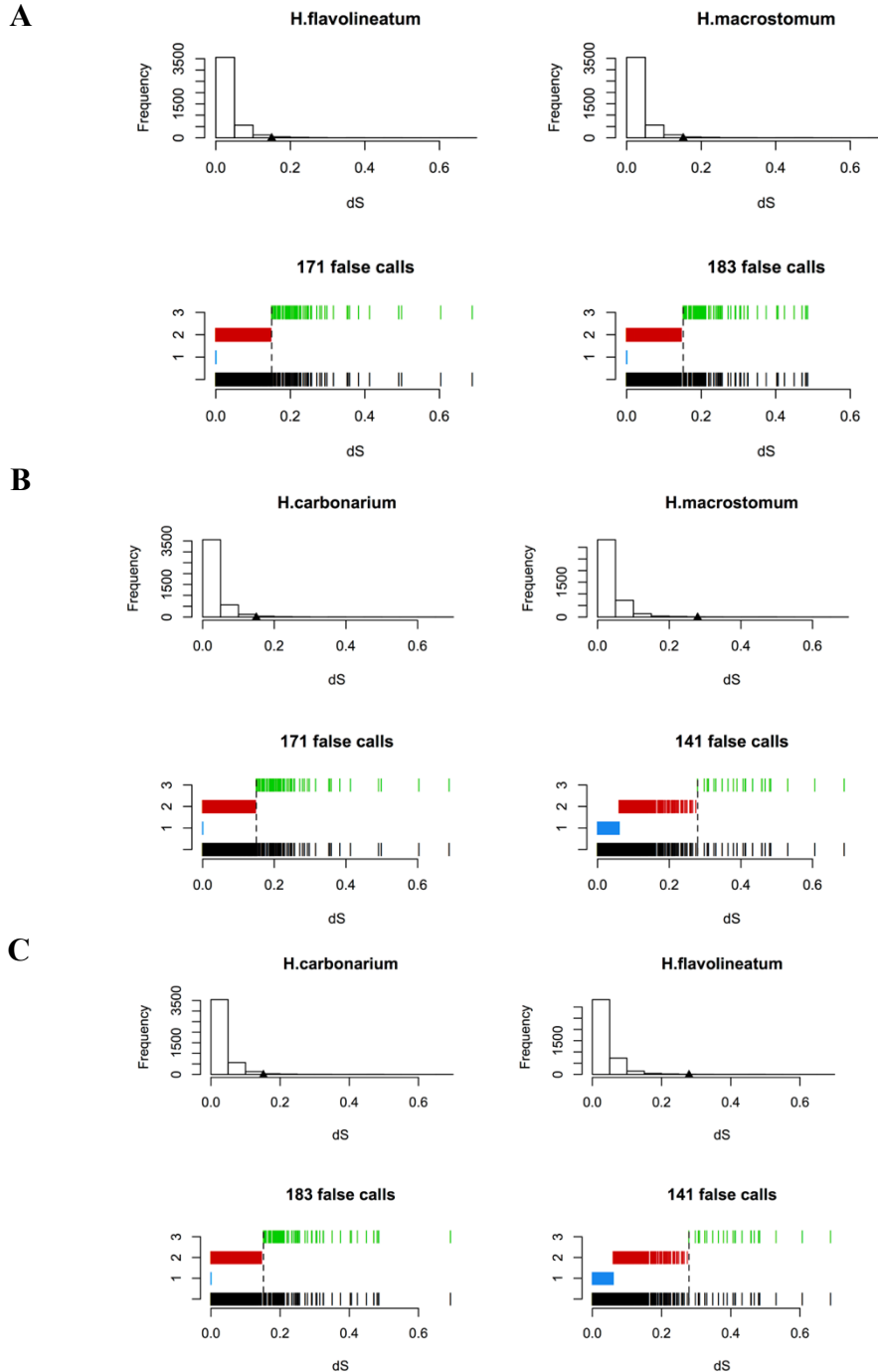


Figure S2 Histograms show the distribution of pairwise $1.0 < dN/dS < 4.0$ for *H. carbonarium* (A), *H. flavolineatum* (B), *H. macrostomum* (C). Colored lines represent the density of four-component mixture models fitted to all dN/dS (between 0 and 20). The fourth component (teal) was assumed to capture false ortholog calls and was removed. For all three species, the transition between the third and fourth component (vertical black dotted line) occurred at a dN/dS value of 3.

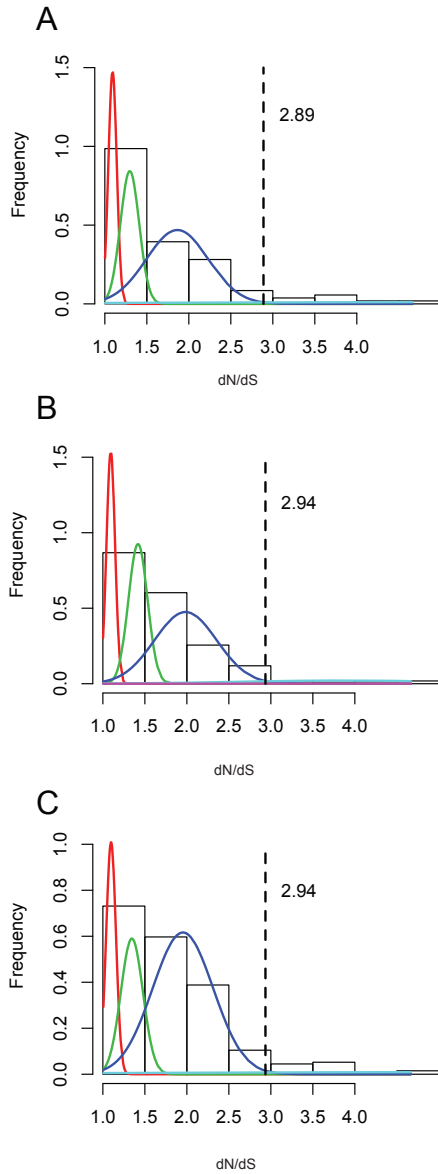


Figure S3 Bar graphs reflecting the completeness of the transcriptome in reference to the BUSCO ortholog database for vertebrates (A) and ray-finned fish (B) for the species *H. carbonarium* (HCAR), *H. flavolineatum* (HFLA) and *H. macrostomum* (HMAC).

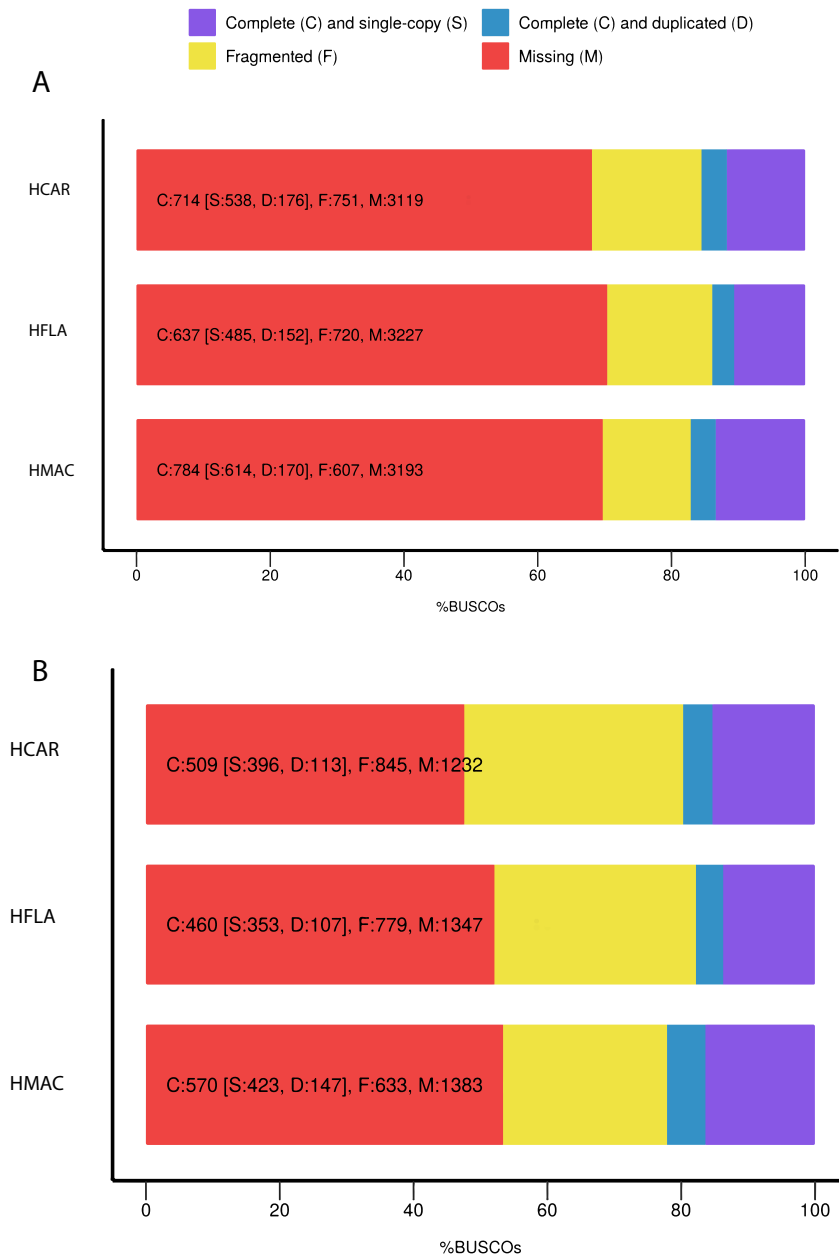


Figure S4 Gene Ontology (GO) terms significantly enriched for the d_N/d_S comparisons between *H. carbonarium* and *H. flavolineatum*, for the categories Biological Process (A), Cellular Component (B) and Molecular Function (C). These results include only genes under positive selection, and size and brightness of GO terms depends on the level of significance. Numbers that precede the category name are the genes present in this particular analysis over the total number of genes that belong to that category.

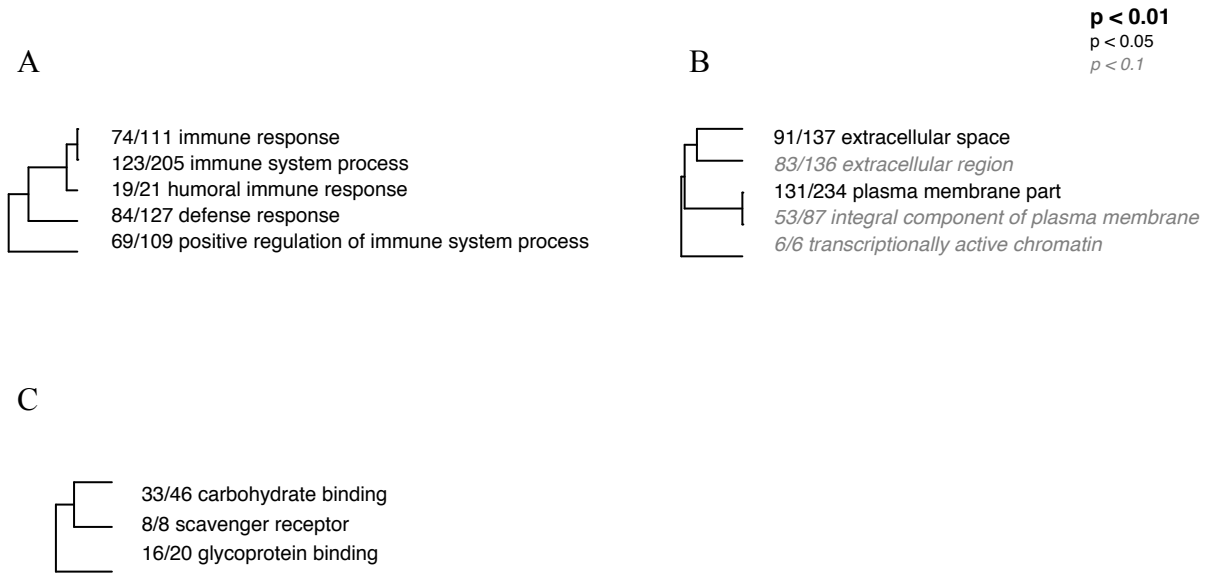


Figure S5 Gene Ontology (GO) terms significantly enriched for the d_N/d_S comparisons between *H. carbonarium* and *H. macrostomum*, for the categories Biological Process (A), Cellular Component (B) and Molecular Function (C). These results include only genes under positive selection, and size and brightness of GO terms depends on the level of significance. Numbers that precede the category name are the genes present in this particular analysis over the total number of genes that belong to that category.

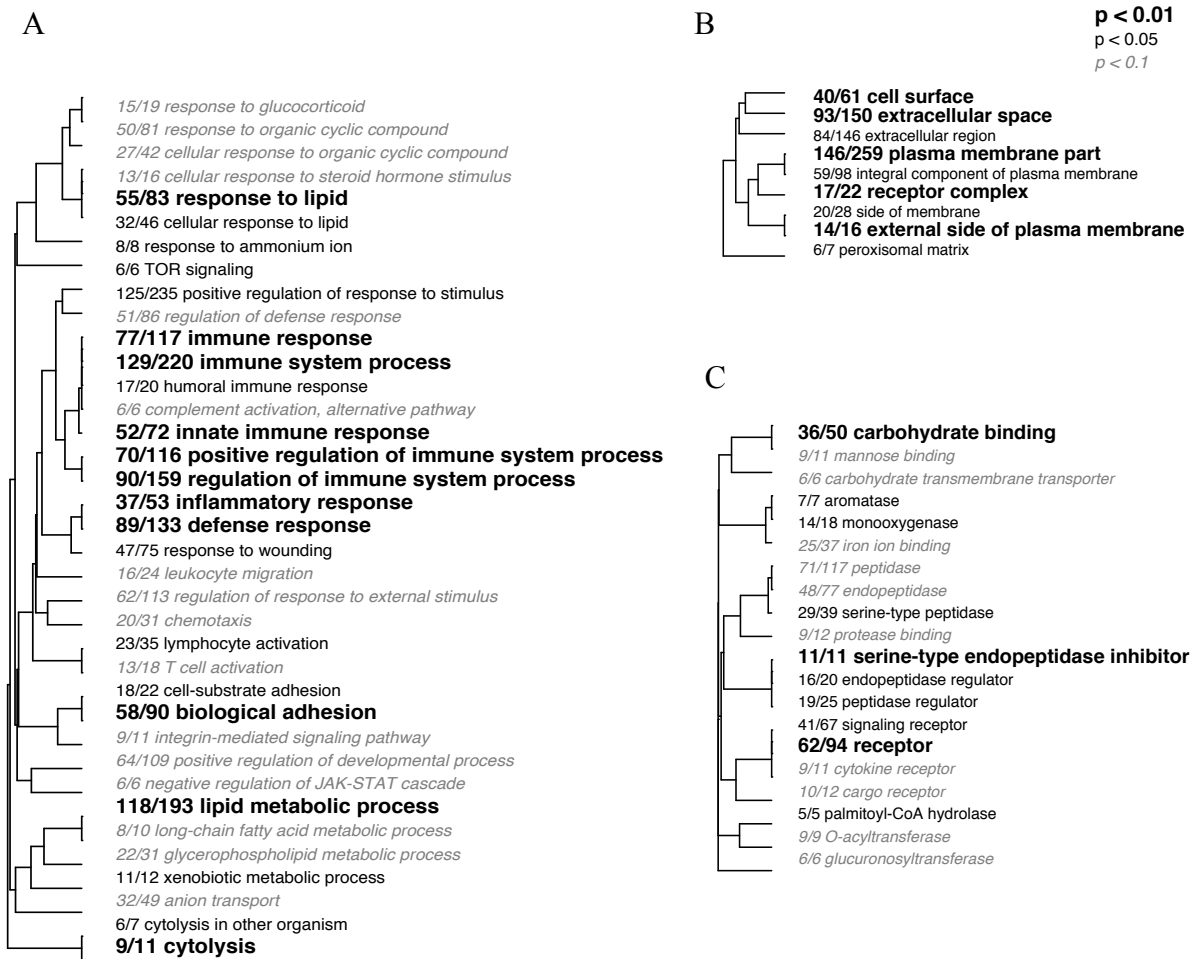


Figure S6 Gene Ontology (GO) terms significantly enriched for the d_N/d_S comparisons between *H. flavolineatum* and *H. macrostomum*, for the categories Biological Process (A), Cellular Component (B) and Molecular Function (C). These results include only genes under positive selection, and size and brightness of GO terms depends on the level of significance. Numbers that precede the category name are the genes present in this particular analysis over the total number of genes that belong to that category.

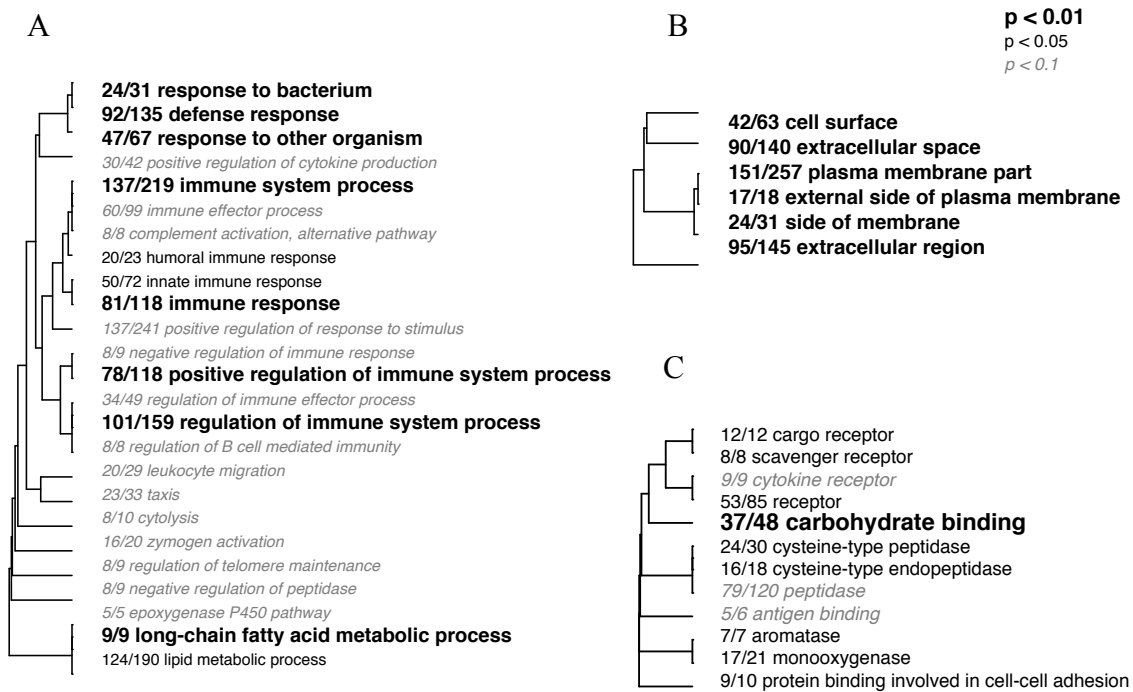


Figure S7 Example of a heatmap for transcripts of Vitellogenin and Zona Pellucida genes, which were highly upregulated for seven individuals of *Haemulon flavolineatum* (HFL; in bold). These individuals were removed from the analyses in order to get more accurate measures of differential expression and GO enrichment. The other individuals of *H. flavolineatum* have similar levels of expression to all the collected individuals of *H. macrostomum* (HMCR).

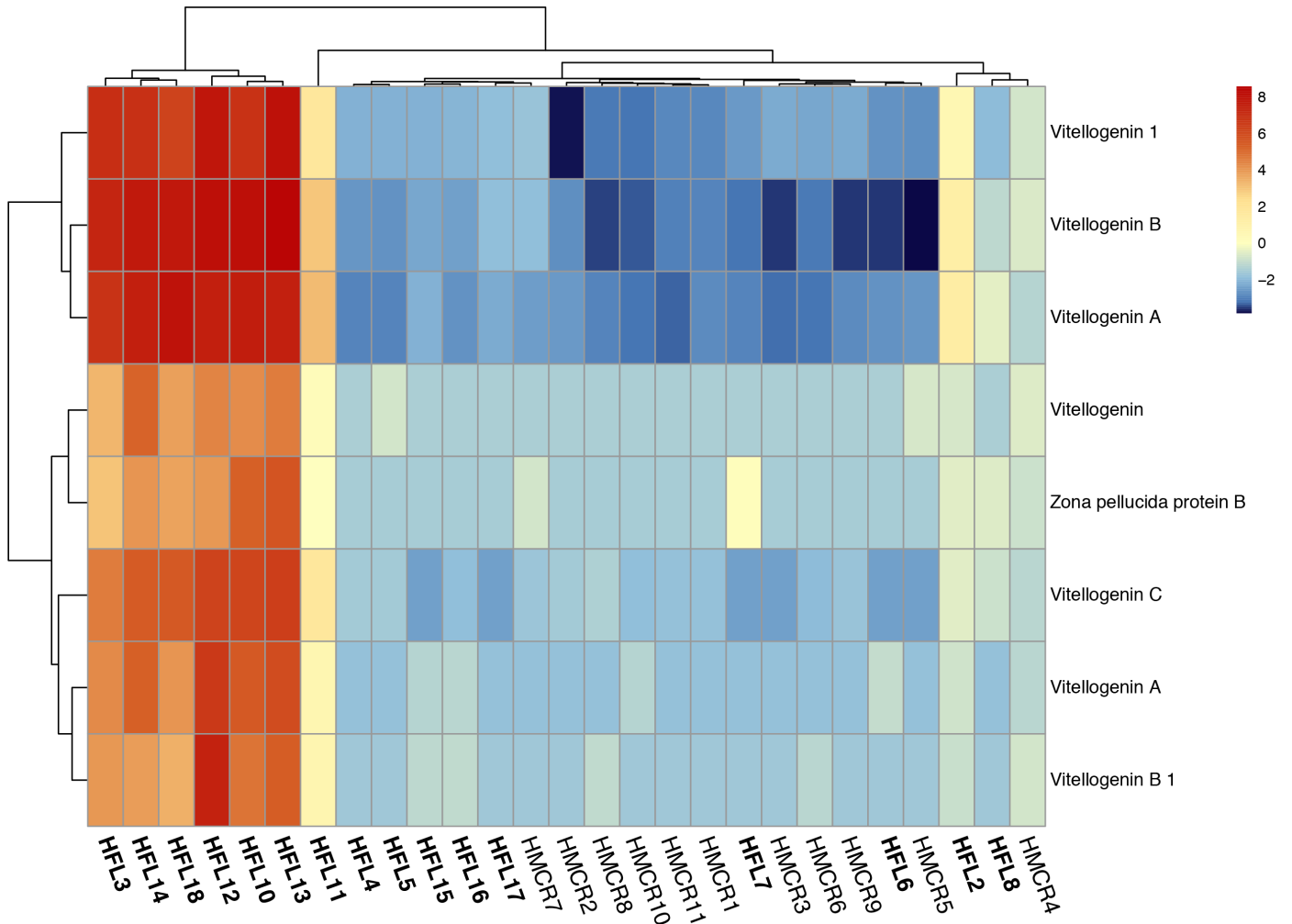


Figure S8 Gene Ontology (GO) terms significantly enriched in the comparison of gene expression of *H. carbonarium* and *H. flavolineatum*, for the categories Cellular Component (A) and Molecular Function (B). Size and brightness of GO terms depends on the level of significance, and numbers that precede the category name are the genes present in this particular analysis over the total number of genes that belong to that category. GO terms in red are upregulated categories for *H. carbonarium*, while blue ones are downregulated. The category of Biological Process only had two significant terms: Translation (downregulated, 182 sequences) and Immune Responses (upregulated, 177 sequences).

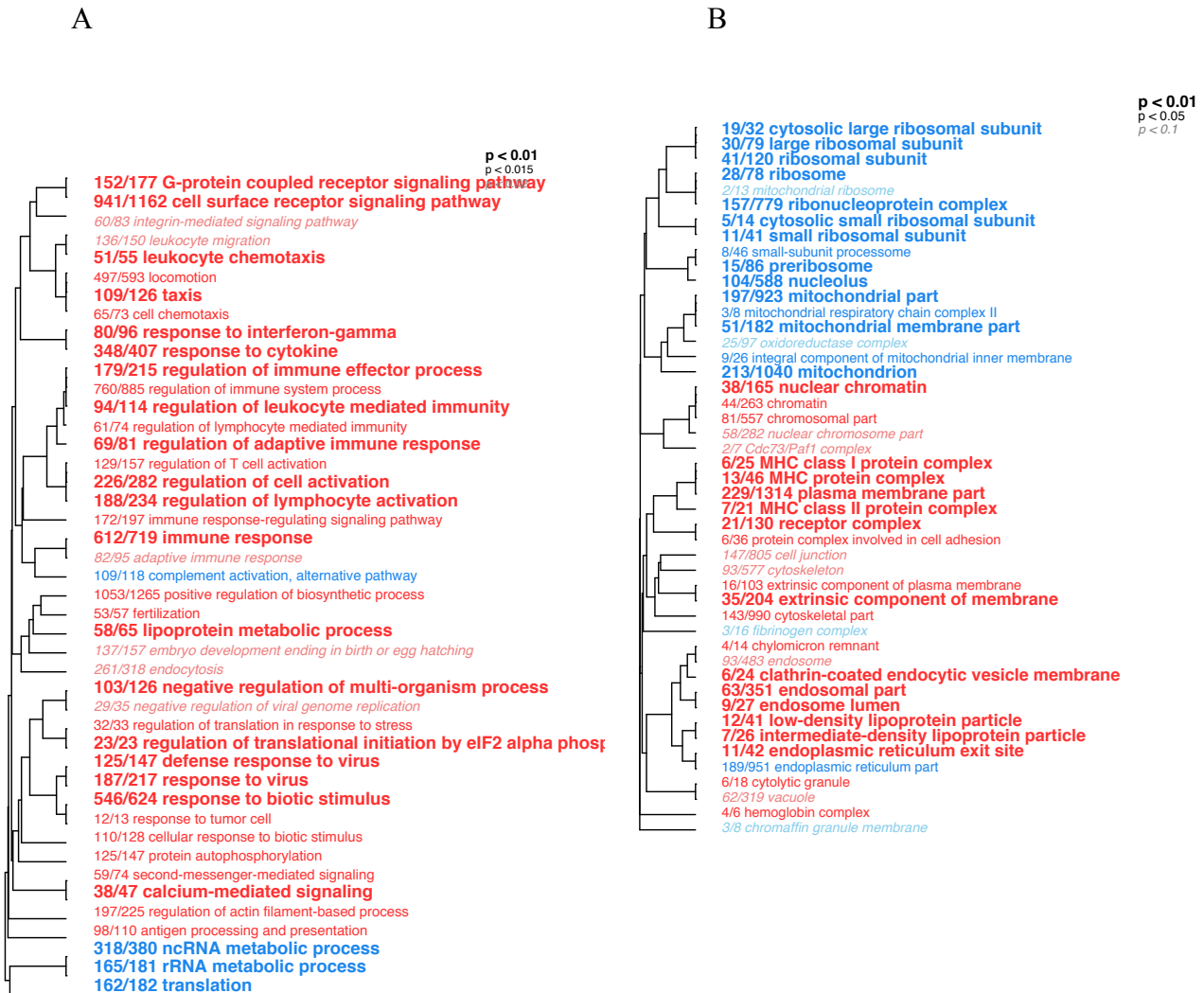
A



B



Figure S9 Gene Ontology (GO) terms significantly enriched with the MWU Test of gene expression for *H. carbonarium* and *H. macrostomum*, for the categories Biological Process (A), Cellular Component (B) and Molecular Function (C). Due to the large number of significant GO terms for BP (171), only those with a FDR<0.02 were included in the tree. Size and brightness of GO terms depends on the level of significance, and numbers that precede the category name are the genes present in this particular analysis over the total number of genes that belong to that category. GO terms in red are upregulated categories for *H. carbonarium*, while blue ones are downregulated.



C

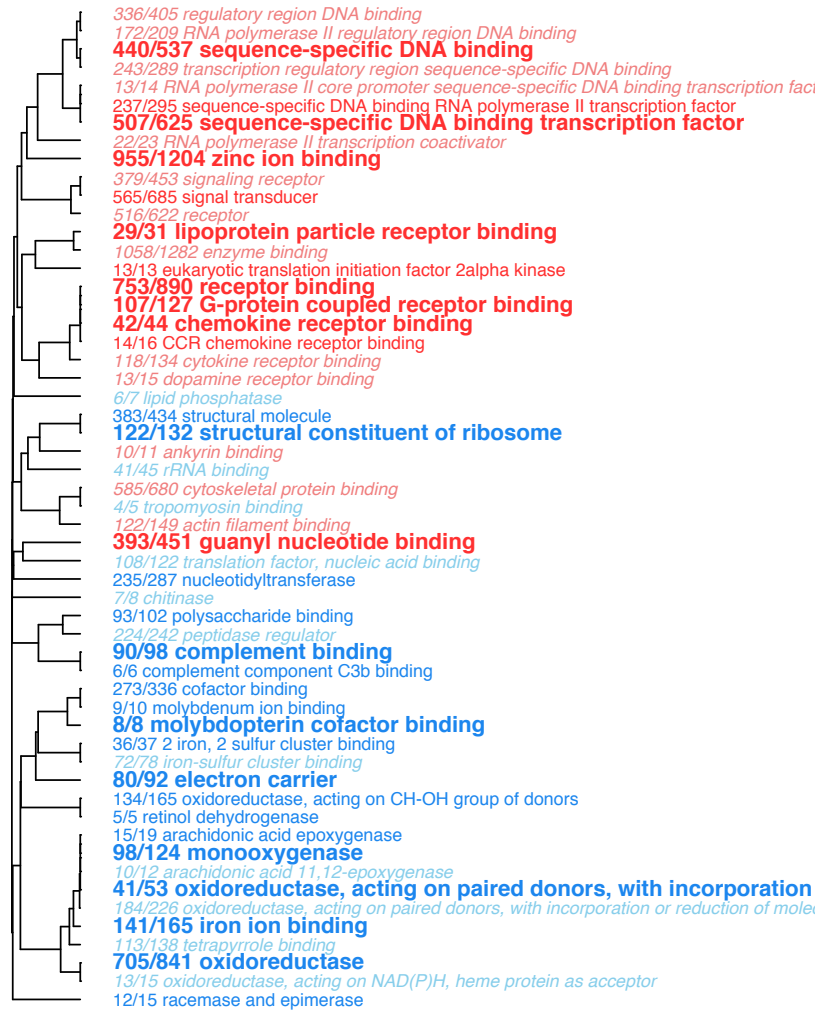


Figure S10 Gene Ontology (GO) terms significantly enriched with the MWU Test of gene expression for *H. flavolineatum* and *H. macrostomum*, for the categories Biological Process (A), Cellular Component (B) and Molecular Function (C). Size and brightness of GO terms depends on the level of significance, and numbers that precede the category name are the genes present in this particular analysis over the total number of genes that belong to that category. GO terms in red are upregulated categories for *H. flavolineatum*, while blue ones are downregulated.

