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# Frogs and toads (Anura)

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## Abstract

Anura (frogs and toads) constitute over 90% of living amphibian diversity. Recent timetree constructions have shown that their diversification was a highly episodic process, with establishment of the major clades in three periods: Triassic (251–200 million years ago, Ma), end of Jurassic to early Cretaceous (~150–100 Ma), and end of Cretaceous to early Paleogene (~70–50 Ma). The early diversification of anurans predated the initial north–south breakup of Pangaea, and resulted in distinct assemblages in both hemispheres. The subsequent radiation of neobatrachian frogs has been largely determined by Gondwanan fragmentation and resulted in recurrent patterns of continent-scale endemism.

Anura (Fig. 1) (“tail-less amphibians”) represent the largest living order of amphibians, and currently include ~5400 described species (1). Most of them undergo the typical amphibious life history and are dependent on the presence of water for their reproduction and development. Multiple lineages, however, show an evolutionary trend toward increased terrestriality in larval or adult frog stages. Despite an evolutionarily conserved body plan, anurans have diversified into a myriad of ecomorphs and have adapted to life in habitats as distinct as rainforest canopies, mangroves, and sand dune burrows. In addition, anurans have attained a subcosmopolitan distribution and are currently only absent in extreme northern latitudes, Antarctica, and most oceanic (non-continental) islands (2). The independent occupation of similar ecological niches by frog taxa in different geographic regions has resulted in extraordinary cases of evolutionary convergence. The consequent high levels of morphological homoplasy have complicated anuran systematics for decades. However, a major ongoing upsurge

of molecular phylogenetic studies is now leading to an increasingly resolved consensus for the anuran tree. In this chapter, we review the relationships and divergence times among 59 anuran families and argue that their evolutionary history is largely congruent with major geological and environmental changes in Earth’s history. We mostly implement clade and family names derived from the taxonomy recently proposed by Frost *et al.* (3). However, we believe that evolutionary time is an important parameter in conveying useful comparative information in biological classification (4). We therefore treat Ascaphidae, Discoglossidae, Nasikabatrachidae, some subfamilies in Nobleobatrachia, and all subfamilies of Microhylidae *sensu lato* as distinct families.

The sequence of early divergences in Anura has been the subject of major controversy. Most of the debate focused on the phylogenetic position of archaeobatrachian families (taxa with primitive or transitional characters, covering ~4% of all extant species) with respect to neobatrachian families (“advanced” taxa, covering



Fig. 1 A tree frog (*Rhacophorus lateralis*) from India. Credit: F. Bossuyt.

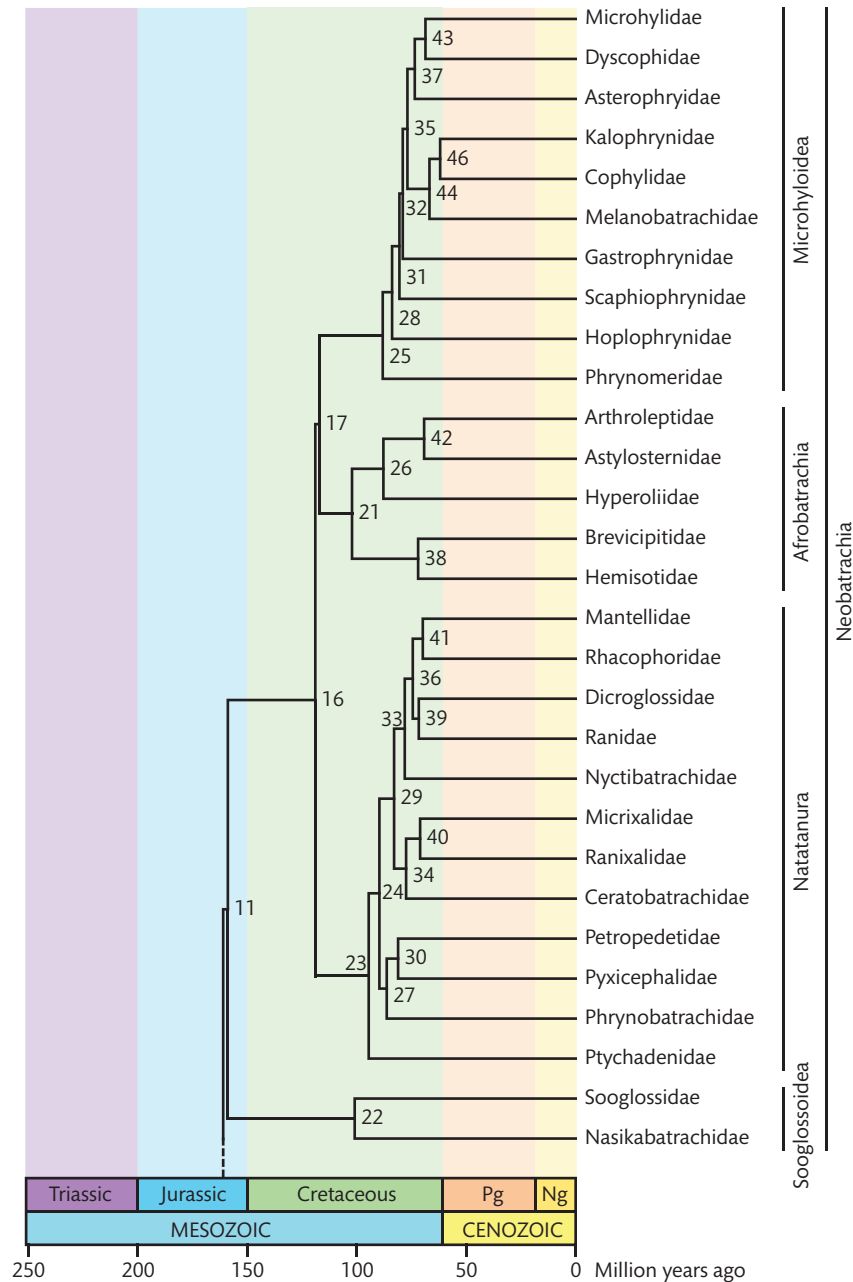
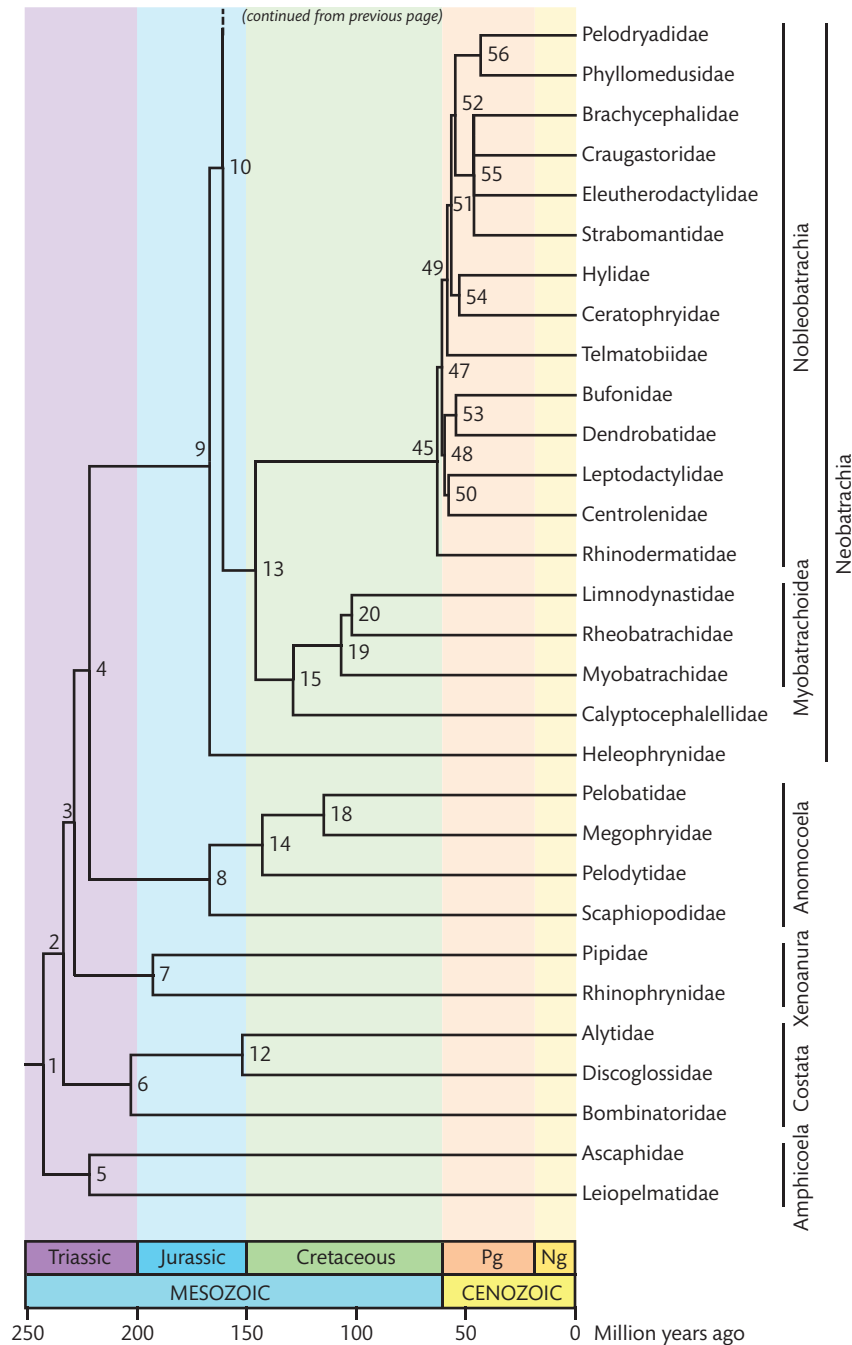


Fig. 2 *Continues*

the remaining 96% of extant species). Morphological studies have supported diverse paraphyletic arrangements of archaeobatrachian families (5–10). Although Neobatrachia have traditionally been considered to constitute a single-nested clade, analyses of combined larval and adult characters have recently questioned their monophyly (9, 10).

Early analyses of ribosomal DNA sequences clustered the archaeobatrachian families in a single clade

(Archaeobatrachia) as the closest relatives of Neobatrachia (11–13). Recent phylogenetic studies, implementing expanded taxon sampling, nuclear and mitochondrial protein-coding DNA sequences, and model-based reconstruction methods, have provided robust support for a paraphyletic arrangement of four major archaeobatrachian lineages: (i) Amphicoela: Ascaphidae + Leiopelmatidae, (ii) Costata (previously known as Discoglossoidae), (iii) Xenanura (Pipoidea), and (iv)



**Fig. 2** A timetree of frogs and toads (Anura). Divergence times are shown in Table 1 Ng (Neogene), Pg (Paleogene), and Tr (Triassic)..

Anomocoela (Pelobatoidea) (3, 14–18). Consistent with most morphological evidence, they supported the basal divergence of Amphicoela, and identified Anomocoela as the closest relative of Neobatrachia. A remaining point of ambiguity is the phylogenetic position of Xenoanura. Molecular studies have variously resolved Xenoanura as the closest relative of Costata (17, 19), of Neobatrachia

(14), of an Anomocoela + Neobatrachia clade (16–18), and of a Costata + Anomocoela + Neobatrachia clade (3, 15).

The monophyly of Neobatrachia has always received strong support from molecular data (3, 12–22). Various arrangements of the following four well-supported lineages have been published: (i) Heleophryinae,

**Table 1.** Divergence times (Ma) and their 95% confidence/credibility intervals (CI) among anurans.

Timetree		Estimates							
Node	Time	Ref. (15)		Ref. (18)		Ref. (26)	Ref. (30)		Ref. (37)
(Fig. 2)	(Ma)	Time	CI	Time	CI	Time	Time	CI	Time
1	243	262	304-223	243	264-217	-	-	-	-
2	234	245	288-204	234	258-210	183	-	-	-
3	229	-	-	229	253-204	-	-	-	-
4	222	216	260-176	222	247-197	-	-	-	-
5	203	237	281-193	203	227-177	172	-	-	-
6	203	199	244-155	203	228-180	132	-	-	-
7	193	-	-	193	218-170	-	-	-	-
8	167	164	208-121	167	191-146	127	-	-	-
9	167	162	199-128	167	186-149	125	-	-	-
10	161	150	186-117	161	180-143	-	-	-	-
11	159	131	167-99	159	178-141	110	-	-	-
12	152	152	199-105	152	179-129	-	-	-	-
13	146	138	172-108	146	164-129	106	-	-	-
14	143	142	187-101	143	166-121	112	-	-	-
15	129	120	154-91	129	147-113	94.4	-	-	-
16	119	99	132-70	119	131-106	-	133	154-115	-
17	117	-	-	117	129-104	-	127	148-110	-
18	115	118	162-77	115	136-93	93.3	-	-	-
19	107	-	-	107	123-89	-	-	-	-
20	102	-	-	102	118-85	-	-	-	-
21	102	-	-	102	115-90	-	107	126-89	-
22	101	-	-	101	121-83	-	-	-	-
23	94.4	-	-	82.5	95-71	-	94.4	113-78	-
24	89.7	-	-	-	-	-	89.7	108-74	-
25	88	-	-	75.6	83-70	-	88	102-77	-
26	87.7	-	-	87.7	100-75	-	96	117-78	-
27	86.3	-	-	-	-	-	86.3	104-71	-
28	83.9	-	-	-	-	-	83.9	97-74	-
29	82.9	-	-	-	-	-	82.9	100-68	-
30	81.1	-	-	72.6	85-62	-	81.1	99-66	-
31	80.5	-	-	-	-	-	80.5	93-72	-
32	79	-	-	-	-	-	79	91-70	-
33	78.1	-	-	-	-	-	78.1	95-64	-
34	77.6	-	-	-	-	-	77.6	95-63	-
35	76.8	-	-	73.0	79-68	-	76.8	89-69	-
36	74.4	-	-	70.3	81-60	-	74.4	91-61	-
37	73.6	-	-	70.2	76-66	-	73.6	85-67	-
38	72.1	-	-	72.1	85-59	-	74.6	94-58	-
39	71.7	-	-	-	-	-	71.7	88-58	-
40	71.2	-	-	-	-	-	71.2	89-56	-

Table 1. Continued

Timetree		Estimates							
Node	Time	Ref. (15)		Ref. (18)		Ref. (26)	Ref. (30)		Ref. (37)
(Fig. 2)	(Ma)	Time	CI	Time	CI	Time	Time	CI	Time
41	69.7	-	-	62.8	74-53	-	69.7	86-56	-
42	69.1	-	-	69.1	81-57	-	-	-	-
43	68.7	-	-	67.1	71-66	-	68.7	78-65	-
44	66.8	-	-	-	-	-	66.8	80-56	-
45	63	-	-	63	77-53	-	-	-	-
46	61.8	-	-	-	-	-	61.8	76-49	-
47	61.1	-	-	61.1	76-51	-	-	-	-
48	59.7	-	-	59.7	74-50	-	-	-	-
49	58.4	-	-	58.4	72-49	-	-	-	-
50	57.9	-	-	57.9	72-49	-	-	-	-
51	56.7	-	-	56.7	70-47	-	-	-	-
52	55	-	-	55	68-46	-	-	-	-
53	54.6	-	-	54.6	68-45	-	-	-	-
54	53.2	-	-	53.2	66-44	-	-	-	-
55	46.5	-	-	-	-	-	-	-	46.5
56	43.2	-	-	43.2	55-36	-	-	-	-

Note: Node times in the timetree are based on refs. (18) and (30) for Natatanura and Microhyloidea, because the use of time estimates averaged across all studies would be incompatible with the depicted topology.

(ii) Sooglossoidea: Nasikabatrachidae + Sooglossidae, (iii) Nobleobatrachia (the “Hyloidea” of 14, 15, 21) + Myobatrachoidea + Calyptocephalellidae, and (iv) Ranoides: Afrobatrachia + Microhyloidea + Natatanura. Most studies of the past few years have converged on a basal split between the South African endemic Heleophrynidae and the remaining neobatrachians (3, 14–18). An important addition to the amphibian tree resulted from the discovery of a new frog lineage (Nasikabatrachidae) in the Western Ghats of India (21). All molecular studies have found this lineage to be the closest relative of Sooglossidae, a small family endemic to the Seychelles (3, 18, 21, 22).

Despite the absence of derived morphological characters supporting Nobleobatrachia, its monophyly is strongly supported by DNA sequence evidence of different loci, and includes a unique codon insertion in the *RAG-1* gene. Within this clade, Leptodactylidae and Hylidae as traditionally defined (1, 2) are now known to be polyphyletic (3, 18, 23, 24) and several of their lineages have been assigned to separate families (3). Most of these studies also identified the ex-leptodactylid genera

*Calyptocephalella* (= *Caudiverbera*) and *Telmatobufo* (both now Calyptocephalellidae) of Chile as the closest relatives of the Australo-Papuan Myobatrachoidea (3, 15, 18, 24). The same analyses recovered this previously unrecognized clade (Australobatrachia) as the closest relatives of Nobleobatrachia. Several controversies however remain in nobleobatrachian phylogeny: first, Leptodactylidae as defined here was not found monophyletic in a recent molecular study (25), although this clade is supported by a unique insertion of two codons in the *Ncx1* gene (18). Second, Hemiphractidae were variously found to be monophyletic (26) or polyphyletic (3). Most important, the sequence of rapid diversification in the radiation of nobleobatrachian families can be considered largely unresolved. Ranoides are composed of three highly supported family assemblages: Afrobatrachia, Microhyloidea, and Natatanura. Afrobatrachia represents a well-resolved African endemic clade and has recently been shown to include the Brevicipitidae (27), which were long considered part of the microhyloid clade. Studies of natatanuran phylogeny incorporating both mitochondrial and nuclear genes agreed on the

basal divergence of several African lineages and corroborated morphological evidence for a close relationship between Mantellidae and Rhacophoridae (3, 28–30). Recent analyses of Microhyloidea using similar multi-gene data sets have demonstrated the non-monophyly of at least five out of nine traditionally recognized subfamilies (2). A consensus for early microhyloid relationships is yet unavailable, but at least three studies have provided evidence for a close relationship of Asian Microhylidae and Madagascan Dyscophidae (18, 30, 31).

During the past few years, molecular divergence time analyses have resulted in increasingly comprehensive timetrees for Anura (Table 1, Fig. 2). The earliest studies incorporated Thorne *et al.*'s (32) relaxed molecular clock model (*divtime*) to date primary divergences in Natatanura, suggesting mid- to late Cretaceous diversification of this clade (33). Subsequent studies implementing different relaxed-clock models, calibration points, and sampling strategies have corroborated the late Cretaceous radiation of both Natatanura and Microhyloidea (29–31, 34). Similar application of Thorne and Kishino's (35) upgraded model, adapted to accommodate rate variation across multiple loci (Multidivtime), resulted in late Jurassic–early Cretaceous time estimates for the basal divergences of Neobatrachia (based on five genes and five calibration points) (21) and Triassic estimates for basal anuran divergences (based on five genes and seven calibration points) (16).

San Mauro *et al.* (15) constructed the first family-level timetree for amphibians using Multidivtime analyses of *RAG1* sequences and nine calibration points. Their analyses were based on broad phylogenetic sampling of frogs and provided confidence intervals for the age of the major anuran clades. An expanded study using penalized likelihood analyses of 84 anuran *RAG1* sequences and 11 calibration points (26) produced overall younger time estimates (Table 1) when the Caudata–Anura split was arbitrarily fixed at 300 Ma. A parallel study, applying both Multidivtime and penalized likelihood analyses using 24 calibration points on a five-gene data set including 120 anuran taxa (18), resulted in divergence time estimates that were very similar to those of San Mauro *et al.* (15), with strong overlap of 95% credibility intervals. These studies indicate that the evolutionary rise of anuran diversity was a highly episodic process, with the establishment of archaeobatrachian clades in the Triassic–early Jurassic (251–199.6 Ma), of the primary neobatrachian lineages in the late Jurassic–early Cretaceous, of the natatanuran and microhyloid radiations in the late Cretaceous, and of Nobleobatrachia

around the Cretaceous–Paleogene (K-P) boundary (65.5 Ma). Zhang *et al.* (19), based on *multidivtime* analyses of mitogenomic data in combination with a single external calibration point, recovered noticeably older age estimates for several nodes, including a Carboniferous–Permian (299 Ma) origin of living anurans and a mid-Cretaceous (99.6 Ma) age for the nobleobatrachian clade. However, because mitochondrial genes evolve much faster than most nuclear genes used in other studies, it is likely that they pose increased risks of mutational saturation and biases in branch length estimation.

The rise of living anurans shows strong overlap with major shifts in vertebrate faunal compositions in the late Permian and Triassic. Both the end Permian mass extinction and the Triassic extinction episodes represented severe losses of amphibian diversity (36) and in parallel to amniote groups, anurans may have taken opportunistic advantage of ecological niche vacancy in the redeveloping and increasingly complex vertebrate ecosystems. Molecular divergence time estimates of all studies also imply that the major archaeobatrachian lineages were present on Pangaea before its Jurassic north–south breakup into Laurasia and Gondwana (15, 16, 18). The subsequent formation of distinct anuran faunas in both landmasses is illustrated by three independent divergences between Laurasian and Gondwanan taxon pairs: (i) Ascaphidae of North America vs. Leiopelmatidae of New Zealand, (ii) Rhinophrynidae of North–Central America vs. Pipidae of South America–Africa, and (iii) Anomocoela of North America–Eurasia vs. Neobatrachia, originally a Gondwanan group. These results reinforce the predicted importance of Pangaea breakup in shaping distinct amphibian faunas in both hemispheres (13).

The late Jurassic or early Cretaceous divergences of the four major neobatrachian lineages (Fig. 2) constitute a second distinct wave of anuran radiation. Two of the four major lineages are now only represented by few species endemic to restricted geographic regions on different ex-Gondwanan landmasses. Heleophrynidae (167 Ma) consist of six extant species that occur in the mountain ranges of the Cape and Transvaal regions of Republic of South Africa; Sooglossoidea (159 Ma) consists of only five described species, one of which (Nasikabatrachidae) is endemic to the Indian Western Ghats, and four of which (Sooglossidae) occur only on the Seychelles. The deep split between both families (101 Ma) identifies each of them as relict lineages that testify for a mid-Cretaceous biogeographic link between the Seychelles and the Indian subcontinent. A similar deep-time



intercontinental link is represented by the Cretaceous divergence of the Chilean Calyptocephalellidae and the Australo-Papuan Myobatrachoidea (129 Ma). This suggests that Australobatrachia once had a trans-Gondwanan distribution, ranging from South America over Antarctica to Australia.

Although the nobleobatrachian radiation produced approximately half of the currently living anuran species, molecular divergence time estimates suggest that its initial diversification commenced relatively late, near the K-P boundary (15, 18). The rapid establishment of a large number of lineages that currently represent a broad range of ecomorphs (including toads, litter frogs, glass frogs, poison arrow frogs, fossorial frogs, and several lineages of tree frogs) fits the pattern of opportunistic radiation in the aftermath of the K-P extinction episode. Given the neotropical distribution of most nobleobatrachian families, this radiation is likely to have taken place primarily in South America. At least four lineages dispersed to other continents in the Tertiary: one lineage of Eleutherodactylidae reached North America (37, 38), Hylidae and Bufonidae attained widespread distributions, probably by dispersing through North America and Eurasia (39, 40), and the occurrence of Pelodyadidae in the Australo-Papuan realm provides evidence for a Tertiary trans-Antarctic range extension (15).

The prevalence of continent-scale endemism in Ranoides, that is, the clear historical association of families with a single Gondwanan landmass, suggests that continental breakup has played a key role in the distribution of these frogs (29, 30). The late Cretaceous radiations of Natatanura and Microhyloidea indicate that dispersal between Gondwanan landmasses took place at least until the end Cretaceous (30, 31). In addition, comparable temporal and spatial divergence patterns within the microhyloid and natatanuran radiations suggest that the isolation of their daughter lineages on different continents were determined by the same geological events. The disruption of terrestrial passages that persisted between continents long after they started drifting apart may have resulted in parallel instances of vicariance in both clades (30).

The late Cretaceous diversification of Natatanura and Microhyloidea imply a survival of multiple lineages across the K-P boundary. Some of the surviving lineages are represented by only few relict species (e.g., Phrynomeridae in Africa, Melanobatrachidae on the Indian subcontinent), but several of their largest families radiated substantially in the Paleogene (e.g., Dicroglossidae,

Mantellidae, Rhacophoridae, Ranidae, Asterophryidae, and Microhylidae) (18, 29).

The timetree that is emerging from the rapid succession of molecular analyses provides an increasingly detailed temporal framework for anuran evolution. Besides shedding light on historical biogeography, this framework allows us to study patterns and rates of evolutionary change in morphological, ontogenetic, and genomic data. A remaining challenge for future phylogenetic studies is represented by the explosive radiations of Natatanura, Microhyloidea, and Nobleobatrachia, and several of their families. Resolving these radiations will most likely require alternative (and more expensive) strategies, provided by the expanding field of phylogenomics (e.g., using SINES or EST data). The credibility of the anuran timetree is reinforced by the relative consistency among independent studies, despite the use of different data sets, calibration points, and methods. In addition, although molecular time estimates for some anuran nodes are notably older than those derived from the fossil record, there are no major incompatibilities between the two types of data (41). Rather, molecular and fossil analyses can be considered complementary tools to understand the paleobiological processes and events that shaped the present-day anuran diversity.

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