New frog family from India reveals ancient biogeographic link with the Seychelles

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Supplementary Material

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a. Measurements on the holotype

Measurements and terminology mainly follow Bossuyt (2002)[1]. The following measurements were taken, to the nearest 0.1 mm, using a digital slide-caliper or a binocular microscope with a micrometer ocular. SVL (snout-vent length), HW (head width, at the angle of the jaws), HL (head length, from rear of mandible to tip of snout), SL (snout length, from tip of snout to anterior orbital border of eye), EL (eye length, horizontal distance between bony orbital borders of eye), IUE (inter upper eyelid width, the shortest distance between the upper eyelids), UEW (maximum upper eyelid width), IN (distance between internal border of nostrils), IFE (internal front of eyes, shortest distance between anterior orbital border of eyes), IBE (internal back of eyes, shortest distance between posterior orbital border of eyes), NS (distance from nostril to tip of snout), EN (distance from nostril to anterior orbital border of eye), FLL (forelimb length, from elbow to base of outer palmar tubercle), HAL (hand length, from base of outer palmar tubercle to tip of third finger), TFL (third finger length, from base of first subarticular tubercle), TL (tibia length), TW (maximum tibia width), FL (femur length), FOL (foot length, from base of inner metatarsal tubercle to tip of fourth toe), FTL (fourth toe length, from base of first subarticular tubercle to tip of fourth toe), TFOL (distance from heel to tip of fourth toe), MTTF (distance from distal edge of metatarsal tubercle to maximum incurvation of web between third and fourth toe), MTFF (distance from distal edge of metatarsal tubercle to maximum incurvation of web between fourth and fifth toe), TFTF (distance from maximum incurvation of web between third and fourth toe to tip of fourth toe), FFTF (distance from maximum incurvation of web between fourth and fifth toe to tip of fourth toe), IMT (inner metatarsal tubercle length), ITL (inner toe length). All measurements are in mm.

Description of the holotype:

Size relatively large (SVL = 70.1); head (Fig. 1b) broader than long (HW = 14.5, HL = 11.9); outline of snout in dorsal view pointed, in profile pointed, with a distinct protrusion; snout (SL = 10.1) much longer than horizontal diameter of eye (EL = 2.2); canthus rostralis distinct and rounded, loreal region concave; interorbital space slightly concave in the middle, much wider (IUE = 8.5) than upper eyelid (UEW = 1.7), much wider than internasal distance (IN = 3.5); distance between posterior margin of eyes (IBE = 12.6) slightly wider than distance between anterior margin of eyes (IFE = 11.1); nostrils oval, without flap or ridge, much closer to tip of snout (NS = 2.2) than to eye (EN = 8.1); pupil rounded, horizontal; tympanum inconspicuous; Elongated horizontal ridge at the roof of upper jaw, behind the choanae. Tongue attached posteriorly, free toward the snout; lingual papilla absent; tooth-like projections on maxilla absent; supratympanic fold absent; no co-ossified skin on head.

Forelimb (FLL = 12.0) shorter than hand (HAL = 18.1, TFL = 7.4) (Fig. 1c). Relative length of fingers: I < IV < II < III; tips of fingers rounded, without disks, without a circummarginal groove; all fingers with dermal fringe on both edges; webbing on fingers rather rudimentary, but clearly present; subarticular tubercles inconspicuous; prepollex oval, distinct; palmar tubercles indistinct; no supernumary tubercles.

Hind limbs short, heels do not touch with limbs folded at right angles to the body; tibia 2.7 times longer (TL = 25.8) than wide (TW = 9.6), shorter than thigh (FL = 30.7), shorter than distance from base of internal metatarsal tubercle to tip of Toe IV (FOL = 32.9); distance from heel to tip of Toe IV (TFOL = 45.6) 2.5 times length of Toe IV (FTL = 17.9); relative length of toes when opposed: I < II < V < III < IV; tips of toes rounded, without disks, without a circummarginal groove; webbing on toes rather extensive (MTTF = 16.7, MTFF = 14.5, TFTF = 8.1, FFTF = 13.2); Subarticular tubercles inconspicuous, flat; inner metatarsal tubercle very large (IMT = 10.1), shovel-shaped, almost twice as long as Toe I (ITL = 5.6) (Fig. 1d); Outer metatarsal tubercle, supernumary tubercles and tarsal tubercle absent.

Skin completely (*i.e.*, snout, side of head, entire dorsum and flanks, forelimbs, thighs, calves, throat, chest, belly, and ventral side) smooth. Overall coloration of dorsal parts light bluish black with a brown tinge, of ventral parts dark gray. Tips of fingers and toes, base of hands, protrusion on snout, nostrils and internal metatarsal tubercle white. Eye has light blue border, orange iris and black pupil.

b. Amplexus - a preliminary diagnosis

The variety of male clasping of the female during mating in frogs can be roughly classified in three major positions: the primitive inguinal amplexus (around the waist, present in Archaeobatrachia, Sooglossidae, Myobatrachinae and several Limnodynastinae) and two derived modes, axillary (behind the forelimbs, present in most Neobatrachia) and cephalic (clasping of the head, present in some Neobatrachia). On one occasion, we have observed an amplexus of *Nasikabatrachus sahyadrensis* in the wild (unfortunately, these animals could not be collected). As a preliminary diagnosis, we can state that the male of this species is similar in general shape, but smaller (SVL about 40 mm) and lighter in color. *Nasikabatrachus sahyadrensis* seems to exhibit an amplexus that is largely inguinal (but tends to glued, as in *Breviceps*), thus supporting an early origin in advanced frog evolution. It must be noted, however, that the bloated shape of both male and female, and the smaller size of the male, may be factors that physically preclude an axillary amplexus. Further study on amplexus and reproduction of this new species are in progress.

c. A new example of convergence - Comparison with other 'balloon-frogs'

The amazing amount of convergence in frogs has been demonstrated already several times and the discovery of *Nasikabatrachus sahyadrensis* adds one more vivid instance. Indeed, this new species is at first sight morphologically very close to other so-called 'balloonfrogs', such as *Hemisus* (Hemisotidae, African balloon frogs) and *Uperodon* (Microhylidae). However, although similar in general appearance, *Nasikabatrachus* is distinct from *Hemisus* by characters such as the horizontal pupil (vertical in *Hemisus*), the absence of a fold across the head, and by being primarily a leg-burrower (versus snout-burrowing in *Hemisus*). Indeed, burrowing in *Nasikabatrachus* is mainly accomplished by 'shuffling' the feet with the well-developed metatarsal tubercle. However, in loose soil, this species sometimes reverses and uses the sharp and hardened snout, thereby shuffling the hind limbs for additional force.

The Indian balloon frogs of the genus *Uperodon* differ by the round snout (without protrusion), the presence of palatal folds, the presence of a papilla posterior to each choana, and by having an unreduced lower jaw.

d. List of species included in the phylogenetic analyses

* = purchased from dealer (no exact locality known); n.a. = no voucher available; Collection abbreviations are: ABTC: Australian biological tissue collection; CAS: California Academy of Sciences; DCC: David C. Cannatella; MVZ: Museum of Vertebrate Zoology, Berkeley; RAN: Ronald A. Nussbaum; VUB: Vrije Universiteit Brussel; ZFMK: Zoologisches Forschungsinstitut und Museum A. Koenig; ZSIM/SRS: Zoological Survey of India, Madras. Species in green were included in dating estimate analyses.

Family	Ref.	Genus	Species	Locality	Voucher
Hynobiidae		*Hynobius*	*formosanus*	Nantou County, Taiwan	MVZ 197237
Salamandridae		*Salamandra*	*salamandra*	Belgium	VUB 0912
Discoglossidae	1	*Alytes*	*obstetricans boscai*	Spain	VUB 0095
Discoglossidae	2	*Discoglossus*	*pictus auritus*	Spain	VUB 0528
Bombinatoridae	3	*Bombina*	*orientalis*	Asia*	VUB 0906
Pipidae	4	*Pipa*	*pipa*	South America*	VUB 0539
Pelobatidae	5	*Pelobates*	*cultripes*	Spain	VUB 0510
Pelodytidae	6	*Pelodytes*	*punctatus*	Spain	VUB 0529
Nasikabatrachidae	7	*Nasikabatrachus*	*sahyadrensis*	India	BNHS 4202
Sooglossidae	8	*Nesomantis*	*thomasetti*	Seychelles	RAN 25171
Heleophrynidae	9	*Heleophryne*	*purcelli*	South Africa	n.a.
Myobatrachidae	10	*Limnodynastes*	*salmini*	Australia	DCC 2898
Myobatrachidae	11	*Myobatrachus*	*gouldii*	Australia	ABTC 63400
Bufonidae	12	*Bufo*	*melanostictus*	India	VUB 0052
Dendrobatidae	13	*Dendrobates*	*auratus*	South America*	VUB 0986
Centrolenidae	14	*Centrolene*	*prosoblepon*	Prov. Herrera, Panama	MVZ 232752
Hylidae	15	*Hyla*	*arenicolor*	North America	DCC 3043
Hylidae	16	*Phrynohyas*	*venulosa*	South America*	VUB 0987
Leptodactylidae	17	*Ceratophrys*	*ornata*	South America*	VUB 1006
Leptodactylidae	18	*Leptodactylus*	*melanonotus*	Prov. Guanacaste, Costa Rica	MVZ 207294
Rhinodermatidae	19	*Rhinoderma*	*darwinii*	Prov. Neuquen, Argentina	MVZ 164829
Arthroleptidae	20	*Arthroleptis*	*variabilis*	Equatorial Guinea	CAS 207822
Hyperoliidae	21	*Leptopelis*	*kivuensis*	Kabale Dist., Uganda	CAS 201700
Astylosternidae	22	*Trichobatrachus*	*robustus*	Cameroon	ZFMK 66453
Hyperoliidae	23	*Hyperolius*	*sp.*	Kenya	VUB 0924
Hemisotidae	24	*Hemisus*	*marmoratus*	Kilifi Dist., Kenya Coast Prov.	CAS 214843
Mantellidae	25	*Boophis*	*xerophilus*	Madagascar	n.a.
Rhacophoridae	26	*Philautus*	*wynaadensis*	India	VUB 0075
Ranidae	27	*Meristogenys*	*kinabaluensis*	Sabah, Malaysia	VUB 0627
Petropedetidae	28	*Petropedetes*	*cf. parkeri*	Monako	n.a.
Microhylidae	29	*Microhyla*	*ornata*	India	VUB 0066
Microhylidae	30	*Scaphiophryne*	*marmoratus*	unknown (dealer)	VUB 0540

e. Uncorrected ("p") distance matrix

Uncorrected ("p") distance (%) matrix for nuclear genes (above the diagonal) and mitochondrial genes (below the diagonal). Numbers refer to species as given in the list above. The divergence between *Nasikabatrachus sahyadrensis* (7) and *Nesomantis thomasetti* (8) is fairly high, *e.g.*, higher than that of all pairwise comparisons of families in Hyloidea s.s (12-19).

	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
1	-	11.67	16.87	19.33	21.03	19.82	18.97	20.54	18.40	20.60	20.27	20.30	21.44	20.94	20.76	21.21	20.21	20.34	19.73	21.40	21.59	20.97	20.94	21.45	20.13	20.76	19.63	21.45	21.45	21.31
2	6.87	-	15.69	18.55	20.22	20.17	19.51	20.07	18.19	21.15	20.97	20.86	21.25	21.29	20.86	21.31	20.62	19.99	20.37	21.34	21.10	21.10	19.40	21.33	20.52	20.94	19.33	21.16	20.93	20.90
3	7.93	6.10	-	19.35	20.41	20.59	19.67	20.55	19.19	20.96	20.67	21.06	21.77	21.35	21.33	19.88	19.94	21.08	20.23	21.80	22.67	22.44	22.11	21.37	20.00	20.91	19.11	21.66	21.77	21.65
4	9.66	8.92	8.89	-	19.95	20.71	19.68	20.15	18.91	19.81	20.31	21.74	21.64	20.89	21.38	21.08	20.36	20.82	21.10	20.38	21.78	22.16	21.38	22.12	20.41	20.22	19.10	20.83	21.14	21.51
5	9.28	8.66	9.63	11.21	-	16.93	21.09	21.69	19.19	20.82	19.58	20.93	21.98	21.30	21.51	20.68	20.23	22.36	21.10	22.65	22.31	23.10	21.43	23.25	21.01	21.02	20.71	21.30	22.10	22.31
6	9.16	8.42	9.02	10.73	6.62	-	20.24	21.59	19.42	21.51	21.04	22.24	23.02	22.47	21.33	21.37	21.72	23.55	22.13	22.41	21.60	22.93	21.67	22.82	22.18	22.00	21.89	21.73	21.93	21.13
7	13.75	13.84	14.62	17.11	15.16	12.76	-	13.05	14.63	16.60	15.75	16.98	17.42	16.21	16.69	17.00	15.14	16.67	15.95	18.03	19.17	17.95	18.06	19.03	17.85	17.47	15.62	17.08	19.57	18.62
8	14.74	14.70	15.12	16.89	15.42	15.07	12.40	-	15.91	17.14	16.65	18.18	18.02	17.42	17.56	17.54	17.02	18.28	16.75	17.43	18.97	17.61	18.61	19.03	16.64	17.88	16.16	16.55	19.17	18.82
9	11.34	12.03	12.07	13.62	12.76	11.43	12.15	13.98	-	14.29	13.87	15.66	16.05	15.71	16.19	15.64	14.10	15.78	15.04	17.47	17.59	17.03	17.01	18.40	16.89	16.92	15.94	16.18	18.30	17.33
10	12.90	11.43	12.79	14.70	11.43	10.47	10.23	13.97	10.35	-	13.63	17.31	18.42	17.47	17.36	17.47	16.41	17.47	16.41	19.11	18.95	19.37	18.87	19.16	17.98	18.74	17.37	18.55	18.68	18.69
11	14.01	13.25	12.93	14.12	13.97	12.77	11.93	15.20	10.12	7.95	-	16.54	17.74	16.41	17.59	16.64	15.22	17.58	16.04	18.69	18.96	18.10	17.98	18.55	17.02	18.06	16.68	18.22	17.92	18.34
12	13.50	12.52	13.03	14.59	13.24	11.43	10.95	13.97	11.07	9.03	10.24	-	10.14	9.53	11.22	10.27	7.85	11.01	9.27	19.43	18.13	18.12	18.56	19.15	17.61	17.59	16.27	17.52	17.12	17.11
13	13.87	13.84	13.52	14.34	14.08	12.40	12.64	12.65	11.07	10.95	11.45	7.58	-	9.59	11.61	11.19	8.58	11.19	10.19	19.46	18.31	18.70	19.30	19.25	17.94	18.41	16.71	17.24	18.42	17.96
14	12.42	11.55	13.03	14.34	12.40	10.59	10.83	13.02	11.55	9.51	11.09	6.02	7.58	-	9.86	9.91	7.51	10.99	8.85	17.97	17.96	17.75	18.59	18.63	17.26	17.21	16.30	16.35	17.08	18.41
15	12.30	12.40	12.91	13.26	12.88	11.07	10.35	12.77	10.95	9.87	10.85	5.30	6.74	4.93	-	8.45	9.19	11.73	10.20	19.25	19.12	18.04	18.43	19.32	18.47	18.36	16.85	17.98	17.96	19.04
16	12.78	11.79	12.91	13.62	12.52	10.83	10.11	12.77	10.47	8.66	9.40	5.05	6.50	5.05	3.97	-	7.70	11.78	9.92	18.90	18.56	18.62	19.28	19.17	17.99	17.74	16.76	17.62	18.67	18.75
17	13.74	12.27	13.64	14.71	12.76	11.79	10.23	14.09	10.95	9.27	10.61	6.62	7.10	5.90	4.93	3.73	-	9.51	7.18	17.71	17.49	17.69	17.78	18.44	16.92	17.15	15.30	16.28	17.53	17.61
18	12.54	12.03	13.15	14.34	13.24	11.79	10.83	13.13	11.79	9.99	11.57	6.38	7.22	6.62	5.54	5.30	5.17	-	10.39	19.04	19.17	18.63	18.40	20.11	17.65	18.21	17.02	17.76	17.51	18.48
19	13.62	12.88	13.40	13.99	13.24	11.19	11.55	13.85	11.79	10.11	10.37	6.98	8.06	6.50	5.54	5.42	5.17	6.74	-	17.86	17.90	17.08	18.08	18.86	16.47	17.08	15.10	16.02	16.70	17.29
20	14.11	14.56	14.98	15.31	13.48	13.24	13.36	14.11	13.36	12.88	13.97	11.43	12.27	10.59	11.55	10.47	11.79	11.55	12.27	-	9.91	9.77	13.14	14.42	14.25	14.21	12.76	14.42	16.78	16.90
21	14.34	13.00	13.74	15.07	14.20	13.84	13.72	15.30	13.60	13.12	13.74	10.83	12.64	11.19	10.59	10.95	11.91	11.31	12.15	9.15	-	9.46	12.85	14.94	14.97	15.13	14.20	15.21	15.72	15.73
22	12.54	11.20	12.41	12.66	12.52	11.07	13.36	13.87	12.52	11.68	12.54	10.83	11.08	10.23	9.51	10.11	10.47	9.87	11.32	8.55	8.19	-	12.87	13.56	14.33	14.97	12.28	13.90	14.59	15.71
23	14.94	14.44	15.21	17.24	13.72	14.08	15.16	16.28	15.65	12.88	14.70	14.08	14.32	13.36	13.60	13.72	14.20	13.96	15.16	11.55	10.83	11.32	-	14.49	14.50	15.88	13.47	15.35	16.55	15.68
24	15.44	15.54	15.22	15.81	14.93	14.81	15.05	16.52	14.69	15.17	15.80	14.33	14.81	14.94	14.09	14.69	15.18	13.97	15.18	12.17	12.17	12.78	14.22	-	14.23	16.48	13.96	16.16	16.67	16.22
25	14.71	14.08	15.09	14.94	13.48	12.64	14.32	16.14	13.72	13.24	13.98	12.88	13.12	12.64	12.03	11.55	12.52	12.64	13.60	12.15	12.76	9.76	14.08	16.14	-	9.66	7.33	9.20	14.79	13.75
26	16.28	15.04	14.61	15.43	15.52	14.32	15.28	17.59	14.80	14.32	15.54	13.24	13.00	12.88	12.40	12.76	13.00	12.40	13.96	12.88	13.12	10.35	15.28	15.06	11.31	-	9.73	11.19	15.72	15.33
27	15.55	14.32	15.10	13.50	14.32	13.24	13.72	15.06	13.84	13.00	13.50	11.31	11.79	12.15	10.71	10.95	11.19	11.79	10.95	10.11	11.19	9.63	12.88	14.46	10.35	11.67	-	8.26	14.10	13.54
28	14.35	13.60	14.25	14.59	13.84	13.96	16.13	15.54	14.44	13.84	13.62	12.76	12.76	12.64	12.40	12.88	13.72	12.40	13.36	12.40	10.71	10.59	13.60	13.61	12.64	13.48	11.43	-	15.60	15.20
29	13.02	12.52	13.03	13.50	12.15	12.15	11.91	13.97	12.27	11.79	11.81	11.79	12.27	10.59	10.23	10.11	10.23	10.59	11.19	8.91	10.23	7.94	11.67	12.41	11.67	13.60	10.11	11.67	-	10.46
30	13.14	12.76	13.39	14.10	11.31	11.91	11.43	13.01	10.95	10.23	11.08	9.75	11.19	9.51	8.79	8.66	9.75	10.23	10.47	8.79	9.87	8.07	11.31	12.53	9.63	13.36	9.27	11.43	5.42	-

f. Bayesian analyses of individual genes

Bayesian analyses of individual genes were performed using the software MrBayes 2.01 under the GTR+Γ+I model. Four Markov chains were run simultaneously for 1,000,000 generations and were sampled every 100 cycles. Likelihood scores reached stationarity well before 100,000 generations but to be on the safe side, we discarded the first 2,000 trees as the "burn in". Hence, in the trees shown below, Bayesian posterior probabilities (numbers above branches) were estimated as the 50% majority-rule consensus tree of the 8,000 last sampled trees.

Colors in the trees are: Blue = Hyloidea s.s; Red = (Nasikabatrachidae, Sooglossidae) Green = Ranoidea; Gray = Heleophrynidae; Purple = Myobatrachidae.

Fig. f.1. Bayesian analysis based on 831 characters of mitochondrial DNA, of which 480 are constant and 245 are parsimony-informative.

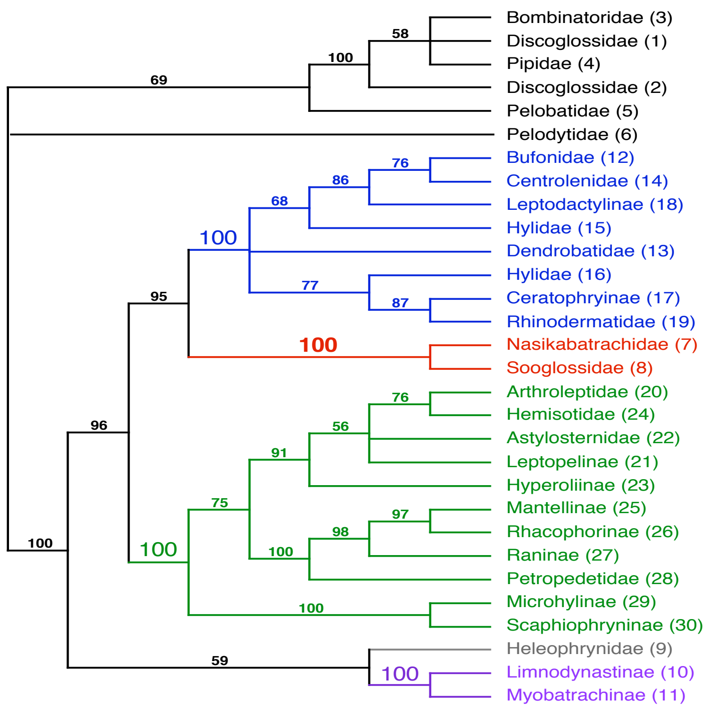


Fig. f.2. Bayesian analysis based on 645 characters of CXCR4 (nuDNA), of which 313 are constant and 279 are parsimony-informative.

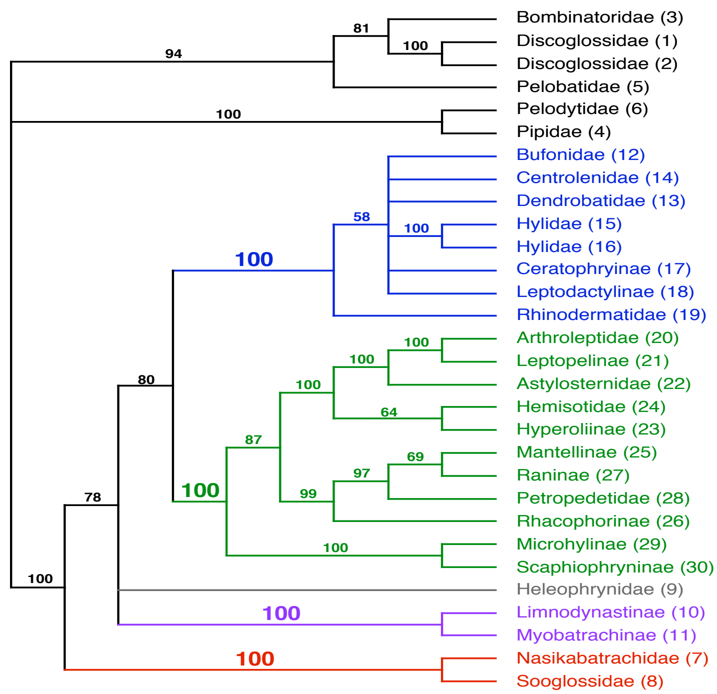


Fig. f.3. Bayesian analysis based on 315 characters of rhodopsin exon 1 (nuDNA), of which 160 are constant and 122 are parsimony-informative.

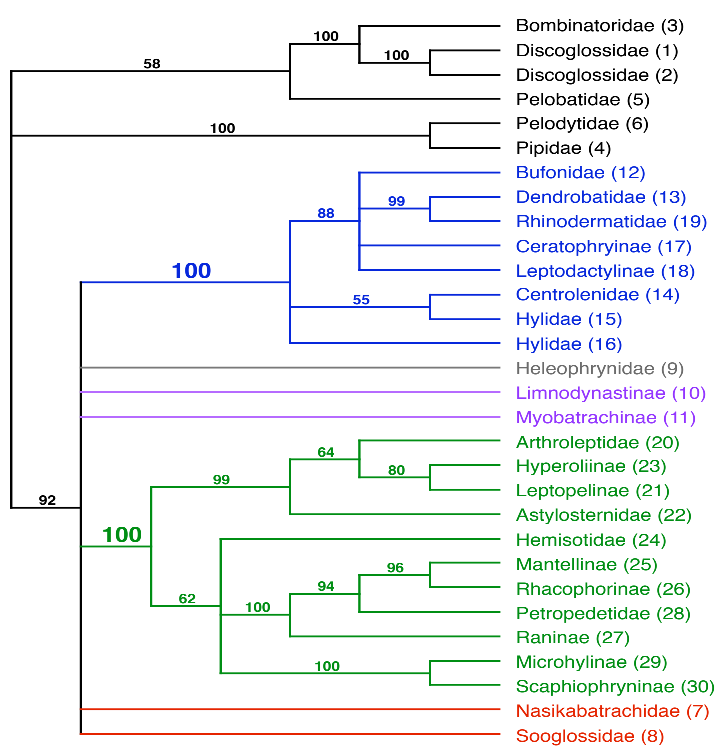
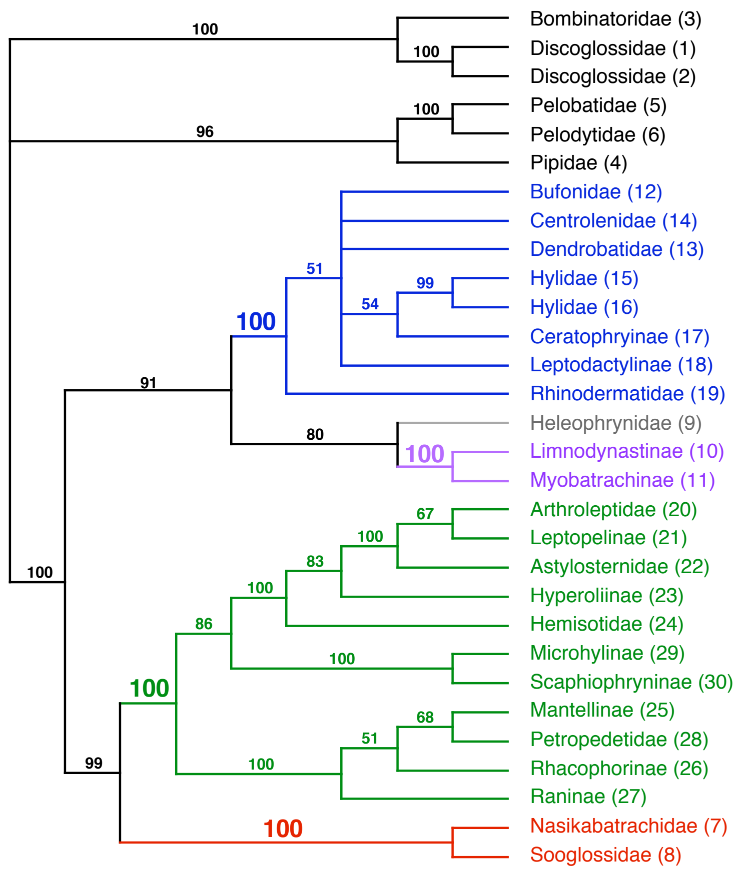


Fig. f.4. Bayesian analysis based on 534 characters of RAG1 (nuDNA), of which 274 are constant and 224 are parsimony-informative.



f. Divergence time estimates

The software multidivtime (available from Jeff Thorne) estimates divergence times from multilocus DNA sequence data in which the assumption of constant rates over time is not required[2]. This program allows to incorporate multiple constraints on node ages. A minimum hypothesis for the divergence of amniotes from amphibians is marked by the age of the East Kirkton tetrapod fauna, the diversity of which makes the date-estimate of 338 mya robust with respect to numerous alternative tree topologies[3]. Our minimum age of Cryptobranchoidea at 164 Myr ago is based on the recent discovery of basal members of the Cryptobranchidae from the volcanic deposits of the Jiulongshan Formation (Bathonian), Inner Mongolia, China[4]. The earliest ancestors of mammals (synapsids) and birds (diapsids) appear at ~310 Myr ago, a timing that is unlikely to be a large underestimate[5] for this split. However, since calibration is essential for estimating node times from molecular data, we also calculated node ages for the diapsid-synapsid split constrained at 338-300 Myr ago (*i.e.*, allowing this divergence to be considerably older or slightly younger, following the suggestion of an anonymous reviewer). The results show that both time estimate and 95% credibility interval for node times remain very similar under these conditions.

Calibration:

(*Homo*, *gallus*) vs. amphibians: Lower bound at 338 Myr ago.
Homo vs. *gallus*: 338-300 Myr ago.
Hynobius vs. *Salamandra*: Lower bound at 164 Myr ago.

Bayesian divergence time estimates

Divergence	Time estimate
Origin of Ranoidea and Hyloidea s.s.	155 Myr ago (111,207)
Origin of Heleophrynidae and Myobatrachidae	153 Myr ago (111,203)
Origin of (Sooglossidae-Nasikabatrachidae) lineage	182 Myr ago (133, 238)
Sooglossidae - Nasikabatrachidae split	134 Myr ago (94, 181)

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