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Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group

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The *Rana catesbeiana* species group consists of seven species, each variously distributed across eastern North America. We estimated the evolutionary relationships among 31 exemplars and used the phylogenetic hypothesis to examine the potential modes of speciation and relative role of dispersal in the evolution and zoogeography of this species group. Phylogenetic relationships based on 1554 combined base pairs of the cytochrome *b* and ND2 mitochondrial genes suggest that the species are closely related, having undergone rapid radiation from a common ancestor during the late Miocene or Pliocene. A Pleistocene origin for the rare *R. okaloosae* is suggested by its pattern of paraphyly with *R. clamitans* and by its geographically restricted distribution, although hybridization as the explanation for paraphyly cannot be ruled out. Dispersal–vicariance analysis suggested a Coastal Plain biogeographical region origin of the species group, supporting the notion that the region was an important centre of anuran diversification, with post-speciation dispersal playing a major role in explaining the distribution of the widespread species, *R. catesbeiana*, *R. clamitans,* and *R. septentrionalis*. High sea levels during the late Tertiary, greatly reducing and insularizing parts of the southern Coastal Plain region may have played a major role in the diversification of this group.

ADDITIONAL KEYWORDS: Bayesian – biogeography – dispersal–vicariance analysis – maximum likelihood – maximum parsimony – Pleistocene – Pliocene – speciation.

INTRODUCTION

Resolution of phylogenetic relationships among closely related organisms is fundamental to our understanding of the evolution and radiation of biological diversity, including our interpretation of biological phenomena such as character evolution, behaviour, or community structure (e.g. Brooks & McLennan, 1991; Harvey & Pagel, 1991; Titus & Larson, 1995; Harvey, 1996; Graybeal, 1997). Further, the distribution of taxa, whether considered from an ecological or historical perspective, and their phylogenetic history are intimately linked (Crisci, 2001). Molecular systematics, coupled with recently developed analytical approaches for determining biogeographical patterns, has been a boon to the study of species distributions and origins.

Vicariance is now considered by many to have been the dominant force underlying biogeographical patterns of modern taxa. Dispersal theory, once the prominent explanation, has been criticized for being, among other things, resilient to rejection (Rosen, 1978; Nelsen & Platnick, 1981; Voelker, 1999). However, both vicariance and dispersal are natural processes, leading recent biogeographers to point out that dispersal, as an explanation of biogeographical pattern, should not be rejected a priori (Morrone & Crisci, 1995; Ronquist, 1997). Further, testing purely vicariant theories is often difficult. Finding congruence between the distribution of groups of taxa and geological events assumes that a singular history is shared by both taxa and the geological history of the area. Because the history of areas may be multiple or even

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reticulate (Ronquist, 1997), this poses a danger of 'pseudocongruence' (Cunningham & Collins, 1994). Another important assumption of vicariance theory is that ancestral taxa were widespread and that speciation occurred with the subdivision of the ancestral range. Inherently, subsequent dispersal is needed to explain current widespread and overlapping distributions of taxa. In contrast, a central tenet of dispersal theory is that of 'centres of origin', from which groups of taxa become peripherally isolated, speciate, and undergo subsequent secondary contact and divergent selection (Dobzhansky, 1937; Mayr, 1954). Despite criticism of the dispersalist paradigm, advances in analytical approaches to determining biogeographical patterns (e.g. Bremer, 1992; Maddison, Ruvolo & Swofford, 1992; Ronquist, 1997) allow us to test both vicariant and dispersal hypotheses where we once relied solely on descriptions of pattern. Such developments in phylogenetics and biogeography are of particular interest for understanding the evolutionary history of organisms that do not fit a 'typical' single branching pattern among areas of endemism, a major assumption of cladistic biogeography. The *Rana catesbeiana* species group (Boulenger, 1920; Hillis & Davis, 1986) is a good example of organisms with a complex pattern of geographical ranges that do not fit into a straightforward, vicariance pattern. Further, given the propensity of vicariance biogeographers to reject dispersalist explanations (e.g. Cracraft, 1986; Mayden, 1988), the *R. catesbeiana* group represents an opportunity to apply analytical techniques to the basic question of what mechanisms (e.g. dispersal, vicariance, sympatric speciation) were prominent in the diversification of this group.

The *Rana catesbeiana* species group consists of seven closely related species of frogs native to eastern North America (for common names refer to Table 1): *Rana catesbeiana* Shaw (1802); *R. clamitans* Latreille, 1801 (in Sonnini & Latreille, 1801), *R. grylio* Stejneger (1901); *R. heckscheri* Wright (1924); *R. okaloosae* Moler (1985), *R. septentrionalis* Baird (1854); and *R. virgatipes* Cope (1891). Support for the monophyly of this species group includes enzyme restriction data on rDNA (Hillis & Davis, 1986; Hillis, 1988), albumin differences (Wallace, King & Wilson, 1972), 12S and 16S ribosomal sequence data (D. Hillis, pers. comm.), and morphology (Boulenger, 1920; Moler, 1985). Four of these species (*R. heckscheri, R. virgatipes, R. grylio,* and *R. okaloosae*) are endemic to the Coastal Plain physiographic region (Fig. 1, region A); one of which, *R. okaloosae*, is restricted to two drainage basins in the Florida panhandle (Moler, 1993). *Rana catesbeiana* and *R. clamitans* have widespread, sympatric geographical ranges that include the Coastal Plain, Appalachian Highlands, Interior Plains, and partially extending into the Laurentian Uplands physiographic regions. *Rana clamitans* has been separated into two subspecies: *R. c. clamitans* in the south, and *R. c. melanota* (Rafinesque, 1820) in the north, based on external coloration and relative body size (Mecham, 1954). The final species, *R. septentrionalis,* is at its southernmost extent in the Northern Appalachians, with most of its distribution between 50° and 55°N latitude in eastern North America (Fig. 1). North American ranids, within which the *Rana catesbeiana* species group is basally positioned (Hillis & Davis, 1986), are believed to have diverged from the Eurasian brown frogs during the Oligocene (25–35 Mya) (Duellman & Trueb, 1986). Case (1978) estimated a common ancestor of the species group at approximately 15 Mya.

Under the assumptions of the dispersalist school of biogeography, the Coastal Plain region represents the 'centre of origin' for the species group. This is inferred from the region's high level of endemism and geographical range overlap (Fig. 1). However, quantitative evidence for this pattern will depend in part on the phylogenetic relationships among the seven species, because, despite their assumed monophyly, little is know about the phylogenetic relationships within the group. No previously published phylogeny has included more than five of the species, and none have included *R. okaloosae*.

Given the incomplete knowledge of the evolutionary history of the *Rana catesbeiana* group, one of our objectives is to estimate the phylogenetic relationships of the seven species using multiple exemplars. Assuming widespread species harbour, on average, greater genetic variation than geographically restricted taxa (Omland, Lanyon & Fritz, 2000), intraspecific diversity should be assessed when reconstructing phylogenies (Melnick *et al*., 1993; see Methods). Using the phylogenetic hypothesis generated from our DNA sequence data, we test whether the Coastal Plain represents the geographical origin of the species group, and determine the possible roles of dispersal and vicariance in shaping the evolutionary trajectory of the *Rana catesbeiana* species group. In light of the prominent role placed on vicariance among biogeographical methods, we also explore an analytical method for distinguishing between modes of speciation based on species relative range size and overlap of sister–species (Lynch, 1989).

MATERIAL AND METHODS

Evolutionary units sampled

Thirty-one individuals representing the seven species within the *Rana catesbeiana* species group were included in our analysis. Including single representatives from such species may negatively affect phylogenetic reconstructions by over-representing genetic

Table 1. Geographic locations for specimens used in this study. Map sites correspond to Fig. 1. Sources are given as: collector[1], toe clip (T) or voucher specimen (V), collection number

	Haplotype	Map site	Geographic area	GenBank Accession #s	Source, specimen No.
Rana catesbeiana (bullfrog)	RcatM95	1	Muskrat Lk., Pembroke, ON; 45°44′N 077°02′W	AY083295, AY206468	TX, T, JDA98-M95
	Rcat978	2	Holden Arboretum, OH; 41°37′N 081°18′W	AY083293, AY206472	JDA, T, JDA00-978
	Rcat1365	3	Chapel Hill, NC; 35°54′N 079°01′W	AY083291, AY206470	JDA, T, JDA01-1365
	Rcat627	4	Walker Mines, AL; 33°19′N 087°20′W	AY083292, AY206471	JDA, T, JDA00-627
	Rcat624	5	Starkville, MS; 33°25′N 088°47′W	AY083294, AY206473	JDA, T, JDA00-624
	Rcat1319	6	Gainesville, FL; 29°44′N 082°25′W	AY083288, AY206469	JDA, T, JDA01-1319
R. clamitans (green frog)					
R. c. clamitans	Rcla1344	7	Dermorest, GA; 34°34′N 083°33′W	AY083274, AY206474	JDA, V, JDA01-1344
	Rcla1334	8	Savannah, GA; 31°60′N 081°16′W	AY083276, AY206475	JDA, T, JDA01-1334
	Rcla1393	9	Walton County, FL; 30°42′N 086°02′W	AY083277, AY206476	PM, T, JDA01-1393
	Rcla1394	10	Escambia County, FL; 30°56′N 087°21′W	AY083279, AY206477	PM, T, JDA01-1394
R. c. melanota	Rcla042	11	Stump Lake, Frontenac Co., ON	AY083280, AY206478	JDA, T, JDA98-42
	Rcla986	2	Holden Arboretum, OH; 41°37′N 081°18′W	AY083281, AY206479	JDA, T, JDA00-986
	Rcla1237	12	Columbia, MO; 38°45′N 092°12′W	AY083282, AY206480	JDA, T, JDA01-1237
R. okaloosae (Florida bog frog)	Rcla1393	13	Okaloosa Co., FL; 30°42′N 086°02′W	AY083277, AY206498	PM, T, JDA01-1408
	Roka1403	13	Okaloosa Co., FL; 30°31′N 086°40′W	AY083283, AY206481	PM, JDA01-1403
	Roka1405	14	Santa Rosa Co., FL; 30°38′N 086°48′W	AY083284, AY206483	PM, JDA01-1405
	Roka1409	13	Okaloosa Co., FL; 30°31′N 086°40′W	AY083285, AY206484	PM, T, JDA01-1409
	Roka1404	14	Santa Rosa Co., FL; 30°38′N 086°48′W	AY083286, AY206482	PM, T, JDA01-1407
R. septentrionalis (mink frog)	Rsep185	15	Big Gull Lake, ON; 44°50′N 76°56′W	AY083272, AY206485	JDA, T, JDA98-185
	Rsep185	16	Little Joeseph, ON; 45°12′N 79°40′W	AY083272, AY206486	JDA, T, JDA98-135
	Rsep24410	17	Caledon, Peel Co., ON	AY083273, AY206487	JPB, V, 24410
R. virgatipes (carpenter frog)	Rvir1348	18	Wood's Bay, SC; 33°57′N 79°59′W	AY083301, AY206488	JDA, T, JDA01-1348
	Rvir1352	18	Wood's Bay, SC; 33°57′N 79°59′W	AY083302, AY206489	JDA, T, JDA01-1352
	Rvir1351	18	Wood's Bay, SC; 33°57′N 79°59′W	AY083303, AY206490	JDA, T, JDA01-1351
	RvirA	19	Cape May Co., NJ	AY083304, AY206491	RA, T, JDA01-A
R. heckscheri (river frog)	Rhec1308	20	Reed Bingham SP, GA; 31°10′N 83°33′W	AY083298, AY206492	JDA, V, JDA01-1308
	Rhec1407	21	Deep Cr., Columbia Co., FL; 30°21′N 082 °32′W	AY083299, AY206493	PM, T, JDA01-1407
	Rhec1407	21	Deep Cr., Columbia Co., FL; 30°21′N 082 °32′W	AY083299, AY206494	PM, T, JDA01-1411
	Rhec1307	20	Reed Bingham SP, GA; 31°10′N 83°33′W	AY083300, AY206495	JDA, V, JDA01-1307
R. grylio (pig frog)	Rgry1396	9	Walton County, FL; 29°70′N 081°23′W	AY083296, AY206496	PM, T, JDA01-1396
	Rgry1332	8	Savannah, GA; 31°60′N 081°16′W	AY083297, AY206497	JDA, T, JDA01-1332
R. sylvatica	Rsyl	–		AY083271, AY206466	JDA, T
R. luteiventris	Rlut	–		AY083270, AY206467	DC

Collector initials: JDA = J. Austin; PM = P. Moler; RA = R. Arndt; TH = T. Hunsinger; TX = T. Haxton; JPB = J. Bogart; DC = D. Call.

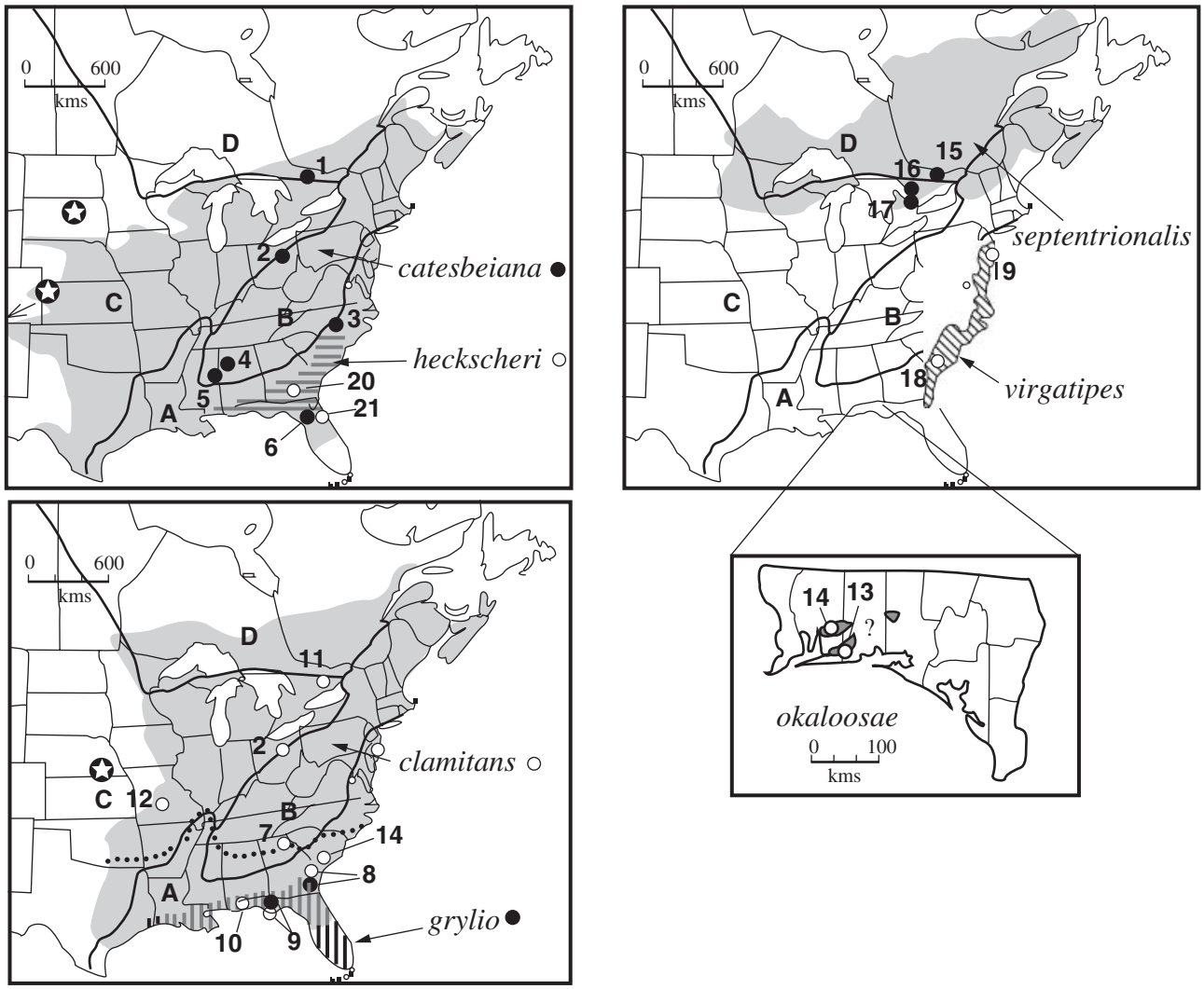

Figure 1. Geographic range and sample location for the seven species of the *Rana catesbeiana* species group. Location numbers correspond to those in Table 1. Borders of major physiographic regions are delimited by solid lines and are identified as follows: A, Coastal Plain; B, Appalachian; C, Interior Plains; D, Laurentian Uplands (after Duellman & Sweet, 1999). Extra-limital fossil records for *Rana catesbeiana* and *Rana clamitans* are indicated with ✪ (see Holman, 1995; references therein). Approximate division between putative subspecies *Rana clamitans clamitans* (south) and *Rana clamitans melanota* (north) is indicated with a dotted line.

distances between species and enforcing assumptions of monophyly among species (e.g. Lanyon, 1994). Therefore, we attempted to include multiple exemplars from each species, including representatives from the two putative subspecies of *R. clamitans* (Mecham, 1954). We included more specimens from the widely distributed species, *R. clamitans* and *R. catesbeiana*, to try to capture a greater proportion of within species genetic diversity, but were limited to relatively few samples of *R. septentrionalis* (see Table 1 and Fig. 1 for sample locations and geographical ranges). For outgroup taxa we chose two North American *Rana* species representing two separate and evolutionarily distinct lineages, *R. sylvatica* and *R. luteiventris* (Hillis & Davis, 1986). Specimen numbers and source localities for specimens are listed in Table 1.

Marker choice and laboratory procedure

We chose cytochrome *b* (cyt *b*) and ND2 to determine the phylogenetic relationships of these closely related species. Both genes have proven useful for genus level (e.g. Lanyon, 1994; Macey *et al*., 1998) and intraspe-

cific phylogenies (e.g. Omland *et al.*, 1999; Weisrock & Janzen, 2000; Macey *et al.*, 2001; Neilson, Lohman & Sullivan, 2001).

Total genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen) following manufacturer's instructions and stored at –20°C. Polymerase chain reaction (PCR) amplifications of double stranded product were performed in a Perkin-Elmer 9600 thermocycler. Amplification of a 1047 base pair (bp) segment of cytochrome *b* (cyt *b*) corresponding to positions 14739–15574 of the human mtDNA was done using primers MVZ15-L (Moritz, Schneider & Wake, 1992) and cytbAR-H (Goebel, Donnelly & Atz, 1999). A 650-bp segment of ND2 and $tRNA^{TRP}$ was amplified using the primers L4882 (Macey *et al.*, 2000) and H5532 (Macey *et al.*, 1998), corresponding to positions 4882–5532 on the human mtDNA. A negative control was included for all PCR reactions. For cyt *b*, 25 μL reactions were used, containing 25–50 ng of template, 1 μM of each primer, 2 mM $MgCl_2$, 1 × Gibco BRL reaction buffer, 10 mM dNTPs, and 0.4 units of Gibco BRL Taq polymerase. PCR volumes were doubled for ND2 in order to increase the amount of product for sequencing (see below). Amplification conditions for both genes involved a denaturation stage at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 20 s, primer annealing at 52°C for 30 s, and elongation at 72°C for 30 s. A final elongation of 5 min at 72°C was followed by cooling to 4°C. Product was run out on 2% agarose gels (Boehringer-Mannheim) in 1× TBE buffer, with subsequent staining in ethidium bromide and visualization under UV light. Bands were cut out of the agarose and cleaned using QIAquick Gel Extraction Kit (Qiagen) following the manufacturer's instructions. Cytochrome *b* PCR product was cycle-sequenced (Amersham–Thermo-Sequenase) using MVZ15-L and cyt *b* br-H, as well as an internal primer, MVZ25-L (Moritz *et al.*, 1992), using the following profile: 35 cycles of 94, 55, and 72°C for 30, 30, and 70 s, respectively, followed by a final extension of 70°C for 7 min. Product was run out on 6% PAA gels for 2–6 h, blotted, vacuum dried, and placed on film (Kodak–Biomax) for 24–48 h. Autoradiograms were read by eye, and sequences were aligned using GeneWorks (IntelliGenetics Inc.) with subsequent verification by eye using MacClade 4.0 (Maddison & Maddison, 1996). ND2 PCR product was column-cleaned with a PCR Purification Kit (Qiagen), eluted using 50 μL of elution buffer, and vacuum concentrated to a final volume of 25 μL. PCR products were cycle sequenced following standard protocols for sequencing kit from Applied Biosystems. Cycle sequencing products were analysed on an ABI-377 Automated Sequencer (Applied Biosystems). Base calls were verified by eye and aligned using GeneWorks, and both cyt *b* and ND2 partitions were imported into MacClade.

Phylogenetic signal

To determine whether the data partitions could be combined and their power to resolve phylogenetic relationships, we: (1) compared the evolutionary rates of the two genes by plotting the uncorrected pair wise distances of ND2 against those of cyt *b*, and (2) performed a partition homogeneity test (Farris *et al.*, 1994) using the two gene partitions under equal weighting. To test explicitly for the possibility of saturation (i.e. multiple substitutions at a base-pair position) we plotted raw sequence divergence (*p*) vs. Kimura's (1980) 2-parameter distance (see Berbee *et al.*, 1995; Burns, 1997) for 1st, 2nd, and 3rd codon positions separately.

Phylogenetic signal was examined in the in-group taxa for both cyt *b* and ND2 considered separately and combined using relative apparent synapomorphy analysis (RASA 3.0.4, Lyons-Weiler, Hoelzer & Tausch, 1996; Lyons-Weiler, 2001) and permutation-tail-probability (PTP) tests (Archie, 1989; Faith & Cranston, 1991). PTP tests compare the tree lengths of the most parsimonious tree(s) to the lengths of trees drawn randomly from the same data set. The PTP value is the proportion of times the randomly generated tree is shorter than the most parsimonious tree(s). A PTP value of less than 0.05 is taken as indicating that significant cladistic covariation (i.e. phylogenetic signal) exists in the data set (Archie, 1989; Faith & Cranston, 1991). PTP tests were run in PAUP* 4.10 (Swofford, 1998) with heuristic searches using random taxon addition and 100 replicates for 1000 permutations.

RASA calculates the 'apparent synapomorphy' (RAS_{ij}) which is the parsimony informative character states shared between taxa *i* and *j* to the exclusion of other taxa, for all characters (Lyons-Weiler *et al.*, 1996; Lyons-Weiler & Hoelzer, 1997). RAS_{ij} is plotted against a phenetic measure of similarity (E_{ij}, which includes all potentially informative characters); the slope is the rate at which cladistic measure increases as a function of the phenetic measure, and is an indication of character covariation (Lyons-Weiler *et al.*, 1996). The slope of this model is compared to a null model of random character covariation to test the null hypothesis of no significant difference between β_{obs} and β_{null}. Unlike other tests for phylogenetic signal, RASA is a tree-independent approach, therefore not linked to the underlying assumptions and potential errors of the tree-building algorithm (Lyons-Weiler & Hoelzer, 1997). RASA is useful in identifying long-branch taxa (a lineage whose character states have become effectively randomized with respect to other taxa in the matrix) (Lyons-Weiler & Hoelzer, 1997), and which tend to disrupt phylogenetic signal (Hendy & Penny, 1989). Long-branch taxa exhibit greater 'cla-

distic' signal than predicted by their 'phenetic' similarity, thus appearing as outlier points in the regression analysis (Lyons-Weiler, 2001). Taxon variance ratio plots of the in-group were examined to identify any taxa with greater than average variance ratio values, indicating long-branches (Lyons-Weiler, 2001).

Phylogenetic analysis

We used three methods of phylogenetic reconstruction: two involving a molecular evolution approach (maximum likelihood and Bayesian analyses), and maximum parsimony. Maximum parsimony (MP, Camin & Sokal, 1965) was implemented using two weighting schemes: all nucleotide positions weighted equally, and differentially weighted transitions and transversions (see Edwards, 1997). The transition/transversion ratio was estimated on the combined data set using maximum likelihood (ML) under the HKY85+I+Γ model of nucleotide substitution, as implemented in MR.MODELTEST vers. 1.1 (Nylander, 2002). Heuristic search strategies were conducted in PAUP*. For MP we used stepwise addition with 100 random addition replicates and TBR branch swapping. Relative support for nodes in resulting trees was evaluated using 200 bootstrap (Felsenstein, 1985) replicates with 100 random additions per replicate.

Under maximum likelihood (ML, Felsenstein, 1981), the optimal likelihood score is dependent upon tree topology as well as the model of DNA substitution and the parameter values used (Huelsenbeck & Crandall, 1997). Therefore, to evaluate the ability of various models of DNA substitution to infer ML topologies with our data, we first compared nested models using a hierarchical framework as implemented by MR.MODELTEST 1.1 (Nylander, 2002). MR.MODELTEST calculates the likelihood scores of each of 24 models based on a NJ topology using the Jukes-Cantor model of evolution (Jukes & Cantor, 1969). The models are nested in that the null model is a special case of the alternative, more parameter rich model (see Posada & Crandall, 1998). Testing the goodness of fit is $\delta = -2 \log \Lambda$ (where Λ = log likelihood of null model/log likelihood of the alternative model). Beginning with the simplest null model, alternative nesting models are tested for goodness of fit; the null model is rejected in favour of the nesting model if the latter has significantly lower log likelihood. The δ statistic is approximately χ^2 distributed (Yang, Golman & Friday, 1995) with q degrees of freedom based on the difference in the number of free parameters between the alternative and null models of DNA evolution (Goldman, 1993). The best model is found when the addition of alternative parameters fails to reject the more simple model. Thus, the likelihood scores provide an objective criterion of goodness of fit of the models and the data (Posada & Crandall, 1998). A ML heuristic search with 'as-is' random additions was conducted using the fully defined model determined above. Support was determined using 100 non-parametric bootstraps, using 'as-is' stepwise addition.

Bayesian approaches to phylogenetic reconstruction are recognized as an important advance because of their ability to analyse large data sets relatively quickly, while incorporating complex models of molecular evolution that can make traditional ML approaches computationally prohibitive with large sample sizes (Sanderson & Kim, 2000; Leaché & Reeder, 2002). The Bayesian approach approximates the posterior probability distribution of the tree topology, branch lengths, and nucleotide substitution model being assumed, using the Markov chain Monte Carlo (MCMC) method (Yang & Rannala, 1997; Mau, Newton & Larget, 1999). Bayesian inference implements likelihood functions to extract information and has been shown to generate similar results to traditional ML, but with drastically reduced computational time (e.g. Yang & Rannala, 1997; Larget & Simon, 1999; Leaché & Reeder, 2002).

Bayesian analysis was implemented with MR.BAYES 2.01 (Huelsenbeck & Ronquist, 2001). We used the same molecular model as for the ML analysis (GTR+I+Γ). Model parameters (base pair frequencies, substitution rate matrix) were treated as unknown variables to be estimated by MR.BAYES. Analyses began with random starting trees and ran for 2.0×10^5 generations, with Markov chains sampled every 100 generations. Metropolis-coupled MCMC, using four incrementally heated Markov chains per generation, were implemented as the default in MR.BAYES. Stationarity of the Markov chain was determined by plotting the sampled ln$L$ scores against generation time to determine when equilibrium was reached. The 'burn-in' generations (random points generated prior to stationarity) were discarded and subsequent generations were used to form the posterior probability distribution. The analysis was conducted twice, using identical settings, to ensure convergence on the same ln$L$ values (Huelsenbeck & Bollback, 2001; Leaché & Reeder, 2002). The remaining trees from both analyses were used to create a majority rule consensus tree, where the percent of samples recovering the same clade represent the posterior probability of that clade. Because these represent the true probabilities of the clades (Rannala & Yang, 1996), posterior probabilities greater than 95% were considered significant (Leaché & Reeder, 2002). For non-parametric bootstrap analyses we used 70% as the criterion for evidence of good support.

Congruence among methods (MP, ML, and Bayesian) was assessed qualitatively by comparing similarity of topologies and corresponding nodal support. Quantitative examination of congruence was done

using the Shimodaira-Hasegawa test, which statistically compares topology likelihoods among multiple competing hypotheses (Shimodaira & Hasegawa, 1999; Goldman, Anderson & Rodrigo, 2000). The Shimodaira-Hasegawa test was implemented in PAUP* using the RELL resampling method of Kishino, Miyata & Hasegawa (1990), with 10 000 bootstrap replicates.

Testing the tempo of diversification

Unresolved evolutionary relationships are considered soft polytomies in that they are multiple dichotomous branching events occurring in rapid succession. To differentiate between poorly supported clades (soft polytomies) vs. zero-length branches (hard polytomies), we used the likelihood ratio test recently proposed by Slowinski (2001). Using the 'describe trees' command following our ML run (with 'Perform likelihood-ratio test for zero branch lengths' selected in the likelihood settings menu), this test returns the probability that each branch length is non-negative. However, the χ^2 distribution with one degree of freedom is inapplicable when the null hypothesis fixes the branch length to zero, which is on the boundary of the set of values permitted under the alternative hypothesis (i.e. $[0,\infty]$) (Whelan & Goldman, 1999; Slowinski, 2001). Goldman & Whelan (2000) have shown that a statistically rigorous distribution is a 50 : 50 mixture of the χ^2 with zero degrees of freedom and with one degree of freedom. Significance for the likelihood ratio test for each branch in the phylogeny was determined using the percentage point values under the Goldman & Whelan (2000) mixed model (their Table 2). We used a conservative α level (0.01) to account for possible Type I error.

To examine temporal divergence within the *Rana catesbeiana* species group, we examined rates of molecular evolution by assuming an evolutionary rate similar to published mtDNA estimates of rate variation in similar-sized anurans, because absolute rate heterogeneity is related primarily to body size and metabolic rate (Martin & Palumbi, 1993). Ideally, such rates are calibrated using fossil records and known vicariant biogeographical events (Rand, 1994; Zamudio & Greene, 1997). Unfortunately, the fossil record for this species group is poor. Further, most of the species in this group are broadly sympatric, making it difficult to tie geological events to vicariant modes of speciation. We looked for mtDNA divergence rates from literature in which anuran amphibians of similar body size were calibrated using vicariant dates, and applied these rates to the *Rana catesbeiana* species group. Rate variation in mtDNA evolution (e.g. Avise *et al*., 1992; Martin, Naylor & Palumbi, 1992; Martin & Palumbi, 1993; Rand, 1994; Avise, Walker & Johns, 1998) underscores the necessity for caution when

Table 2. Distribution of phylogenetically informative and variable positions for cyt *b*, ND2, and $tRNA^{TRP}$ gene regions. See text for sequence lengths of each data partition. Base pair frequency for each gene region is included

		Variable characters (proportion)				Parsimony informative (proportion)				Base pair frequency			
		1st	2nd	3rd	Total	1st	2nd	3rd	Total	A	C	G	T
Cyt *b*	Ingroup only	33 (.162)	8 (.006)	212 (.029)	253 (.274)	28 (.091)	6 (.019)	203 (.657)	237 (.256)	0.2412	0.3079	0.1404	0.3105
	Overall	45 (.146)	9 (.029)	253 (.819)	307 (.332)	28 (.091)	6 (.195)	216 (.699)	250 (.270)	0.2410	0.3083	0.1402	0.3105
ND2	Ingroup only	38 (.191)	11 (.055)	119 (.595)	168 (.280)	36 (.181)	10 (.050)	113 (.565)	159 (.265)	0.2767	0.2827	0.1084	0.3323
	Overall	66 (.332)	19 (.095)	165 (.825)	250 (.417)	38 (.191)	10 (.050)	127 (.635)	175 (.292)	0.2770	0.2810	0.1088	0.3331
$tRNA^{TRP}$	Ingroup only	–	–	–	0	–	–	–	0	0.3667	0.2667	0.2000	0.1677
	Overall	–	–	–	2 (.067)	–	–	–	1 (.033)	0.3677	0.2657	0.1980	0.1677

attempting to interpret biogeographical scenarios using a molecular clock calibrated for other groups (Zamudio & Greene, 1997). We therefore use any estimates of divergence times cautiously. Finally, incorporating intraspecific diversity may have a profound effect on determining interspecific divergence (Melnick *et al*., 1993). Accordingly, we corrected for within species genetic distances following Avise *et al*. (1992), using the corrected values to estimate divergence times.

Dispersal–vicariance analysis

Current distribution (excluding regions where some species are introduced) and fossil records (Holman, 1995) were examined to assign each of the seven species of *Rana* to four major physiographic regions of Eastern North America (Coastal Plain, Appalachian, Interior Plain, and Laurentian Upland). These four regions are large geographical areas encompassing various natural regions within each (see descriptions in Duellman & Sweet, 1999). Given the limited size of the species group in question, we chose to limit the number of geographical regions to this broad scale. Because substantial geographical overlap exists in many of the species' ranges, increasing the geographical resolution (i.e. using natural areas rather than major physiographic regions) would simply decrease the resolution of our analysis.

The present Appalachian region is largely the result of the most recent major regional orogeny that ending during the Triassic (140–210 Mya). Here the Appalachians refers to the Highlands, Piedmont, and Appalachian Plateaux. The Interior Plain separates the Appalachians from the Rocky Mountains and consists of the Great Plains, Interior Lowlands, and Interior Highlands. The Laurentian Upland is characterized by a peneplain developed on low-lying granite rock. This region was repeatedly and completely glaciated during the Pleistocene. Finally, the Coastal Plain consists of lowlands along the Gulf of Mexico and Atlantic Ocean, ranging from Long Island to Mexico. There is very little increase in elevation inland until the Fall Line, which demarcates the beginning of the Piedmont and Appalachian Plateaux (Duellman & Sweet, 1999).

Dispersal–vicariance analysis (Ronquist, 1996, 1997) was used to reconstruct the ancestral distributions and identify possible dispersal events in the history of the *Rana catesbeiana* species group. A number of characteristics of dispersal–vicariance analysis improve upon traditional biogeographical analytical approaches (Ronquist, 1997). For example, dispersal–vicariance does not require an area-relationship hypothesis. Over evolutionary time, geographical areas are not necessarily hierarchically related to one another. Rather, the formation and loss of barriers, fragmentation of ecological regions, and so on, reflect reticulate relationships among area biota (Ronquist, 1997). Dispersal–vicariance neither shares many of the constraining assumptions associated with traditional methods (such as dispersal events obligately associated with speciation), nor assumes that ancestors were restricted geographically to a single unit area (Ronquist, 1996). DIVA (version 1.1; Ronquist, 1996) reconstructs the optimal ancestral distributions of a group of taxa of a known phylogeny based on a three-dimensional cost matrix with two main assumptions:

1. Speciation is generally due to vicariance. If the ancestral species is restricted to a single unit area, it speciates allopatrically and the two descendant species co-occur in the same unit area. This scenario could be equally explained by geographical allopatry, peripatry (*sensu* Mayr, 1954, 1982), or even sympatric speciation (Ronquist, 1996). Alternatively, a widespread ancestor (one associated with two or more unit areas) speciates allopatrically (assumes a geographical separation), resulting in the distribution of daughter species into two mutually exclusive areas. The cost of either scenario is zero.
2. The addition (i.e. via dispersal) or loss (extinction) of an area to a distribution has a cost of one per unit area.

The algorithm searches for the biogeographical reconstruction that minimizes dispersal cost following two rules: (1) the optimal ancestral distribution cannot contain an area not occupied by any descendant, and (2) the ancestral node must include at least one unit area from each of the two descendant species (Ronquist, 1997). Weaknesses associated with the dispersal–vicariance approach include its inability to realistically model extinction events (extinctions will not be interpreted without constraints on the cost-assignment rules; Ronquist, 1996). Also, uncertainties inherent in tree optimization mean that the root node (i.e. the ancestral area) will be the least reliable in the tree, usually manifesting itself as the root node being widely distributed (Ronquist, 1996). Since one objective is to estimate the distribution of the common ancestor of the *Rana catesbeiana* species group, we approached this problem in two ways. (1) We included both outgroup species in our biogeographical reconstruction. (2) Excluding outgroups, we constrained the optimization to give a maximum of two unit areas at the ancestral node. Both of these methods allowed us to determine the most likely distribution of the common ancestor, assuming it had a limited distribution.

Mode of speciation

In light of the interesting distributional patterns among species of the *Rana catesbeiana* group, we

applied the method of Lynch (1989), which assigns modes of speciation based on the assumption that relative species distributions of sister taxa contain information on the mode of speciation. Lynch's method compares relative range size and range overlap of sister-groups across speciation levels. Here, speciation level refers to phylogenetic branching order; level one is sister-species, level two is inclusive of level one, and so on. Lynch somewhat arbitrarily (Lynch, 1989; Chesser & Zink, 1994) assigned range overlap of greater than 20% among level one comparisons to represent substantial sympatry; at levels two and above, greater than 20% was indeterminate between sympatry and allopatric speciation with subsequent dispersal. Ranges with less than 20% overlap were considered to reflect allopatric speciation, and subsequent examination of range sizes was used to differentiate between vicariance and peripheral isolation. Evidence for peripheral isolate speciation was taken when the smaller range of the sister-species was less than 5% of the larger. The distributions are well documented for the *Rana catesbeiana* group (see Fig. 1). The amount of range overlap was conservatively estimated by eye to the nearest 10%. We deemed this method sufficient given the known patterns of distribution. In other words, none of the estimated range overlaps approached the 20% overlap criteria (see Results).

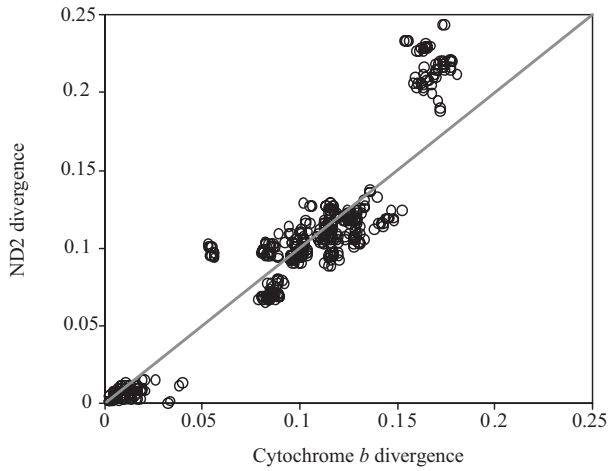

Figure 2. Scatterplot of Nei's uncorrected pairwise sequence divergence for cyt *b* vs. ND2 for all sequences. The solid line ($x = y$) corresponds to equal divergence rates for both genes. ND2 accumulates mutations faster than cyt *b* only at divergences greater than 15%.

RESULTS

Phylogenetic signal

For phylogenetic analyses we obtained 925 bp of cyt *b* and 629 bp of ND2 (including 30 bp of the 5′ flanking $tRNA^{TRP}$ region). Of the 925 bp of cyt *b*, 33% were variable, 27% being potentially parsimony informative. For ND2, 42% were variable and 29% potentially parsimony informative. The 30 bp of the tRNA flanking region had no variable positions within the in-group. (see Table 2 for complete list of variable sites and base pair content). The absence of insertions, misplaced stop codons, or indels; the presence of a strand bias against guanine on the light strands of both coding genes, and a notable third codon bias suggest that our sequences are not nuclear homologues. Plots of Kimura 2-parameter vs. raw genetic distance suggest slight saturation for both cyt *b* and ND2 at the third bp position only (not shown). ND2 was slightly more variable than cyt *b* across pairwise comparisons of in-group taxa (mean uncorrected *p* distances 0.0848 and 0.0808, respectively, see Table 3). This difference was significant (paired sample *t*-test: $t_{464d.f.} = 6.335$, $P < 0.001$). The difference in the rate of evolution (or saturation) increased in ND2 relative to cyt *b* only at levels of divergence greater than 15% (Fig. 2). The partition homogeneity test indicated a lack of significant heterogeneity between the two partitions ($P = 0.158$).

Results from Faith's permutation analysis suggested that phylogenetic structure was significantly different from random for both data partitions considered separately and combined (all PTP = 0.001). RASA also indicated statistically significant phylogenetic signal for both partitions (ND2–$t_{RASA,431d.f.} = 11.33$, $P < 0.001$; cyt *b*–$t_{RASA,431d.f.} = 10.55$, $P < 0.001$) and the combined data set ($t_{RASA,431d.f.} = 11.00$, $P < 0.001$). Despite the strong phylogenetic signal, taxon variance plots for both data partitions, considered separately and combined, suggested a potential long-branch problem attributable to *R. grylio* (Fig. 3). Phenetic (e.g. neighbour-joining) and MP methods may be particularly sensitive to long-branch taxa (Felsenstein, 1978; Hillis, Huelsenbeck & Swofford, 1994). Therefore, we conducted weighted and unweighted MP analysis with and without *R. grylio* to determine the effect of the problem taxon on the resultant topology and nodal support.

Phylogenetic relationships

Maximum parsimony resulted in slightly different tree topologies depending on weighting scheme and taxon inclusion. Unweighted MP resulted in six equally most parsimonious trees of 1096 steps, with *R. grylio* basal in the species group with high bootstrap support (100%, Fig. 4A). Exclusion of the long-branch taxa (Fig. 4B) resulted in convergence of the tree topology close to that of the weighted MP (Fig. 5) and minimum evolution trees (Fig. 6), where

Table 3. Uncorrected pairwise raw distances of ND2 (above diagonal) and cyt *b* (below diagonal) among *Rana catesbeiana* species group and outgroup taxa. Haplotypes as in Table 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Rcat1319	–	0.010	0.008	0.008	0.008	0.005	0.067	0.068	0.073	0.068	0.067	0.067	0.067	0.072	0.072	0.067	0.072
2. Rcat1365	0.009	–	0.015	0.015	0.015	0.008	0.077	0.078	0.080	0.078	0.077	0.077	0.077	0.078	0.078	0.077	0.078
3. Rcat627	0.016	0.019	–	0.007	0.000	0.010	0.068	0.070	0.072	0.070	0.068	0.068	0.068	0.070	0.070	0.068	0.070
4. Rcat978	0.021	0.026	0.006	–	0.007	0.010	0.068	0.070	0.072	0.070	0.068	0.068	0.068	0.070	0.070	0.068	0.070
5. Rcat624	0.015	0.021	0.001	0.005	–	0.010	0.068	0.070	0.072	0.070	0.068	0.068	0.068	0.070	0.070	0.068	0.070
6. RcatM95	0.007	0.009	0.017	0.019	0.016	–	0.068	0.070	0.072	0.070	0.068	0.068	0.068	0.070	0.070	0.068	0.070
7. Rcla1344	0.084	0.089	0.086	0.086	0.085	0.086	–	0.005	0.007	0.007	0.003	0.003	0.003	0.005	0.005	0.003	0.005
8. Rcla1334	0.087	0.091	0.086	0.086	0.085	0.089	0.013	–	0.012	0.012	0.008	0.008	0.008	0.010	0.010	0.008	0.010
9. Rcla1393	0.084	0.089	0.086	0.086	0.085	0.086	0.004	0.009	–	0.013	0.010	0.010	0.010	0.002	0.002	0.010	0.002
10. Rcla1394	0.084	0.089	0.086	0.086	0.085	0.086	0.013	0.017	0.011	–	0.003	0.003	0.003	0.012	0.012	0.007	0.012
11. Rcla042	0.081	0.085	0.083	0.083	0.082	0.083	0.014	0.021	0.016	0.010	–	0.000	0.000	0.008	0.008	0.003	0.008
12. Rcla986	0.079	0.083	0.081	0.081	0.080	0.081	0.014	0.018	0.012	0.005	0.004	–	0.000	0.008	0.008	0.003	0.008
13. Rcla1237	0.081	0.085	0.084	0.084	0.083	0.083	0.013	0.019	0.011	0.004	0.010	0.005	–	0.008	0.008	0.003	0.008
14. Roka1408	0.084	0.089	0.086	0.086	0.085	0.086	0.004	0.009	0.000	0.011	0.016	0.012	0.011	–	0.000	0.008	0.000
15. Roka1403	0.084	0.089	0.086	0.086	0.085	0.086	0.006	0.011	0.002	0.011	0.016	0.012	0.011	0.002	–	0.008	0.000
16. Roka1405	0.088	0.092	0.090	0.090	0.089	0.090	0.011	0.011	0.006	0.017	0.018	0.016	0.017	0.006	0.009	–	0.008
17. Roka1409	0.087	0.091	0.089	0.089	0.088	0.089	0.009	0.009	0.004	0.015	0.016	0.014	0.015	0.004	0.006	0.004	–
18. Roka1404	0.082	0.086	0.084	0.084	0.083	0.084	0.011	0.017	0.009	0.002	0.008	0.003	0.002	0.009	0.009	0.015	0.013
19. Rsep185	0.115	0.116	0.118	0.120	0.117	0.116	0.102	0.103	0.102	0.104	0.102	0.104	0.102	0.102	0.102	0.102	0.103
20. Rsep135	0.115	0.116	0.118	0.120	0.117	0.116	0.102	0.103	0.102	0.104	0.102	0.104	0.102	0.102	0.102	0.102	0.103
21. Rsep24410	0.113	0.116	0.116	0.120	0.115	0.116	0.100	0.099	0.097	0.097	0.097	0.097	0.095	0.097	0.097	0.100	0.099
22. Rvir1348	0.128	0.129	0.129	0.131	0.128	0.123	0.115	0.119	0.115	0.112	0.116	0.116	0.111	0.115	0.117	0.120	0.117
23. Rvir1352	0.128	0.129	0.129	0.131	0.128	0.123	0.112	0.117	0.112	0.110	0.114	0.114	0.109	0.112	0.115	0.118	0.115
24. Rvir1351	0.130	0.131	0.131	0.133	0.130	0.125	0.115	0.119	0.115	0.112	0.116	0.116	0.111	0.115	0.117	0.120	0.117
25. RvirA	0.092	0.094	0.095	0.098	0.094	0.089	0.101	0.109	0.103	0.101	0.104	0.104	0.100	0.103	0.105	0.108	0.105
26. Rhec1308	0.096	0.097	0.097	0.097	0.096	0.096	0.084	0.089	0.085	0.084	0.083	0.082	0.083	0.085	0.088	0.085	0.086
27. Rhec1407	0.095	0.096	0.096	0.096	0.095	0.095	0.081	0.089	0.082	0.081	0.083	0.081	0.080	0.082	0.084	0.085	0.086
28. Rhec1411	0.095	0.096	0.096	0.096	0.095	0.095	0.081	0.089	0.082	0.081	0.083	0.081	0.080	0.082	0.084	0.085	0.086
29. Rhec1307	0.097	0.098	0.101	0.101	0.099	0.097	0.054	0.056	0.053	0.056	0.056	0.055	0.055	0.053	0.055	0.054	0.054
30. Rgry1396	0.143	0.147	0.142	0.138	0.141	0.144	0.122	0.125	0.124	0.124	0.122	0.123	0.124	0.124	0.126	0.121	0.125
31. Rgry1332	0.148	0.152	0.146	0.143	0.145	0.148	0.128	0.130	0.130	0.130	0.126	0.129	0.130	0.130	0.132	0.125	0.130
R. sylvatica	0.163	0.168	0.166	0.169	0.165	0.163	0.174	0.177	0.177	0.174	0.170	0.171	0.174	0.177	0.177	0.177	0.178
R. luteiventris	0.159	0.165	0.171	0.169	0.170	0.158	0.162	0.163	0.165	0.162	0.160	0.161	0.163	0.165	0.167	0.166	0.166

Table 3. *Continued*

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1. Rcat1319	0.065	0.093	0.095	0.090	0.100	0.102	0.104	0.104	0.092	0.093	0.093	0.092	0.114	0.117	0.200	0.209
2. Rcat1365	0.075	0.104	0.105	0.100	0.110	0.112	0.114	0.114	0.102	0.104	0.104	0.102	0.124	0.124	0.204	0.209
3. Rcat627	0.067	0.095	0.097	0.088	0.105	0.107	0.109	0.109	0.090	0.092	0.092	0.090	0.115	0.119	0.207	0.214
4. Rcat978	0.067	0.095	0.097	0.092	0.102	0.104	0.105	0.105	0.093	0.095	0.095	0.093	0.112	0.115	0.209	0.210
5. Rcat624	0.067	0.095	0.097	0.088	0.105	0.107	0.109	0.109	0.090	0.092	0.092	0.090	0.115	0.119	0.207	0.214
6. RcatM95	0.067	0.095	0.097	0.092	0.105	0.107	0.109	0.109	0.093	0.095	0.095	0.093	0.119	0.119	0.202	0.205
7. Rcla1344	0.005	0.099	0.100	0.095	0.107	0.109	0.110	0.110	0.095	0.097	0.097	0.095	0.117	0.117	0.217	0.227
8. Rcla1334	0.010	0.100	0.102	0.097	0.105	0.107	0.109	0.109	0.093	0.095	0.095	0.093	0.115	0.115	0.215	0.227
9. Rcla1393	0.012	0.105	0.107	0.102	0.114	0.115	0.117	0.117	0.102	0.104	0.104	0.102	0.124	0.124	0.220	0.230
10. Rcla1394	0.005	0.097	0.099	0.093	0.105	0.107	0.109	0.112	0.097	0.099	0.099	0.097	0.119	0.119	0.215	0.227
11. Rcla042	0.002	0.095	0.097	0.092	0.104	0.105	0.107	0.110	0.095	0.097	0.097	0.095	0.117	0.117	0.217	0.225
12. Rcla986	0.002	0.095	0.097	0.092	0.104	0.105	0.107	0.110	0.095	0.097	0.097	0.095	0.117	0.117	0.217	0.225
13. Rcla1237	0.002	0.095	0.097	0.092	0.104	0.105	0.107	0.110	0.095	0.097	0.097	0.095	0.117	0.117	0.217	0.225
14. Roka1408	0.010	0.104	0.105	0.100	0.112	0.114	0.115	0.115	0.100	0.102	0.102	0.100	0.122	0.122	0.219	0.229
15. Roka1403	0.010	0.104	0.105	0.100	0.112	0.114	0.115	0.115	0.100	0.102	0.102	0.100	0.122	0.122	0.219	0.229
16. Roka1405	0.005	0.097	0.099	0.093	0.104	0.105	0.107	0.107	0.095	0.097	0.097	0.095	0.120	0.120	0.219	0.227
17. Roka1409	0.010	0.104	0.105	0.100	0.112	0.114	0.115	0.115	0.100	0.102	0.102	0.100	0.122	0.122	0.219	0.229
18. Roka1404	–	0.097	0.099	0.093	0.102	0.104	0.105	0.109	0.097	0.099	0.099	0.097	0.119	0.119	0.215	0.227
19. Rsep185	0.104	–	0.002	0.010	0.114	0.115	0.114	0.120	0.112	0.114	0.114	0.112	0.135	0.135	0.202	0.214
20. Rsep135	0.104	0.000	–	0.012	0.115	0.117	0.115	0.122	0.112	0.114	0.114	0.112	0.137	0.135	0.202	0.214
21. Rsep24410	0.097	0.013	0.013	–	0.114	0.115	0.114	0.120	0.112	0.114	0.114	0.112	0.132	0.132	0.205	0.217
22. Rvir1348	0.112	0.115	0.115	0.117	–	0.005	0.007	0.013	0.124	0.125	0.125	0.124	0.124	0.130	0.194	0.210
23. Rvir1352	0.110	0.112	0.112	0.115	0.002	–	0.002	0.012	0.125	0.127	0.127	0.125	0.119	0.125	0.189	0.205
24. Rvir1351	0.112	0.115	0.115	0.117	0.004	0.002	–	0.013	0.127	0.129	0.129	0.127	0.119	0.125	0.187	0.204
25. RvirA	0.101	0.119	0.119	0.123	0.040	0.038	0.040	–	0.127	0.129	0.129	0.127	0.120	0.127	0.199	0.215
26. Rhec1308	0.084	0.115	0.115	0.116	0.117	0.115	0.117	0.105	–	0.002	0.002	0.000	0.122	0.119	0.219	0.232
27. Rhec1407	0.081	0.114	0.114	0.115	0.116	0.114	0.116	0.102	0.003	–	0.000	0.002	0.124	0.120	0.219	0.232
28. Rhec1411	0.081	0.114	0.114	0.115	0.116	0.114	0.116	0.102	0.003	0.000	–	0.002	0.124	0.120	0.219	0.232
29. Rhec1307	0.056	0.110	0.110	0.113	0.118	0.116	0.118	0.106	0.032	0.034	0.034	–	0.122	0.119	0.219	0.232
30. Rgry1396	0.124	0.136	0.136	0.140	0.129	0.126	0.129	0.127	0.116	0.116	0.116	0.115	–	0.007	0.214	0.242
31. Rgry1332	0.130	0.136	0.136	0.140	0.133	0.131	0.133	0.133	0.119	0.119	0.119	0.120	0.011	–	0.210	0.242
R. sylvatica	0.174	0.160	0.160	0.160	0.171	0.172	0.172	0.168	0.172	0.174	0.174	0.173	0.177	0.181	–	0.209
R. luteiventris	0.164	0.174	0.174	0.173	0.163	0.162	0.163	0.164	0.154	0.155	0.155	0.156	0.173	0.174	0.170	–

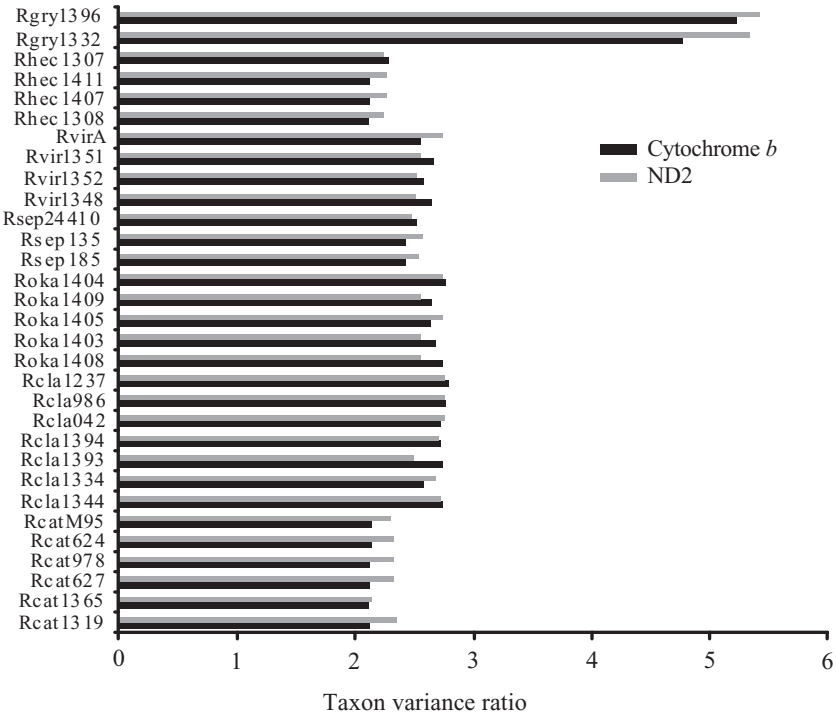

Figure 3. Taxon variance ratio plots generated by RASA 3.0.4. For both data partitions, *Rana grylio* contributes significantly greater variance to the RAS (i.e. cladistic measure) than to the phenetic variance (see text), indicating that *R. grylio* may be long-branched.

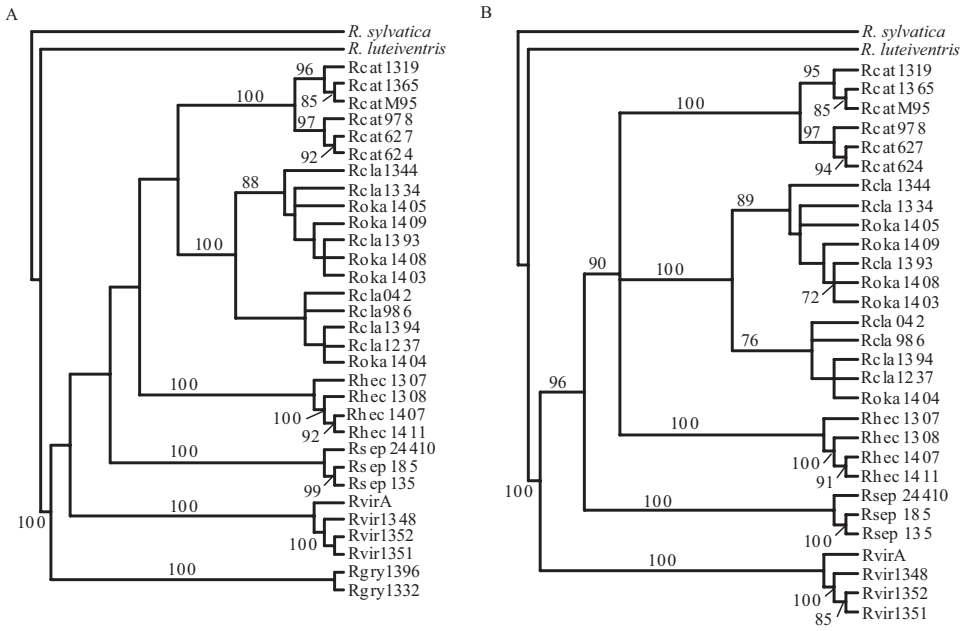

Figure 4. Phylogenetic reconstructions derived from equally weighted maximum parsimony, using 100 random additions and TBR branch swapping. (A) All taxa included: consensus of six most parsimonious trees (1096 steps, CI = 0.630 RI = 0.836). (B) *Rana grylio* excluded due to potential long-branch state: consensus of 12 most parsimonious trees (958 steps, CI = 0.672, RI = 0.858). Numbers above branches indicate per cent nodal support following 200 bootstraps.

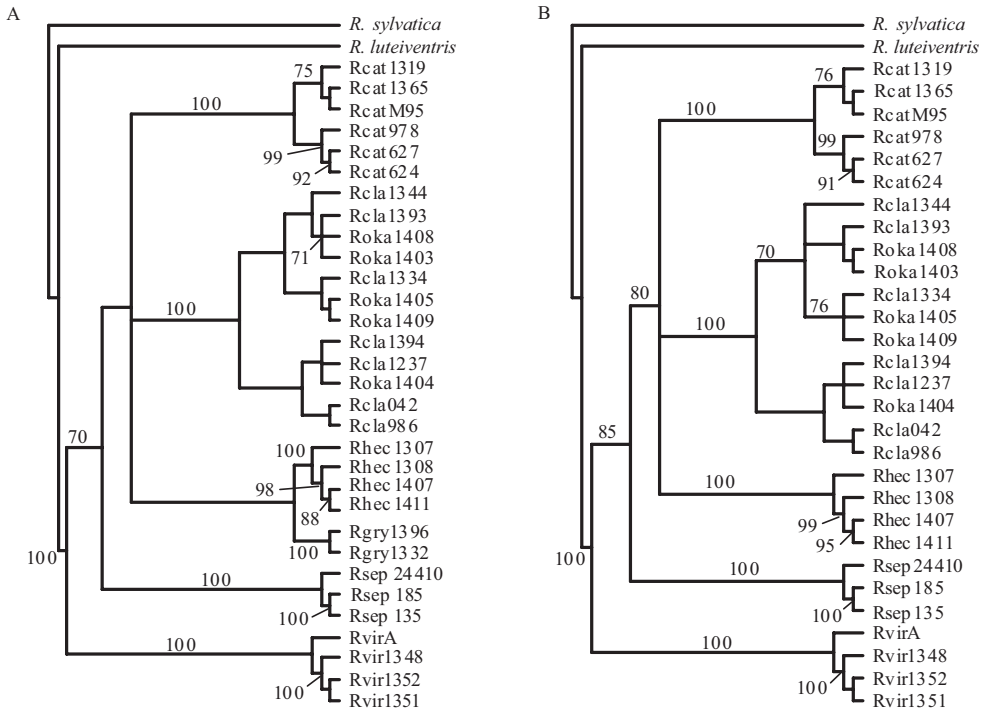

Figure 5. Phylogenetic reconstructions derived from maximum parsimony analysis with transitions weighted 6× transversions, based on the empirically derived estimate. Analysis used 100 random additions and TBR branch swapping. (A) All taxa included: consensus of two most parsimonious trees (2283 steps, CI = 0.692, RI = 0.840). (B) *Rana grylio* excluded due to potential long-branch state: single most parsimonious trees (2013 steps, CI = 0.737, RI = 0.864). Numbers above branches indicate per cent nodal support following 200 bootstraps.

R. virgatipes was basally placed. MP analysis with transitions weighted 6× transversions (based on empirical estimate of 6.3) also produced a topology similar to that of the minimum evolution trees, placing *R. grylio* sister to *R. heckscheri* (Fig. 5A). Removal of *R. grylio* under the weighting scheme (Fig. 5B) did not have a major topological effect but did increase the bootstrap support at basal nodes.

The best fit model for mtDNA evolution based on the nested likelihood ratio test was the general time reversible model (Rodríguez *et al*., 1990) assuming unequal base frequencies, a proportion of invariable sites, and that rates of change are γ distributed (GTR+I+Γ; see Table 5). ML tree topology was derived using empirically estimated nucleotide frequencies and incorporating the proportion of invariable sites ($\theta = 0.462$) and γ distribution of rate heterogeneity across variable sites ($\alpha = 0.858$) estimated using MR.MODELTEST. The log likelihood for the single ML tree obtained (Fig. 6) was $-\ln L = 7038.10$. Bootstrap analysis resulted in 15 nodes with bootstrap support greater than 70%.

Two independent Bayesian analyses under the GTR+I+Γ model converged on the same ln*L* scores (mean = –ln 7013.348 ± 0.424 SD) after 20 000 generations (not shown). Therefore, the first 20 000 trees were omitted from analyses. The topology for both ML and Bayesian analyses were virtually identical, with the mean likelihood of two Bayesian runs being 7023.35 (0.424 SD). Posterior probability values from Bayesian analysis were highly congruent with ML bootstrap support. The strict consensus of the 3.6×10^5 trees resulted in 16 nodes with posterior probabilities greater than or equal to 95% (Fig. 6). Together, similarity in ln*L* values and nodal support suggest the two methods successfully converged on the same tree space.

Phylogenetic analyses using MP, ML, and Bayesian methods all supported a pattern of paraphyly among lineages of *R. clamitans* and *R. okaloosae*. The level of divergence within and between *R. clamitans* and *R. okaloosae* was low (mean intraspecific pairwise uncorrected distances: 0.93% ± 0.4 and 0.7% ± 0.35, respectively; mean interspecific distances: 0.88% ± 0.48). One haplotype was shared by both species (see Table 1). The Shimiodaira–Hasegawa test rejected the monophyly of *R. clamitans* and *R. okaloosae* (Table 4). All other species were monophyletic. Further, *R. clamitans* haplotypes do not reflect subspecies designations (i.e. they are not mono-

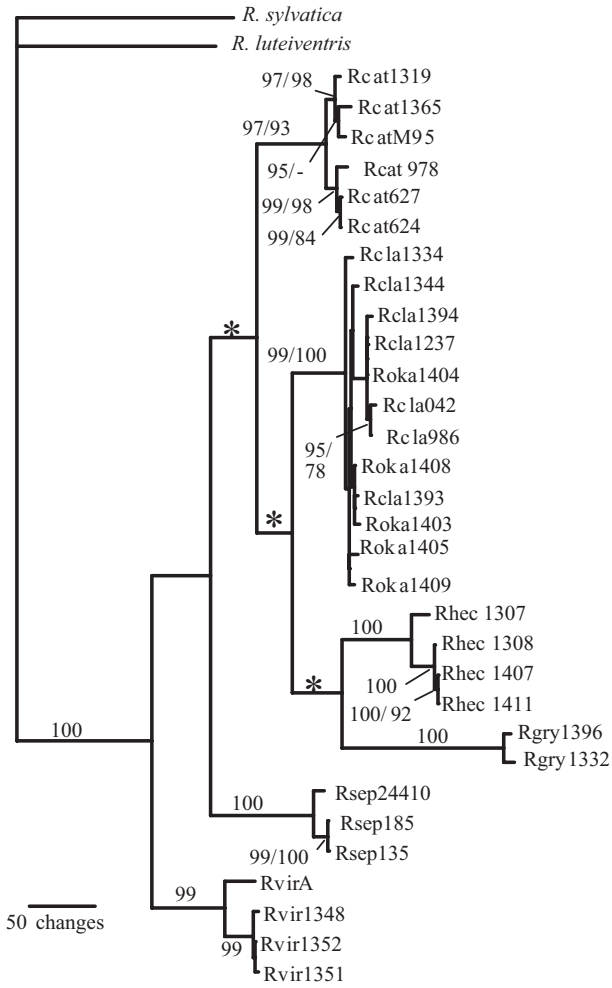

Figure 6. Phylogenetic reconstruction resulting from both maximum likelihood and Bayesian analyses. Both approaches implemented a GTR+Γ+I model using user-specified γ distribution, estimated proportion of invariable sites, substitution rate matrix, and empirical nucleotide frequencies. Asterisks indicate branches that are not significantly different from zero-length (only interspecific zero-length branches are indicated). Per cent posterior probability and per cent bootstrap support are indicated, respectively, at nodes.

phyletic) afforded by Mecham (1954) and others (Ferguson, 1961; Jenssen, 1968). One Florida haplotype (Rcla1394) is more similar to 'northern' haplotypes, despite its location well south of the proposed intergrade zone between races.

Timing of divergence in the *Rana catesbeiana* group

Bayesian posterior probability of the node separating *R. septentrionalis* from the rest of the species was nearly significant (94%), but non-parametric bootstrap support was low at less than 70%. However, the branch length separating *R. septentrionalis* from the more terminal nodes was significantly greater than zero, suggesting that the lack of support does not reflect a hard polytomy, but rather the tendency of bootstrap support in particular, but also Bayesian posterior probabilities, to underestimate phylogenetic accuracy at higher levels of support (Wilcox *et al*., 2002). The relationship between *R. catesbeiana*, *R. grylio*, *R. heckscheri* and *R. clamitans* remains unresolved. All three phylogenetic methods resulted in a trichotomy where bootstrap and posterior probabilities are low and branch lengths not significantly larger than zero. The Shimiodaira–Hasegawa test failed to reject the commonly assumed *Rana clamitans–Rana catesbeiana* sister relationship (Case, 1978; Hillis & Davis, 1986), nor did it reject the alternative topologies suggested by the weighted and unweighted MP trees (Table 4).

A calibrated rate of mtDNA evolution (using 1063 bp consisting of partial sequence of ND1, transfer RNAs, and the complete ND2 gene) for *Bufo bufo* of 0.69% sequence change per million years (Macey *et al*., 1998) was applied to our data set. This calibration was based on the uplift and aridization of the Tibetan Plateau, separating European *Bufo* from Asia conspecifics. The level of variation was similar between their ND1 and ND2 sequences, with slightly more variation measured in ND2, similar to our cyt *b*–ND2 sequence. Omission of *R. grylio* and application of this rate to interspecific distances corrected for within lineage divergence

Table 4. Results of multiple comparisons of log-likelihoods using Shimodaira–Hasegawa test

Alternative hypothesis	–ln*L*	Difference –ln*L*	*P*
Bayesian tree	7038.101	0	0.923
R. clamitans & *R. okaloosae* monophyletic	7103.457	65.356	0.004
R. catesbeiana + *R. clamitans*/*R. okaloosae* sister clades	7042.674	4.573	0.704
MP unweighted	7055.917	17.816	0.291
MP weighted	7046.532	8.432	0.584

Table 5. Results of the nested likelihood ratio tests on models of DNA substitution from combined cyt *b* + ND2 data partitions. L_0 and L_1 denote the likelihoods under the null and alternative hypotheses, respectively. The likelihood ratio test statistic ($-2 \log \Lambda$) and the corresponding *P*-value represent the likelihood of the null hypothesis being true. The degrees of freedom are determined by the difference in the number of free parameters between the two hypotheses. Because multiple tests were performed, the significance value for rejection was conservatively set at 0.01. See text for details

Null hypothesis	Models compared	$-\log L_0$	$-\log L_1$	$-2 \log \Lambda$	d.f.	*P*
Equal base frequencies	H0: JC H1: F81	7966.31	7800.75	331.12	3	<<0.001
Transition rate equals transversion rate	H0: F81 H1: HKY	7800.45	7377.73	846.04	1	<<0.001
Two transversion rates	H0: HKY H1: GTR	7377.73	7357.89	39.68	4	<<0.001
Equal rates among sites	H0: GTR H1: GTR+Γ	7357.89	6989.88	736.02	1	<<0.001
No invariable sites	H0: GTR+Γ H1: GTR+Γ+I	6989.88	6981.09	17.57	1	<<0.001

Models of DNA substitution evaluated: JC (Jukes & Cantor, 1969); F81 (Felsenstein, 1981); HKY (Hasegawa, Kishino & Yano, 1985); GTR (Yang, 1994); I = proportion of invariable sites; Γ = shape parameter of the gamma distribution.

yielded an age of approximately 14 million years for the common ancestor of the mitochondrial lineages examined in our study. This is very close to the estimate of divergence times based on immunological data (Case, 1978: fig. 6). Based on these distances, the *R. septentrionalis* lineage diverged approximately 11 Mya, with *R. catesbeiana*, *R. clamitans*, and *R. heckscheri* radiating approximately 9 Mya. Divergence within the *R. clamitans–R. okaloosae* lineage suggests a more recent, Pleistocene divergence. A second data set of *Rana* ND3 sequence (Plötner *et al*., 2001) calibrated by the isolation of the island of Crete 5 Mya, and subsequent differentiation of *R. cretensis* from mainland conspecifics, suggests a molecular rate of approximately 2.4% sequence change per million years. The ND3 gene has a similar rate of substitution as the ND2 gene in brown frogs (J. Plötner, pers. comm.); application of this rate suggests that the species group originated entirely (excluding *R. okaloosae*) within the Pliocene.

Dispersal–vicariance analysis

We used the phylogenetic hypothesis presented in Figure 6 for our biogeographical analysis. However, DIVA is unable to incorporate non-bifurcating phylogenies (Ronquist, 1996). Therefore, we repeated the analysis for two plausible phylogenetic scenarios assuming our tree represents a soft polytomy: (1) *R. catesbeiana* sister to *R. clamitans*, and (2) *R. catesbeiana* basal to *R. clamitans* (see Fig. 7). These phylogenetic hypotheses assume that *R. grylio* and *R. heckscheri* are true sister-species as has been suggested in other studies (e.g. Hillis & Davis, 1986).

All dispersal–vicariance analyses resulted in multiple combinations of optimal reconstructions. The number of inferred dispersal events did not differ between the two topologies tested (see Table 6 for results). As predicted (Ronquist, 1996), the omission of outgroups resulted in an inferred widespread ancestral range, including all four geographical regions (Table 6, scenario 1 and 4). Constraining the number of ancestral areas to a maximum of two (Table 6, scenario 2 and 5, node 6) resulted in three possible ancestral distributions that did not differ between the two topologies tested. For both phylogenetic hypotheses, the three possible ancestral areas inferred were: Coastal Plain only, Coastal Plain + Appalachians, Coastal Plain + Laurentian. The latter hypothesis requires the ancestor to have gone extinct in the Appalachian region or undergone long distance dispersal; both unlikely scenarios. The hypothesis suggesting the Coastal Plain as the sole ancestral area supports the dispersal–vicariance results with the inclusion of outgroups (Table 6, scenario 3 and 6).

Both vicariance and dispersal played a major role in describing the distribution of *R. septentrionalis*. Assuming the Coastal Plain represents the ancestral area of the species group, the optimal solution indicates that a vicariant event separated the ancestor of *R. virgatipes* and the ancestor of *R. septentrionalis*, with both lineages co-occurring within the Coastal Plain, with subsequent dispersal by the ancestor of *R. septentrionalis*. Dispersal may have been widespread, extending into the Appalachian and Laurentian regions prior to the vicariant event leading to the separation of the ancestor of *R. septentrionalis* from the rest of the lineage. Alternatively, dispersal

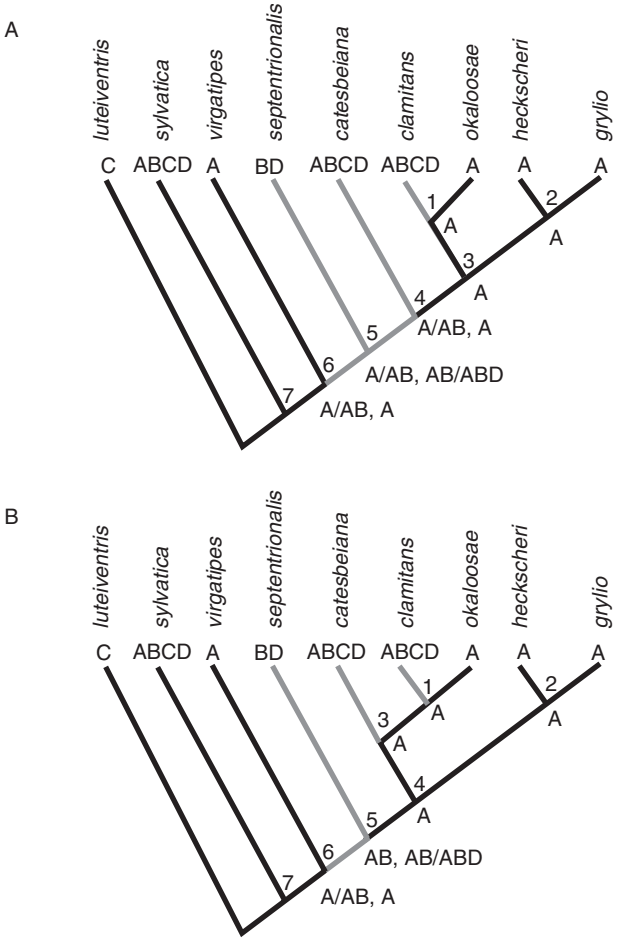

Figure 7. Phylogenetic hypotheses used in reconstruction of ancestral areas. (A) *Rana catesbeiana* is basally positioned relative to *R. clamitans*. (B) *Rana catesbeiana* sister to *R. clamitans*. Letters at terminal nodes relate to geographical area(s). Results from analyses constraining ancestral areas to a maximum of two regions and results including outgroup taxa (corresponding to Table 6) are, respectively, indicated at nodes, separated by a comma. Hatched lines indicate possible dispersal events. Ambiguous results are indicated by a '/'symbol. Redundant results are not shown.

into the Appalachians prior to the separation of *R. septentrionalis*, with subsequent dispersal into the Laurentians, explains the distribution of *R. septentrionalis* with equal likelihood (see Fig. 7). Even if the ancestor was widespread, located in the Coastal Plain + Appalachian regions (Table 6, hypotheses 2 or 5), vicariance followed by widespread dispersal is required to explain the current distribution of *R. septentrionalis*. Either way, the analysis infers that both dispersal and vicariance played important roles in the early diversification of the group.

The current distributions of *R. catesbeiana* and *R. clamitans* are best explained by independent, wide-

Table 6. Biogeographical inference of ancestral areas. Presented are results of dispersal-vicariance analysis for each node corresponding to numbered nodes in Fig. 7. Min. dispersal refers to the minimum number of inferred dispersal events required under the optimal reconstruction. Letters correspond to four major physiographic areas (A = Coastal Plain, B = Appalachians, C = Interior Highlands, D = Laurentian). For some nodes, dispersal-vicariance results suggest multiple equally likely distributions indicated by areas, or combinations of areas, separated by commas. Distributions shown in parentheses are considered to be unlikely due to the disjunct distribution of the regions (see Results). Outgroup nodes (7 and 8) are shown together

		Node						
Phylogenetic hypothesis	Min. dispersal	1	2	3	4	5	6	7, 8
1 *R. catesbeiana* ancestral (Fig. 7A)	7	A	A	A	A, AC, (AD), ACD, ABCD	ABD, BCD, ABCD	ABD, ABCD	–
2 As 1, ancestor constrained to max. two areas	8	A	A	A	A, AB (AD)	A,AB, (D), (AD)	A, AB (AD)	–
3 As 1, including outgroup	10	A	A	A	A	AAB, (AD), ABD	A	AC, ABCD
4 *R. clamitans-R. catesbeiana* sister (Fig. 7B)	7	A	A	A	A	ABD	ABD	–
5 As 4, ancestor constrained to max. two areas	8	A	A	A	A	AB, (AD)	A, AB (AD)	–
6 As 4, including outgroup	10	A	A	A	A	AB, (AD), ABD	A	AC, ABCD

spread dispersal by both species. This conclusion is evident regardless of which phylogenetic hypothesis is invoked, or what the ancestral distribution of the species group is. Independent dispersal is more parsimonious than if the common ancestor (Fig. 7A, node 3) was widespread. That is to say, had dispersal into the Appalachian, Laurentian, and Interior Plains occurred prior to the split of *R. catesbeiana* and *R. clamitans*, this scenario would require at least four more dispersal events to explain the widespread distributions of *R. catesbeiana* and *R. clamitans*. Finally, the remainder of the species group is inferred to be native to the Coastal Plain region.

Mode of speciation

Ignoring the status of *R. okaloosae* for the moment, the sister-species pairs of *R. catesbeiana* + *R. clamitans* and *R. grylio* + *R. heckscheri* (Fig. 7A) both consist of species with similar range sizes and are largely sympatric in distribution (Fig. 1), suggesting sympatric speciation according to Lynch's (1989) definitions. This pattern for *R. catesbeiana* + *R. clamitans* becomes indeterminant if we treat *R. okaloosae* as the true sister-species to *R. clamitans*. In turn, *R. okaloosae* and *R. clamitans* would be assumed to be the product of sympatric speciation due to their complete overlap in range. Alternatively, ignoring the range overlap criterion, the relative geographical position of *R. okaloosae* suggests peripheral isolation as a possible mode of speciation. Overlap of *R. catesbeiana–R. clamitans* and *R. grylio–R. heckscheri* sister-groups is also high (only the lower portion of the Florida peninsula is non-overlapping) (Fig. 1). At higher taxonomic levels this pattern is considered indeterminant since these may be the result of sympatric speciation or allopatric speciation with subsequent dispersal (see Discussion). Overlap of the range of *R. septentrionalis* exceeds 50%, attributable to the *R. catesbeiana-R. clamitans* distributions, and *R. virgatipes* is partially or entirely sympatric with *R. catesbeiana, R. clamitans, R. heckscheri,* and *R. grylio*. The only difference in interpretation based on the phylogenetic hypothesis in Figure 7B is in the placement of *R. catesbeiana* basal to the group including *R. clamitans*. This results in an indeterminant inference of speciation mode for this group. Overall, there is no definitive interpretation of allopatric speciation; however, sympatric speciation is inferred among sister-species comparisons.

DISCUSSION

Overview of phylogenetic analysis

Results from our phylogenetic analyses confirm that members of the *Rana catesbeiana* species group are closely related. It has generally been assumed that two sister-species pairs included *R. catesbeiana* + *R. clamitans* and *R. grylio* + *R. heckscheri* (Case, 1978; Hillis & Davis, 1986). Our results, based on over 1500 bp of mtDNA, could not confidently resolve these relationships. The possibility that the molecular polytomy representing *R. catesbeiana, R. clamitans, R. heckscheri,* and *R. grylio* truly reflects underlying organismal evolutionary relationships (i.e. species polytomy) requires a series of independent gene trees (Slowinski & Page, 1999); because mtDNA genes are genealogically linked they are inappropriate for such application. However, our results suggest that mtDNA lineages representing the four species coalesce within a brief span, sometime between the late Miocene and Pliocene.

Consistency across molecular evolution analyses was not surprising given that both ML and Bayesian approaches are based on likelihood principles and that for both we employed the same model of molecular evolution. Congruence between the two methods is increasingly reported in the literature (e.g. Leaché & Reeder, 2002; Wilcox *et al*., 2002). The convergence of results from weighted MP, and MP without long-branch lineages, on that of the molecular evolution methods lends support to the basal placement of *R. virgatipes* and *R. septentrionalis*. Long-branches may result from differential lineage origination or extinction, or simply biases in taxon sampling (Lyons-Weiler & Hoelzer, 1997). Thus, the results from MP analyses may improve with additional geographical representation of *R. grylio* and other, under-represented taxa. Combining data partitions did not clarify the long-branch problem as suggested by Xiang *et al*. (2002). In other words, addition of further characters may improve support generated for nodes, but may not improve the phylogenetic signal of long-branch taxa (the 'Felsenstein zone', Felsenstein, 1978; Lyons-Weiler & Hoelzer, 1997).

Reciprocal paraphyly in *R. clamitans* and *R. okaloosae* is particularly interesting given that their status as species is generally not questioned by herpetologists. Although these two species share some common diagnostic features (e.g. dorsolateral folds), other features (e.g. relative extent of webbing, call characteristics, relative size, larval colouration) support their distinctiveness (see Moler, 1985, 1993; for descriptions). Given the apparent rapid evolution of the group, most phenotypic characters used to diagnose these species appear to be evolutionarily labile. The question remains whether or not the observed paraphyly is the result of hybrid origin, incomplete lineage sorting, or recent hybridization (Weckstein *et al*., 2001).

Rana okaloosae may be a result of hybridization of female *R. clamitans* and a male from another member

of the species group. Hybrid taxa are typically intermediate in phenotype and carry the maternal mtDNA (Demarais *et al*., 1992). However, as previously mentioned, many external morphological characters appear to be labile within the species group, and it is difficult to identify the 'paternal' contribution in the hybrid origin hypothesis. However, this possibility may be testable with the inclusion of polymorphic nuclear markers (e.g. microsatellites, allozymes). Alternatively, the phylogenetic pattern seen here may be due to more recent hybridization between female *R. clamitans* and male *R. okaloosae* (one *R. okaloosae* shared an identical haplotype, Rcla1393, with a *R. clamitans* from an adjacent county in Florida; Table 1). As species become rare, particularly under stressful environmental conditions, the likelihood of hybridization increases (Lamb & Avise, 1986; Lehman *et al*., 1991; Wayne *et al*., 1992). *Rana okaloosae* is a rare, geographically restricted species (Fig. 1), making it a candidate for hybridization with *R. clamitans*. The breeding phenology of the two species overlaps broadly and putative hybrids have been found (P. E. Moler, unpubl. data). Further, *R. clamitans* larvae represent the only other member of the species group commonly found syntopically (Moler, 1993). Finally, if the pattern represents the retention of ancestral mtDNA, then this would suggest fairly recent reproductive isolation. *Rana okaloosae* contains both 'northern' and 'southern' *R. clamitans* haplotypes (see below). This supports the hypothesis of incomplete lineage sorting because hybridization would be less likely to result in 'northern' haplotypes being represented in the geographically restricted *R. okaloosae*. Frequent hybridization would require the mtDNA haplotypes found in *R. okaloosae* to exist in areas of sympatry. Our samples include *R. clamitans* haplotypes from as far north as Canada, and west to Missouri (Table 1). Therefore, unless these shared 'northern' haplotypes are actually widespread and found in the Coastal Plain Region, hybridization seems the less likely mechanism explaining the pattern of paraphyly seen here. Ongoing work on the molecular support for subspecies status of *R. clamitans* and the phylogeographical pattern of *R. clamitans* and *R. okaloosae* will help to clarify this pattern.

Overview of patterns of speciation

A general outcome of our dispersal–vicariance analysis is that, despite the lack of resolution among derived species of *Rana*, we have a fairly complete picture of the biogeographical history of the species group. Our analyses strongly suggest that the widespread distributions of *R. catesbeiana*, *R. clamitans,* and *R. septentrionalis* are the result of independent widespread dispersal. Alternate topologies among derived taxa (*R. clamitans, R. catesbeiana, R. heckscheri, R. grylio*) do not affect the biogeographical reconstruction (not shown). In each case, the ancestral distribution of the node leading to these species is always inferred to be the Coastal Plain, with independent dispersal in *R. clamitans* and *R. catesbeiana*. Finally, the occurrence of fossil *R. catesbeiana* throughout its current range (reviewed in Holman, 1995) refutes the possibility that this species may not be native to the Coastal Plain, or any other assigned region.

Diagnosing a 'centre of origin' using dispersal–vicariance analysis is somewhat problematic in that the method is sensitive to the current distribution of outgroups. Although the Coastal Plain appears to be an important area in the evolution of the species group, it is not a certainty that the ancestor was geographically restricted to this area. However, a Coastal Plain origin is in general agreement with patterns of amphibian species diversity and endemism. The Coastal Plain represents the greatest concentration of endemic anuran (including four of the species studied here) and obligatory neotenic salamanders in eastern North America (Duellman & Sweet, 1999). This suggests that the area was a major centre of speciation in these aquatic groups. In contrast, the Appalachian region was an important area of speciation for terrestrial plethodontid salamanders during the Pliocene (Highton, 1995). The general aquatic nature of the *Rana catesbeiana* group and drier Pliocene climate in the Appalachians (Van Valkenburgh & Janis, 1993; Larsen *et al*., 1994) strongly suggest the exclusion of the Appalachian region as part of a wider 'centre of origin' for the *Rana catesbeiana* group.

Inferences of speciation using Lynch's (1989) method strongly conflict with dispersal–vicariance interpretations in their relative importance of sympatric speciation and the directional pattern of species dispersion. Previous studies implementing Lynch's (1989) method have found that allopatric speciation is inferred more commonly over sympatric speciation (Grady & LeGrande, 1992; Chesser & Zink, 1994; Green, vanVeller & Brooks, 2002), reflecting theoretical arguments indicating the dominance of allopatric speciation in animals (Mayr, 1963). For our data, allopatric speciation is never identified due to the pattern of species' range overlap. Lynch's (1989) method assumes that the geographical patterns of speciation are not obscured by dispersal. Brown & Gibson (1983) predicted that geographical overlap among sister-lineages should increase with speciation level (Chesser & Zink, 1994); however, Lynch found no significant correlation. Similarly, Brown & Gibson's (1983) prediction does not seem to fit our data well, given that the widespread species (*R. catesbeiana, R. clamitans,* and to a lesser extent *R. septentrionalis*) are not basal, and because of the apparent rapid radiation of the species

group. In contrast, dispersal–vicariance allows for either sympatric or allopatric speciation to explain the origin of *R. catesbeiana* and *R. clamitans* within the Coastal Plain region, attributing their large ranges to postspeciation dispersal. Given the degree of species overlap in the Coastal Plain region, sympatric speciation remains a possible mode of diversification according to Lynch (1989). However, given the large range sizes, similarity in distribution, and large degree of syntopy of *R. catesbeiana* and *R. clamitans*, wide-scale sympatric speciation seems unlikely. Evolution to exploit 'empty niches' (Wilson & Turelli, 1986) would not have occurred over such a large geographical area without multiple speciation origins. Polyploidy seems the most plausible mechanism for widespread sympatric speciation in many amphibian taxa (Kuramoto, 1990; Tymowska, 1991). For example, polyploidy has arisen three separate times in the widespread, *Hyla chrysoscelis–H. versicolor* (Ptacek, Gerrhardt & Sage, 1994). However, no polyploid species are known to exist among ranids of North America (Kuramoto, 1990; J. Bogart, pers. comm.).

Does postspeciation dispersal better explain the geographical patterns of the *Rana catesbeiana* group? Chesser & Zink (1994) argued that the dispersal capability of birds explains the overestimate of sympatric speciation in their and Lynch's (1989) study, stating that the admittedly arbitrary 20% range overlap was too liberal for some taxa. For the frogs in our study, the 20% overlap is well exceeded across sister-species and sister-grouping, suggesting that dispersal capability is underestimated in these anurans.

Undoubtedly, current distributions are only a rough indication of historical ranges. However, we can be fairly confident that during the Pleistocene, populations of *R. catesbeiana, R. clamitans*, and *R. septentrionalis* were displaced southward; this is particularly evident in *R. septentrionalis,* whose current range was entirely glaciated. The current geographical ranges of these three species suggest that they were capable of closely tracking their suitable climate north during postglacial expansion (Holman, 1995). Examining genetic patterns of postglacial expansion in these species may provide better indication of their dispersal ability. Other phylogeographical studies have demonstrated rapid postglacial dispersal ability in widespread anurans (e.g. Green *et al*., 1996; Austin *et al*., 2002). However, dispersal ability alone may not explain the differential ranges of members of the *Rana catesbeiana* species group; differences in range limits across the *Rana catesbeiana* species group may also depend on individual species adaptive ability (Davis & Shaw, 2001).

Variation in ecological and demographic characteristics across latitudes in *R. catesbeiana* (e.g. George, 1940; Klimstra, 1949; Willis, Moyle & Baskett, 1956; Emlen, 1976; Collins, 1979; Shirose *et al*., 1993) underscores the potential for local adaptation to environments in this species, as does the success and spread of introduced *R. catesbeiana* across the globe (Europe: Lanza, 1962; Gasc *et al*., 1997; Japan: Telford, 1960; West Indies: Perez, 1951; Mahon & Aiken, 1977; Hedges, 1999; western North America: Clarkson & DeVos, 1986; Moyle, 1973; Green, 1978; Hammerson, 1982). The idea of the 'general purpose genotype' (Baker, 1965) to explain broad distributions has been refuted by demonstrating local adaptation in 'weedy' plants (Law, Bradshaw & Putwain, 1977; Teramura, 1983; Reinartz, 1984), and may not be the best explanation for the success of species such as *R. catesbeiana*.

Given the evidence from our phylogenetic and biogeographical analyses, the most logical scenario explaining the evolution and distribution of the *R. catesbeiana* species group involves widespread dispersal following speciation. This is due to the large amount of species overlap, not only for widespread species, but also for those endemic to the Coastal Plain. The alternative, frequent sympatric speciation, is not supported by any of the evidence discussed here. Allopatric speciation in the *R. catesbeiana* species group may have been related to late Tertiary sea-level fluctuations (Riggs, 1983). Following an abrupt fall, sea levels rose and fluctuated in the Miocene, and peaked at roughly 80 m above sea level briefly during the Pliocene, vastly decreasing the Coastal Plain area all the way to the Fall Line along the Atlantic coast, and creating a series of interbasin peninsulas throughout present day Georgia and Alabama (see fig. 7.3 in Swift *et al*., 1986). Evidence for a prolonged insularization of peninsular Florida during high Pliocene sea-levels has been implicated in the diversification of freshwater fish faunas (Gilbert, 1987) and paedomorphic salamanders of the genus *Pseudobranchus* (Moler & Kezer, 1993). Such isolation of populations within the Coastal Plain region during this period may have been important in speciation in the *R. catesbeiana* species group, and the abrupt reduction in area may have encouraged dispersal in some early lineages. Similar fluctuations in sea level during the Pleistocene may have permitted the divergence of *R. okaloosae*. Many of the streams where *R. okaloosae* occurs are steep-valleyed and surrounded by inhospitable, xeric uplands. During the Pleistocene, these valleys would have been cut off from other freshwater systems during periods of elevated sea level (Lambeck & Chappell, 2001), effectively isolating populations of *R. clamitans* long enough for divergence to take place.

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