

## Background

Baraminology is the study of God's created kinds as described in Genesis 1 (Wise, 1990). Holobaramins are scientific approximations of these kinds, identified by building up monobaramins (smaller, continuous groups of organisms) and dividing apobaramins (larger, discontinuous groups of organisms). The lungless salamander family (Caudata: Plethodontidae), composed of over 500 species in 29 genera, have been grouped together by herpetologists for almost 200 years. Our previous research indicates that lungless salamander holobaramin(s) range from the genus to family levels. The goal of this study is to further investigate the baraminology of these organisms by reanalyzing a prominent study of lungless salamander phylogeny (Chippindale et al., 2004) using molecular baraminology techniques.

## Research Question

- How many created kinds are found within the lungless salamander family based on molecular data?

## Methods

- DNA sequences from two mitochondrial (CYTB, ND4) and one nuclear gene (RAG-1) were gathered from the GenBank database ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)):
  - Cytochrome b (CYTB)
  - NADH dehydrogenase subunit 4 (ND4)
  - Recombination Activating Gene 1 (RAG-1)
- Sequences from each gene were aligned across all taxa using MEGA software ([www.megasoftware.net](http://www.megasoftware.net)) and corrected distance matrices were created with the TN93 + Gamma model in R software ([www.r-project.org](http://www.r-project.org)).
- Analyses were performed on the corrected distance matrices for each gene, plus a concatenated dataset of all genes combined, using R (Blaschke, 2022; Wheeler & Blaschke, 2022) and BARCLAY (Wood, 2020):
  - Hierarchical Clustering (HC)
  - Classic Multidimensional Scaling (MDS)
  - Distance Correlation Analysis (DCA)
- Other salamander families were included in all analyses, as outgroups, alongside the lungless salamanders:
  - Amphiumas (Amphiumidae), Sirens (Sirenidae), and Mudpuppies (Proteidae)
  - Giant (Cryptobranchidae) and Pacific Giant (Dicamptodontidae) salamanders
  - Newts (Salamandridae), Mole (Ambystomatidae) and Torrent salamanders (Rhyacotritonidae)

## Results and Conclusions

- All analyses indicate that lungless salamanders cluster into one large group, separate from the outgroups, and are therefore a potential holobaramin (Figs. 1-3).
- Data also suggest the presence of at least five major groups of genera, clustering in two subgroups that correspond to modern subfamilies (Figs. 1-3):
  - Tribe Hemidactyliini** (clusters separately from members of Tribe Spelerpini)
  - Tribe Spelerpini**
  - Tribe Batrachosepini** (clusters separately from members of Tribe Bolitoglossini)
  - Tribe Bolitoglossini**
  - Subfamily Plethodontinae** – Tribe Desmognathini clusters with all other members of this subfamily
- These five groups, at the tribe and subfamily levels, represent monobaramins and possibly holobaramins according to DCA analyses (Fig. 3).

**Conclusions** – Overall, these data indicate that lungless salamander holobaramin(s) range from tribe to family levels. Morphological, hybridization, and historical taxonomic research by our team are consistent with these results. Despite the preliminary nature of our conclusions, we are the first to conduct baraminological analyses in this family of salamanders.

## Future Work

- We will continue to identify holobaramins by increasing the number of species we include in our molecular analyses and using genetic distance data to expand our hybridization analysis.

## References

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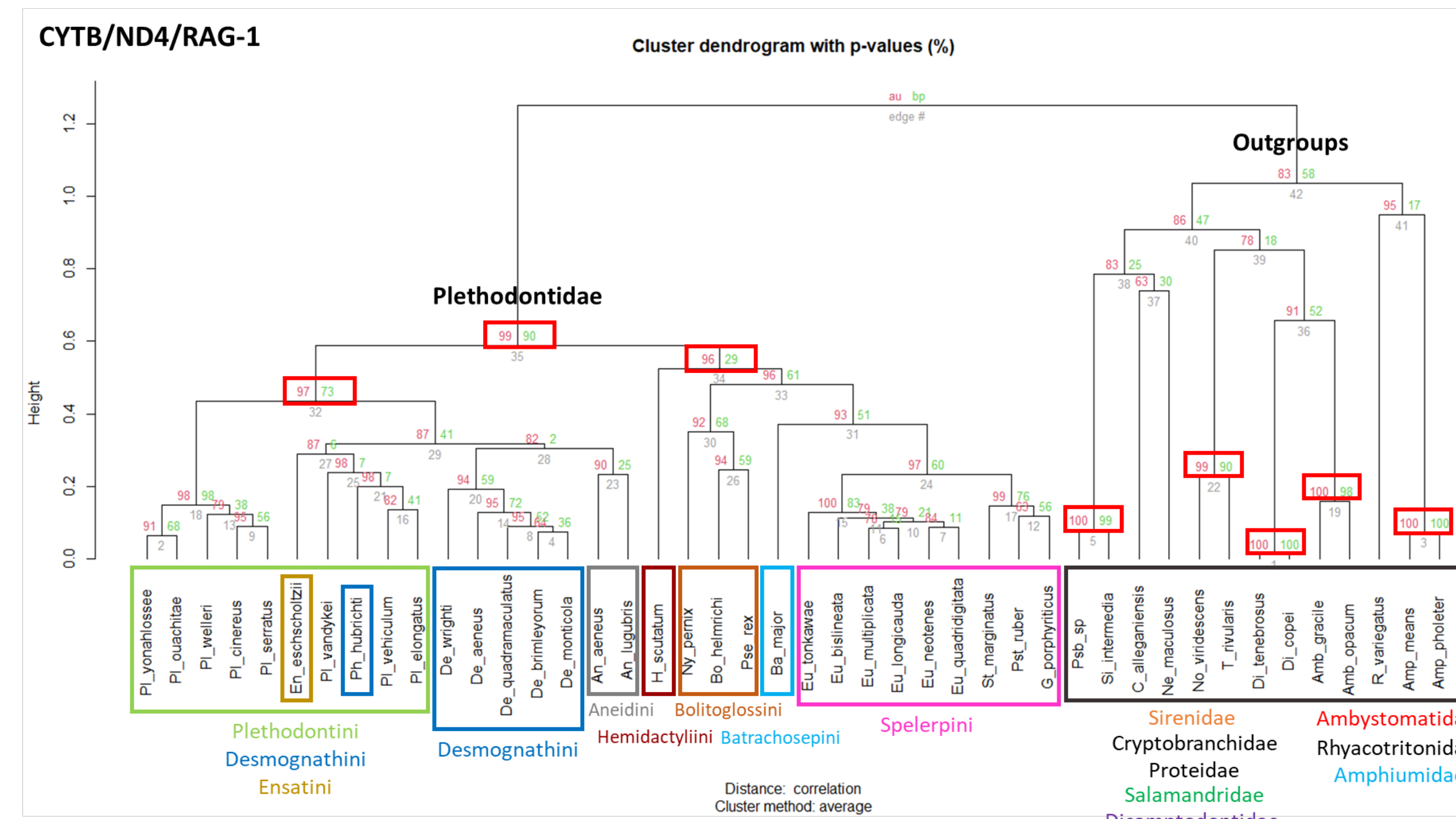
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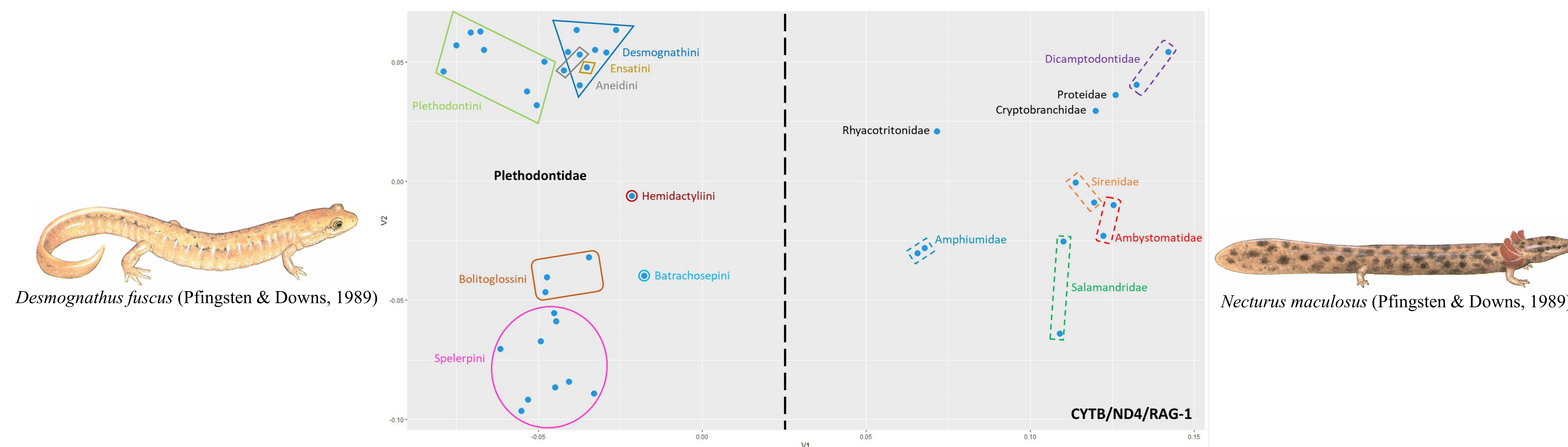
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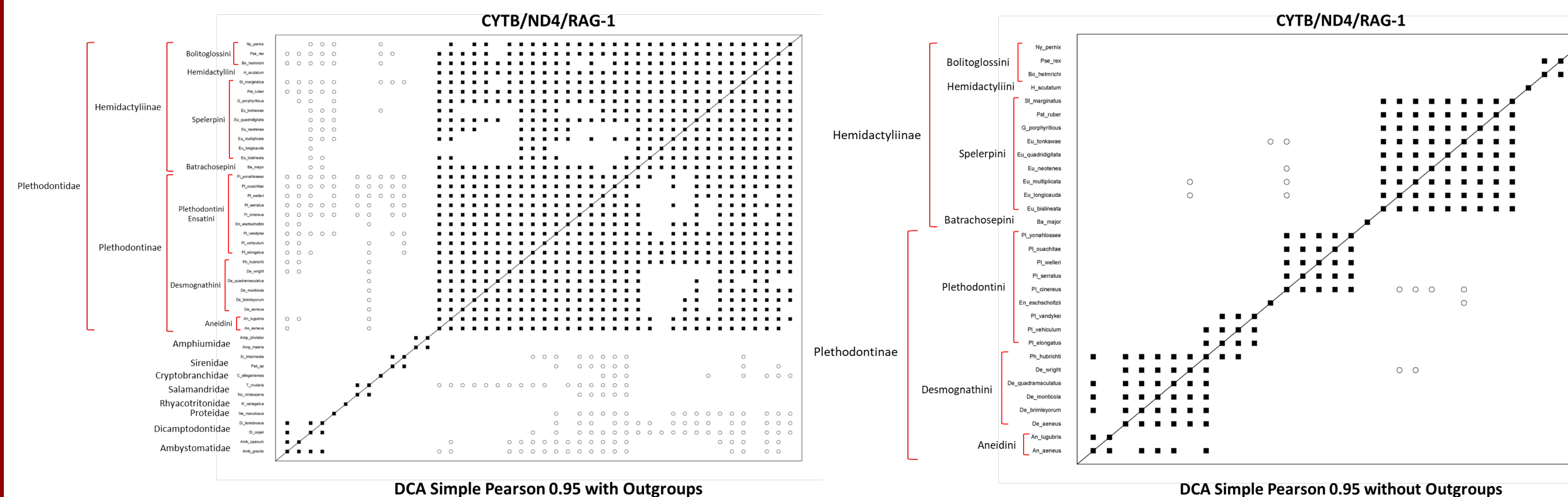
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**Figure 1.** Hierarchical clustering analysis on corrected distance matrices (TN 93 + Gamma) for concatenated dataset (CYTB, ND4, and RAG-1 genes) of plethodontid salamander species and several outgroup taxa. Taxonomy follows Vieites et al. (2011) and Wake (2012). Red numbers indicate Approximately Unbiased p-values (au) for each cluster.



**Figure 2.** Classic multidimensional scaling analysis on corrected distance matrices (TN 93 + Gamma) for concatenated dataset (CYTB, ND4, and RAG-1 genes) of plethodontid salamander species and several outgroup taxa. Taxonomy follows Vieites et al. (2011) and Wake (2012).



**Figure 3.** DCA analysis on corrected distance matrices (TN 93 + Gamma) for concatenated dataset (CYTB, ND4, and RAG-1 genes) of plethodontid salamander species and several outgroup taxa. Simple Matching distances, Pearson correlations, and character relevances of 0.95 are utilized. Taxonomy follows Vieites et al. (2011) and Wake (2012).