

Additional file 11

Supplementary results - phylogenetic analysis using nuclear gene orthologs

This file displays 12 single-gene phylogenies (Figures 1-12) of *Pristimantis euphronides* and other Hyloidea species, inferred from nuclear gene orthologs BDNF, BMP2, CXCR4, MC1R, NTF3, POMC, RAG2, RHO, SIAH1, SLC8A3, TNS3, and TYR retrieved from NCBI GenBank. All trees were inferred using IQ-TREE v2.2.0.3 [1] with best-fit substitution models selected by ModelFinder, 1,000 ultrafast bootstrap replicates, 1,000 SH-aLRT tests, and the --bnni option, and are rooted with *Rana temporaria* (Ranidae) as outgroup. Trees were visualized and annotated using iTOL v6 [2]. In all figures, colours indicate taxonomic family (legend at left), *P. euphronides* (family Strabomantidae) is highlighted in red and bold, branch support values are shown as ultrafast bootstrap percentages (%), and tree scale is 0.1 substitutions per site. Family-level taxonomy follows the NCBI Taxonomy database (taxdump downloaded 17 April 2025; taxonkit v0.19.0 [3]). For legibility, clades with average branch length distance to their leaves below 0.05 were collapsed in the trees for the genes CXCR4, POMC, RHO, SIAH1, and TYR. Full-resolution PNG images of all figures are available at Zenodo (doi: 10.5281/zenodo.15298547, folder: Phylogenetic_analysis_using_nuclear_gene_orthologs/figures/).

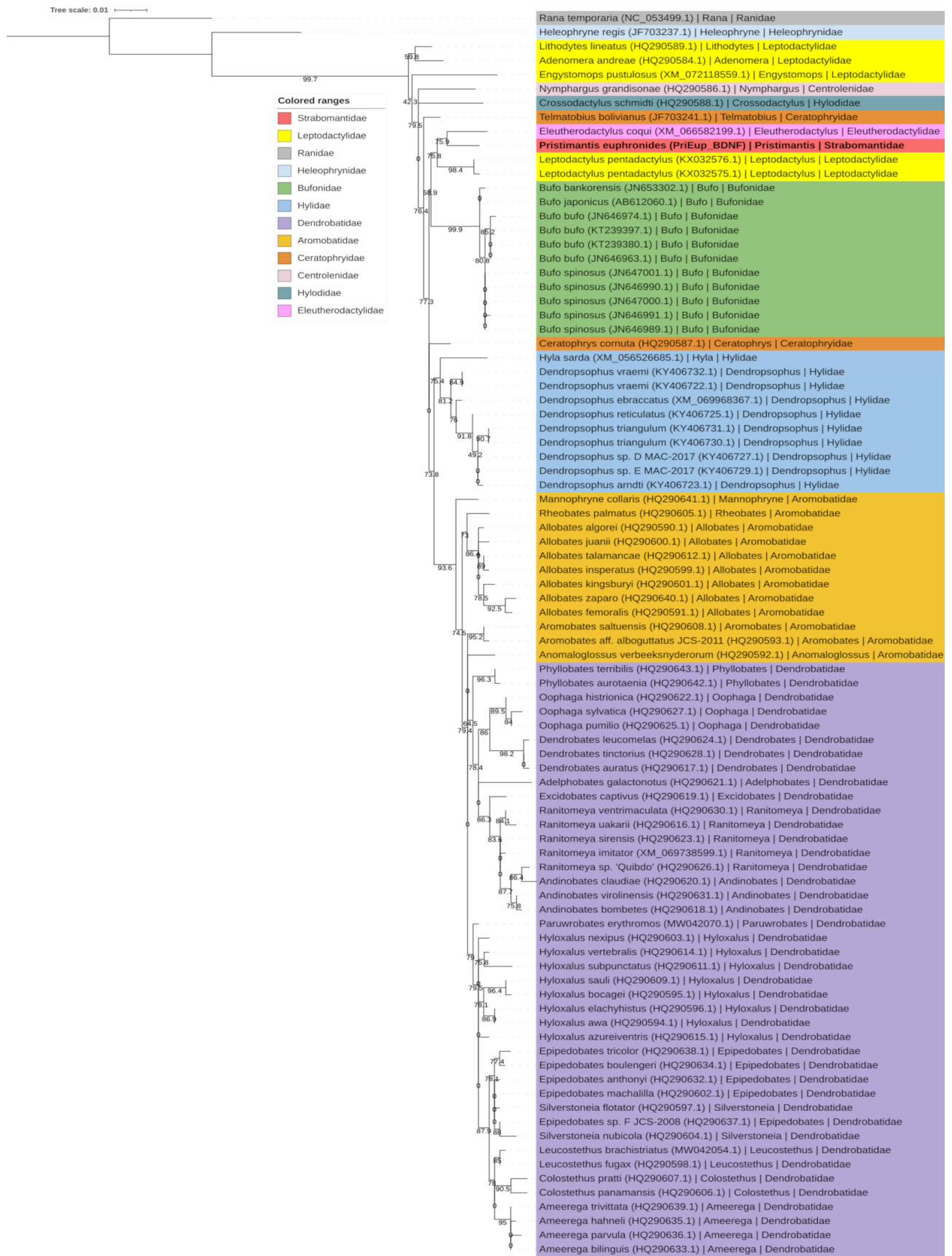


Figure 1. Single-gene phylogeny of BDNF for *Pristimantis euphronides* and other species across 11 Hyloidea families in total.



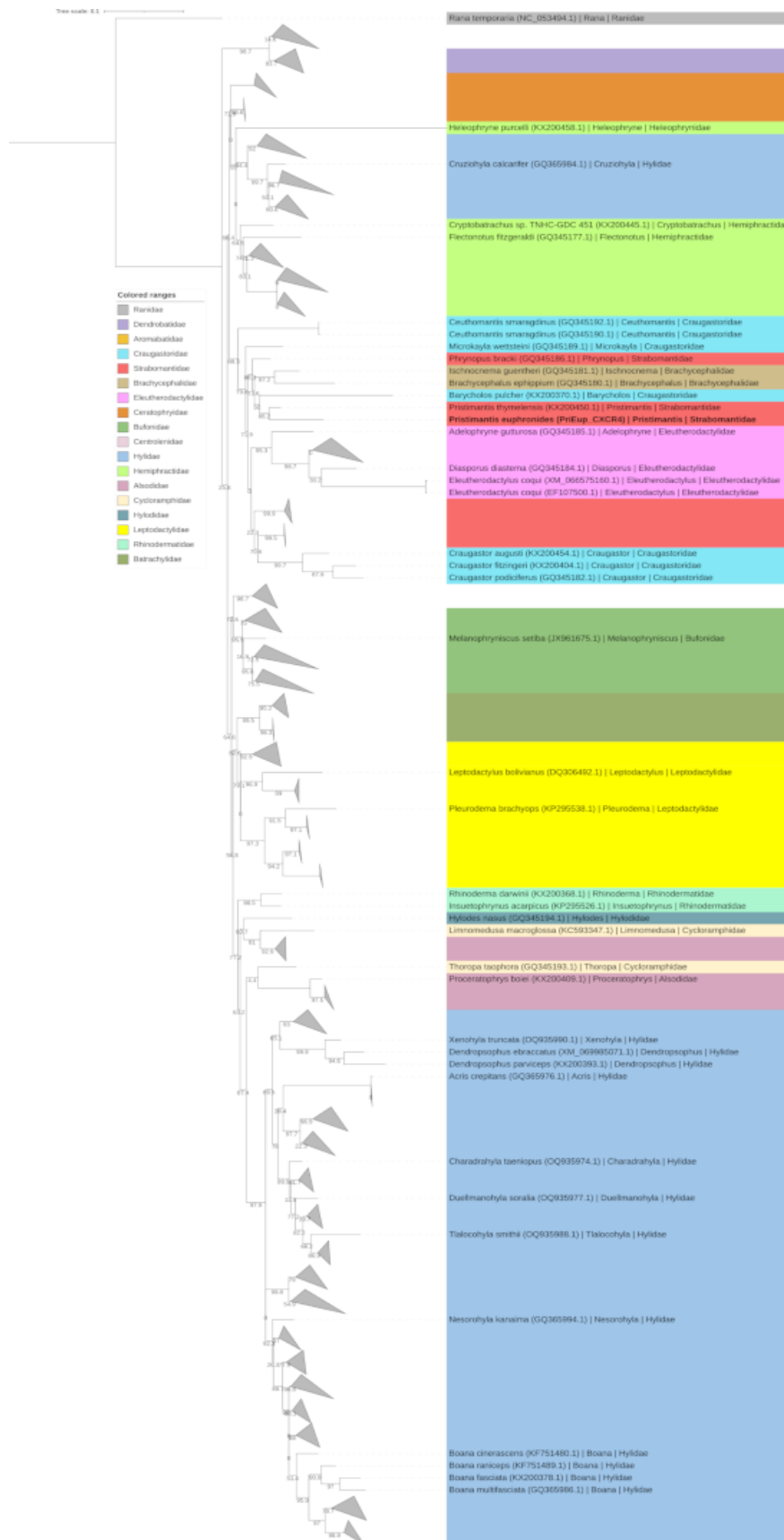


Figure 3. Single-gene phylogeny of CXCR4 for *Pristimantis euphronides* and other species across 18 Hyloidea families in total.

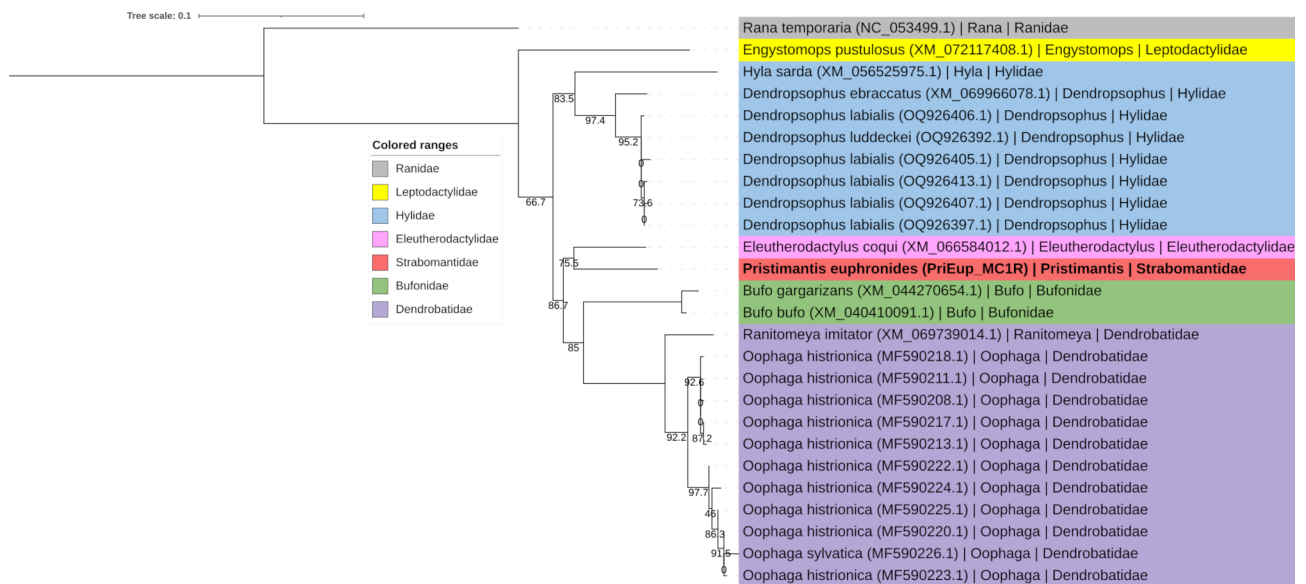


Figure 4. Single-gene phylogeny of MC1R for *Pristimantis euphronides* and other species across six Hyloidea families in total.



Figure 5. Single-gene phylogeny of NTF3 for *Pristimantis euphronides* and other species across ten Hyloidea families in total.

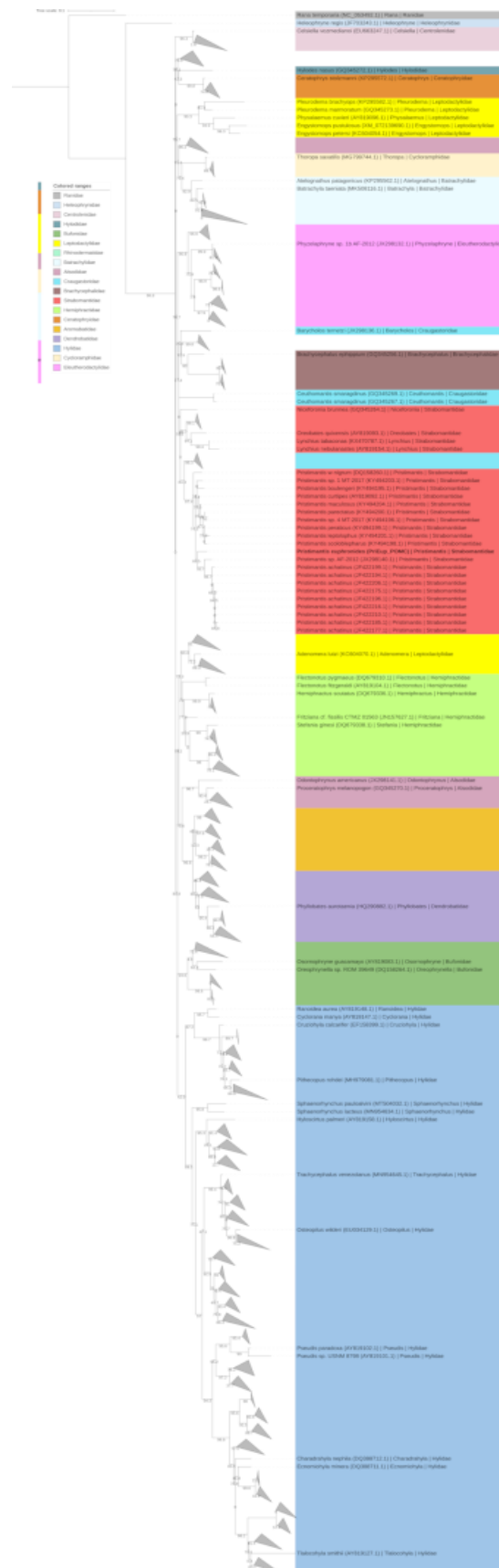


Figure 6. Single-gene phylogeny of POMC for *Pristimantis euphronides* and other species across 19 Hyloidea families in total.

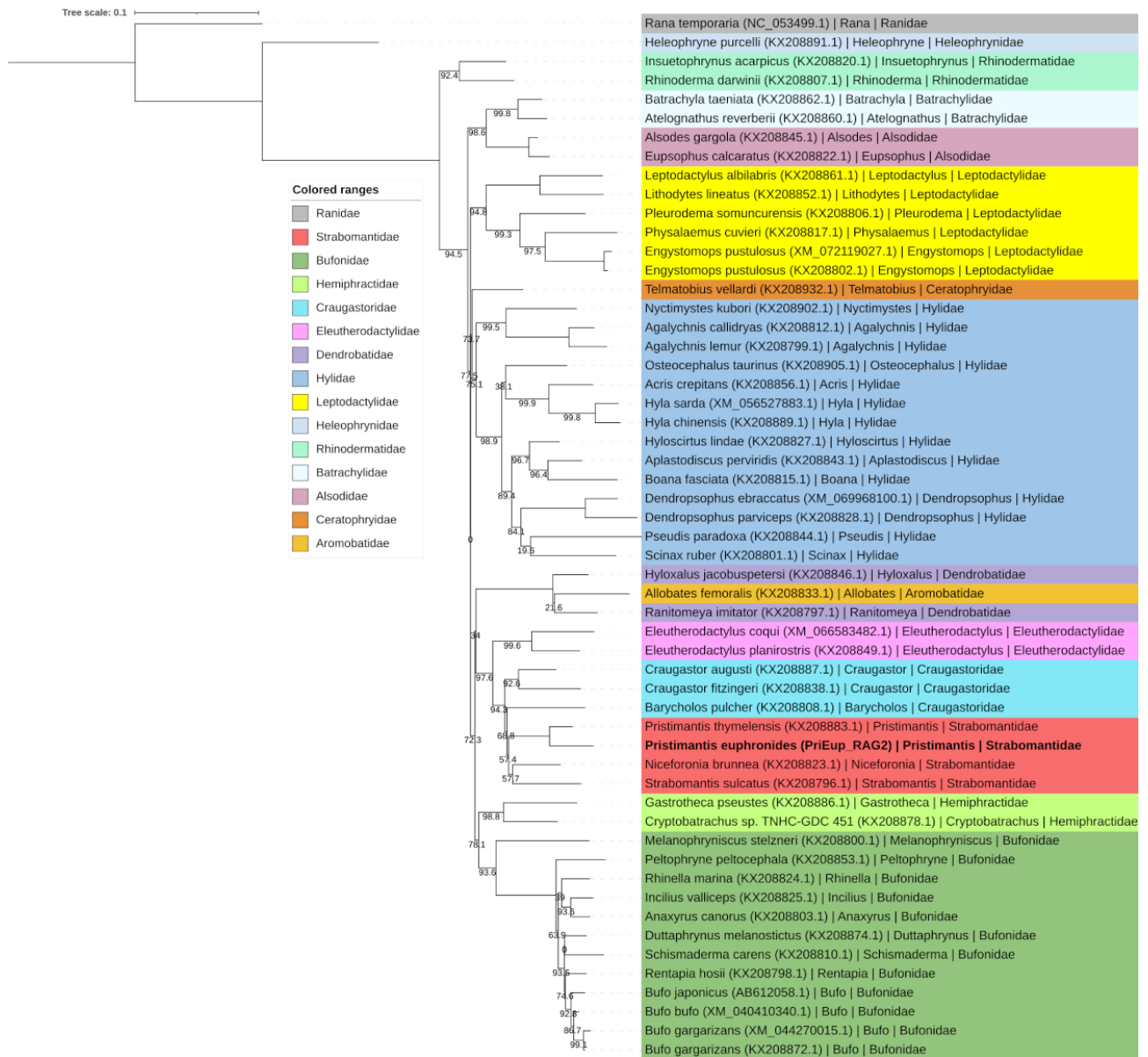


Figure 7. Single-gene phylogeny of RAG2 for *Pristimantis euphronides* and other species across 14 Hyloidea families in total.

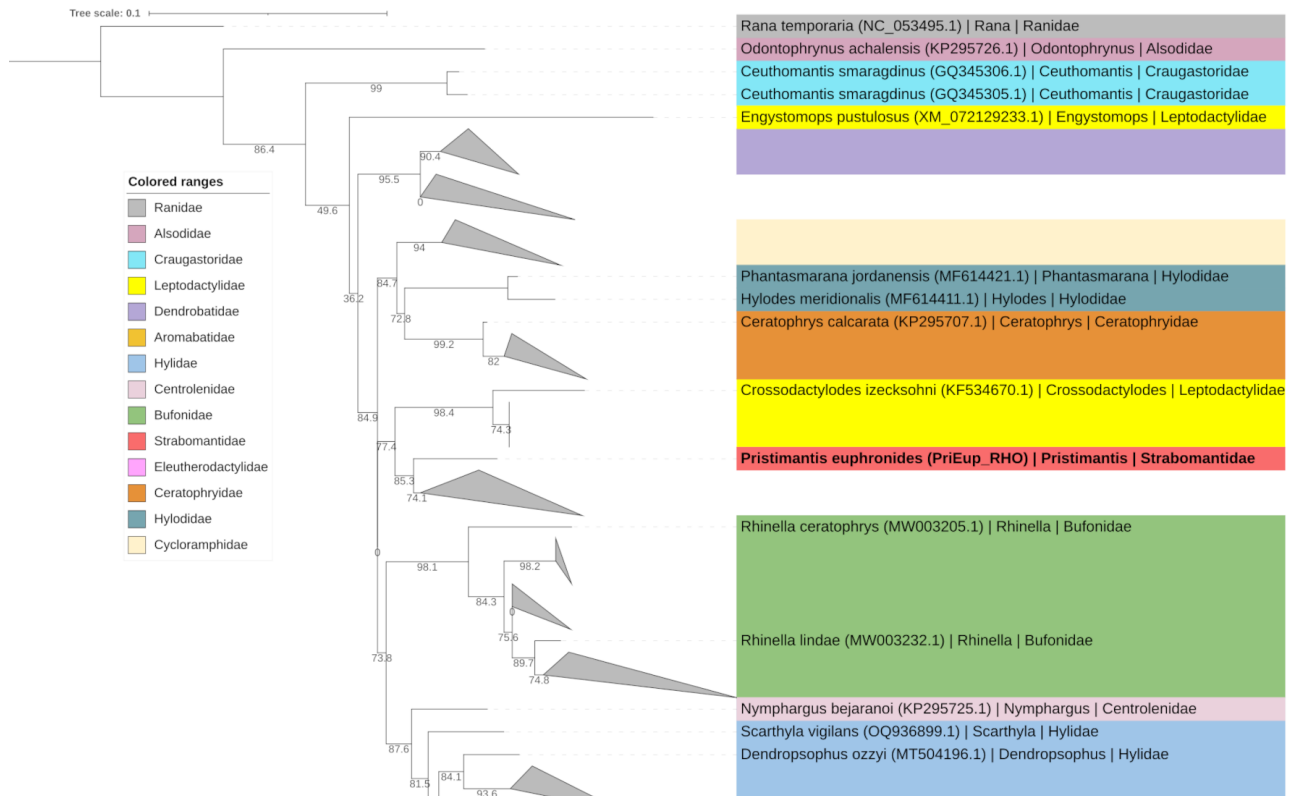


Figure 8. Single-gene phylogeny of RHO for *Pristimantis euphronides* and other species across 13 Hyloidea families in total.

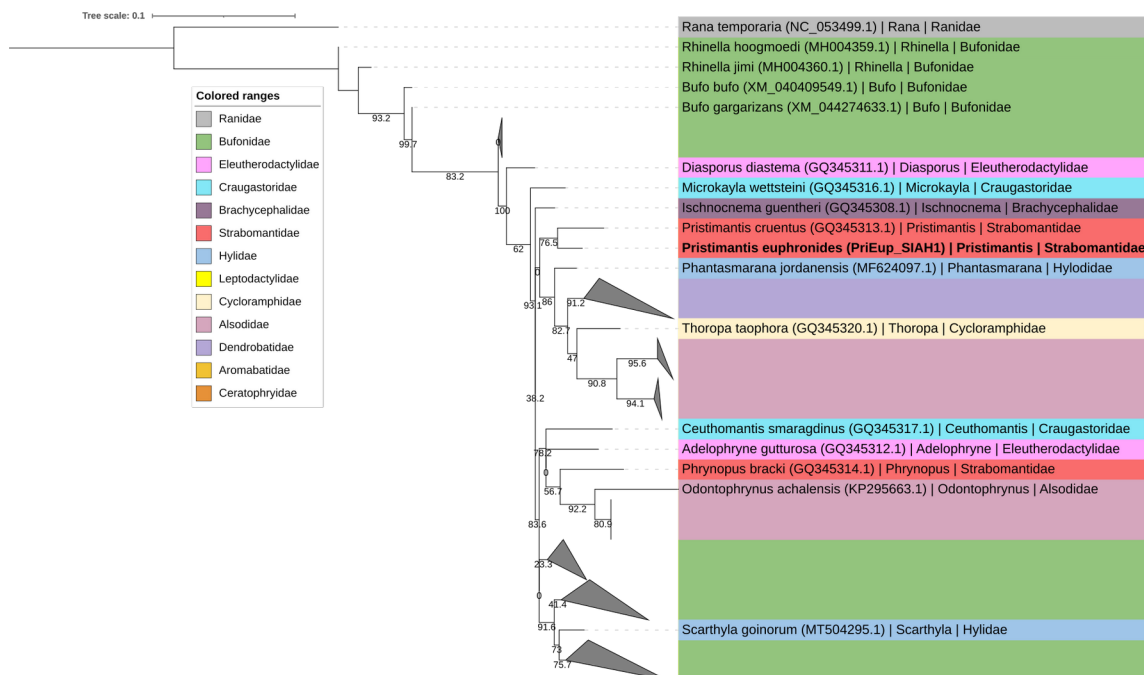


Figure 9. Single-gene phylogeny of SIAH1 for *Pristimantis euphronides* and other species across 13 Hyloidea families in total.



Figure 10. Single-gene phylogeny of SLC8A3 for *Pristimantis euphronides* and other species across 20 Hyloidea families in total.



Figure 12. Single-gene phylogeny of TYR for *Pristimantis euphronides* and other species across 15 Hyloidea families in total.

References

1. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol.* 2020;37(5):1530–4.
2. Letunic I, Bork P. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Res.* 2024 Jul 5;52(W1):W78-W82.
3. Shen W, Ren H. TaxonKit: A practical and efficient NCBI taxonomy toolkit. *J Genet Genomics.* 2021 Sep 20;48(9):844-850.