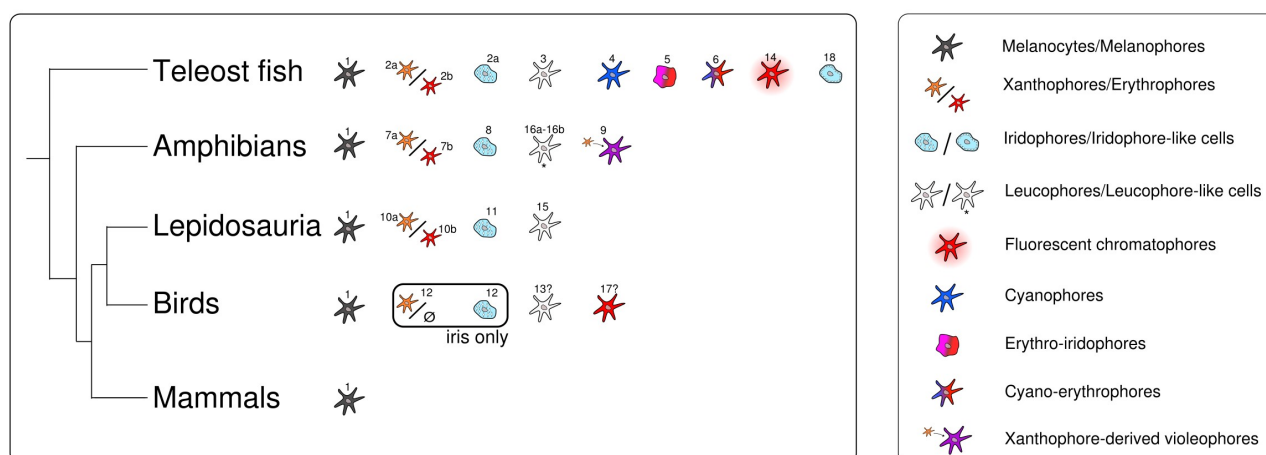


SUPPLEMENTARY FIGURES AND TABLES

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Supplementary figure 1: An overview of the different pigment cells in the major vertebrate lineages.

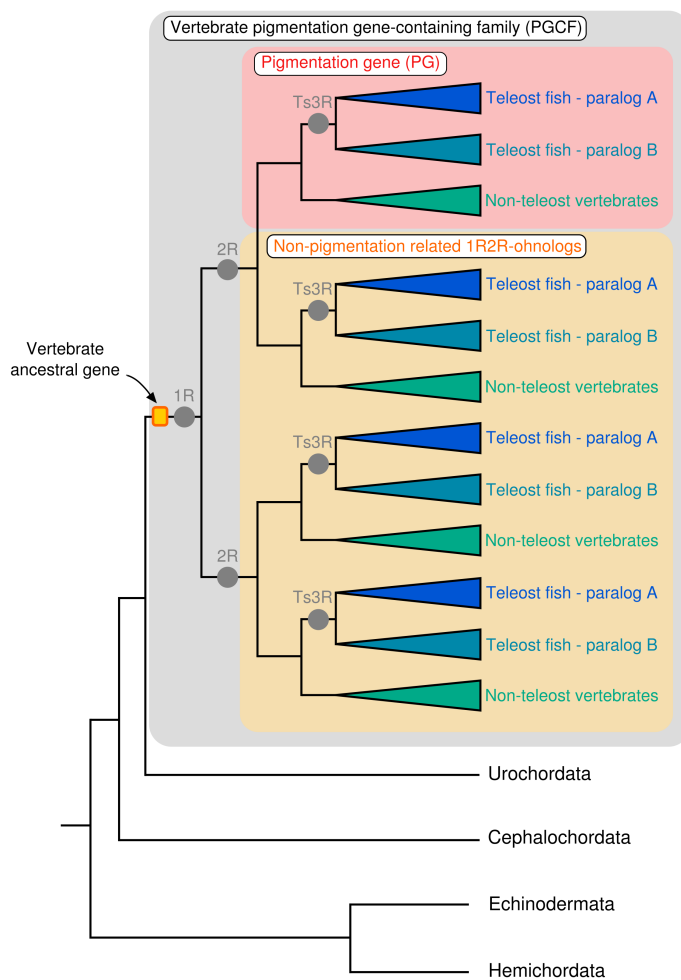
Numbers indicate the corresponding literature references (see below). Apart from melanocytes (first column), cells in the same column may not correspond to homologous cell types (inter-taxa ontogenetic or genetic evidence is missing).

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Supplementary figure 2. Theoretical evolution of a vertebrate "pigmentation family" and definitions of terms used in this study.

The vertebrate pre-WGD ancestral gene, theoretically present as a single copy in non-vertebrate deuterostomes, has been duplicated at the basis of vertebrate evolution and subsequently in teleost fish, leading to a total of four non-teleost vertebrate paralogs (1R/2R-ohnologs) or to eight teleost paralogs. Pigmentation genes (PGs) are defined as all vertebrate genes that are ortholog to a gene related to pigmentation in at least one vertebrate species. A pigmentation gene-containing family (PGCF) is a gene family encompassing a PG as well as all its 1R/2R-ohnologs, if any. After 1R/2R but before Ts3R, PGCFs can harbor only 1 gene (the PG) if gene losses occurred after the 1R/2R event, but most PGCFs harbor 2 genes or more. Note that 15 PGCFs harbor two paralogous PGs (see Supplementary Table 3). The 4th round of WGD occurring at the basis of the salmonid lineage is not represented here.

Supplementary figure 4A-J (see below): Paralogy and orthology relationships for chordate pigmentation gene families. Filled boxes correspond to gene presence. Overlapping boxes (such as for Coelacanth ALDOC) represent small scale duplications (SSDs). Boxes one above the other correspond to Ts3R-duplicates (such as for zebrafish *aldoc*: *aldoca* and *aldocb*) or to Ss4R-duplicates when there was only one Ts3R ohnolog in fish (such as for salmon *adrbk2*). Smaller boxes one next to the other correspond to Ss4R-duplicates of a Ts3R-retained gene. For instance, *aldoa* has been duplicated in fish, leading to *aldoaa* and *aldoab*; both *aldoaa* and *aldoab* were subsequently duplicated at Ss4R and all paralogs are present in trout. Empty boxes with dashed contour lines correspond to absence of gene. *P* means that the gene was annotated as a pseudogene in Ensembl.

Genes with red contours correspond to PGs (labeled with a star symbol). All genes in the same PGCF have the same fill color. For instance, *aldoc* was duplicated in fish, leading to *aldoca* and *aldocb*; only *aldocb* was subsequently kept after Ss4R, while only one copy of *aldoca* remained in salmon but was lost in trout. For multigenic PGCFs, there is only one gene copy in non-vertebrate chordates; hence, “aldo” is present in a single copy in non-vertebrate chordates and was further duplicated at 1R/2R; one ohnolog was lost leading to three extant genes.

Animal symbols are taken from PhyloPic (<http://phylopic.org/>). Divergence time between species is taken from the TimeTree website (<http://www.timetree.org/>) and from Berna et al. (2014). The exact timing of the main WGD events in the tree is not known but these events are positioned on the appropriate branches. Image credits of *L. oculatus*, *A. mexicanus*, *G. aculeatus*, *T. nigroviridis*, *X. maculatus*, *O. niloticus*: Milton Man. Image credits of *O. anatinus*, *A. carolinensis*: Sarah Werning. Image credit of *O. mykiss*: Servien (vectorized by T. Michael Keesey). Ages in chronological time scale are indicated as millions of years before present. Cmb.: Cambrian, O: Ordovician, S: Silurian, D: Devonian, Crb.: Carboniferous, P: Permian, T: Triassic, J: Jurassic, Cret.: Cretaceous, P: Paleogene, N: Neogene, Q: Quaternary. (This figure was generated with an in-house Python code available on request.)

References for supplementary figure 4 :

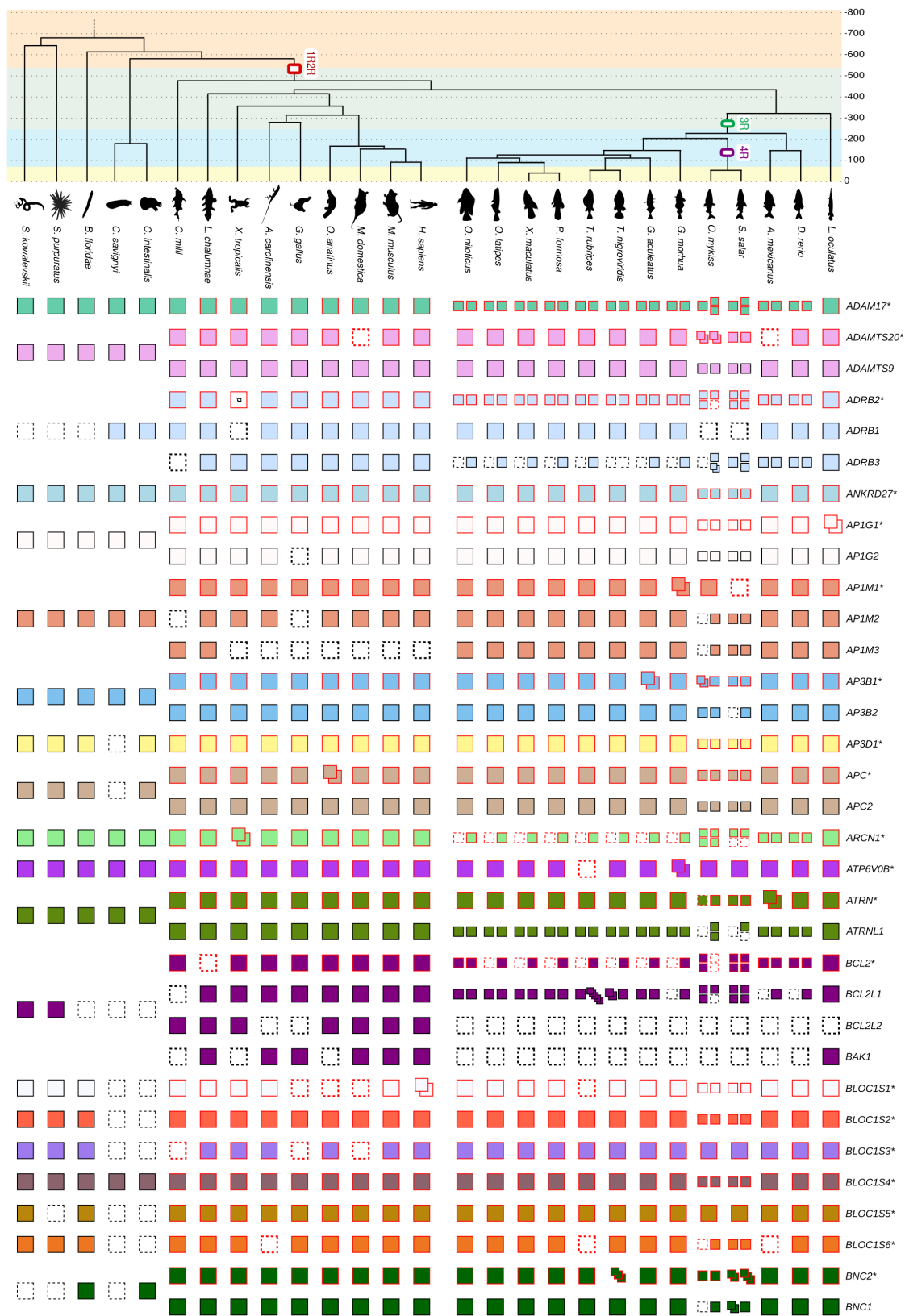
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Hedges SB et al. 2006. TimeTree: a public knowledge-base of divergence times among organisms. *Bioinformatics*. 22:2971–2972. doi: 10.1093/bioinformatics/btl505.

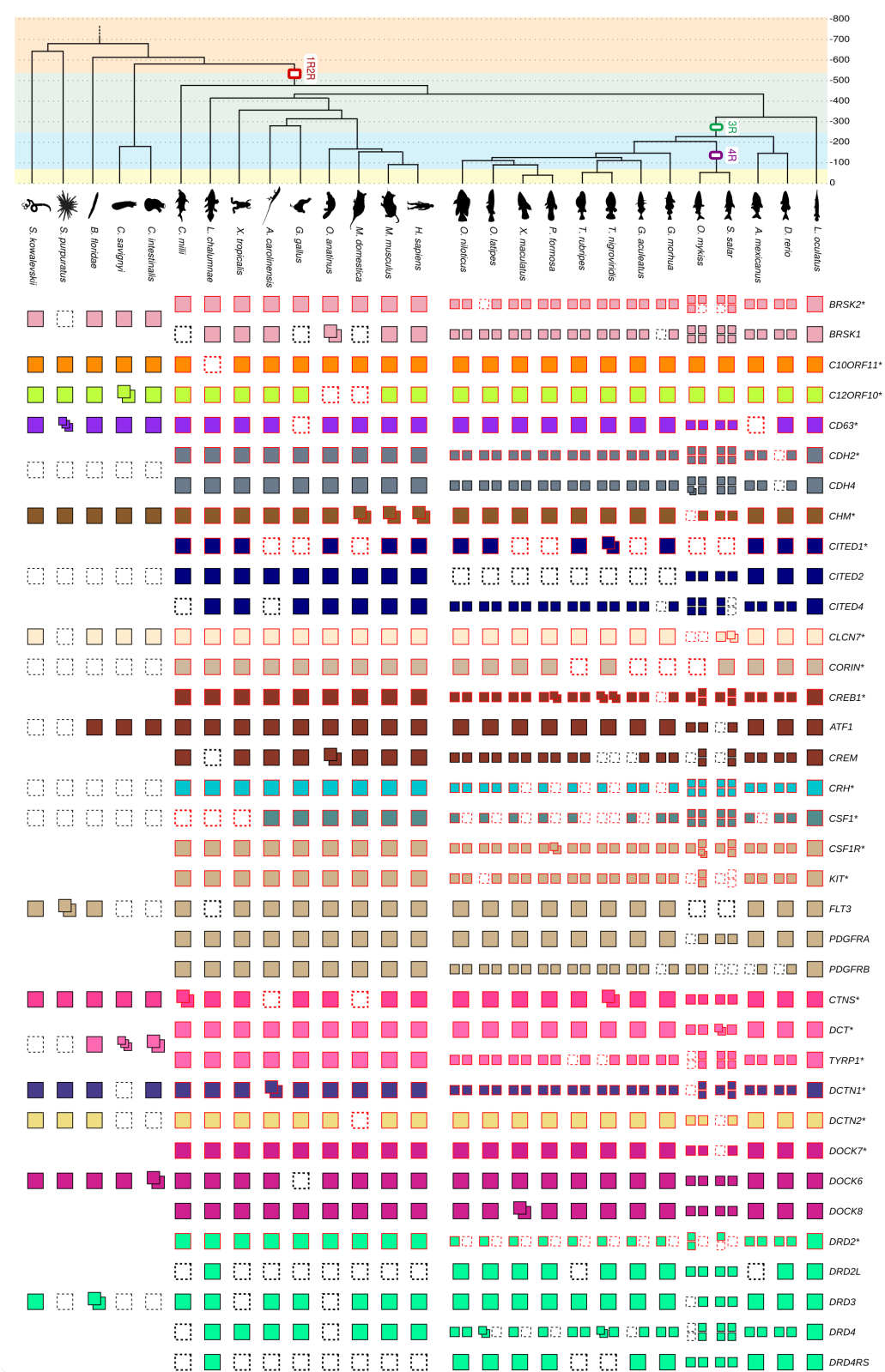
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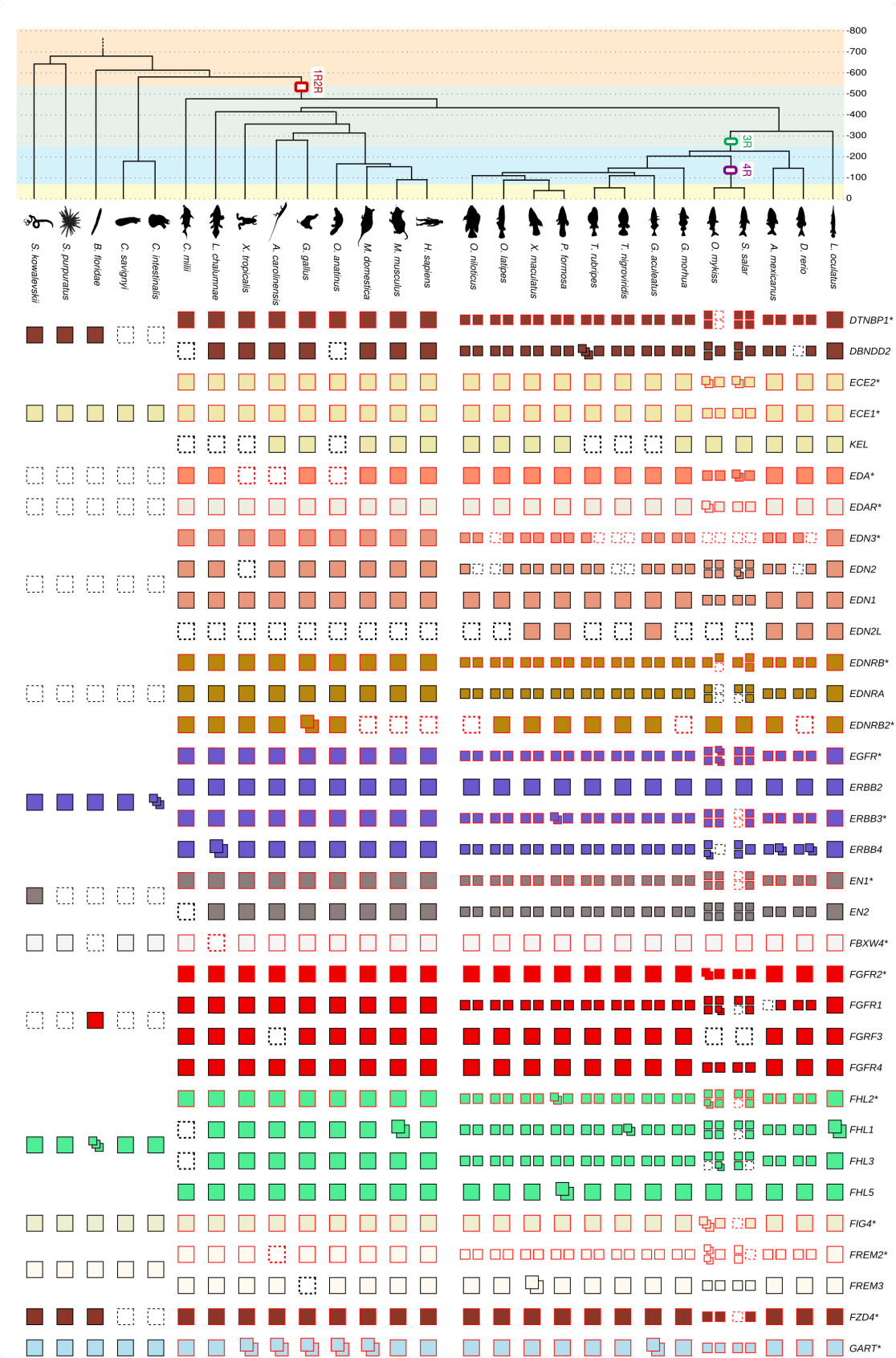
Supplementary figure 4A



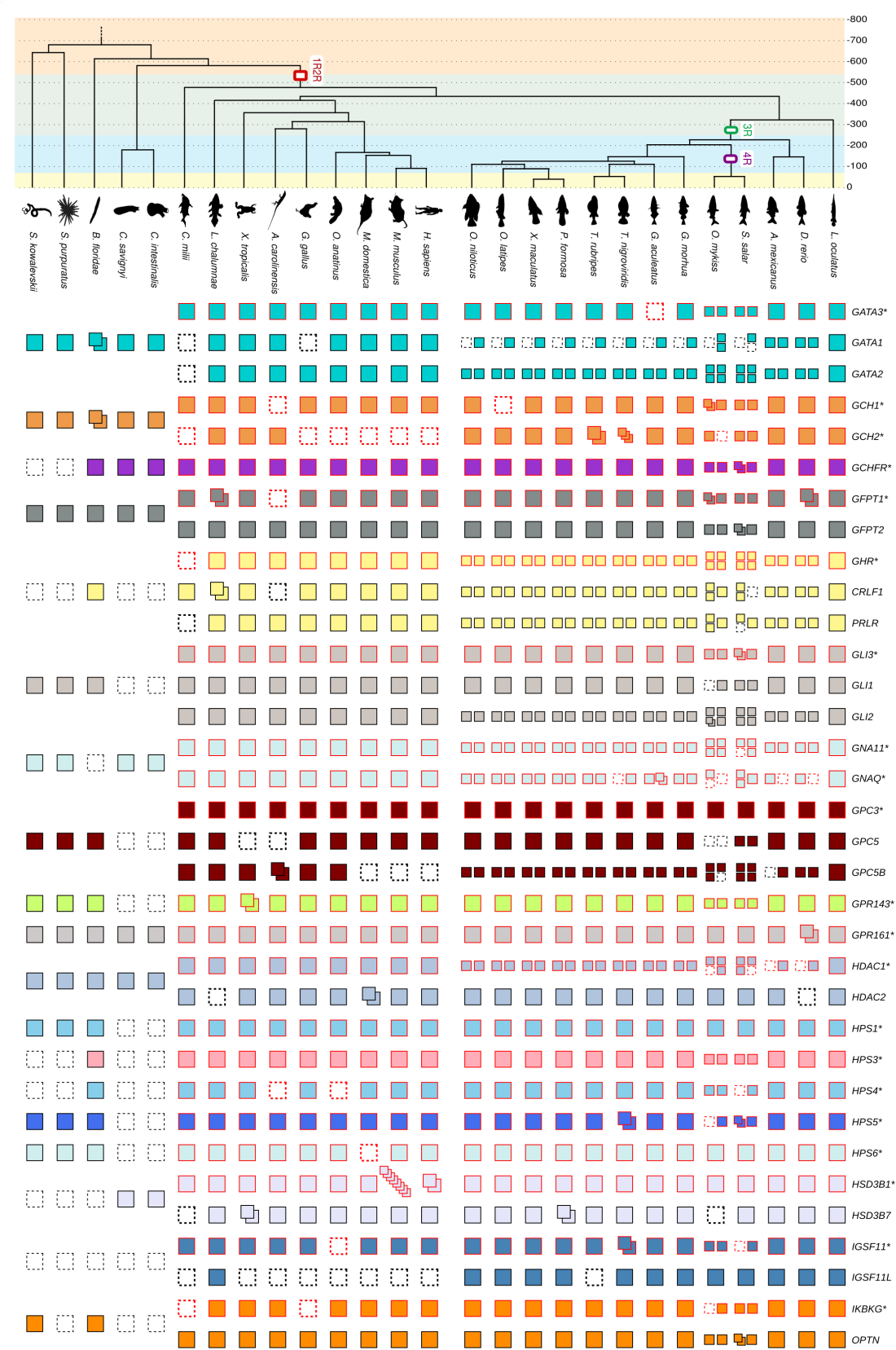
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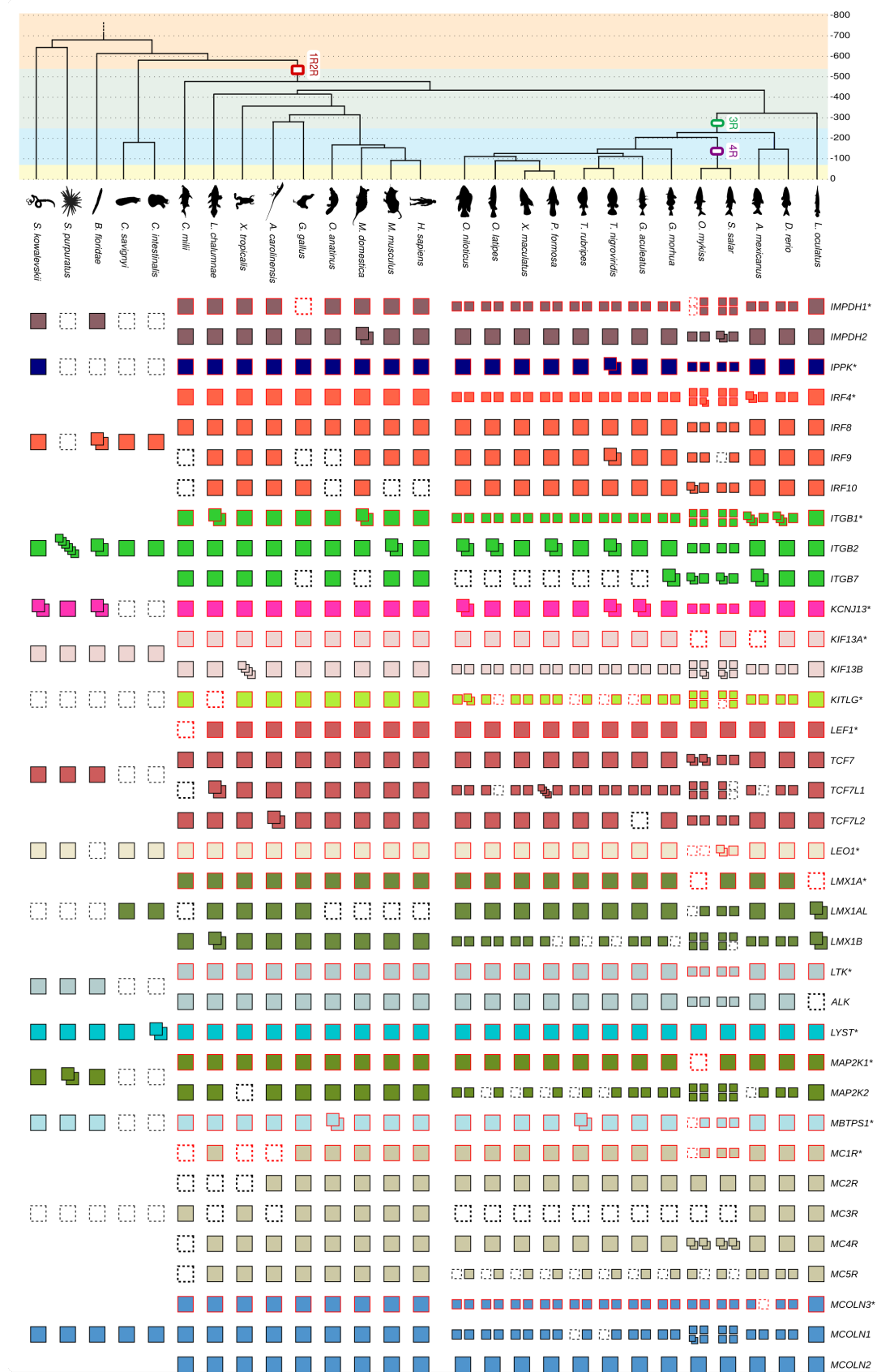
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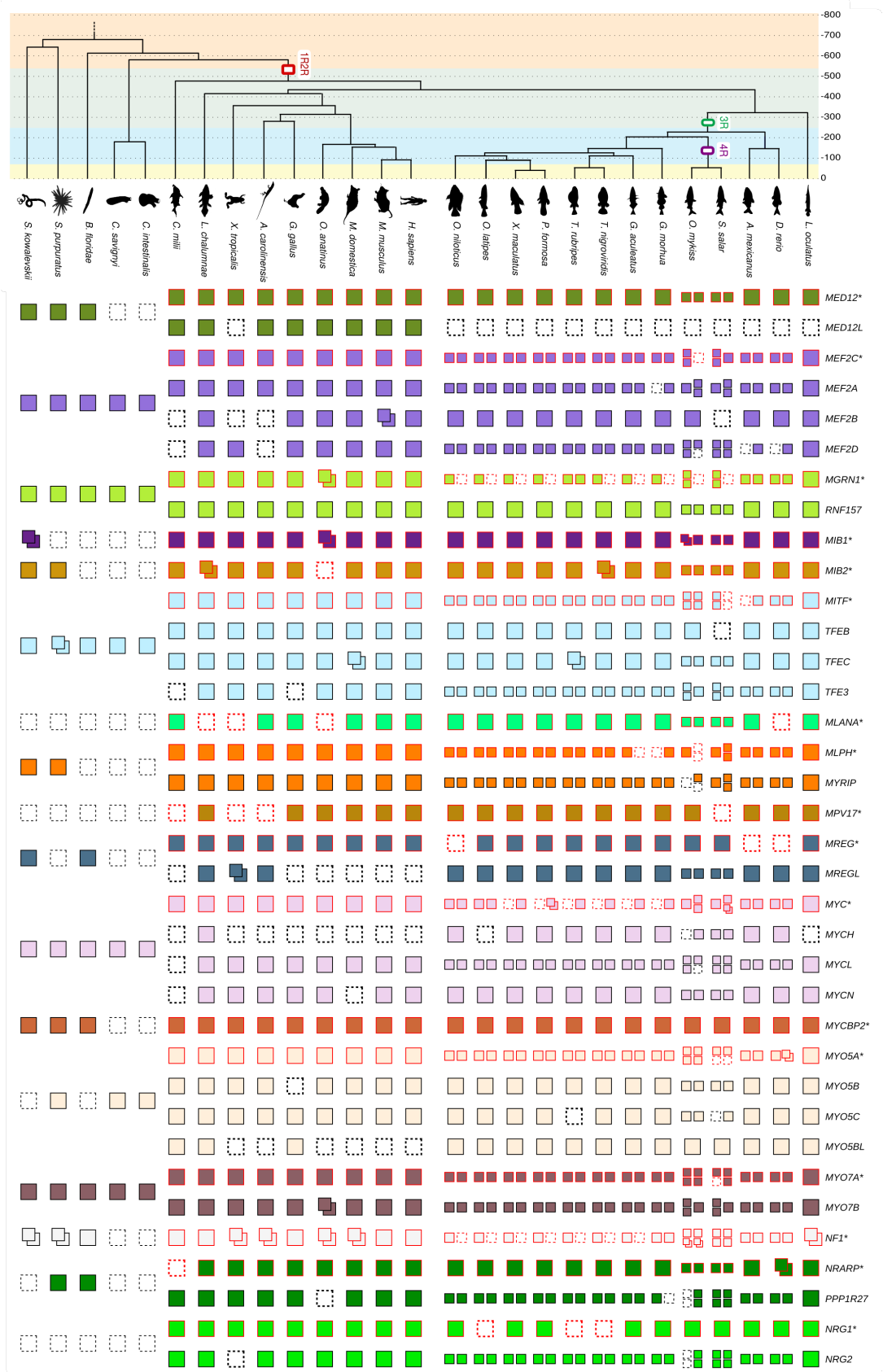
Supplementary figure 4D



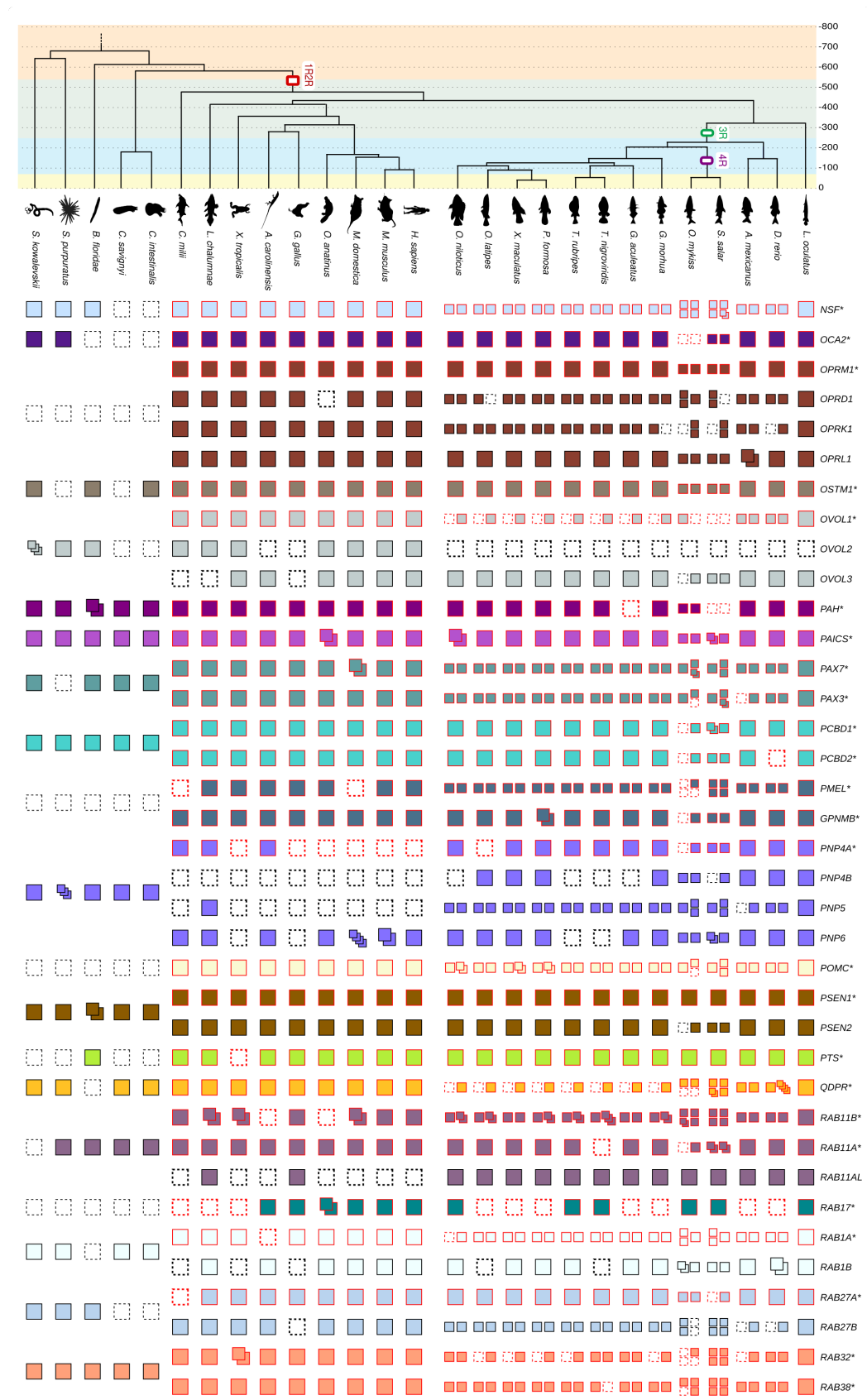
Supplementary figure 4E



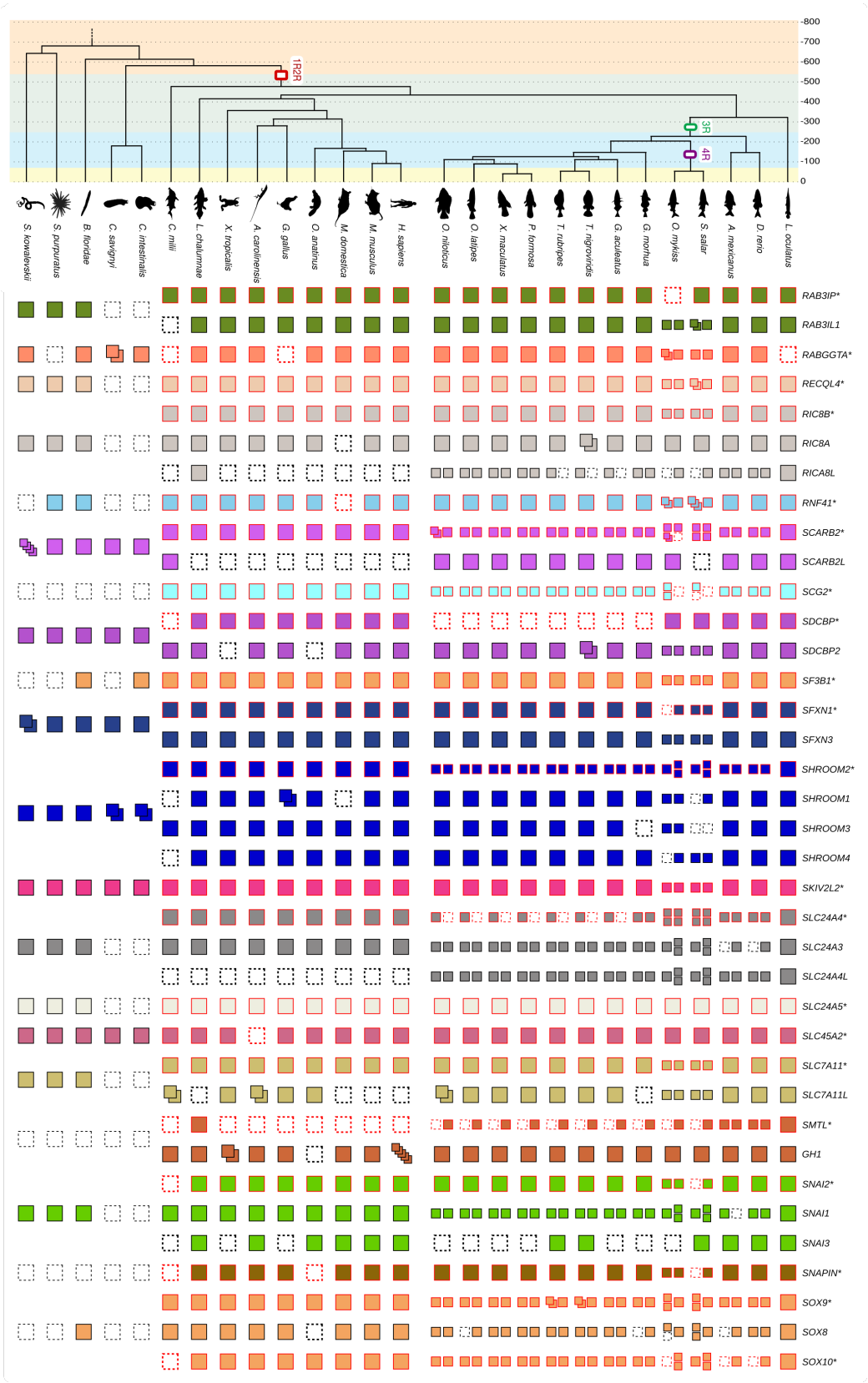
Supplementary figure 4F



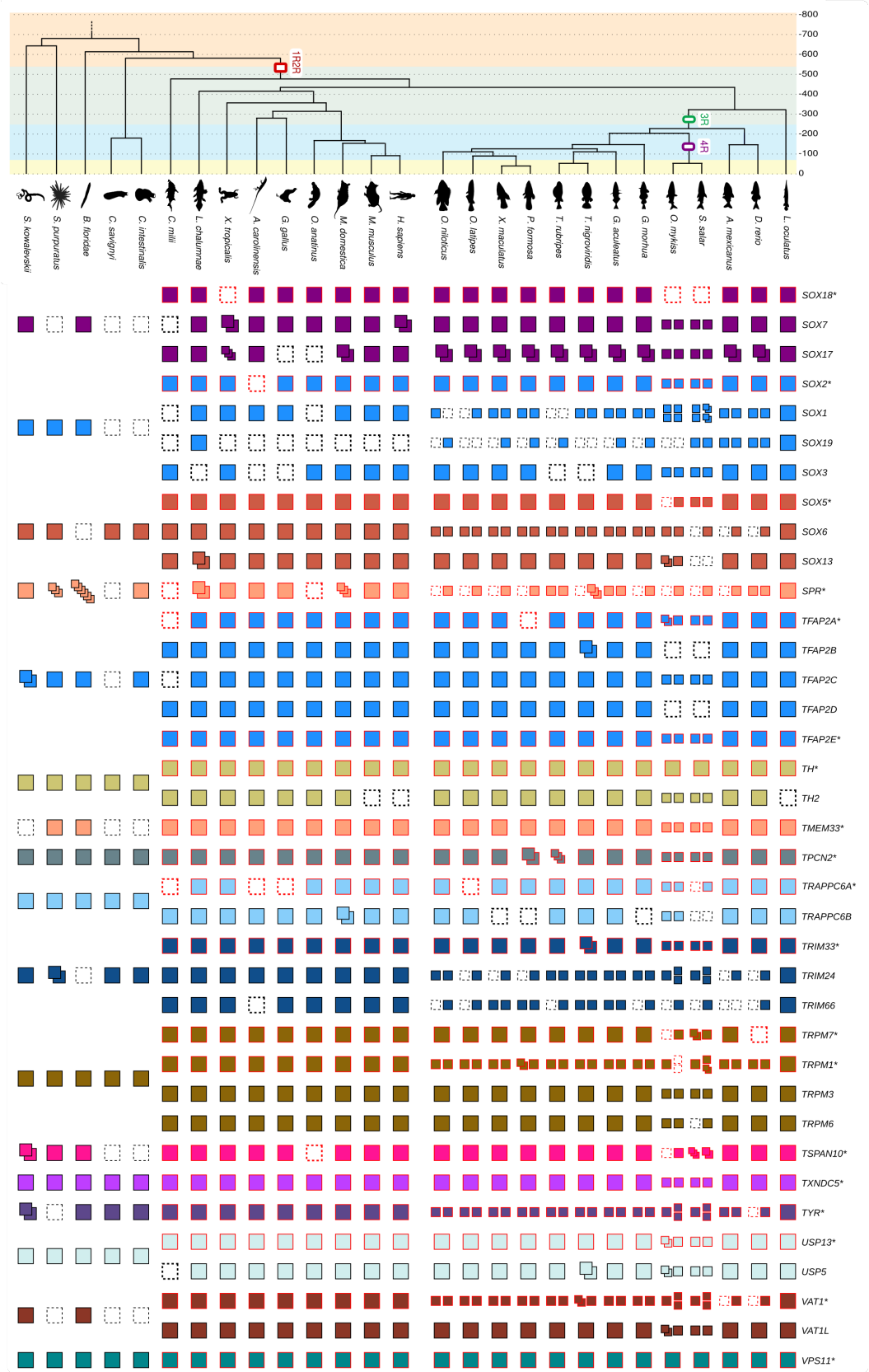
Supplementary figure 4G



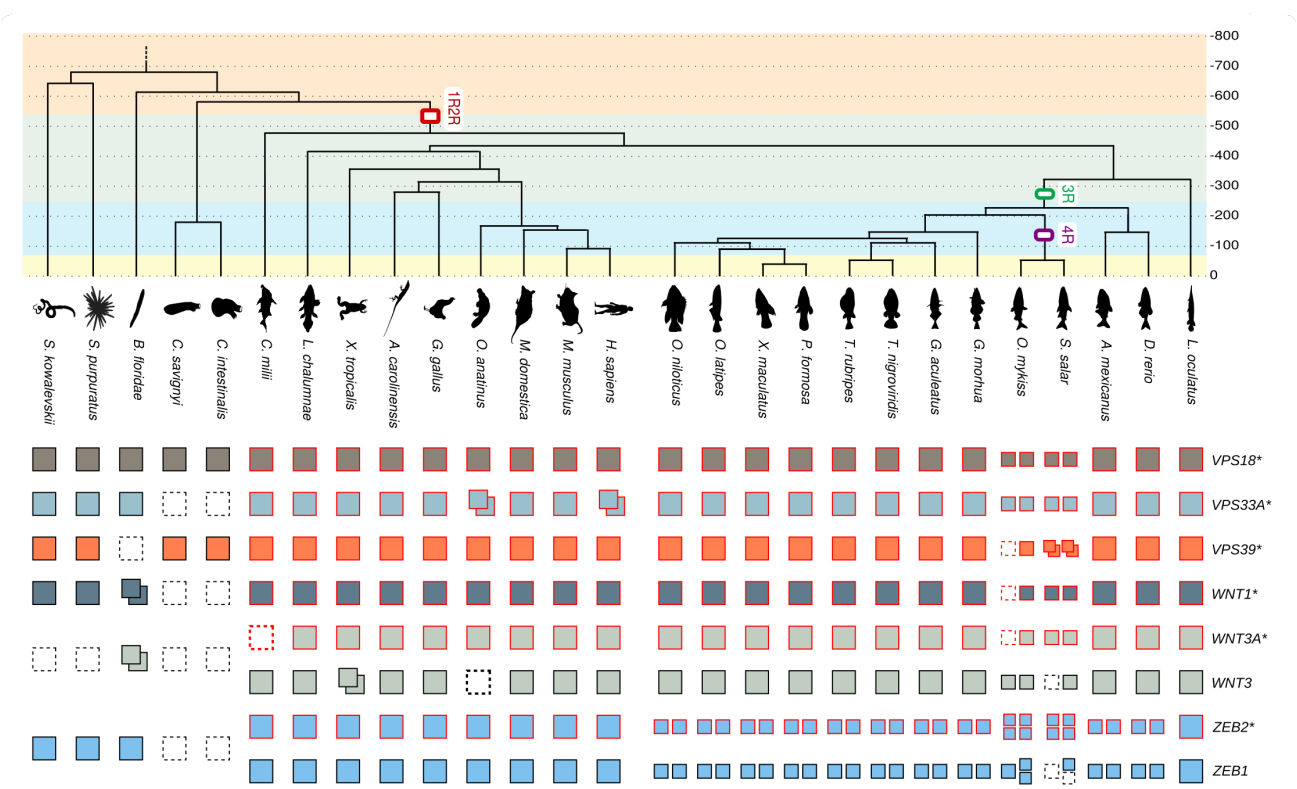
Supplementary figure 4H



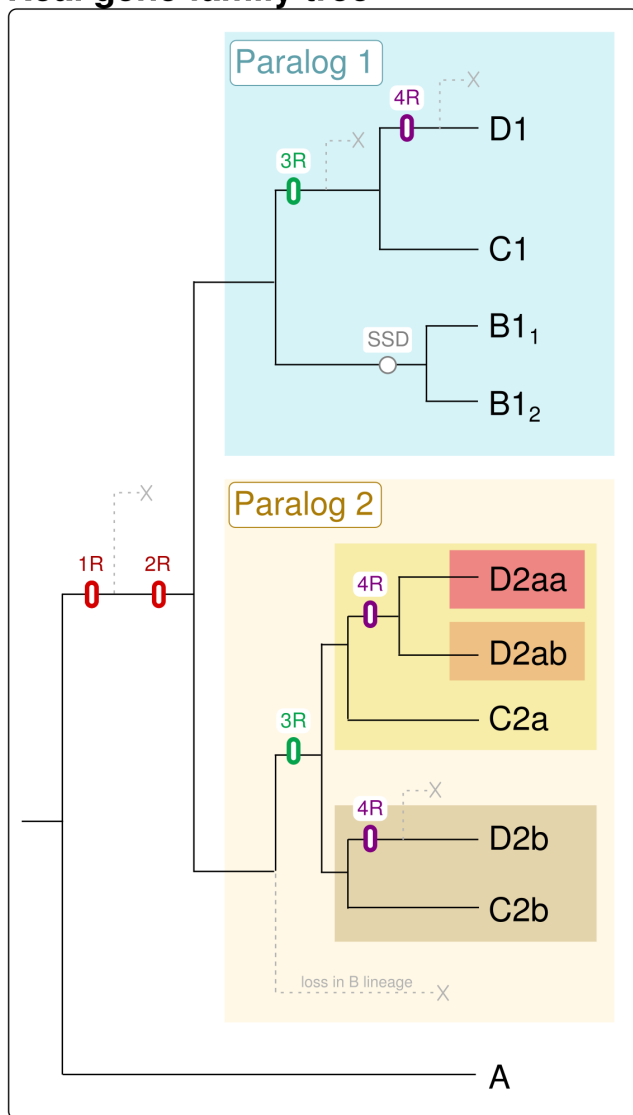
Supplementary figure 4I



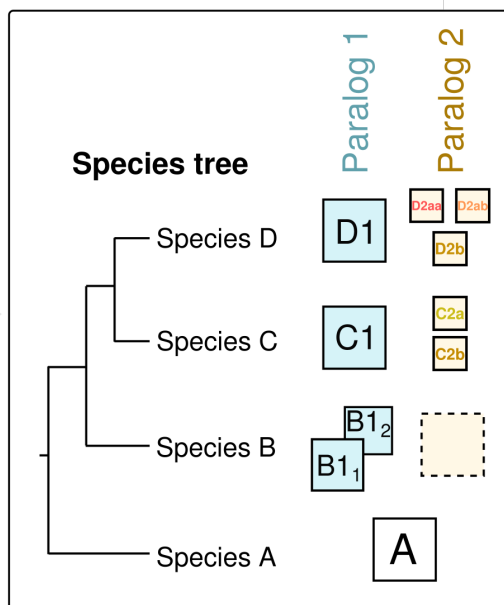
Supplementary figure 4J



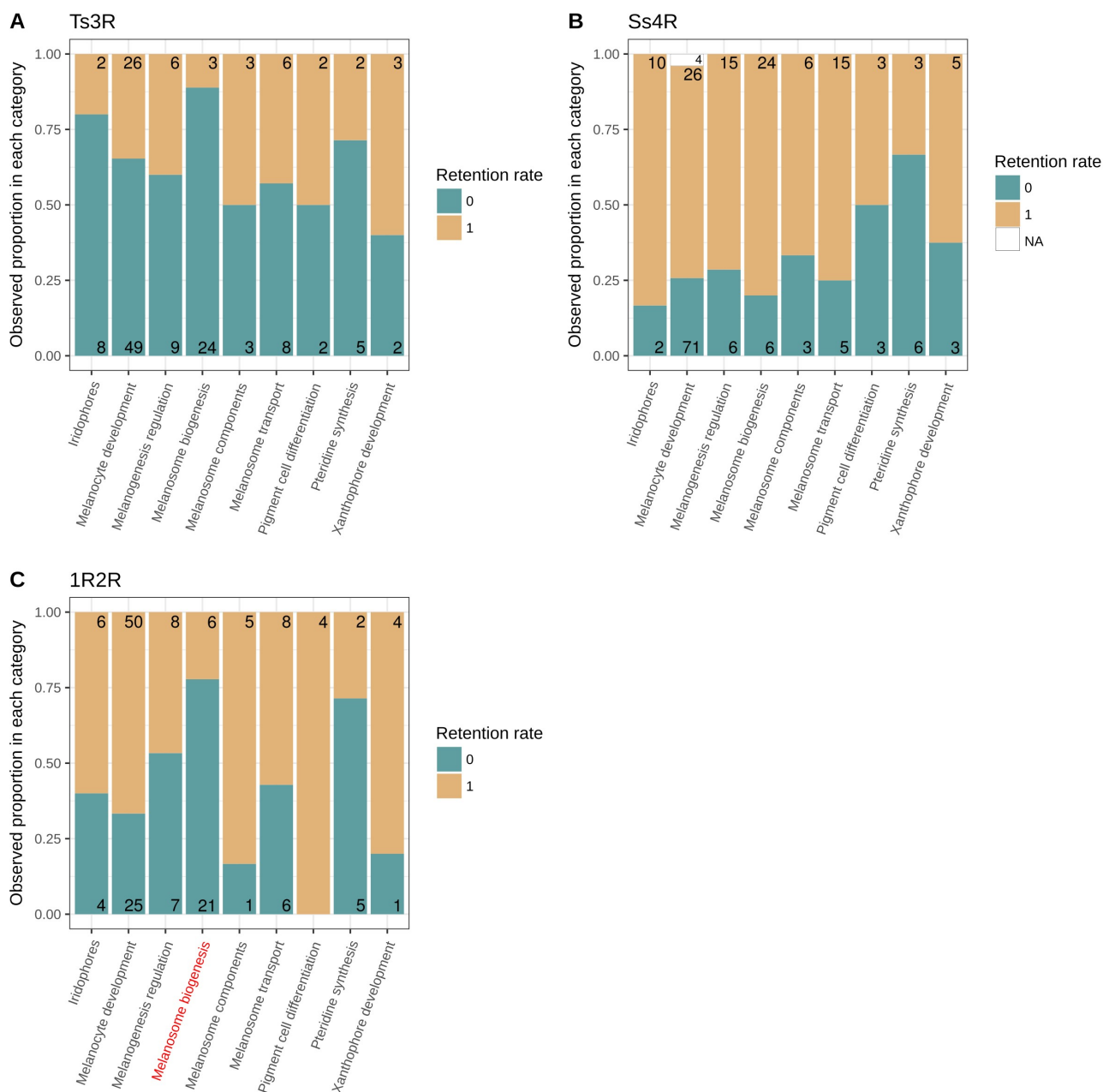
Real gene family tree



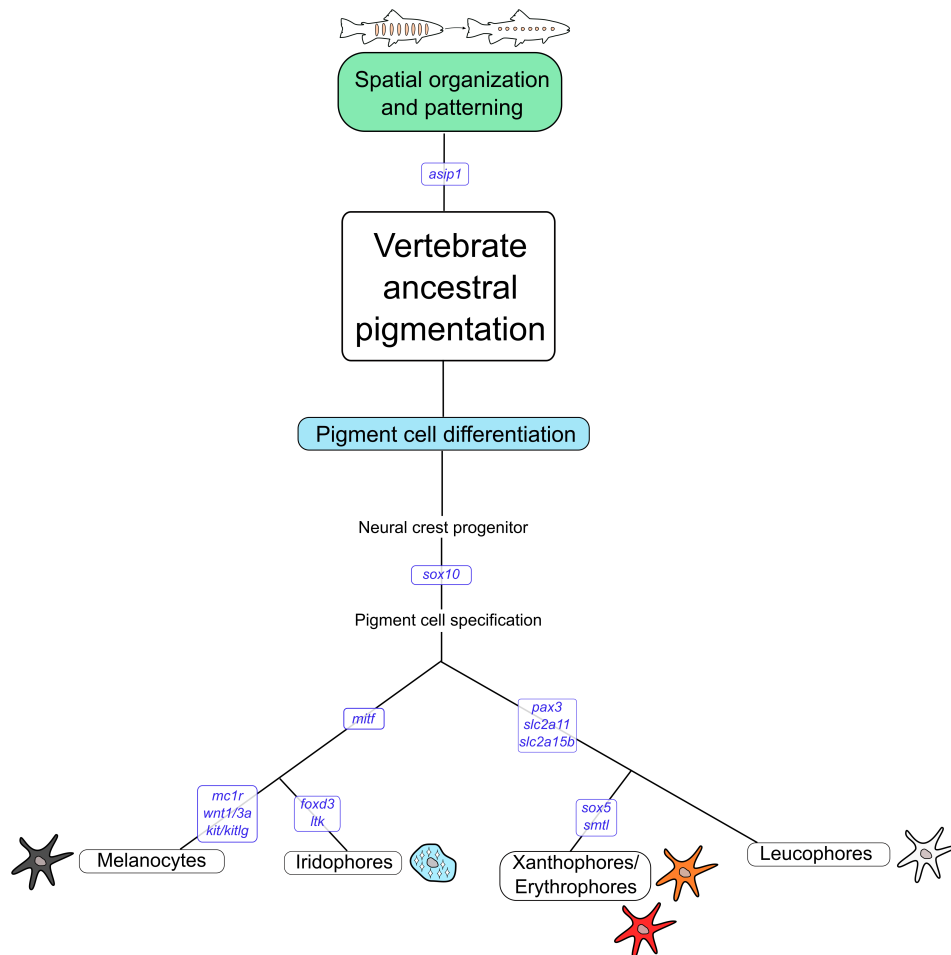
Summarized tree



Supplementary figure 5. Graphical illustration of the method used to build the representation of the gene relationships within a family.



Supplementary figure 6. Retention rates after Ts3R (A), Ss4R (B) and 1R/2R (C) for each functional category of pigmentation gene (see Fig. 2). There was no significant group effect after Ts3R and Ss4R (p-value of Wald test = 0.3 and 0.34, respectively). However, we found a group effect after 1R/2R (p-value of Wald test = 0.015). The "melanosome biogenesis" group (in red) was found to be enriched in monogenic PGCFs compared to other groups. Numbers indicate the number of genes in each category.



Supplementary figure 7. A putative ancestral vertebrate pigmentation pathway. According to the phylogenetical studies performed in this study, genes that are widely conserved among vertebrates are highlighted (blue). Ontogenetic relationships between 4 major pigment cell types in vertebrates are provided based on Kimura (2014). Evidence for positioning of:

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Supplementary table 1: List of all pigmentation genes (PGs) considered in this study. For each PG, its known function(s) and the linked reference are documented. The gene list under the Gene Ontology term “pigmentation” (GO:0043473) parsed for vertebrates (taxon ID: 7742) was manually verified.

Supplementary table 2: Best-fitting tree model for each sequence alignment (Bayesian Information Criterion).

Supplementary table 3: Gene pairs for which both pigmentation genes (PGs) belong to the same pigmentation gene-containing family, and the function of each paralogue. For some families such as *kit/csf1r* or *ece1/ece2* there is evidence for a specialization of each 1R/2R-ohnolog in a different pigmentation function.

Supplementary table 4. Gene Ontology terms enrichment for:

- A. all genes in multigenic PGCFs.
- B. all PGs in monogenic PGCFs.

In all cases, “pigmentation” is the most significant BP term, as expected. Results correspond to the GO terms collapsed at a high hierarchical level. BP: Biological Process; CC: Cellular Component; MF: Molecular Function. ‘P-values’ refer to the Benjamini-Hochberg False Discovery Rate. Only terms for which p-value is below 0.05 are shown. T is the number of term genes, Q the number of query genes, Q&T the number of common genes between term and query.