

A MITOGENOMIC EXPLORATION OF THE PHYLOGENY AND EVOLUTION OF EXTANT ANURANS

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Phylogenetic relationships among major clades of anuran amphibians were analyzed using mitogenome sequences of 54 frog species from 41 genera. Anurans are under two major groups Archaeobatrachia and Neobatrachia. Mesobatrachia was introduced later this classification. Our phylogenetic study based on mitogenome authenticated four groups (1. Bombinatoridae + Alytidae; 2. Megophryidae + Pelobatidae + Pelodytidae; 3. Bombinatoridae + Alytidae cluster with Leiopelmatidae; 4. Pipidae + Rhinophrynidae). Our phylogenetic study confirms Neobatrachia is monophyletic and Archaeobatrachia is paraphyletic.

Keywords: Anura; Archaeobatrachia; Neobatrachia; phylogeny.

The largest estimate of extant amphibian phylogeny to date is that of Frost et al. (2006). Though the study has also been disapproved on various grounds, including concerns about taxon sampling and methodological strategies (Marjanovič and Laurin, 2007; Pauly et al., 2009; Wiens, 2008). Pyron and Weins (2011) combined available data and produce a novel estimate of extant amphibian phylogeny, containing 2871 species (40% of the known extant species) from 432 genera (85% of the 500 currently recognized extant genera). Numerous studies with limited taxon sampling have generated large amounts of partially overlapping sequence data for many species. Mitochondrial DNA (mtDNA) is well accepted as a marker to establish molecular diversity (Galtier et al., 2009). Studies also indicated that the mitogenome had adaptive selection but the selective role for genome rearrangement is under debate. The evolutionary trends of duplicated and rearranged mt genes in animals have not well been explored because of their low numbers and, particularly, because very few examples exist of rearranged and non-rearranged mt genomes within closely-related taxa (Kurabayashi and Sumida, 2013). As data accumulated, it has become apparent that animal mtDNA evolves at a rate 5 to 10 times faster than single-copy pro-

tein coding nuclear genes, although this differ extremely across genes and taxa (Meyer, 1993). Traditionally two major anuran groups, Archaeobatrachia and Neobatrachia, are well recognized. The former is widely accepted as a paraphyletic group of basal anurans. The latter is a monophyletic taxon of modern anurans and contains over 95% of extant frogs. A phylogenetic framework is important for discovering, understanding, and preserving extant amphibian diversity. We have constructed an anuran tree of life with 54 species of 41 genera. Among them three species of three genera (*Leptobrachium bowringii*, *Breviceps adpersus*, *Lechriodus melanopyga*) of those 54 species of 41 genera deserve novelty. Still now extant amphibian phylogeny and classification is in need of additional study.

All desired full mitochondrial nucleotide sequence occurs in a series within a single text file in FASTA format and compiled together in a single text file. The Clustal W (Thompson et al., 1997) algorithm was used to perform an initial multiple sequence alignment. Multiple alignments were performed using the program CLUSTAL_X (version 1.83, The European Molecular Biology Laboratory (EMBL) EMBL-EBI, Hinxton, Cambridgeshire, UK) (Thompson et al., 1997) which is a windows interface. Nucleotide substitution rates were determined (Kimura, 1980). Phylogenetic trees were constructed using the neighbor joining method (Saitou and Nei, 1987) with CLUSTAL_X and the maximum-parsimony method (Fitch, 1971) with the software MEGA3 (MEGA Inc., Englewood, NJ, USA) (Kumar et al., 2004).

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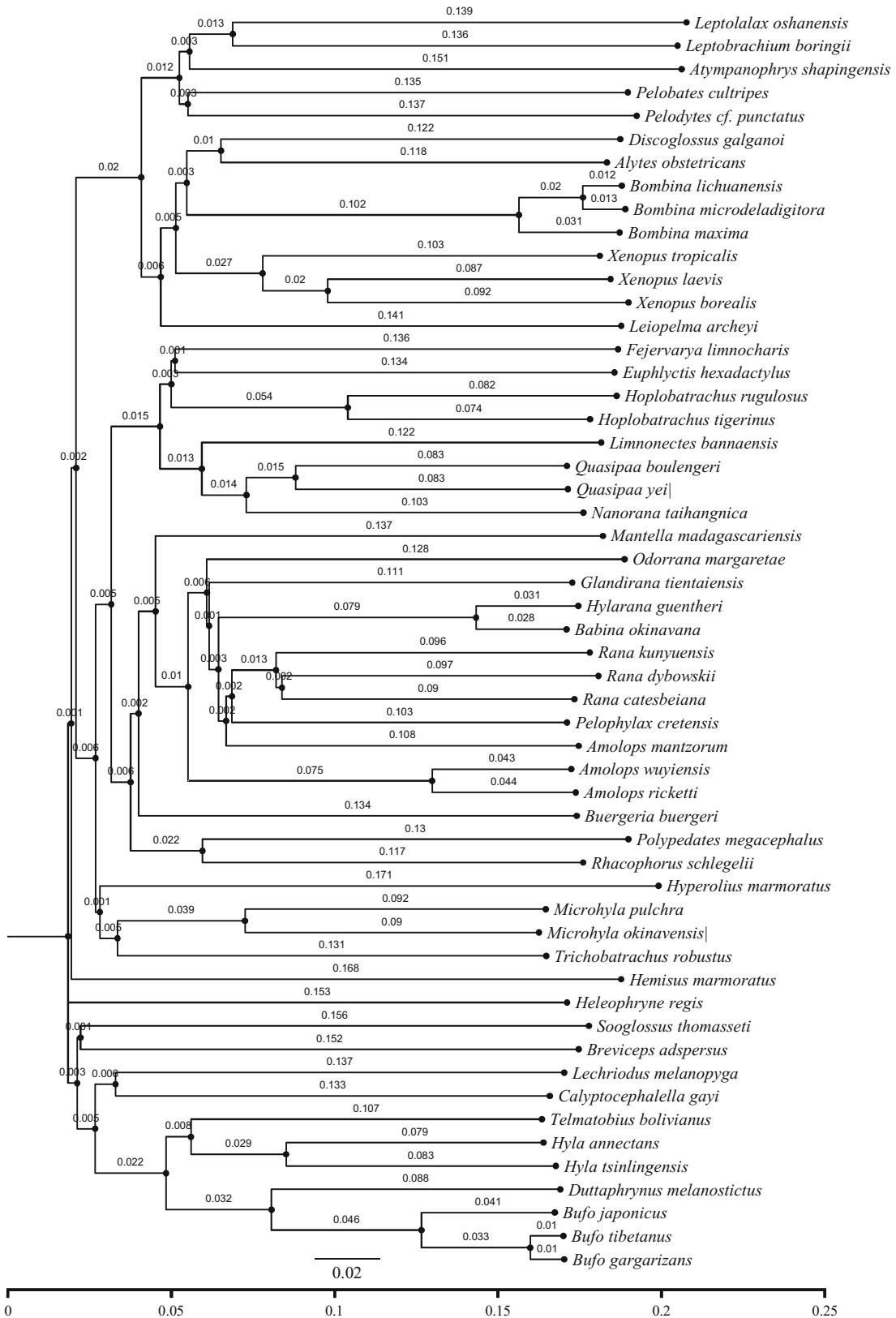


Fig. 1. Phylogenetic tree constructed with 54 species of 41 anuran genera.

Archaeobatrachia

Our phylogenetic study confirms the followings: 1. Neobatrachia is monophyletic; 2. “Archaeobatrachia” is paraphyletic with respect to Neobatrachia. Anurans were divided into the “archaic” frogs, the Archaeobatrachia, and the “advanced” frogs, the Neobatrachia (Reig, 1958). In 1975 Duellman redefined Archaeobatrachia. In 1979 Laurent raised the suborder Mesobatrachia for those archaeobatrachians (Pelobatidae, Pelodytidae, Pipidae, and Rhinophrynidae) whose morphology transitional between this two suborders. Ford and Cannatella’s study in 1993 based on morphological characters went a step further and made the Mesobatrachia a sister group to the Neobatrachia (to form the Papanura) and put an end to the Archaeobatrachia as a taxonomic entity. They projected that the Discoglossidae, Bombinatoridae, *Leiopelma*, and *Ascapus* successively fall outside of the rest of the anurans, rather than forming a monophyletic unit. Although no statistical tests were performed to show the significance of these findings.

Our phylogenetic study based on mitogenome authenticated the following groups (Fig. 1):

1. The Bombinatoridae are grouped with the Alytidae; 2. Megophryidae (*Leptobrachium boringii*, *Leptolalax oshanensis*, and *Atympanophrys shapingensis*) + Pelobatidae (*Pelobates cultripes*) + Pelodytidae (*Pelodytes cf. punctatus*) (Mesobatrachia); 3. Bombinatoridae (*Bombina maxima*, *Bombina microdeladigitora*, and *Bombina lichuanensis*) + Alytidae (*Alytes obstetricians* and *Discoglossus galganoi*) cluster with Leiopelmatidae (*Leiopelma archeyi*); 4. Pipidae (*Xenopus tropicalis*, *Xenopus borealis*, and *Xenopus laevis*) (Mesobatrachia) + Rhinophrynidae (not shown in this tree).

Neobatrachia

The Neobatrachia clearly form a monophyletic group that is sustained by methods applied. A placement of Heleophryne among archaeobatrachians as suggested by larval characters (Haas, 2003) is unlikely. Based on data mining and distance values we suggested: 1. Hyloidae + Myobatrachidae (*Lechriodus melanopyga*) + Calyptocephalellidae (*Calyptocephalella gayi*); 2. Ranoidae (*Rana dybowskii*) [Discoglossidae (*Euphlyctis hexadactylus*) + Mantillidae (*Mantella madagascariensis*) + Ranidae (*Rana kunyuensis*) + Rhacophoridae (*Rhacophorus schlegelii*, *Polypedates megacephalus*, and *Buergeria buergeri*) + Microhylidae (*Microhyla pulchra*) + Anthropletidae (*Trichobatrachus robustus*) + Hemisotidae (*Hyperolius marmoratus*)]; 3. Sooglossidae (*Sooglossus thomasseti*) (including Nasikabatrachidae); 4. Heleophrynidae (*Heleophryne regis*) as a sister group of all other neobatrachians. Sooglossids are secretive frogs that hide in leaf litter and rock crevices. There is no fossil record

TABLE 1. List of Taxa Studies with Sequences Accession Numbers

No.	Species name	Accession
1	<i>Xenopus tropicalis</i>	NC_006839.1
2	<i>Xenopus borealis</i>	NC_018776.1
3	<i>Hemisis marmoratus</i>	AB777217.1
4	<i>Pelobates cultripes</i>	NC_008144.1
5	<i>Bufo gargarizans</i>	NC_008410.1
6	<i>Xenopus laevis</i>	NC_001573.1
7	<i>Fejervarya limnocharis</i>	NC_005055.1
8	<i>Rhacophorus schlegelii</i>	NC_007178.1
9	<i>Hoplobatrachus tigerinus</i>	NC_014581.1
10	<i>Euphlyctis hexadactylus</i>	NC_014584.1
11	<i>Duttaphrynus melanostictus</i>	NC_005794.2
12	<i>Bufo japonicus</i>	NC_009886.1
13	<i>Polypedates megacephalus</i>	NC_006408.1
14	<i>Alytes obstetricans</i>	NC_006688.1
15	<i>Discoglossus galganoi</i>	NC_006690.1
16	<i>Limnonectes bannaensis</i>	NC_012837.1
17	<i>Hoplobatrachus rugulosus</i>	NC_019615.1
18	<i>Atympanophrys shapingensis</i>	JX458090.1
19	<i>Bufo tibetanus</i>	JX878885.1
20	<i>Bombina maxima</i>	JX893181.1
21	<i>Bombina microdeladigitora</i>	NC_021476.1
22	<i>Bombina lichuanensis</i>	NC_021477.1
23	<i>Rana catesbeiana</i>	KF049927.1
24	<i>Leptolalax oshanensis</i>	NC_020610.1
25	<i>Rana dybowskii</i>	NC_023528.1
26	<i>Quasipaa boulengeri</i>	NC_021937.1
27	<i>Amolops ricketti</i>	NC_023949.1
28	<i>Amolops mantzorum</i>	NC_024180.1
29	<i>Nanorana taihangnica</i>	NC_024272.1
30	<i>Leptobrachium boringii</i>	NC_024427.1
31	<i>Hylarana guentheri</i>	KM035413.1
32	<i>Microhyla pulchra</i>	NC_024547.1
33	<i>Rana kunyuensis</i>	NC_024548.1
34	<i>Odorrana margaritae</i>	NC_024603.1
35	<i>Quasipaa yei</i>	NC_024843.1
36	<i>Glandirana tientaiensis</i>	NC_025226.1
37	<i>Hyla annectans</i>	NC_025309.1
38	<i>Pelophylax cretensis</i>	NC_025575.1
39	<i>Amolops wuyiensis</i>	NC_025591.1
40	<i>Trichobatrachus robustus</i>	AB777219.1
41	<i>Hyperolius marmoratus</i>	AB777218.1
42	<i>Mantella madagascariensis</i>	AB212225.1
43	<i>Breviceps adspersus</i>	NC_023379.1
44	<i>Babina okinavana</i>	NC_022872.1
45	<i>Buergeria buergeri</i>	AB127977.1
46	<i>Microhyla okinavensis</i>	AB303950.1
47	<i>Hyla tsinlingensis</i>	NC_026524.1
48	<i>Leiopelma archeyi</i>	NC_014691.1
49	<i>Sooglossus thomasseti</i>	NC_020001.1
50	<i>Telmatobius bolivianus</i>	NC_020002.1
51	<i>Pelodytes cf. punctatus</i>	NC_020000.1
52	<i>Lechriodus melanopyga</i>	NC_019999.1
53	<i>Heleophryne regis</i>	NC_019998.1
54	<i>Calyptocephalella gayi</i>	NC_019997.1

for the family. Ancestors diverged when the Seychelles Islands split from India plate about 100 million years ago. The Sooglossidae previously has been placed in the Ranoidea (Duellman, 1975), Bufonoidea (Laurent, 1979), Microhyloidea (Blommers-Schlösser, 1993), and also as a sister group with the myobatrachines (Lynch, 1973; Ford and Cannatella, 1993). Morphologically the Sooglossidae is primitive to other neobatrachians (Duellman and Trueb, 1986). Our tree also placed Sooglossidae in the same cluster with Bufonidae and Hylidae. The Leptodactylidae (*Telmatobius bolivianus*) is also move away from present Hylidae (*Hyla* species in this tree).

Our phylogenetic tree (Fig. 1) favored with alternative relationships {Bufonidae (*Bufo gargarizans*, *B. japonicas*, *B. tibetanus*, and *Duttaphrynus melanostictus*) + [Leptodactylidae (*Telmatobius bolivianus*) + Hylidae (*Hyla annectans* and *Hyla tsinlingensis*)]} within Neobatrachia. Our molecular data have placed *Heleophryne* (*Heleophryne regis*) as the sister-group of all other Neobatrachia and placed this species basal in this clade. Duellman and Trueb in 1986 treated *Hemisus* as a subfamily of ranids. Our tree also supports this idea. In 1981 the arthroleptids (*Trichobatrachus robustus*) were separated as a distinct family by Dubois. Duellman and Trueb (1986) recognized Arthroleptinae and Astylosterninae as subfamilies of Ranidae. According to Laurent in 1986 they are distinctive among ranids, but similar to hyperoliids. Our tree placed Microhylidae, Hyperoliidae, and Arthroleptids in a cluster.

REFERENCES

- Blommers-Schlösser R. M. A.** (1993), "Systematic relationships of the Mantellinae Laurent 1946 (Anura, Ranoidea)," *Ethol. Ecol. Evol.*, **5**, 199 – 218.
- Dubois A.** (1981), "Liste des genres et sous-genres nominaux de ranoidea (Amphibiens Anoures) du monde, avec identification de leurs especes-types: Consequences nomenclaturales," *Monit. Zool. Ital.*, Suppl. XV, 225 – 284.
- Duellman W. E. and Trueb L.** (1986), *Biology of Amphibians*, McGraw-Hill, New York.
- Duellman W. E.** (1975), "On the classification of frogs," *Occas. Pap. Mus. Nat. Hist. Kansas*, **42**, 1 – 14.
- Fitch W. M.** (1971), "Toward defining the course of evolution: Minimum change for a specific tree topology," *Syst. Biol.*, **20**, 406 – 416.
- Ford L. S. and Cannatella D. C.** (1993), "The major clades of frogs," *Herpetol. Monogr.*, **7**, 94 – 113.
- Frost D. R., Grant T., Faivovich J., Bain R. H., Haas A., Haddad C. F. B., De Sa R. O., Channing A., Wilkinson M., Donnellan S., Raxworthy C. J., Campbell J. A., Blotto B. L., Galtier N., Nabholz B., Glémin S., and Hurst G. D.** (2009), "Mitochondrial DNA as a marker of molecular diversity," *Mol. Ecol.*, **8**(22), 4541 – 4550.
- Haas A.** (2003), "Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura)," *Cladistics*, **19**, 23 – 89.
- Kimura M.** (1980), "A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences," *J. Mol. Evol.*, **16**, 111 – 120.
- Kurabayashi A. and Sumida M.** (2013), "Afrobatrachian mitochondrial genomes: genome reorganization, gene rearrangement mechanisms, and evolutionary trends of duplicated and rearranged genes," *BMC Genom.*, **14**, 633.
- Laurent R. F.** (1979), "Esquisse d'une phylogénèse des anoures," *Bull. Soc. Zool. Fr.*, **104**, 397 – 422.
- Laurent R. F.** (1986), "Sous Classe de Lissamphibiens (Lissamphibia). Syste'matique," in: P.-P. Grassé, M. Delsol (eds.), *Traité de Zoologie: Anatomie, Systématique, Biologie*, Tome XIV, Masson, Paris, pp. 594 – 797.
- Marjanović D. and Laurin M.** (2007), "Fossils, molecules, divergence times, and the origin of lissamphibians," *Syst. Biol.*, **56**, 369 – 388.
- Meyer A.** (1993), "Evolution of mitochondrial DNA in fishes," in: P. W. Hochachka and T. P. Mommsen (eds.), *The Biochemistry and Molecular Biology of Fishes. Vol. 2*, Elsevier Science Publishers, pp. 1 – 38.
- Moler P., Drewes R. C., Nussbaum R. A., Lynch J. D., Green D. M., and Wheeler W. C.** (2006), "The amphibian tree of life," *Bull. Am. Mus. Nat. Hist.*, **297**, 8 – 370.
- Pauly G. B., Hillis D. M., and Cannatella D. C.** (2009), "Taxonomic freedom and the role of official lists of species names," *Herpetologica*, **65**, 115 – 128.
- Pyron R. A. and Wiens J. J.** (2011), "A large scale phylogeny of Amphibia including over 2800 species and a revised classification of frogs, salamanders and caecilians," *Mol. Phylogen. Evol.*, **61**(2), 543 – 583.
- Reig O. A.** (1958), "Proposiciones para una nueva macrosistemática de los anuros. Nota preliminary," *Physis.*, **21**, 109 – 118.
- Saitou N. and Nei M.** (1987), "The neighbor-joining method: A new method for reconstructing phylogenetic trees," *Mol. Biol. Evol.*, **4**, 406 – 425.
- Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F., and Higgins D. G.** (1997), "The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools," *Nucl. Acids Res.*, **25**, 4876 – 4882.
- Wiens J. J.** (2008), "Systematics and herpetology in the age of genomics," *Bioscience*, **58**, 297 – 307.