Supplementary Information for

Natatanuran frogs used the Indian Plate to step-stone disperse and radiate across the Indian Ocean

Zhi-Yong Yuan[1, 2#], Bao-Lin Zhang[1, 3#], Christopher J. Raxworthy[4], David W. Weisrock[5], Paul M. Hime[5,6], Jie-Qiong Jin[1, 7], Emily M. Lemmon[8], Alan R. Lemmon[9], Sean D. Holland[8], Michelle L. Kortyna[8], Wei-Wei Zhou[1, 7], Min-Sheng Peng[1,10], Jing Che[1, 7,11*], and Elizabeth Prendini[4]

[1] *State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China*
[2] *Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, Kunming 650224, China*
[3] *Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming 650204, China*
[4] *Department of Herpetology, American Museum of Natural History, New York, NY 10024, USA*
[5] *Department of Biology, University of Kentucky, Lexington, KY 40506, USA*
[6] *Biodiversity Institute, University of Kansas, Lawrence, KS 66045, USA*
[7] *Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw 05282, Myanmar*
[8] *Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA*
[9] *Department of Scientific Computing, Florida State University, Tallahassee, FL 32306, USA*
[10] *Sino-Africa Joint Research Center, Chinese Academy of Sciences, Kunming 650224, China*
[11] *Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming 650223, China*
[#]*Equal contribution*
[*]Corresponding Author: Jing Che;
Email: chej@mail.kiz.ac.cn

This PDF file includes:

Supplementary Note
Figures 1 to 3
Tables 1 to 4

Supplementary Note

Taxon Sampling We sampled 86 Natatanuran species (13 of 14 recognized Natatanura families) and 20 outgroup taxa (Supplementary Table 1). The only family unsampled in this study (Micrixalidae) was previously placed as sister group to

Ranixalidae [1,2]. Our data included representatives of all major Natatanura clades extant on the landmasses that were part of Laurasia and Gondwana, based on the combined results of Bossuyt *et al*. [1], Pyron [3] and Feng *et al*. [2]. Frogs of genus *Rana* from South America were excluded from this study as they are a known dispersal from North American *Rana* [4].

Probe Design Following Barrow *et al*. [5] and Heinicke *et al*. [6], we mined the publicly available genome sequences for the model frog *Xenopus tropicalis* [7], complete transcriptomes for the salamanders *Ambystoma mexicanum* [8], and *Notophthalmus viridescens* [9]. To increase taxon representation in our probe design, we also developed and mined genomic resources *de novo* for six additional frogs (*Ascaphus montanus*, *Gastrophryne carolinensis*, *Mixophes schevilli*, *Pseudacris feriarum*, *Pseudacris nigrita*, and *Rana sphenocephala*), one salamander (*Desmognathus fuscus*), and one caecilian (*Ichthyophis multicolor*), as well as transcriptomic resources for two additional salamanders (*Cryptobranchus alleganiensis* and *Ensatina eschscholtzii*). For each of these 13 amphibian taxa, we attempted to identify putative orthologs to a subset of 403 of the original 512 anchored hybrid enrichment loci. Although not all of these target loci were identified in all 13 model taxa, each locus was represented by on average 11.1 model taxa.

We then designed a set of 120-mer DNA probes tiled across each of these loci for each of the 4,061 locus-by-model-taxon combinations. The tiling density of probes over target regions ranged from 1.0 to 2.0. Each locus consists of an evolutionarily conserved core region flanked by more variable regions on either side. Probes for each model taxon covered these core regions and extended into the flanks in order to increase the lengths of captured loci across diverse taxa. Across all 13 model taxa and 403 target loci, the region covered by the probes was ~1,090 bp per locus on average. In practice, longer assemblies were generated from this due to the use of paired-end sequencing, allowing for the extension of sequenced regions beyond the core conserved regions covered by the probes. This set of 57,750 unique 120-mer probes was synthesized by Agilent Technologies.

Library Preparation Total genomic DNA was extracted using a standard phenol–chloroform extraction with ethanol precipitation [10]. Library preparation and sample enrichment was conducted at the Center for Anchored Phylogenomics at Florida State University (FSU; www.anchoredphylogeny.com). Genomic DNA was sonicated to a fragment size of ~200–600 bp via a Covaris E220 Focused-ultrasonicator. Libraries were prepared and indexed using a modified protocol from Meyer and Kircher [11] as described in Prum *et al*. [12]. Indexed samples were pooled in equimolar quantities, and the pools were enriched using an Agilent Custom SureSelect Kit (Agilent Technologies) with Anchored Hybrid Enrichment probes designed for amphibians above. Sequencing was performed on 10 PE150 Illumina HiSeq2500 lanes at the FSU Translational Science Laboratory. Up to 48 samples were run in each lane.

Read Assembly To increase read accuracy and length, paired reads were merged before assembly, following Rokyta *et al*. [13]. Reads were mapped to the probe regions using *P. nigrita*, *G. carolinensis*, and *R. spenocephala* as references, combined with a de novo assembly approach to extend the assembly into flanking regions [12,14]. Read files were traversed repeatedly until no additional mapped reads were produced. Following read assembly, consensus bases were called from assemblies either as ambiguous or unambiguous bases, depending on the relative probability of sequencing error and heterozygosity. Assembled contigs resulting from fewer than 109 reads were removed to mitigate the effects of rare sequencing errors and mis-indexing.

Orthology Assessment For each locus, orthology was determined following procedures described in Prum *et al*. [12] and Hamilton *et al*. [14]. A pairwise distance matrix among all homologous sequences was calculated using an alignment-free approach and used to cluster sequences with a neighbor-joining algorithm, constraining each resulting cluster to contain at most one sequence from each individual. Resulting clusters containing fewer than 53 individuals were removed from further analysis.

Alignment and Trimming Sequences in each orthologous cluster were first aligned using MAFFT v. 7.023b [15], then trimmed and masked following the procedure

established in Prum *et al*. [12] and Hamilton *et al*. [14]. Sites with the same character in at least 21 of the sequences were considered "conserved". A 20 bp sliding window was then moved across the alignment, and regions with <12 characters matching the most common base at the corresponding conserved site were masked. Sites with <52 unmasked bases were removed. Finally, the masked alignments were inspected by eye and regions considered obviously misaligned or paralogous were removed.

Phylogenetic Inference A subset of 376 of the originally targeted 512 anchored hybrid enrichment loci were ultimately used in the phylogenetic analysis. Phylogenetic trees were inferred by both concatenation and coalescent methods. For the concatenation analyses, we performed maximum likelihood (ML) and Bayesian inference (BI) methods on the concatenated matrix using both unpartitioned and partitioned strategies. PartitionFinder v. 2 [16] was used to select the best-fitting partitioning scheme under the Bayesian Information Criterion. This approach suggested that 93 partitions of our data set represented the best-fitting partitioning scheme. The ML analyses were performed in RAxML v. 8.0.15 [17], using 100 rapid bootstrapping searches (-# 100 -f a) with GTRGAMMA model assigned to each partition. Bayesian analyses were conducted using ExaBayes v. 1.5 [18]. Two independent ExaBayes runs were executed with four coupled Markov chains (temperature set to 0.1) for 1,000,000 generations with sampling every 500 generations. Branch lengths among partitions were linked. The first 25% of the samples were discarded as burnin. Convergence on the posterior distribution of parameter values among runs was examined by checking the effective sample sizes (>200) in the Tracer v1.5 [19]. The consensus trees were obtained using the *consense* utility, which was a part of ExaBayes. Species-trees were generated using two coalescent methods: ASTRAL [20] and STAR [21], both of which are computationally efficient and exhibit good performance with phylogenomic-scale data. The input gene trees for each anchored locus were generated using RAxML v. 8.0.15 [17] with the GTRGAMMA model with 100 bootstrap replicates.

Divergence time estimation Due to large alignments, we utilized the approximate likelihood method implemented in MCMCTREE [22] to estimate a time-calibrated

tree. The inferred ExaBayes tree was used as the reference topology. We first used BASEML program (in PAML, [22]) to estimate the mean substitution rate for all partitions derived from PartitionFinder. These calculations were conducted under the GTR + Γ substitution model, using the strict molecular clock, assuming a 173 Ma root age [2]. The average estimated rates were then used to set the prior of overall substitution rate (rgene gamma = 1, 11.59, 1) and rate-drift parameter (sigma2 gamma =1, 1, 1). We used five well-justified fossils and one secondary calibration (Table 2) to calibrate the date of nodes. Fossils constraints were scaled to units of 100 million years. The constraints of minimum and maximum bounds were soft, with a default 2.5% probability that those bounds can be violated. The MCMC chain was first run for 5,000,000 generations as burnin, then sampled every 1,000 generations until a total of 10,000 samples was generated. This analysis was executed twice to check for convergence using randomly generated seeds.

Biogeographical Reconstruction Based on the presumed history of Laurasia and Gondwana, and the current distribution pattern of Natatanura, seven biogeographic areas were defined: (A) Africa, (B) Madagascar, (C) India (including Sri Lanka), (D) Australia-New Guinea, (E) Asia, (F) Europe and (G) North America (including the Neotropics) (Table 1). Biogeographic analyses were performed using BioGeoBEARS [23].

We used dispersal matrices to indicate the probability of dispersal events between two areas: 0.01 representing disallowed dispersal between well-separated areas by oceans or other land masses (0.0 generates computational difficulties); 0.5 representing moderate dispersal probability between adjacent, but not connected areas; 1.0 representing unrestricted dispersal between well-connected land masses or connected by a land bridge. Four time slices (130–88 Ma, 88–55 Ma, 55–25 Ma, 25–0 Ma) were delimited to reflect the dispersal probabilities, caused by three major palaeogeographical events: 1) separation of India from Madagascar, *ca.* 88 Ma ago [24]; 2) India colliding with Asia, *ca.* 55 Ma ago [25]; and 3) Australia colliding with Asia, *ca.* 25 Ma ago [26].

Whether the Indian Plate acted as an isolated biotic "ferry" or as a stepping stone route for biota during its northward journey towards Asia in the late Cretaceous, has been contentious [27,28]. Thus, we tested these hypotheses specifically. For the biotic ferry hypotheses, we assigned a value of 0.01 for disallowed dispersal between India-Africa, India-Madagascar, and India-Asia during the 88–55 Ma time slices, while assigning a value of 0.5 for moderate dispersal probability for the above three pairs in the stepping stone hypotheses (Table 3). We simulated ancestral area reconstructions for all six models implemented in BioGeoBEARS under DEC and DEC+J [23]. The best-fitting model to the data was assessed using likelihood-ratio tests and the Akaike Information Criterion (AIC). The maximum number of areas allowed at each node was set to two in all analyses. The node to all Ranoidea and Microhylidae was fixed to an African distribution according to Feng *et al*. [2] using the BioGeoBEARS "fixlikes" option.

Results of Sequence Characteristics A total of 376 high quality loci were assembled from the target sequencing data for the 86 Natatanura species and 20 outgroup taxa (Table 1). These loci had a concatenated length of 545,165 base pairs (bp) and an average length of 1,445 bp (170–2,466 bp), of which an average of 58.0% of sites (33.9–82.2%) were parsimony informative. The total percentage of missing data was 9.5%. These data are available on the Dryad Digital Repository (will provide upon acceptance).

Results of Phylogeny, Temporal, and Spatial Diversification The concatenated and species-tree analyses provided strong and concordant support (bootstrap proportions = 100; Bayesian posterior probabilities = 1.0) for all the relationships, except for one node (Clade 4, Fig. 1) with low support (bootstrap proportions < 70%; Bayesian posterior probabilities < 0.95) with maximum-likelihood and Bayesian analyses (Fig. 1), and moderately high support with species trees (Fig. 2). There was strong support for the sister relationship of Natatanura and Afrobatrachia, which separated *ca.* 98.5 Ma ago [104.8–90.0 Ma, 95% highest posterior density (HPD), Fig.4. Within

Natatanura, all six African families (Odontobatrachidae, Ptychadenidae, Phrynobatrachidae, Conrauidae, Petropedetidae, and Pyxicephalidae) formed a clade (Clade 1, Fig. 1 which separated from other Natatanura frogs (Clade 2, Fig. 3 *ca.* 75.6 Ma ago (85.1–66.3 Ma, 95% HPD). The two endemic Indian families formed Clade 3, which was sister to Clade 4 (uniting Ranidae, Mantellidae, Rhacophoridae, Dicroglossidae, and Ceratobatrachidae), which separated *ca.* 72.8 Ma ago (82.6–63.4 Ma, 95% HPD; Fig.3). Clade 5 united Ranidae, Dicroglossidae, Mantellidae, Rhacophoridae, and Dicroglossidae. The Madagascan Mantellidae and Asian Rhacophoridae formed Clade 7, which was sister to Ranidae. These two clades diverged *ca.* 62.4 Ma (71.7–54.0 Ma, 95% HPD, Fig. 1 and Fig. 3) and were united with strong support. All Natatanuran families diversified rapidly, within a short period from *ca.* 75.6 Ma to 62.4 Ma (85.1–54.0 Ma, 95% HPD). The Australia-New Guinea genus *Cornufer* diverged from its sister clade of Asian frogs (*Platymantis*) within Ceratobatrachidae *ca.* 30.2 Ma (40.0–21.3 Ma, 95% HPD). A second Australia-New Guinea clade (*Papurana*) was also sister to an Asian clade (*Hydrophylax*), and separated *ca.* 14.9 Ma (19.5–10.4 Ma, 95% HPD). The remaining three African taxa included single lineages from within otherwise Asian clades; these included the African genera *Amnirana*, *Chiromantis* and *Hoplobatrachus*, which were sister to Asian frogs within Ranidae, Rhacophoridae and Dicroglossidae, respectively. The North American *Rana* were sister to Asian *Rana* frogs within Ranidae.

The DEC+J class of models produced a much higher likelihood for the data than DEC (AIC; Table 4). Regardless, the “stepping stone” India model was favored over the “ferry” India model within each class (Table 4), and we thus used this model in our evaluation of ancestral range estimates. The most recent common ancestor (MRCA) of extant Natatanura inhabited Africa, as recently shown by Feng *et al*. [2]. The first dispersal involved a range expansion to India, and then from India across to Asia *ca.* 72.8 Ma (82.6–63.4 Ma, 95% HPD, Fig.1 and Fig. 3), resulting in one clade uniting two endemic Indian families (Ranixalidae and Micrixalidae), and the other clade uniting all other Asian families. However, the latter then underwent dispersal from Asia to Madagascar *ca.* 62.4 Ma (71.7–54.0 Ma, 95% HPD, Fig. 1 and Fig. 3),

yielding the endemic Madagascan frog radiation (Mantellidae). We identified two dispersal events from Asia to Australia-New Guinea *ca.* 30.2 Ma (21.3–40.0 Ma, 95% HPD,) and 14.9 Ma (10.4–19.5 Ma, 95% HPD), resulting in *Cornufer* and *Papurana* emerging from Ceratobatrachidae and Ranidae, respectively. Three dispersals from Asia to Africa were identified at *ca.* 21.6 Ma (18.1–25.6 Ma, 95% HPD), 20.6 Ma (14.2–27.0 Ma, 95% HPD) and *ca.* 12.7 Ma (6.6–21.3 Ma, 95% HPD), by a single lineage from Ranidae (*Amnirana*), a lineage of Rhacophoridae (*Chiromantis*), and a lineage of Dicroglossidae (*Hoplobatrachus*), respectively.

Vertebrate Fossil Records Consistent with the "Indian Stepping Stone Hypotheses" These data include: 1) the Madagascar bothremydid turtles, e.g. *Kurmademys* and Indian *Sankuchemys* [29], 2) the gondwanatherian mammals, e.g. *Lavanify* and *Bharattherium* from the Late Cretaceous of Madagascar and India [30], 3) the discovery of *Simosuchus*-like notosuchian crocodiles from India, which is the first report of the group outside the Late Cretaceous of Madagascar [31], and 4) the presence of myliobatid (*Igdabatis*) and rhombodontid (*Rhombodus*) fishes and troodontid dinosaurs from the Maastrichtian of India (72.1 to 66.0 Ma) related to taxa from Africa and/or Laurasia [32–34].

References

1. Bossuyt F, Brown RM and Hillis DM *et al.* Phylogeny and biogeography of a cosmopolitan frog radiation: Late cretaceous diversification resulted in continent-scale endemism in the family Ranidae. *Syst Biol* 2006; **55**: 579–594.
2. Feng YJ, Blackburn DC and Liang D *et al*. Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary. *Proc Natl Acad Sci USA* 2017; **29**: 5864–5870.
3. Pyron RA. Biogeographic analyses reveals ancient continental vicariance and recent oceanic dispersal in Amphibia. *Syst Biol* 2014; **63**: 779–797.
4. Yuan Z, Zhou W and Chen X *et al*. Spatiotemporal diversifcation of the true frogs (genus *Rana*): a historical framework for a widely studied group of model organisms. *Syst Biol* 2016; **65**: 824–842.

5. Barrow LN, Lemmon AR and Lemmon EM. Targeted sampling and target capture: assessing phylogeographic concordance with genome-wide data. *Syst Biol* 2018; syy021, https://doi.org/10.1093/sysbio/syy021.

6. Heinicke MP, Lemmon AR and Lemmon EM *et al*. Phylogenomic support for evolutionary relationships of New World direct-developing frogs (Anura: Terraranae). *Mol Phylogenet Evol* 2018; **118**:145–155.

7. Hellsten U, Harland RM and Gilchrist MJ *et al*. The genome of the Western clawed frog *Xenopus tropicalis*. *Science* 2010; **328**:633–636.

8. Wu CH, Tsai M and Ho C *et al*. De novo transcriptome sequencing of axolotl blastema for identification of differentially expressed genes during limb regeneration. *BMC Genomics* 2013; **14**: 434.

9. Abdullayev I, Kirkham M and Björklund AK *et al*. A reference transcriptome and inferred proteome for the salamander *Notophthalmus viridescens*. *Exp Cell Res* 2013; **319**: 1187–1197.

10. Sambrook J, Fritsch EF and Maniatis T. *Molecular cloning: a laboratory manual*. 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.

11. Meyer M and Kircher M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *CSH Protocols* 2010; **2010**:6.

12. Prum RO, Berv JS and Dornburg A *et al*. A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. *Nature* 2015; **526**:569–573.

13. Rokyta DR, Lemmon AR and Margres MJ *et al*. The venom-gland transcriptome of the eastern diamond ack rattlesnake (*Crotalus adamanteus*). *BMC Genomics* 2012; **13**: 312.

14. Hamilton, CA, Lemmon AR and Lemmon EM *et al*. Expanding anchored hybrid enrichment to resolve both deep and shallow relationships within the spider tree of life. *BMC Evol Biol* 2016; **16**: 212.

15. Katoh K and Standley DM. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol Biol Evol* 2013; **30**: 772–780.

16. Lanfear R, Calcott B and Ho SY *et al*. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol* 2012; **29**: 1695–1701.
17. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014; **3**: 1312–1313.
18. Aberer AJ, Kobert K and Stamatakis A. ExaBayes: massively parallel Bayesian tree inference for the whole-genome era. *Mol Biol Evol* 2014; **31**: 2553–2556.
19. Rambaut A and Drummond AJ. *Tracer 1.5* http://tree.bio.ed.ac.uk/software/tracer/, 2007.
20. Mirarab S, Reaz R and Bayzid MS *et al*. ASTRAL: genome-scale coalescent-based species tree estimation. *Bioinformatics* 2014; **30**: i541–i548.
21. Liu L, Wu S and Yu L *et al*. Estimating species phylogenies using coalescence times among sequences. *Syst Biol* 2009; **58**: 468–477.
22. Yang Z. PAML 4: phylogenetic analysis by maximum likelihood. *Mol Biol Evol* 2007; **24**: 1586–1591.
23. Matzke NJ. BioGeoBEARS: Biogeography with Bayesian and likelihood evolutionary analysis in R scripts. Available at cran.r-project.org/web/packages/BioGeoBEARS/, 2013. (Accessed April 6, 2017).
24. Chatterjee S, Goswami A and Scotese CR. The longest voyage: Tectonic, magmatic, and paleoclimatic evolution of the Indian plate during its northward flight from Gondwana to Asia. *Gondwana Res* 2013; **23**: 238–267.
25. Aitchison JC, Ali JR and Davis AM. When and where did India and Asia collide? *J Geophys Res* 2007; **112**: B05423.
26. Hall R. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *J Asian Earth Sci* 2002; **20**: 353–431.
27. Briggs JC. The biogeographic and tectonic history of India. *J Biogeogr* 2003; **30**: 381–388.

28. Verma O, Khosla A and Goin FJ *et al*. Historical biogeography of the Late Cretaceous vertebrates of India: comparison of geophysical and paleontological data. *New Mexico Mus Nat Hist Sci Bull* 2016; **71**: 317–330.
29. Gaffney ES, Krause DW and Zalmou IS. *Kinkonychelys*, a new side-necked turtle (Pelomedusoides: Bothremydidae) from the Late Cretaceous of Madagascar. *Am Mus Novit* 2009; **3662**: 1–25.
30. Krause DW, Prasad GVR and Koenigswald WV *et al*. Cosmopolitanism among Gondwanan Late Cretaceous mammals. *Nature* 1997; **390**: 504–507.
31. Prasad GVR, Verma O and Flynn JJ *et al*. A new Late Cretaceous vertebrate fauna from the Cauvery Basin, South India: Implications for Gondwanan paleobiogeography: *J Vert Paleontol* 2013; **33**: 1260–1268.
32. Prasad GVR and Sahni A. Late Cretaceous continental vertebrate fossil record from India: palaeobiogeographical insights. *Bulletin de la Société Géologique de France* 2009; **180**:369–381.
33. Goswami A, Prasad GV and Upchurch P *et al*. A radiation of arboreal basal eutherian mammals beginning in the Late Cretaceous of India. *Proc Natl Acad Sci USA* 2011; **108**: 16333–16338.
34. Goswami A, Prasad GVR and Verma O *et al*. A troodontid dinosaur from the latest Cretaceous of India. *Nat Commun* 2013; **4**: 1–5.

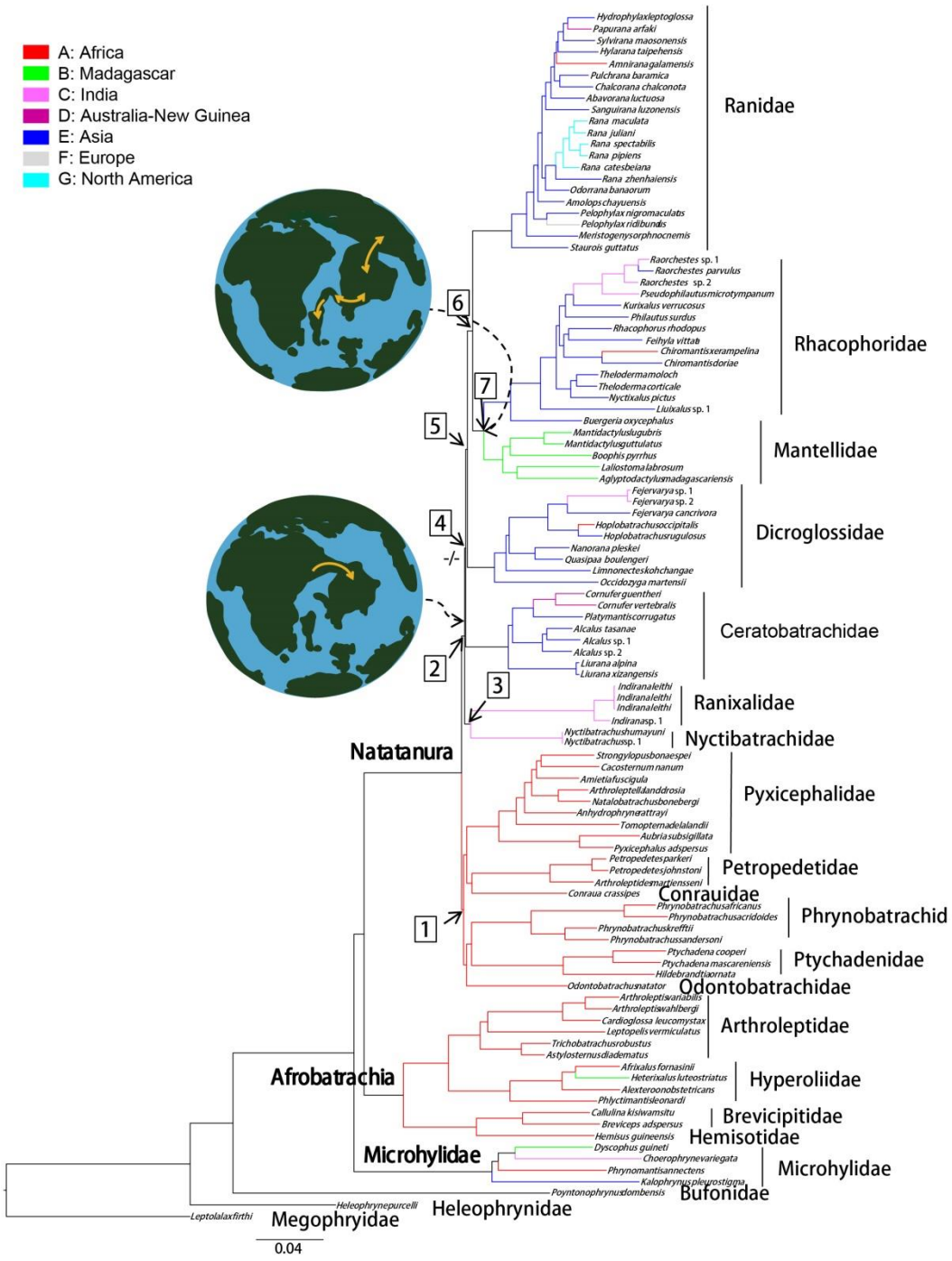

Figure 1 The Bayesian inference tree derived from 376 nuclear loci. Branches without support symbols were strongly supported in both Bayesian and maximum-likelihood phylogenetic analyses. Bootstrap proportions <70% and Bayesian posterior probabilities < 0.95 were treated as weakly supported (−). Colors of branches indicate the geographic distribution of extant species. Paleogeographic reconstructions are modified from Chatterjee et al. (24) and Briggs (27). Clades of interest are numbered in boxes.

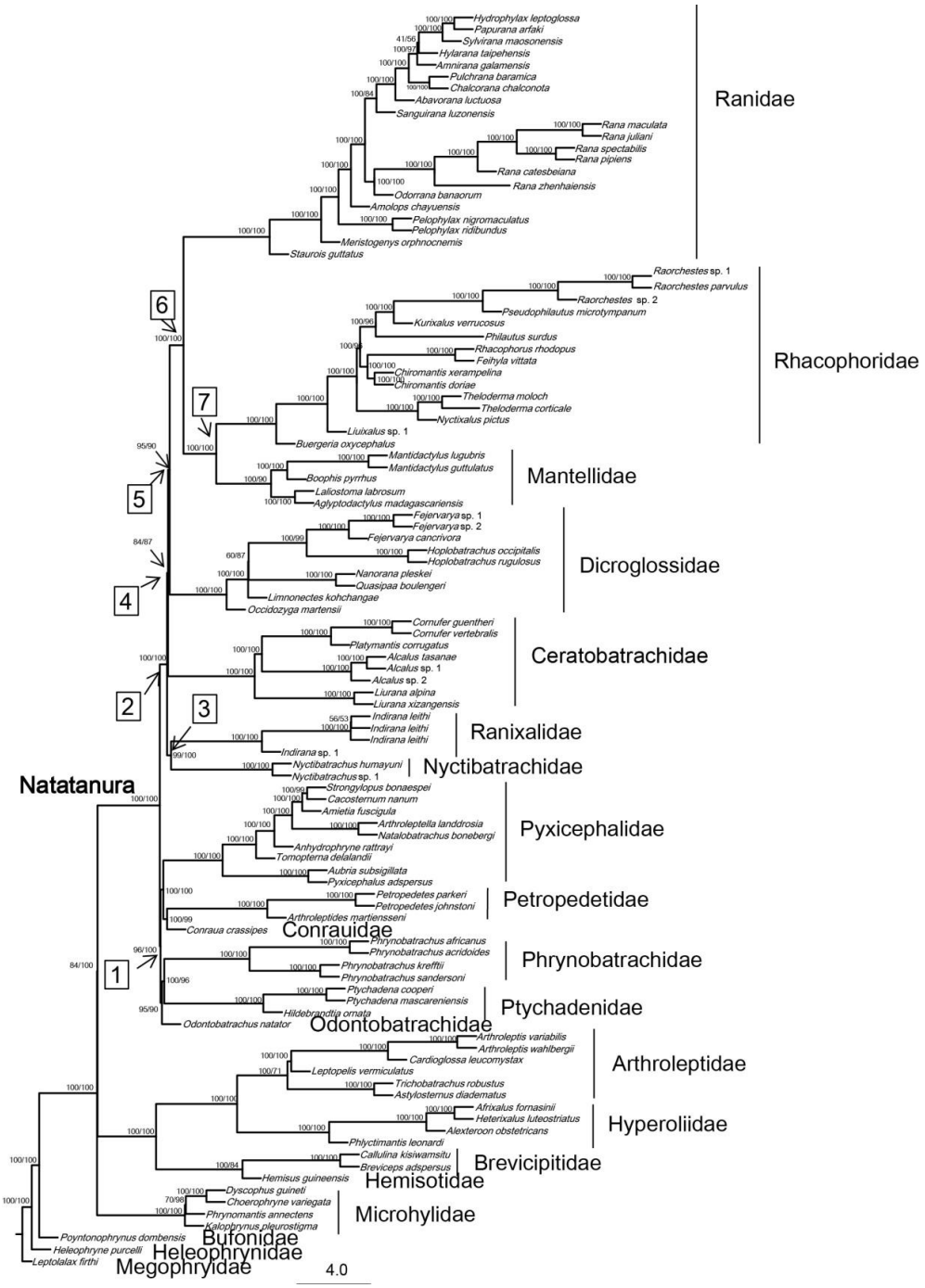

Figure 2 The species tree inferred from ASTRAL analyses based on gene trees estimated from 376 nuclear loci. Numbers near nodes are bootstrap proportions inferred by ASTRAL analyses and STAR analyses, respectively. Clades of interest are numbered in boxes.

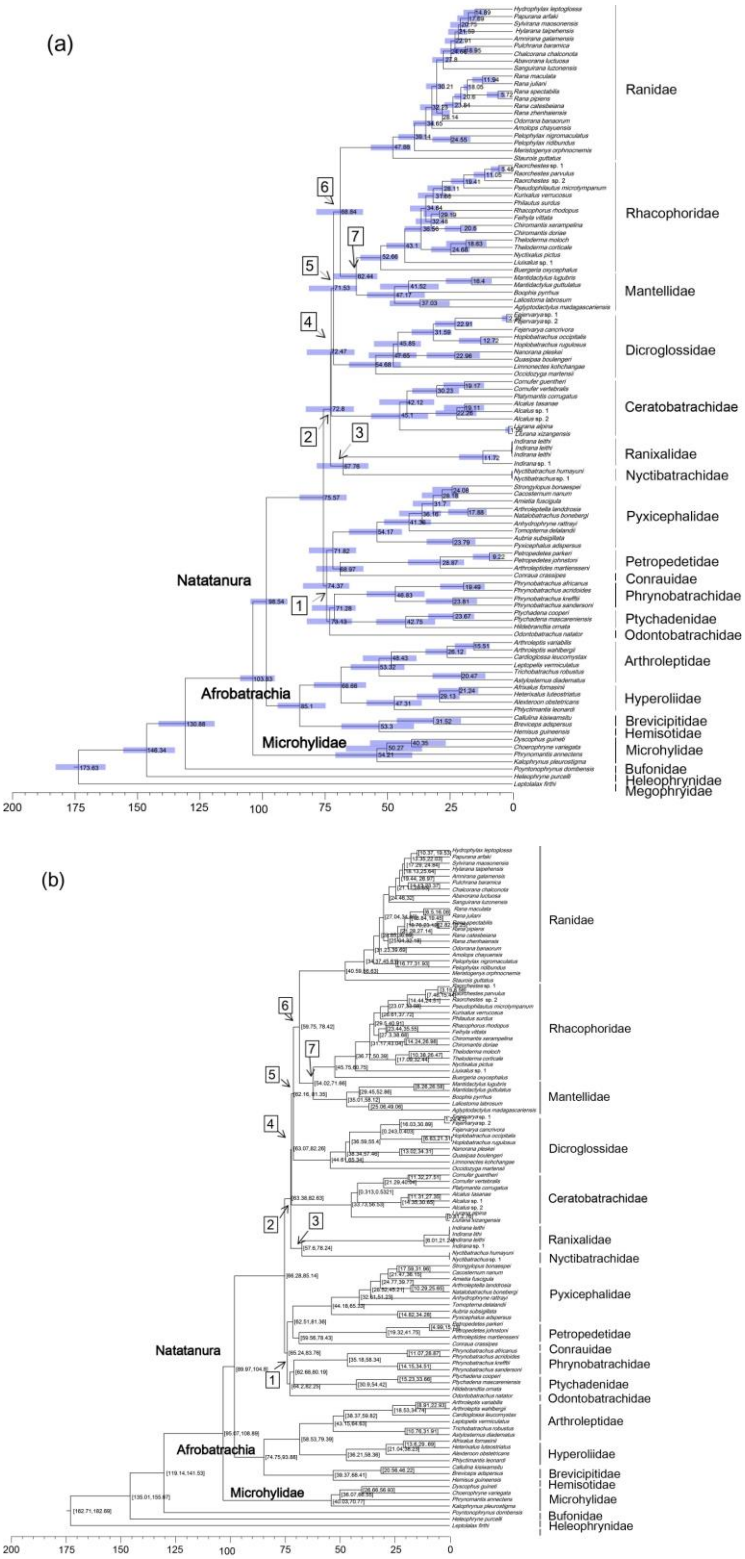

Figure 3 Time-calibrated phylogeny of Natatanura inferred by MCMCTREE based on 376 nuclear loci. (a) Mean ages for each node are shown. Node bars represent the 95% highest posterior density for node ages. (b) 95% HPD intervals of node ages are presented. Clades of interest are numbered in boxes.

Table 1 Sampling information for Natatanura and outgroups used, including voucher specimens, localities, and biogeographic region affiliation. "-" denote the lineages were not used to biogeographical reconstruction..

Voucher	**Family**	**Genus**	**Epithet**	**Locality**	**Biogeographic region**
UTA-A44451/DPL3597	Arthroleptidae	*Arthroleptis*	*variablis*	Cameroon: Eastern Province	Africa
TMSA84361	Arthroleptidae	*Arthroleptis*	*wahlbergii*	South Africa: KZN: Mondi Sjonajona Plantation	Africa
MCZH136805	Arthroleptidae	*Astylosternus*	*diadematus*	Cameroon: Banyang Mbo F.R.	Africa
MCZH139626	Arthroleptidae	*Cardioglossa*	*leucomystax*	Nigeria: Adamawa: Kwano: Gashaka Gumti N.P.	Africa
TMSA84038	Arthroleptidae	*Leptopelis*	*vermiculatus*	Tanzania: E. Usambara Mtns: Armani F.R.	Africa
MCZH136806	Arthroleptidae	*Trichobatrachus*	*robustus*	Cameroon: Banyang Mbo F.R.	Africa
JDV95069	Brevicipitidae	*Breviceps*	*adspersus*	Shankara	Africa
MCZH138534	Brevicipitidae	*Callulina*	*kisiwamsitu*	Tanzania: W. Usambara Mtns: Mazumbai F.R.	Africa
CAS243929	Ceratobatrachidae	*Alcalus*	sp. 1	Myanmar: Tanintharyi: Dawei: Yephyu	Asia
CAS225248	Ceratobatrachidae	*Alcalus*	sp. 2	Myanmar: Kachin: Putao: Nagmung	Asia
FMNH/THNHM268828	Ceratobatrachidae	*Alcalus*	*tasanae*	Thailand: Eurathami: Khao Sok N.P.	Asia
AMNH-A168953	Ceratobatrachidae	*Cornufer*	*vertebralis*	Solomon Is: New Georgia Gr.: Vella Lavella: Oula River	Australia-New Guinea
AMNH-A161638	Ceratobatrachidae	*Cornufer*	*guentheri*	Solomon Islands	Australia-New Guinea
KIZ11104	Ceratobatrachidae	*Liurana*	*xizangensis*	China: Tibet: Medog	Asia
KIZ11141	Ceratobatrachidae	*Liurana*	*alpina*	China: Tibet: Medog	Asia
FMNH266259	Ceratobatrachidae	*Platymantis*	*corrugatus*	Philippines: Luzon: Zambales: Sitio Dampa	Asia
MVZB253198	Conrauidae	*Conraua*	*crassipes*	Nigeria: Cross River: Cross River N.P.	Africa
FMNH263189	Dicroglossidae	*Limnonectes*	*kohchangae*	Cambodia: Kampot: Kampot: Bokor N. P.	Asia

ROM35975	Dicroglossidae	*Occidozyga*	*martensii*	China: Yunnan: Simao	Asia
MVZB239400	Dicroglossidae	*Fejervarya*	*cancrivora*	Indonesia: Sulawesi: Desa Abelisawah: Kota Kendari	Asia
R1427	Dicroglossidae	*Fejervarya*	sp. 1	India: Maharashtra: Aurangabad	India
R1207	Dicroglossidae	*Fejervarya*	sp. 2	Sri Lanka: Wilpattu National Wildlife P.	India
CAS241469	Dicroglossidae	*Hoplobatrachus*	*rugulosus*	Myanmar: Kachin: Mohnyin: Indawgyi W. S.	Asia
MVZB245101	Dicroglossidae	*Hoplobatrachus*	*occipitalis*	Ghana: Volta: Togo Hills: Kue River	Africa
MVZB231208	Dicroglossidae	*Nanorana*	*pleskei*	China: Xingdugiao: Kingding	Asia
AMNH-A163869	Dicroglossidae	*Quasipaa*	*boulengeri*	Vietnam: Ha Giang: Vi Xuyen: Cao Bo	Asia
AMB8787	Hemisotidae	*Hemisus*	*guineensis*	Namibia: Kavango Region	Africa
AMNH-A153305	Hyperoliidae	*Afrixalus*	*fornasinii*	Tanzania: Morogoro: Udzungwa Mtns N.P.	Africa
AMCC125880	Hyperoliidae	*Alexteroon*	*obstetricans*	Cameroon	Africa
MVZB238721	Hyperoliidae	*Heterixalus*	*luteostriatus*	Madagascar: Ambalavao	Madagascar
AMCC124754	Hyperoliidae	*Phlyctimantis*	*leonardi*	Cameroon: South West Prov.	Africa
MVZB238723	Mantellidae	*Aglyptodactylus*	*madagascariensis*	Madagascar: Andasibe	Madagascar
MVZB238732	Mantellidae	*Boophis*	*pyrrhus*	Madagascar: Andasibe	Madagascar
MVZB241453	Mantellidae	*Laliostoma*	*labrosum*	Madagascar: Ifaty	Madagascar
AMNH-A168365	Mantellidae	*Mantidactylus*	*lugubris*	Madagascar: Fianarantsoa: Vatoharanana	Madagascar
AMNH-A167544	Mantellidae	*Mantidactylus*	*guttulatus*	Madagascar: Mahajanga: Antsohihy: Irony River Relic F.	Madagascar
ID7602	Nyctibatrachidae	*Nyctibatrachus*	*humayuni*	India: Maharashtra: Matheran	India
R1432	Nyctibatrachidae	*Nyctibatrachus*	sp. 1	India: Maharashtra: Pune	India
CAS230053	Odontobatrachidae	*Odontobatrachus*	*natator*	Sierra Leone: Western: Regent-Grafton: Peninsula Mtns	Africa
AMNH-A151341	Petropedetidae	*Arthroleptides*	*martiensseni*	Tanzania: Morogoro: Udzungwa Mtns N.P.	Africa
UTA-A44414	Petropedetidae	*Petropedetes*	*johnstoni*	Cameroon: South West Prov.	Africa
MCZH139541	Petropedetidae	*Petropedetes*	*parkeri*	Nigeria: Adamawa	Africa
MCZH138214	Phrynobatrachidae	*Phrynobatrachus*	*acridoides*	Tanzania: Dar es Salaam: Univ. of, nr Zoology	Africa

CAS207782	Phrynobatrachidae	*Phrynobatrachus*	*africanus*	Equatorial Guinea: Bioko: Luba	Africa
MCZH-A138314	Phrynobatrachidae	*Phrynobatrachus*	*krefftii*	Tanzania: Tanga: W. Usambara Mtns	Africa
MCZH136791	Phrynobatrachidae	*Phrynobatrachus*	*sandersoni*	Cameroon: S.W.Prov.: Banyang Mbo F.R.	Africa
AC1110	Ptychadenidae	*Hildebrandtia*	*ornata*	Mozambique: Beira	Africa
AMNH-A[AMCC]158394	Ptychadenidae	*Ptychadena*	*cooperi*	Ethiopia: Bale: E. of Dinsho	Africa
AMCC117640/DPL3601	Ptychadenidae	*Ptychadena*	*mascareniensis*	Cameroon: East Prov.	Africa
ESP1134	Pyxicephalidae	*Amietia*	*fuscigula*	South Africa: W. Cape: Grootwinterhoek Mtns	Africa
AC1206	Pyxicephalidae	*Anhydrophryne*	*rattrayi*	South Africa: E. Cape: Hogsback	Africa
AMNH-A144967	Pyxicephalidae	*Arthroleptella*	*landdrosia*	South Africa: W. Cape: Landdroskop	Africa
AMCC122774/DPL3941	Pyxicephalidae	*Aubria*	*subsigillata*	Cameroon: East Province	Africa
ESP90	Pyxicephalidae	*Cacosternum*	*nanum*	South Africa: W. Cape: Storms River Mouth	Africa
AACRG0025	Pyxicephalidae	*Natalobatrachus*	*bonebergi*	South Africa: KZN: Vernon Crookes N.R.	Africa
JDV9268	Pyxicephalidae	*Pyxicephalus*	*adspersus*	Namibia: Otjiwarongo	Africa
AMCC105565	Pyxicephalidae	*Strongylopus*	*bonaespei*	South Africa: W. Cape: Landdroskop	Africa
AMNH-A144982	Pyxicephalidae	*Tomopterna*	*delalandii*	South Africa: W. Cape: Stellenbosch	Africa
YPM-HERA13758	Ranidae	*Abavorana*	*luctuosa*	Brunei Darussalam: Temburong: Bukit Pagon	Asia
BMNH2000.853	Ranidae	*Amnirana*	*galamensis*	Tanzania: E. Usambaras: Kwamguni F.R.	Africa
KIZ014016	Ranidae	*Amolops*	*chayuensis*	China: Xizang	Asia
FMNH268535	Ranidae	*Chalcorana*	*chalconota*	Thailand: Surat Thani: Kaeng Krung N.P.	Asia
CAS221687	Ranidae	*Hydrophylax*	*leptoglossa*	Myanmar: Sagging: Mon Ywa: AK P.	Asia
AMCC163973	Ranidae	*Hylarana*	*taipehensis*	Vietnam: Ha Giang	Asia
FMNH243341	Ranidae	*Meristogenys*	*orphnocnemis*	Malaysia: Sabah: Tenom: Purulon	Asia
MVZB258265	Ranidae	*Odorrana*	*banaorum*	Cambodia: Virachey N.P.	Asia
CCA17046	Ranidae	*Papurana*	*arfaki*	Papua New Guinea: Central Prov.: Laranu	Australia-New Guinea
YPX18689	Ranidae	*Pelophylax*	*nigromaculatus*	China: Chongqing: Wangsheng	Asia
ROM26861	Ranidae	*Pelophylax*	*ridibundus*	Armenia: Ankavan Field Station	Europe

FMNH248213	Ranidae	*Pulchrana*	*baramica*	Brunei: Tutong Dist: Tasek Merimbun: Sg Merimbun	Asia
KIZ0803271	Ranidae	*Rana*	*zhenhaiensis*	China: Zhejiang: Ningbo	Asia
DMH91-87	Ranidae	*Rana*	*juliani*	no data	North America
KUH195258	Ranidae	*Rana*	*maculata*	Mexico: Oaxaca: Colonia Rodulfo Figueroa	North America
KUH195186	Ranidae	*Rana*	*spectabilis*	Mexico: Hidalgo: La Estanzuela	North America
CAS212740	Ranidae	*Rana*	*catesbeiana*	USA: CA: Colusa: Mendocino N.F.	North America
CAS223402	Ranidae	*Rana*	*pipiens*	USA: CA: Spring Valley	North America
FMNH263491	Ranidae	*Sanguirana*	*luzonensis*	Philippines: Luzon: Kalinga	Asia
FMNH248431	Ranidae	*Staurois*	*guttatus*	Brunei: Belait: Labi	Asia
FMNH255637	Ranidae	*Sylvirana*	*maosonensis*	Vietnam: Nghe An	Asia
R1429	Ranixalidae	*Indirana*	*leithi*	India: Maharashtra: Pune	India
R1428	Ranixalidae	*Indirana*	*leithi*	India: Maharashtra: Pune	India
AMB7600	Ranixalidae	*Indirana*	*leithi*	India: Maharashtra: Matheran	India
R1417	Ranixalidae	*Indirana*	sp. 1	India: Kerala: Wayanad	India
MVZB241442	Rhacophoridae	*Buergeria*	*oxycephalus*	China: Hainan: Hongzha	Asia
MVZB236697	Rhacophoridae	*Chiromantis*	*doriae*	China: Hainan: Bawangling	Asia
ESP1091	Rhacophoridae	*Chiromantis*	*xerampelina*	South Africa: KZN: Bonamanzi N.R.	Africa
KIZ9387	Rhacophoridae	*Feihyla*	*vittata*	Laos: XeKong: XeKong	Asia
KIZ22320	Rhacophoridae	*Kurixalus*	*verrucosus*	China: Guizhou: Libo: Weng Ang	Asia
KIZ010760	Rhacophoridae	*Liuixalus*	sp. 1	China: Hainan	Asia
MVZB239460	Rhacophoridae	*Nyctixalus*	*pictus*	Indonesia: Sumatra: Propinsi Bengkulu	Asia
FMNH259485	Rhacophoridae	*Philautus*	*surdus*	Philippines: Luzon: Kalinga: Balbalan	Asia
R1284	Rhacophoridae	*Pseudophilautus*	*microtympanum*	Sri Lanka: Bambarakanda	India
R1260	Rhacophoridae	*Raorchestes*	sp. 2	India: Tamil Nadu: Katagiri	India
R1422	Rhacophoridae	*Raorchestes*	sp. 1	India: Karnataka: Belagavi	India
CAS233160	Rhacophoridae	*Raorchestes*	*parvulus*	Myanmar: Chin: Haka	Asia

CAS224676	Rhacophoridae	*Rhacophorus*	*rhodopus*	Myanmar: Kachin: Putao: Nagmung	Asia
MVZB225131	Rhacophoridae	*Theloderma*	*corticale*	Vietnam: Vinh Phuc: Vinh Yen: Tam Dao	Aisa
KIZ029453	Rhacophoridae	*Theloderma*	*moloch*	China: Tibet: Medog	Asia
R1208	Microhylidae	*Choerophryne*	*variegata*	Sri Lanka: Sigiriya	India
MVZB238744	Microhylidae	*Dyscophus*	*guineti*	Madagascar: Toamasina	Madagascar
CAS247917	Microhylidae	*Kalophrynus*	*pleurostigma*	Myanmar: Tanintharyi: Dawei: Yaephyu	Asia
ESP808	Microhylidae	*Phrynomantis*	*annectens*	Namibia: Erongo: Brandberg Mtn: Longipoele	Africa
ESP934	Bufonidae	*Poyntonophrynus*	*dombensis*	Namibia: Warmquelle: Ongongo C.C.	-
ZMMU-NAP-02264	Megophryidae	*Leptolalax*	*firthi*	Vietnam: Gia Lai: Kon Ka Kinh N.P.	-
SANBI1954	Heleophrynidae	*Heleophryne*	*purcelli*	South Africa: W. Cape: Marloth N.R.	-

Table 2 Calibrations used in this study.

No.	Calibrations	Fossils	References
1	Second calibration for Ranoidea from 92.8 Ma to 108.6 Ma	—	Feng et al. (1)
2	MRCA of Neobatrachia at least 66 Ma	*Beelzebufo ampinga*	Rogers et al. (2)
3	MRCA of Rhacophorinae at least 45 Ma	*Indorana prasadi*	Folie et al. (3)
4	Stem of *Pelophylax* at least 32 Ma	*Pelophylax* sp.	Rage and Roček (4)
5	Stem of *Rana pipiens* group at least 18 Ma	*Rana cf. Rana pipiens*	Holman (5, 6)
6	Node between *Ptychadena* + *Phrynobatrachus* at 25 Ma	Ptychadenidae fossil	Blackburn et al. (7)

References

1. Feng YJ, Blackburn DC and Liang D *et al*. Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary. *Proc Natl Acad Sci USA* 2017; **29**: 5864–5870.
2. Rogers RR, Krause DW and Kast SC *et al*. A new, richly fossiliferous member comprised of tidal deposits in the Upper Cretaceous Maevarano Formation, northwestern Madagascar. *Cretaceous Res* 2013; **44**: 12–29.
3. Folie A, Rana RS and Rose KD *et al*. Early Eocene frogs from Vastan ignite Mine, Gujarat, India. *Acta Palaeontol Pol* 2013; **58**:511–524.
4. Rage J and Roček Z. Evolution of anuran assemblages in the Tertiary and Quaternary of Europe, in the context of palaeoclimate and palaeogeography. *Amphibia-Reptilia* 2003; **24**: 133–167.
5. Holman JA. Early Miocene anurans from Florida. *J Florida Acad Sci* 1965; **28**: 68–82.
6. Holman JA. Additional Miocene anurans from Florida. *J Florida Acad Sci* 1968; **30**:121–140.
7. Blackburn DC, Roberts EM and Stevens NJ. The earliest record of the endemic African frog family Ptychadenidae from the Oligocene Nsungwe Formation of Tanzania. *J Vert Paleontol* 2015; **35**: e907174.2

Table 3
Dispersal multipliers for four time slices under the Biotic-ferry (1–4) and Stepping-stone biogeographic models (5–8). Dispersal rates of 0.01 represent disallowed dispersals between well-separated areas, by oceans or other land masses; 0.5 represents moderate dispersal probability between adjacent, but not connected areas; 1 represents unrestricted dispersal between well-connected areas. A = Africa, B = Madagascar, C = India, D = Australia-New Guinea, E = Asia, F = Europe, G = North America.

(1) 130–88 Ma

	A	B	C	D	E	F	G
A	1	0.5	0.5	0.01	0.01	0.5	0.01
B	0.5	1	1	0.5	0.01	0.01	0.01
C	0.5	1	1	0.5	0.01	0.01	0.01
D	0.01	0.5	0.5	1	0.01	0.01	0.01
E	0.01	0.01	0.01	0.01	1	0.5	0.5
F	0.5	0.01	0.01	0.01	0.5	1	0.5
G	0.01	0.01	0.01	0.01	0.5	0.5	1

(2) 88–55 Ma

	A	B	C	D	E	F	G
A	1	0.5	0.01	0.01	0.01	0.5	0.01
B	0.5	1	0.01	0.01	0.01	0.01	0.01
C	0.01	0.01	1	0.01	0.01	0.01	0.01
D	0.01	0.01	0.01	1	0.01	0.01	0.01
E	0.01	0.01	0.01	0.01	1	0.5	0.5
F	0.5	0.01	0.01	0.01	0.5	1	0.5
G	0.01	0.01	0.01	0.01	0.5	0.5	1

(3) 55–25 Ma

	A	B	C	D	E	F	G
A	1	0.5	0.01	0.01	0.5	0.5	0.01
B	0.5	1	0.01	0.01	0.01	0.01	0.01
C	0.01	0.01	1	0.01	1	0.5	0.01
D	0.01	0.01	0.01	1	0.5	0.01	0.01
E	0.5	0.01	1	0.5	1	0.5	0.5
F	0.5	0.01	0.5	0.01	0.5	1	0.5
G	0.01	0.01	0.01	0.01	0.5	0.5	1

(4) 25–0 Ma							
	A	B	C	D	E	F	G
A	1	0.5	0.01	0.01	1	1	0.01
B	0.5	1	0.01	0.01	0.01	0.01	0.01
C	0.01	0.01	1	0.01	1	0.01	0.01
D	0.01	0.01	0.01	1	0.5	0.01	0.01
E	1	0.01	1	0.5	1	1	0.5
F	1	0.01	0.01	0.01	1	1	0.5
G	0.01	0.01	0.01	0.01	0.5	0.5	1
(5) 130–88 Ma							
	A	B	C	D	E	F	G
A	1	0.5	0.5	0.01	0.01	0.5	0.01
B	0.5	1	1	0.5	0.01	0.01	0.01
C	0.5	1	1	0.5	0.01	0.01	0.01
D	0.01	0.5	0.5	1	0.01	0.01	0.01
E	0.01	0.01	0.01	0.01	1	0.5	0.5
F	0.5	0.01	0.01	0.01	0.5	1	0.5
G	0.01	0.01	0.01	0.01	0.5	0.5	1
(6) 88–55 Ma							
	A	B	C	D	E	F	G
A	1	0.5	0.5	0.01	0.01	0.5	0.01
B	0.5	1	0.5	0.01	0.01	0.01	0.01
C	0.5	0.5	1	0.01	0.5	0.01	0.01
D	0.01	0.01	0.01	1	0.01	0.01	0.01
E	0.01	0.01	0.5	0.01	1	0.5	0.5
F	0.5	0.01	0.01	0.01	0.5	1	0.5
G	0.01	0.01	0.01	0.01	0.5	0.5	1
(7) 55–25 Ma							
	A	B	C	D	E	F	G
A	1	0.5	0.01	0.01	0.5	0.5	0.01

B	0.5	1	0.01	0.01	0.01	0.01	0.01
C	0.01	0.01	1	0.01	1	0.5	0.01
D	0.01	0.01	0.01	1	0.5	0.01	0.01
E	0.5	0.01	1	0.5	1	0.5	0.5
F	0.5	0.01	0.5	0.01	0.5	1	0.5
G	0.01	0.01	0.01	0.01	0.5	0.5	1
(8) 25–0 Ma							
	A	B	C	D	E	F	G
A	1	0.5	0.01	0.01	1	1	0.01
B	0.5	1	0.01	0.01	0.01	0.01	0.01
C	0.01	0.01	1	0.01	1	0.01	0.01
D	0.01	0.01	0.01	1	0.5	0.01	0.01
E	1	0.01	1	0.5	1	1	0.5
F	1	0.01	0.01	0.01	1	1	0.5
G	0.01	0.01	0.01	0.01	0.5	0.5	1

Table 4 The results of likelihood-ratio tests and AIC comparisons for alternative biogeographic models, based on different dispersal matrices of Table 3.

Biogeographic Hypotheses	Model	LnL	d	e	j	AIC	AIC_wt
Biotic ferry	DEC	-156.7	0.24	0.29	0	317.4	4.1E-29
	DEC+J	-91.46	1E-12	1E-12	0.051	188.9	0.33
Stepping stone	DEC	-139.6	0.2	0.17	0	283.2	2.1E-24
	DEC+J	-85.18	1E-12	1E-12	0.048	176.4	0.33