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- Cnidarians (jellyfish, sea anemones, and corals) are characterized by complex life histories and cnidocytes: explosive stinging cells.
- Many **anthozoans** (sea anemones and corals) host dinoflagellate symbionts.
- Many **medusozoans** (jellyfish) possess an energetically demanding medusa stage.
- The **Endocnidozoa** are a parasitic lineage that have undergone extensive genome reduction.
- Gene gain and loss can be inferred using **orthogroups** (families of orthologous genes) and **dollo parsimony**, which assumes that orthologous genes originate once, but can be lost repeatedly in different lineages.
- Comparative **single-cell RNA sequencing** (scRNA-seq) analysis enables the identification of cell-type specific expression patterns of genes gained at key evolutionary nodes.

1. Quantify orthogroup (gene family) gain and loss across cnidarian phylogeny.
2. Characterize the functions associated with bursts of gene gain and loss.
3. Identify cell types with elevated expression of genes gained at focal nodes.
4. Identify instances of gene gains that lead to novel cell types.

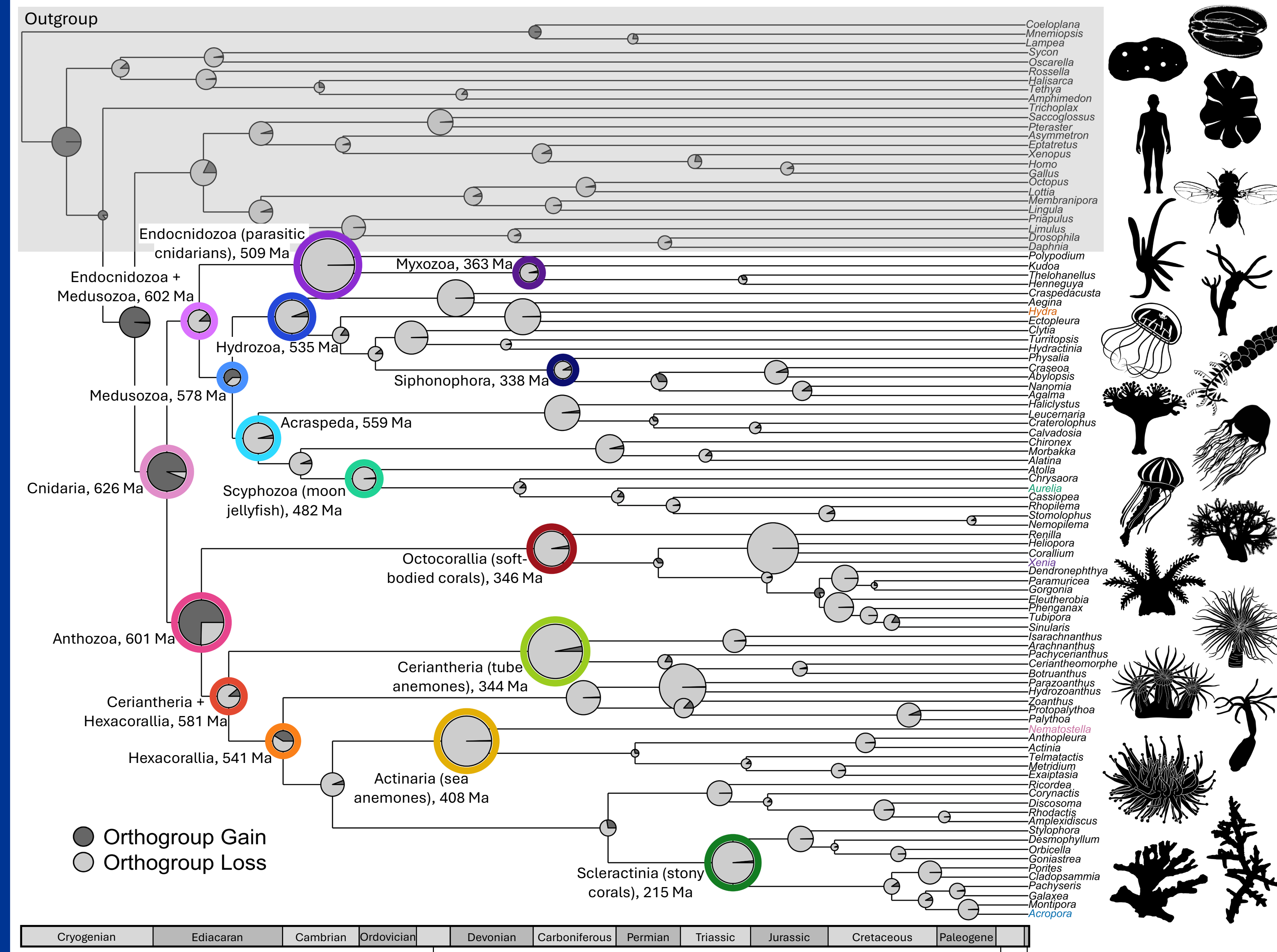
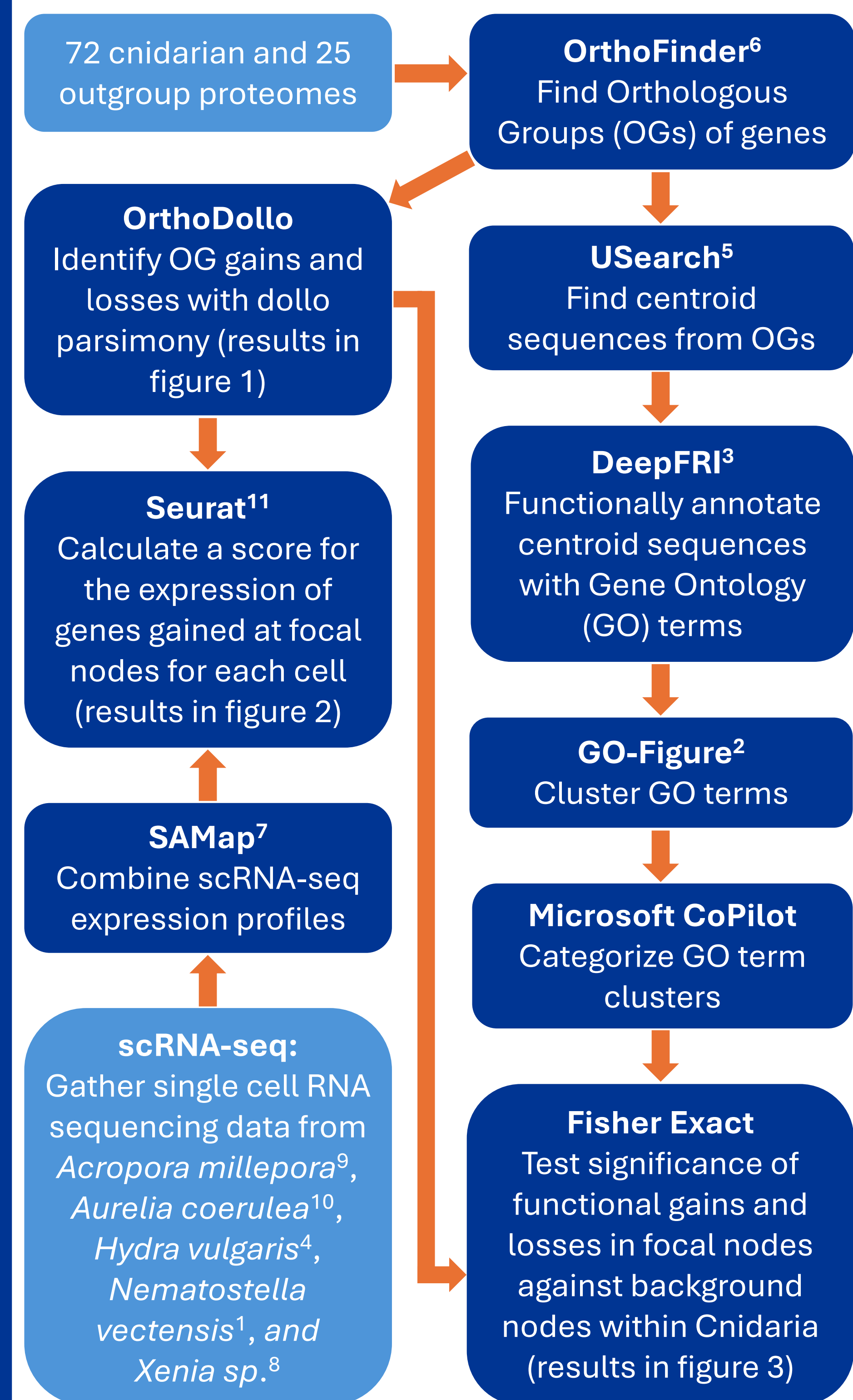


Figure 1: Orthogroup gain and loss in Cnidaria. A time-calibrated phylogenetic tree showing the relationships between 72 cnidarians and 25 outgroup taxa. Node pie charts reflect the proportion of gene family gains and losses in ancestral genomes; sizes are scaled to the total number of changes. Focal nodes are circled, and their divergence times (in millions of years) are indicated. Species included in the SAMap gene expression analysis have colored tip labels that match the color scheme in Figure 2B. Silhouette images are from PhyloPic (<https://www.phylopic.org/>).

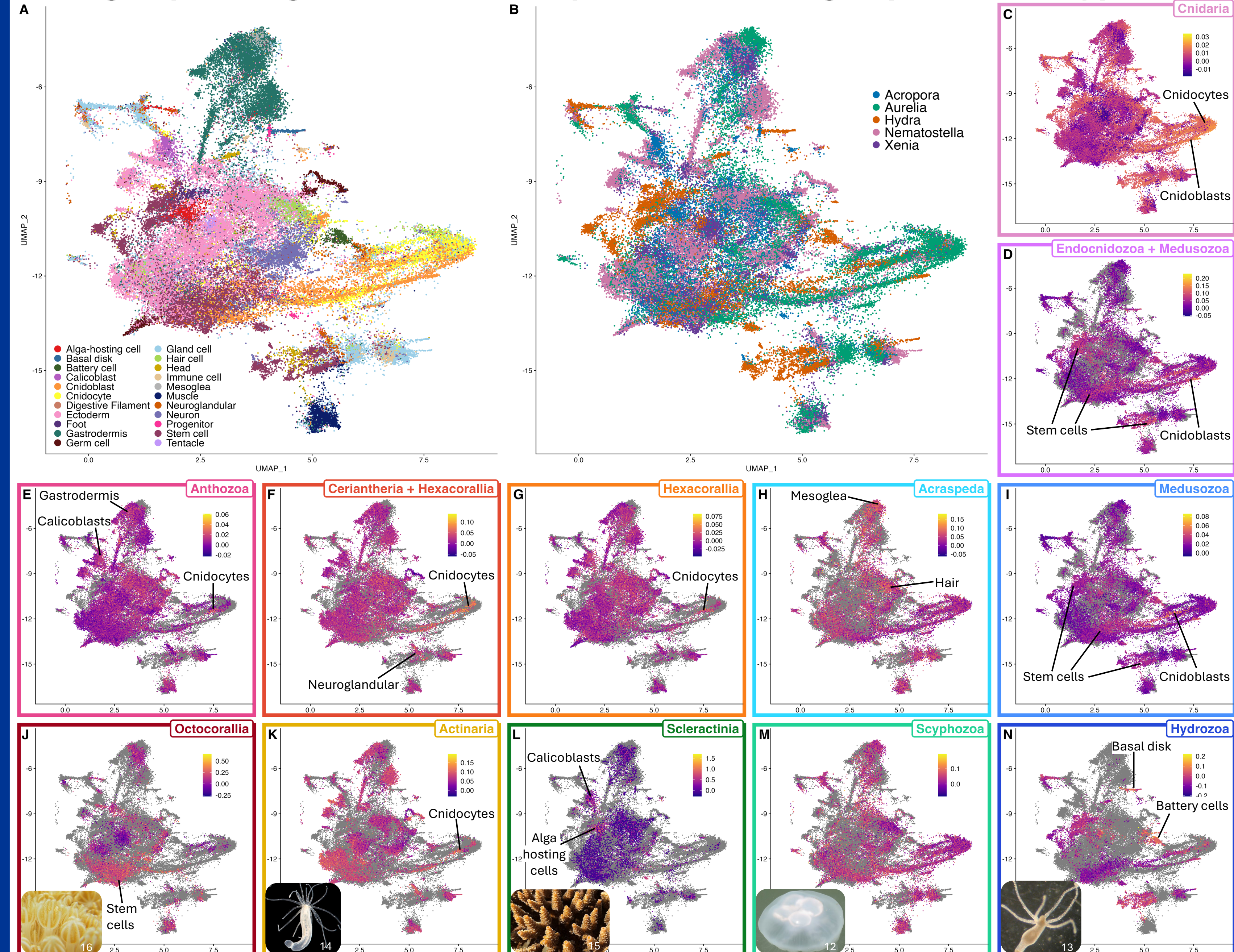


Figure 2: SAMap of 5 cnidarians. A UMAP representation of single cells from: *Acropora millepora*, *Aurelia coerulea*, *Hydra vulgaris*, *Nematostella vectensis*, and *Xenia* sp. Each point represents one cell. A) is colored by cell type, and B) is colored by species. C-M) A collective expression score was calculated for genes gained at specified nodes. Scores are comparable across panels, but the **color is scaled to each node** for visualization. Select cell types in each node are indicated.

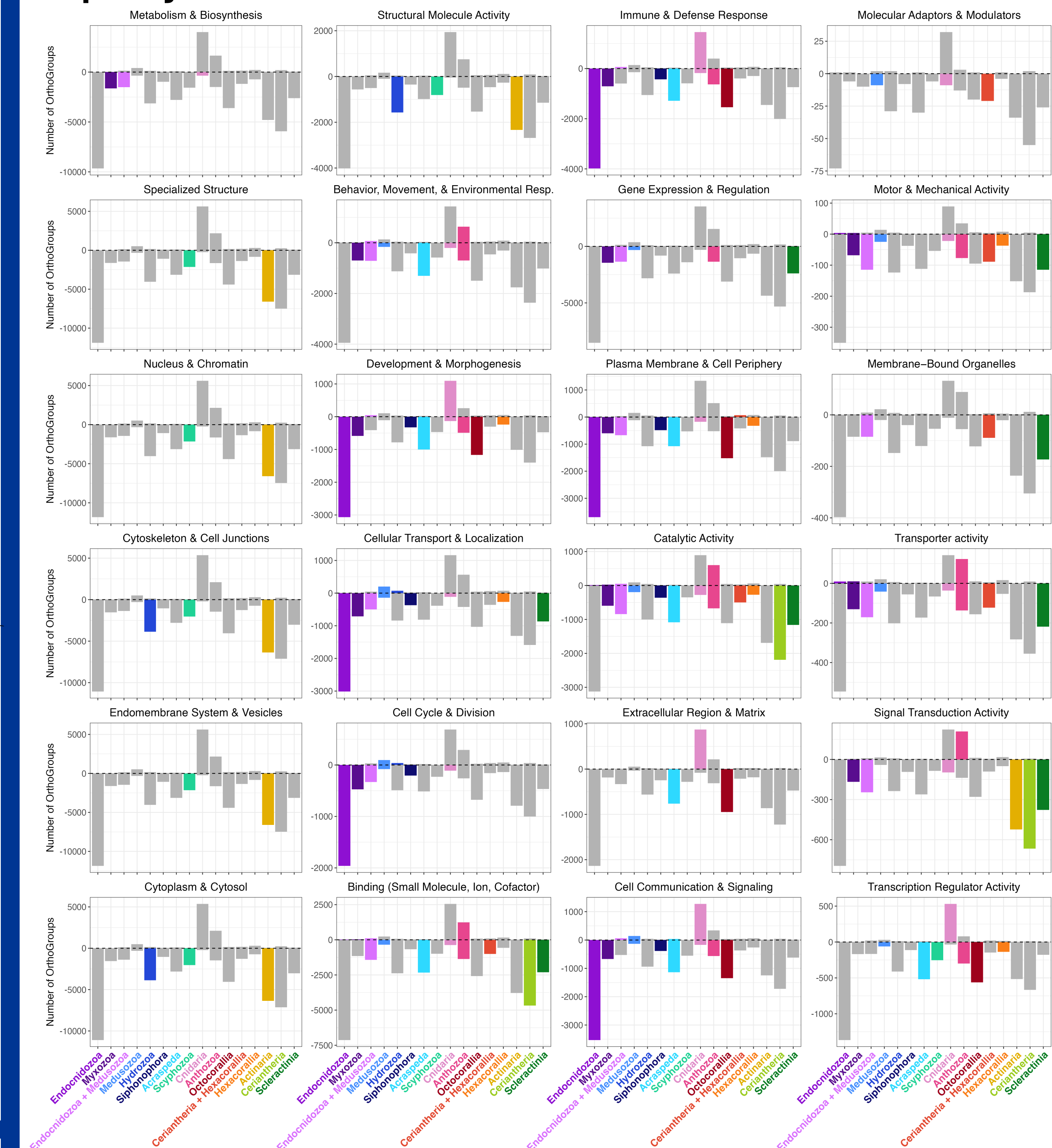


Figure 3: Functional categorization of orthogroup gain and loss. Each panel shows gene family (OG) gains (bars above the x-axis) and losses (bars below) in key nodes in Cnidaria for a specific function. Nodes with significant enrichment in gains or losses are highlighted.

- Cnidarian gains in **regulation, development, extracellular matrix, and signaling** enabled the emergence of diverse life histories and are highly expressed in cnidocytes.
- Anthozoan gains are expressed in immune, muscle, and gastrodermal cells and include functions associated with **transporters, signal transduction, and binding**, which support nutrient exchange, symbiont identification, and physical association with symbionts.
- Gains in **cell communication and signaling, cell cycle and division, and transport and localization** in Medusozoa are expressed in stem cells and support the cell proliferation and organization of medusae.
- Some gains in Hydrozoa and Scleractinia are highly expressed in lineage specific cell types such as battery cells and calicoblasts.
- Endocnidozoa and Myxozoa exhibit major losses in **localization, cell periphery, immune system, development, and the cell cycle**, reflecting reduced functions of free-living cnidarians, while gaining **transporters** that support nutrient exchange with their host.

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