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# Using phylogenomics to understand the link between biogeographic origins and regional diversification in ratsnakes

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## ARTICLE INFO

## ABSTRACT

Globally distributed groups may show regionally distinct rates of diversification, where speciation is elevated given timing and sources of ecological opportunity. However, for most organisms, nearly complete sampling at genomic-data scales to reduce topological error in all regions is unattainable, thus hampering conclusions related to biogeographic origins and rates of diversification. We explore processes leading to the diversity of global ratsnakes and test several important hypotheses related to areas of origin and enhanced diversification upon colonizing new continents. We estimate species trees inferred from phylogenomic scale data (304 loci) while exploring several strategies that consider topological error from each individual gene tree. With a dated species tree, we examine taxonomy and test previous hypotheses that suggest the ratsnakes originated in the Old World (OW) and dispersed to New World (NW). Furthermore, we determine if dispersal to the NW represented a source of ecological opportunity, which should show elevated rates of species diversification. We show that ratsnakes originated in the OW during the mid-Oligocene and subsequently dispersed to the NW by the mid-Miocene; diversification was also elevated in a subclade of NW taxa. Finally, the optimal biogeographic region-dependent speciation model shows that the uptick in ratsnake diversification was associated with colonization of the NW. We consider several alternative explanations that account for regionally distinct diversification rates.

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## 1. Introduction

Exploring the spatial and temporal modes of diversification as well as the factors influencing their patterns is critical for understanding the processes leading to biodiversity accumulation (Fritz et al., 2013). For species-rich assemblages with a global distribution, diversification is expected to be influenced by several mechanisms involving both biotic and abiotic factors (Moore and Donoghue, 2007). Adaptive radiation, defined as rapid diversification of descendants from a common ancestor into distinct environments, is considered one of the main mechanisms shaping biodiversity on earth (Simpson, 1953; Schluter, 2000). Ecological opportunity generated by the appearance of new resources, the mass extinction of competitors, or the colonization of new areas has typically been the prime motivator for adaptive radiation

(Losos, 2010; Yoder et al., 2010). Adaptive radiation via ecological opportunity predicts diversity-dependent diversification, in which speciation rates are initially rapid, filling abundant unoccupied niches and declining as available niches become saturated (Schluter, 2000; Losos, 2010). Furthermore, enhanced diversification rates should be found only in diverse taxa experiencing adaptive radiation when compared to other groups not showing heightened diversity (Glor, 2010).

Inferring diversification processes requires a comprehensive phylogeny that incorporates clade age and biogeographic information (Moore and Donoghue, 2007). Using divergence-time estimation and ancestral area reconstruction, while considering events such as dispersal, geographic isolation and mass extinction, provide a context for understanding how diversification rates change across temporal and geographic dimensions. For example, *Anolis* lizards are an important case where dispersal to new areas accelerated diversification by colonizing the abundant open niches of the West Indies, permitting a set of ecomorphs to evolve repeatedly on

distinct islands that share similar environmental conditions (Harmon et al., 2003; Losos, 2009).

However, just because regions were free from competitors when colonized does not guarantee that rates of speciation were elevated early in the history of a particular group. For instance, a signature of rapid early radiation was not found in Caribbean alsophiine snakes despite colonizing unoccupied regions that share a similar distribution and ecological opportunity with the *Anolis* lizards (Burbrink et al., 2012). Explanations for the lack of elevated diversification in alsophiines involve the young age of this group (they may not have had sufficient time to show a reduction in speciation rates), and waiting time between island colonization (offsetting early bursts of speciation; Burbrink et al., 2012). Therefore, it is important to properly estimate divergence time, ancestral area, and species diversification so that a comprehensive view of biodiversity accumulation is properly assessed.

While many examples of diversification and adaptive radiation have occurred in geographically more controlled areas such as islands, several continentally distributed examples are relevant as well. The ratsnakes (Coronellini), which historically have been used for systematics, ecological, behavioral, and physiological research (Boulenger, 1894; Underwood, 1967; Lawson and Dessauer, 1981; Schulz, 1996; Schulz and Gumprecht, 2013), are important examples of continental-level adaptive radiation, given their rapid diversification into unique ecological niches in biogeographically distinct regions. The ratsnakes, composed of 88 species (Table S1; Uetz, 2014), are widely distributed throughout the Palearctic, northern part of the Oriental, the Nearctic and portions of the Neotropical Zoogeographic regions (Fig. 1). Given their global distribution, ratsnakes occupy very heterogeneous habitats, including mountain forests, grassland, deserts, and tropical rain and dry forests (Schulz, 1996), which likely provided ecological opportunity for rapid divergence within this group. Unlike many other ectothermic animals, ratsnakes have attained their highest diversity in both the Old World (OW) and the New World (NW) temperate regions.

Previous biogeographic studies supported a tropical Asian origin of ratsnakes with dispersal to OW temperate regions and subsequent Beringial dispersal to the NW (Burbrink and Lawson, 2007; Burbrink and Pyron, 2010; Chen et al., 2013). This Cenozoic Beringian Dispersal Hypothesis (CBDH; Guo et al., 2012) is supported in several squamate groups as well as various plant and other animal groups. This unidirectional dispersal was likely important in shaping temperate Eurasian and North American faunas and floras (Enghoff, 1995; Wen, 1999; Smith et al., 2005; Burbrink and Lawson, 2007; Brandley et al., 2011). Importantly, diversification of the NW clade of ratsnakes, Lampropeltini, occurred rapidly upon arrival in the Americas (Burbrink and Lawson, 2007; Burbrink and Pyron, 2010). However, under similar environmental conditions, it is possible that the rapid bursts of diversification in the NW lineages were an extension of broadly rapid Holarctic diversification and not a phenomenon isolated to the Americas. Alternatively, after divergence between OW and NW clades, these lineages may have diversified uniquely in terms of tempo and trajectory of species accumulation in their respective regions. Nevertheless, neither of these hypotheses was tested in a biogeographical context where rates of diversification were examined across the phylogeny of ratsnakes while at the same time considering region of origin.

We use the Anchored phylogenomics platform to sample and sequence hundreds of loci across the entire ratsnake genome (Lemmon et al., 2012) to infer a dated species tree using coalescent-model based methods to overcome potential gene-tree/species-tree conflicts from incomplete-lineage sorting (Pamilo and Nei, 1988; Maddison, 1997; Page and Charleston, 1997; Slowinski et al., 1997; Slowinski and Page, 1999; Edwards, 2009). This represents the first attempt to infer phylogeny using genomic data across most species and all 20 genera of ratsnakes (Utiger et al., 2002, 2005; Burbrink and Lawson, 2007). Specifically, with this dated tree we examine monophyly of all genera and tribes (Coronellini and Lampropeltini) and estimate ancestral area and dispersal probabilities to test the CBDH, previously examined only using 2 loci (Burbrink and Lawson, 2007). With phylogenomic estimates, we examine species diversification as the interaction between speciation and extinction for understanding the buildup of biodiversity (Ricklefs, 2007; Morlon et al., 2010; Pyron and Burbrink, 2013). With clade age, diversity, branch length, and topology available, we use time- and taxon-dependent models to examine speciation and extinction rate changes to understand the potential factors influencing diversification patterns (Rabosky and Lovette, 2008a, 2008b; Morlon et al., 2010, 2011; Etienne et al., 2011; Stadler, 2011). Finally, linking diversification back to biogeography, we determine if diversification processes are heterogeneous across different lineages (Alfaro et al., 2009; Rabosky, 2014) and test correlation between diversification
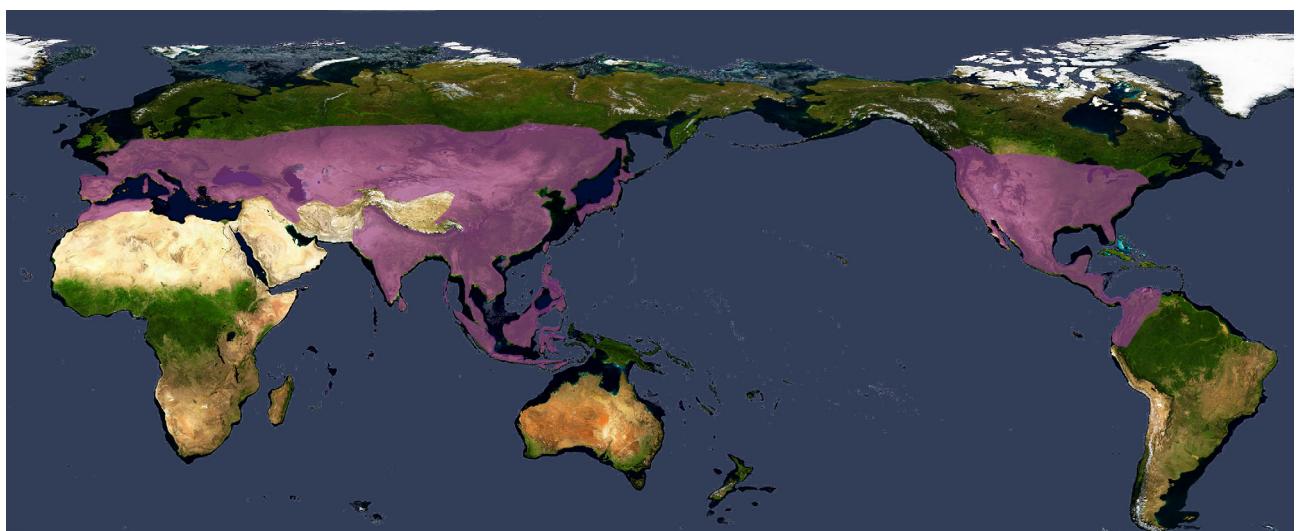


**Fig. 1.** The global distribution of ratsnakes (colored in purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

changes and geographic regions. Ultimately, investigating the tempo and mode of diversification, we find that (a) the radiation of the entire ratsnake group shows a diversity-dependent pattern of diversification, and (b) speciation rate- heterogeneity is prominent within the NW subclade. We discuss potential alternative scenarios that link diversification variation and the inter-continental colonization event.

## 2. Methods

### 2.1. DNA sample preparation

We collected tissue or DNA samples from 79 ratsnake species (91% of 88 putative members of this clade) as well as 9 outgroups (7 taxa representing main clades in Colubrinae and 2 non-colubrine species; Table S1). This includes all OW genera of ratsnakes, *Archelaphe*, *Coronella*, *Elaphe*, *Euprepiophis*, *Oocatochus*, *Oreocryptophis*, *Rhinechis*, *Zamenis*, *Gonyosoma* and *Coelognathus*, and New World genera, *Arizona*, *Bogertophis*, *Cemophora*, *Lampropeltis*, *Pantherophis*, *Pituophis*, *Pseudelaphe*, *Rhinocheilus* and *Senticolis* (Fitzinger, 1833; Boulenger, 1894; Pope, 1935; Dowling, 1958; Underwood, 1967; Schulz, 1996; Utiger et al., 2002, 2005; Burbrink and Lawson, 2007). Genomic DNA of all tissue samples was extracted using the standard protocol included with QIAGEN DNeasy Kits (QIAGEN, Valencia, CA). The concentrations of DNA extractions were checked with NanoDrop Thermo scientific 2000c and the extent of DNA degradation was shown with electrophoresis. All of the samples (88 species included) used for sequencing contained over 2.6 µg of DNA with bright bands in the high molecular-weight region on the electrophoresis gel, which is required by the high-throughput sequencing anchored enrichment approach (Lemmon et al., 2012).

### 2.2. DNA sequencing and alignment generation

Data were collected following the general methods of Lemmon et al. (2012) through the Center for Anchored Phylogenomics at Florida State University (www.anchoredphylogeny.com). Briefly, each genomic DNA sample was sonicated to a fragment size of ~150–350 bp using a Covaris E220 Focused-ultrasonicator with Covaris microTUBES. Subsequently, library preparation and indexing were performed on a Beckman-Coulter Biomek FXp liquid-handling robot following a protocol modified from Meyer and Kircher (2010). One important modification is a size-selection step after blunt-end repair using SPRIselect beads (Beckman-Coulter Inc.; 0.9× ratio of bead to sample volume). Indexed samples were then pooled at equal quantities (typically 12–16 samples per pool), and enrichments were performed on each multi-sample pool using an Agilent Custom SureSelect kit (Agilent Technologies), which contained the probes designed from *Anolis carolinensis* and *Calamaria pavimentata* (see Supplemental materials for details). After enrichment, the 6 enrichment pools were combined in equal quantities for sequencing on 2 PE150 Illumina HiSeq2000 lanes. Sequencing was performed in the Translational Science Laboratory in the College of Medicine at Florida State University.

To generate usable alignments, raw reads were first filtered with Illumina standard CASAVA v1.8 pipeline (with quality ensured using a high chastity filter) and demultiplexed based on 8 bp index. Reads were processed downstream following the methods of Prum et al. (2015), with the following adaptation: paired reads from library fragments less than 280 bp were identified and merged following Rokyta et al. (2012). Reads were assembled using probe region sequences from *Anolis carolinensis* and *Calamaria pavimentata* as references. Consensus sequences were then generated from assemblies containing >60 mapped reads. Orthol-

ogy was assessed using a neighbor-joining clustering approach that utilizes a common-kmer distance matrix. Sets of orthologous sequences were then aligned using MAFFT v7.023b (Katoh, 2013), and trimmed/masked to remove problematic regions. Detailed protocol of the raw data analysis is described in the supplemental materials Anchored Phylogenomics Methods and in Prum et al. (2015). All data collection and preliminary analysis was performed at the Center for Anchored Phylogenomics at Florida State University (http://anchoredphylogeny.com/).

### 2.3. Phylogenetic inference

#### 2.3.1. Gene annotations
Prior to phylogenetic inference, we determined the potential homology of each locus using the bioinformatics tool BLAST 2.2.31 (Altschul et al., 1990; Altschul, 1997) with the NCBI nucleotide database (nt). The expect threshold was setup as $10^{-6}$ and only the top hit with bits-score over 200 was saved for each query.

#### 2.3.2. Gene-tree inference
We determined the appropriate substitution model for all loci using *Bayesian information criterion (*BIC) among the 24 commonly used models with the program jModeltest 2.1.4 (Guindon and Gascuel, 2003; Darriba et al., 2012).

To reduce the negative influence of gene-tree inference error on our species tree, we generated gene trees using both maximum likelihood (ML) and Bayesian inference (BI) methods, compared the gene-tree variations from the two methods and used the gene-tree set with less conflicts to build the species tree. To estimate the support values of nodes on the species tree, ML trees with 1000 bootstrap (BS) replicates for all loci were generated in the program RAxML 8.0.20 (Stamatakis et al., 2008; Stamatakis, 2014) using the GTRCAT model, with a single taxon, the natricine *Nerodia sipedon*, designated as the outgroup (Stamatakis, 2006). Bayesian inference gene trees were estimated in program Mrbayes 3.2.1-v2 (Ronquist et al., 2012). We used the best-fit substitution models for all loci based on the model test result (Table S2) with *Nerodia sipedon* defined as an outgroup. Every MrBayes run was conducted using four chains (one cold chain, three hot chains) over two simultaneous runs with 20 million generations for each, sampling every 1000 generations. Convergence was assessed and confirming effective sample size (ESS) were >200 for all parameters. After removing burn-in, we randomly sampled 1000 trees from posterior (PP) tree distribution for species-tree inference.

#### 2.3.3. Gene-tree comparisons
We examined the distances between gene trees among different loci (among-locus distances) for both BS and PP tree sets and compared those distances to within-locus distances from BS and PP replicates. Theoretically, because the topological variation within each BS or PP set is estimated from a single locus, we expect that the within-locus distances should be smaller than among-locus distances. Therefore, if a large portion of gene trees have excessive within-locus distances in the final dataset, it is then expected that they will contribute to lower the reliability of the estimated species tree.

Tree distance comparison tests were performed using program TreeCmp v1.0-b291 (Bogdanowicz et al., 2012). The triple distance metric (TT; Critchlow et al., 1996) was used to quantify tree distance. The matrix comparison mode (-m) was used, which allowed all tree pairs to be compared. The results from our empirical data were reported as normalized distances, which were corrected by comparing the distances of a simulated random tree distribution under the Yule model (McKenzie and Steel, 2000). The value of normalized distances at "0" means tree topologies were identical, "1" if the distance distribution of the tree topologies were the same as

randomly generated trees, and >1 if the dissimilarity of tree topologies was greater than that of the randomly generated trees.

### 2.3.4. Species-tree inference

Species-tree inference was performed using a coalescent-based parametric method MPEST v1.4 (Liu et al., 2010) and the nonparametric method ASTRAL-II (Mirarab and Warnow, 2015). Both programs take gene trees as the input and are highly computational efficient and statistically consistent for handling phylogenetic inference with dataset of numerous loci and taxa, unlike BEST (Liu, 2008) or ∗BEAST (Heled and Drummond, 2010). Both MPEST and ASTRAL-II methods maintain accuracy even with a high level of incomplete lineage sorting and potentially low rates of horizontal gene transfer (Liu et al., 2010; Mirarab and Warnow, 2015).

We estimated species trees using the gene-tree set with both relatively small among-loci distances and within-locus distances (see Section 2.3.2 for gene-tree comparison method). Species trees were summarized by majority rule consensus, including topological support values, using the python script SumTrees.py in the package DendroPy (Sukumaran and Holder, 2010). All phylogenetic analyses were performed using computer clusters at the CUNY High Performance Computing Center.

### 2.4. Divergence-time estimation

With the topology of species tree available, we estimated the divergence time in the program MCMCTree (dos Reis and Yang, 2011) in the PAML package (Yang, 2007), which is a computationally feasible method to deal with phylogenomic dataset (Chiari et al., 2012; Hedin et al., 2012). The substitution rates for all 304 loci were first estimated in BASEML (Yang, 2006); substitution models for each locus were obtained using the best-fit model determined in jModeltest. Then, to increase computational speed, loci with similar substitution rates were concatenated (M. dos Reis, pers. comm.). By calculating the Euclidean distances of the substitution rates for all loci, we clustered loci into 15 groups using Ward Hierarchical clustering method (Ward, 1963). Next, branch lengths were estimated using the gradient and Hessian features in BASEML (dos Reis and Yang, 2011). Five fossil calibrations were used to date the phylogeny with soft bounds (Table S2; Yang and Rannala, 2006). The range of the stem-group age of Colubridae is from 33.3 Ma, based on the oldest known colubrid *Texasophis galbreathi* (Holman, 1984), to 65 Ma, the upper boundary of the colubroids estimated in Pyron and Burbrink (2012b). The most recent common ancestor (MRCA) of the clade including *Zamenis situla*, *Z. lineatus* and *Z. longissima* ranged from a minimum 6 Ma, based on fossils of direct ancestors to this group (Ivanov, 1997) to a maximum 20 Ma, when the first ratsnake (genus *Elaphe*) appeared in Europe (Ivanov, 2002). The date of the MRCA of the Lampropeltini ranged from 11.4 to 37.1 Ma with a mean of 20.6 Ma based on the oldest known putative lampropeltinine, *Pseudocemophora* cf. *antiqua* (Holman, 2000). The mean date of the divergence between *Pantherophis* and *Pituophis* was 15.5 Ma (9.5–25.3 Ma) based on the oldest known ratsnake, *Elaphe* (*Pantherophis*) *kansensis* (Holman, 2000). The divergence date between the genera *Lampropeltis* and *Cemophora* is based on the oldest known kingsnake, *Lampropeltis similis* with a mean of 13.75 Ma (8.4–24.4 Ma, Holman, 2000). These calibrations have been applied successfully to estimate divergence dates across various colubroid groups (Burbrink and Lawson, 2007; Pyron and Burbrink, 2009a, 2012a). The MCMC chain length was run for 1.5 million generations sampled every 100 generations, with the first 500,000 samples removed as burnin. Convergence and ESS for all parameters was checked in Tracer v1.6 (Rambaut et al., 2014).

### 2.5. Ancestral area estimation

The ratsnakes have a global distribution covering five biogeographically distinct regions (Lomolino et al., 2010): Western Palearctic, Eastern Palearctic, Oriental, Nearctic, Neotropical. Using the dated species tree, we estimated the ancestral area of this group and all major clades and tested the CBDH. Results supporting the CBDH should show a geographically monophyletic NW group originating from either Western Palearctic or Eastern Palearctic or both, and all events would necessarily have occurred within the Cenozoic. Ancestral area reconstruction analysis was performed in the R package BioGeoBears (Matzke, 2013a) using DEC (Dispersal-Extinction-Cladogenesis; Ree and Smith, 2008) and DEC + J (Matzke, 2013b) models. The former is a stochastic model accounting for the evolution of geographic range along the phylogeny considering dispersal, extinction, and speciation effects; the latter is based on the DEC model while adding the parameter J to describe a founder effect. We constrained the possible area combinations for dispersal based on the information of node ages in the phylogeny with corresponding adjacent continental distributions. Because the root age of Colubridae ranges from 33.3 to 65 Ma, after the breakup of Laurasia and Gondwanaland (Sanmartín et al., 2001; Lomolino et al., 2010), dispersal probabilities were constrained to zero between Neotropical/Western Palearctic, Neotropical/Eastern Palearctic, Neotropical/Oriental, Nearctic/Oriental. Additionally, we also relaxed all constraints, which assumed dispersal probabilities were equal among all biogeographic regions. Maximum likelihood values were generated given the parameters for each model and performance of two models was evaluated using Akaike weights (Wagenmakers and Farrell, 2004).

### 2.6. Fitting diversification models

To examine diversification patterns and estimate speciation and extinction rates within Coronellini, we fit the time-calibrated species tree to multiple diversification models. We examine the overall fit of the tree to various time-dependent measures of diversification as a general fit to adaptive radiation theory and then determine diversification rate heterogeneity across clades and regions (next section). We first compared the nine models from Morlon et al. (2010) and determined the best-fit model using the corrected Akaike information criterion (AICc; Akaike, 1974). Models 1 and 2 assume a constant diversity and a positive extinction rate with a constant turnover rate for model 1 and a declining turnover rate for model 2. Models 3–6 accommodate expanding diversity. Models 3, 4a–4c allow a positive extinction rate and models 5–6 assume no extinction. Models 3 and 5 assume a constant speciation rate and models 4a–c and 6 allow speciation rates to vary through time. For all of these, the likelihood function is expressed for the node distance distribution based on a coalescent model. We performed a model selection test by modifying the function MISFITS (Burbrink et al., 2012) in R. Then, we compared the diversity-dependent models and the time-dependent models for this phylogeny using the R package TreePar (Stadler, 2011). TreePar estimates speciation and extinction rates under a constant birth-death model with rate shifts and a diversity-dependent model. The maximized likelihood value for each model was estimated, and we compared and selected the best-fit model based on Akaike weights.

### 2.7. Diversification rate heterogeneity across lineages and biogeographic regions

Considering that the ratsnakes have an intercontinental distribution covering highly heterogeneous habitats, we tested specia-

tion rate heterogeneity across lineages and determined the rate-region correlation.

First, we ran the test using R function MEDUSA in package Geiger (Harmon et al., 2008), which fits a constant birth-death model based on both the branching times and the species richness of the target group and selects the optimal model using a stepwise AIC approach (Alfaro et al., 2009). Corrected AIC values were calculated to identify significant shifts in nodes and the threshold of AICc was estimated based on the tree size. To contrast these results, we performed the test using the program BAMM v2.1.0 (Rabosky et al., 2013), which allows speciation and extinction rates to vary through time and uses Bayesian statistical methods with the reversible-jump Markov chain Monte Carlo (rjMCMC) algorithm to estimate posterior probability for the possible number of shift points and shift models (Rabosky, 2014). We first estimated the priors in the R package BAMMtools (Rabosky et al., 2013; Rabosky, 2014) and ran the MCMC chain 20 million generations with sampling frequency every 10,000 generations. Chain convergence was checked using the R package Coda (Plummer et al., 2006). Results were summarized and visualized using the package BAMMtools to assess probabilities of speciation, extinction and diversification rate changes across the tree.

To determine if significant diversification rate shifts were associated with particular zoogeographic regions, we constructed geographic distribution-dependent diversification models in the program GEOSSE using the R package diversitree (Goldberg et al., 2011; FitzJohn, 2012). We realized that power for testing this family of statistics (SSE methods) may be somewhat ambiguous (see Rabosky and Goldberg, 2015), however we use this test to contrast to the results from BAMM and MEDUSA described above. Two geographic regions were defined representing the Old World (A) and New World (B), respectively. Using this likelihood method, we first defined two models with one ($m_0$) assuming the same diversification rate in OW and NW (speciation rate sA = sB; extinction rate xA = xB); the other one ($m_1$) having different diversification rates for OW and NW. Two models were compared using an ANOVA. Then, we also estimated the parameters in $m_1$ using Bayesian method with MCMC chain. After running 50,000 generations, we removed first 90% steps and checked for burnin in the R package CODA (Plummer et al., 2006). Posterior distribution of all the parameters (speciation [sA, sB], extinction [xA, xB], dispersal [dA, dB] and the between-region speciation rate sAB) as well as the rate difference between region A and B were estimated and plotted.

All the sequence data and the scripts running the pipeline of phylogenomic and downstream analyses are available at Dryad (http://dx.doi.org/10.5061/dryad.r43s0).

# 3. Results

## 3.1. Gene-tree comparisons and species-tree inference

We obtained 304 loci (total length: 452,698 bp; mean length: 1489 bp/locus) for tree inference. The BLAST results showed that 97% of the loci matched to known genes, particularly from the *Python bivittatus* genome (Castoe et al., 2011; Table S3). The best-fit substitution model for 58% of the loci was HKY, including either gamma or the invariable sites parameter or both (Table S3).

The gene-tree distance distributions show that the among-loci distance of PP trees sets were smaller than the distances from the BS tree sets (Fig. 2; p < 0.05) with a median of 0.42 and 0.55 respectively. The within-locus distances for the BS and PP replicates revealed that the topological variation within PP replicates was generally smaller than those from BS replicates. Both of the within-locus distance distributions had long right tails, implying excessive gene-tree topological variation for some loci; certain loci exceed the among-locus distances (Fig. 3). The number of the loci
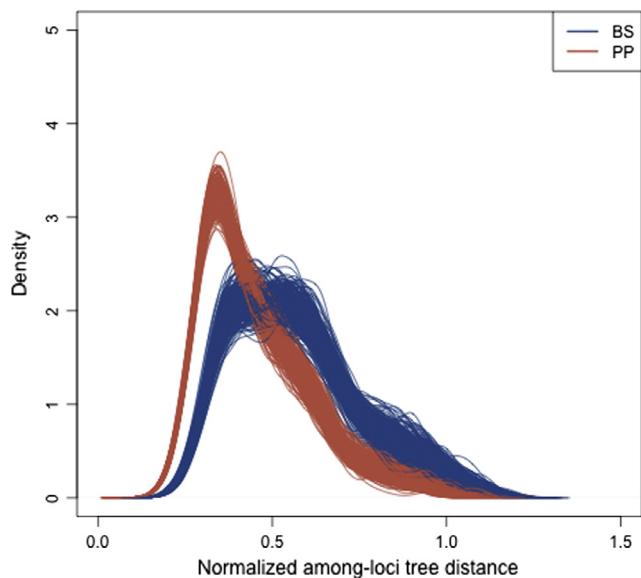


**Fig. 2.** Gene-tree distance distribution calculated using the triple metric. BS: bootstrapping trees; PP: posterior trees.

where the within-locus tree distances were greater than the among-locus tree distances in BS replicates (53 loci) was greater than those from PP replicates (40 loci). Large topological variation could increase the uncertainty of the gene-tree inference, thus, we used the species tree reconstructed from the PP gene trees for further analysis.

The species tree estimations from MPEST and ASTRAL-II were highly consistent (Fig. 4). The results suggested that the genera *Gonyosoma* and *Coelognathus*, mainly distributed in tropical and subtropical regions of Southeast and East Asia, were not part of the least-inclusive ratsnake group (gray box in Fig. 4). Therefore, the core group of what were typically considered ratsnakes included the most recent common ancestor uniting the genera *Archelaphe*, *Coronella*, *Elaphe*, *Euprepiophis*, *Oocatochus*, *Oreocryptophis*, *Orthriophis*, *Rhinechis*, *Zamenis* and the NW tribe Lampropeltini. This ratsnake clade (70 taxa in this study; Fig. 4 blue and orange boxes) was considered as our ingroup for all remaining analyses in the following discussion. Additionally, the typical support for the NW ratsnake group as monophyletic was also supported (orange box in Fig. 4).

## 3.2. Divergence time and ancestral area estimation

After discarding burnin, convergence was confirmed in Tracer with an ESS for all the parameters >200 for the divergence time estimation using MCMCTree. Our results support that Coronellini originated in mid-Oligocene (27–33 Ma) and the divergence between OW and NW clades occurred during the mid-Miocene (15–18 Ma, Fig. S1). Ancestral area models prefer the DEC to DEC + J model assessed by Akaike weights (73%); both models with constrained region combinations and relaxed constraints showed similar results. The results suggested that ratsnakes originated from Eastern Palearctic and dispersed to Western Palearctic and Nearctic ∼ 20 Ma (Fig. 5), during which, the Beringian land bridge was likely the most available connection between the OW and the NW with suitable habitat for reptile migration (Pielou, 1979), supporting CBDH.

## 3.3. Patterns of diversification

Diversification model tests selected Morlon model 6, 2, 4a, and 4d as the top-four models (Akaike weights 45%, 35%, 15%, 4.9%,
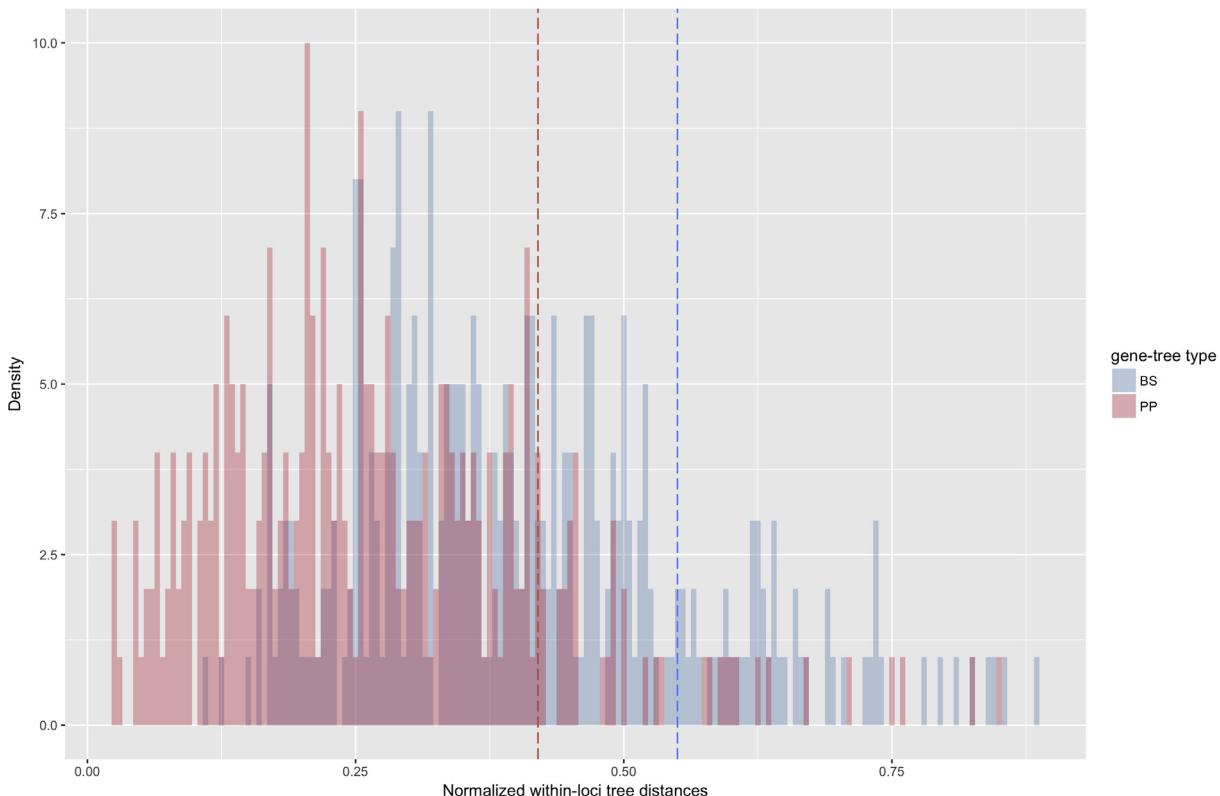
**Fig. 3.** Distribution of within-locus distance medians of BS and PP replicates for each locus. BS: bootstrapping replicates; PP: posterior replicates. Blue dashed line: median of among-loci distances for PP gene trees; orange dashed line: median of among-loci distances for BS gene trees. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1). Results using the package TreePar suggested the diversity-dependent model excluding extinction rates as the best-fit model (Akaike weights 44%). We note that despite that both the best fit model from the Morlon method estimated the extinction rate as zero, the models (2, 4a, 4d) with 54.9% weights supported positive extinction rates. On the other hand, among the top-five models in TreePar, only one estimated a positive extinction rate (Table 1).

Both BAMM and MEDUSA detected significant diversification rate change in a subclade in the NW group. From the BAMM results, we removed the first 30% of samples as burn-in and the effective sample sizes of the number of shifts and the likelihood were >1200. The results showed four shift models with an accumulative probability = 96% (Fig. S2). The best rate-shift model has one primary rate increase on the phylogeny for a subclade of the NW ratsnakes which includes the genera *Pantherophis*, *Pituophis*, *Arizona*, *Cemophora*, *Rhinocheilus* and *Lampropeltis* with 65% probability (Fig. 6a). To compare net diversification between the rate-shifting clade and non-shifting clades, estimates for speciation, extinction and net diversification rates were examined for the entire ratsnake tree, the subclades with significant rate shifts, and the remaining ratsnakes exclusive of rate shifts (Figs. S3 and 6b). Generally, the speciation and net diversification rates for the entire ratsnake group gradually declined from the root toward the tips and reached a plateau around 14 Ma (Fig. S3). The trend of a declining speciation rate towards the present for both the rate-shifting clade and non-shifting clades were similar (Fig. S3). However, the significant rate-shift speeds up diversification within in the NW subclade (Fig. 6). The results from MEDUSA also supported the significant rate shift at the root of this NW subclade (Fig. S4), with the mean diversification rates changing from 0.078 to 0.21 before and after the shift.

The GeoSSE analysis using maximum likelihood selected the model which permits diversification rates to change between the NW and OW ($m_1$, $\chi^2 = 11.975$, p-value = 0.0025). Based on this model, the speciation rate in the NW region is higher than the rate of OW ($sA < sB$) and the extinction and dispersal rates for both regions ($xA$, $xB$, $dA$, $dB$) were low or zero. Bayesian estimation also supported the diversification rate within NW region significantly higher than the rate in the OW (p-value < 2.2e−16, Figs. 7 and S5).

## 4. Discussion

### 4.1. Biogeography

Ancestral area reconstruction showed that the ratsnakes (Coronellini) originated in the Eastern Palearctic and subsequently dispersed to the Western Palearctic and likely crossed Beringia into Nearctic (Fig. 5). This result supports the CBDH, which indicates that OW taxa seeded the diversification of ratsnakes in the NW. All extant colubroid snake groups occurring in the NW, originated in the OW and dispersed to the Americas during the Oligocene or Miocene (Chen et al., 2014a, 2014b). This pattern is confirmed for ratsnakes in our study using mtDNA-free NGS phylogenies for the first time. Interestingly, considering the inverse latitudinal diversity gradient of ratsnakes (Pyron and Burbrink, 2009b), where the highest diversity is in temperate regions, adaptation from an OW temperate origin may have been a potential constraint for the rise of diversity in both the OW and the NW tropical regions.

While testing the CBDH at the genomic level is so far unique to this study, origins in the OW and unidirectional dispersal to the NW from the Eocene to the Miocene is not uncommon among
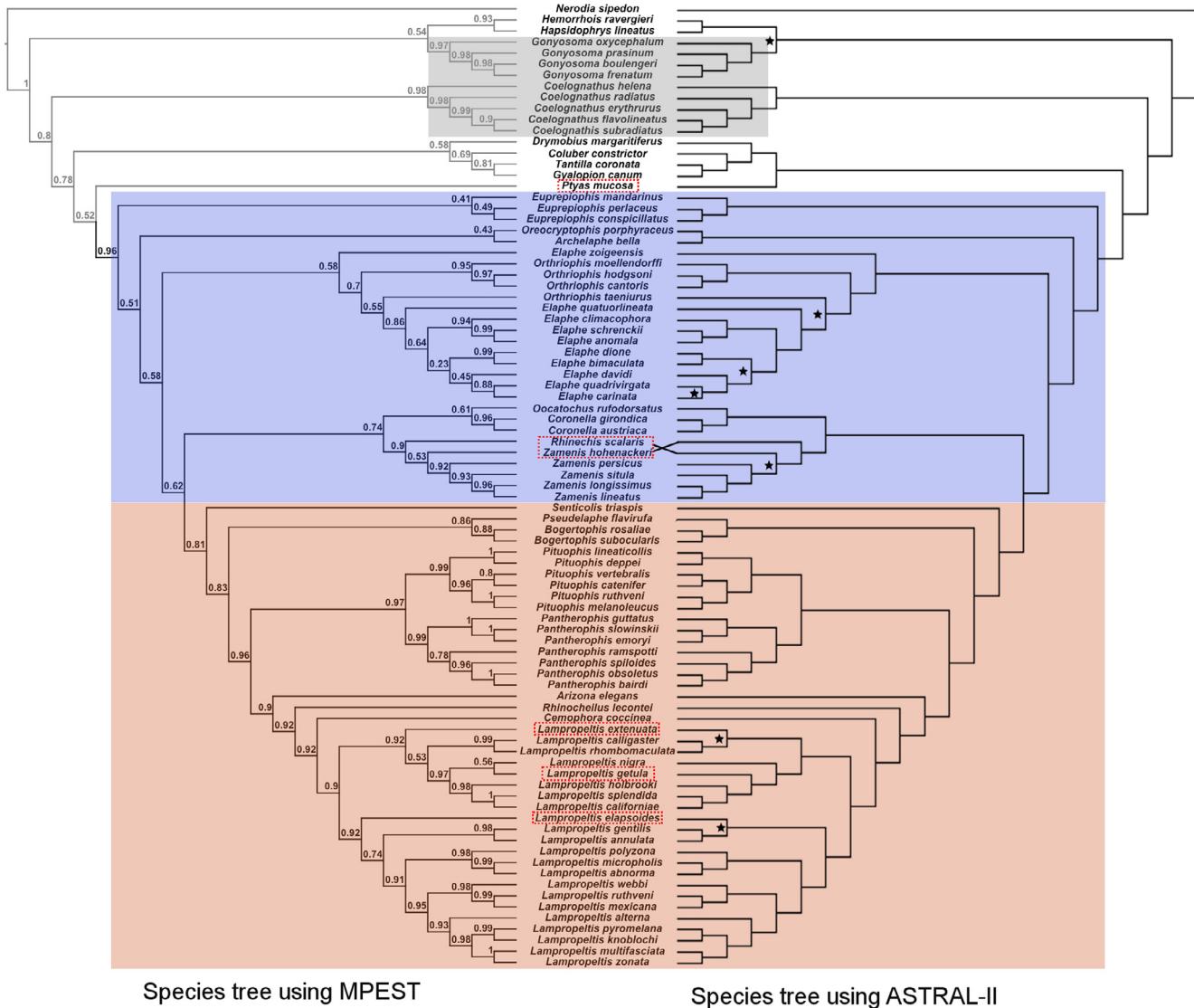
**Fig. 4.** The species trees generated using 1000 posterior probability gene-tree set and the programs MPEST (left) and ASTRAL-II (right). The former ratsnake genera *Gonyosoma* and *Coelognathus* are colored in the gray box. Old world ratsnakes are colored in blue box and new world ratsnakes are colored in the orange box. For the ASTRAL-II species tree, the inter-nodes with support values lower than 0.9 are labeled with a star. The lineages with incongruous topologies on two species trees are noted in the red dashed boxes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

other groups of organisms (Lomolino et al., 2010; Sanmartín et al., 2001; Wen, 1999), particularly in snakes, viperids (Wüster et al., 2008), ratsnakes and other colubrines (Burbrink and Lawson, 2007; Chen et al., 2014a, 2014b), watersnakes (Guo et al., 2012), sibynophiines, dipsadines, and elapids (Chen et al., 2014a, 2014b), as well as eublepharid geckos (Gamble et al., 2011), and *Plestiodon* skinks (Brandley et al., 2011). Given that this pattern of origin and dispersal was found in ratsnakes using either two loci (Burbrink and Lawson, 2007) or 304 loci (this study), we suspect that this general trend among squamates may be credible even among those studies relying on trees generated from a small sample of the genome.

The positions of the continents were similar at the timing of origin of ratsnakes as they are now, which therefore indicates that two routes of dispersal from the OW to the NW were possible: Asia to North America via the Beringia or Europe to North America via the Greenland-Faeroes Bridge (Sanmartín et al., 2001). While it is beyond the ability of any of the methods used here to determine which route, or even if overwater dispersal was possible, it is gen-

erally thought that the Bering Land Bridge provided more suitable habitats to sustain populations of ectotherms (Burbrink and Lawson, 2007). Studies like this and others showing unidirectional dispersal to the NW bring up an intriguing question. Why did species from these groups not disperse back to the OW? Testing why reverse dispersal was not found in these groups is likely difficult, but three hypotheses should be considered to explain this pattern: (1) closing of Beringia and then subsequently cooling at the end of the Miocene eliminated dispersal, (2) competitive exclusion and niche occupation by species in the OW eliminated successful colonization of similar taxa from the NW, or (3) the OW was colonized by taxa from the NW, but extinction eliminated evidence of this trend. Hypotheses 1 and 2 are fairly self-explanatory. Hypothesis 3, however, suggests that if extinction probabilities are the same for all species, then ancestral area reconstructions and estimates of unidirectional dispersal from trees of extant species are therefore biased by those monophyletic groups which diversify rapidly and regionally, thus eliminating the probability of detecting single dispersals in the opposite direction.
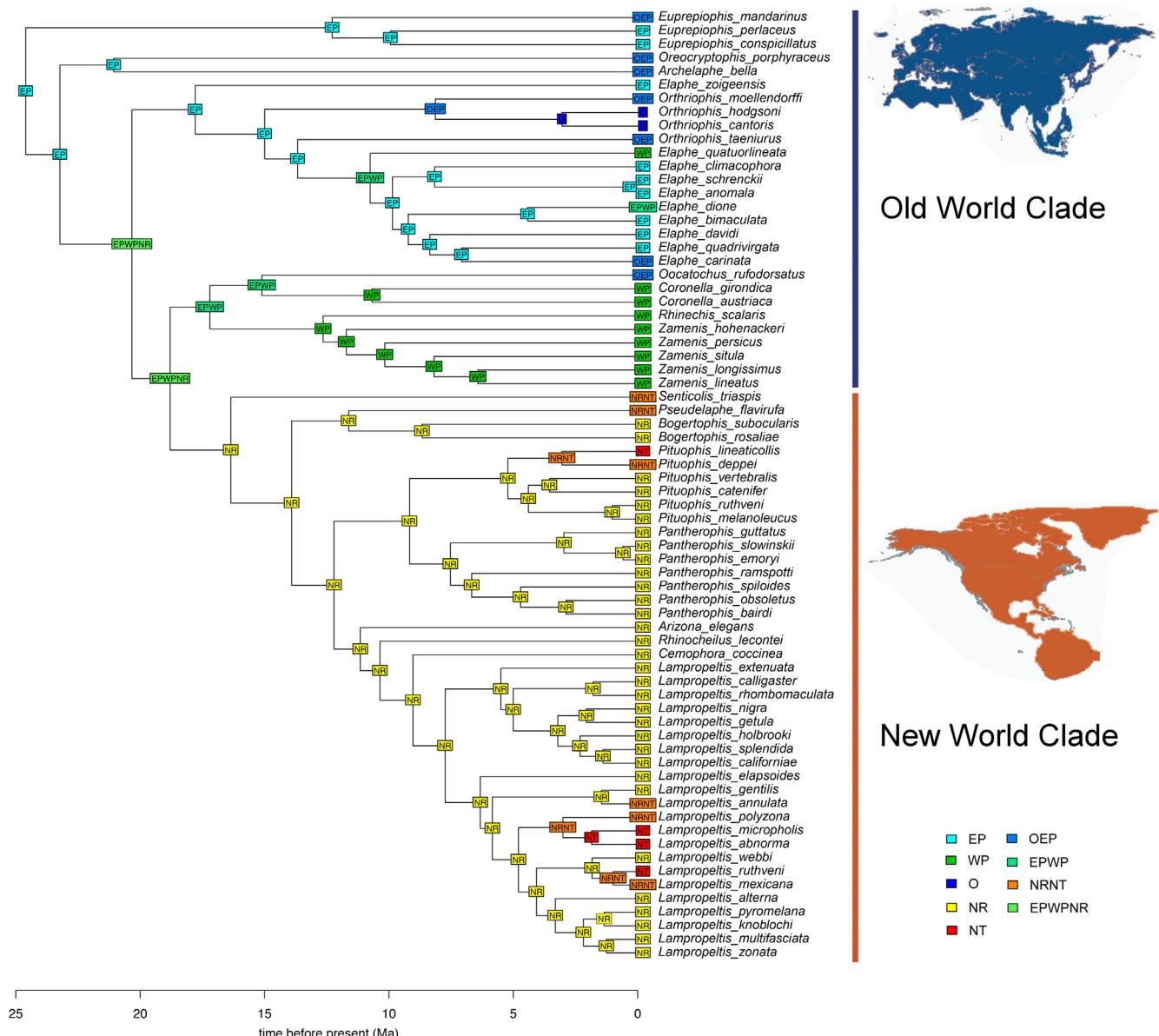
**Fig. 5.** The time-calibrated species phylogeny with estimated ancestral area states. EP: Eastern Palearctic; WP: Western Palearctic; O: Oriental; NR: Nearctic; NT: Neotropical.

**Table 1**

The best-fit diversification models selected from the Morlon models (row 1–4; Morlon et al., 2010) using MISFITS function (Burbrink et al., 2012) and TreePar models using R package TreePar (row 5–9; Stadler, 2011).

| Models[a] | AIC | Akaike weights |
|---|---|---|
| Morlon Model 6 | −32.3870 | 0.45 |
| Morlon Model 2 | −31.8815 | 0.35 |
| Morlon Model 4a | −30.1966 | 0.15 |
| Morlon Model 4d | −27.9367 | 0.049 |
| Diversity-dependent without extinction | 424.7633 | 0.44 |
| Yule with one rate shift | 426.4205 | 0.19 |
| Diversity-dependent with extinction | 426.7633 | 0.16 |
| Yule with two rate shift | 428.1999 | 0.078 |
| Yule without shift | 428.3048 | 0.074 |

[a] Morlon Model 6: expanding diversity model with an exponential decreasing in the speciation rate and no extinction rate; Morlon Model 2: saturated diversity model with positive extinction rate and an exponential decreasing in the turnover rate; Morlon Model 4a: expanding diversity model with an exponential decreasing in the speciation and positive constant extinction rate; Morlon Model 4d: both speciation and extinction rates changing over time.

## 4.2. Rapid diversification in ratsnakes

Part of adaptive radiation theory predicts lineages with increased rates of speciation should show either a diversity-dependent pattern of diversification or an exceptional rate of diversification relative to the non-radiating clades (Simpson, 1953; Givnish, 1977; Glor, 2010). For the entire ratsnake group, the diversity-dependent model of diversification was the best fit to our phylogenies, indicating a general rapid radiation (Table 1). The programs BAMM and MEDUSA each assigned a rate elevation within the NW group near the node that includes the genera *Pantherophis*, *Pituophis*, *Arizona*, *Cemophora*, *Rhinocheilus* and *Lampropeltis*, excluding the four species in the genera *Senticolis*, *Bogertophis* and *Pseudelaphe* (Fig. 6, red circle; Fig. S4). The dynamic of speciation rate for this NW subclade (Fig. 6; Fig. S4) also shows a diversity-dependent slow down pattern (Fig. S3). This implies sequential rapid radiation events happened for the ratsnake group at the early stage of origin in OW region and later in the NW after intercontinental dispersal, respectively. Diversity dependence sug-
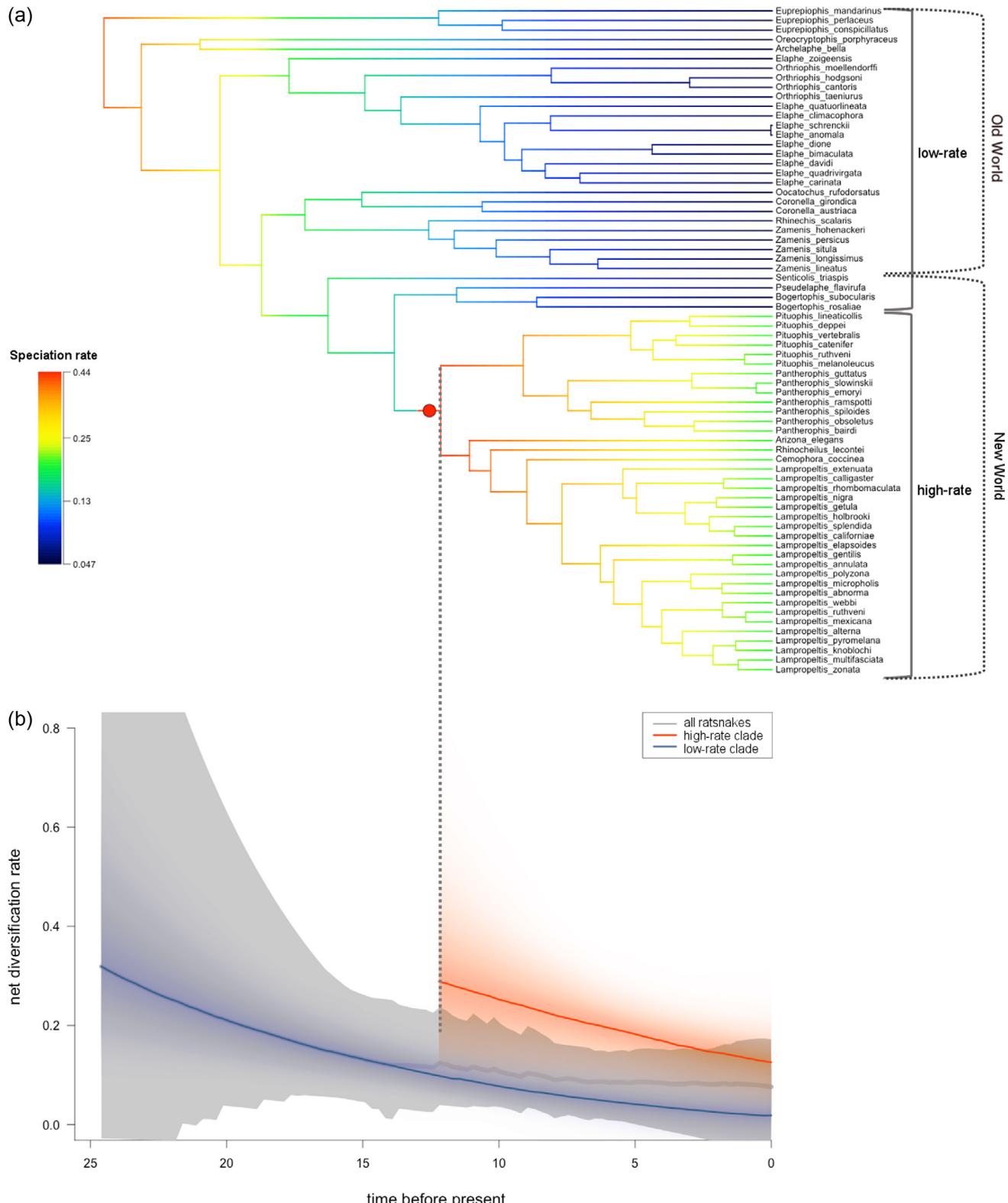
**Fig. 6.** (a) The best-fit speciation rate shift configuration for Coronellini. The node of rate shift is highlighted with red circle. (b) The dynamics of net diversification rate for the complete phylogeny (gray), the clade with diversification rate elevation (red) and the remainder of clades excluding the rate-shifting group (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

gests that species even occupying different continents diversify rapidly to fill unoccupied niches and slow as ecological opportunity diminishes and competition increases (Rabosky and Lovette, 2008a; Phillimore and Price, 2008; Rabosky, 2009; Pyron and Burbrink, 2013). Interestingly, our results suggest that, even with different rates of diversification across subclades of ratsnakes occurring on different continents and habitats, diversity dependence is still likely. This indicates that these diversity trajectories
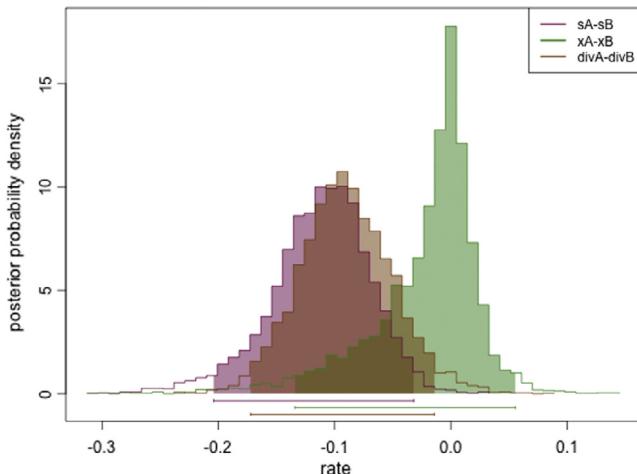
**Fig. 7.** Posterior probability distribution of speciation rate difference (sA-sB), extinction rate difference (xA-xB) and net diversification rate (speciation rate – extinction rate) difference (divA-divB) between Old World (A) and New World (B) areas using GeoSSE.

for ratsnakes are likely deterministic regardless of area or initial rates of speciation.

While a diversity dependence relationship has been found among different groups of organisms (Phillimore and Price, 2008; Burbrink et al., 2012; Kennedy et al., 2012), the existence of processes accounting for reduced rates of speciation when niches become saturated has been debated (Rabosky and Hurlbert, 2015; Harmon and Harrison, 2015). However, these models may be subject to error, particularly in cases with the ratsnakes where they fail to provide a realistic and consistent extinction rate (Table 1). Alternatively, diversification rates may also appear to slow down but are artifacts of methodological problems associated with insufficient taxon sampling (Pybus and Harvey, 2000), failure to sample cryptic species (Weir, 2006), failure to account for protracted speciation processes (Rosindell et al., 2010), large phylogenies (Pennell et al., 2012), underparameterized substitutional models (Revell et al., 2005), and general problems estimating timing of divergence (Burbrink et al., 2011; see Morlon and Moen, 2014).

Slow downs in diversification may have several geographic and environmental causes not linked to saturated ecological space. For instance, the size of the geographic area required for speciation could limit these rates as diversity increases and size of ranges decreases and extinction increases (Rosenzweig, 1995; Pigot et al., 2010). Rates of speciation could be elevated due to rapid changes in habitat at particular times and then reduced as climate ameliorates (Vrba, 1985), such as during the drastic environmental changes during the Miocene and Pliocene in North America. Diversification may also be time dependent and slow as species fail to keep pace with environment change (Quental and Marshall, 2013; Pyron and Burbrink, 2012b). Suggestions on how to tease apart causes for slow downs in diversification have been made (Morlon and Moen, 2014), which often require data beyond just phylogenetic information (e.g., paleontological, morphological); to our knowledge these have not been applied in the context of a unified model. Future research on slow downs in diversification on ratsnakes regionally should therefore attempt to model these alternative explanations to adaptive radiation.

In terms of the CBDH, to test if dispersal to the NW triggered exceptional diversification given an abundance of novel open niches, previous studies detected diversity-dependent diversification showing a rapid initial speciation rate for the NW ratsnakes, watersnakes, viperids and *Plestiodon* skinks (Burbrink and Pyron,

2010; Burbrink et al., 2012). However, these studies did not specifically determine if diversification rates were substantially different from the OW groups. Here, by fitting the models using program GeoSSE, we detect that diversification rate increases in the NW group compared to the OW group (Figs. 7 and S5). This uptick in diversification in the NW for ratsnakes is consistent with fossil record, where many genera of ratsnakes and other colubrines appeared suddenly in the mid-Miocene in North America (Holman, 2000). The majority of species of NW ratsnakes occur in the Nearctic; during the Miocene this region was characterized by several features that may have enhanced the diversification of these snakes, such as the rapid change in climate, the rise of grasslands, the reworking of forests, and noticeable increases in tectonic activity in the western US and Mexico (Zachos et al., 2001; Dickinson, 2006; Spencer et al., 2008).

We also highlight several issues regarding the use of these diversification models. First, the programs BAMM and MEDUSA have identified a point near the root of NW group where speciation is significantly elevated, but not at the root of NW group; GeoSSE's parameterization will limit our ability to detect rate changes at a fine geographic scale within each region (Rabosky and Goldberg, 2015). A constant speciation rate assumed by the GeoSSE model could also be an oversimplified model for ratsnakes since the plot of speciation rates of OW and NW groups from BAMM demonstrates non-linear changes over time (Fig. S6).

Second, considering the intercontinental distribution covering various environments of ratsnakes, the dynamics of diversification in this group could be heterogeneous over time and across multiple linages. In this study, the lineage-specific diversification models in the programs BAMM and MEDUSA did identify significant speciation rate changes within the NW clade, which the time-dependent models (Morlon et al., 2010; Stadler, 2011), including diversity-dependent models, would not be able to detect. However, considering the recent criticisms about statistical methods for the lineage-specific diversification models (May and Moore, 2016; Moore et al., 2016), which have potentially high false-positive rates, we believe it is more appropriate to not completely ignore the output from time-dependent models.

Third, despite a general slow-down in speciation rates supported by time-dependent and lineage-specific models, the estimation of extinction rates from different models are inconsistent. Multiple models from Morlon et al. (2010) support positive extinction rates either as a constant value or changing through time (Table 1). But models from Stadler (2011) barely support a positive extinction rate (Table 1), while lineage-specific models in BAMM show a nearly constant extinction rate (Fig. S3). Estimating a realistic extinction rate has been a huge challenge based on molecular phylogenies and development in this direction will greatly improve the robustness of diversification models in the future.

Additionally, while often not recognized in most diversification studies, it is possible that uneven taxonomic investigation among clades has a detrimental effect on estimating diversification processes. For instance, this rapid increase in diversification rate in the NW may be biased by the inclusion of the genus *Lampropeltis* (Fig. 6). In recent years, intensive phylogeography studies have determined that this genus is much more diverse than formerly considered (Burbrink et al., 2011; Myers et al., 2013; Ruane et al., 2014). These types of phylogeography studies are generally not paralleled in OW taxa, though some examples exist (Chen et al., 2014a, 2014b). For example, after removing species in genus *Lampropeltis*, GeoSSE could not distinguish the models with and without diversification rate changes between NW and OW groups ($p = 0.25$). Therefore, future studies on ratsnake diversification should also include detailed phylogeographic examination of a majority of species.

## 4.3. Taxonomy of the ratsnakes

We developed a pipeline to estimate specie trees of ratsnakes using genome-wide sampled markers (304 loci) to assesses the quality of gene tree inferences under a coalescent-model based framework to produce a well-resolved and robust phylogeny. The species trees inferred here confirm that the genera *Gonyosoma* and *Coelognathus* should not be included within the most inclusive group of traditional ratsnakes. Therefore, the ratsnakes should be defined as the most recent common ancestor that includes the genera *Archelaphe, Coronella, Elaphe, Euprepiophis, Oocatochus, Oreocryptophis, Orthriophis, Rhinechis, Zamenis, Arizona, Bogertophis, Cemophora, Lampropeltis, Pantherophis, Pituophis, Pseudelaphe, Rhinocheilus,* and *Senticolis.* This monophyletic group is sister to a group containing the Asian racers (genus *Ptyas*) and mainly the NW colubrines (*Coluber, Drymobius, Gyalopion* and *Tantilla,* Fig. 4).

We also found several changes in the phylogenetic positions with this ratsnake group relative to the last taxonomic revision from Utiger et al. (2002, 2005; Fig. S1). The genus *Euprepiophis* is subtended by the basal node separating them from the remainder of the ratsnake lineages. The genera *Coronella, Oocatochus, Rhiechis* and *Zamenis,* including most of the West Palearctic lineages, form a monophyletic group, which is sister to the NW lineages. To name this historically important group of inclusive ratsnakes and distinguish them from the rest of the taxa in Colubrinae, the tribe Coronellini Jan, 1863 was proposed by Utiger et al. (2005) to represent both OW and NW ratsnakes. This would then indicate that the rank of the existing tribe Lampropeltini Dowling, 1975 should be demoted to the subtribe Lampropeltina Dowling, 1975, which includes the monophyletic NW ratsnakes, composed of the nine NW genera *Arizona, Bogertophis, Cemophora, Lampropeltis, Pantherophis, Pituophis, Pseudelaphe, Rhinocheilus, Senticolis.* However, this would then require the other four OW monophyletic groups to be named as subtribes (1) Coronellina Jan, 1863 including the genera *Coronella, Oocatochus, Rhinechis* and *Zamenis;* (2) "Elapheina", including *Elaphe* and *Orthriophis;* (3) "Oreocryptophina" including *Archelaphe* and *Oreocryptophis,* and (4) "Euprepiophina" including *Euprepiophis* (Fig. 4). The name of each subtribe was composed of modifying the oldest genus name in each subtribe. Unfortunately, officially naming these groups may be premature given that two of the proposed clades, Eupreophina and Oreocryptophina, generally have low support (Fig. 4). Therefore, the possibility remains that they could be sister clades to the other remaining named and well-supported clades and thus could be subsumed as a more inclusive previously named group.

Additionally, *Orthriophis* renders *Elaphe* paraphyletic, where *Elaphe zoigeneesis* is sister to all the other *Elaphe* and *Orthriophis,* and *Orthriophis taeniurus* is sister to the rest of *Elaphe* species (Figs. 4, S1, 5). Thus, to maintain monophyly, we suggest merging genus *Orthriophis* into the genus *Elaphe,* based on the priority rule, that the genus *Elaphe* Fitzinger, 1833 has precedence over *Orthriophis* Utiger, Helfenberger, Schätti, Schmidt, Ruf and Ziswiler, 2002.

This study provides a robust framework for understanding and resolving the evolutionary history of ratsnakes. Given the number of newly described ratsnakes in the New World (Burbrink et al., 2011; Myers et al., 2013; Ruane et al., 2014), rediscovery of taxa in the OW (Chen et al., 2013), it is likely that the diversity of this group is much greater than currently recognized. These newly described taxa should easily fit within the taxonomic framework presented here.

## Acknowledgments

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2017.03.017.

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