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the **TIMETREE** *of* **LIFE**

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Amphibians (Lissamphibia)

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Abstract

Living amphibians (6449 species) include three distinctive orders: salamanders (Caudata), caecilians (Gymnophiona), and frogs (Anura). Each is supported as monophyletic in molecular phylogenetic analyses, with frogs + salamanders forming the clade Batrachia. Molecular time estimates of the origin of Lissamphibia vary greatly (367 -282 million years ago; latest Devonian to Early Permian), although recent analyses favor the youngest ages. Divergences among the three orders likely occurred during the Permian, 300 -251 million years ago. Debates about the origin, relationships to extinct taxa, and monophyly of living amphibians are ongoing.

Lissamphibia, a subclass of Amphibia, includes all living representatives, which form three clades, frogs (Salientia), salamanders (Caudata), and caecilians (Gymnophiona), each readily recognizable based on their highly distinctive body plans (Fig. 1). Frogs generally have large mouths and bulging eyes, but short vertebral columns and no tail. These squat creatures have powerful hind limbs for jumping. They are the most speciose clade with about 5700 species. Most of the 576 species of living salamanders are more typical-looking tetrapods, with a tail and four legs. Some aquatic or fossorial species have reduced limbs and girdles and elongated trunks. Living caecilians are elongate, limbless, tail-less or nearly so, and have grooved rings encircling the body. A distinctive tentacle anterior to and below the typically inconspicuous eye is used for chemoreception. Although the majority of the 176 species are fossorial, one lineage has invaded aquatic habitats.

The phylogenetic relationships among the three lissamphibian orders have been controversial for decades.

A close relationship between caecilians and salamanders (Procera hypothesis) was supported by earlier analyses of mitochondrial and nuclear ribosomal DNA sequences (1–3) and mitochondrial genomes (4). The Procera hypothesis has advantages for interpreting distribution patterns and fossil records of the three orders: frogs are distributed worldwide but salamanders and caecilians have strong Laurasian and Gondwanan distribution patterns, respectively; frog-like fossils can be traced back to the Triassic (~250 Ma) but no salamander or caecilian fossils have been found before the Jurassic (~190 Ma). However, most recent analyses, using larger databases of either nuclear genes, mitochondrial genes (including mitochondrial genomes), or a combination of both, have found frogs and salamanders to be closest relatives, a group called Batrachia; the earlier molecular analyses were misled by poor performance of data or insufficient taxon sampling (5–8). Furthermore, analyses of morphological data, including fossil taxa (9–14), have also found the Batrachia hypothesis to be more strongly supported than the Procera hypothesis, and we follow that conclusion here.



Fig. 1 Representative lissamphibians. *Pseudotriton ruber* (Plethodontidae), a salamander (upper); *Hypsiboas helprini* (Hylidae), an anuran (lower left); and *Schistometopum thomense* (Caeciliidae), a caecilian (lower right). Credits: S. B. Hedges.

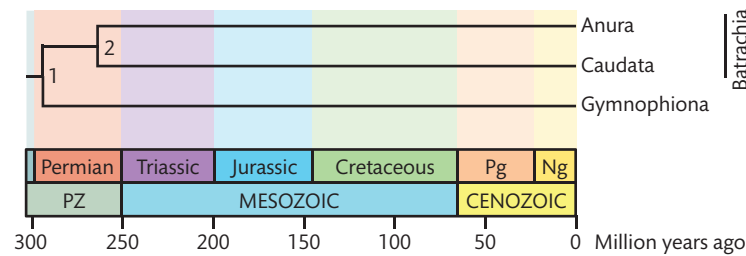


Fig. 2 A timetree of amphibians (Lissamphibia). Divergence times are shown in Table 1. *Abbreviations:* Ng (Neogene), Pg (Paleogene), and PZ (Paleozoic).

The identity of the Paleozoic relatives of modern amphibians is controversial (15). Three hypotheses have been proposed. The Temnospondyl hypothesis suggests that frogs, salamanders, and caecilians form a clade that is nested within dissorophoid temnospondyls. Dissorophoidea (Moscovian of the late Carboniferous to upper portion of the Early Permian) is a large superfamily of temnospondyl amphibians, including some small, paedomorphic forms, such as *Doleserpeton*, amphibamids, and branchiosaurids, which share many derived features with modern amphibians (9–12, 16). The most recent study found the dissorophoid amphibians *Amphibamus* and *Doleserpeton* to be most closely related to modern amphibians (13).

In diametric opposition to the earlier is the Lepospondyl hypothesis. Modern amphibians are nested within the lepospondyls; their closest relatives are Lysorophia (*Brachydectes*), known from the Bashkirian of the late Carboniferous to the upper part of the Early Permian, with the next successive relatives being microsaurids (17, 18). According to this hypothesis, modern amphibians are more closely related to amniotes than they are to temnospondyls.

A third view, the Polyphyletic hypothesis, incorporates elements of both of the earlier hypotheses. It argues for polyphyly of modern amphibians with respect to the major Palaeozoic lineages (14). As in the Lepospondyl hypothesis, living caecilians are seen as most closely related to lepospondyls, which are in turn are closer to amniotes than to other modern amphibians. Furthermore, as in the Temnospondyl hypothesis, salamanders and frogs are derived from temnospondyls, specifically the branchiosaurids. This hypothesis was strengthened by the discovery of the Lower Jurassic taxon *Eocaecilia*, a putative limbed caecilian that may be nested between goniorhynchid microsaurids, such as *Rhynchonkos* (Lower Permian), and the extant caecilians (19). However, the *Eocaecilia*–microsaur relationship has

recently been rejected (20). A monophyletic origin of modern amphibians with respect to living amniotes is strongly supported in all molecular phylogenetic studies to date (1–8, 21). Thus, if the lepospondyls are a clade most closely related to amniotes, the Polyphyletic hypothesis of modern amphibians can be indirectly rejected because it requires a closer relationship of caecilians to amniotes than to the Batrachia.

Using numbers of nuclear genes and a molecular-clock-based method, Kumar and Hedges (22) provided the first time estimate for the origin of lissamphibians at about 360 Ma, using average evolutionary rates of rate-constant genes to apply a clock. More recent analyses use relaxed-clock methods, which allow evolutionary rates to vary among genes and lineages.

Zhang *et al.* (8) analyzed 14 complete lissamphibian mitochondrial genomes and obtained an estimate of the age of the lissamphibian ancestor (337 Ma, Table 1). By comparing the confidence intervals to the temporal distribution of related fossil taxa, the Temnospondyl hypothesis was found to be most compatible with these results. This finding was challenged because of imprecise characterization of the stratigraphic ranges of the groups of Palaeozoic tetrapods, and these estimates would be more compatible with a polyphyletic origin of lissamphibians (23), which is questioned (24).

There are few early lissamphibian fossils, the oldest being the proto-frogs *Triadobatrachus* and *Czatko-batrachus*, from the Early Triassic (~250 Ma; 25, 26). Dates estimated from molecular data typically are much older than the fossil record suggests. The summary of published dates for the nodes Lissamphibia and Batrachia (Table 1; Fig. 2) indicates substantial lack of consensus. For example, three published dates based on nuclear sequences place the split of Lissamphibia (into Gymnophiona and Batrachia) at 367 Ma (27), 369 Ma (7), and 360 Ma (22), approximately at the

Table 1. Divergence times and credibility/confidence intervals (CI) among amphibians (Lissamphibia).

Timetree		Estimates							
Node	Time	Ref. (6)(a)	Ref. (6)(b)	Ref. (7)(a)		Ref. (7)(b)		Ref. (8)	
		Time	Time	Time	CI	Time	CI	Time	CI
1	294	322	292	368.8	396–344	351.6	370–304	337	353–321
2	264	267–266	267–266	357.8	385–333	332.9	353–289	308	328–289

Timetree		Estimates (Continued)							
Node	Time	Ref. (22)	Ref. (24)		Ref. (27)		Ref. (29)		
		Time	Time	CI	Time	CI	Time	CI	
1	294	360	282	356–250	367	417–328	294	319–271	
2	264	-	254	257–246	357	405–317	264	276–255	

Note: Node times in the timetree are from ref. (29). Estimates from ref. (6) are from r8s penalized likelihood analyses of (a) 2613 basepairs and (b) 871 amino acids of the *RAG1* gene. Estimates from ref. (7) are from (a) Multidivtime and (b) r8s penalized likelihood analysis of 3747 base pairs from *16S*, *CXCR4*, *NCX1*, *RAG1*, and *SLC8A3* genes. Estimates from ref. (8) are from Multidivtime analysis of mitochondrial genomes. Estimates from ref. (22) are sequence divergence analysis of multiple nuclear genes. Estimates from ref. (24) are from r8s penalized likelihood analysis of the dataset from ref. (27). Estimates from ref. (27) are from Multidivtime analysis of the 1368 basepairs of *RAG1*. Estimates from ref. (29) are from BEAST analysis of mitochondrial genomes.

Devonian–Carboniferous boundary, the time of the occurrence of the first tetrapods (Table 1). At the other extreme, a reanalysis (24) of the mitogenome data set (8) reported several dates for Lissamphibia and Batrachia, based on various combinations of topology, analytical parameters in r8s, and use of external (outside of amphibians) or internal (within) calibrations. The youngest of the estimates for lissamphibians (267 Ma), based only on internal calibration points, is just slightly older than the oldest lissamphibian fossils, from the Triassic.

Recent molecular studies are reducing the discrepancies between molecular dating and the fossil record. An analysis of *RAG1* from all tetrapods (6) estimated ages for Lissamphibia and Batrachia of 322 and 274 Ma, respectively (no internal amphibian calibration dates were used). Another analysis of complete mitochondrial genomes using several internal amphibian calibration points and a “soft” bound calibration strategy (28) produced dates for Lissamphibia and Batrachia of 294 and 264 Ma, respectively (Zhang and Wake, unpublished results). Improved analytical methods (30–32) and larger databases are producing dates more in line with what might be expected from extrapolating from fossils. Thus, the 264 Ma date estimated from molecular data for Batrachia is close to the age (250 Ma) and phylogenetic position of the Lower Triassic fossils *Triadobatrachus* and *Czatkobatrachus*.

In general, available time tree estimates for the origin of Lissamphibia and Batrachia are controversial, with about

87 and 103 million years difference between the youngest and oldest estimates, respectively (Table 1). Robust time estimation using different methods with multiple markers should clarify how the group originated and evolved. For example, if the split of the caecilians from Batrachia happened after the time the Permian microsauro *Rhynchonkos* lived, the Polyphyletic hypothesis will be rejected. The most recent analyses with the largest data sets are tending to favor younger times of divergence.

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