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Phylogeny and Biogeography of a Cosmopolitan Frog Radiation: Late Cretaceous Diversification Resulted in Continent-Scale Endemism in the Family Ranidae

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Abstract.—Ranidae is a large anuran group with a nearly cosmopolitan distribution. We investigated the phylogenetic relationships and early biogeographic history of ranid frogs, using 104 representatives of all subfamilies and families, sampled from throughout their distribution. Analyses of ~1570 bp of nuclear gene fragments (*Rag-1*, *rhod*, *Tyr*) and ~2100 bp of the mitochondrial genome (12S *rRNA*, $tRNA^{VAL}$, 16S *rRNA*) indicate that the monophyly of several taxa can be rejected with high confidence. Our tree is characterized by a clear historical association of each major clade with one Gondwanan plate. This prevalence of continent-scale endemism suggests that plate tectonics has played a major role in the distribution of ranid frogs. We performed dispersal-vicariance analyses, as well as analyses constrained by paleogeographic data, to estimate ancestral distributions during early ranid diversification. Additionally, we used molecular clock analyses to evaluate whether these scenarios fit the temporal framework of continental breakup. Our analyses suggest that a scenario in which the ancestors of several clades (Rhacophorinae, Dicroglossinae, Raninae) reached Eurasia via the Indian subcontinent, and the ancestor of Ceratobatrachinae entered via the Australia–New Guinea plate, best fits the paleogeographic models and requires the fewest number of dispersal/vicariance events. However, several alternatives, in which part of the ranid fauna colonized Laurasia from Africa, are not significantly worse. Most importantly, all hypotheses make clear predictions as to where to expect key fossils and where to sample other living ranids, and thus constitute a strong basis for further research. [Anura; biogeography; convergence; radiation; Ranidae; Ranoidea.]

With more than 1000 extant species, Ranidae *sensu lato* (i.e., *sensu* Dubois, 2005) is one of the largest extant families of anurans. This cosmopolitan group of frogs evolved an enormous diversity in all kinds of habitats, such as forests, savannas, grasslands, and deserts. The characters associated with this ecological, morphological, and developmental diversity have been utilized extensively by systematists for generating hypotheses on ranid relationships (Clarke, 1981; Dubois, 1992; Blommers-Schlösser, 1993; Inger, 1996). In recent years, the integration of molecular phylogenetic studies (e.g., Bossuyt and Milinkovitch, 2000; Emerson et al., 2000; Roelants et al., 2004) led to revised classifications, recognizing several subfamilies with a wide distribution (e.g., Dicroglossinae, Raninae) (Dubois, 2003; Frost, 2004). However, because most investigations were based on relatively short gene sequences, or were limited to taxonomically or geographically restricted groups, a hypothesis for all major lineages of Ranidae is still lacking. In this study, we provide the first comprehensive estimate of the phylogenetic relationships of ranid frogs, with the secondary goal of evaluating concordance between evolutionary relationships and affinities suggested by current taxonomies (Dubois, 2003; Frost, 2004). We used species sampled extensively from all currently and previously recognized taxonomic groups, and from all the continents. Second, we applied several combinations of calibration points, and used the union of these individual estimates as confidence intervals to obtain a conservative approximation of the timing of the major ranid diversifications. Third, we sought to evaluate the early biogeographic history of ranid frogs by evaluating our tree topologies and divergence time estimates in light of prevailing paleogeographic models.

MATERIALS AND METHODS

Taxon Sampling and DNA Sequencing

This study includes 104 representatives of all frog subfamilies and families recognized in Ranoidea (Frost, 2004), sampled from throughout the distribution of the group (Appendix 1). Six hyloid and one sooglossid species served as outgroups. The following nuclear protein-coding gene fragments were PCR-amplified and cycle-sequenced on both strands: (i) a region of ~555 base pairs (bp) in the recombinase activating gene 1 (*Rag-1*); (ii) a region of ~534 bp in exon 1 of tyrosinase (*Tyr*); and (iii) a region of ~490 bp in exon 1 and 4 of the rhodopsin gene (*Rhod*). A fourth fragment covers ~2100 bp of 12S *rRNA*, $tRNA^{VAL}$, and 16S *rRNA* of the mitochondrial genome. Primers used in this study are given elsewhere (Bossuyt and Milinkovitch, 2000; Darst and Cannatella, 2004; Hillis and Wilcox, 2005).

Sequence Alignment and Phylogeny Inference

Sequences were aligned using ClustalX 1.64 (Thompson et al., 1997), and ambiguous sections, identified by eye for mtDNA and by comparison with amino acid sequences for nuDNA, were excluded for subsequent analyses. The data matrix has been deposited in TreeBase under accession number SN2787. Phylogeny estimations were obtained under the maximum parsimony (MP) and the maximum likelihood

(ML) criteria, and in a Bayesian framework. MP searches were performed using the parsimony ratchet as implemented in PAUPRAT (Sikes and Lewis, 2001) and PAUP* (Swofford, 1998) (200 iterations of the ratchet, with TBR branch swapping). Clade support under MP was evaluated using 10,000 replicates of nonparametric bootstrapping. Appropriate likelihood models were determined using the Akaike Information Criterion implemented in ModelTest 3.06 (Posada and Crandall, 1998). ML analyses were first performed with PHYML 2.1b1 using a GTR+Γ+I model of sequence evolution. Using the best tree found by PHYML as a starting tree, heuristic ML searches were executed with PAUP* 4.0b10, with TBR branch swapping, and all parameter values estimated. Clade stability was estimated by non-parametric bootstrapping in 500 replicates with PHYML. Because distant outgroups can influence inferred relationships among ingroup taxa (Lyons-Weiler et al., 1998), independent ML analyses were conducted on a taxon set composed of the ingroup alone. We also conducted 250 replicated metaGA searches (Lemmon and Milinkovitch, 2002) using MetaPIGA 1.0.2b with probability consensus pruning among four populations, using a HKY + rate heterogeneity + I model (the most parameter-rich model in MetaPIGA 1.0). The metaGA is an evolutionary computation heuristic (i.e., implementing a set of operators that mimic processes of biological evolution) that vastly improves the speed and efficiency with which ML trees are found and yields a probability index for each branch. It has been suggested that a metaGA search with a finite number of populations provides an estimate of the posterior probability distribution of possible trees (Lemmon and Milinkovitch, 2002). The 1000 resulting trees were therefore used to compute a majority-rule consensus tree and calculate posterior branch support (PBS) values. Bayesian analyses were performed using MrBayes 3.0 (Ronquist and Huelsenbeck, 2003), both under mixed models per locus and under the GTR+Γ+I model for all loci, using default settings as priors. We conducted two independent analyses (with and without outgroup), in which four chains were run simultaneously for 2×10^7 generations and trees were sampled every 1000 cycles. Bayesian posterior probabilities (PP) were estimated as the 50% majority-rule consensus tree of the 18,000 last sampled trees (2000 samples discarded as burn-in), and also of the 10,000 last sampled trees (10,000 samples discarded as burn-in). Given that the dynamic of searching tree space using the metaGA is quite different from that using MCMCMC, high support values generated by the two above methods increases our confidence in the validity of that support.

Evaluation of Alternative Phylogenetic Hypotheses

Thirty-two alternative phylogenetic hypotheses for ranid frogs, represented by candidate trees estimated under ML using conventional, backbone, or reversed constraints in PAUP*, were compared using the approximately unbiased (AU) test (Shimodaira, 2002). Site-wise log-likelihoods for all trees were estimated using PAUP* and used as input for CONSEL 0.1g (Shimodaira and Hasegawa, 2001). Multiscale bootstrap resampling was conducted in ten sets of 10,000 replicates each, with scale parameters ranging from 0.5 to 1.4. Because sampling density is a function of likelihood score in our Bayesian analyses, we additionally assessed support by examining the proportion of trees (in the accepted sample) that support or reject a particular hypothesis (Pauly et al., 2004).

Setting the Biogeographic Framework

For biogeographic analyses, two aspects concerning the complex geological history of tectonic plates should be considered. First, there can be a time interval of tens of million of years between the first indications of continental rift and the subsequent sea-floor spreading (Chatterjee and Scotese, 1999). Clearly, the time of actual separation is most relevant in biogeographic analyses. Second, the relative position of Gondwanan landmasses during the late Cretaceous is debated, and extensive plate reshuffling was probably accompanied by the formation of multiple temporary landbridges (Fig. 1a–f) (e.g., Gunnerus Ridge, Kerguelen Plateau, Greater Somalia) (Chatterjee and Scotese, 1999; Briggs, 2003; Hay et al., 1999; Rage, 2003; Sereno et al., 2004). With these points in mind, we briefly review recent progress in paleogeographic models to set a conservative framework for evaluating hypotheses on ranid biogeography. The geological events that can be important for frog evolution are here briefly discussed in three major classes of terrestrial connections: intra-Gondwanan routes, Gondwana-Laurasia connections, and intra-Laurasian routes.

Intra-Gondwanan routes.—Madagascar-India-Seychelles started rifting from Africa about 165 Mya, and complete disjunction was probably attained in the early Cretaceous (121 Mya), with the opening of the Somali Basin (Sanmartin and Ronquist, 2004). However, faunal dispersal between these landmasses may have remained possible via the tenuous "Central corridor" (Fig. 1c) until 90 Mya (Chatterjee and Scotese, 1999). Furthermore, at a time when Madagascar was separated from India-Seychelles, a nearly continuous landbridge reconnected Madagascar to Africa from the Middle Eocene (approximately 45 Mya) until the early Miocene (approximately 26 Mya) (McCall, 1997). Although most models accept that India has been isolated for a considerable time, the standard "biotic ferry" model (Hedges, 2003), which shows India isolated from other Gondwanan landmasses by large expanses of water for several tens of million years (Fig. 1d), has been challenged by both geological and paleontological data (Patriat and Segoufin, 1988; Chatterjee and Scotese, 1999; Briggs, 2003; Rage, 2003). First, Greater India rifted from Antarctic-Australia-New Guinea ca. 130 Mya (Briggs, 2003), but the latter plate may have remained joined by the Kerguelen Plateau (connection with India) and the Gunnerus Ridge (connection with Madagascar) until 80 Mya (Hay et al., 1999) (Fig. 1b). Second, contact between

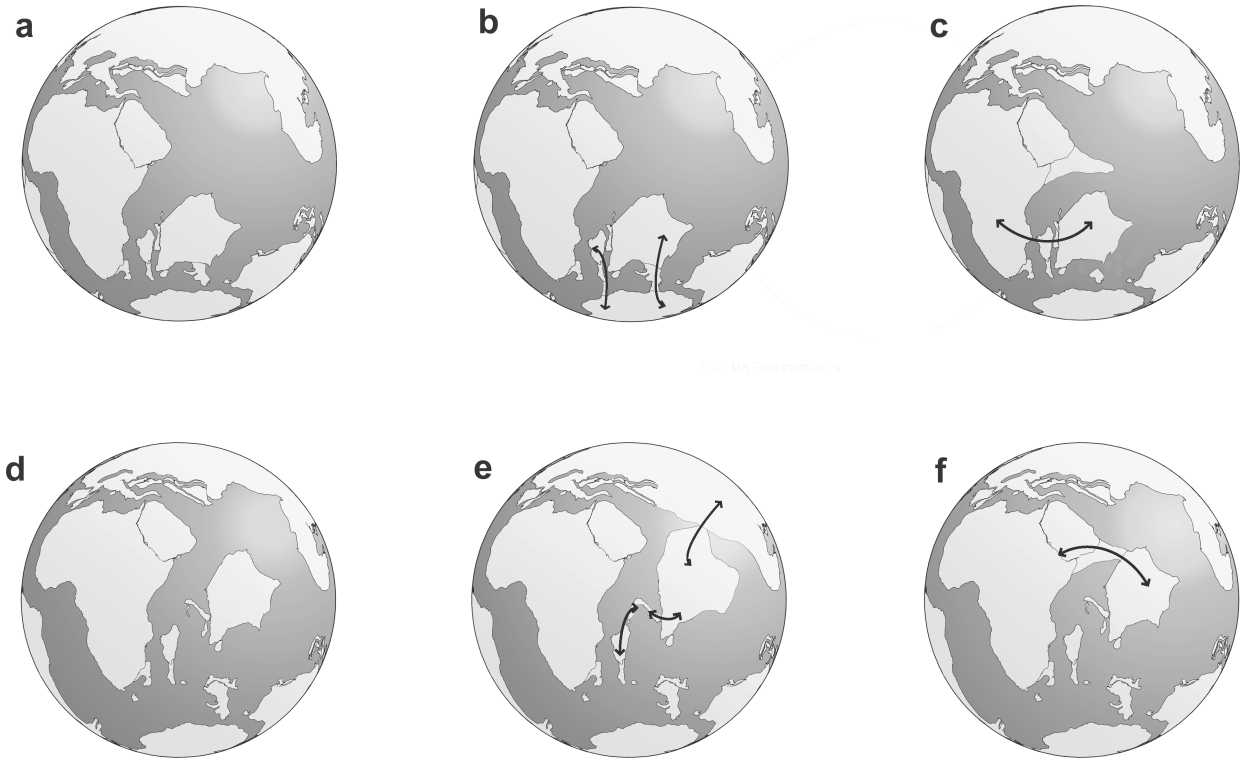

FIGURE 1. Schematic representation of different hypotheses on land connections and corridors for dispersal (indicated by arrows) between landmasses about 88 Mya (a–c) and 65 Mya (d–f). (a) The breakup of Madagascar and India-Seychelles (Storey et al., 1995; Scotese, 2001), no more connections between the major Gondwanan units. (b) Prolonged dispersal between Antarctica and Indo-Madagascar over the Gunnerus ridge (left arrow) and the Kerguelen plateau (right arrow) (Hay et al., 1999). (c) Closer distance between between Africa and Indo-Madagascar (Briggs, 2003), with possible dispersal via a Central Corridor (Chatterjee and Scotese, 1999). (d) The standard biotic ferry model (Hedges, 2003): India and Madagascar are completely isolated at the KT boundary (Scotese, 2001). (e) Different position of India and Madagascar, with dispersal between Eurasia and Madagascar possible over India and the Seychelles plateau (Patriat and Segoufin, 1988; Rage, 2003). (f) Reconnection of Africa and India over Greater Somalia (Chatterjee and Scotese, 1999).

India and Madagascar remains controversial: most models agree on initial fragmentation around 88 Mya (Storey et al., 1995) (Fig. 1a) and complete disjunction by 80 Mya (Chatterjee and Scotese, 1999). However, a land bridge between India and Madagascar via the Seychelles Plateau (Patriat and Segoufin, 1988) (Fig. 1e) until the close of the Cretaceous (65 Mya), perhaps because of a different longitudinal position for India (Briggs, 2003), has also been conceived (Rage, 2003). Third, Chatterjee and Scotese (1999) suggested that India and Africa became joined by a landbridge, termed Greater Somalia (Fig. 1f), at the end of the Cretaceous, about 70 to 75 Mya. Plate tectonic models indicate that this connection may have allowed biotic interchange until about 60 Mya.

Gondwana-Laurasia connections.—There have been four primary routes for faunal interchange between Gondwana and Laurasia: first, North America was probably connected to South America by a terrestrial route during the latest Cretaceous (Rage and Rocek, 2003). Second, throughout the Mesozoic, Africa was probably separated from Eurasia by the Tethys Sea, which continued to form a barrier until collision of the body of the Afro-Arabian plate with Eurasia in the Late Eocene (approximately 40 Mya) (Van Yperen et al., 2005). The Mediterranean Sill may have formed routes (stepping stones) between Europe/southwestern Asia and Africa in the Cretaceous and early Tertiary for some taxa, but this route must have been discontinuous (Rage and Rocek, 2003) and dispersal during that period must be regarded as transoceanic. Third, the timing of the collision of India with Eurasia is controversial, but a vast northern extension of Greater India may have brought about contact with Eurasia earlier than generally thought (Beck et al., 1995). A recent compilation of data indicate that the initial collision took place between 55 and 50 Mya in the Early Eocene, although other estimates range from the Late Maastrichtian (>65) to 40 Mya (Beck et al., 1995, and references therein). Fourth, the Australia–New Guinea landmass collided with the Phillipine Sea plate at ca. 25 Mya, and the continued rotation of the latter subsequently caused accretion of fragments from the northern Australian margin into the southeast Asian margin (Hall, 1996).

Intra-Laurasian routes.—During the Late Mesozoic, Laurasia was divided into two palaeocontinents, Euramerica and Asiamerica, that were separated by two epicontinental seaways, the Turgai Strait and the

Mid-Continental Seaway (Sanmartin et al., 2001). The latter closed around the KT transition, but Europe connected to Asia only by the end of the Eocene, when the Uralian or Western Siberian Sea (Obik Sea + Turgai Strait) became partially closed (Rage and Rocek, 2003), resulting in possible terrestrial dispersal routes. Throughout the Tertiary, Trans-Atlantic and Trans-Beringian landbridges have been periodically available for animal dispersal between Eurasia and North America (reviewed in Sanmartin et al., 2001).

Spatial and Temporal Diversification

Reconstruction of early spatial diversification.—In our biogeographic analyses, we consider seven Mesozoic and Cenozoic areas that correspond to historically persistent landmasses within which dispersal (and speciation) can happen at no cost (Sanmartin and Ronquist, 2004): (1) Africa, (2) Madagascar, (3) India (including Sri Lanka), (4) Australia–New Guinea, (5) Eurasia, (6) North America, and (7) South America. Because our primary goal is to evaluate the *early* biogeographic history, strongly supported subfamilial clades for which dispersal-vicariance analysis (implemented using DIVA; Ronquist, 1997) and parsimony optimization (conducted in MacClade; Maddison and Maddison, 2000) show an unambiguous place of origin were lumped into a single ancestral area. Evaluation of biogeographic scenarios for early ranid evolution was then done on the ML tree of both the total data set and the unrooted analysis. Reconstruction of ancestral areas or composites of these individual areas at each node was done in two independent analyses. First, we used the software DIVA (Ronquist, 1997), which assumes that speciation is caused by vicariance and only imposes a cost for a dispersal or extinction event. This method has the advantage of not being constrained by uncertainties or inaccuracies in geological models and allows the kind of reticulate area relationships that is indicated by paleogeographic data (Ronquist, 1997). However, because the above analyses are not restricted by plate tectonic evidence, the suggested dispersal events are not necessarily between adjacent plates and can constitute long-distance oceanic dispersal (followed by vicariance between two areas that have never been connected according to plate tectonic evidence). Therefore, for each node, we evaluated a posteriori whether the proposed event fits prevailing paleogeographic models. For example, a reconstruction that suggests an Asia to Madagascar dispersal, followed by vicariance between these two landmasses, is scored as an improbable dispersal/vicariance event (anomaly), because no geological model has ever suggested a direct Asia-Madagascar connection (India not being involved). As a second, independent approach, we evaluated multiple scenarios by reconstructing ancestral distributions under the a priori constraint that vicariance events follow at least one of the prevailing models of continental breakup. Finally, because a temporal framework is extremely important for correct interpretation, all of the above scenarios are evaluated against divergence time estimates. As a conservative approach, we use an interval that is the union of three independent 95% credibility intervals for nodal ages (see below). If this interval does not overlap with any tectonic evidence for terrestrial connections, the initially presumed terrestrial dispersal event is secondarily scored as an anomaly. For example, although India-to-Asia dispersal is allowed, it can be scored as improbable if the nodal estimate predates the late Maastrichtian, i.e., the earliest proposed terrestrial connection between both landmasses (Beck et al., 1995).

Divergence time estimates.—We estimated nodal ages and 95% credibility intervals from our nuclear DNA sequence data using the Bayesian molecular clock method implemented in the software MultiDivtime (Thorne and Kishino, 2002). The assumption of constant rates over time is not required for this method, and multiple constraints on node ages are allowed. We extended our data set with frogs (*Ascaphus truei*, *Alytes obstetricans*, *Rhinophrynus dorsalis*, *Pipa* sp., *Hymenochirus boettgeri*, *Pelobates cultripes*, *Mantidactylus wittei*, and *Mantidactylus* sp. and *Boophis* sp. from the Comores), salamanders (*Salamandra salamandra* and *Hynobius* sp.), human, and chicken to obtain additional calibration points for estimation of divergence ages. We added coelacanth as an outgroup, and additionally included *Chirixalus cf. doriae* to estimate the most recent divergence time between Eurasian and African rhacophorines (Wilkinson et al., 2002). Uncertain phylogenetic relationships of nonranid lineages were constrained as polytomies. Based on fossil and tectonic evidence, we used a prior of 344 ± 20 Mya for the split between Lissamphibia and Amniota (Ruta et al., 2003), and imposed the following time constraints on internal nodes:

1. A minimum age of 306.1 Mya and maximum age of 332.3 Mya for the split between mammals (synapsids) and birds (diapsids) (Van Tuinen et al., 2004; Graur and Martin, 2004).
2. A minimum age of 245 Mya for the frog-salamander split, based on †*Triadobatrachus* (Rage and Rocek, 1989).
3. A minimum age of 164 Mya for the origin of the Cryptobranchoidea, based on the fossil †*Chunerpeton tianyiensis*, of Bathonian age (Gao and Shubin, 2003).
4. A minimum age of 164 Mya for the origin of Discoglossoidea, based on the fossil †*Eodiscoglossus oxoniensis*, of Bathonian age (Evans et al., 1990; Rocek, 2000).
5. A minimum age of 151 Mya for the origin of Rhinophrynidae, based on the fossil †*Rhadinosteus parvus*, of Kimmeridgian age (Henrici, 1998).
6. A minimum age of 86 Mya for the divergence of the South American *Pipa* from the African *Hymenochirus*. Our calibration point is conservative, since it corresponds to the final separation (i.e., permanent equatorial seaway of significant depth) of the respective continents (Pitman et al., 1993; Sereno, 2004).
7. A maximum age of 15 Mya (the volcanic origin of the oldest Comoro island Mayotte) for the split between

Boophis tephraeomystax and its sister species (Vences et al., 2003b) on Mayotte.

8. A maximum age of 15 Mya for the split between *Mantidactylus wittei* and its sister species on Mayotte.

Because most of our calibration points are outside Ranidae, and no reliable fossil evidence is available to circumvent this problem, we repeated the analyses with three combinations of calibration points: (1) all calibration points; (2) calibration point 1; (3) calibration points 2 to 8. Because the second and third analysis are completely independent, and because we use an interval that is the union of the three individual estimates to further test hypotheses, this approach can be considered conservative. All analyses were duplicated to confirm successful convergence towards the proper distributions for divergence ages.

RESULTS

Phylogenetic Relationships

Phylogeny of Ranoidea.—Sequences were deposited in GenBank under accession numbers DQ346956 to DQ347411. The concatenated data set consisted of a matrix of 3679 characters, of which 2659 could be reliably aligned. MP analyses produced 14 equally parsimonious trees (length = 12,369 steps, not shown). Maximum likelihood analyses of the total data set produced a single ML tree (lnL = −59,664.54). Unrooted ML analyses, i.e., including only frogs of the family Ranidae, produced a very similar topology, differing mainly in the fact that the Ceratobatrachinae and the (Ranixalinae, Micrixalinae) lineage switched positions. Our analyses of ranoid relationships (Fig. 2) show that three main clades receive strong statistical support. First, a clade of six subfamilies (Arthroleptinae, Astylosterninae, Leptopelinae, Hyperoliinae, Hemisotinae, and Brevicipitinae; node A, Brevicipitidae) received strong support. With the exception of a few nested hyperoliine representatives from Madagascar (*Heterixalus*) and the Seychelles (*Tachycnemis*), this clade is tightly associated with Africa (Vences et al., 2003a). Second, Microhylidae (node C), with the exclusion of Brevicipitinae, form a clade with a wide distribution. The third clade (node D) contains all remaining ranoid sub(families), hereafter referred to as Ranidae (cf. AmphibiaWeb). With the exception of southern South America and most of Australia, this clade has a cosmopolitan distribution. At the base of the ranid subtree, the mainly African-distributed subfamily Ptychadeninae forms the sister group of all remaining ranids with high support (node O).

Unexpected high degree of continent-scale endemism in frogs of the family Ranidae.—Several earlier taxonomic hypotheses based on morphology, molecules, or combined evidence are corroborated by our analyses. Conversely, many currently recognized taxa do not form clades (Table 1, hypotheses 19 to 32), and monophyly of most of these taxonomic hypotheses can be rejected with a high degree of confidence. Most intriguingly, our tree is characterized by a combination of both known and unexpected radiations having occurred each in a single biogeographic region, with few dispersal events across these regions. This endemism (in terms of earlier or current tectonic plates) is extensive at several levels of divergence, indicating a much wider continental scale of regional radiation than previously assumed for ranids (Savage, 1973; Inger, 1999; Dubois, 2003; Frost, 2004). For example, *Batrachylodes* from the Solomon Islands, which is currently classified in Raninae (Dubois, 2003), is nested within a strongly supported clade (Fig. 2, first split after node M) grouping several genera (*Batrachylodes*, *Ceratobatrachus*, *Discodeles*, *Platymantis*) essentially restricted to New Guinea, Fiji, Bismarck, Admiralty, Palau, and/or Solomon Islands (i.e., Australia–New Guinea area) and the Philippines. Second, our ML, MetaPIGA, and Bayesian trees demonstrate a large clade of ranids associated with subsaharan Africa (Fig. 2, node R). This suggests an extensive radiation of African frogs, containing species that are currently (incorrectly in some cases) classified in up to seven different subfamilies (Cacosterninae, Dicroglossinae, Raninae, Petropedetinae, Phrynobatrachinae, Pyxicephalinae, Tomopterninae; Dubois, 1992; Dubois, 2003). This African clade receives high Bayesian (PP = 97) and moderate MetaPIGA (PBS = 72) support, and although monophyly of this group is not significant under the AU test (Table 1, hypothesis 18), the clustering of "dicroglossine" and "ranine" constituents with other representatives of their traditional subfamily (Table 1, hypotheses 24 and 30) is strongly rejected ($P < 0.001$). If future research corroborates monophyly of this group, this African assemblage would contain some of the smallest anurans (10 mm among some *Phrynobatrachus* and *Arthroleptella*) as well as the world's largest frog, *Conraua goliath* (adult size up to 320 mm) (Dubois, 2003). Even if this assemblage as a whole does not form a clade, a sub-clade (node Q) of the former is supported by all analyses. Its members fill a broad range of niches, both in terms of adult ecomorphs (e.g., burrowing and torrential frogs) and developmental modes (e.g., most have aquatic larval stages but direct development is present in *Arthroleptella*) (van der Meijden et al., 2005). Most of the African ecomorphs have counterparts with similar adaptations in other radiations that have occurred on different land masses (Bossuyt and Milinkovitch, 2000). Nested in this group is a strongly supported clade (node P) that is restricted to the Cape Province, and includes at least four genera (but possibly also *Anhydrophryne* and *Microbatrachella*, not in our analyses). Ranid evolution is clearly characterized by Gondwanan break-up and geographically restricted adaptive radiations associated with striking ecological/morphological convergences among different regions.

Alternative hypotheses.—Cacosterninae and Petropedetinae (or Petropedetidae) have been recognized as groups that include various frog genera confined to sub-Saharan Africa. Although these species indeed cluster with other African species in our tree, monophyly of these taxa as strictly defined by various authors (Duellman and Trueb, 1986, largely followed by Frost,

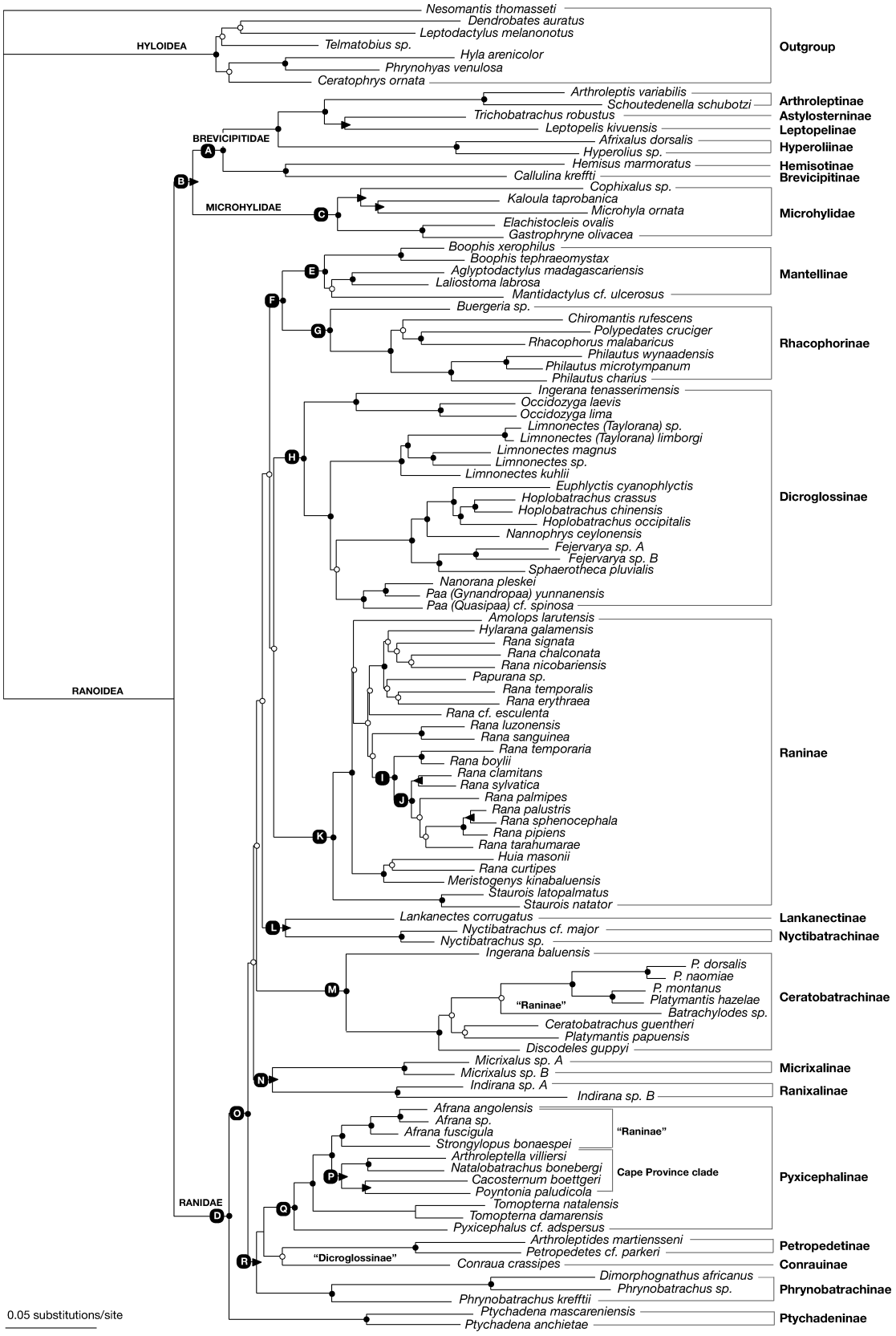
Nesomantis thomasseti
Dendrobates auratus
Leptodactylus melanonotus
HYLOIDEA
Telmatobius sp.
Hyla arenicolor
Phrynohyas venulosa
Ceratophrys ornata
Outgroup
Arthroleptis variabilis
Schoutedenella schubotzi
Arthroleptinae
Trichobatrachus robustus
Astylosterninae
Leptopelis kivuensis
Leptopelinae
BREVICIPITIDAE
A
Afrixalus dorsalis
Hyperolius sp.
Hyperoliinae
Hemisus marmoratus
Hemisotinae
B
Callulina kreffti
Brevicipitinae
Cophixalus sp.
Kaloula taprobanica
MICROHYLIDAE
C
Microhyla ornata
Elachistocleis ovalis
Microhylidae
Gastrophryne olivacea
Boophis xerophilus
Boophis tephraeomystax
E
Aglyptodactylus madagascariensis
Laliostoma labrosa
Mantellinae
Mantidactylus cf. ulcerosus
F
Buergeria sp.
Chiromantis rufescens
G
Polypedates cruciger
Rhacophorus malabaricus
Philautus wynaadensis
Rhacophorinae
Philautus microtympanum
Philautus charius
Ingerana tenasserimensis
Occidozyga laevis
Occidozyga lima
Limnonectes (Taylorana) sp.
Limnonectes (Taylorana) limborgi
H
Limnonectes magnus
Limnonectes sp.
Limnonectes kuhlii
Euphlyctis cyanophlyctis
Hoplobatrachus crassus
Hoplobatrachus chinensis
Hoplobatrachus occipitalis
Dicroglossinae
Nannophrys ceylonensis
Fejervarya sp. A
Fejervarya sp. B
Sphaerotheca pluvialis
Nanorana pleskei
Paa (Gynandropaa) yunnanensis
Paa (Quasipaa) cf. spinosa
Amolops larutensis
Hylarana galamensis
Rana signata
Rana chalconata
Rana nicobariensis
Papurana sp.
RANOIDEA
Rana temporalis
Rana erythraea
Rana cf. esculenta
Rana luzonensis
Rana sanguinea
Rana temporaria
Rana boylii
I
Rana clamitans
Rana sylvatica
J
Rana palmipes
Rana palustris
Rana sphenocephala
K
Rana pipiens
Rana tarahumarae
Huia masonii
Rana curtipes
Meristogenys kinabaluensis
Staurois latopalmatus
Staurois natator
Raninae
Lankanectes corrugatus
Lankanectinae
L
Nyctibatrachus cf. major
Nyctibatrachinae
Nyctibatrachus sp.
Ingerana baluensis
P. dorsalis
P. naomiae
M
P. montanus
Platymantis hazelae
"Raninae"
Batrachylodes sp.
Ceratobatrachinae
Ceratobatrachus guentheri
Platymantis papuensis
Discodeles guppyi
Micrixalus sp. A
Micrixalinae
Micrixalus sp. B
N
Indirana sp. A
Ranixalinae
Indirana sp. B
O
Afrana angolensis
Afrana sp.
Afrana fuscigula
"Raninae"
Strongylopus bonaespei
Arthroleptella villiersi
Natalobatrachus bonebergi
P
Cacosternum boettgeri
Cape Province clade
Poyntonia paludicola
Pyxicephalinae
Tomopterna natalensis
RANIDAE
Q
Tomopterna damarensis
D
Pyxicephalus cf. adspersus
Arthroleptides martiensseni
Petropedetinae
Petropedetes cf. parkeri
R
"Dicroglossinae"
Conraua crassipes
Conrauinae
Dimorphognathus africanus
Phrynobatrachinae
Phrynobatrachus sp.
Phrynobatrachus krefftii
Ptychadena mascareniensis
Ptychadeninae
Ptychadena anchietae
0.05 substitutions/site

FIGURE 2.

TABLE 1. Hypothesis testing. Results of the AU test (Shimodaira, 2002) and Bayesian posterior probabilities (BPP) for 32 alternative hypotheses. The nodes A to R correspond to the clades in Figure 1. Δ lnL is the difference of likelihood between the ML tree and the best tree compatible with the constraint indicated under "hypothesis"; "++" score indicates rejection by the AU test and BPP at $\alpha = 0.05$; "−+" indicates rejection by the BPP alone; "−−" indicates failure of rejection by either test.

Ref.	Hypothesis	Node	Δ ln L	AU	BPP	Rejection
1	Non-monophyly of Brevicipitidae	A	6.35	0.473	0.021	−+
2	No Brevicipitidae-Microhylidae clade	B	1.87	0.840	0.220	−−
3	Non-monophyly of Microhylidae	C	96.80	<0.001	<0.001	++
4	Non-monophyly of Ranidae (as defined here)	D	23.30	0.124	<0.001	−+
5	Non-monophyly of Mantellinae	E	47.66	0.023	<0.001	++
6	No Mantellinae-Rhacophorinae clade	F	6.98	0.409	0.005	−+
7	Non-monophyly of Rhacophorinae	G	37.78	0.001	<0.001	++
8	Non-monophyly of Dicroglossinae	H	31.07	0.020	<0.001	++
9	Non-monophyly of New World *Rana* + *Rana temporaria*	I	33.28	0.011	<0.001	++
10	Non-monophyly of New world *Rana* (excl.*Rana boylii*)	J	27.91	0.038	<0.001	++
11	Non-monophyly of Raninae	K	71.73	<0.001	<0.001	++
12	Non-monophyly of Indian (Micrixalinae + Ranixalinae)	N	4.66	0.553	0.038	−+
13	Non-monophyly of Ceratobatrachinae	M	64.42	0.007	<0.001	++
14	Non-monophyly of (Lankanectinae + Nyctibatrachinae)	L	11.85	0.151	<0.001	−+
15	No basal position for Ptychadeninae in Ranidae	O	5.65	0.473	0.001	−+
16	Non-monophyly of African Cape ranids	P	14.74	0.072	<0.001	−+
17	Non-monophyly of Pyxicephalinae	Q	21.44	0.088	<0.001	−+
18	Non-monophyly of Large African clade	R	4.71	0.549	0.032	−+
19	Cacosterninae *sensu* Dubois, 2003 (1)	n.a.	16.99	0.182	<0.001	−+
20	Cacosterninae *sensu* Dubois, 2003 (2)	n.a.	237.11	<0.001	<0.001	++
21	Petropedetinae *sensu* Dubois, 2003	n.a.	155.45	0.002	<0.001	++
22	Petropedetidae *sensu* Frost	n.a.	126.77	0.001	<0.001	++
23	Amolopinae *sensu* Fei, Ye & Jiang, 2000	n.a.	57.82	0.004	<0.001	++
24	Dicroglossinae *sensu* Dubois, 2003	n.a.	494.18	<0.001	<0.001	++
25	Ceratobatrachini *sensu* Dubois, 2003	n.a.	651.92	<0.001	<0.001	++
26	Limnonectini *sensu* Dubois, 2003	n.a.	197.84	<0.001	<0.001	++
27	Paini *sensu* Dubois, 2003	n.a.	15.10	0.179	<0.001	−+
28	Ranidae *sensu* Dubois, 2003	n.a.	24.07	0.114	<0.001	−+
29	Ranidae *sensu* Frost, 2004	n.a.	234.03	<0.001	<0.001	++
30	Ranini *sensu* Dubois, 2003	n.a.	842.69	<0.001	<0.001	++
31	Blommers-Schlosser 1993 as backbone constraint	n.a.	38.05	0.122	<0.001	−+
32	Haas 2003 as backbone constraint	n.a.	742.45	<0.001	<0.001	++

2004; Dubois, 2003) is mostly rejected (Table 1, hypotheses 19 to 22). The subfamily Amolopinae groups four genera (*Amo*, *Amolops*, *Huia*, and *Meristogenys*) with an exclusively southeast Asian distribution. Our tree shows that *Rana curtipes* also falls in this clade and that monophyly of Amolopinae can be rejected (Table 1, hypothesis 23). This extends the distribution of this clade as a whole to the Western Ghats of the Indian subcontinent. The Dicroglossinae (hypothesis 24), as well as the tribes provisionally recognized in this group (hypotheses 25 to 27) (Dubois, 2003), are also challenged by our analyses. With the exception of the AU test for Paini ($P = 0.179$), monophyly of these groups is rejected at $P < 0.001$ by both tests. The family Ranidae has been defined in several ways (e.g., Dubois, 2003; Frost, 2004), which differ mainly in inclusion/exclusion of Petropedetinae, Phrynobatrachinae, Mantellinae, and Rhacophorinae. Monophyly of Ranidae *sensu* Dubois (2003) (hypothesis 28) is rejected by BPP ($P < 0.001$), but not by the AU test ($P = 0.114$), whereas Ranidae *sensu* Frost (2004) (hypothesis 29) is rejected by both methods ($P < 0.001$). Species in the subgroup Ranini, which was already considered a catch-all tribe (Dubois, 2003), also do not form a clade (hypothesis 30, $p < 0.001$; BPP < 0.001). Two morphology-based hypotheses (Table 1, hypothesis 31 and 32) (Blommers-Schlösser, 1993; Haas, 2003) on ranoid relationships were tested by constraining hypothesized relationships as a backbone. The former is rejected by BPP ($P < 0.001$), but not by the AU-test ($P = 0.122$), whereas the latter is rejected by both methods ($P < 0.001$).

Spatial and Temporal Diversification

Based on our ML tree, inference of the most likely ancestral distribution is straightforward for many clades. Within Rhacophorinae, Dicroglossinae, Raninae, and

FIGURE 2. Maximum likelihood phylogram ($-\ln L = 59{,}664.54$) for the total (mitochondrial + nuclear gene fragments) data set. Analyses without outgroup species gave very similar results, but Ceratobatrachinae and the (Micrixalinae, Ranixalinae) clade switched places. For each clade, branch support is indicated as follows: black circles: BPP and PBS ≥ 95%; triangle pointing to the right: BPP > 95%, PBS < 95%; triangle pointing to the left: BPP < 95%, PBS > 95%; white circles: BPP and PBS < 95%. Clades discussed in the text and included in the AU test are indicated with letters A to R in black circles. Suggested taxonomic recognition of clades is indicated at the right of the tree, and lineages currently classified differently are indicated between full quotes.

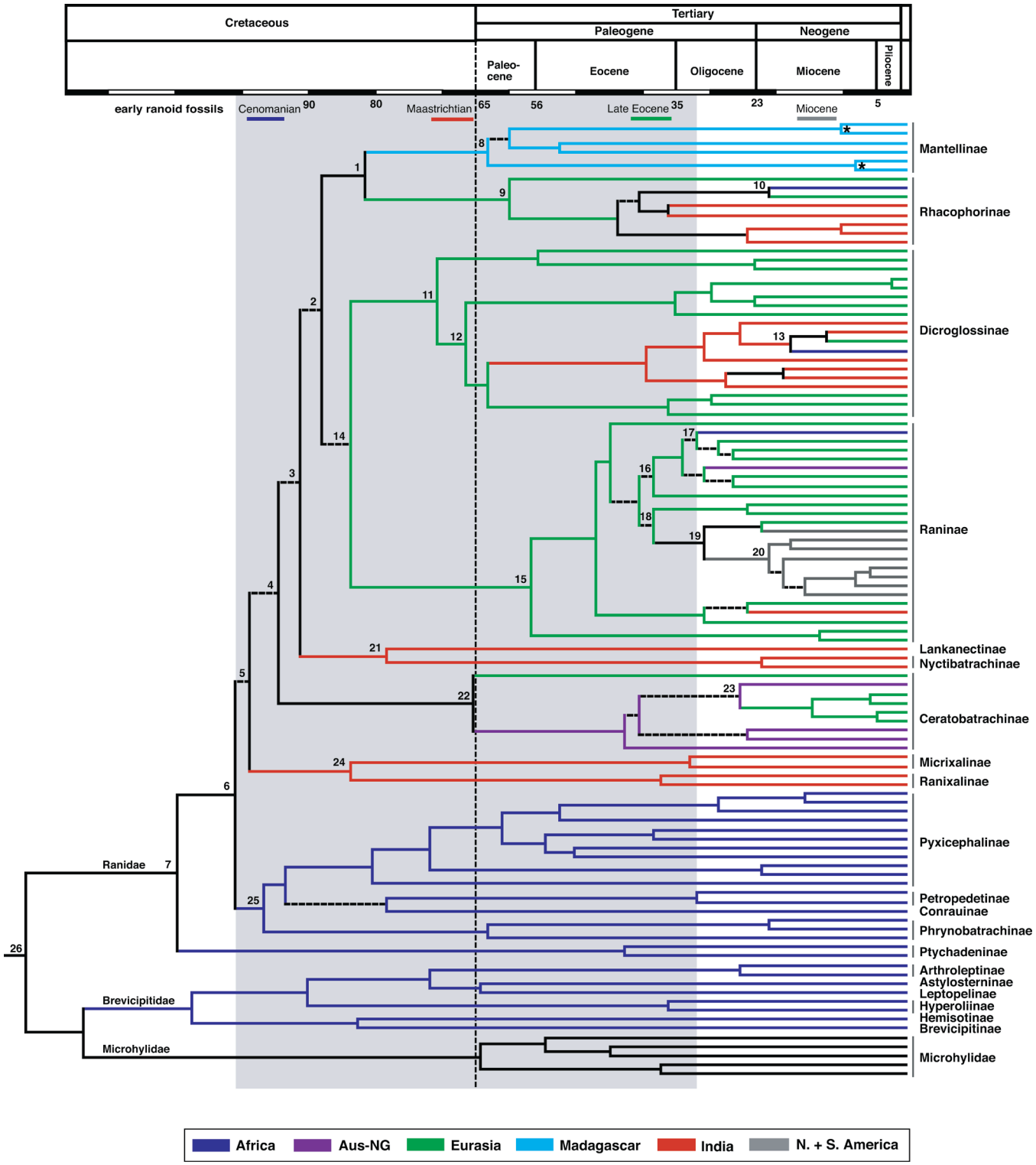

FIGURE 3. A molecular timescale for ranid evolution. The dashed branches indicate lineages whose phylogenetic position is ambiguous (PB and PBS support values <95%). Colored bars at the top of the tree indicate the age of Ranoidea fossils from the respective continents: (1) undetermined Ranoidea from the Cenomanian of Africa (Báez and Werner, 1996); (2) Ranidae from the Maastrichtian of India (Prasad and Rage, 2004); (3) Raninae from the Late Eocene of Europe (Rage and Rocek, 2003); (4) Raninae from the Miocene of North America (Rage and Rocek, 2003). The grey background indicates the possible absence of dispersal between Africa and any other biogeographical unit (between nodes 6 and node 17) for about 70 million years. The dotted vertical line at 65 Mya represents the K-T boundary. Numbers on the nodes are cross-referenced in Figure 4. The two internal calibration points are indicated with an asterisk.

TABLE 2. Divergence time estimates (mean ± standard deviation, and 95% confidence interval) for 26 relevant nodes in our ML tree. The three estimates represent use of the following calibrations: (1) all calibration points; (2) calibration points 2 to 8 (i.e., excluding the mammal-bird calibration; (3) calibration point 1 (i.e., only the mammal-bird calibration). The accepted interval (used to test the hypotheses discussed in the present paper) is the union of the three individual estimates.

	Estimate 1		Estimate 2		Estimate 3		Accepted interval
Node	Mean ± SD	95% interval	Mean ± SD	95% interval	Mean ± SD	95% interval	
1	82.7 ± 13.6	58.5–111.8	81.4 ± 13.7	57.0–110.8	85.0 ± 15.0	58.6–117.8	57.0–117.8
2	89.9 ± 14.3	64.6–120.6	88.5 ± 14.5	62.9–118.7	92.2 ± 15.7	64.5–126.2	62.9–126.2
3	92.9 ± 14.7	67.0–124.3	91.5 ± 14.9	65.1–122.8	95.2 ± 16.0	66.8–129.8	65.1–129.8
4	95.9 ± 15.1	69.4–128.3	94.5 ± 15.3	67.3–126.7	98.3 ± 16.4	69.0–133.7	67.3–133.7
5	101.0 ± 15.6	73.3–134.6	99.5 ± 15.7	71.5–132.2	103.5 ± 16.8	73.6–139.3	71.5–139.3
6	102.9 ± 15.8	74.7–136.9	101.2 ± 15.9	72.9–134.5	105.3 ± 17.0	74.9–141.7	72.9–141.7
7	111.3 ± 16.9	81.4–147.7	109.6 ± 17.0	79.1–145.7	113.7 ± 18.2	81.5–152.6	79.1–152.6
8	64.1 ± 11.5	43.7–89.1	63.1 ± 11.6	42.6–88.5	66.7 ± 13.1	43.6–95.8	42.6–95.8
9	60.8 ± 11.6	40.7–86.0	60.0 ± 11.7	39.8–85.7	62.8 ± 12.6	41.0–90.3	39.8–90.3
10	21.5 ± 6.1	11.7–35.5	21.1 ± 6.0	11.5–34.7	22.1 ± 6.5	12.0–37.3	11.5–37.3
11	72.3 ± 12.7	50.2–100.2	71.2 ± 12.8	48.2–99.1	74.2 ± 13.9	49.7–104.6	48.2–104.6
12	67.4 ± 12.2	46.3–93.8	66.4 ± 12.2	44.7–93.2	69.1 ± 13.2	46.0–98.0	44.7–98.0
13	17.3 ± 5.6	8.7–30.4	17.2 ± 5.7	8.5–30.2	17.8 ± 5.9	8.7–31.5	8.5–31.5
14	85.6 ± 13.9	61.0–115.6	84.2 ± 14.1	59.1–114.1	87.8 ± 15.2	60.8–120.6	59.1–120.6
15	57.1 ± 11.6	37.2–82.1	56.2 ± 11.6	36.1–81.1	58.8 ± 12.5	37.7–85.9	36.1–85.9
16	39.0 ± 8.9	23.8–58.4	38.3 ± 8.8	23.5–57.6	40.2 ± 9.4	24.5–61.0	23.5–61.0
17	32.0 ± 7.8	19.0–49.5	31.5 ± 7.7	18.6–48.6	33.1 ± 8.3	19.4–51.8	18.6–51.8
18	38.8 ± 8.9	23.7–58.5	38.2 ± 8.8	23.3–57.6	40.0 ± 9.4	24.4–61.0	23.3–61.0
19	31.2 ± 8.1	18.0–49.3	30.7 ± 7.9	17.4–48.2	32.3 ± 8.5	18.6–51.3	17.4–51.3
20	21.4 ± 6.4	11.3–35.9	21.0 ± 6.2	11.0–35.6	22.1 ± 6.7	11.6–37.3	11.0–37.3
21	79.7 ± 13.7	55.7–109.3	78.4 ± 13.8	54.2–108.0	81.7 ± 14.9	55.7–113.9	54.2–113.9
22	66.7 ± 12.9	44.3–95.3	65.7 ± 12.9	43.3–93.8	68.4 ± 13.8	44.2–99.3	43.3–99.3
23	25.7 ± 7.2	14.1–42.4	25.4 ± 7.3	13.8–42.2	26.4 ± 7.6	14.4–43.7	13.8–43.7
24	84.8 ± 14.6	59.0–116.0	83.5 ± 14.7	57.3–114.3	86.7 ± 15.5	59.5–120.3	57.3–120.3
25	98.8 ± 15.4	71.5–132.0	97.2 ± 15.6	69.5–130.1	101.2 ± 16.6	71.8–136.2	69.5–136.2
26	135.4 ± 19.1	100.9–176.6	133.2 ± 19.3	98.6–173.2	137.5 ± 20.3	101.4–180.8	98.6–180.8

Ceratobatrachinae, however, dispersal events between different areas (for example, in *Polypedates*, *Fejervarya*, *Sylvirana*, *Platymantis*, respectively) makes inference of the ancestral distribution more problematic, and our sampling is insufficient to allow accurate reconstruction of the number and direction of dispersal events within these groups. However, in the first three of these clades, Eurasian ancestry is supported by dispersal-vicariance analysis (DIVA) and parsimony optimization (MacClade), and we therefore attributed a strictly Eurasian (non-Indian) distribution to the basal node in these clades (see also Kosuch et al., 2001). In Ceratobatrachinae, relationships among species within the sister group of *Ingerana baluensis* are weakly supported, and we acknowledge that the basal node can be either Eurasia or Australia–New Guinea.

Our molecular clock analyses suggest that the main ranid lineages diversified relatively rapidly, probably during changing geological configurations in the Late Cretaceous (Fig. 3). This result is robust to choice of calibration points (Table 2), and the union of the three individual estimates is used below to evaluate the likelihood of biogeographic scenarios.

DIVA analyses.—Reconstruction of ancestral distributions using DIVA (Fig. 4a, b) show ranids ancestrally widespread on Africa, Australia–New Guinea, India, and Eurasia for both ML topologies. Vicariance between an African clade and a group that is distributed on a larger area that includes Eurasia (node 6) suggests a Pangaean distribution. However, because Pangaean breakup has been estimated between 180 to 150 Mya (Scotese, 2001), our time estimate for node 6 (72.9 to 141.7 Mya) suggests that this ancestral distribution should be considered improbable. Furthermore, reconstructions on both ML trees contain two vicariance events that do not fit any of the paleogeographic models. First, the vicariant split between Rhacophorinae and Mantellinae (node 1) is made possible through a preceding dispersal directly from Eurasia to Madagascar. Such a dispersal may have been terrestrial under some palaeographic scenarios (Fig. 1e), but would have required the Indian subcontinent (and the Seychelles) as a transitional landmass. Therefore, the suggested vicariance between Rhacophorinae on Eurasia and Mantellinae on Madagascar is an anomaly. Second, the basal split in Ceratobatrachinae is considered a vicariant event between Australia–New Guinea and Eurasia. However, there are no paleogeographic models that are in agreement with such an event, because all accept that the Australia–New Guinea landmass moved towards Eurasia, not the opposite. The tree in Figure 4b (node 5) shows an additional vicariance event between an Australia–New Guinea-Eurasia landmass and India, which similarly must be considered unlikely.

Paleogeographic evidence-constrained analyses.—Contrary to what DIVA analyses suggest, Neobatrachia shows an undeniable association with landmasses of Gondwanan origin (Feller and Hedges, 1998; Biju and Bossuyt, 2003), and the sister-group relationship of Ptychadeninae to all other ranids strengthens our conviction

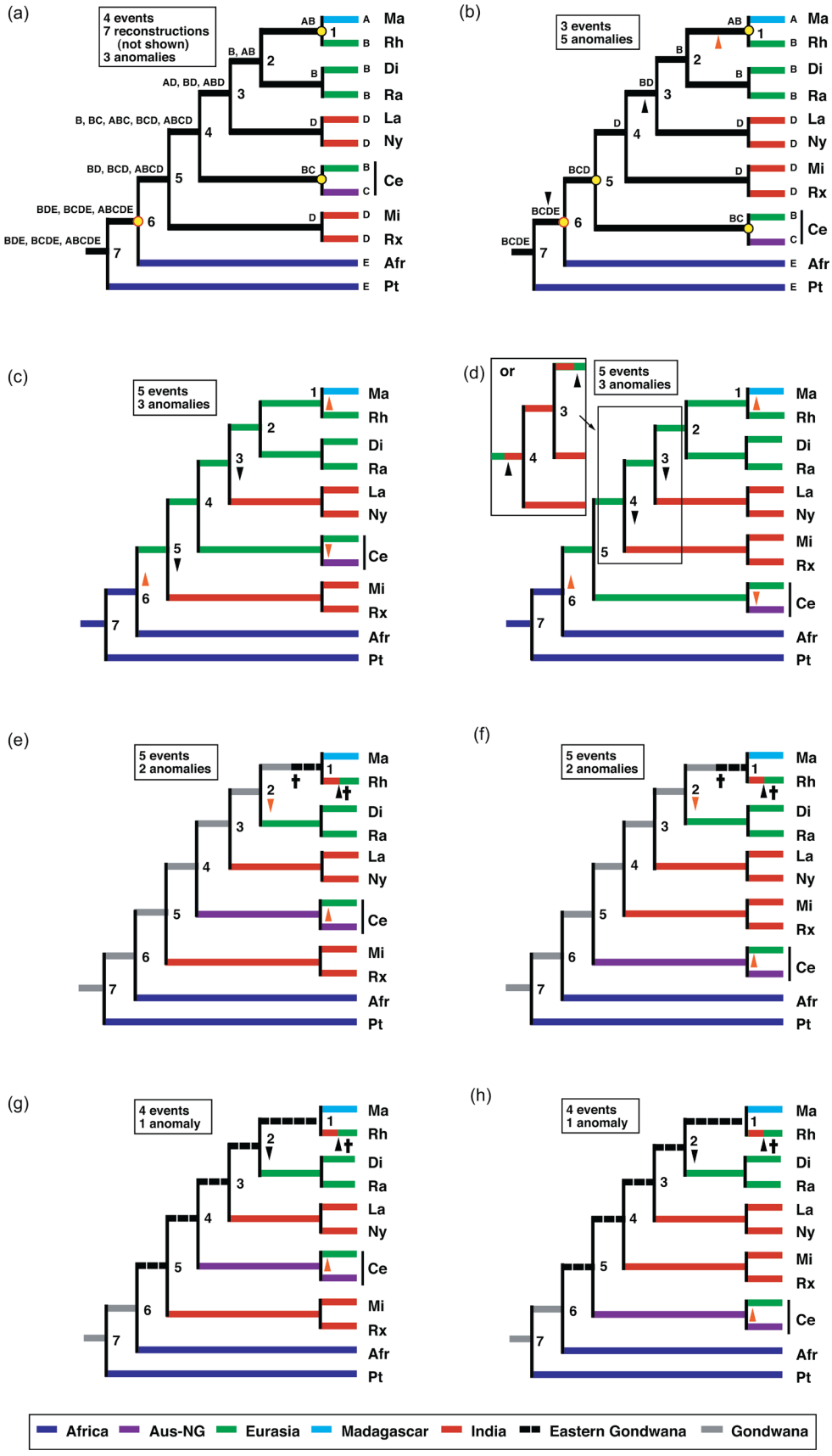
(a)
4 events
7 reconstructions
(not shown)
3 anomalies
AB
B, AB
AD, BD, ABD
B, BC, ABC, BCD, ABCD
BD, BCD, ABCD
BDE, BCDE, ABCDE
BDE, BCDE, ABCDE
BC
(b)
3 events
5 anomalies
BD
BCD
BCDE
BCDE
(c)
5 events
3 anomalies
(d)
or
5 events
3 anomalies
(e)
5 events
2 anomalies
(f)
5 events
2 anomalies
(g)
4 events
1 anomaly
(h)
4 events
1 anomaly
Ma
Rh
Di
Ra
La
Ny
Ce
Mi
Rx
Afr
Pt
Africa
Aus-NG
Eurasia
Madagascar
India
Eastern Gondwana
Gondwana

FIGURE 4.

that frogs currently assigned to the family Ranidae have an origin in the southern hemisphere. Accepting this premise, there are four possible Gondwanan landmasses through which ranids might have entered Laurasia: (1) South America, (2) Africa, (3) the Indian subcontinent, (4) Australia–New Guinea. We dismiss the first option, because the deeply nested position of South American *Rana*, together with our dating estimates, clearly indicate a recent colonization (see below) of South America (through North America). Australia–New Guinea to Asia dispersal as the *only* route of Laurasian colonization is extremely unlikely because of the nearly complete absence of Ranidae on Australia (a single ranine species, *Rana daemeli*), a requirement for six dispersal events (not shown) to explain the current distribution of Ranidae, and the incompatibility of our time estimates for several of these nodes with available terrestrial routes at that time. We therefore further explore in detail the best scenarios that indicate full or partial colonization from Africa (Rage, 2001), and the best scenarios with no out-of-Africa dispersal, i.e., basically the out-of-India hypothesis (as proposed by Bossuyt and Milinkovitch, 2001).

Our ML tree shows a single split between African and mostly non-African lineages (Fig. 4c, d, node 6). Therefore, the area-based cladogram is in agreement with a single dispersal from Africa to Eurasia. Both trees can be explained by five dispersal events, but they require two of these to be transoceanic: first, node 6 is estimated to be between 72.9 and 141.7 Mya, and there was probably no terrestrial route available at that time. Second, node 1 represents Eurasia to Madagascar dispersal, which must have been overseas (see above). This colonization could have happened using India as an intermediate landmass (Fig. 1e), but this scenario would require an extra extinction event on India. A better explanation for a partial out-of-Africa hypothesis (Fig. 4e, f) is to accept a Gondwanan (Eastern + Western Gondwana) distribution for nodes 2 to 7. Because node 2 is always older than node 1, and because India was far from Eurasia before the initial East-West breakup of Gondwana, dispersal from Africa to Asia at node 2 would constitute the first arrival of ranids on Eurasia. Under this scenario, the fragmentation into Eastern and Western Gondwana must have happened between nodes 2 and 1, because all geological models accept complete disjuntion of Africa and Madagascar when the latter starts rifting from Indo-Seychelles. This event should therefore be older than 121 Mya (the complete disjuntion of Africa from Indo-Madagascar; Scotese, 2001), or at the very least 90 Mya (end of contact via the Central Corridor; Chatterjee and Scotese, 1999). Although our estimate of 62.9 to 126.2 Mya for node 2 is in accordance with this scenario, the first terrestrial contact between Africa and Eurasia is only much later, around 40 Mya (Van Yperen et al., 2005). Under currently accepted paleogeographic models, such an early Africa-Eurasia faunal interchange should be regarded as transoceanic dispersal. Furthermore, accepting an all-Gondwana distribution at node 2 requires that the lineage that goes to node 1 continues to be all-Gondwana for some time. However, because all geological models agree on a complete separation of Africa and Madagascar at the time of the break-up of Indo-Madagascar (node 1), this scenario also requires an extra extinction event on Africa (indicated by a cross in Fig. 4e and f).

The fewest dispersal/vicariance events is required when assuming that Ranidae entered Laurasia only via India and Australia–New Guinea. Indeed, our ML tree is in agreement with the presence of Ranidae on India during its northward drift (Duellman and Trueb, 1986; Bossuyt and Milinkovitch, 2001) and our 95% estimate of 72.9 to 141.7 Mya (Fig. 4g, h, node 6) allows a vicariant event between Africa and Indo-Madagascar (at that time likely connected to Antarctica-Australia–New Guinea). Frogs currently assigned to Ceratobatrachinae then probably evolved from ancestors that originated on the Antarctica-Australia–New Guinea landmass, which may have been connected to Greater India until 80 Mya via the Kerguelen Plateau (connection with India) and the Gunnerus Ridge (connection with Madagascar) (Hay et al. 1999; Case, 2002; Rage, 2003). Our estimate of 71.5 to 139.3 Mya (Fig. 4g, node 4; Fig. 4h, node 5) indicates that this split can represent a vicariant event. The ranid lineages currently endemic to the Western Ghats and/or Sri Lanka (Micrixalinae, Ranixalinae, Nyctibatrachinae, Lankanectinae) indicate a considerable period of isolation (Roelants et al., 2004), and are in agreement with an origin on Greater India. The split between Mantellinae

FIGURE 4. Comparison of the minimum number of events (dispersal and extinction) and anomalies ("impossible" vicariance, or oceanic dispersal) necessary under different reconstructions of ancestral distributions for Ranidae, shown on the best ML tree of the total dataset (left) and the reduced (i.e., no outgroup) data set (right). Abbreviations are the first two letters of the subfamilies shown in Figure 2, except for Rx, Ranixalinae and Af, the African radiation (Pyxicephalinae, Petropedetinae, Conrauinae, and Phrynobatrachinae). Abbreviations for geological units are A = Madagascar (light blue); B = Eurasia (green); C = Australia–New Guinea (purple); D = India (red); and E = Africa (dark blue); gray = Gondwana, dashed = Eastern Gondwana. Black arrows indicate terrestrial dispersal, orange arrows indicate oceanic dispersal (i.e., not fitting paleogeographic models in light of our accepted interval of time estimates). Oceanic dispersal is here used to denote dispersal across all kinds of not strictly terrestrial routes, and thus ranges from dispersal via stepping stones to long-distance oceanic dispersal. Yellow circles at nodes indicate that the split is spatially (black border) or temporally (red border) not supported by paleogeographical models. (a) Reconstruction of ancestral distributions on the best ML tree gives seven possible reconstructions (not shown) with four dispersals. (b) DIVA gives a single reconstruction with three dispersals on the reduced ML tree. (c and d) A single colonization of Eurasia from Africa requires five dispersals, three of which are oceanic. (e and f) A triple colonization of Eurasia, i.e., from Africa, India, and Australia–New Guinea requires five events (three dispersals, two extinctions), two of which constitute anomalies. (g and h) A colonization of Laurasia via India and Australia–New Guinea requires four events (three dispersal, one extinction), one being an anomaly. Note that DIVA analyses are not directly comparable with the other analyses.

and Rhacophorinae (Fig. 4g, h, node 1) can be explained as a vicariance event between India and Madagascar. Although complete disjunction of these plates remains controversial, all models agree on an initial fragmentation around 88 Mya (Storey et al., 1995), and complete disjunction, including a possible connection via the Seychelles plateau (Patriat and Segoufin, 1988), at the close of the Cretaceous. Our divergence estimate of 57.0 to 117.8 Mya for node 1 is compatible with this split being caused by this vicariant event. Given the above relationship, the colonization of Eurasia under this scenario must have happened in at least two waves. The first dispersal event from India to Eurasia (Fig. 4g, h, node 2) is estimated to have happened between 62.9 and 126.2 Mya. This must have been before the complete separation of India and Madagasar at node 1, and preferably when India already formed a land connection with Eurasia. Both conditions possibly existed for a short period during the Late Maastrichtian (Fig. 1e; Patriat and Segoufin, 1988; Rage, 2003). Second, Rhacophorinae must have dispersed to Eurasia, somewhere between node 1 (Fig. 4g, h) and the basal split within Rhacophorinae (Fig. 3, node 9), i.e., between 39.8 and 117.8 Mya (Table 2). The strictly Asian (i.e., non-Indian) origin for node 1 additionally requires a subsequent extinction on India.

Colonization of the New World.—The earliest ranids in Europe are known at least from the late Eocene (∼56–35 Mya) and belong to the ranine clade, whereas the earliest ranine fossil in North America is from the Miocene (∼23–5 Mya) (Rage and Rocek, 2003). Our ML tree and dating estimates indicate that Ranidae colonized the New World (subgenus *Rana*) and Europe (subgenera *Rana* and *Pelophylax*) from Asia in the Oligocene or Miocene. Dispersal of ancestral green frogs (*Rana*, subgenus *Pelophylax*) to Europe may have been quick, because several fossils are known from Miocene localities in Europe (Rage and Rocek, 2003). The ancestor(s) of the American *Rana* reached the New World in the Late Eocene, Oligocene, or early Miocene (Fig. 3), in one or two waves. In the former case, a back-dispersal event to Eurasia is required to explain the occurrence of brown frogs (subgenus *Rana*) in Eurasia.

Discussion

The high degree of historical association of each major clade with a single Gondwanan plate (or two in the case of India and Eurasia), and divergence times that are contemporary with the breakup of these plates, suggest that plate tectonics has played a major role in the current distribution of ranid frogs. Our analyses imply that the early biogeographic history of Ranidae can be largely explained with minimal dispersal across saltwater barriers, although oceanic dispersal has been demonstrated (Vences et al., 2003b; Evans et al., 2003). Our topologies and divergence time estimates are best in agreement with two colonization routes of Laurasia from Gondwana for ranid frogs, one via India, and a second via the Australia–New Guinea plate. The relative period of isolation of India from other landmasses in the Cretaceous and early Tertiary, and its impact on the development of an endemic fauna and flora, has been heavily debated. Whereas some studies seem to find clear evidence for phylogenies that reflect the break-up of Gondwana (e.g., Hedges et al., 1996; Ericson et al., 2003), such patterns are not always evident in other groups (Vences, 2004). The KT boundary was marked by extensive Deccan Traps volcanism on India, with lava outflows that reached as far as the east coast (Knight, 2003) and thus spanned the entire width of the Indian subcontinent. Still, our analyses suggest that the Western Ghats' endemic lineages survived in situ, possibly far from the lava flows, on the edge of the subcontinent, i.e., southern India and Sri Lanka.

Most paleogeographic models agree that Africa also was isolated for a long time (but see Chatterjee and Scotese, 1999), approximately between 105–90 Mya (separation from South America and gradual disappearance of the Central corridor, respectively) and 45–40 Mya (connection to Madagascar, and reconnection with Eurasia, respectively). If the split between the large African ranid clade and its sister group (Fig. 4g, h, node 6) represents a vicariance event, the absence of dispersal between Africa and any other continent for a long period (Fig. 3, grey area, defined by nodes 6 and 17) may be the result of this extended isolation. As such, the large African clade of Ranidae shows analogy with Afrotheria, a clade of mammals that probably evolved on Africa during its isolation (Hedges, 2001). Furthermore, no dispersal between Africa and Eurasia is obvious from our tree since the mid-Miocene. This may be related to the formation of the Sahara, which began its shift from a tropical to an arid environment in that period, following the Neogene warmth climax (17 to 15 Mya) (Douady et al., 2003).

It must be noted that several other hypotheses, e.g., partial or full out-of-Africa dispersal, require only few extra dispersal or extinction events, and that these alternatives cannot be rejected with significance. The hypotheses compared here make clear predictions about where one should expect to find key fossils and where additional living ranids should be sampled. For example, any finding of an early ranine or dicroglossine could support or reject some of the hypotheses, or could indicate that extinction has obfuscated patterns assessed from living species. A discovery of such a fossil in Eurasia before the India plate collision would be evidence for partial colonization from Africa, whereas discovery in India would confirm their evolution on the subcontinent. At present, the fossil record of Ranidae is too poor to allow reconstruction of diversification for the main ranid lineages, but their temporal appearance on each of the continents is in congruence with our tree topology. The earliest presumed ranoid fossils extend back to the Cenomanian (∼99–93.5 Mya) of Africa (Báez and Werner, 1996), where frogs of the family Ranidae probably originated. The description of a frog "belonging to the ranid-rhacophorid assemblage" from the Maastrichtian (∼71–65 Mya) of Naskal, India (Prasad and Rage, 2004) is evidence for the presence of ranids on India in that period. The late appearance of Ranidae in the fossil record of Europe, and later in the New World (Rage and Rocek,

2003), completes the "logical" sequence of ranid fossil discoveries. Future refinement of paleogeographic models, together with reinterpretation of morphological evolution through detailed morphological studies of extant and fossil species, will likely help to further clarify the biogeographic history of Ranidae.

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APPENDIX 1. List of taxa included in this study and their sampling locality, with corresponding tissue reference or voucher specimen numbers. Abbreviations: CMNH, Cincinnati Museum of Natural History; DLSUD, De La Salle University Reference Collection; FMNH, Field Museum of Natural History; MVZ, Museum of Vertebrate Zoology, Berkeley; NMP: National Museum of the Philippines; NMT, National Museum of Taiwan; TMM, Texas Memorial Museum; TNHC Texas Natural History Collections; UW, University of Wisconsin (Madison) Natural History Museum; VUB, Vrije Universiteit Brussel; ZFMK, Zoologisches Forschungsinstitut und Museum A. Koenig; CAS, California Academy of Sciences; CCA, Chris C. Austin; RMB, Rafe M. Brown; DCC, David C. Cannatella; RAN, Ronald A. Nussbaum; CR, Christina Richards; MV, Miguel Vences; ES, Elizabeth Scott.

Genus	Species	Locality	Tissue reference/voucher
Ceratophrys	*ornata*	*Unknown (pet shop)*	VUB1006
Dendrobates	*auratus*	*Unknown (pet shop)*	VUB0986
Hyla	*arenicolor*	USA	TNHC 61118
Leptodactylus	*melanonotus*	Costa Rica	MVZ 207294 (FC14298)
Nesomantis	*thomasseti*	Seychelles	RAN 25171
Phrynohyas	*venulosa*	*Unknown (pet shop)*	VUB0987
Telmatobius	*niger*	South America	TNHC 62930
Afrana	*angolensis*	Kenya	CAS 191519
Afrana	*fuscigula*	Africa	CR 1073
Afrana	sp.	Rep. South Africa	CAS 211668
Afrlxalus	*dorsalis*	Equatorial Guinea	CAS 207523
Aglyptodactylus	*madagascariensis*	Madagascar	VUB0933 (MV)
Amolops	*larutensis*	Thailand	TNHC 57944-JAM 1371
Arthroleptella	*villiersi*	Africa	CR 1070
Arthroleptides	*martiensseni*	Africa	CR 10898
Arthroleptis	*variabilis*	Equatorial Guinea	CAS 207822
Batrachylodes	sp.	Solomon Islands	VUB0799
Boophis	*xerophilus*	Madagascar	ZFMK 66705
Boophis	*tephraeomystax*	Madagascar	VUB0936 (MV)
Buergeria	*sp*	Taiwan	VUB0797
Cacosternum	*boettgeri*	Namibia	ZFMK 66727
Callulina	*kreftti*	Tanzania	TNHC 62491
Ceratobatrachus	*guentheri*	Solomon Islands	VUB1017 (SR5543)
Chiromantis	*rufescens*	Equatorial Guinea	CAS 207599
Conraua	*crassipes*	Cameroon	ZFMK 75446
Cophixalus	sp.	New Guinea	TNHC 51333
Dimorphognathus	*africanus*	Equatorial Guinea	CAS 207779
Discodeles	*cf. guppyi*	New Britain Island	UW-JF189
Elachistocleis	*ovalis*	South America	TNHC-DCC 3301
Euphlyctis	*cyanophlyctis*	India	VUB0039
Fejervarya	sp. *A*	India	VUB0017
Fejervarya	sp. *B*	India	VUB0054
Gastrophryne	*olivacea*	USA	TNHC 61952
Hemisus	*marmoratus*	Kenya	CAS 214843
Hoplobatrachus	*crassus*	Sri Lanka	VUB0107
Hoplobatrachus	*occipitalis*	*unknown (pet shop)*-Africa	VUB0537
Hoplobatrachus	*chinensis*	Vietnam	VUB0684
Huia	*masonii*	Java Isl., Indonesia	TNHC 59912-RMB 2122
Hylarana	*galamensis*	Kenya	CAS 214840
Hyperolius	sp.	Kenya	VUB0924
Indirana	sp. *A*	India	VUB0037
Indirana	sp. *B*	India	VUB0023
Ingerana	*tenasserimensis*	Myanmar	CAS 205064
Ingerana	*baluensis*	Mt. Kinabalu, Borneo, Malaysia	FMNH 44690
Kaloula	*taprobanica*	Sri Lanka	VUB0102
Laliostoma	*labrosa*	Madagascar	ZFMK 66698
Lankanectes	*corrugatus*	Sri Lanka	VUB0106
Leptopelis	*kivuensis*	Uganda	CAS 201700
Limnonectes	*kuhlii*	Vietnam	VUB0930 (MV)
Limnonectes	*magnus*	Mindanao Isl., Philippines	VUB0965
Limnonectes	sp.	Sulawesi Isl., Indonesia	TNHC 59014
Limnonectes (Taylorana)	sp.	Yunnan Province, S. China	NMT-nn
Limnonectes (Taylorana)	*limborgi*	Laos	VUB1218
Mantidactylus	*cf. ulcerosus*	Madagascar	ZFMK 66659
Meristogenys	*kinabaluensis*	Mt. Kinabalu, Borneo, Malaysia	VUB0627
Micrixalus	sp. *A*	India	VUB0013
Micrixalus	sp. *B*	India	VUB0268
Microhyla	*ornata*	India	VUB0066
Nannophrys	*ceylonensis*	Sri Lanka	VUB0172
Nanorana	*pleskei*	China	VUB0722 (NJNU F97034)
Natalobatrachus	*bonebergi*	Rep. South Africa	VUB0952
Nyctibatrachus	*cf. major*	India	VUB0006
Nyctibatrachus	sp.	India	VUB0089

(Continued on next page)

APPENDIX 1. *(Continued)*

Genus	Species	Locality	Tissue reference/voucher
Occidozyga	*laevis*	Luzon Isl., Philippines	VUB0967 (DLSUD 002)
Occidozyga	*lima*	Java Isl., Indonesia	TNHC 59864-RMB 2134
Paa	*yunnanensis*	Vietnam	VUB0691
Paa	*cf. spinosa*	Vietnam	VUB0713
Papurana	sp.	New Guinea	TNHC 54732
Petropedetes	*cf. parkeri*	Africa	VUB0955 (MV)
Philautus	*microtympanum*	Sri Lanka	VUB0158
Philautus	*wynaadensis*	India	VUB0070
Philautus	*charius*	India	VUB0075
Phrynobatrachus	sp.	Rep. South Africa	VUB0953
Phrynobatrachus	*krefftii*	Tanzania	VUB1068 (ES700)
Platymantis	*dorsalis*	Luzon Isl., Philippines	NMP-RMB2240
Platymantis	*montanus*	Luzon Isl., Philippines	NMP-ACD1013
Platymantis	*hazelae*	Negros Isl, Philippines	CMNH-RSK3918
Platymantis	*naomiae*	Luzon Isl., Philippines	NMP-ACD1001
Platymantis	*papuensis*	New Guinea	TNHC 51978
Polypedates	*cruciger*	Sri Lanka	VUB0125
Poyntonia	*paludicola*	Cape Province, Rep. South Africa	VUB1066 (ES175b)
Ptychadena	*mascareniensis*	Kenya	VUB0957
Ptychadena	*anchietae*	Kenya	VUB0958
Pyxicephalus	*cf. adspersus*	Africa	ZFMK 66446
Rana	*temporalis*	India	VUB0046
Rana	*curtipes*	India	VUB0085
Rana	*signata*	Borneo, Malaysia	VUB0606
Rana	*erythraea*	Borneo, Malaysia	VUB0609
Rana	*chalconata*	Borneo, Malaysia	VUB0610
Rana	*luzonensis*	Luzon isl., Philippines	VUB0798
Rana	*temporaria*	Belgium	VUB0920
Rana	*cf. esculenta*	Belgium	VUB0940
Rana	*clamitans*	USA	TNHC-JSF 1118
Rana	*palmipes*	South America	TNHC-WED 54116
Rana	*palustris*	USA	TNHC-JSF 1110
Rana	*pipiens*	USA	TNHC-JSF 1119
Rana	*sphenocephala*	USA	TNHC-JSF 845
Rana	*tarahumarae*	USA	TNHC-JSF 1071
Rana	*sanguinea*	Palawan Isl., Philippines	RMB 3011
Rana	*nicobariensis*	Java Isl., Indonesia	TNHC 59856
Rana	*boylii*	California, USA	MVZ 148929
Rana	*sylvatica*	New York, USA	MVZ 137426
Rhacophorus	*malabaricus*	India	VUB0001
Schoutedenella	*schubotzi*	Uganda	CAS 201736
Sphaerotheca	*pluvialis*	Sri Lanka	VUB0182
Staurois	*latopalmatus*	Borneo, Malaysia	VUB0652
Staurois	*natator*	Mindanao Isl., Philippines	CMNH-H1626
Strongylopus	*bonaespei*	Rep. South Africa	VUB1221
Tomopterna	*natalensis*	Rep. South Africa	ZFMK 68815
Tomopterna	*damarensis*	Namibia	ZFMK 66403
Trichobatrachus	*robustus*	Cameroon	ZFMK 66453