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A Preliminary Analysis of Lungless Salamander Baraminology (Caudata: Plethodontidae)

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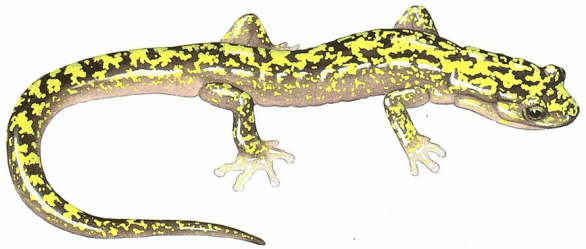
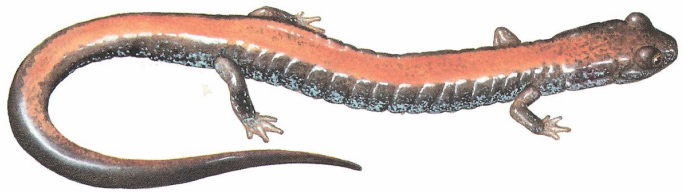


Aneides aeneus (Pfingsten & Downs, 1989)



Plethodon cinereus (Pfingsten & Downs, 1989)

Lungless Salamander Taxonomy (Wake 2012)

Order Caudata
- Suborder Salamandroidea
  - Family Plethodontidae
    - Subfamily Plethodontinae
      - Tribe Aneidini
      - Tribe Desmognathini
      - Tribe Ensatinini
      - Tribe Hydromantini
      - Tribe Plethodontini
    - Subfamily Hemidactyliinae
      - Tribe Batrachosepini
      - Tribe Bolitoglossini
      - Tribe Hemidactyliini
      - Tribe Spelerpini

Desmognathus fuscus (Pfingsten & Downs, 1989)

Background

- The lungless salamander family (Caudata: Plethodontidae) is currently composed of over 500 species in 29 genera, nine tribes, and two subfamilies (Wake 2012)

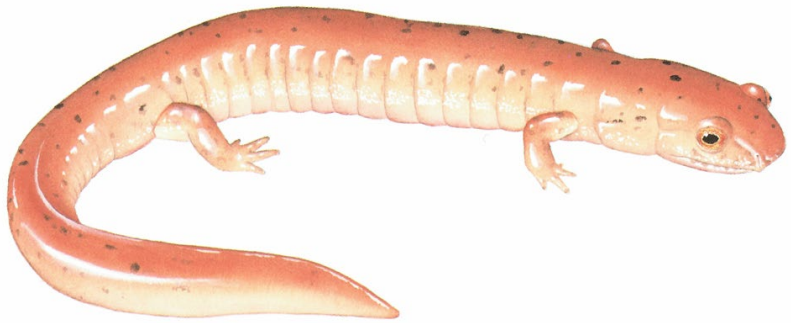
- Have been grouped together for almost 200 years, suggesting they form a distinct cognitum and quite possibly an apobaramin

- Despite their abundance and diversity, no formal baraminological analyses have been conducted for this family

- Hennigan (2013), however, tentatively defaulted to the genus level for lungless salamander kinds and suggested future research would probably lump them into larger taxonomic groupings

Goals of the Study

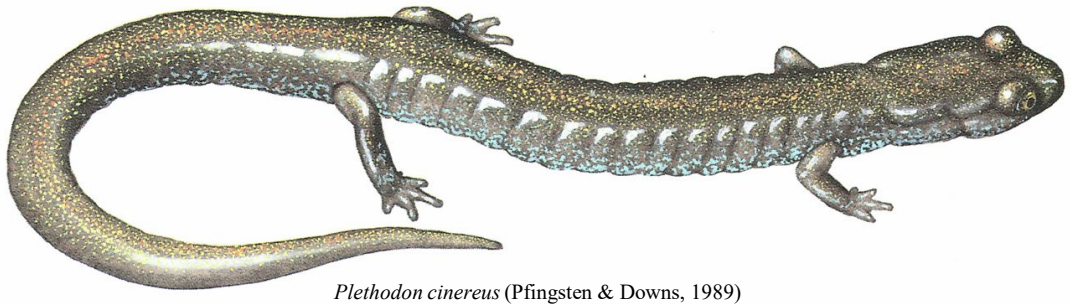
- We attempted to estimate the number and identity of lungless salamander kinds using:
  1) Taxonomic Data
     a) Survey of Shared & Unique Characteristics
     b) Survey of Consistent Groupings over Time
  2) Hybridization with Genetic Distance Data
  3) Morphological Data
  4) Molecular Data



Gyrinophilus porphyriticus (Pfingsten & Downs, 1989)

1a) Taxonomic Data – Shared & Unique Characteristics

- A survey of published literature suggests that all or most lungless salamanders share a considerable number of characteristics, several of which are unique to the family:
  - Duellman & Trueb 1986 – *Biology of Amphibians*
  - Petranka 1998 – *Salamanders of the United States and Canada*
  - Heying 2003 – Animal Diversity Web (https://animaldiversity.org)



Plethodon cinereus (Pfingsten & Downs, 1989)

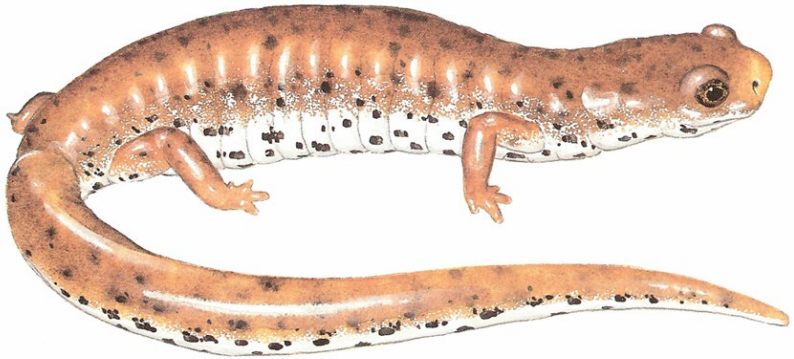
Present in All	Common in Many
Bone & Muscle Structure	
Operculum replaced by columella footplate*	Premaxillae paired or fused
Pterygoid absent*	Maxillae and premaxillae present
Vertebrae opisthocoelous	Opercularis muscle derived from m. cucullaris major
All but first three spinal nerves exit intravertebrally	
Lacrimals absent	
Exoccipital, prootic, and opisthotic fused	
Internal carotid foramen absent	
Pedicellate teeth	
Palatal teeth extend posteriorly along medial edges of vomers	
Nasals ossified from lateral analgen	
One or more flexures of periotic canal from junction with protrusion of periotic cistern to fenestra ovalis	
Reproduction	
Tail straddle walk during courtship*	Egg-guarding
	Terrestriality and direct development
	Cirri (males)
	Papillose cloacal lips (males)
	Mental gland
Miscellaneous	
Nasolabial grooves near nares*	4 toes on forelimbs, 5 on hindlimbs
Lunglessness (also found in small number of Family Hynobiidae)	Projectile tongue and hyoid apparatus
Ypsiloid cartilage absent (associated with lunglessness)	Distribution in the Americas
Diploid number 26 or 28	

*Unique to Family Plethodontidae

- 15 characteristics shared by all members of family, four of which are unique to family
- Additional 15 characteristics common in many members of family
- Combination of shared and unique characteristics suggests entire family may represent a holobaramin

1b) Taxonomic Data – Consistent Groupings

- An investigation of three “taxonomic waves” over the past 60 years identifies seven sets of consistent taxonomic groupings:
  - Morphological Taxonomies from 1960s-80s (e.g., Wake 1966)
  - Molecular Taxonomies from the 2000s (e.g., Chippindale et al. 2004)
  - More Molecular Taxonomies from the 2010s (e.g., Vieites et al. 2011)



Hemidactylium scutatum (Pfingsten & Downs, 1989)

Consistent Groups	Morphological 1960s-80s	Molecular 2000s	Molecular 2010s
Plethodontamin (MB): *Plethodon, Aneides,* and *Ensatina*	SF Plethodontinae Tribe Plethodontini	SF Plethodontinae+ (includes Desmognathamin and Hydromantamin)	SF Plethodontinae+ Tribe Plethodontini Tribe Aneidini Tribe Ensatinini
Desmognathamin (MB): *Desmognathus* and *Phaeognathus**	SF Desmognathinae	SF Plethodontinae (includes Plethodontamin and Hydromantamin) Supergenus Desmognathus	SF Plethodontinae Tribe Desmognathini
Hydromantamin (MB): *Hydromantes* and *Karsenia**	SF Plethodontinae Tribe Bolitoglossini Supergenus Hydromantes	SF Plethodontinae+ (includes Plethodontamin and Desmognathamin)	SF Plethodontinae Tribe Hydromantini (includes *Karsenia*)
Batrachosepamin (MB): *Batrachoseps**	SF Plethodontinae Tribe Bolitoglossini Supergenus Batrachoseps	SF Bolitoglossinae+ (includes Bolitoglossamin)	SF Hemidactyliinae Tribe Batrachosepini
Bolitoglossamin (MB): *Bolitoglossa, Bradytriton, Chiropterotriton, Cryptotriton, Dendrotriton, Ixalotriton, Nototriton, Nyctanolis, Oedipina, Parvimolge, Pseudoeurycea, Thorius, Aquiloeurycea,* and *Isthmura*	SF Plethodontinae Tribe Bolitoglossini Supergenus Bolitoglossa	SF Bolitoglossinae+ (includes Batrachosepamin)	SF Hemidactyliinae Tribe Bolitoglossini
Hemidactylamin (MB): *Hemidactylium**	SF Plethodontinae Tribe Hemidactyliini+ (includes Spelerpinamin)	SF Hemidactylinae	SF Hemidactyliinae Tribe Hemidactyliini
Spelerpinamin (MB): *Eurycea, Gyrinophilus, Psuedotriton, Stereochilus,* and *Urspelerpes*	SF Plethodontinae Tribe Hemidactyliini+ (includes Hemidactylamin)	SF Spelerpinae	SF Hemidactyliinae Tribe Spelerpini
*Groups differently/inconsistently among taxonomic schemes			

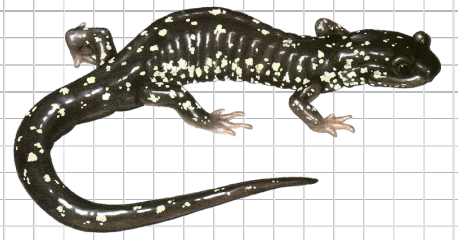
- These seven sets of consistent taxonomic groupings range from supergenus (sometimes ≈ tribe) to tribe and subfamily levels
- Represent seven monobaramins within the family

2) Hybridization & Genetic Distance Data

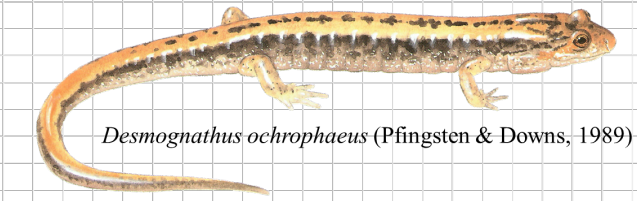
- Records of interspecific hybridization discovered for eight of 29 lungless salamander genera (Melander and Mueller 2020)
- DNA sequences for one mitochondrial (CYTB) and one nuclear gene (RAG-1) gathered from GenBank (www.ncbi.nlm.nih.gov/genbank)
- Sequences from each gene aligned across all hybridizing taxa using ClustalW in MEGA (www.megasoftware.net) and corrected distance matrices created with TN93 + Gamma model in R (www.r-project.org)
- Two species considered part of same monobaramin if:
  - Hybridized directly
  - Hybridized with same 3rd species
  - Fell within range of genetic distances of hybridizing species

Genus	Species		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Plethodon	*metcalfi*	1																																																	
	teyahalee	2																																																	
	cheoah	3																																																	
	chlorobryonis	4																																																	
	chattahoochee	5																																																	
	shermani	6																																																	
	aureolus	7																																																	
	jordani	8																																																	
	cylindraceus	9																																																
	yonahlossee	10																																																
	montanus	11																																																
	petraeus	12																																																
	kentucki	13																																																
	glutinosus	14																																																
	hoffmani	15																																																
	virginia	16																																																
	fourchensis	17																																																
	ouachitae	18																																																
	dorsalis	19																																																
	ventralis	20																																																
	cinereus	21																																																
	electromorphus	22																																																
	richmondi	23																																																
Desmognathus	*conanti*	24																																																
	ochrophaeus	25																																																
	fuscus	26																																																
	santeetlah	27																																																
	orestes	28																																																
	carolinensis	29																																																
Aneides	*flavipunctatus*	30																																																
	klamathensis	31																																																
Ensatina	*eschscholtzii platensis*	32																																																
	e. croceater	33																																																
	e. eschscholtzii	34																																																
	e. oregonensis	35																																																
	e. klauberi	36																																																
	e. picta	37																																																
	e. xanthoptica	38																																																
Hydromantes	*ambrosii*	39																																																
	italicus	40																																																
Eurycea	*bislineata*	41																																																
	cirrigera	42																																																
	wilderae	43																																																
	neotenes	44																																																
	tridentifera	45																																																
Batrachoseps	*gavilanensis*	46																																																
	luciae	47																																																
Bolitoglossa	*lincolni*	48																																																
	franklini	49																																																

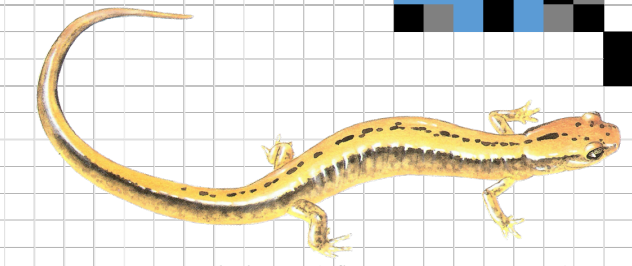
Interspecific Hybrids
Hybridizes with Same Third Species
Genetic Distance within Range of Hybridizing Species



Plethodon glutinosis (Pfingsten & Downs, 1989)



Desmognathus ochrophaeus (Pfingsten & Downs, 1989)



Eurycea bislineata (Pfingsten & Downs, 1989)

- Eight monobaramins at genus level ranging from 2-23 species
- No intergeneric crosses discovered
- Genetic distance data only joined members of same genus

3) Morphological Data

- Recoded published cladistic dataset (Lombard & Wake 1986) of 30 tongue characters across eight lungless salamander feeding modes/groupings and one composite outgroup (some analyses)

- Character states for composite outgroup "gleaned" from text of paper and added to character matrix

TABLE 1. Tongue modes and structure/behavior categories in plethodontid salamanders. Letters designating a subfamily or tribe are indicated.

Tongue mode and structure/behavior category	Mode symbol	Genera
	Subfamily Desmognathinae – D	
I Attached protrusible	DES	*Desmognathus* *Leurognathus* *Phaeognathus*
	Subfamily Plethodontinae **Tribe Plethodontini – P**	
II Attached protrusible	PLE	*Plethodon* *Aneides*
III Attached projectile	ENS	*Ensatina*
	Tribe Hemidactyliini – H	
IV Attached or free projectile	EUR	*Stereochilus*[a] *Typhlomolge*[a] *Haideotriton*[a] *Typhlotriton*[a] *Eurycea*[b] *Gyrinophilus*[b] *Pseudotriton*[b]
V Attached projectile	HEM	*Hemidactylium*
	Tribe Bolitoglossini – B	
VI Free projectile	BOL	*Bolitoglossa* *Nyctanolis* *Pseudoeurycea* *Chiropterotriton* *Lineatriton* *Parvimolge* *Thorius* *Dendrotriton* *Bradytriton* *Oedipina* *Nototriton*
VII Free projectile	HYD	*Hydromantes*
VIII Attached projectile	BAT	*Batrachoseps*

[a] Attached projectile tongue, symbolized in paper by EUR[1].
[b] Free projectile tongue, symbolized in paper by EUR[2].

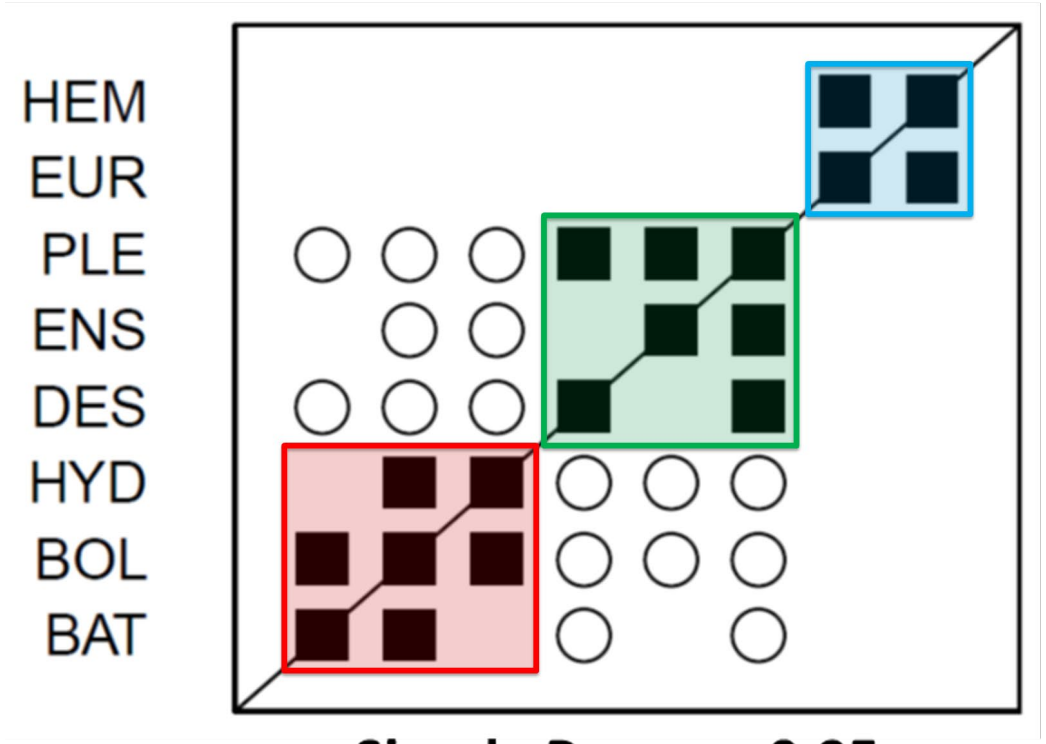
3) Morphological Data

- Analyzed using several statistical baraminology clustering techniques (Wood, 2020, 2021):
  - Distance Correlation Analysis (DCA) & Multidimensional Scaling (MDS)
  - Medoid Partitioning (PAM) & Fuzzy Analysis (FANNY)

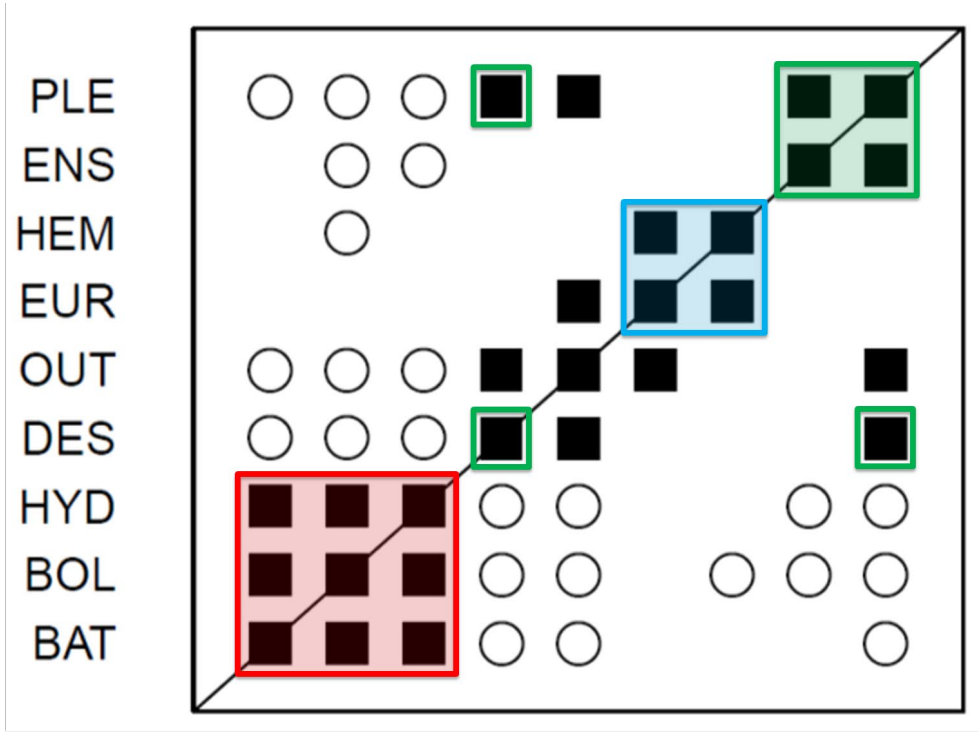
- Simple matching & Jaccard distances with character relevance of 0.95 (all 30 characters) for all analyses
  - Pearson & Spearman correlations for DCA
  - 2-5 cluster analyses with average silhouette widths for PAM & FANNY

- Heatmap created for all DCA, PAM, and FANNY analyses combined (n=32), summarizing continuity between related groups

3) Morphological Data – DCA

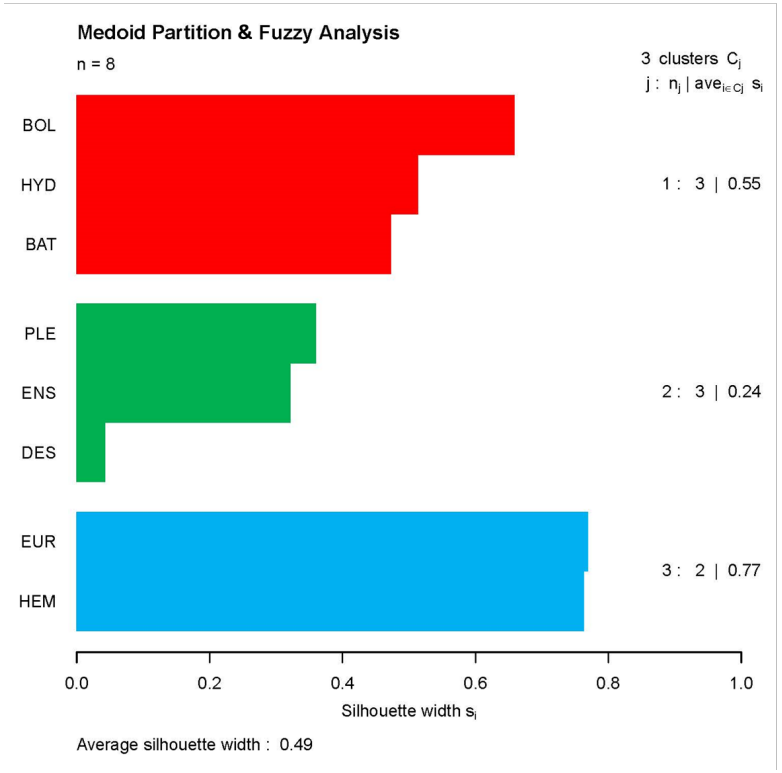


Simple Pearson 0.95
Without Outgroup

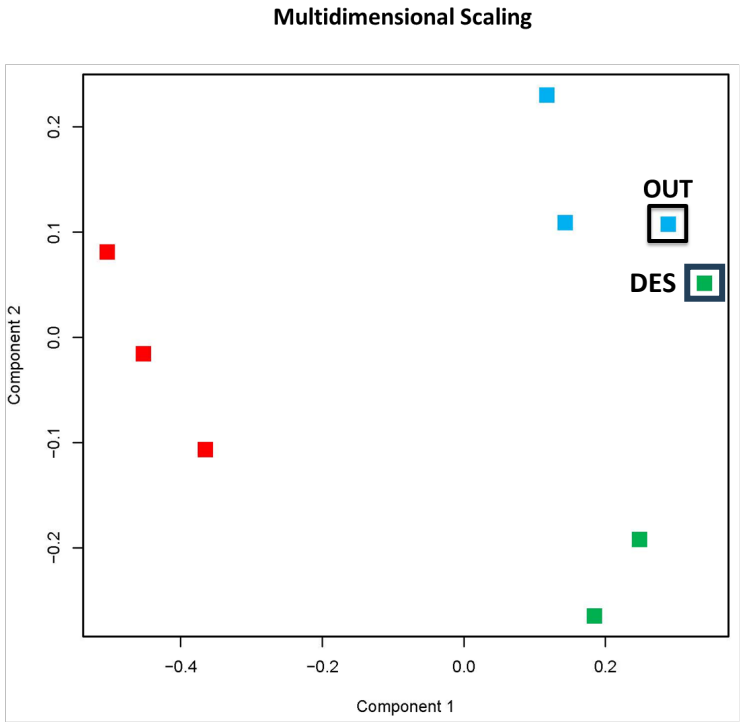


Simple Pearson 0.95
With Outgroup

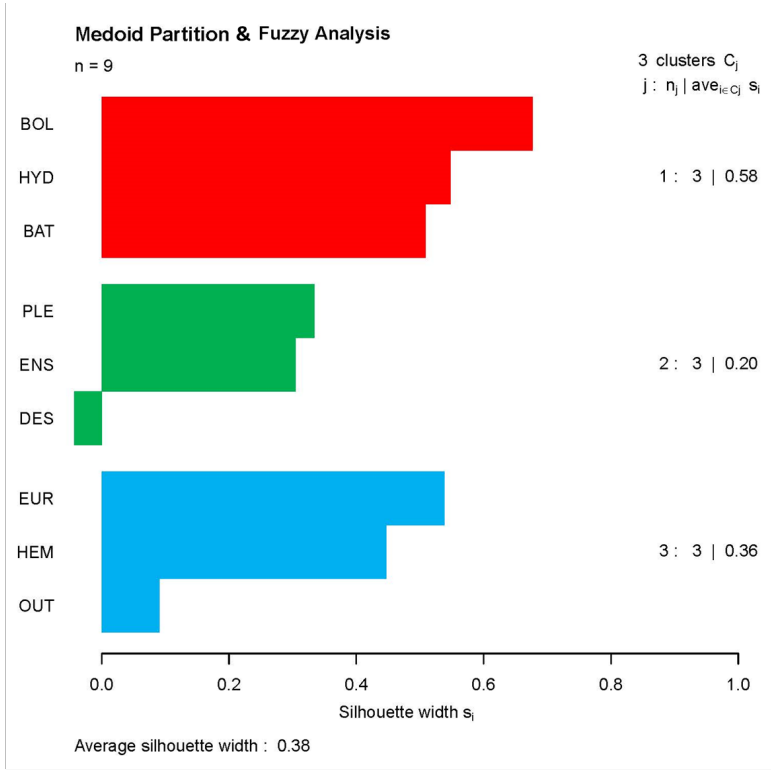
3) Morphological Data – PAM/FANNY/MDS (3 clusters)



Simple 0.95
Without Outgroup

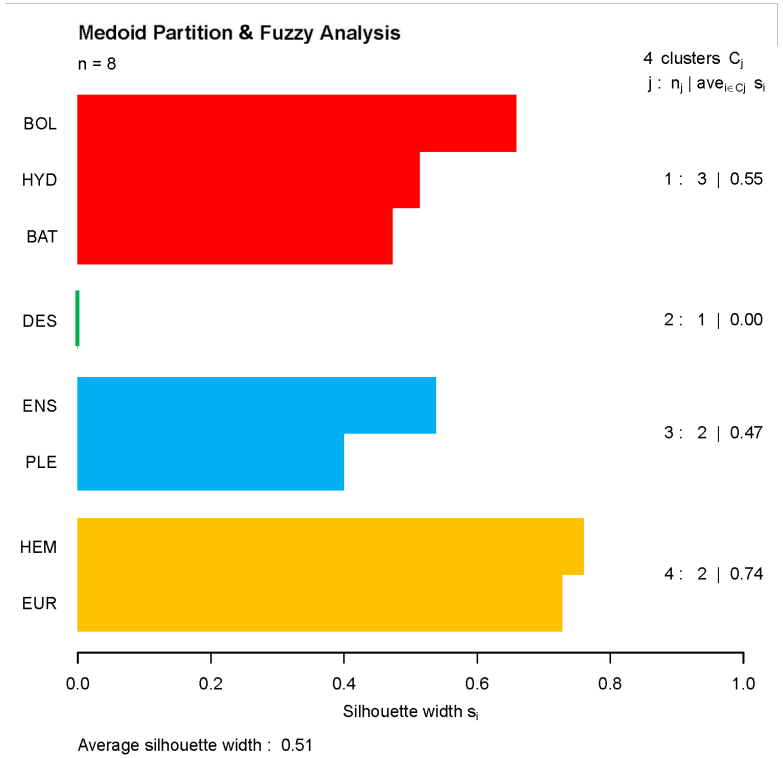


Simple 0.95

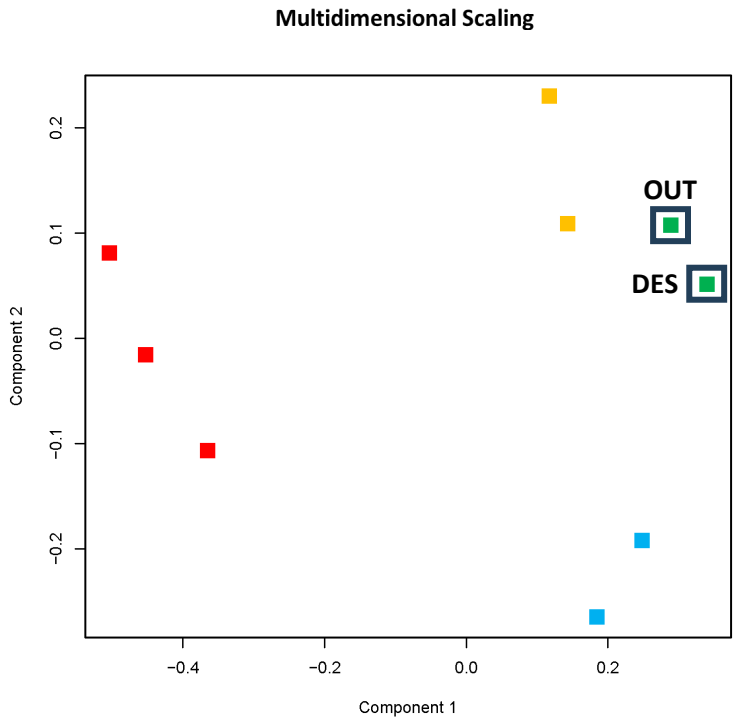


Simple 0.95
With Outgroup

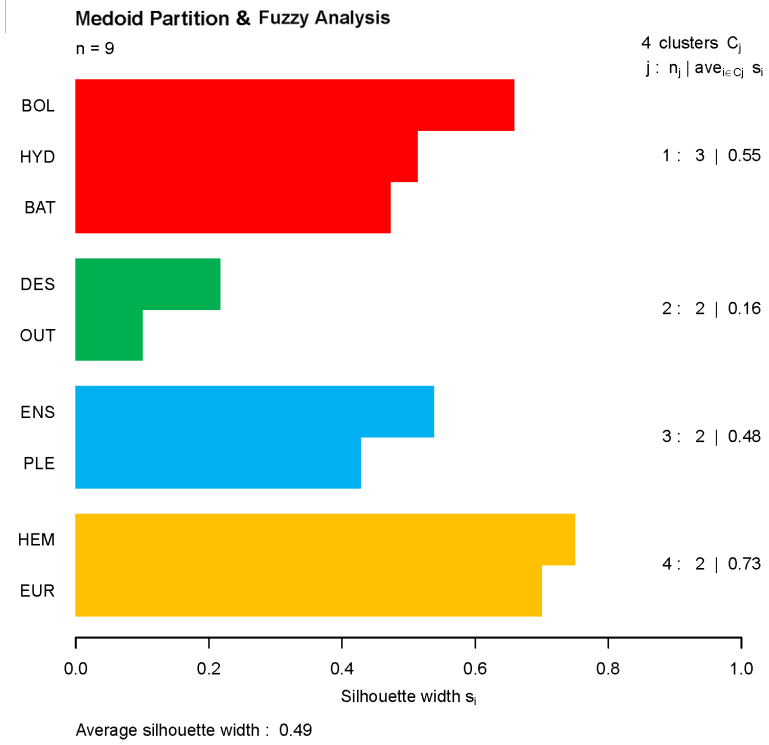
3) Morphological Data – PAM/FANNY/MDS (4 clusters)



Simple 0.95
Without Outgroup



Simple 0.95



Simple 0.95
With Outgroup

3) Morphological Data – Heatmap (3 clusters)

	DES	PLE	ENS	EUR	HEM	BOL	HYD	BAT
DES		75	53	34	25	0	0	0
PLE	75		100	25	25	0	0	0
ENS	53	100		25	25	0	0	0
EUR	34	25	25		100	0	0	0
HEM	25	25	25	100		0	0	0
BOL	0	0	0	0	0		94	97
HYD	0	0	0	0	0	94		85
BAT	0	0	0	0	0	97	85	

Percent of DCA, PAM, and FANNY analyses (n=32) where taxa clustered together. Light yellow = high percentage; Dark red = low percentage

3) Morphological Data – Heatmap (4 clusters)

	DES	PLE	ENS	EUR	HEM	BOL	HYD	BAT
DES		75	53	34	25	0	0	0
PLE	75		100	25	25	0	0	0
ENS	53	100		25	25	0	0	0
EUR	34	25	25		100	0	0	0
HEM	25	25	25	100		0	0	0
BOL	0	0	0	0	0		94	97
HYD	0	0	0	0	0	94		85
BAT	0	0	0	0	0	97	85	

Percent of DCA, PAM, and FANNY analyses (n=32) where taxa clustered together. Light yellow = high percentage; Dark red = low percentage

3) Morphological Data – Monobaramins

- Three (or four) monobaramins are evident in morphological analyses:
  - Tribe Plethodontini + Aneidini + Ensatinini + Desmognathini (Subfamily Plethodontinae minus Hydromantini)
  - Tribe Spelerpini + Hemidactyliini
  - Tribe Bolitoglossini + Hydromantini + Batrachosepini
  - Desmognathini may or may not be its own monobaramin (3 vs. 4 clusters)?

- The [Plethodontini + Aneidini + Ensatinini + Desmognathini] monobaramin is also discontinuous from the [Bolitoglossini + Hydromantini + Batrachosepini] monobaramin in several DCA analyses, indicating these may be separate holobaramins

4) Molecular Data

- DNA sequences for two mitochondrial (CYTB, ND4) and one nuclear gene (RAG-1) gathered from GenBank:
  - Cytochrome B (CYTB)
  - NADH Dehydrogenase Subunit 4 (ND4)
  - Recombination Activating Gene 1 (RAG-1)

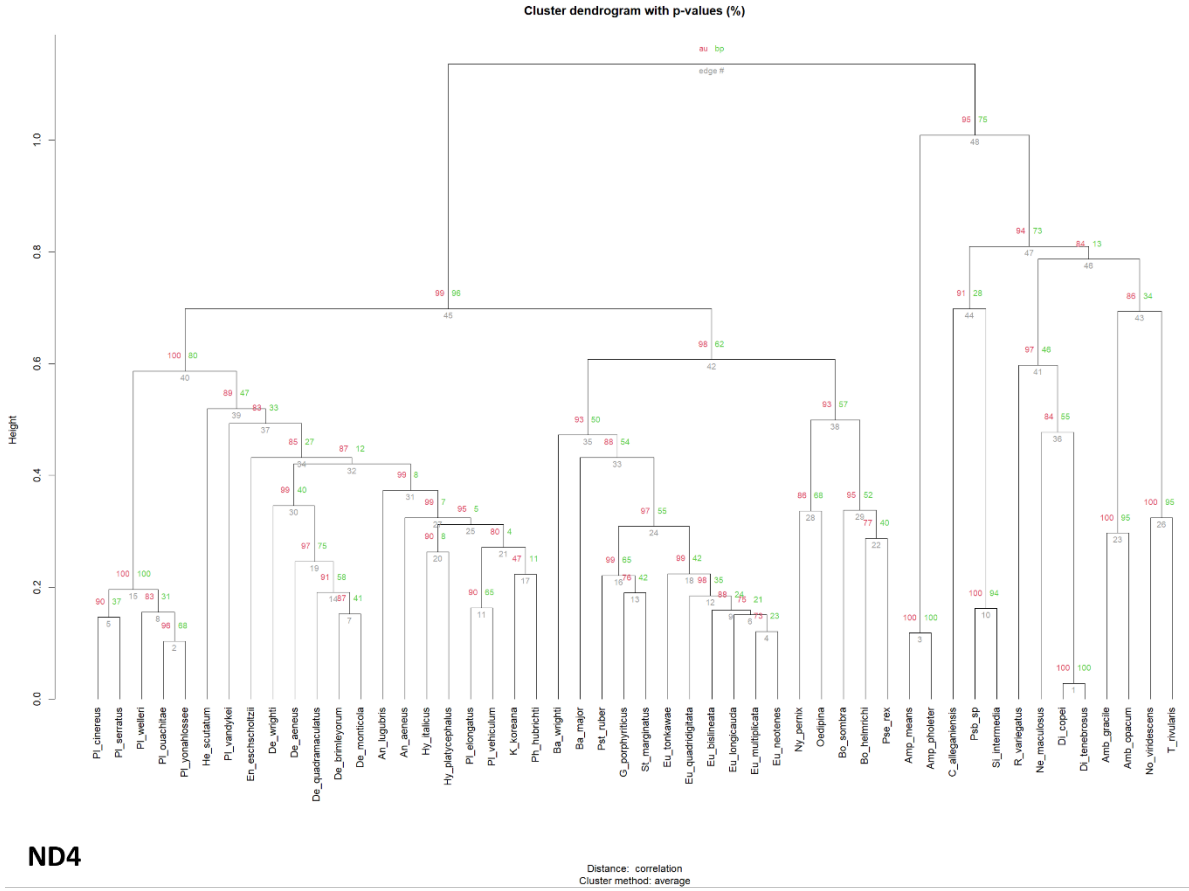
- Dataset included 37 representative plethodontid taxa from both subfamilies and all nine tribes

- 13 outgroup salamander taxa, from eight families, also included

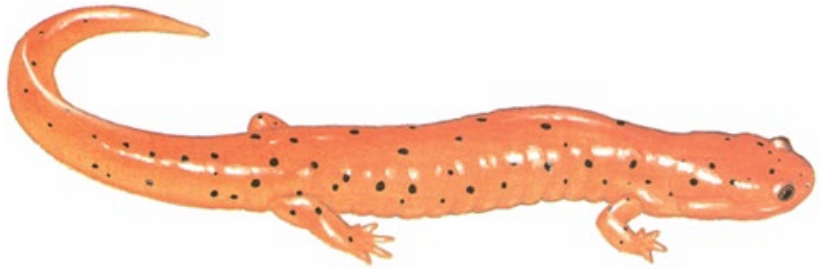
4) Molecular Data

- Sequences from each gene aligned across all taxa using ClustalW in MEGA
- Corrected distance matrices for each gene, plus a concatenated sequence of all genes combined, created with TN93 + Gamma model in R
- Distance matrices analyzed using hierarchical clustering, MDS, and DCA in R (Blaschke 2022; Wheeler and Blaschke 2022) and BARCLAY (Wood 2020)

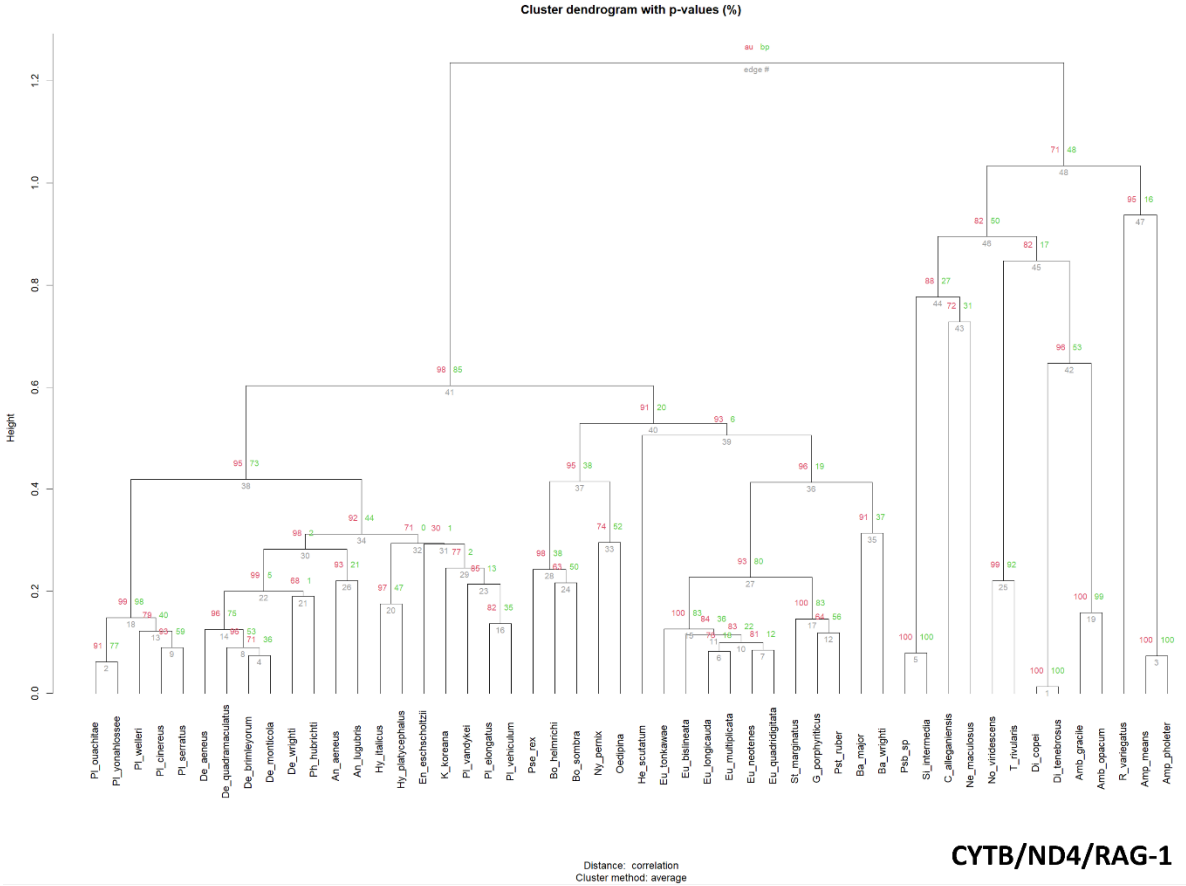
4) Molecular Data – HC



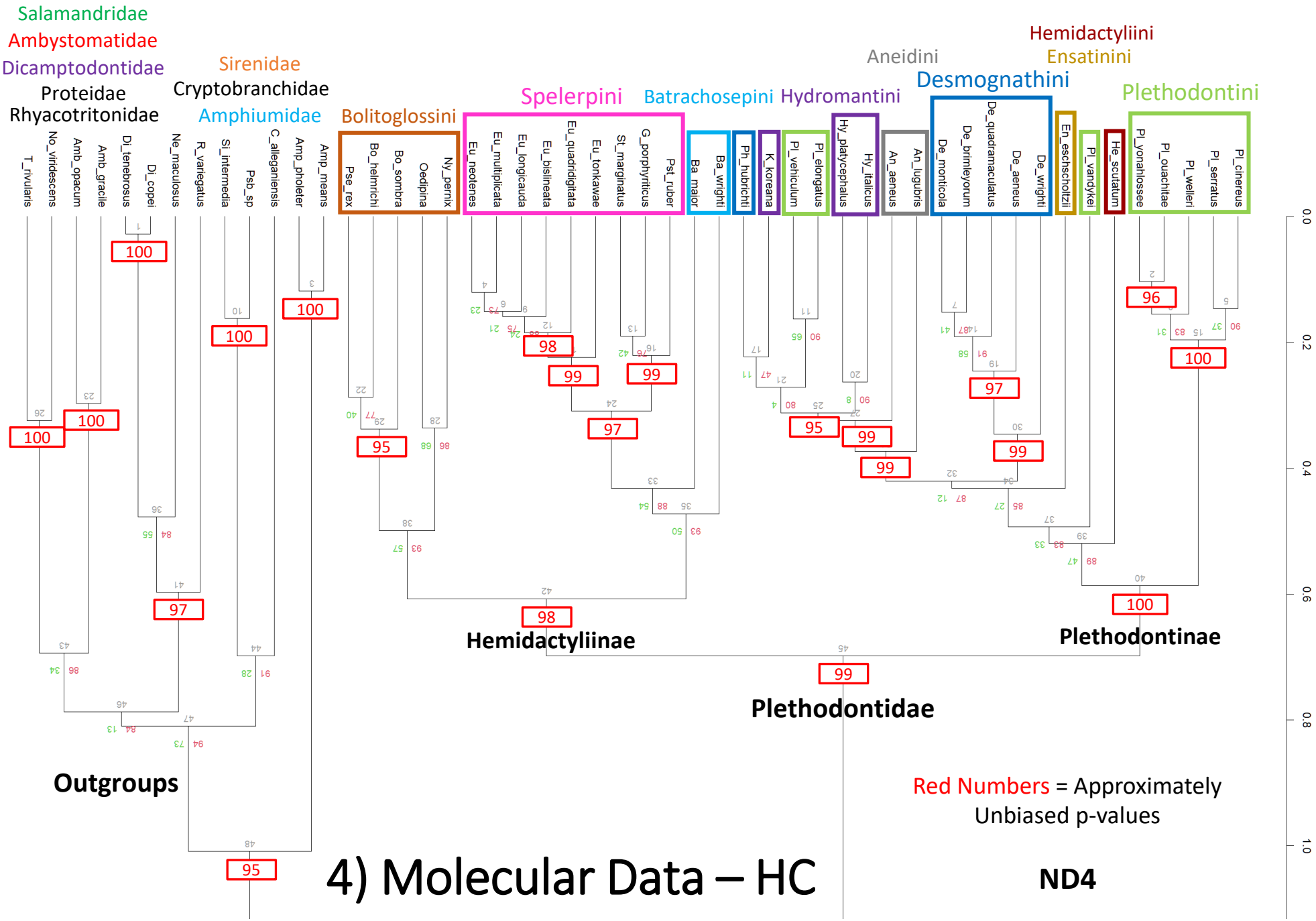
ND4



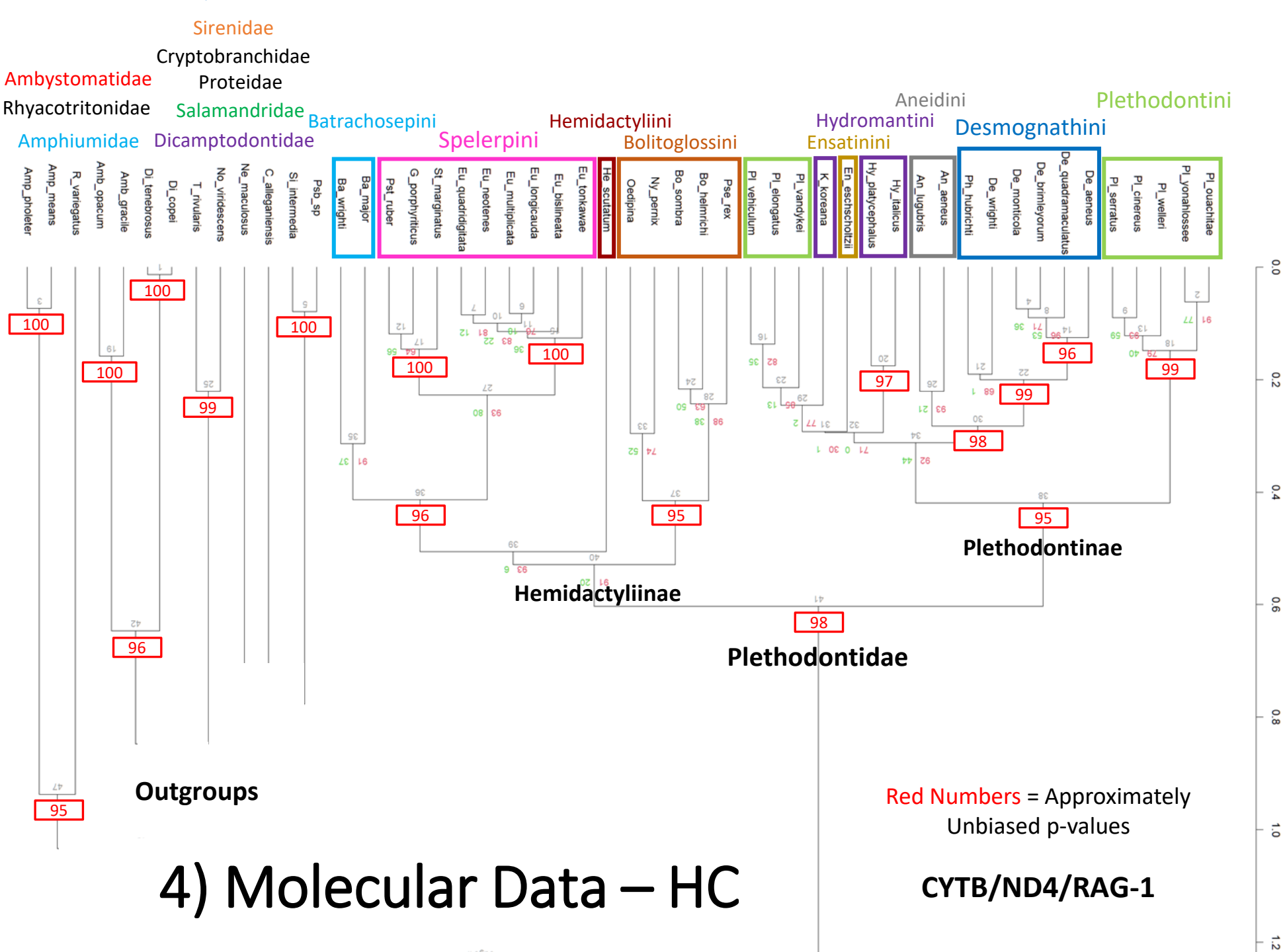
Pseudotriton montanus (Pfingsten & Downs, 1989)



CYTB/ND4/RAG-1



4) Molecular Data – HC

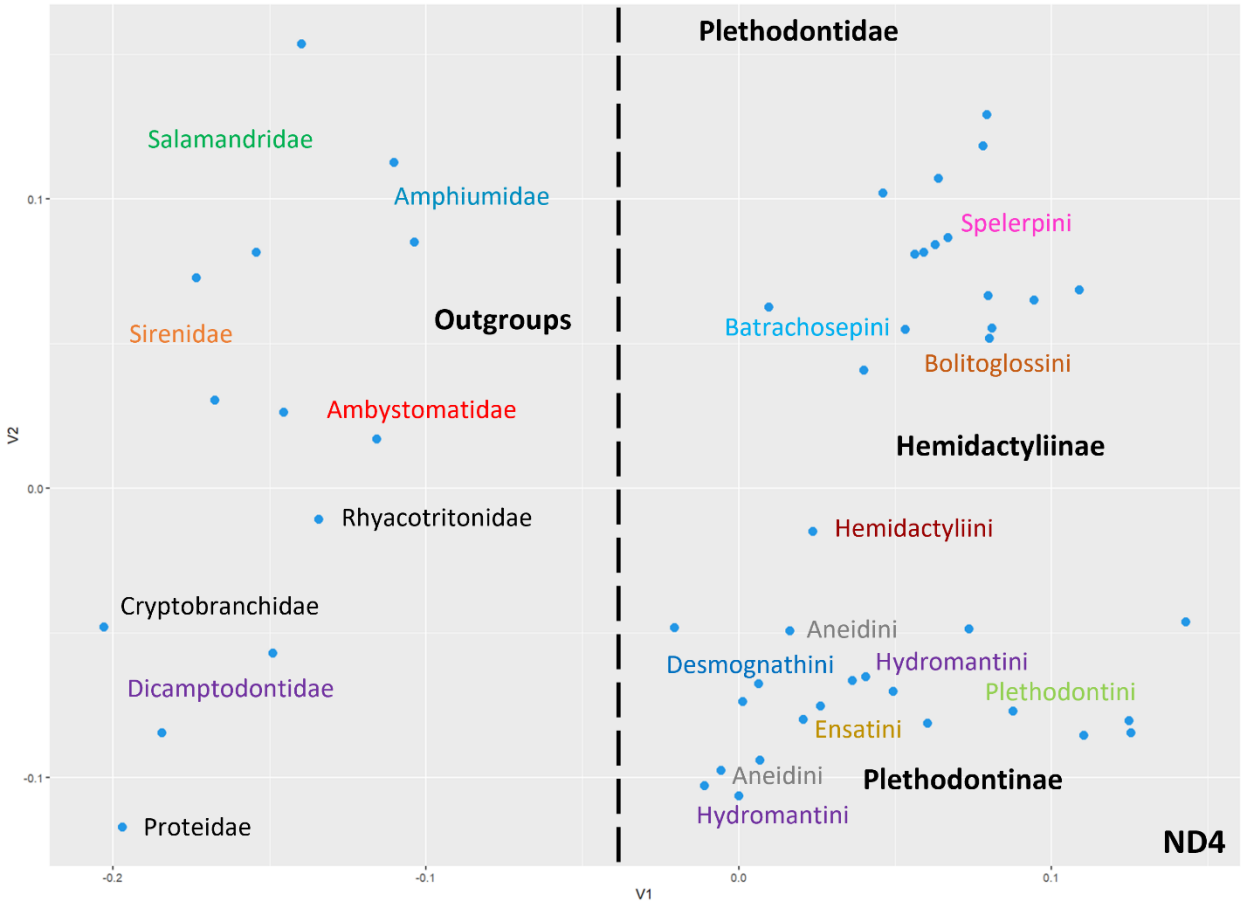


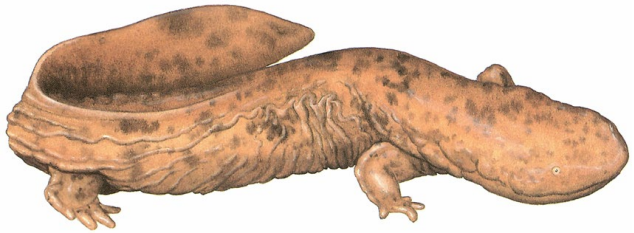
Red Numbers = Approximately Unbiased p-values

4) Molecular Data – HC

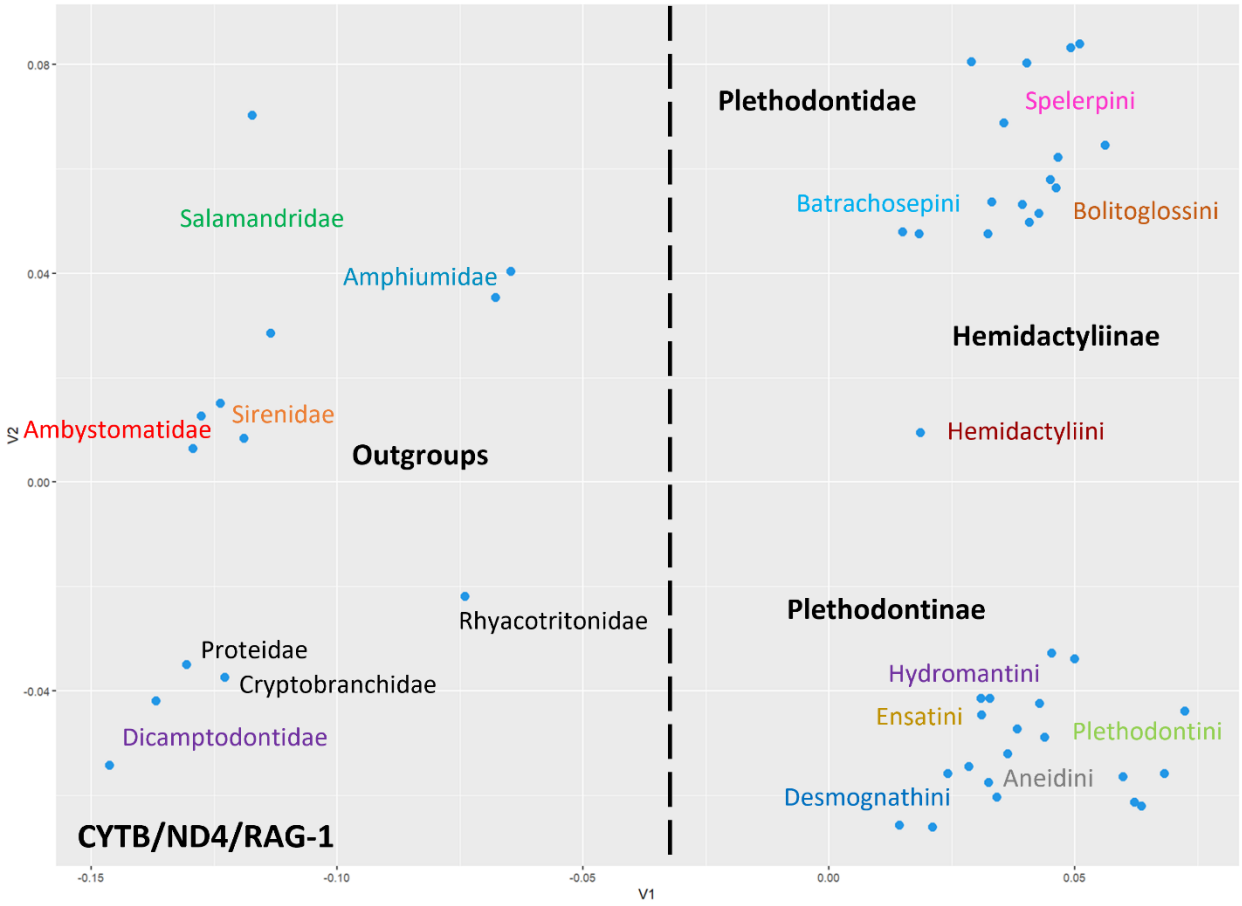
CYTB/ND4/RAG-1

4) Molecular Data – MDS

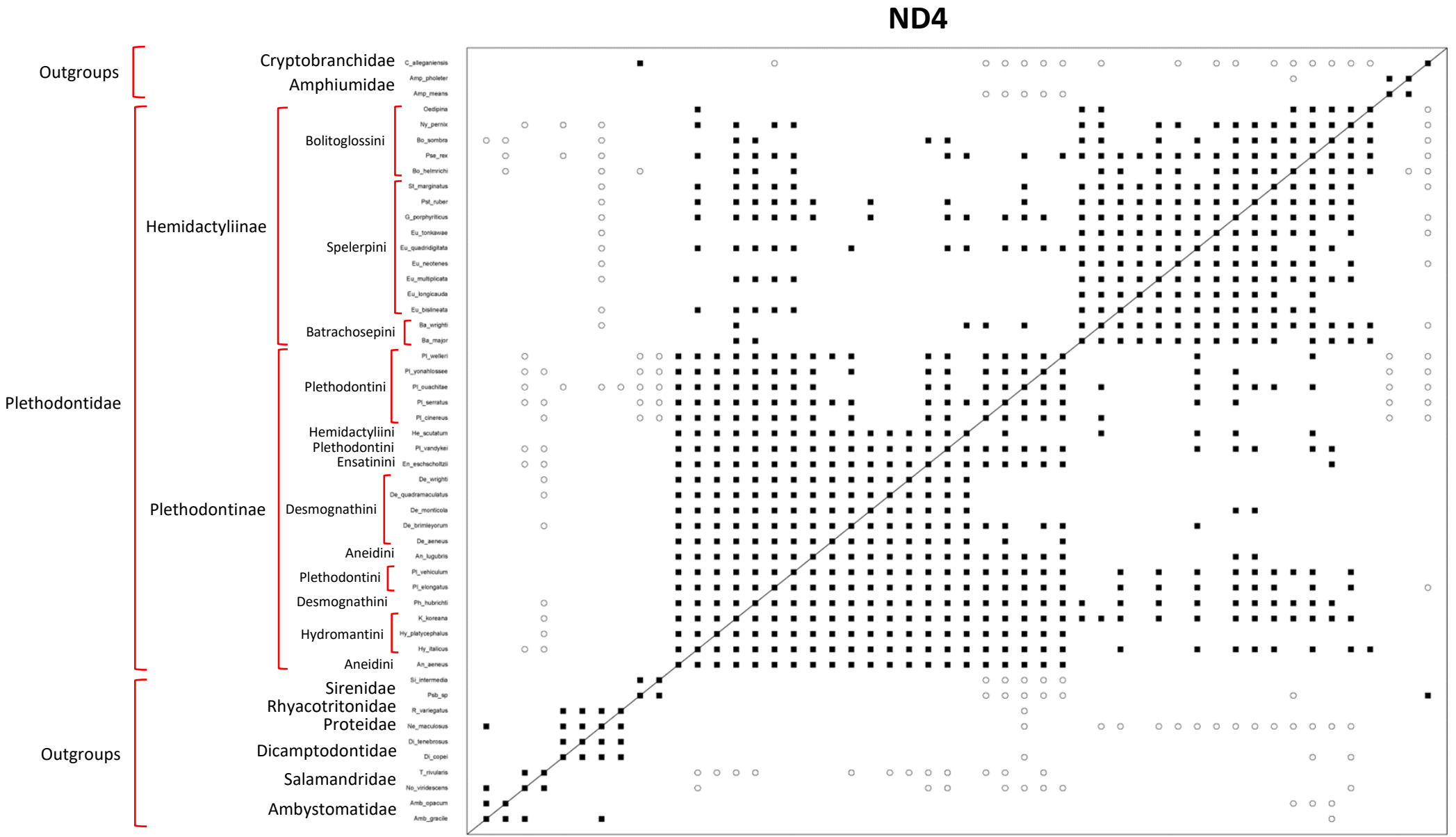




Cryptobranchus alleganiensis (Pfingsten & Downs, 1989)

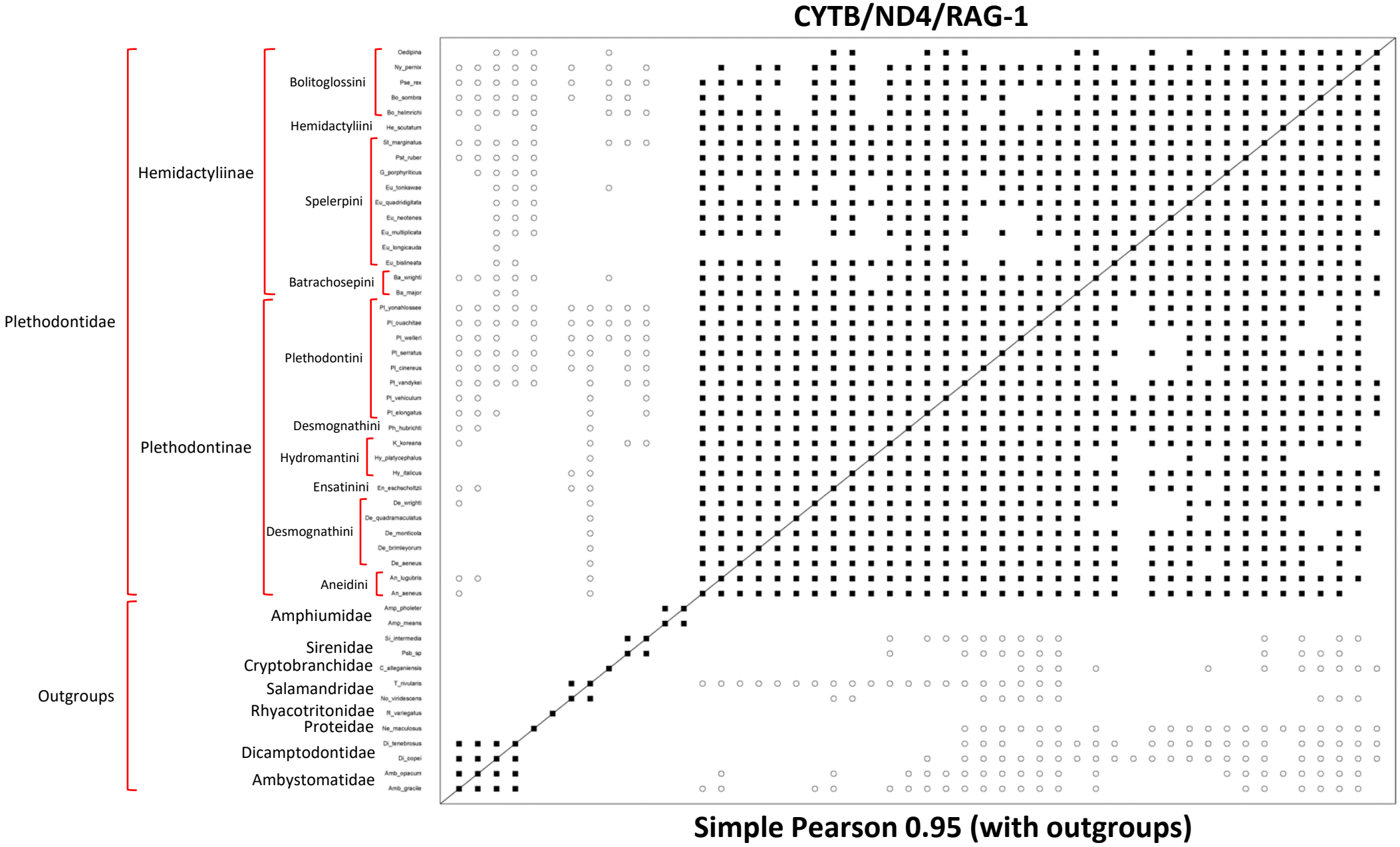


4) Molecular Data – DCA



Simple Pearson 0.95 (with outgroups)

4) Molecular Data – DCA



4) Molecular Data

- Analyses of DNA sequences suggest the presence of five lungless salamander monobaramins that further cluster into two subfamily groupings:
  - Tribe Hemidactyliini
  - Tribe Spelerpini
  - Tribe Batrachosepini
  - Tribe Bolitoglossini

  } Subfamily Hemidactyliinae

  - All five tribes in Subfamily Plethodontinae

- These monobaramins also cluster into one large family group, separate from outgroups, and may represent a single holobaramin

Overall Conclusions

- The genus-level monobaramins indicated by interspecific hybridization are joined into tribe and/or subfamily groupings, ranging from two to seven monobaramins/holobaramins overall, by certain taxonomic, morphological, and molecular analyses

- Some taxonomic and molecular analyses also suggest the possibility of a single family-level holobaramin

- Future research will include:
  - Additional morphological and molecular analyses
  - Investigations of fossils, biogeography, and biblical passages related to potential Flood/post-Flood dispersal mechanisms

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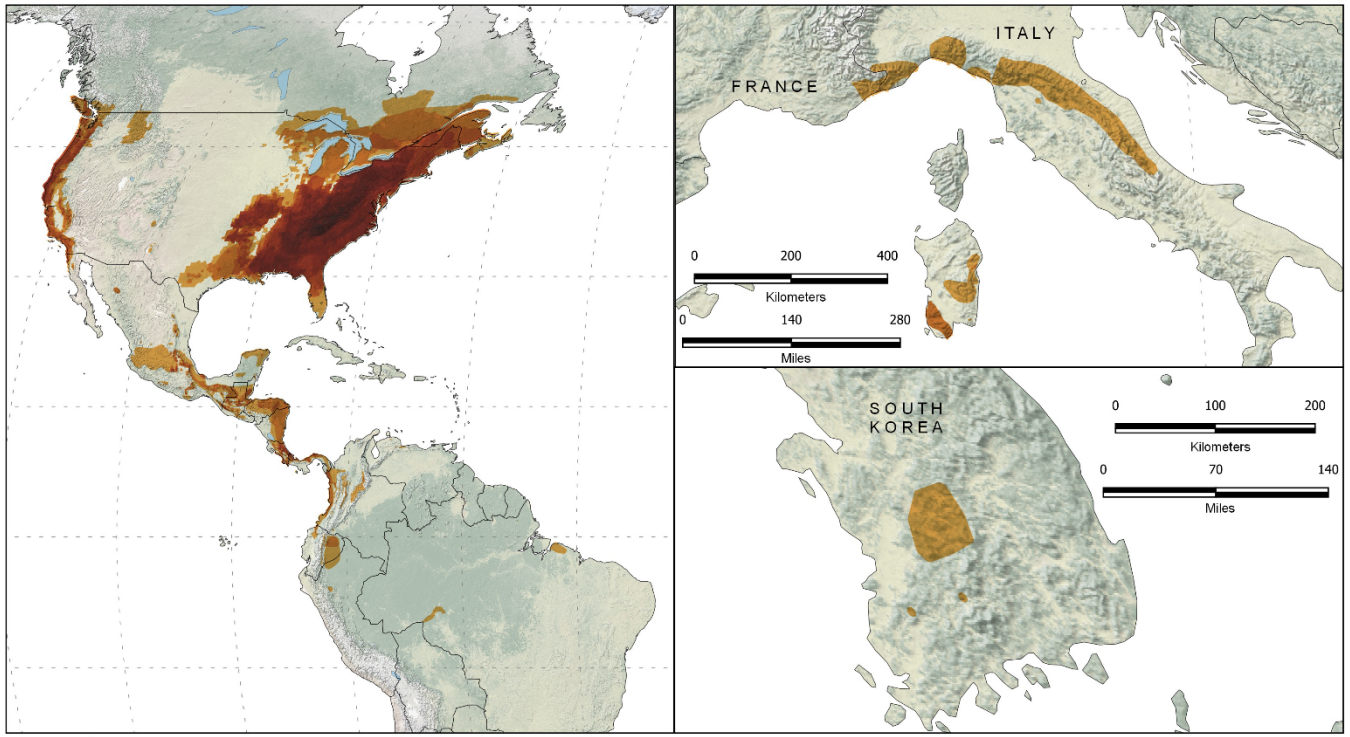
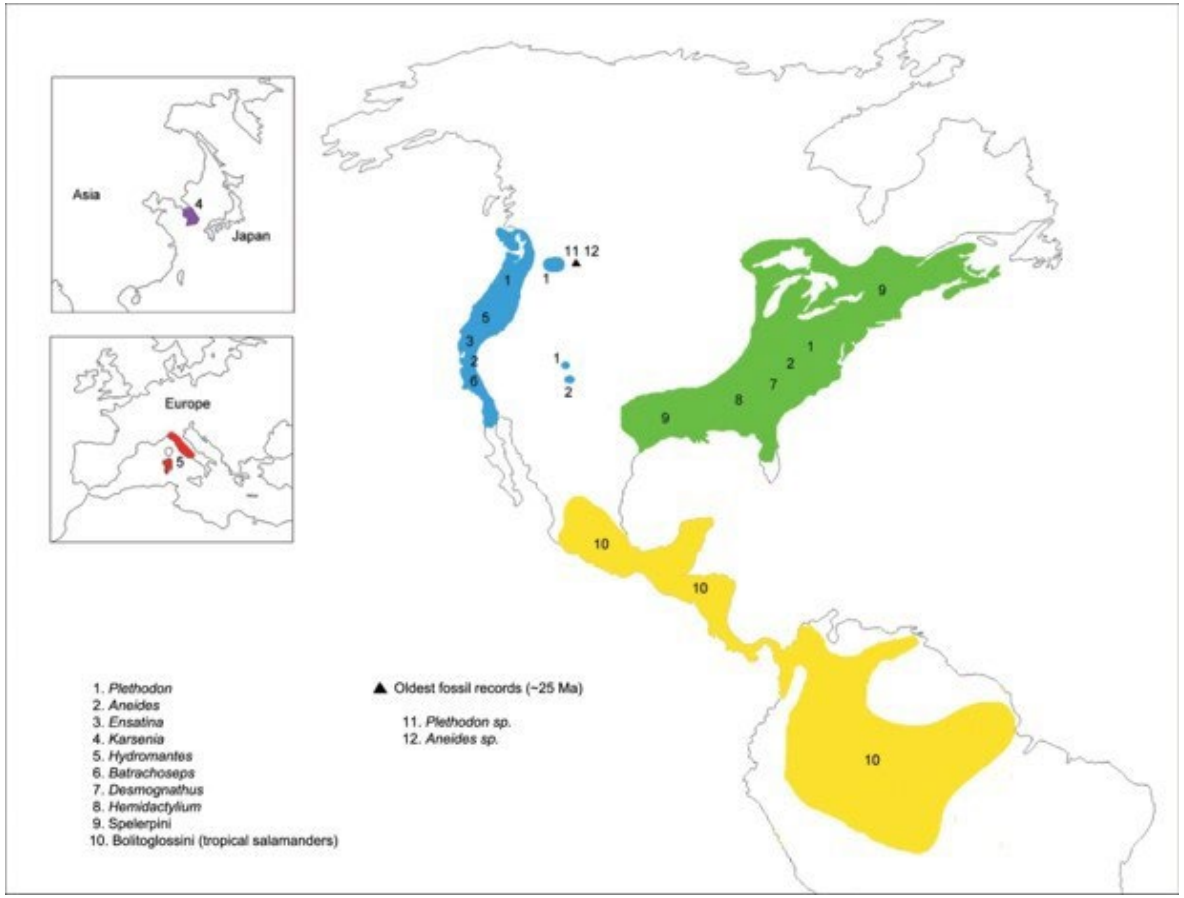
Additional References

Pfingsten, R.A., and F.L. Downs (eds.). 1989. Salamanders of Ohio: Bulletin of the Ohio Biological Survey 7(2). Ohio State University, College of Biological Sciences, Columbus, OH.

FAMILY PLETHODONTIDAE





Mapped by AmphibiaWeb, December 2020

452 species ranges showing, with overlapping areas increasing in darkness.

Graticules at 30 degrees on world range, and 15 degrees on inset.

Projection is World Robinson, WGS84 (EPSG: 54030)

Data Sources: Natural Earth, AmphibiaWeb, IUCN Red List

0 2,000 4,000 6,000
Kilometers
0 900 1,800 2,700 3,600
Miles

Disclaimer: Species ranges may be missing, incomplete, or inaccurate and are produced with best available data and thus subject to change.