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Salamanders (Caudata)

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Abstract

Living salamanders (~570 species) are placed in 10 families, comprising the Order Caudata. Their classification is relatively stable, but phylogenetic relationships among families are contentious. Recent molecular phylogenetic analyses have found five major clades. The salamander timetree shows the deepest divergence, between Cryptobranchioidea and all other families, in the Triassic (251–200 million years ago, Ma) with subsequent diversification occurring in the Jurassic (200–146 Ma) and early Cretaceous (146–100 Ma).

Salamanders form a monophyletic group, constituting one of the three orders of modern amphibians (Lissamphibia), together with frogs and caecilians. Salamanders comprise the second most species-rich order of amphibians (1) and are typically classified in 10 families, with ca. 68% of the species belonging to the Family Plethodontidae. The body plan has remained relatively stable since the Jurassic (2, 3) (Fig. 1), displaying several features that in combination distinguish it from the body plan of other amphibians: presence of a tail both in larval and adult phases, two pairs of limbs of equal size (when present) set perpendicular to the body, presence of teeth on both jaws, presence of ribs on most trunk vertebrae, and absence of several skull bones (4). Here we review the phylogenetic relationships and the divergence times of salamander families.

The families are grouped into five suborders: Cryptobranchioidea (Cryptobranchidae and Hynobiidae), Sirenoidea (Sirenidae), Salamandroidea (Salamandridae, Ambystomatidae, Dicamptodontidae), Proteoidea (Proteidae), and Plethodontioidea (Plethodontidae, Rhyacotritonidae, and Amphiumidae). Despite the increasing number of studies and data addressing the phylogeny of salamander families, their relationships are difficult to resolve. Several relationships are consistently recovered

with different data sets, while the positions of others, in particular the sirenids and proteids, have remained contentious.

A monophyletic Cryptobranchioidea and a clade consisting of Dicamptodontidae and Ambystomatidae are recovered in every molecular phylogenetic study (Vieites and Wake, submitted; Zhang and Wake, submitted; 2, 5–10). Salamandridae is usually recovered as the closest relative of the clade constituted by dicamptodontids and ambystomatids (Vieites and Wake, submitted; Zhang and Wake, submitted; 2, 5–7, 9, 10). Frost *et al.* (9) proposed placing Dicamptodontidae in Ambystomatidae because the two families form a clade and each contains only a single living genus, but divergence between the lineages is great and the two are very old (115.8 Ma, Table 1). Furthermore, dicamptodontids have a long and rather rich fossil record so the recognition of only a single family is misleading.

The position of Proteidae has been contentious. North American and European species form a clade. Several molecular studies recovered proteids as closest relatives of sirens and nested within the crown (2, 6, 8, 9). Recent molecular phylogenetic studies have found that Proteidae is closest to Salamandroidea (7, 9), although with low statistical support. In contrast, a recent study using one mitochondrial and four nuclear markers found that Proteidae is the closest relative of Plethodontioidea (10). The same relationship was found, with high statistical support, with a data set of 19 nuclear markers as well as with complete mitochondrial genomes (Fig. 2; Vieites and Wake, submitted; Zhang and Wake, submitted). Plethodontioidea is monophyletic and Rhyacotritonidae is closest to a clade constituted by amphiumids and plethodontids (Vieites and Wake, submitted; Zhang and



Fig. 1 A plethodontid salamander (*Karsenia koreana*) female from the type locality. Credit: D. R. Vieites.

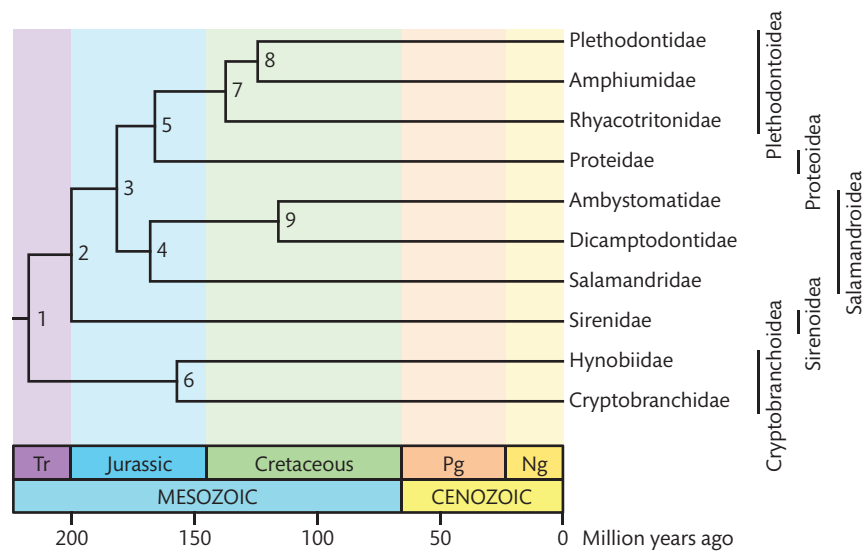


Fig. 2 A timetree of salamanders (Caudata). Divergence times are shown in Table 1. Ng (Neogene), Pg (Paleogene), and Tr (Triassic).

Wake, submitted; 7, 9, 10). The large Family Plethodontidae always has been found to be monophyletic (e.g., 11). Multiple nuclear markers and complete mitochondrial genomes found that amphiumids are closest to plethodontids (Vieites and Wake, submitted; Zhang and Wake, submitted; 7, 10). One study that combined analyses of nuclear rRNA and mtDNA data suggested that amphiumids are at the base of the salamander tree, as closest relative of the remaining salamanders (8).

The base of the salamander tree has been subject to controversy for decades. The Sirenidae is a small clade restricted to the southeastern United States and extreme northeastern Mexico that has a characteristic morphology. The species are greatly elongated, permanently aquatic, and gilled. They lack hind limbs and have jaws that lack teeth (except on the small coronoid bone on the inner surface of the lower jaw) and are covered by hardened keratinized “beaks.” Their reproduction is external but they differ from all other salamanders in lacking pelvic glands. They were once considered not to be salamanders and placed in a separate Order Trachystomata (12), later becoming the Order Meantes (13). In contrast, a paleontological study found Sirenidae to be a deeply nested clade, closest to Salamandridae (14). All relevant molecular studies have clustered sirenids with other salamanders. Analyses of complete mitochondrial genomes of all families found Sirenidae to be the closest relative of all other salamanders with high statistical support (Zhang and Wake, submitted). One study found them to be part of a basal polytomy

based on partial *RAG1* sequences (15). An early study that combined morphological and molecular data suggested that sirenids represent a basal stem group (5). However, another study that combined nuclear RNA (based on the relatively scanty data then available) and morphological data found that Cryptobranchioidea is the closest relative of all other families (2). The same result was found with recent analyses of single (7) and multiple nuclear loci (10) data sets, but without strong statistical support. Bayesian and maximum likelihood analyses of a data set comprising 19 nuclear markers (Vieites and Wake, submitted) (Fig. 2) strongly support Cryptobranchioidea as the first branch, closest to a group including Sirenoidea and the remaining suborders. Cryptobranchoids display several traits that appear to be ancestral. They have external fertilization, like sirenids, and they are the only salamanders known to have a separate angular bone in the lower jaw, and high numbers of microchromosomes.

Relationships of the extinct taxa Batrachosauroididae, Prosirenidae, and Scapherpetontidae are unclear because the fossil record is incomplete, but Karauridae is widely accepted as the closest relative of the living and extinct salamanders (14). Salamander fossils are scarce, and few phylogenetic studies have estimated divergence times among all salamander families. San Mauro *et al.* (15) estimated the divergence of Caudata and Anura at 271 ± 19 million years ago (Ma), based on a partial fragment of *RAG1*. Using several nuclear and a mitochondrial marker for representatives of all living families,

Table 1. Divergence times (Ma) and confidence/credibility intervals among salamanders (Caudata).

Timetree		Estimates							
Node	Time	Ref. (10)(a)		Ref. (10)(b)		Vieites and Wake (submitted)		Zhang and Wake (submitted)	
		Time	CI	Time	CI	Time	CI	Time	CI
1	217.5	248.7	282–220	220.1	247–196	218.3	234–204	183.0	201–167
2	200.1	232.2	266–199	194.8	232–169	202.2	221–184	171.0	186–158
3	181.7	209.8	242–178	176.0	211–149	181.1	200–162	160.0	177–144
4	168.0	198.6	231–165	162.6	196–131	165.8	186–146	145.0	165–125
5	166.2	190.0	221–160	154.0	188–123	169.8	189–151	151.0	168–134
6	157.2	174.0	208–150	145.5	168–146	158.3	166–155	151.0	162–145
7	137.3	156.5	185–127	119.5	147–92	137.3	157–119	136.0	153–118
8	124.4	144.6	175–115	106.0	130–80	123.1	143–104	124.0	143–107
9	115.8	136.4	170–107	107.6	151–80	113.2	134–94	106.0	137–74

Note: Node times in the timetree represent the mean of time estimates from different studies. Divergence times calculated from an analysis of four nuclear and one mitochondrial markers using Bayesian (a) and penalized likelihood (b) methods (10) are shown. In another study (Vieites and Wake, submitted), 19 nuclear markers were used, and in a third study (Zhang and Wake, submitted) complete mitochondrial genomes were analyzed.

Roelants *et al.* (10) provided a younger estimate, using both Bayesian and penalized likelihood approaches (Table 1). A 19-nuclear-marker study (Vieites and Wake, submitted) using a Bayesian approach and minimum constraints, instead of fixed calibration points, yielded divergence times that were on average close to the penalized likelihood estimates from Roelants *et al.* (10). An analysis of complete mitochondrial genomes using a rate-uncorrelated dating technique and a “soft bound” calibration strategy yielded an estimate of ~183 Ma (Zhang and Wake, submitted). A comparison of results for all salamander families, from studies using the same method of divergence time estimation (Table 1), shows discrepancies between the different estimates averaging 25 million years. The nuclear (Vieites and Wake, submitted) and mitochondrial (Zhang and Wake, submitted) data sets provided overlapping divergence time estimates in young nodes, while the mitochondrial data set gave much younger estimates for older nodes.

All available data sets suggest that most of the families of salamanders diversified during the Jurassic (Fig. 2). Sirenoidea diverged from the crown group ~200 Ma. Salamandridae split from Ambystomatidae + Dicamptodontidae at about the same time as Proteoidea split from Plethodontidae, during the mid-Jurassic (Table 1). The estimated time of divergence of hynobiids and cryptobranchids (157.2 Ma, Table 1) is in agreement with the oldest known fossils (3). The divergence time estimates of Ambystomatidae and Dicamptodontidae

are older than the oldest fossil (55.8 Ma, 16), suggesting an mid-Cretaceous split, 115.8 Ma. A similar result was found with respect to the split of Plethodontidae and Amphiumidae (124.4 Ma, Table 1), much older than the oldest amphiumid fossil (65.5 Ma, 17).

In summary, the salamander timetree (Fig. 2) suggests that the diversification of extant salamander families happened during the Jurassic to mid-Cretaceous. Cryptobranchoids are known from fossils dating from 155 Ma in northeastern China (3, 18). The earliest sirenooid fossils are from the Cretaceous of Sudan, Africa, ca. 100 Ma (19), and the late Cretaceous, 83 Ma, and Paleocene of western North America (Alberta to Wyoming, 20). The earliest proteoid is from the late Paleocene of North America (20), the earliest plethodontoid is from the late Cretaceous, 66 Ma, of Montana (17), and the earliest salamandroid is from the latest Paleocene, 56 Ma, of Alberta and Montana (14, 16). Extant species of all families except Hynobiidae today occur in North America, and five families (Ambystomatidae, Amphiumidae, Dicamptodontidae, Rhyacotritonidae, and Sirenidae) are restricted to that continent. Both families of the Cryptobranchioidea occur in East Asia, where they cooccur with Salamandridae and Plethodontidae.

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References

1. Amphibia Web, *Information on Amphibian Biology and Conservation*, <http://amphibiaweb.org/> (Berkeley, California, 2008).
2. K. Gao, N. H. Shubin, *Nature* **410**, 574 (2001).
3. K. Gao, N. H. Shubin, *Nature* **422**, 424 (2003).
4. W. Duellman, L. Trueb, *The Biology of Amphibians* (Johns Hopkins University Press, Baltimore, 1994), pp. 461–475.
5. A. Larson, W. Dimmick, *Herp. Monogr.* **7**, 77 (1993).
6. A. Larson, D. W. Weisrock, H. H. Kozak, in *Reproductive Biology and Phylogeny of Urodela (Amphibia)*, D. M. Sever, Ed. (NH: Science Publishers, Enfield, 2003), pp. 31–108.
7. J. J. Wiens, R. M. Bonett, P. T. Chippindale, *Syst. Biol.* **54**, 91 (2005).
8. D. W. Weisrock, L. J. Harmon, A. Larson, *Syst. Biol.* **54**, 758 (2005).
9. D. R. Frost *et al.*, *Bull. Am. Mus. Nat. Hist. New York* **297**, 1 (2006).
10. K. Roelants *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 887 (2007).
11. R. L. Mueller, J. R. Macey, M. Jaekel, D. B. Wake, J. L. Boore, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 13820 (2004).
12. C. Goin, O. Goin, *Introduction to Herpetology*, 2nd ed. (Freeman & Co., San Francisco, 1971).
13. C. Goin, O. Goin, G. Zug, *Introduction to Herpetology*, 3rd ed. (Freeman & Co., San Francisco, 1978).
14. R. Estes, in *Handbuch der Paläoherpetologie* (Pfeil Verlag, 1981), pp. 1–115.
15. D. San Mauro, M. Vences, M. Alcobendas, R. Zardoya, A. Meyer, *Am. Nat.* **165**, 590 (2005).
16. B. G. Naylor, R. C. Fox, *Can. J. Earth Sci.* **30**, 814 (1993).
17. J. D. Gardner, *J. Vert. Paleont.* **23**, 769 (2003).
18. D. Marjanovic, M. Laurin, *Syst. Biol.* **56**, 369 (2007).
19. S. E. Evans, A. R. Milner, C. Werner, *Paleontology* **39**, 77 (1996).
20. J. A. Holman, *Fossil Salamanders of North America* (Indiana University Press, Bloomington, 2006).