Molecular Evidence for the Early History of Living Amphibians

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The evolutionary relationships of the three orders of living amphibians (lissamphibians) has been difficult to resolve, partly because of their specialized morphologies. Traditionally, frogs and salamanders are considered to be closest relatives, and all three orders are thought to have arisen in the Paleozoic (>250 myr). Here, we present evidence from the DNA sequences of four mitochondrial genes (2.7 kilobases) that challenges the conventional hypothesis and supports a salamander–caecilian relationship. This, in light of the fossil record and distribution of the families, suggests a more recent (Mesozoic) origin for salamanders and caecilians directly linked to the initial breakup of the supercontinent Pangaea. We propose that this single geologic event isolated salamanders and archaeobatrachian frogs on the northern continents (Laurasia) and the caecilians and neobatrachian frogs on the southern continents (Gondwana). Among the neobatrachian frog families, molecular evidence supports a South American clade and an African clade, inferred here to be the result of mid-Cretaceous vicariance.   © 1998 Academic Press

INTRODUCTION

Living amphibians (lissamphibians) form three distinctive groups with divergent body plans. Frogs (Order Anura) have relatively long hindlimbs and a modified skeleton adapted for saltatory locomotion (jumping). Salamanders (Order Caudata) are terrestrial generalists with slender bodies, relatively short limbs, and poorly formed skeletons. Caecilians (Order Gymnophiona) are limbless burrowers with reinforced skulls (Romer, 1966; Duellman and Trueb, 1986; Duellman, 1988). The conventional hypothesis, based on morphological characters of living and fossil species, is that lissamphibians arose from a single lineage of late Paleozoic (300–250 myr) amphibians and that frogs and salamanders are closest relatives (Duellman and

Trueb, 1986; Duellman, 1988; Gardiner, 1983; Trueb and Cloutier, 1991; Milner, 1988, 1993a). However, some fossil evidence has suggested a multiple origin for lissamphibians (Carroll and Curie, 1975; Carroll and Holmes, 1980; Smithson, 1985), and a salamander–caecilian relationship has appeared in several molecular studies of nuclear genes (Larson and Wilson, 1989; Hedges *et al.,* 1990; Hay *et al.,* 1995). A key to understanding the early evolutionary history of living amphibians and their biogeography is the relationships of the three orders. Therefore, we have collected new DNA sequence data to address this question.

MATERIALS AND METHODS

We sequenced representatives of three distantly related families from each order, including those considered to be morphologically primitive (basal) (Duellman and Trueb, 1986; Duellman, 1988). The complete small (12S) and large (16S) subunit mitochondrial (mt) rRNA genes, intervening tRNA^{VAL} gene, and a portion of the tRNA$^{LEU(UUR)}$ gene were analyzed, totaling 2.7 kilobases. Smaller portions of the two rRNA genes had been sequenced in a previous study of amphibian family relationships (Hay *et al.,* 1995) and the same DNA samples were used here to extend the sequences of representative species. The frogs include *Xenopus laevis* (Pipidae), *Eleutherodactylus cuneatus* (Leptodactylidae), and *Rana pipiens* (Ranidae); the salamanders are *Siren intermedia* (Sirenidae), *Ambystoma mexicanum* (Ambystomatidae), and *Plethodon yonahlossee* (Plethodontidae); and the caecilians are *Epicrionops* sp. (Rhinatrematidae), *Ichthyophis bannanicus* (Ichthyophiidae), and *Typhlonectes natans* (Caeciliidae). Published sequences of three amniotes were included to root the tree: a mammal (*Homo sapiens*) (V00662; Anderson *et al.,* 1981), a bird (*Gallus gallus*) (X52392; Desjardins and Morais, 1990), and a turtle (*Trachemys scripta*) (L28077; Hedges, 1994).

Amplification and sequencing was performed as described elsewhere (Hedges, 1994). DNA was amplified with the use of 31 primers (Table 1) designed from conserved regions among vertebrates, and those primers were used for sequencing of both complementary

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TABLE 1

Primers Used in Amplification and Sequencing

| Primer (5′ to 3′)	Laboratory name	Location of 3′ position
GTATRACCGCGGTGGCTGGCA	12H6	888
ACCGCGGYGGCTGGCACGARRTTKRCCR	12H7	876
GGDKTATCGATTAYAGAACAGGCTCCTCTA	12H8	1195
GAAGGWGGATTTAGYAGTAAA	12L7	1415
AAAGCAHRRCACTGAARATGYYDAGA	12L9	623
CMCAMGGGAMWCAGCAGTGATWAAHATT	12L10	831
GTGTAGCMWATRRRRTGGRAGARATGGGCTACA	12L11	1367
AAAGAAGAGGAAAGTCGTAACATGGTA	12L13	1572
TTAGGGAGAGGATTTGAACCTCTG	16H12	3279
CCGGTCTGAACTCAGATCACGTA	16H13	3058
AYYCTTGTTACTCATWTTARCA	16H14	2299
GCWRRRGGRKATGTTTTTGGTAAACA	16H17	2495
ATGCAAAAGGTABRAGGKTWRRTCTYTGCT	16H18	1825
AACCCKTCTCTGTKGCAAAAGAGTGRGA	16L16	1971
CCWAMCGARCYTRGTGATAGCTGGTT	16L17	2021

Note. Other primers used, but described elsewhere, are 12H2–12H3, 12L2–12L4, 16H3–16H5, 16H11, 16L1–16L4, 16L8, and 16L11 (Hedges, 1994) and 16L10 (Hay *et al.,* 1995). The reference for the location of the 3′ position is the human mtDNA sequence (Anderson *et al.,* 1981).

strands. Although the complete mtDNA sequence of the frog *X. laevis* (X02890) already was available (Roe *et al.,* 1985), we sequenced the 12S rRNA gene, tRNA^{VAL} gene, and 5′ portion of the 16S rRNA gene in that species in order to verify an unusual 140-bp gap near beginning of 12S rRNA gene and a series of short insertions near the end of the 12S rRNA gene (in the published sequence) not present in other organisms. We did not find the gap or insertions and therefore we have used our revised sequence of *X. laevis* for the analyses.

Sequences were aligned by eye (Cabot and Beckenbach, 1989) with reference to secondary structure (Gutell *et al.,* 1994). Phylogenetic analyses were done using minimum evolution (neighbor joining with Kimura transversion distance) (Saitou and Nei, 1987; Kumar *et al.,* 1993), maximum likelihood ($\alpha/\beta = 100$) (Adachi and Hasegawa, 1996), and maximum parsimony (Swofford, 1993). Four-cluster analysis (Rzhetsky *et al.,* 1995) was performed to calculate interior branch support and test rate constancy and alternative topologies under minimum evolution.

RESULTS

The DNA sequences yielded an initial alignment of 2899 nucleotide sites for the 12 taxa (9 families of amphibians and the outgroup of 3 amniotes). The following regions of uncertain alignment (432 sites) were removed before analyses: 940–987, 1140–1187, 1556–1603, 1745–1803, 1922–1990, 2328–2399, 2443–2499, and 2822–2852. An additional 427 sites containing gaps or missing information also were removed. Of
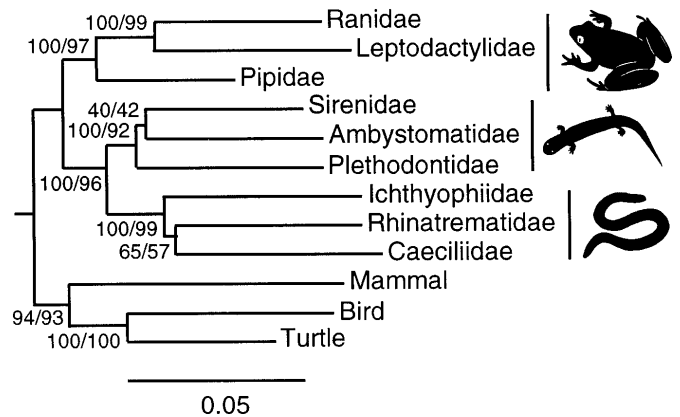
FIG. 1. Phylogenetic relationships of frogs, salamanders, and caecilians inferred from analyses of mitochondrial DNA sequences (2.7 kb region). Minimum evolution, maximum likelihood, and maximum parsimony analyses yielded the same ordinal phylogeny (Table 2). Confidence values on nodes are from interior branch and bootstrap tests (respectively); node without value is root (see text); branch lengths were estimated using neighbor joining.

2040 remaining sites analyzed, 1198 were variable and 868 were informative for parsimony. The sequences (Y10943-951) and alignment (DS32253) have been deposited in the EMBL database.

Phylogenetic analyses of the aligned sequence data using minimum evolution, maximum likelihood, and maximum parsimony methods support a salamander–caecilian clade at high confidence values (Fig. 1, Table 2). Those analyses were done with transitions excluded in order to reduce effects of saturation concomitant with large pairwise distances (0.3–0.5) among taxa. However, inclusion of transitions in the analyses, and use of a gamma distance ($\alpha = 0.8$, calculated from data) yielded the same ordinal topology but at lower confidence values. When transitions were included, intraordinal relationships strongly supported (99%) the following family pairs: Ranidae + Leptodactylidae, Sirenidae + Ambystomatidae, and Rhinatrematidae + Ichthyophiidae. The use of lungfish sequences (EMBL Accession Numbers Z21923, Z21927, and Z48715; Hedges *et al.,* 1993a) to root the tree, although less desirable because of greater sequence divergence, also resulted in the same ordinal topology (at lower confidence). A four-

TABLE 2

Statistical Confidence (Bootstrap *P* Values) for Alternative Relationships of the Three Orders of Living Amphibians

Method of analysis	Frogs + salamanders	Caecilians + salamanders	Frogs + caecilians
Minimum evolution	0.01	0.98	<0.01
Maximum likelihood	0.04	0.96	<0.01
Maximum parsimony	0.12	0.88	<0.01

cluster analysis (Rzhetsky *et al.,* 1995), constraining each order to be monophyletic (Duellman and Trueb, 1986; Hay *et al.,* 1995), identified the salamander–caecilian tree as significantly better than the salamander–frog tree ($P = 0.977$) and frog–caecilian tree ($P = 0.998$). Rate constancy was rejected ($P < 0.01$) and therefore divergence times were not estimated.

DISCUSSION

Amphibian Phylogeny and the Breakup of Pangaea

The joining of salamanders and caecilians in this sequence analysis contrasts with phylogenetic analyses of morphological data, which have consistently supported a frog–salamander clade (Duellman and Trueb, 1986; Duellman, 1988; Gardiner, 1983; Trueb and Cloutier, 1991; Milner, 1988, 1993a; McGowan and Evans, 1995). Shared-derived osteological characters supporting a frog–salamander relationship include large orbit, moderate-sized external naris, absence of postorbital and surangular bones, and separation of pterygoid (anterior ramus) and palatine (Trueb and Cloutier, 1991; Milner, 1988). Some soft anatomical characters are presence of a carotoid labyrinth, absence of a papilla neglecta, choanal tube opening into archenteron during development, and modification of the pronephros for sperm transport (van der Horst *et al.,* 1991). Apparently, there is no support from morphology or molecules for the third alternative, a close relationship between frogs and caecilians.

Morphologically, the salamander–caecilian clade can be diagnosed by the following shared-derived characters: dermal folds reflecting body segmentation, intrinsic narial musculature, lobular testes, reduced clavicles, stapes with otic and quadrate processes, coossification of scapula and coracoid, similarities in cephalic venous drainage, and sperm ultrastructure (Trueb and Cloutier, 1991; Milner, 1988; van der Horst *et al.,* 1991; McGowan and Evans, 1995). There are also neuroanatomical traits that support this grouping (Roth *et al.,* 1993). We propose the name Procera (Latin, for slender, long) as a superorder to include salamanders and caecilians.

The monophyly of living amphibians with respect to other living vertebrates is well supported with both morphological and molecular evidence (Hedges *et al.,* 1990; Szarki, 1962; Parsons and Williams, 1963). However, there are numerous fossils representing extinct groups of Paleozoic tetrapods that cannot be examined for soft anatomy or by DNA sequence analysis. Even in the context of those fossil groups, lissamphibians have been considered to be monophyletic and descendants of temnospondyls (Duellman and Trueb, 1986; Duellman, 1988; Gardiner, 1983; Trueb and Cloutier, 1991; Milner, 1988, 1993a), although a multiple origin has been suggested by some analyses (Carroll and Curie, 1975; Carroll and Holmes, 1980; Smithson, 1985). More recently, a phylogenetic analysis of 38 taxa and 157 osteological characters yielded a single origin for lissamphibians from lepospondyls (Laurin and Reisz, 1997). The conclusion common to most of these morphological studies is that lissamphibians constitute a single clade with respect to a diversity of fossil Paleozoic groups. The studies differ as to which particular fossil group contains the closest relatives of this lissamphibian clade.

Albanerpetontids are extinct amphibians that existed from at least the Jurassic to the Miocene (170–15 myr) (Milner, 1993b) and resembled salamanders. They are considered to be either salamanders (Trueb and Cloutier, 1991; Estes and Sanchíz, 1982) or a sister group to the salamanders and frogs (McGowan and Evans, 1995). However, the latter possibility would be contradicted by the molecular phylogeny (Fig. 1). Also, two characters of the albanerpetontid atlas found in salamanders and caecilians, spinal nerve foramina (Milner, 1988) and an interglenoid tubercle (McGowan and Evans, 1995; Jenkins and Walsh, 1993), may have additional diagnostic value for the salamander–caecilian clade. Albanerpetontids also share a Laurasian distribution with salamanders. For these reasons we tentatively agree with the previous association of albanerpetontids and salamanders (Trueb and Cloutier, 1991; Estes and Sanchíz, 1982).

A salamander–caecilian relationship has implications for a possible mechanism to explain their distribution. A Paleozoic origin for all three orders is required under the conventional hypothesis of relationships because frogs (or their salientian relatives) were present near the beginning of the Mesozoic (240 myr) (Milner, 1993a,b; Benton, 1990). However, if salamanders and caecilians are closest relatives, then the fact that they first appear later in the Mesozoic (190–160 myr) (Milner, 1993b; Jenkins and Walsh, 1993) may reflect a later evolutionary origin rather than their absence in the early Mesozoic fossil record (Fig. 2).

The Jurassic appearance of salamanders and caecilians roughly coincides with the initial breakup of Pangaea, timed at 195–157 myr (Hallam, 1994). This event may explain the Laurasian distribution of salamanders (and albanerpetontids) and the Gondwanan distribution of caecilians. Likewise, the primary distributions of the two suborders of frogs (Archaeobatrachia, Laurasia; Neobatrachia, Gondwana) may have the same explanation. Families endemic to either Laurasia or Gondwana follow this pattern with only one exception, the family Pipidae (Fig. 3).

The presence of the earliest known caecilian, *Eocaecilia* (Jenkins and Walsh, 1993), in Laurasia at a time when Pangaea was only just beginning to rift (190 myr) is not predicted by the hypothesis proposed here. That this fossil has limbs and some salamander traits may indicate that it was close to the divergence of salamanders and caecilians. It is possible that it represents a lineage-sorting event in which the phylogenetic divergence preceded the geologic divergence. Some lineage sorting should be expected to occur when very large land areas, involving faunas with multiple species,

A

B
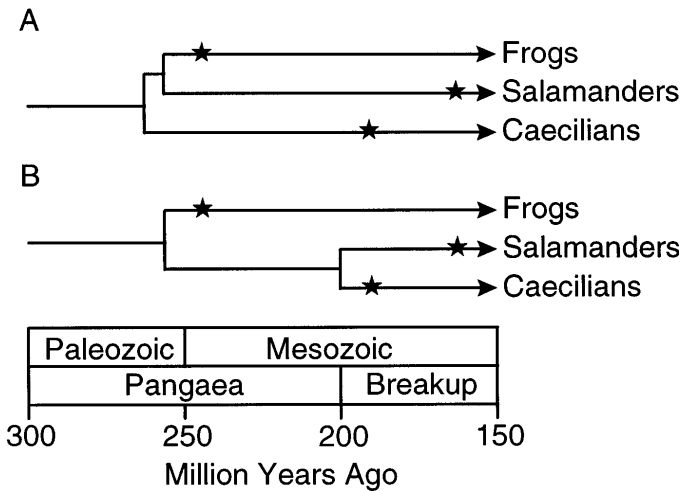
FIG. 2. Effect of phylogeny on inferring the time of origin of amphibian orders. The oldest fossil representative of each order is indicated by a star. (A) The early Mesozoic fossil *Triadobatrachus,* on the frog lineage, forces a minimum time for the origin of all three orders under the conventional hypothesis of relationships. (B) This constraint is relaxed in the molecular phylogeny permitting a later origin for salamanders and caecilians at a time when the supercontinent Pangaea was splitting into Laurasia and Gondwana.

undergo vicariance (Fig. 4). There are no other limbed caecilians (fossil or recent) and the common ancestor of living caecilians presumably was limbless. The broad Gondwanan distribution of caecilians suggests that a limbless form became isolated in Gondwana in the Jurassic before that southern land mass broke into smaller fragments.

Critics of the above hypothesis may point out some apparent geographic inconsistencies in our biogeographic argument. Regarding the proposition that Archaeobatrachia originated on Laurasia, the distributions of the pipids and leiopelmatids would require dispersal explanations. Pipid frogs are Gondwanan, not Laurasian, and fossils indicate that they were on Africa and South America in the Mesozoic (Milner, 1993b; Evans *et al.,* 1996). However, the closest relatives (Rhynophrynidae, Paleobatrachidae) are known only from Laurasian areas, and that clade is nested among other Laurasian families in the suborder Archaeobatrachia, suggesting that the pipids dispersed to Gondwana, probably in the late Jurassic or early Cretaceous.
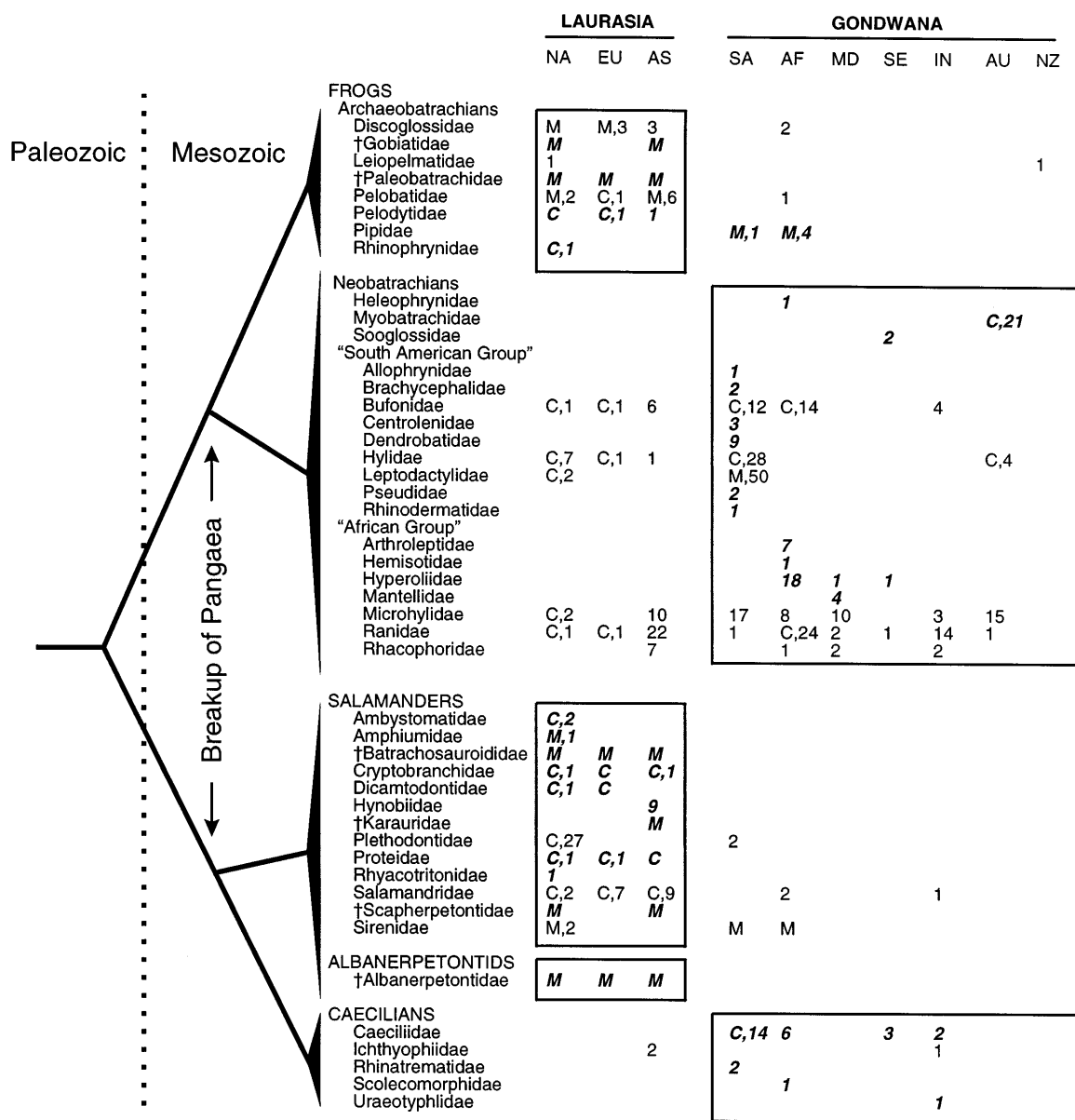
The Leiopelmatidae has an unusual distribution in that it occurs in North America and New Zealand. Unfortunately, the only fossil (*Notobatrachus,* from South America) has been reclassified as a more basal anuran along with *Vieraella* (Milner, 1993b; Ford and Cannatella, 1993), leaving little evidence of the past biogeographic history of this family. Leiopelmatids may have reached the Australasian region at the same time as the marsupials and by the same route. Marsupials are believed to have dispersed from North to South America and then to Antarctica and Australia by way of intercontinental corridors or filters in the late Creta-

ceous and/or early Tertiary (Woodburne and Case, 1996). Leiopelmatids thus should be expected from the late Cretaceous and/or Cenozoic fossil record of South America, Antarctica, and Australia.

Alternative explanations require a much greater number of dispersal events and geographic anomalies. For example, if it is postulated that the archaeobatrachian families arose on Pangaea, then what is the explanation for the striking Laurasian distributional pattern? Where are the Gondwanan discoglossids (Mesozoic), gobiatids, paleobatrachids, pelobatids (Mesozoic), pelodytids, and rhynophrynids? Even under a paraphyletic Archaeobatrachia (Ford and Cannatella, 1993), a more complex biogeographic scenario is needed to explain the evolution of frogs regardless of whether the lineage splitting happened before or after the breakup of Pangaea.

Salamanders and caecilians show even a greater concordance with geography. For example, there is no evidence that 10 of the 13 families of salamanders ever existed on Gondwanan land areas, despite the Mesozoic age and broad northern distribution of some families. Two of the families that occur on both northern and southern continents, Plethodontidae and Salamandridae, arose in Laurasia based on phylogenetic and fossil evidence (Duellman and Trueb, 1986). The third family, Sirenidae, is known from Laurasia as well as the mid-Mesozoic of South America and Africa, but the Gondwanan distribution is believed to represent a mid-Mesozoic dispersal from Laurasia (Evans *et al.,* 1996). In discussing the historical biogeography of salamanders, Milner (1983) recognized the difficulty in reconciling their supposed Permian (Pangaean) origin with a strong Laurasian pattern of distribution. His explanation was that the early forms were cold adapted and were confined to the northern latitudes of Pangaea for at least 50 million years, until the separation of Laurasia and Gondwana. Such an explanation is not needed if they arose by vicariance.

Of the five families of caecilians, four are endemic to Gondwanan areas and the fifth (Ichthyophiidae) is found on Gondwanan (India) and Laurasian (southeast Asia) land masses. The origin of the southeast Asian ichthyophiids has presented a biogeographic problem. Duellman and Trueb (1986) suggested that they arrived (along with the uraeotyphlids and caeciliids of India) by continental drift on the Indian subcontinent. Hedges *et al.* (1993b) proposed two other alternatives based on the large molecular divergence of ichthyophiids from other caecilians (Hass *et al.,* 1993): that they originated (1) on Laurasia at the time of the initial breakup of Pangaea or (2) on Gondwana and dispersed to Asia in the early Cretaceous. The relationships of caecilians still are not well known, but if the Rhinatrematidae is the most basal family (Nussbaum, 1977; Hedges *et al.,* 1993b), and given the new evidence here for ordinal relationships, the Asian caecilians probably originated on Gondwana.

Left of the table (phylogeny diagram axis labels): Paleozoic · Mesozoic · Breakup of Pangaea

| Taxon	LAURASIA			GONDWANA						
	NA	EU	AS	SA	AF	MD	SE	IN	AU	NZ
FROGS										
Archaeobatrachians										
Discoglossidae	M	M,3	3	2						
†Gobiatidae	M		M							
Leiopelmatidae	1									1
†Paleobatrachidae	M	M	M							
Pelobatidae	M,2	C,1	M,6							
Pelodytidae	C	C,1	1		1					
Pipidae				M,1	M,4					
Rhinophrynidae	C,1									
Neobatrachians										
Heleophrynidae					1					
Myobatrachidae									C,21	
Sooglossidae						2				
"South American Group"										
Allophrynidae				1						
Brachycephalidae				2						
Bufonidae	C,1	C,1	6	C,12	C,14			4		
Centrolenidae				3						
Dendrobatidae				9						
Hylidae	C,7	C,1	1	C,28					C,4	
Leptodactylidae	C,2			M,50						
Pseudidae				2						
Rhinodermatidae				1						
"African Group"										
Arthroleptidae					7					
Hemisotidae					1					
Hyperoliidae					18	1	1			
Mantellidae						4				
Microhylidae	C,2		10	17	8	10		3	15	
Ranidae	C,1	C,1	22	1	C,24	2		1	14	1
Rhacophoridae			7		1	2		2		
SALAMANDERS										
Ambystomatidae	C,2									
Amphiumidae	M,1									
†Batrachosauroididae	M	M	M							
Cryptobranchidae	C,1	C	C,1							
Dicamptodontidae	C,1	C								
Hynobiidae			9							
†Karauridae			M							
Plethodontidae	C,27			2						
Proteidae	C,1	C,1	C							
Rhyacotritonidae	1									
Salamandridae	C,2	C,7	C,9		2			1		
†Scapherpetontidae	M		M							
Sirenidae	M,2			M	M					
ALBANERPETONTIDS										
†Albanerpetontidae	M	M	M							
CAECILIANS										
Caeciliidae				C,14	6		3	2		
Ichthyophiidae			2					1		
Rhinatrematidae				2						
Scolecomorphidae					1					
Uraeotyphlidae								1		

FIG. 3. Distribution of the families of lissamphibians. Relationships (on left) are from the molecular phylogeny (Fig. 1). Listed are first appearance in the fossil record (M, Mesozoic; C, Cenozoic) and number of native extant genera in each geographic region: NA (North America), EU (Europe), AS (Asia, excluding India), SA (South America), AF (Africa), MD (Madagascar), SE (Seychelles), IN (India), AU (Australia and New Guinea), and NZ (New Zealand) (Duellman and Trueb, 1986; McGowan and Evans, 1995; Duellman, 1993; Roček and Nessov, 1993). Distributions restricted to either Laurasia or Gondwana are in bold and italics; boxes circumscribe the paleolandmass of hypothesized origin for each group. Occurrences outside boxed areas are postulated to represent dispersal events after the separation of Laurasia and Gondwana. Central America is treated as either North or South America depending on the continent with which that distribution is shared. *Triadobatrachus* preceded, and *Eocaecilia* and *Prosalirus* were contemporaneous with, the breakup of Pangaea and are not shown. Two Middle and Late Jurassic anuran fossils (*Viaraella* and *Notobatrachus*) also are not shown because they are considered to be basal among anurans (Milner, 1993b; Ford and Cannatella, 1993) and presumably diverged from the anuran lineage before the separation of Archaeobatrachia and Neobatrachia.

The Africa–South America Split

Of the 19 families of neobatrachian frogs, seven are known from multiple land masses (Fig. 3). Other than a late Cretaceous leptodactylid fossil, all known fossil neobatrachians are from the Cenozoic (Milner, 1993b). However, molecular estimates of divergence times within families (e.g., Maxson, 1984; Maxson and Myers, 1985; Maxson and Heyer, 1988) have suggested that the families are significantly older than indicated by the fossil record. Unfortunately, divergences much older than 60–80 myr are beyond the resolution of albumin immunological distance estimation (Maxson, 1992), and therefore there is no accurate estimate of when the families of neobatrachian frogs actually arose.

Duellman and Trueb (1986) proposed that the presence of some anuran families on multiple tectonic

EARTH HISTORY

CLASSIC VICARIANCE

LINEAGE SORTING
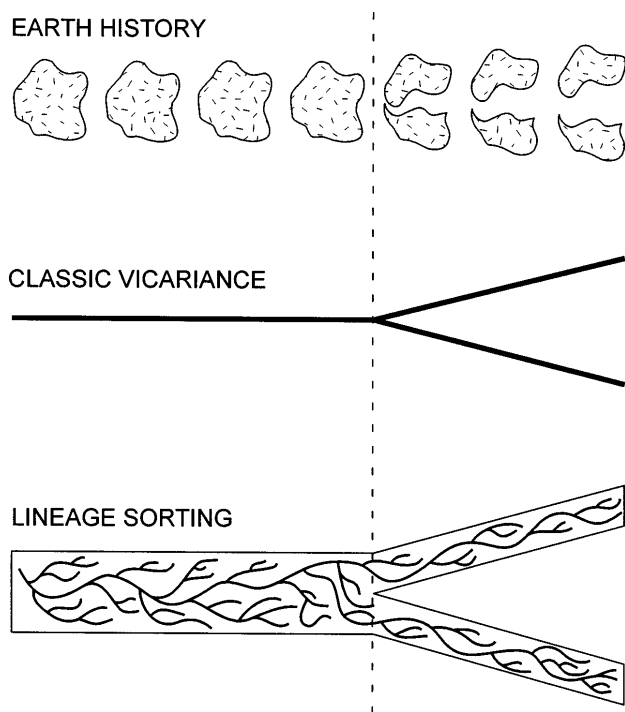
FIG. 4. A model for continental vicariance. A single large land mass with considerable topographic and environmental heterogeneity, and correspondingly diverse fauna, diverges into two land masses gradually over millions of years. In the case of classic vicariance, the phylogenetic divergence is predicted to have occurred at the same time as the physical separation. However, when continents with large faunas are involved, some lineage sorting is expected. The coalescence of lineages on each land mass does not correspond exactly to the time of geologic separation but to a slightly earlier speciation event that most likely took place on one or the other land mass.

plates is the result of a Jurassic pan-Gondwana distribution of those families. However, this does not explain the current absence of hylids and leptodactylids in Africa and the absence of hyperoliids and rhacophorids in South America. They suggested that these families must have been restricted to only certain parts of the single Africa–South America land mass before it separated 100 myr ago (Smith *et al.,* 1994). Although that is plausible, the same pattern could be explained, perhaps more easily, by vicariance.

The relationships of anuran families from molecular evidence (Hay *et al.,* 1995; Ruvinsky and Maxson, 1996) shows concordance with geography. All endemic Neotropical families examined (Centrolenidae, Rhinodermatidae, Dendrobatidae, Pseudidae, and Leptodactylidae) clustered in one group and the endemic African (region) families, Hyperoliidae and Mantellidae, clustered in another group. The association of the remaining families with those two phylogenetic groups agreed with other data (e.g., subfamilial or generic diversity patterns) indicating their place of origin. For example, more ranid and microhylid subfamilies occur in Africa than in South America (Duellman, 1993), and the

molecular evidence places those two families in the African Group.

The existing superfamily names Hyloidea (for the South American Group) and Ranoidea (for the African Group) are appropriate for the these two groups of neobatrachian frog families. The relationships of the three remaining families, Heleophrynidae (South Africa), Myobatrachidae (Australasia), and Sooglossidae (Seychelles), are unclear and therefore their superfamily status remains undetermined.

We suggest that Hyloidea and Ranoidea diverged when South America separated from Africa. This implies a considerably younger date for the origin of those families but is a simpler explanation for continental endemism, and it is supported by the molecular evidence (Hay *et al.,* 1995; Ruvinsky and Maxson, 1996). Ruvinsky and Maxson suggested that divergences among some of these families were related to earlier tectonic events in the breakup of Gondwana but this again brings up the same distributional problems (continental endemism) as in the scenario of Duellman and Trueb (1986).

The myobatrachids and pelodryadine hylids probably reached Australia via the connection with Antarctica and South America in the late Cretaceous (Maxson *et al.,* 1975) following the same route as the marsupials (Woodburne and Case, 1996). The heleophrynids, known only from cold mountain streams in extreme southern Africa, could have arrived by dispersal from Antarctica in the late Cretaceous when the two continents were closer and the ocean currents were flowing in a favorable (northward) direction.

How did the more wide-ranging neobatrachian families reach the northern continents? In the case of the hyloids (bufonids, hylids, and leptodactylids), they probably dispersed northward from South America across the proto-Antilles in the late Cretaceous. From there, the bufonids and hylids could have reached Asia and Europe via Beringia, with the bufonids dispersing to nearby Africa as that continent approached Asia. At the same time, the ranoids (microhylids, ranids, and rhacophorids) probably dispersed northward out of Africa to Asia and, in the case of ranids and microhylids, to North America and South America. Although the ranids probably arrived to South America late in the Cenozoic, the microhylids may have arrived earlier based on their diversification into 17 genera on that continent. Geologically, Madagascar, India, and the Seychelles separated from Africa at too early a time (130 myr) for vicariance to easily explain the origin of their endemic ranoid taxa under the model proposed here. Thus, the groups inhabiting Madagascar and the Seychelles probably arrived by dispersal from nearby Africa.

ACKNOWLEDGMENTS

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