Biodiversity is distributed heterogeneously across Earth. Consequently, the determinants of spatial patterns of diversity are of paramount interest for biologists (Gaston 2000; Ricklefs 2004). Broadly distributed, species-rich clades provide an opportunity to explore the evolutionary processes that drive diversity across large spatiotemporal scales (e.g., Derryberry et al. 2011; McGuire et al. 2014). Methods combining the dynamics of diversification (e.g., speciation, extinction) with biogeographic history allow biologists to test hypotheses of diversification within and between regions (Ricklefs 2004; Wiens and Donoghue 2004; Mittelbach et al. 2007).

If speciation and extinction rates (ignoring dispersal for the moment) are roughly constant across geographic areas, then species diversity is influenced by time-independent processes (McPeek and Brown 2007). However, changes in speciation rate (e.g., by adaptive radiations) or extinction rate (e.g., by climate effects) can quickly disrupt the correlation between clade age and species diversity. In many radiations, a pattern of early, rapid cladogenesis followed by a slowdown in diversification rate is thought to be related to ecological constraints (Schluter 2001; Rabosky 2009). Further, increases in cladogenesis are often associated with broad-scale environmental changes or geological processes (e.g., orogenesis).

Diverse clades that are distributed across several major continental areas provide excellent opportunities to compare patterns of distribution and diversification in different regions (Qian and Ricklefs 2000; McGuire et al. 2014). In this article we investigate the spatial and temporal patterns of regional and continental-scale biodiversity in a widely studied clade that is broadly distributed across Eurasia and the Americas, the true frogs (genus Rana, sensu AmphibiaWeb 2015).

True frogs are extensively used as model organisms in studies of development, genetics, physiology, ecology, behavior, and evolution. Comparative studies among the more than 100 species of Rana rely on an understanding of the evolutionary history and patterns of diversification of the group. We estimate a well-resolved, time-calibrated phylogeny further suggests that previous classifications were misled by morphological homoplasy and plesiomorphic color changes and especially intensive tectonic movements along the Asian margin from the Oligocene to early Miocene. Our phylogeny further suggests that previous classifications were misled by morphological homoplasy and plesiomorphic color patterns, as well as a reliance primarily on mitochondrial genes. We provide a phylogenetic taxonomy based on analyses of multiple nuclear and mitochondrial gene loci. [Amphibians; biogeography; diversification rate; Holarctic; transcontinental dispersal;].
in *Rana* (Briggs and King 1952), and various species of *Rana* continue to be used widely in studies of physiology and genetics. Many species of *Rana* are also commonly studied in the field as well as the laboratory. One of the most extensive monographs on the ecology and life history of an amphibian was based on *Rana temporaria* (Savage 1962), and studies of *Rana* ecology, behavior, conservation, and evolution have accelerated in recent years (reviewed by Hillis and Wilcox 2005).

More than 100 described species of *Rana* (AmphibiaWeb 2015) range across Europe (12 species) and Asia (32 species), and from North America to the northern half of South America (57, plus several recognized but not yet described species; Hillis and Wilcox 2005). Species occur in a wide variety of habitats including tundra, temperate coniferous and deciduous forests, grasslands, deserts, brackish-water marshes, freshwater streams and lakes, montane cascades, semitropical cloud forests, and tropical rainforests (Hillis and Wilcox 2005). Many species are of conservation concern, and several species are recently extinct or threatened with extinction. Despite the diversity of the genus and its importance in many biological investigations, no comprehensive analysis of the spatial patterns and drivers of diversity for *Rana* has been published.

The Eurasian species are morphologically conservative. They possess prominent dorsolateral folds, a dark temporal mask, and a body that is countershaded in various shades of brown, leading to the common English name “brown frogs” (Boulenger 1920; Liu and Hu 1961). This color pattern occurs in many species in Europe, Asia, and North America, although several New World species groups show far greater morphological differentiation. This greater morphological diversity correlates with their varied ecologies, physiologies, behaviors, and morphological structures associated with mating calls and habitat (Hillis and Wilcox 2005). These differences in patterns of species diversity and biological divergence make *Rana* an excellent group for studying the processes of biodiversity generation.

Most previous studies of *Rana* have been restricted to particular geographic regions, or have used very limited sampling of global taxa, or have been limited to analyses of mtDNA sequences (sometimes with small amounts of nuclear DNA (nuDNA)) of *Rana* have included species in Europe (e.g., Veith et al. 2003), the New World (e.g., Hillis and Wilcox 2005), the mainland of East Asia (e.g., Che et al. 2007a), and the Asian islands (e.g., Tanaka-Ueno et al. 1998; Matsu 2011). We expand on these studies by analyzing six nuclear and three mitochondrial genes for a large majority of the extant species (90 species; Supplementary Table S1, available on Dryad) across the entire distribution in Eurasia and the Americas, and by estimating divergence times and patterns of net diversification.

The family Ranidae began to diversify about 57 Ma (Bossuyt et al. 2006; Wiens et al. 2009), long after the breakup of Pangaea (>120 Ma, Sanmartin et al. 2001). Therefore, the global distribution of the family must have resulted from intercontinental dispersal. Based on limited sampling of *Rana*, Bossuyt et al. (2006) suggested two alternative hypotheses: two dispersals from Asia to America via Beringia, or one dispersal from Asia to America with a second back-dispersal to Asia. We asked the following questions:

(1) When did the intercontinental dispersal of *Rana* occur between Eurasia and the Americas? Were these dispersals via trans-Atlantic land bridges (Case 1978) or by trans-Beringian (Pacific) land bridges (Macey et al. 2006)? Was there only one dispersal from Eurasia to the Americas, with a dispersal back to Eurasia, or were there two distinct dispersal events into the New World? When did major dispersal events within Eurasia and the Americas occur (e.g., into Europe, and into the Neotropics)?

(2) To what extent was dispersal into new areas accompanied by increased diversification rates? Were speciation rates highest when a lineage entered new regions, or were they relatively constant?

(3) How did geologic events and environmental changes influence the diversification of *Rana* in the Old World (especially the diversity in East Asia) and the New World?

**Materials and Methods**

**Taxon Sampling**

Complete sampling of all species in *Rana* was a challenge due to the large number of species, their intercontinental distribution, and rarity or recent extinction of some species. Our analyses included 82 of the currently recognized species of *Rana* as well as eight undescribed taxa (Supplementary Table S1, available on Dryad). All major lineages were included (Tanaka et al. 1996; Veith et al. 2003; Hillis and Wilcox 2005; Che et al. 2007a; Matsu 2011). Based on other studies, the relationships of the unsampled species (Supplementary Table S2, available on Dryad) are uncontroversial and the species are distributed evenly across the tree. Thus, our sampling bias is negligible. Four species from *Odorrana*, *Pelophylax*, *Hylanna*, and *Rugosa* (Ranidae) were chosen as outgroup taxa based on Che et al. (2007b). New sequences from 80 species were analyzed, along with 14 species from GenBank (Veith et al. 2003; Hillis and Wilcox 2005; Che et al. 2007a) (Supplementary Table S1, available on Dryad).
DNA Extraction, Amplification, and Sequencing

We extracted DNA from muscle or liver tissues (either frozen or preserved in 95% ethanol) using standard phenol-chloroform extraction protocols (Sambrook et al. 1989). We amplified and sequenced three fragments of mitochondrial DNA (mtDNA) and six nuDNA loci (Supplementary Table S1, available on Dryad). The mitochondrial genes included 12–16S (partial sequence of 12S ribosomal RNA gene), complete sequence of tRNAVal and partial sequence of 16S ribosomal RNA, CYTB (cytochrome b) and ND2 (partial sequence of NADH dehydrogenase subunit 2; complete sequence of tRNAAla, tRNAArg, tRNAPhe; and partial sequence of the light strand replication origin). The nuDNA markers (Supplementary Table S3, available on Dryad) included RAG1 (partial sequence of recombinese activating 1 protein gene), RAG2 (partial sequence of recombinese activating 2 protein gene), BDNF (partial sequence of brain-derived neurotrophic factor gene), SLC8A3 (partial sequence of solute carrier family 8 member 3), TTR (exon 1 of tyrosine precursor gene), and POMC (partial sequence of pro-opiomelanocortin A gene). We used standard polymerase chain reactions (PCRs) in 25-µL reactions with the following protocol: initial denaturation step with 5 min at 95°C, 35 cycles of 94°C for 1 min, 41–57°C (depending on primers; Supplementary Table S3, available on Dryad) for 1 min, 72°C for 1 min and a single final extension at 72°C for 10 min. PCR products were purified with a Gel Extraction Mini Kit (Watson BioTechnologies, Shanghai). The purified product was used as the template DNA for cycle-sequencing reactions performed using Big Dye Terminator Cycle Sequencing Kit (v2.0, Applied Biosystems). Sequencing was conducted on an ABI PRISM 3730 (Applied Biosystems) automated DNA sequencer. We sequenced amplified fragments with PCR primers in both directions and subjected the raw sequences to BLAST searches in GenBank to verify the amplifications.

Nucleotide sequences were aligned using ClustalX v1.81 (Thompson et al. 1997) with default parameters, and checked for ambiguously aligned regions in MEGA 5.0 (Tamura et al. 2011). Saturation tests for each gene and gene partitions were done using DAMBE (Xia and Xie 2001).

Phylogenetic Analyses

Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) analyses were conducted using the nine concatenated gene fragments. BI analyses were performed in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) using the optimal partitioning strategy and best-fit nucleotide substitution model for each region (Supplementary Table S4, available on Dryad) selected by PartitionFinder v1.1.1 (Lanfear et al. 2012). Four incrementally heated Markov chains (using the default heating value of 0.1) were run for 10 million generations each while sampling the chains at intervals of 1000 generations. Two independent runs were carried out. We discarded the first 50% of the samples as burn-in, and log-likelihood scores were tracked to assure convergence (effective sample size, ESS, values >200). We assessed topological convergence using AWTY (Nylander et al. 2008) to visualize the cumulative split frequencies in the set of posterior trees, as recommended by Moyle et al. (2012). The average split frequency value was set to <0.01 to assure convergence. The samples were summarized as a majority-rule consensus tree and the frequencies of nodal resolution were interpreted as Bayesian posterior probabilities (BPPs).

The ML trees were estimated using RAxML v7.0.4 (Stamatakis 2006). We used GTR+I as the substitution model, based on the partitioning strategy selected using PartitionFinder (Supplementary Table S4, available on Dryad). Support values were estimated from 1000 nonparametric bootstrap pseudoreplicates.

MP analyses were implemented using PAUP* 4.0b10a (Swofford 2003). The heuristic MP searches were executed for 1000 replicates using tree-bisection-reconnection branch swapping. All characters were treated as unordered and equally weighted. We used bootstrap analysis (BP) conducted with 1000 pseudoreplicates to assess nodal support. Gaps were treated as missing data.

We conducted exploratory BI analyses of a concatenated mitochondrial data set and a concatenated nuclear data to assess the separate contributions of each data type to the tree derived from all concatenated genes. We also conducted species tree estimation using the gene-tree-based coalescent methods ASTRAL (Mira rab et al. 2014) and MP-EST analyses were conducted using the ASTRAL server (Shaw et al. 2013), and ASTRAL analyses conducted using ASTRAL v4.7 (Mira rab et al. 2014).

Divergence Times

Divergence times were estimated using BEAST v1.7.5 (Drummond et al. 2012) using a relaxed uncorrelated clock (Drummond et al. 2006). The substitution models and partitions were the same as used in the BI analyses. A Yule process was chosen for the tree prior. We used two independent analyses of 40 million generations each with sampling every 1000 trees. Convergence of the chains was determined with Tracer v1.5 (Rambaut and Drummond 2007), with target ESS values more than 200 for all parameters; 25% of samples were discarded as burn-in.

Nodes 2, 9, 5, and 17 (as shown in Fig. 1) were used for calibration. Node 2 was calibrated using a previous age estimate from Bossuyt et al. (2006; Fig. 3, their node 19). A normal prior distribution with mean of 31.2 and SD of 8.1 Ma yielded 2.5% and 97.5% quantiles of 15.3
FIGURE 1. The BI tree derived from the combination of six nuDNA and three mtDNA loci. Branches without support symbols were strongly supported in all three phylogenetic analyses (BI, ML, and MP); we treated bootstrap proportions ≥70% and BPPs ≥95% as significantly supported (+). Bootstrap proportions <70% and BPPs <95% were considered weakly supported (−). Support values are shown only for branches that were weakly supported in at least one of the three analyses, in which case support values are shown in order for BI, ML, and MP analyses. Colors of branches indicate the geographic distribution of extant taxa. Numbers on branches correspond to clades discussed in this study. Numbers with a black box correspond to the nine subgenera (plus *Rana sylvatica*) referred to in Supplementary Table S2, available on Dryad. Photographs depict the morphological variation of *Rana* across clades 1, 4, 5, 7, 8, 9, 11, 13, 15, 17, and 18. Frog illustrations are used with permission from David Hillis, Hui Zhao, Todd Pierson, Andreas Noellert, and Richard Sage.

and 47.1 Ma. Three node calibrations were based on fossil *Rana* representing the *R. pipiens* group (node 9), the *Rana catesbeiana* group (node 5), and the *R. temporaria* group (node 17). The last is based on a single articulated individual. The first two are represented primarily by ilia, one of the most commonly preserved elements of Tertiary fossil frogs. As noted by Parmley et al. (2010), it is relatively easy to diagnose *Rana* ilia from other genera due to the extremely prominent iliac crest. However, the ilia are less easily distinguished among species within *Rana*. Assignment of the fossils to species groups by previous authors was based on overall similarity based on comparisons of several species of *Rana*. Because
FIGURE 2. Map showing the generalized distribution areas of the extant Rana species and schematic drawings that illustrate possible dispersal events among the four major Holarctic areas under H0, H1, and H2. Seven major geographic regions (A–G) are indicated using colors. H0 is the null model, which assumes equal rates of dispersal between any two regions. H1 is the trans-Beringian hypothesis, which is further subdivided into H1a, with dispersals between East Asia and western North America and eastern North America, in either direction; H1b, two independent dispersals from East Asia to western North America and eastern North America (Macey et al. 2006); H1c, one dispersal from East Asia to eastern North America and one back to East Asia from western North America (Macey et al. 2006). H2 is the trans-Atlantic hypothesis: dispersal(s) occurred between Europe and western North America or eastern North America.

the authors explicitly allocated each fossil to a well-circumscribed clade of species, we inferred that the fossil is nested within that group, and used it to calibrate the most recent common ancestor (MRCA) of that group. The exception was the R. temporaria group fossil (see below).

We used a lognormal distribution to sample the prior for the age of each node. To parameterize the priors we used the BEAUti user interface to set the “offset” as the minimum age of the fossil, the mean at 2.0 (standard scale), and the standard deviation at 1.0 (natural log). The mean and standard deviation were chosen so that the upper 97.5% quantile limit of each calibration age was 9–10 Ma older than the minimum age. Because no fossils that can be clearly allocated to Rana are older than 20 Ma, we view these limits as reasonable.

The calibration of node 9 was based on several “Rana cf. Rana pipiens” individuals from the Early Miocene Thomas Farm locality in Florida (18 Ma; Holman 1965, 1968). Holman concluded that the fossil ilia were not distinguishable from some extant species within the R. pipiens group (although they are from others), so we infer that the fossil is nested within the R. pipiens clade (node 9) and thus the clade had begun to diversify at least 18 Ma. The 2.5% and 97.5% quantiles of the prior distribution are 18.2 and 26.6 Ma.

Calibration of node 5 was based on Hottell Ranch Site fossils (15 Ma, Barstovian, Miocene), identified as Rana near R. “clamitans” (Voorhies et al. 1987), a species within the R. catesbeiana clade. Therefore, the MRCA of the R. catesbeiana group was calibrated at a minimum age of 15 Ma with 2.5% and 97.5% quantiles of 15.2 and 23.6 Ma. Voorhies et al. (1987) also identified “R. pipiens complex” ilia in their sample, suggesting that the authors were confident in the assignment of their R. clamitans fossil.

Calibration of node 17 was based on the single articulated fossil (R. tempora group) from Dietrichsburg, Germany, dated at 19–20 Ma, early Miocene (Böhme 2001). Because the author did not state whether the fossil was nested within the R. tempora clade, as opposed to being its sister species, we used it to calibrate the node immediately ancestral to the MRCA of the European clade; that is, the divergence of Rana asiatica from the European species of the R. tempora group (node 17). We set the minimum age at 19 Ma; 2.5% and 97.5% quantiles are 19.2 and 27.6 Ma.

To test the sensitivity of our time calibration to particular calibration points, we used a jackknife analysis (Near et al. 2005). We successively deleted each of the individual fossil calibration points, repeated the calibration analyses, and compared the median age estimates with those ages from the complete analysis (Supplementary Table S5: Analyses 1–4, available on Dryad). Removing the R. catesbeiana group fossil yielded age estimates that were on average 8% younger than the estimates based on all fossil calibrations (Analysis 1). Similarly, removing the R. tempora group fossil
(Analysis 4) yielded age estimates that on average were 9% younger than those of Analysis 1. In contrast, jackknife deletion of the R. pipiens group fossil (Analysis 3) yielded ages that were an average of 4% older than those of Analysis 1. Despite the modest systematic differences (older or younger) between the jackknife estimates and the complete analysis, the 95% credible intervals of the ages overlapped broadly, and so we used the calibration based on all fossils for our biogeographical analyses.

Biogeographic Multimodel Inference and Ancestral Range Estimation

To estimate the ancestral range for Rana, we defined seven major geographic regions (Fig. 2): (A) East Asia (mainland + islands), (B) Central Asia, (C) Europe, (D) western North America, (E) eastern North America, (F) Mexican Plateau and surrounding region, and (G) the Neotropics. We distinguished region B based on its arid continental climate and distinct anuran fauna compared with region A, and separated region C from regions A and B along the Ural Mountains and Caspian Sea, the area of the ancient Turgai Straits (Tiffney 1985a; Briggs 1995). We first divided the Americas into temperate and tropical regions, and then further divided North American temperate regions into eastern NA (E) and western NA (D) along the Rocky Mountains (Tiffney 1985b; Sanmartín et al. 2001; Donoghue and Smith 2004). We also defined an important transition area between the temperate and tropical regions that is centered on the Mexican Plateau (F), a hotspot of diversity in Rana. This region includes the montane areas of southern Arizona and New Mexico, from the Mogollon Rim southward, as well as the Edwards Plateau in Texas southward. The southern boundary of this region is defined by the montane regions of Mexico north of the Isthmus of Tehuantepec (Fig. 2). The Neotropics (G) lies south of this boundary, and along the lowlands of both the Atlantic and Pacific coasts. Areas A–C are collectively referred to as Old World, and D–G as New World.

We estimated model parameters and ancestral range probabilities using the DEC and DEC+J models in the BioGeoBEARS R package (Matzke 2013a; R Core Development Team 2014). The DEC model describes the temporal change in the range of a species, and distinguishes anagenetic change (between nodes) from cladogegetic change (at a node). It estimates two free parameters describing anagenesis: $d$, the rate of dispersal (range expansion) and $x$, the rate of extinction (range contraction) (Ree and Smith 2008). The DEC+J model includes a third parameter, $j$, which describes the relative weight of founder event speciation during cladogenesis (Matzke 2013b). Under this model, which resembles founder event speciation, an ancestor in area A instantaneously “jumps” to area B, leaving one descendant in A and one in B. An increase in $j$ reduces the weight of the traditional DEC cladogenesis processes.

We followed the terminology of Matzke (2014) for cladogenetic events. Vicariance is the splitting of a species’ range such that the two descendants have non-overlapping ranges. In sympatric-subset cladogenesis, the range ABC yields two descendant ranges that overlap, such as (ABC, A), or (ABC, B). Under the Trans-Beringian hypothesis (H1), we considered (a) dispersals only between East Asia and western NA and eastern NÁ, in either direction; (b) two independent dispersals from East Asia to western NA and eastern NA (Macey et al. 2006); and (c) one dispersal from East Asia to eastern NA and one back to East Asia from western NA (Macey et al. 2006). Under the Trans-Atlantic hypothesis (H2), we tested whether dispersals(s) occurred between Europe and western NA or eastern NA.

We assigned a value of 1 for unrestricted dispersal between two areas and 0.001 for disallowed dispersal (0.0 causes computational difficulties; Supplementary Table S6, available on Dryad). For all analyses the maximum number of areas was set at four. We specified allowed connections with an area adjacency matrix (Supplementary Table S7, available on Dryad). The fit of all models to the data was compared using AIC, and the combination of connectivity model (dispersal matrices) and DEC/DEC+J was determined using relative model weights (Supplementary Table S9, available on Dryad).

The ML estimates for each model were then used to calculate the ancestral range probabilities at each node of the tree. Given that all species of Odorrana, the immediate outgroup of Rana, the immediate outgroup of Rana (Che et al. 2007b; Stuart 2008), occur in East Asia, we fixed the node below Rana to have an East Asian distribution using the BioGeoBEARS “fixlikes” option in all analyses.

Diversification Patterns

Diversification analyses used (1) the entire genus Rana, and (2) subsets of species within a region of interest. We used the R package ape (Paradis et al. 2004) to generate a lineage-through-time (LTT) plot based on the maximum clade credibility tree and 95% credible interval obtained by random sampling of 1000 trees from the post-burnin posterior distribution of the BEAST analysis using Burntrees v.0.1. (http://www.abc.se/~nylander/). Outgroups were excluded. In addition, we explored regional diversification of Rana using scripts provided by Mahler et al. (2010) to visualize the accumulation of lineages within different regions. These differ from LTT plots in that they reflect diversity due to both in situ diversification and independent immigration events (see McGuire et al. 2014).
We performed four LTT analyses to explore regional patterns of diversity. First, we compared Old World and New World species. Second, we compared individual regions: East Asia, Europe (including central Asia considering their close relationships), western NA, and eastern NA. Third, we partitioned the East Asia region into mainland and islands to examine the contribution of species from East Asian islands, for example, Japan and Taiwan. Last, we compared western NA, eastern NA, the Mexican Plateau, and the Neotropics to identify which regions contributed the lineage accumulation in clade 3 (Fig. 1).

Because the LTT plots measure only the accumulation of species and not speciation/extinction rates, we used BAMM (Rabosky 2014) and MEDUSA (Alfaro et al. 2009) to examine diversification rate heterogeneity along the branches. Analyses were conducted using three groups: all Rana, Old World Rana (Fig. 1: clades 1 + 11 + 14), and New World Rana (using clade 3 only because clade 13 represents an independent dispersal into the New World).

The BAMM analyses used 200 million generations, sampling every 10,000 generations, and sampling event data every 10,000 generations. We simulated the prior phylogeny (Alfaro et al. 2009) and detects significant rate shifts along the branches. We also estimated specification/extinction rates using MEDUSA, part of the R package geiger (Harmon et al. 2008). The former fits a birth–death model to the phylogeny (Alfaro et al. 2009) and detects significant rate shifts along the branches. We ran analyses using the default AIC threshold. The MEDUSA algorithm first estimates likelihood and AIC scores with the default AIC threshold. The MEDUSA algorithm is a nonrandom incomplete taxon-sampling correction. We removed the first 30% of generations as burn-in after checking MCMC convergence using BAMMtools (Rabosky et al. 2013; Rabosky 2014). We checked the ESS (target value of 300) in the fragment of 12S–16S and regions of ambiguous alignment were excluded. No indication of a saturation effect was detected (Supplementary Table S8, available on Dryad). The preferred substitution models for each partition are listed in Supplementary Table S4, available on Dryad.

The BI, ML, and MP trees were very similar (Fig. 1 and Supplementary Fig. S1, available on Dryad). The main conflict between mtDNA and nuclear genes involved the position of Rana sylvatica and the relationships among the three major New World groups within clade 6 (Supplementary Fig. S2, available on Dryad). Our species-tree analyses (Supplementary Fig. S3, available on Dryad) produced results that were consistent with our concatenated analyses (Fig. 1), but with somewhat less resolution. Given the greater resolution of the concatenated analysis, and its general agreement with the species-tree analysis, we used the concatenated analyses for our biogeographic assessment (Figs. 1 and 3). Our analyses resolved the following seven major geographic lineages of Rana with strong support: Rana weiningensis (clade 1) from southwestern China was the sister group of the remaining taxa, which together included a New World clade 3 (eastern North America, Mexican Plateau, and Neotropics), southwestern China (clade 11; only Rana shuchinae), western North America (clade 13), southwestern China and northern Vietnam (clade 15), Central Asian R. asiatica plus Europe (clade 17), and East Asia (clade 18), including the mainland and adjacent islands.

We found that species of Old World Rana are paraphyletic with respect to the two New World clades. The major well-differentiated, morphologically and ecologically distinct clades within New World Rana largely support the traditional subgeneric designations for the genus (Dubois 1992; Hillis and Wilcox 2005; Hills 2007; AmphibiaWeb 2015). The New World clade 13 consists of the Rana boylii group in western North America (the subgenus Amerana). The second New World clade (clade 3) includes the remaining North and South American taxa, with five strongly differentiated subclades (Fig. 1): R. sylvatica (clade 4), which forms the sister group of the remaining species; the R. catesbeiana group (clade 5; the subgenus Aquarana); the Rana palmarum group (clade 7; the subgenus Lithobates, although that subgenus was further subdivided by Hillis and Wilcox 2005); Rana sierranuadrensis plus the Rana tarahumarae group (clade 8; the subgenus Zweifelia, although that name was used for the R. tarahumarae group alone by Hillis and Wilcox 2005); and the R. pipiens
FIGURE 3. Spatiotemporal reconstruction of the true frogs based on the Trans-Beringia hypothesis (H1b). Results based on the (a) DEC and (b) DEC+J likelihood methods implemented in BioGeoBEARS are presented. The tree topology, derived from BEAST analyses, is consistent with the Bayesian tree of Figure 1. Branch lengths are proportional to divergence times. Means and 95% confidence intervals of divergence times are presented in Supplementary Figure S5, available on Dryad. Colors reflect biogeographic designations (for species at tips) and most probable states at each node. Gray colors indicate nodes with multiple ancestral states. States at nodes represent the most probable ancestral area before the instantaneous speciation event, whereas those on stems represent the state of the descendant lineage immediately after speciation. Some node and stem labels are omitted to reduce clutter; in all cases these are identical to the state at both the ancestral and descendant nodes. The corresponding pie charts for the proportional likelihoods of ancestral area states at each node and stem are presented in Supplementary Figure S6, available on Dryad. Abbreviations: y, sympatric range-copying speciation; s, sympatric-subset speciation; v, vicariance; j, jump-dispersal or founder event speciation. Arrows show the dispersal events.

group (clade 9; the subgenus Pantherana). Although each of these distinct groups is strongly supported, the higher relationships among groups have weaker support.

The East Asian taxa (clades 1, 11, 15, and 18) formed a polyphyletic group. Clade 18 included five well-supported subclades (Fig. 1 and Supplementary Fig. S4, available on Dryad); clades 19 and 22 included exclusively the East Asian species from Taiwan and Japan; 20, 21, and 23 included the remaining insular species and closely related taxa from the East Asian mainland. The unresolved relationships among the five subclades suggest rapid radiation.

Divergence time estimates from the BEAST chronograms are shown in Figure 3 (with details in Supplementary Fig. S5, available on Dryad). These are described in concert with the historical biogeography below.

**Historical Biogeography**

The DEC+J class of models always conferred a much higher likelihood on the data than DEC (log-likelihood differences of 11–16 units; Supplementary Table S9 and Supplementary Fig. S6, available on Dryad). Within each class, all submodels that allowed dispersals across Beringia (H1) were favored over the Trans-Atlantic dispersal hypothesis (H2) (Supplementary Table S9, available on Dryad). Among the Beringian dispersal hypotheses, hypothesis H1b (two independent dispersal events from East Asia to North America) received the strongest support. Thus, we used this model in our evaluation of ancestral range estimates.

We contrasted the results of the DEC and DEC+J models (Fig. 3) under hypothesis H1b. DEC (Fig. 3a) estimated the dispersal rate ($r$) as 0.0023 and the extinction rate ($e$) as 0.0. By definition, the jump-dispersal rate ($j$) was 0.0 under this model. We identified nine dispersal events (horizontal arrows on Fig. 3a) that split the ancestral range. We inferred 10 vicariance and 7 sympatric-subset events ($s$). The remaining events we inferred were sympatric range-copying events, in which the ranges of both descendants were identical to
FIGURE 4. Summary of major range shifts (dispersal and vicariance) of *Rana* over three time periods (a–c). For details of the range shifts see Figure 3. (a1) Dispersal to eastern North America within the interval 48–43 Ma, followed by vicariance at 43 Ma. (a2) Dispersal to western North America 43–34 Ma, followed by vicariance at 34 Ma. (b1) Dispersal to Europe + Central Asia (composite area) during 29–25 Ma (or possibly earlier; see text). (b2) Europe and Central Asia split at 20 Ma by vicariance. (b3) Dispersal to the Mexican Plateau + Neotropics composite area during 37–32 Ma, the Neotropics split from its sister-group (eastern North America + Mexican Plateau clade) by vicariance at 32 Ma, divergence at 31 Ma of the first of several Mexican Plateau clades. (c1) Repeated dispersals among East Asia Islands and the mainland during 20–5 Ma. (c2) At least two dispersals from the Mexican Plateau to the Neotropics during 13–10 Ma. Taxon names in panels are subgenera.

We focus first on the divergences of the earliest-diverging branches. Under the DEC model (Fig. 3a), range expansions can be described by a combination of dispersal followed by vicariance or sympatric-subset events. Because the reconstruction of areas at some nodes is not definitive, we report general patterns rather than enumerating each event.

The MRCA of *Rana* dated to the Eocene at about 48 Ma (95% credibility interval [CI]: 55–40 Ma). The first dispersal involved a range expansion through Beringia and then across high latitudes of North America into eastern North America about 48–43 Ma (Fig. 4a), followed by vicariance at 43 Ma resulting in an eastern North American clade 3 and an Asian clade 10 whose ancestral range is reconstructed as East Asia (Area A). The latter then underwent a second range expansion across Beringia and then south into western North America 43–34 Ma (Fig. 4a), followed by vicariance (node 12) at 34 Ma, yielding a western North American clade (13) and a clade of Eurasian species (clade 14). The Eurasian clade subsequently split into an East Asian clade and a clade in Europe and Central Asia (Fig. 4b).

In the New World radiation, the first invasion of *Rana* from Asia underwent a primary split into two lineages: *R. sylvatica* versus the remaining species. Then another split occurred among the majority of species between clade 5 (*Aquarana*; the North American water frogs) and a clade that expanded into the Mexican Plateau and the Neotropics (Fig. 4b). Shortly thereafter (~32 Ma), this latter clade split to form clade 7 (*Lithobates*; the Neotropical true frogs), and in turn a second lineage split at approximately 31 Ma, yielding clade 8 (*Zweifelia*; the torrent frogs) on the Mexican Plateau, and the large clade 9 (*Pantherana*; the leopard frogs; Fig. 4c).

Within *Pantherana*, two lineages, *R. forreri*, and a clade comprising *R. macroglossa*, sp. 4, sp. 5, and sp. 6, diverged from their close relatives at approximately 11
Ma to occupy the Neotropics. The remaining species of *Pantherana* are found in eastern and northern North America and the Mexican Plateau. In contrast, clade 13 (*Amerana*) gave rise to several species beginning at 23 Ma, but none of the descendants expanded east or south into other areas. Ambiguities of ancestral range estimation (Fig. 3) make precise interpretation of the biogeographic history difficult, but at least four dispersal/vicariance events occurred between eastern North America and the Mexican Plateau.

Under the DEC+J model (Fig. 3b), these patterns can be explained by 14 jump dispersals (j symbol at the node, and vertical arrows). The j parameter was estimated to be 0.0279, and d and e were 0.0. Under this model, no vicariance events are inferred. We inferred seven sympatric range-copying cladogenetic (y) events, but no sympatric-subset cladogenetic events. Although the estimated ranges and inferred mechanisms differed, the locations of range shifts on the tree were similar under both models.

**Diversification Patterns**

The standard LTT plot for all *Rana* depicted a near-constant accumulation of lineages through time until about 12 Ma, after which there was a gradual slowdown (Supplementary Fig. S7, available on Dryad). However, diversification plots of regions (Fig. 5) indicated several region-specific patterns.

Generally, the Old World species (Fig. 1, clades 1 + 11 + 14) show a different trajectory of lineage accumulation compared with taxa of the New World (Fig. 1, clade 3 + 13) (Fig. 5a). Further regional plotting indicates that East Asian clades 1 + 11 + 18 and the larger clade 3 from
FIGURE 6. Temporal dynamics of diversification rate through the evolutionary history of Rana revealed by BAMM analyses. Rate-through-time curves depict clade-specific net diversification trajectories for (a) all Rana species, (b) the New World clade 3, and (c) the Old World species (clades 1, 11, and 14; Fig. 1). Shading intensity reflects relative probability of a given diversification trajectory, with upper and lower bounds denoting the 90% Bayesian credible interval on the distribution of rates through time. A period of elevated diversification rate in (c) is highlighted by a rectangle.

eastern NA, the Mexican plateau, and the Neotropics are important in explaining the diversity of the Old World and New World, respectively (Fig. 5b). The Old World Rana began species accumulation about 48 Ma ago, slowly at first, until 29 Ma; this was followed by continuous cladogenesis, and acceleration of the rate of lineage accumulation (Fig. 5a). Plots of the East Asian lineages (Fig. 5b,d) indicate that East Asian islands account for a substantial portion of Old World Rana diversity, especially given the small size of the islands relative to the mainland.

Generally, lineage accumulation in the New World showed a faster rate than in the Old World (Fig. 5a,b), and especially an increase in the rate of cladogenesis around 14 Ma. This increase is associated with separate radiations in eastern North America, the Mexican Plateau, and the Neotropics, of which the radiation on the Mexican Plateau had the greatest impact (Fig. 5c). In contrast, species accumulation in western North America was relatively slow and constant, resulting in only eight extant species after the second dispersal from East Asia (Fig. 5b).

Unlike the regional LTT analyses, BAMM analysis of all Rana indicated an initial high net diversification rate that gradually decreased through time (Fig. 6a and Supplementary Figs. S8a and S9a, available on Dryad). Analyses further supported strongly heterogeneous diversification dynamics between the Old World and New World species (clade 3) supports diversity-dependent speciation, with a pattern of initial high diversification that gradually decreased through time (Fig. 6b). In contrast, both BAMM and MEDUSA analyses detected a more sudden rate shift among the Old World species (Fig. 6c and Supplementary Figs. S8c, S9c, and S10, available on Dryad). The net diversification rate from BAMM shows a hump-shaped curve for the Old World species, with an increased rate from 29 to 18 Ma (about 0.14 species/Ma; Fig. 6c). The MEDUSA analysis also suggested that the current diversity of Old World Rana (clades 1 + 11 + 14) is best explained by one rate shift (AICc threshold = 2.01; Supplementary Fig. S10, available on Dryad); the average speciation rate increases significantly from 0.013 to 0.093 after the split of K. schuchiniae from other Rana at approximately 37 Ma.

DISCUSSION

Phylogeny and Classification of Rana

Our analyses yield a comprehensive and well-resolved phylogeny for the genus Rana and strongly confirm its monophyly. Our treatment of Rana is largely consistent with that used by AmphibiaWeb, except that R. cangyuanensis is now considered part of Odorrana (Fei et al. 2012). Previous studies have been hampered by sampling restricted geographic regions or limited species groups with limited gene markers, mostly based on mtDNA analyses (e.g., Tanaka et al. 1996, Tanaka-Ueno et al. 1998; Veith et al. 2003; Hillis and Wilcox 2005; Frost et al. 2006; Che et al. 2007; Matsui 2011). In contrast, our tree is based on deep taxon sampling and multiple nuclear and mitochondrial loci. Prior studies did not recover most of the phylogenetic relationships because they did not simultaneously consider both Eurasian and American species.

The two New World clades of Rana (3 and 13 in Fig. 1) clearly nest within East Asian clades (1, 11, 15, and 18 in Fig. 1), and thus the simple sister pairs of intercontinental disjunct Rana lineages (e.g., Macey et al. 2006) cannot be assumed. This finding for Rana mirrors the conclusions of Wen (1999), who considered similar intercontinental distributions of several plant groups, and found that many taxa traditionally treated as disjunct sister groups were polyphyletic or paraphyletic. Our study highlights the importance of thorough sampling for phylogenetic history constructions.
The phylogenetic relationships we recovered do not support some of the taxonomic assignments suggested by Fei et al. (2012) and Frost (2015) (Supplementary Table S2, available on Dryad). For example, Pseudorana from both Fei et al. (2012) and Frost (2015) is paraphyletic. Clade 15 contains Rana johnsi, Rana ziegeli, and Rana sangzhiensis (Fig. 1 and Jing Che unpublished data) and these are not the sister taxa of clade 1 (R. winningenesis).

Morphological homoplasy has led to some of the taxonomic confusion surrounding the genus *Rana*. The presence of grooves on the toe pads of adults in the species in clades 1, 15, and *Rana sauteri* (within clade 19), and an abdominal sucker in the tadpole of *R. sauteri* have led to various taxonomic proposals for these species. For example, *Pseudorana* (Fei et al. 1990) and *Pseudoamolops* (Jiang et al. 1997) were established as separate genera but with various compositions of species (Dubois 1992; Ye et al. 1993; Fei et al. 2012). The recognition of *Pseudoamolops* has long been rejected by phylogenetic studies (e.g., Tanaka-Ueno et al. 1998; Che et al. 2007a, 2007b) and our results corroborate these findings. Frost et al. (2006) included both *Pseudorana* and *Pseudoamolops* in *Rana*, and at the same time, recognized clade 3 of this study as *Lithobates* based on limited species and gene sampling. However, that arrangement makes the remaining *Rana* paraphyletic. Later, largely based on the phylogeny of Che et al. (2007b), Frost (2015) revived the use of *Pseudorana* and recognized genera *Pseudorana, Lithobates, and Rana* as subgenera. However, this action still does not result in entirely monophyletic taxa (Supplementary Table S2, available on Dryad).

Even if these groups were fixed by transferring species among these taxa, the major morphological, ecological, and behavioral differentiation within *Rana* does not occur among these poorly differentiated taxa (*Pseudorana, Rana, and Lithobates sensu Frost 2015*), but within them. For example, biologists have a much greater need for names of well-differentiated groups including Pantherana (the leopard frogs), Zwiefelia (the torrent frogs), Lithobates (the Neotropical true frogs), and Aquarana (the North American water frogs), than for a taxon that combines these groups. Hillis and Wilcox (2005) provided nested, clade-based names at both levels, but Dubois (2006) argued that such nested subgeneric names were incompatible with the rules of the International Code of Zoological Nomenclature (ICZN). Hillis (2007) disagreed with Dubois’ interpretation of the ICZN rules, but nonetheless suggested a compromise classification that recognized the distinct, nonoverlapping clades within *Rana* as subgenera.

Based on our phylogenetic analyses and the lack of any diagnostic morphological characters for the putative genera recognized by Fei et al. (2012) or Frost et al. (2006), and the clear monophyly of the larger group, we retain all these species in the traditional genus *Rana* (Supplementary Table S2, available on Dryad). This also has the desirable outcome of retaining the traditional binomial species names for these well-studied species, and thus retaining the connections of these names to their corresponding extensive literatures.

We support the recognition of the major diverse lineages within *Rana* (the groups that show significant molecular, morphological, ecological, and behavioral divergences) as subgenera (Supplementary Table S2, available on Dryad), which largely match the traditional major distinct species groups long recognized by numerous authors (Zweifel 1955; Case 1978; Farris et al. 1980, 1983; Hillis et al. 1983, 1984; Hillis 1988, 2007; Hillis and de Sá 1988; Dubois 1992; Hillis and Wilcox 2005). The subgenera of *Rana* are *Pseudorana* (clade 1), *Aquarana* (clade 5), *Lithobates* (clade 7), Zwiefelia (clade 8), *Pantherana* (clade 9), Liuhurana (clade 11), Amerana (clade 13), and *Rana* (clade 14) (Fig. 1, Supplementary Table S2, available on Dryad).

The inferred relationships of *R. sylvatica* differed in the trees derived from nuclear versus mitochondrial genes (Supplementary Fig. S2, available on Dryad), and different studies and data sets have placed this species at various places within clades 3 and 10 (Case 1978; Farris et al. 1980, 1983; Post and Uzzell 1981; Hillis and Davis 1986; Hillis and Wilcox 2005). In our combined analysis, *R. sylvatica* forms the sister group of all the other remaining New World *Rana* (Fig. 1), which is consistent with Case (1978) based on immunological data. However, the support is weak (Fig. 1), because mtDNA sequences conflict with the signal from the six nuclear genes regarding the placement of *R. sylvatica* (Supplementary Fig. S2a,b, available on Dryad). The different resolutions for this species may indicate hybridization or incomplete lineage sorting among the ancestral lineages of clade 3.

### Biogeographic Processes Inferred from DEC and DEC-+J Models

The DEC model most often interprets range evolution as expansion into an unoccupied area (i.e., dispersal) (Matzke 2014), followed by splitting of the ancestral range by vicariance or sympatric-subset cladogenesis into two daughter species with more restricted ranges. In total, nine dispersals followed by 10 vicariance speciation and 7 sympatric-subset events were inferred, for a total of 16 range changes under DEC compared with 14 under DEC-+J. In some cases, even though DEC and DEC-+J reconstructed the same number of changes in ancestral range, the interpretation (e.g., dispersal vs. vicariance) may differ because of differences in the models.

Although the DEC-+J model was significantly favored over DEC under all scenarios, it is worth considering its suitability for this analysis given that it was developed to accommodate founder event speciation (p parameter), which is common in oceanic island systems (Matzke 2014). Under DEC-+J, the ancestral ranges of all nodes involve a single area (Fig. 3b). Pie charts of the relative likelihoods of alternative reconstructions (Supplementary Fig. S6c4, available on Dryad) showed low likelihoods, except for ancestral
node 17. This suggests that founder event speciation was more common than vicariance or sympatric-subset speciation.

In contrast, DEC range reconstructions include 18 nodes with >1 area (not including the “corner” nodes). These reflect classic dispersal into new regions. The reconstructions for several nodes are ambiguous, having alternative possibilities with similar likelihoods.

The DEC+J model typically yields higher overall likelihood scores and the range estimates are unambiguous, as expected (Matzke 2013b, 2014). Thus, DEC+J presents a “cleaner” scenario. However, it is worth considering whether the DEC-J model is biologically meaningful to dispersal between contiguous areas within a continent. An improvement in likelihood score indicates a better fit of data to a particular model only, which does not mean that the model offers a better biological explanation for the data. Notwithstanding, the DEC class of analyses also may be biased depending on how the distributional data were scored. Matzke (2014) pointed out that in his simulations DEC+J tended to be favored in trees with terminal taxa that occupied a single area, whereas DEC was favored when terminal taxa occupied multiple areas. Because we assigned all terminal taxa to a single area, the favoring of DEC-J is not surprising. Clearly, the choice of terminal assignment to one or more areas may affect model selection.

**Historical Biogeography**

The origin and evolution of *Rana* has been of long-standing interest to systematists and biogeographers (e.g., Case 1978; Veith et al. 2003; Macey et al. 2006). Our extensive sampling suggests that *Rana* originated in the East Asia region, perhaps in southwestern China (Figs. 1 and 3) where three distinct, well-differentiated groups occur: *R. weiningensis* (clade 1), *R. shuchiniae* (clade 11), and the sister species *R. shengi* and *R. johnsi* (clade 15) (Fig. 1). The former two species have limited distributions at high elevations on the Yunnan-Guizhou Plateau (1700–2950 m and 2760–3800 m, respectively). Species in clade 15 (including *R. sangzhiensis*) occur in the lowlands of Southwest China and adjacent northern Vietnam.

The DEC and DEC+J models both suggest an “Out of Asia” pattern involving two independent dispersals from East Asia into the New World (Fig. 4a), and a third one into Europe and Central Asia (Fig. 4b). This basic biogeographic pattern was established 48–25 Ma.

Under DEC, a range expansion approximately 48–43 Ma (Eocene) from East Asia into eastern North America (across northern North America) was followed by a vicariance event at 43 Ma. Subsequently, a second range expansion from East Asia to western North America (south along the Pacific coast of North America) occurred approximately 43–34 Ma (Eocene) followed by vicariance at 34 Ma, which restricted the *R. boylii* group (*Amerana*) to western North America. Under DEC+J, range evolution would be explained by jump dispersal, which is “instantaneous” speciation. The first such event (to eastern North America) occurred at 43 Ma, and the second (to western North America) at 34 Ma.

Are these models supported by geological and paleoecological information? During the Eocene (36–34 Ma), a continuous belt of boreotropical forest extended over the entire Northern Hemisphere from Asia through North America across Beringia (Wolfe 1975, 1987). This would have allowed considerable trans-Beringian exchange of terrestrial biota adapted to warm-temperate climates (Tiffney 1985a). Thus, during the early branching of *Rana*, it seems there was no obvious barrier to dispersal across Beringia. In the mid-Tertiary, beginning about 35 Ma, mixed deciduous hardwood and coniferous forests began to dominate in Beringia (Wolfe 1987), which perhaps facilitated the second dispersal of *Rana* (clade 13). The end of the Eocene event marked a dramatic climatic change (Sanmartín et al. 2001) from a “greenhouse” to an “icehouse” world. From 14–10 Ma to 3.5 Ma, cold taiga forest covered the Beringia bridge (Wolfe 1987), rendering additional dispersals of *Rana* unlikely. Asia and North America remained joined by Beringia until the opening of the Bering Strait at 5.5 or 5.3 Ma (Marincovich and Gladenkov 1999; Gladenkov et al. 2002).

The lack of a dispersal barrier suggests that range expansion to North America can be explained by DEC without including the J parameter. Under DEC+J, jump dispersal is typically associated with an inhospitable barrier such as ocean separating two continents or islands (Matzke 2013b).

The third major event, the occupation of Europe and Central Asia (Fig. 3b) from East Asia, took place from 29 to 25 Ma (under DEC; ambiguity in the reconstruction might push the 29 Ma limit back to 34 Ma). This event corresponds with the receding of the Turgai Sea at 30–29 Ma, which likely acted as a barrier for biotic exchanges between Europe and Asia from the Jurassic to the Eocene (Briggs 1995; Sanmartín et al. 2001). The ancestor of clade 17, which occupied Europe + Central Asia, experienced vicariance at 20 Ma. This isolated *R. asiatica* in Central Asia from its sister group, the European *R. temporaria* group. DEC+J depicts the two range shifts as a jump dispersal from East Asia to Europe at 25 Ma, followed by jump dispersal from Europe back to Central Asia at 20 Ma (Fig. 3). Progressive aridification of Central Asia beginning about 22 Ma (Guo et al. 2008) may have contributed to the present disjunction between the European *R. temporaria* group, *R. asiatica*, and clade 18 (the remaining East Asian species). Savage (1973) first proposed that this aridification event led to the range disjunction of the amphibian faunas of Europe and East Asia, and our analyses support this hypothesis for *Rana*.

Other amphibian groups that may fit this pattern include *Bombina* (Zheng et al. 2009), *Hyla* (Li et al. 2015), and two clades within *Salamandridae* (Zhang et al. 2008).

Finally, expansion of *Rana* into South America occurred across the Isthmus of Panama. The three strictly South American species of *Rana* form a single clade that originated 16 Ma, and then diversified by 9 Ma (Fig. 3).
This finding supports dispersal across the Panamanian Isthmus in the middle Miocene, which is consistent with recent geological evidence that supports the formation of the Panamanian Isthmus at this time (Montes et al. 2015).

Although vicariance appears to have played a dominant role in speciation, strict geographic barriers do not appear to have been present in all speciation events. Heterogeneous landscapes, facilitated by differentiation in ecological dimensions, may have also driven speciation. The three New World areas (Fig. 2) are effectively ecological islands for speciation in Rana; almost no widespread species range across any two regions. The Mexican Plateau, as the transition zone between temperate and tropical regions, provides unique and restricted habitats that may drive ecological speciation. This might explain the numerous endemic species associated with this biodiversity hotspot (e.g., Mittermeier et al. 2005). Under DEC+J, dispersals from eastern NA to the Mexican Plateau and Neotropics appear to have occurred since the late Eocene, approximately 36 Ma. In the Neogene, we inferred frequent dispersals and speciation events (Fig. 3b). This jump-dispersal scenario for American Rana taxa should be further tested with other taxa, such as pitvipers and bunchgrass lizards (Castoe et al. 2009; Bryson et al. 2012).

Trans-Atlantic versus Trans-Beringian Dispersal

Our biogeographic analysis supports two independent dispersals of Rana from East Asia to North America via the Beringian land bridges (Fig. 2: H1b). In contrast, the hypothesis of a trans-Atlantic bridge connecting North America and Europe, as suggested by Case (1978) and illustrated in Figure 2 (H2), received little statistical support (Supplementary Table S9, available on Dryad). The trans-Atlantic dispersal route does not seem likely based on geological considerations. According to the ancestral range reconstructions for model H2 (Supplementary Fig. Sbe, available on Dryad), one jump dispersal (DEC+J) or range expansion (DEC) from East Asia to Europe would have had to occur prior to the trans-Atlantic dispersal between Europe and eastern North America. The dispersal from East Asia to Europe would have had to occur by 48 Ma, but the Turgai Sea separated Asia from Europe at that time. Only after 30–29 Ma (Briggs 1995; Sanmartín et al. 2001) were extensive biotic exchanges between Europe and Asia possible. The lack of a sister-group relationship between the Rana of Europe and North America also argues strongly against the trans-Atlantic scenario.

The disjunct distributions of many taxa across the Holarctic region have attracted considerable attention of botanists and zoologists (e.g., Wen 1999; Sanmartín et al. 2001; Donoghue and Smith 2004). Most groups of temperate forest plants originated and diversified in eastern Asia, and then dispersed out of Asia across Beringia, mostly during the last 30 Ma (Donoghue and Smith 2004). In contrast, no general “Out of Asia” pattern has been reported for terrestrial animals. However, most terrestrial animal examples involve insects, birds, and mammals (Sanmartín et al. 2001). Do amphibians and reptiles show similar biogeographic patterns to plants, or to insects, birds, and mammals?

A considerable body of literature exists on the relationships of amphibians and reptiles that are endemic to at least two of the following major Holarctic regions: Europe, East Asia, western North America, and eastern North America. There have been 28 hypothesized dispersal events of amphibians and reptiles among these four regions (Table 1; Fig. 7). Amphibians involved in these dispersal events include groups of Cryptobranchidae, Salamandridae, Proteidae, Plethodontidae, Hylidae, and Ranidae (this study), and reptiles include Elapidae, Viperidae, Natricinae, Lampropeltini, Scincidae, Alligatoridae, Trionychidae, Emydidae, Geoemydidae, and Testudinidae (Table 1; Spinks et al. 2004; Min et al. 2005; Smith et al. 2005; Le et al. 2006, 2014; Macey et al. 2006; Burbbrink and Lawson 2007; Roos et al. 2007; Vieites and Wake 2007; Le and McCord 2008; Pramuk et al. 2008; Wüster et al. 2008; Zhang et al. 2008, Zhang and Wake 2009; Hua et al. 2009; Kelly et al. 2009; Spinks and Shaffer 2009; Van Bocxlaer et al. 2010; Brandley et al. 2011; Garcia-Porta et al. 2012; Guo et al. 2012; Pyron et al. 2013; Pyron 2014; Li et al. 2015). Although Proteidae is also distributed in North America and Europe, the divergence between the relevant taxa is older than the breakup of Laurasia (108 Ma; Pyron 2014), and so Proteidae is not included in these comparisons. The remainder of the estimated dispersal times for the amphibians and reptiles ranged from the Early Eocene to the Middle Miocene (Table 1). The biogeographic origins of these clades largely fit the pattern seen among Holarctic plants (Donoghue and Smith 2004), with strong and repeated support for trans-Beringian over trans-Atlantic dispersal (Fig. 7). In contrast, support exists for just three trans-Atlantic dispersals (Salamandridae and Plethodontidae; Zhang et al. 2008; Zheng et al. 2009; and Emydidae; Spinks and Shaffer 2009).

Lineage Diversification in the Old versus New World

The lineages of Old and New World Rana have different trajectories of diversification (Figs. 5 and 6). Of the two clades that resulted from trans-Beringian dispersals, Amerana (clade 13, western North America), does not seem to have greatly influenced New World diversity. In contrast, clade 3, comprising the species of eastern North America, the Mexican Plateau, and the Neotropics rapidly radiated (Figs. 5b and 6b; 0.17 species/Ma), probably owing to the availability of diverse new ecological opportunities. BAMM analyses suggest a diversity-dependent process (Fig. 6b; Rabosky et al. 2007), resulting in saturation of ecological niche space and a decline in the speciation rate (Walker and Valentine 1984; Valentine 1985; Rabosky and Lovette 2008).
<table>
<thead>
<tr>
<th>Taxa</th>
<th>Disjunction pattern number, direction of dispersal, and estimated divergence time (Ma, in parentheses)</th>
<th>Reference(s)</th>
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<tr>
<td>Cryptobranchidae</td>
<td>1, A to E (43)</td>
<td>Zhang and Wake (2009); Pyron (2014)</td>
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<td>Ranae (Rana)</td>
<td>2, A to E (43)</td>
<td>Zhang et al. (2008); Pyron (2014)</td>
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<td>17, A to C (25)</td>
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<tr>
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<td>18, C to A (27)</td>
<td>Zhang et al. (2008); Pyron (2014)</td>
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<td>Salamandridae (Taricha and Notophthalmus)</td>
<td>22, A to C (35)</td>
<td>Zhang et al. (2008); Pyron (2014)</td>
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<td>Salamandridae (New World salamandrids)</td>
<td>24, C to NA (43.5)</td>
<td>Zhang et al. (2008); Pyron (2014)</td>
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<td>Plesodontidae (Hydromantes)</td>
<td>25, D to C (33)</td>
<td>Pyron (2014); Vieites and Wake (2007)</td>
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<tr>
<td>Plesodontidae (Karsenia)</td>
<td>12, NA to A (53)</td>
<td>Pyron (2014); Min et al. (2005); Vieites and Wake (2007)</td>
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<td>Hylidae (Hyla)</td>
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<td>Li et al. (2015); Pyron (2014)</td>
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<td>Wu et al. (2003); Roos et al. (2007)</td>
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<td>Le et al. (2004)</td>
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<td>Emysidae (Emys)</td>
<td>15, A to C (21)</td>
<td>Le et al. (2004)</td>
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<td>Geoemydidae (Mauremys)</td>
<td>14, A to C (30–38)</td>
<td>Spinks et al. (2004); Le and McCord (2008)</td>
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<td>Testudinidae (Testudo)</td>
<td>16, A to C (7)</td>
<td>Le et al. (2006)</td>
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</table>

Notes: Twenty-two clades with disjunct distributions are assigned to one of the eight two-area categories. Where the divisions of eastern and western North America are ambiguous, we combined them as North America (NA). "?" indicates that the time or place of origin is ambiguous. Numbers in parentheses represent the time of divergence (Ma). Numbered events refer to the disjunctions in Figure 7. Abbreviations: A, East Asia (mainland + islands); C, Europe; D, western North America; E, eastern North America. References refer to the phylogenetic studies from which data were obtained.
A distinct increased rate of lineage accumulation occurred at around 14 Ma (clade 3; Fig. 5b), corresponding to dispersals among the Mexican Plateau, eastern North America, and Neotropics (Figs. 3 and 4c2), as well as in situ diversification of Mexican taxa. The rate increase generally corresponds to the Middle Miocene formation of the Trans-Mexican Volcanic Belt (Ferrari et al. 1999; Gómez-Tuena et al. 2007), which has likely contributed to the diversification of numerous taxa (e.g., Bryson et al. 2011, 2012).

In contrast, lineage accumulation of the Old World clade further diversified in the Mediterranean region around 18 Ma, possibly in association with the onset of Asian monsoons at the Oligocene/Miocene boundary (Sun and Wang 2005).

Lineage accumulation of the East Asian clades 19–23 (Fig. 1) occurred over a short period of time (23–20 Ma) in association with island formation (Fig. 5b,d). From 23 to 15 Ma, rifting and tectonic deformation associated with block rotations and volcanism resulted in the substantial fragmentation of the East Asian margin and formation of islands as a continental sliver (Taira 2001; Itoh et al. 2006). Following the initial opening of the Sea of Japan in the Late Oligocene (Otofuji et al. 2001; Itoh et al. 2006), the archipelagos of Japan rotated into their present configuration about 15 Ma (Taira 2001). The five clades (19–23; Fig. 1) along the Asian margin formed well before the origin of the present island system. Thus, our results support a role for Miocene tectonic events in the diversification of East Asian Rana.

Our analysis provides a framework for understanding the biology of the well-studied frogs of the genus Rana throughout Eurasia and the Americas. Numerous studies of the biology of these frogs can be facilitated by a better understanding of their phylogeny and biogeography.

**AUTHOR CONTRIBUTIONS**


**SUPPLEMENTARY MATERIAL**

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.ck1m7.

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