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Diversification in vipers: Phylogenetic relationships, time of divergence and shifts in speciation rates

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ABSTRACT

Snakes of the cosmopolitan family Viperidae comprise around 329 venomous species showing a striking heterogeneity in species richness among lineages. While the subfamily Azemiopinae comprises only two species, 70% of all viper species are arranged in the subfamily Crotalinae or the "pit vipers". The radiation of the pit vipers was marked by the evolution of the heat-sensing pits, which has been suggested to be a key innovation for the successful diversification of the group. Additionally, only crotalines were able to successfully colonize the New World. Here, we present the most complete molecular phylogeny for the family to date that comprises sequences from nuclear and mitochondrial genes representing 79% of all living vipers. We also investigated the time of divergence between lineages, using six fossils to calibrate the tree, and explored the hypothesis that crotalines have undergone an explosive radiation. Our phylogenetic analyses retrieved high support values for the monophyly of the family Viperidae, subfamilies Viperinae and Crotalinae, and 22 out of 27 genera, as well as well-supported intergeneric relationships throughout the family. We were able to recover a strongly supported sister clade to the New World pit vipers that comprises *Gloydius*, *Ovophis*, *Protobothrops* and *Trimeresurus gracilis*. Our results agree in many aspects with other studies focusing on the phylogenetics of vipers, but we recover new relationships as well. Despite the addition of new sequences we were not able to resolve some of the poor supported relationships previously suggested. Time of divergence estimates suggested that vipers started to radiate around the late Paleocene to middle Eocene with subfamilies most likely dating back to the Eocene. The invasion of the New World might have taken place sometime close to the Oligocene/Miocene boundary. Diversification analyses suggested a shift in speciation rates during the radiation of a sub-clade of pit vipers where speciation rates rapidly increased but slowed down toward the present. Thus, the evolution of the loreal pits alone does not seem to explain their explosive speciation rates. We suggest that climatic and geological changes in Asia and the invasion of the New World may have also contributed to the speciation shift found in vipers.

1. Introduction

Vipers form a monophyletic lineage of venomous snakes comprising about 329 species distributed worldwide. Because vipers are considered a medically important group, different aspects of their biology have been widely studied (e.g. Fenwick et al., 2012; Greene, 1997; Martins et al., 2001) but their macroevolutionary dynamics and some aspects of phylogenetic relationships are still poorly understood. Species are currently arranged in 35 genera belonging to three subfamilies: Viperinae, Azemiopinae, and Crotalinae (Uetz and Hosek, 2014). Viperines, or the "true vipers", comprise 98 species whereas Azemiopinae comprises only two and both subfamilies are restricted to the Old World (Phelps, 2010; Uetz and Hosek, 2014). Crotalinae, or the "pit vipers", is the most diverse and widely distributed lineage of vipers, comprising about 229 species (Campbell and Lamar, 2004; Uetz and Hosek, 2014) occurring both in the Old and New World.

In the past years, the access to new Viperidae DNA sequences has greatly improved the limited phylogenetic inferences done solely based on morphological data (see Castoe and Parkinson, 2006) but the few studies (Fenwick et al., 2012; Pyron et al.,

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2013; Wüster et al., 2008) that have used molecular data investigated the phylogenetic relationships of vipers in a broader phylogenetic context. The pioneering study by Wüster et al. (2008) included all except two Viperidae genera in their molecular analysis but was limited to 87 species and therefore explored the phylogenetic relationships among higher taxa. Although recent works by Fenwick et al. (2012) and Pyron et al. (2013) included 220 and 210 Viperidae terminals respectively, the phylogenetic relationships of more inclusive lineages and the tempo of diversification underlying the divergence among those lineages are still unclear and debatable.

The choice of proper fossils and/or biogeographic events (Benton et al., 2009; Ho and Phillips, 2009; Sauquet et al., 2012; see also the supplementary material) is of central importance in dating analyses because it is not possible to estimate absolute ages from molecular data alone (Ho and Phillips, 2009). Wüster et al. (2008) used four fossils and two biogeographic events to calibrate their genus-level tree and Fenwick et al. (2012) two fossils and one biogeographic event. These authors found different diversification times for some lineages, which could be related to differences in sampling effort or choice of calibration points (Parham et al., 2012; Sauquet et al., 2012). A survey of the snake fossil record suggests it is possible to use additional fossils for conducting dating analysis of vipers (see Section 2 and supplementary material) avoiding biogeographic events, which have been suggested to be problematic in dating studies (Sauquet et al., 2012). A calibration setting comprising only fossils and a wider inclusion of current species should therefore greatly improve our understanding of the tempo of viperid diversification and also allow the first proper investigation of the diversification dynamics that gave rise to the current diversity of the family.

Vipers show a striking heterogeneity in diversity among different lineages, and a number of hypothesis have been proposed to explain the differential species richness of particular clades (e.g. Greene, 2002; Hendry et al., 2014; Lynch, 2009). The early radiation of these snakes is associated with the evolution of a highly derived venom system, which may have allowed the invasion of new niches (Greene, 1997; Pyron and Burbrink, 2012). Furthermore, the evolutionary history of the subfamily Crotalinae is marked by the evolution of a pair of heat-sensing pits on each side of their heads between the eye and the nostril (“loreal pits”) (Goris, 2011; Roelke and Childress, 2007), which have been suggested to represent a key innovation facilitating the radiation of the clade (Rosenzweig et al., 1987; Rosenzweig and McCord, 1991) even though not directly tested. Crotalines also invaded the New World, an event frequently associated with explosive radiation (Burbrink et al., 2012a; Wüster et al., 2002, 2008).

Explosive radiations or “early bursts” have been frequently reported in molecular phylogenies (e.g. Harmon et al., 2003; Morlon et al., 2012) and are usually characterized by very high diversification rates during the early radiation of a lineage followed by a decrease toward the present. The emergence of a key innovation (e.g. Glor, 2010; Losos and Mahler, 2010) and/or the invasion of new areas might allow a lineage to explore previously unavailable niches (Burbrink et al., 2012a,b) and, in theory, could be associated with explosive radiations. Although the predominant explanation regarding diversification slowdowns rely upon speciation mediated by niche differentiation and the subsequent decrease in ecological opportunities (e.g. Burbrink et al., 2012a; Rabosky and Lovette, 2008), recent studies have suggested alternative processes that can also underlie diversification rates slowdowns (see Moen and Morlon, 2014).

In this paper we assembled the most complete time-calibrated molecular dataset of Viperidae to investigate the phylogenetic relationships and diversification dynamics of vipers. We also explored the hypothesis that crotalines, comprising the most diverse subfamily, have undergone an explosive radiation (Rosenzweig et al., 1987; Rosenzweig and McCord, 1991) by investigating if diversification rates significantly increased during the diversification of the Crotalinae.

2. Material and methods

2.1. Taxon sampling and data acquisition

The Reptile Database (Uetz and Hosek, 2014) currently recognizes 329 species in the family Viperidae. From those, we included 260 species as terminal taxa in the ingroup of our phylogenetic tree. Although some researchers considered *B. colombiensis* and *B. isabelae* as synonyms of *B. atrox* (e.g., Campbell and Lamar, 2004; Rivas et al., 2012) others considered both as valid species (e.g. Salomão et al., 1999; Fenwick et al., 2009; Pyron et al., 2013). We chose to include *Bothrops colombiensis* and *Bothrops isabelae* as distinct species in the present study. Castoe et al. (2007) suggest that *C. tortugensis*, an endemic rattlesnake of Tortuga Island, is nested within *C. atrox*, representing a junior synonym of the latter. Murphy et al. (2002) retrieved *C. tortugensis* within *C. atrox* but they considered paraphyly as acceptable in cases of peripheral isolation. In the present study we followed taxonomic arrangement that considers some of the lineages endemic to islands that recently diverged from their sister taxa as valid species, and thus included *C. tortugensis* as distinct species in our analyses (Grazziotin et al., 2006; Grismer, 1999). It is important to highlight to the reader that diversification studies are always prone to be affected by the taxonomic arrangements they follow, but we are confident that only major taxonomic changes in the taxonomy of vipers could affect our results. Thus, our sampling for Viperidae encompasses 263 taxa corresponding to 79% of the diversity presently described for the family. This taxon sampling comprises all the three known subfamilies with the following sampling schemes (sampled species/number of described species): Azemiopinae (1/2), Viperinae (71/98), and Crotalinae (191/232). Additionally, we included as outgroup 97 species from different families: Boidae, Elapidae, Colubridae, Dipsadidae, Homalopsidae, Natricidae, Atractaspididae, Lamprophiidae, Psamophiidae, and Xenodermatidae. The species *Indotyphlops braminus* (Scolecophidia: Typhlopidae) was used to root our phylogenetic tree (Pyron et al., 2013). Details on our sampling strategy and on the curatorial work performed including a list with several issues faced when using sequences from public databases are available in our supplementary material.

Our molecular matrix is composed of sequences from 11 genes, six mitochondrial (12S, 16S, cytb, cox1, nd2, nd4) and five nuclear (bndf, c-mos, jun, nt3, rag1). We used sequences for species of Viperidae available in GenBank up to April 2014 (Table S1). We also included sequences available in the Barcode of Life Database (BOLDSYSTEMS, http://www.boldsystems.org/) to complement information for the Cox1 gene (Table S1). We provided new DNA sequences for 27 viper species (Table S1) for eight genes, including a species not previously included in GenBank (*Causus lichtensteini*), totaling 167 new sequences. All new sequences were obtained following standard PCR and sequencing protocols as described in Grazziotin et al. (2012). Both strands of the PCR products were sequenced, and the trimming and assembling procedures were performed using the default parameters in the program GENEIOUS v.5 (Biomatters, available at http://www.geneious.com).

Our dataset represents a significant improvement in sampling compared to recently published viper phylogenies. We include 43 more species than Fenwick et al. (2012) and 53 more than Pyron et al. (2013). Moreover, our molecular dataset comprises 11 genes and 1186 sequences whereas Fenwick et al. (2012) and Pyron et al. (2013) comprise 784 and 817 sequences for 4 and 11 genes respectively. Therefore, the study presented herein

comprises the most complete phylogenetic analysis for the family to the present.

2.2. Phylogenetic analyses and divergence time estimation

Sequences were aligned with MAFFT (Katoh et al., 2002) using the E-INS-i algorithm for 12S, 16S, nd4, nd2, cox1 and rag1, and FFT-NS-i for bndf, cmos, cytb, jun and nt3. For both algorithms all the parameters were set as default. Resulting alignments were visually inspected in Mesquite v 2.75 (Maddison and Maddison, 2009). Sequences were concatenated with SequenceMatrix v 1.7.8 (Vaidya et al., 2011) in two different ways: (1) all sequences for all the 11 genes (361 terminals), and (2) all sequences from 16S, 12S, cytb and nd4 genes (genes with sequences representing the majority of the species included in the phylogenetic analysis, 357 terminals). Our main dataset (dataset 1) contained alignments with 10,712 base pairs (bp) and dataset 2 contained 4,419 bp. The proportion of viper species represented within each individual gene matrix varies widely (79% of the 263 species had 12S sequences, 83% had 16S, 92% had cytb, 87% had nd4, 19% had nd2, 22% had cox1, 21% had cmos, 17% had rag1, 13% had nt3, 10% had bndf, 8% had jun) and highlights the heterogeneous completeness of our molecular matrices.

Although recent studies found no evidence that missing data would lead to inaccurate estimates in Bayesian phylogenetic analyses and time divergence estimates (Filipski et al., 2014; Wiens and Morrill, 2011; but see Lemmon et al., 2009), we used dataset 2 to access any potential influence of missing data in our phylogenetic estimates by comparing its results to our main dataset 1. The comparison between datasets also allowed us to evaluate any improvements in the phylogenetic relationships of vipers when adding nuclear genes information (main dataset) given that dataset 2 comprises only mitochondrial genes. We used PartitionFinder 1.0.1 (Lanfear et al., 2012) with the greedy algorithm and linked branch lengths to select the best partition scheme and the best models of nucleotide substitution for our molecular matrix partitioned by gene and codon position for each dataset above. The Bayesian Information Criterion (BIC) was used as the optimality criterion to select the best partition schemes and best models of nucleotide substitution for each dataset (Table S2).

We estimated the phylogenetic relationships and time of divergence between species using a Bayesian framework implemented in the program BEAST v1.8.1 (Drummond and Rambaut, 2007) for each dataset. Substitution rates were estimated under a relaxed uncorrelated lognormal clock (Drummond et al., 2006) that allows different branches to have independent rates. Extinction has played a major role in shaping diversification patterns in the tree of life (Raup, 1986; Quental and Marshall, 2010), so using a Yule model, which assumes extinction rates equal to zero, as our tree prior would be inappropriate. Thus, we used a Birth-Death speciation model accommodating incomplete taxon sampling as our tree prior (Stadler, 2009).

Calibrating a phylogenetic tree is a critical step in molecular dating analyses and caution is required in choosing the points to be used (Sanders et al., 2010; Sauquet et al., 2012). To time calibrate our phylogenetic tree, we followed recent published guidelines and protocols that aim to help researchers in choosing fossils to be used as calibration points (Benton et al., 2009; Parham et al., 2012; Sauquet et al., 2012; see supplementary material). We chose to include in our analyses six fossil records from the literature, two of them described as Viperidae and the other four positioned throughout the outgroup (Table 1). Details and justifications on the choice of calibration schemes are available in the supplementary material.

For each concatenated dataset we ran four replicates of our phylogenetic analyses with different starting trees or random seeds saving the parameters and trees every 1000 interactions. In total, we ran eight phylogenetic analyses. Chain length was set to 300 million generations but we stopped the analyses as soon as they reached convergence. For each concatenated dataset we kept the replicate(s) that achieved the higher likelihood values. We used the program Tracer v 1.6 (Rambaut and Drummond, 2007) to check for convergence, the effective sample size (ESS) of parameters and to verify burn-in. When appropriate we used logCombiner 1.8 to combine posteriors of replicates of the same concatenated dataset that converged to the same higher likelihood values. We used TreeAnnotator v 1.8.1 (Drummond and Rambaut, 2007) to generate maximum credibility trees.

2.3. Patterns of species diversification

To perform diversification analyses while incorporating phylogenetic uncertainty, we randomly sampled 100 trees from the posterior distribution regarding dataset 1 (our main dataset). We performed all diversification analyses on those 100 trees after removing the outgroup. To visually inspect the pattern of diversification across time we generated lineage through time plots (LTT, i.e. the cumulative number of lineages through time, Nee et al., 1992) for the 100 posterior trees. To test if there were temporal changes in diversification rates we first used the gamma statistic while controlling for the statistic bias imposed by species undersampling (Pybus and Harvey, 2000). For that we simulated 1000 phylogenetic trees with the known number of viper species and then removed species in order to represent our sampling scheme. All those analysis were done both for Viperidae as a whole (329 know species plus three subspecies considered here as full species and 263 terminals in our sampling scheme) as well as for Viperinae (98 known species and 71 terminals in our sampling scheme) and Crotalinae (229 known species plus three subspecies considered here as full species and 191 terminals in our sampling scheme). These analyses were performed using the packages Ape (Paradis et al., 2004) and TreeSim (Hartmann et al., 2010; Stadler, 2014) implemented in R (R Development Core Team, 2013).

Given the limitations of the gamma statistic (Quental and Marshall, 2010, 2011) we also used BAMM (Bayesian Analysis of Macroevolution Mixtures, Rabosky, 2014; Rabosky et al., 2013, 2014a), which estimates speciation and extinction rates throughout the different branches in a phylogenetic tree. BAMM is based on the premise that phylogenetic trees are often shaped by heterogeneous mixtures of distinct processes (see Fig. 1 in Rabosky, 2014). This

Table 1
Fossils used as calibration points in time divergence estimates of vipers.

Node	Fossil	Geological age	Source
Stem-Alethinophidia	*Haasiophis terrasanctus*	Cenomanian, Cretaceous	Tchernov et al. (2000)
Stem-Colubroidea	*Procerophis sahnii*	Ypresian, Eocene	Rage et al. (2008)
Stem-Boinae	*Titanoboa cerrejonensis*	Middle-late Paleocene	Head et al. (2009)
Stem-Elapidae	Elapidae gen. & sp. indet.	Aquitanian, Miocene	Kuch et al. (2006)
Stem-Viperidae	Viperidae. gen. & sp. indet.	Aquitanian, Miocene	Kuch et al. (2006)
Stem-*Sistrurus*	*Sistrurus* sp. indet.	Clarendonian, Late Miocene	Parmley and Holman (2007)

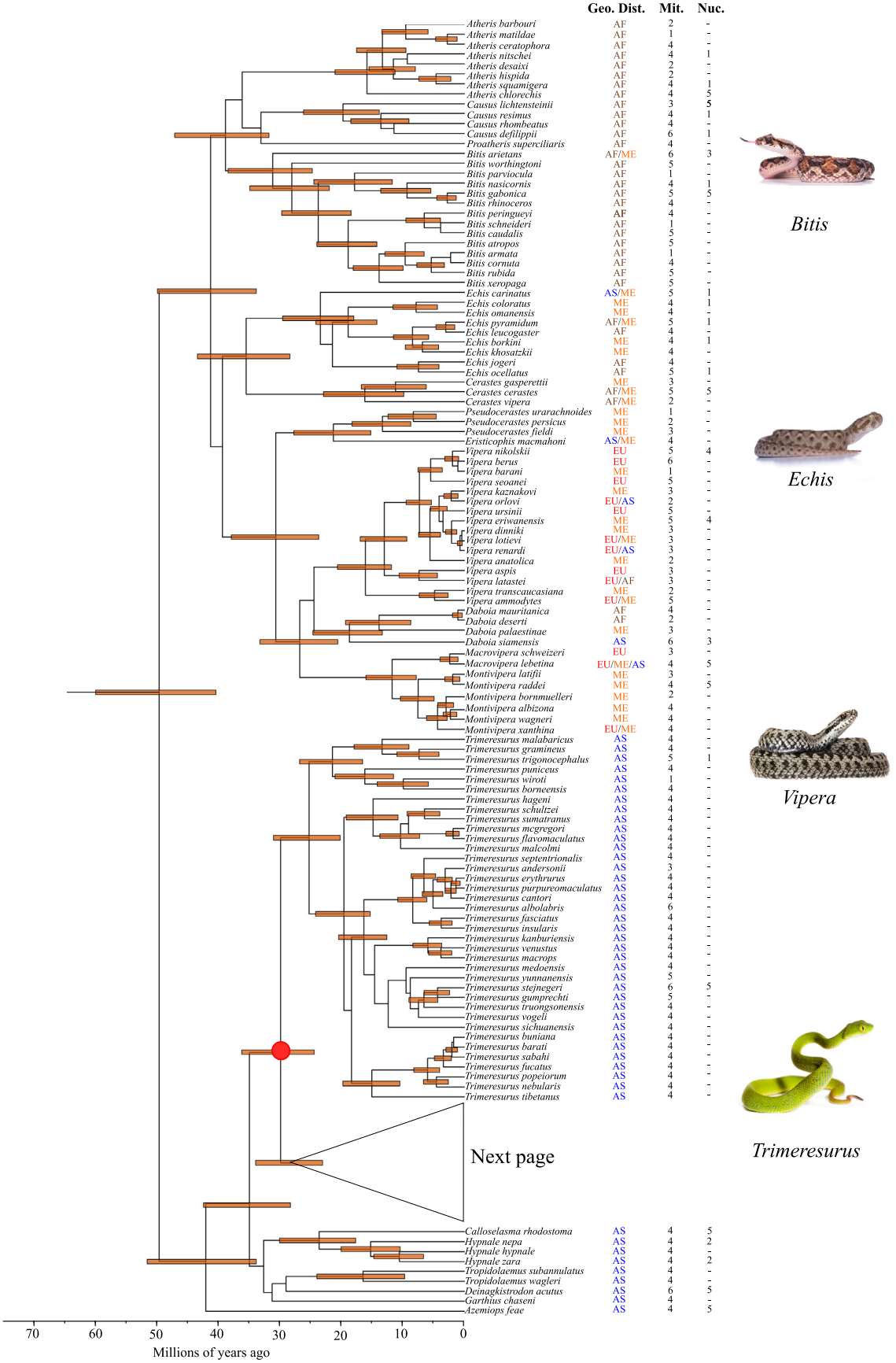

Fig. 1. Maximum credibility tree generated for the family Viperidae comprising all genes and species (dataset 1). Bars showing the 95% high posterior density interval of age estimates were added only for nodes with posterior probability values equal to or higher than 0.95. Geographic distribution and the number of mitochondrial and nuclear gene sequences for each taxon are also shown. AF = Africa, ME = Middle East, AS = Asia, EU = Europe, NW = New World. Red circle indicates the position of the speciation rate shift. Images of vipers by Thor Hakonsen (http://thorhakonsen.com/). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

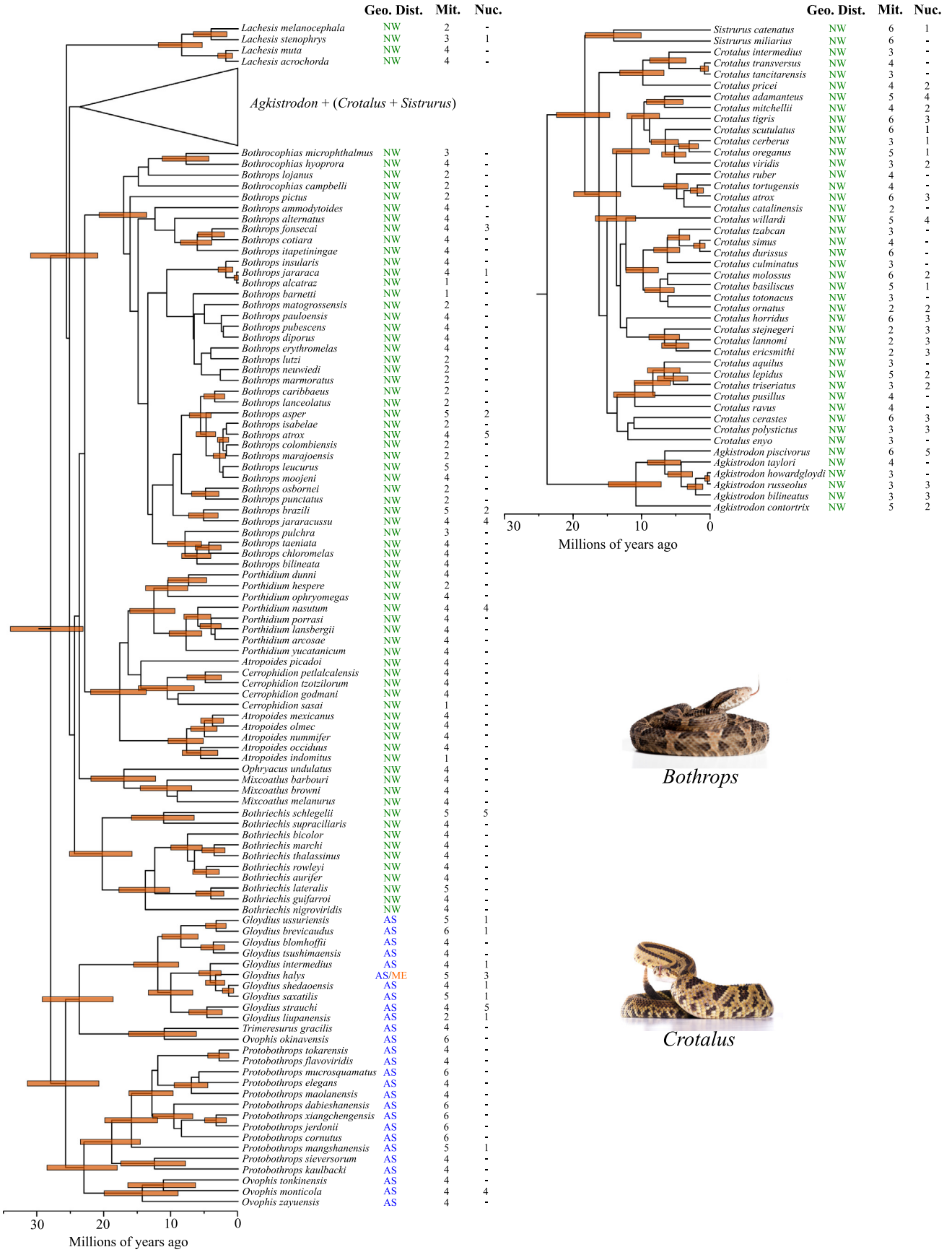

Fig. 1 (*continued*)

rate heterogeneity across lineages and time has been shown to be the case in numerous phylogenetic trees (e.g. Etienne et al., 2012) and failure to accommodate such variation can cause serious bias in results and interpretations (Rabosky, 2010, 2012). Additionally, estimating extinction rates from molecular phylogenies have been heavily criticized (Quental and Marshall, 2010; Rabosky, 2010) and most methods seem unable to estimate negative diversification rates (extinction higher than speciation rates) (e.g. Medusa, Alfaro et al., 2009). However, this problem is in theory relaxed in BAMM, which given its architecture is potentially able to detect clades in decline (see Rabosky, 2014). We note that for our data and proposed hypothesis BAMM might be specially interesting because it: 1 - accounts for incomplete taxon sampling; 2 - allows extinction rates to exceed speciation rates therefore allowing to characterize diversification dynamics where clades are in decline; and 3 - is designed to detect rate shifts across the tree.

In this study we used BAMM to explore if the extant diversity of vipers is the result of a single or multiple diversification regimes, and how speciation and extinction rates varied throughout their evolutionary history using the phylogenetic framework generated herein. With this approach we also explicitly explored the hypothesis that the subfamily Crotalinae has a different diversification dynamic characterizing an explosive radiation. We ran BAMM (version 2.1.0) on the 100 randomly sampled posterior trees for 25 million generations sampling every 10.000 generations. Given that incomplete sampling can bias analyses of diversification and that vipers are not randomly sampled in the phylogeny, we informed BAMM specific sampling fractions of clades included (Table S3). To evaluate how many distinct macroevolutionary regimes characterized the radiation of vipers, we first compiled the overall number of rate shifts found among all trees. We then generated for each tree the rate shift configuration most frequently sampled in BAMM posterior, taking into account the prior probability using a Bayes Factor criterion of 5. By doing this, we are analyzing only those rate shifts that are supported by the data and not by prior alone. Through the shift configuration it is possible to visualize when, during the radiation of vipers, diversification rates significantly changed. Finally, we analyzed how speciation and extinction rates varied through time in the whole family and in crotalines separately. BAMM output was analyzed using the R package BAMMtools (Rabosky, 2014; Rabosky et al., 2014b).

3. Results

We generated maximum credibility trees after discarding burn-in with a posterior probability limit of 95% (Figs. 1, S1 and S2). Both datasets yielded similar topologies and divergent times estimates for the family Viperidae (Figs. 1 and S2, Table S4). Thus, results will focus on our main dataset (dataset 1) comparing with dataset 2 when relevant for the discussion. We also note that the small differences in dating analyses are not significant given that estimates for both datasets show considerable overlap in their 95% high posterior density interval (Table S4, Figs. 1 and S2). The complete maximum credibility tree for dataset 1 including the outgroup and calibration points can be visualized through Fig. S1.

Phylogenetic analyses resulted in a relatively well-resolved tree with most nodes having high posterior probabilities ($pp > 0.95$, represented by nodes with bars of age estimates in Fig. 1) confirming the monophyly of the family and subfamilies with more than one species included in the analyses (Viperinae and Crotalinae). Phylogenetic relationships among subfamilies were also recovered with high support with Viperinae being sister to a clade formed by Azemiopinae and Crotalinae. Of the 27 genera for which we could test for monophyly, we recovered 22 as monophyletic with high posterior probabilities (see Section 4 for details). Among viperines, posterior probability showed high support for a clade comprising the genera *Atheris*, *Causus*, *Proatheris* and *Bitis*. Eurasian vipers (*Pseudocerastes*, *Eristicophis*, *Vipera*, *Macrovipera* and *Montivipera*) also formed a well-supported clade (Fig. 1). Among the remaining lineages of the subfamily Viperinae, *Echis* and *Cerastes* also showed a robust sister relationship (Fig. 1). Regarding the affinities in the subfamily Crotalinae, *Hypnale*, *Garthius*, *Deinagkistrodon*, *Calloselasma* and *Tropidolaemus* formed a weak supported clade sister to the remaining crotalines with *Hypnale* and *Calloselasma* forming a well-supported clade (Fig. 1). We recovered with great confidence a clade comprising the genera *Protobothrops*, *Gloydius*, *Ovophis*, and *T. gracilis* as the sister clade to the New World pit vipers, with almost all nodes showing strongly supported relationships (Fig. 1). Among the New World (NW) pit vipers, we recovered high posterior probabilities for clades comprising the Middle American genera *Atropoides*, *Cerrophidion*, and *Porthidium*, another comprising the Bothropoides (*Bothrops* and *Bothrocophias*), as well as high supported clades formed by *Sistrurus* + *Crotalus*, and *Ophryacus* + *Mixcoatlus* (Fig. 1). However, more inclusive relationships and some higher-level relationships were recovered with low support among New World pit vipers (see Fig. 1).

Divergence time estimates suggest that the ancestral of vipers diverged from its sister group in the Paleocene (around 64.5 Mya) and that vipers started to diversify around the late Paleocene to middle Eocene (59.9–40.4 Mya) (high posterior density interval of the main dataset). During the Eocene (around 49.91–33.86 Mya) and in the middle Eocene to late Oligocene (around 42.45–28.31 Mya), subfamilies Viperinae and Crotalinae started to diversify, respectively. New World pit vipers started to radiate around the middle/late Oligocene to the early Miocene (31.05–20.99 Mya). Information regarding divergence time estimates of vipers is summarized in Fig. 1 and Table S4.

Lineage through time plots show no clear slowdown in lineage accumulation except very close to the present both in Viperidae and in the subfamily Crotalinae respectively (Fig. S3). The negative gamma statistics estimated for the whole family and for crotalines alone suggest a slowdown in lineage diversification, looking even more evident among crotalines (Fig. S3). However, the LTT plot for the subfamily Viperinae suggests that lineage accumulation decelerated right after their initial diversification but seems to have remained constant toward the present (Fig. S3). The gamma statistics test after correcting for incompleteness did not show any evidence for slowdowns among viperines (Fig. S3).

When looking for dynamic heterogeneity, our results indicate that models with one or two shifts in macroevolutionary rate regimes were chosen more frequently during MCMC analyses (Fig. S4). However, after eliminating poorly supported shifts (i.e. those likely to be attributed to the prior alone), the most frequently sampled shift configuration estimated in BAMM for 87 trees (out of 100) comprised only one shift (Fig. 2). Twelve showed no shifts as the most frequent shift configuration, and one tree showed two shifts (Fig. S5A and B). Configurations with one shift (Fig. 2) suggest an increase in speciation rates after the split of the most basal crotaline clade, thus comprising part of the Old World (OW) crotalines and the whole NW crotaline clade, with decay in speciation rates toward the present. The configuration with two shifts includes the shift described above plus a second shift representing an increase in speciation rates inside the genus *Vipera*.

In summary, the great majority of BAMM runs suggest that a distinct macroevolutionary regime characterize not the whole subfamily Crotalinae but a sub-group of crotaline lineages. Although there is uncertainty on the absolute values and in the general trend in speciation rates for the background regime (that is, the lineages not part of the clade covered by the rate shift) (Fig. 2), background lineages comprise a different speciation regime compared to the one inferred after the shift. The uncertainty here is due to two optimum parameter combinations, one with initial high speciation

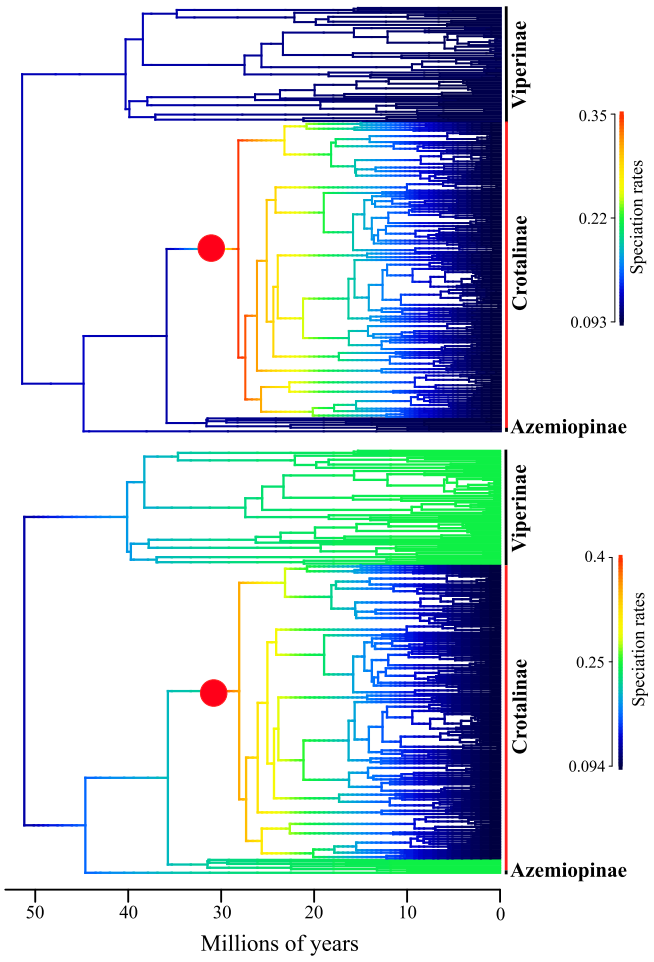

Fig. 2. Best shift configuration sampled by BAMM for 87 phylogenetic trees. For each tree, speciation rates are calculated as follow: in the upper figure instantaneous rates for each posterior sample are calculated along each branch, and these instantaneous rates are then averaged. In the bottom figure BAMM posterior samples corresponding to this best shift configuration are pooled and the mean is taken for each parameter and rates are estimated. Red circle indicates the place where rate shift might have occurred. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rates associated with a decay on it and another with small initial values of speciation and an increase on it (Fig. S6). This suggests an increase or a decrease in speciation rates and even constant speciation rates during the background diversification.

Rate through time plots show that initially speciation rates slightly decreases but after a given amount of time (Fig. 3A) experience a considerable increase. After this burst, speciation rates start to decrease. On the other hand, extinction rates seem to remain roughly constant for most of the time (Fig. 3B). Speciation through time plots generated for the Crotalinae subfamily and the remaining vipers separately (Fig. 3C) showed that at the early radiation of crotalines speciation rates do not differ between the two groups. However, speciation rates among crotalines reach much higher values after this initial radiation. Extinction rates do not show any significant variation along time when plotted for crotalines and remaining vipers separately (Fig. 3D).

4. Discussion

4.1. Phylogenetic inference

Phylogenetic relationships among vipers recovered in the present study agree in many aspects with those from previously published works (e.g. Castoe and Parkinson, 2006; Parkinson, 1999; Parkinson et al., 2002; Pyron et al., 2013; Wüster et al., 2008) but we also found some distinct patterns (see below). Although all subfamilies and the majority of genera were considered monophyletic, we did not recover *Bothrops* and *Bothrocophias*, *Atropoides*, *Trimeresurus* and *Ovophis* as monophyletic as suggested by other studies (e.g. Castoe et al., 2003; Castoe and Parkinson, 2006; Carrasco et al., 2012; Malhotra and Thorpe, 2000, 2004; Malhotra et al., 2010).

The Andean *Bothrops lojanus* has been considered as *incertae sedis* and although morphological analyses recovered the species within *Bothrops* (Carrasco et al., 2012), in the present study *B. lojanus* appears within the genus *Bothrocophias* with weak support. The distribution of *B. lojanus* in terrestrial forested habitats in Ecuador and the occurrence of some *Bothrocophias* species in this same region and habitat (Campbell and Lamar, 2004; Harvey et al., 2005) suggest this might be a possible relationship. However, the few gene sequences available for *B. lojanus* (only two mitochondrial gene sequences) may be preventing us to find a reliable phylogenetic position for the species. Irrespective to the position of *B. lojanus*, the monophyly of Bothropoides (*Bothrops* + *Bothrocophias*) has been recovered with great confidence in both morphological and molecular analyses (Carrasco et al., 2012; Fenwick et al., 2009; Parkinson, 1999; Parkinson et al., 2002; Wüster et al., 2002; present study).

Another genus not recovered as monophyletic was *Atropoides*. The monophyly of *Atropoides* has been controversial because of the uncertainty in the phylogenetic position of *A. picadoi* (Castoe et al., 2003, 2005; Castoe and Parkinson, 2006; Castoe et al., 2009; Kraus et al., 1996; Jadin et al., 2010). While using morphological characters Jadin et al. (2010) found strong support for the monophyly of *Atropoides*, molecular studies failed to find strong support for the monophyletic configuration of the genus (Castoe et al., 2003, 2005; Castoe and Parkinson, 2006; Castoe et al., 2009; Kraus et al., 1996). In the present study, we could not recover the phylogenetic position of *A. picadoi* with confidence. Both *Atropoides picadoi* and the remaining species of the genus are represented by sequences of four mitochondrial genes (except for *A. indomitus* that had a sequence of nd4 gene only). That is, molecular data available for *Atropoides* species seem to be insufficient to elucidate the relationship between *A. picadoi* and the remaining *Atropoides*.

Like previous studies (e.g. Castoe and Parkinson, 2006; Malhotra and Thorpe, 2000, 2004; Malhotra et al., 2010), we found "*Ovophis*" *okinavensis* to be sister to "*Trimeresurus*" *gracilis*. Both species have been suggested to be more closely related to the genus *Gloydius* than to *Ovophis* or *Trimeresurus* (e.g. Castoe and Parkinson, 2006; Malhotra and Thorpe, 2004; Malhotra et al., 2010). Here, we not only found a monophyletic clade comprising "*Ovophis*" *okinavensis*, "*Trimeresurus*" *gracilis* and the genus *Gloydius* but we also recovered this relationship with a very high support value (>0.99) contrasting with the results from other studies (e.g. Castoe and Parkinson, 2006; Malhotra and Thorpe, 2004). Hence our results support previous ideas that those two species should be placed in a genus of its own or perhaps considered to belong to the genus *Gloydius* (Malhotra and Thorpe, 2004; Malhotra et al., 2010).

Regarding viperines, the most striking difference between phylogenetic studies relates to the position of the genus *Causus*. The particular phenotype of *Causus* suggests the genus is sister to the remaining viperines in morphological analyses (Herrmann et al., 1999). However, many molecular studies disagree from morphological ones and found different arrangements suggesting *Causus* to be either sister to *Proatheris* (present study, Fig. 1), *Cerastes* (Wüster et al., 2008; Fig. S2) or *Echis* (Pyron et al., 2013), all with weak support. Additionally, as previously suggested by Wüster

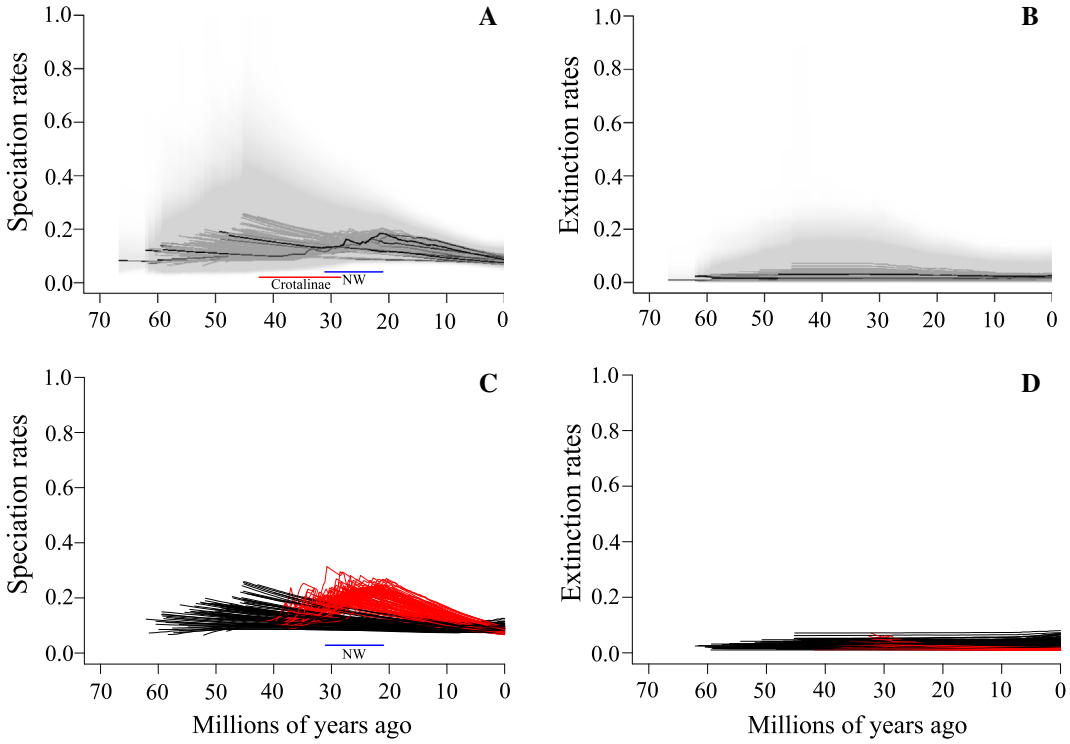

Fig. 3. (A) Speciation and (B) extinction rates through time estimated for 100 phylogenetic trees for all vipers. Shaded regions denote the 95% credible interval of the posterior distribution of rates at a given point in time. (C) Speciation and (D) extinction rates through time estimated for the 87 phylogenetic trees showing the one shift configuration as the most frequent diversification scenario (see Section 3 for details), with the subfamily Crotalinae (red lines) and the remaining vipers (black lines) plotted separately. We preferred to omit the 95% credible intervals in (C and D) for better visualization. The red and blue bars indicate the posterior density interval (dataset 1) of the ages of the Crotalinae subfamily and the NW crotalines respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al. (2008) and Pook et al. (2009), but differently from early viperine studies (e.g. Herrmann et al., 1999; Lenk et al., 2001), we recovered well-supported phylogenetic relationships between *Cerastes* and *Echis*.

Intergeneric relationships among the Crotalinae species poor genera *Tropidolaemus*, *Deinagkistrodon*, *Garthius*, *Hypnale* and *Calloselasma* remained unresolved in the present study. Although these genera form a monophyletic configuration in the present study agreeing with Malhotra et al. (2010) who also analyzed nuclear sequences, both studies recovered a weakly supported configuration. Paraphyletic arrangements were frequently recovered in studies using only mitochondrial genes (Castoe and Parkinson, 2006; Wüster et al., 2008 but see Pyron et al., 2013). Even though our dataset comprising only mitochondrial genes (dataset 2) also suggested a weakly supported monophyletic configuration (as in Malhotra and Thorpe, 2004), posterior probability values doubled when we included nuclear sequences. That is, including nuclear sequences for other species belonging to this crotaline clade result in higher support values and greatly improves estimate the relationship between these genera connected by long-branches. Irrespective of the true configuration of the clade, a strongly supported relationship between *Calloselasma* and *Hypnale* has been recovered here, in early studies (e.g. Kraus et al., 1996; Parkinson, 1999; Malhotra and Thorpe, 2004) and in more recent ones (Pyron et al., 2013).

Although Malhotra et al. (2010) recovered *Gloydius* as the sister clade of NW crotalines with moderate to high support (0.87–0.99), relationships between the other related lineages (*Protobothrops*, *Ovophis*, "*Trimeresurus*" *gracilis* + "*Ovophis*" *okinavensis*) remained unresolved (see also Castoe and Parkinson, 2006; Kraus et al., 1996; Malhotra and Thorpe, 2004; Pyron et al., 2013; Wüster et al., 2008). In the present study, we recovered with great confidence a monophyletic clade as the sister group to NW crotalines comprising not only the previously mentioned clade formed by *Gloydius* and *O. okinavensis* + *T. gracilis*, but also including the genera *Protobothrops* and *Ovophis*. Moreover, great majority of the nodes in the sister clade proposed for the NW crotalines shows strong support. Given the proximity of the current geographic ranges of those lineages, predominantly in southeast to East Asia (Malhotra et al., 2011; Uetz and Hosek, 2014), the NW sister clade proposed here would not be unexpected. Additionally, some *Gloydius* species also occur in more northern parts relatively close to the Beringia (Gloyd and Conant, 1990; Malhotra et al., 2010), where the invasion of the NW is thought to have taken place (Wüster et al., 2008).

Like previous studies on phylogenetics of vipers (e.g. Castoe and Parkinson, 2006; Gutberlet and Harvey, 2002; Parkinson, 1999; Parkinson et al., 2002), relationships among deeper branches in the clade of NW pit vipers were poorly resolved. This difficulty is likely to be related to the suggested rapid cladogenesis of pit vipers after their invasion in the NW (Gutberlet and Harvey, 2002; Wüster et al., 2008; see also analysis below) leading to very short branches among major NW clades (see Fig. 1). However, differently from morphological analyses (e.g. Gutberlet and Harvey, 2002), molecular studies recovered a well-supported clade comprising *Porthidium*, *Cerrophidion* and *Atropoides* (e.g. Castoe and Parkinson, 2006; Castoe et al., 2005, 2009; Kraus et al., 1996; Parkinson et al., 2002; present study). Our results also agree with previously studies (e.g. Castoe and Parkinson, 2006; Jadin et al., 2011; Murphy et al., 2002; Parkinson et al., 2002) in recovering highly supported clades comprising the genera *Crotalus* + *Sistrurus* and *Mixcoatlus* + *Ophryacus*.

4.2. Divergence time estimates

Although the oldest fossil known for vipers dates back to 19.5 Mya (early Miocene, Kuch et al., 2006), our date estimates suggests that the ancestral of vipers diverged from its sister group during the Paleocene. Our dating analyses suggest slightly older ages for Viperidae than those proposed by Wüster et al. (2008) (Table S4). However, our date estimates are much older than those suggested by Fenwick et al. (2012) (Table S4). Age of the initial diversification of vipers vary widely in the literature (e.g. ~25.5 Mya in Fenwick et al., 2012, 35.6 Mya in Pyron and Burbrink, 2012, 47.4 Mya in Wüster et al., 2008) but our results suggest that vipers diversified around the late Paleocene to middle Eocene, with subfamilies most likely dating back to the Eocene. Discrepancies in divergence time estimates can emerge for several reasons (see Ho and Phillips, 2009; Sauquet et al., 2012). The differences in the taxa included among studies, tree prior used (birth-death speciation in the present study vs Yule in the others) and/or the different calibration points and prior distributions chosen, could all be plausible explanations.

Our results suggest that close to the Oligocene/Miocene boundary the subfamily Crotalinae invaded the New World, which has been suggested to have occurred as a single event via the Beringian Land Bridge (e.g. Wüster et al., 2002, 2008), a dispersion route also suggested for other snakes lineages (e.g. Burbrink and Lawson, 2007). The Isthmus of Panama, a strip of land that connects North and Central America to South America (Bacon et al., 2013), has also been suggested as a mechanism of dispersion by which some NW pit vipers, such as the rattlesnake *Crotalus durissus* and some *Porthidium* species, probably invaded South America (Vanzolini and Heyer, 1985; Wüster et al., 2002). Very recently, studies have suggested that migration through the Isthmus of Panama (not necessarily a fully continuous landmass) might have occurred much earlier than previously thought (Bacon et al., 2013, 2015; Farris et al., 2011; Montes et al., 2012) and our date estimates for the South American Bothropoids, *Bothrops* and *Bothrocophias*, are in accordance with an earlier migration than the usually accepted date for the emergence of the Isthmus of Panama of 3.5 Mya.

4.3. Diversification dynamics in vipers

Two distinct diversification regimes characterized the radiation of vipers. The specific details for the diversification regime suggested by BAMM for the background lineages are inconclusive and suggest that different possible scenarios might have taken place after the initial radiation of the family Viperidae. This background regime basically reflects the speciation and extinction dynamics of the subfamily Viperinae, plus the species-poor subfamily Azemiopinae, and very few species from the Crotalinae subfamily. BAMM results indicated that it is either likely that speciation rates increased, declined or remained constant as soon as vipers started to diversify. Additionally, gamma values estimated for the subfamily Viperinae, which comprise the majority of lineages in the background regime, does not indicate any sign of decreasing diversification rate. Given that the deceleration signature depicted by gamma statistics might deteriorate as time goes by Liow et al. (2010), Quental and Marshall (2011) it is indeed difficult to infer what is the most likely dynamics for the lineages that comprises the background diversification regime, and it is likely that only a good fossil record could tease those apart (Quental and Marshall, 2010).

Importantly, however, the data strongly suggests that a different diversification regime took place during the diversification of crotalines when speciation rates started to rapidly increase (Figs. 2 and 3A and C). Surprisingly, this shift in speciation rates took place after the first divergence event among crotalines and not at the moment pit vipers started to radiate as expected. The shift did not comprise the five species-poor genera *Calloselasma*, *Deinagkistrodon*, *Garthius*, *Hypnale* and *Tropidolaemus*. One could argue that a species under-sampling effect on this clade could have biased the shift position in BAMM analyses. However, BAMM takes into account possible species under-sampling effects (see Section 2), and our analysis does not represent a great level of under-sampling where 73% of the species within this clade were included in our analyses. Thus, the distinct diversification regime found comprises only part of the OW crotalines and the whole NW clade (see Figs. 1 and 2). Our results suggest that the emergence of the loreal pits in crotalines might not have immediately triggered an explosive radiation, the diversification pattern usually associated with the evolution of key innovations (Glor, 2010).

It is important to highlight, however, that our results do not mean that the evolution of loreal pits was not important for the increase in speciation rates shown by the sub-group of crotalines. Studies suggest that the loreal pits evolved not only for enhancing prey finding, but also as an efficient defense mechanism and as a powerful tool in the search for optimal thermoregulation sites (e.g. Goris, 2011; Krochmal et al., 2004; Krochmal and Bakken, 2003). That is, this change in the life history of vipers may have allowed crotalines to invade different habitats facing new selective pressures (Greene, 2002; Rosenzweig and McCord, 1991). We suggest that the evolution of loreal pits in combination with other factors might have triggered the rapid speciation events found among most crotalines.

The remarkable shift in the diversification regime of vipers might have started to take place either during the middle Eocene until the Oligocene with speciation rates remaining relatively high until the Miocene. These time periods were characterized by several geographical events that occurred in Asia and in fact, pit vipers are thought to have originated in this continent (Malhotra et al., 2010; Wüster et al., 2008). Almost all extant OW pit vipers are restricted to Asia (see Fig. 1) mainly in the southeast and China (Uetz and Hosek, 2014). The Asian continent experienced a complex geological history during the Cenozoic, and two orogenic events, the India-Asia collision and the Asia-Australia collision, drastically affected its climate, topography, and vegetation (Bruyn et al., 2014; Morley, 2012; Wang, 2004). These events had a dramatic impact on the Asian climate characterizing a significant climate shift toward much wetter climates (Bruyn et al., 2014; Morley, 2012; Wang, 2004). Since the Eocene to Miocene, arid regions retracted to northwest China and forests expanded in Southeast Asia to south and eastern China (Sun and Wang, 2005). We hypothesize that forest expansion allowed crotaline ancestral lineages to expand their ranges culminating in rapid subsequent speciation events. In fact, the great majority of OW crotalines are restricted to forested habitats (Gumprecht et al., 2004) as probably were their ancestors. Speciation events could have occurred as a consequence of the colonization of different areas imposing different selective pressures as well as due to vicariant events (Rosenzweig, 1995).

During the late Oligocene to early Miocene pit vipers also colonized the New World, perhaps favored by the forest expansion and wetter climate during this time period in East Asia. The invasion of the NW has been suggested as a potential driver of species diversification in different groups of organisms (e.g. Barker et al., 2015; Burbrink and Pyron, 2009) and may have contributed to the increase in speciation rates observed in crotalines as already suggested in the literature (Burbrink et al., 2012a; Wüster et al., 2008). The invasion of a new area free of competitors and/or predators can lead to explosive radiations due to the greater ecological opportunities available (Glor, 2010; Rabosky and Lovette, 2008). When pit vipers invaded the NW, the snake fauna at the region was far less diverse than it is today (Burbrink and Pyron, 2009;

Holman, 2000), which may have contributed to the diversification opportunities faced by NW vipers (Wüster et al., 2002).

After the remarkable increase in speciation rates among crotalines however, rates started to decline. Decreases in speciation rates are commonly found both on molecular phylogenies (McPeek, 2008; Morlon et al., 2010) and the fossil record (Alroy, 1996; Quental and Marshall, 2013; Silvestro et al., 2015). These rate slowdowns are usually attributed to diversity dependent diversification dynamics where ecological niches are filled with new taxa, the available ecological space shrinks, and opportunities to speciate decrease (Burbrink and Pyron, 2009; Phillimore and Price, 2008; Rabosky and Lovette, 2008; but see Quental and Marshall, 2010). Therefore it is possible that after pit vipers invaded the NW rapidly dispersing through the continent, populations occupied different environments culminating in the accelerated formation of several new lineages not long after the invasion (see also Burbrink et al., 2012a). Speciation rates would thus slowdown because a large set of geographically spaced and distinct environments was no longer available all at the same time as they once were. A similar scenario could explain speciation slowdowns among OW crotalines with new opportunities emerging with the forest expansion in Asia. After OW pit vipers dispersed through the forests occupying the newly available opportunities, the speed of species formation would have decreased reflecting a drop in speciation rate. It is important to note that we do not mean that after speciation slowdowns environments remained stable or that new opportunities to speciate stopped to emerge. Instead, environments are always changing and new opportunities should always be available (Van Valen, 1973). However, these new opportunities will emerge in a slower rate and/or in much more local scales compared to previous time periods.

A slowdown in speciation, however, does not necessarily imply an association with niche divergence (Moen and Morlon, 2014). An increase in the geographic ranges of lineages after their invasion of new areas (new forested areas and/or the NW) could also be subject to successive vicariant events, and speciation due to isolation may increase speciation rates (Moen and Morlon, 2014). In fact, lineages with larger geographic distributions are more likely to be dissected by geographic barriers (Rosenzweig, 1995) and at least in theory, geographic isolation can lead to an increase in diversification rates (Pigot et al., 2010). The successive division of the larger ranges would produce many new species with smaller ranges, which in turn would be less likely to be subdivided by potential barriers causing speciation rates to decline (Moen and Morlon, 2014). That is, bursts of speciation with subsequent slowdowns could also be caused by purely geographic factors.

5. Conclusions

The phylogenetic relationships among vipers recovered well-supported clades with most genera being monophyletic. However, as in previous studies, we draw attention to the paraphyletic position of *Atropoides picadoi*, *Ovophis okinavensis* and *Trimeresurus gracilis* respective to their current genera, and to the non-monophyletic configuration of *Bothrops* and *Bothrocophias*. Our study helps to clarify previously problematic relationships between Africa-Middle East taxa (e.g. *Echis* and *Cerastes*), and propose a highly supported Asian sister group for NW pit vipers. However, deeper nodes among NW pit vipers remain uncertain. Despite adding new sequences of mitochondrial and nuclear genes, and being able to recover several phylogenetic patterns suggested for the Viperidae, we were not able to resolve some previous problematic issues. We hope that our results help to guide future taxonomic decisions (e.g. *Ovophis okinavensis* and *Trimeresurus gracilis*) and sequencing efforts regarding poorly supported clades (e.g. NW pit vipers).

Our date estimates suggested that vipers started to diversify during the end of the middle Paleocene to middle Eocene epochs. Until early Miocene pit vipers emerged and rapidly colonized the NW. Moreover, date estimates for South American clades totally agree with an earlier migration than the usually accepted date for the emergence of the Isthmus of Panama.

Regarding diversification dynamics in vipers, our results would suggest that vipers are still growing in species number because speciation rates are higher relative to extinction rates. However, extinction rates were estimated to be very low and seem roughly constant through time. Extinction rates estimated from molecular phylogenies are typically very low and extreme caution should be taken when interpreting those estimates (Quental and Marshall, 2010; Rabosky, 2010). Moreover, our results could not recover with confidence the diversification scenario of the background regime, mostly comprised by the subfamily Viperinae. However, irrespective of what is the true extinction rates and diversity trajectory for vipers, our results undoubtedly suggest that two diversification regimes are present and that at some point in their history, vipers experienced a very high speciation rate that was followed by a considerable decline. The evolution of the loreal pits alone does not seem to explain this explosive speciation rates. We suggest that the evolution of the loreal pits coupled with the dispersion to new niches emerged as a consequence of climatic and geological changes in Asia and the invasion of the NW may have spurred the increase in speciation rates. Moreover, the drop in speciation rates in this successful group of snakes may be a consequence of the decrease in opportunities to speciate after their rapid dispersion through the several available environments.

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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.2016.07.029.

References

Alfaro, M.E., Santini, F., Brock, C., Alamillo, H., Dornburg, A., Rabosky, D.L., Carnevale, G., Harmon, L.J., 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc. Natl. Acad. Sci. USA 106 (32), 13410–13414. http://dx.doi.org/10.1073/pnas.0811087106.

Alroy, J., 1996. Constant extinction, constrained diversification, and uncoordinated stasis in North American mammals. Palaeogeogr. Palaeoclimatol. Palaeoecol. 127 (1–4), 285–311. http://dx.doi.org/10.1016/S0031-0182(96)00100-9.

Bacon, C.D., Mora, A., Wagner, W.L., Jaramillo, C.A., 2013. Testing geological models of evolution of the Isthmus of Panama in a phylogenetic framework. Bot. J. Linn. Soc. 171 (1), 287–300. http://dx.doi.org/10.1111/j.1095-8339.2012.01281.x.

Bacon, C.D., Silvestro, S., Jaramillo, C., Smith, B.T., Chakrabarty, P., Antonelli, S., 2015. Biological evidence supports an early and complex emergence of the Isthmus of Panama. Proc. Natl. Acad. Sci. USA 112 (19), 6110–6115. http://dx.doi.org/10.1073/pnas.1423853112.

Barker, F.K., Burns, K.J., Klicka, J., Lanyon, S.M., Lovette, I.J., 2015. New insights into New World biogeography: an integrated view from the phylogeny of blackbirds, cardinals, sparrows, tanagers, warblers, and allies. Auk 132 (2), 333–348. http://dx.doi.org/10.1642/AUK-14-110.1.

Benton, M.J., Donoghue, P.H.J., Asher, R.J., 2009. Calibrating and constraining molecular clocks. In: Hedges, S.B., Kumar, S. (Eds.), The Time Tree of Life. Oxford University Press, Oxford, pp. 35–86.

Bruyn, M., Stelbrink, B., Morley, R.J., Hall, R., Carvalho, G.R., Cannon, C.H., Bergh, G.V. D., Meijaard, E., Metcalfe, I., Boitani, L., Maiorano, L., Shoup, R., Rintelen, T.V., 2014. Borneo and Indochine are major evolutionary hotspots for Southeast Asian biodiversity. Syst. Biol. 63 (6), 879–901. http://dx.doi.org/10.1093/sysbio/syu047.

Burbrink, F.T., Lawson, R., 2007. How and when did old world ratsnakes disperse into the New World? Mol. Phylogenet. Evol. 43 (1), 173–189. http://dx.doi.org/10.1016/j.ympev.2006.09.009.

Burbrink, F.T., Pyron, R.A., 2009. How does ecological opportunity influence rates of speciation, extinction, and morphological diversification in the New World ratsnakes? (Tribe Lampropeltini)? Evolution 64 (4), 934–943. http://dx.doi.org/10.1111/j.1558-5646.2009.00888.x.

Burbrink, F.T., Chen, X., Myers, E.A., Brandley, M.C., Pyron, R.A., 2012a. Evidence for determinism in species diversification and contingency in phenotypic evolution during adaptive radiation. Proc. Roy. Soc. London B 279 (1748), 4817–4826. http://dx.doi.org/10.1098/rspb.2012.1669.

Burbrink, F.T., Ruane, S., Pyron, R.A., 2012b. When are adaptive radiations replicated in areas? Ecological opportunity and unexceptional diversification in West Indian dipsadine snakes (Colubridae: Alsophiini). J. Biogeogr. 39 (3), 465–475. http://dx.doi.org/10.1111/j.1365-2699.2011.02621.x.

Campbell, J.A., Lamar, W.W., 2004. The Venomous Reptiles of the Western Hemisphere. Comstock, Ithaca and London.

Carrasco, P.A., Mattoni, C.I., Leynaud, G.C., Scrocchi, G.J., 2012. Morphology, phylogeny and taxonomy of South American bothropoid pitvipers (Serpentes, Viperidae). Zoolog. Scr. 41 (2), 109–124. http://dx.doi.org/10.1111/j.1463-6409.2011.00511.x.

Castoe, T.D., Chippindale, P.T., Campbell, J.A., Ammerman, L.K., Parkinson, C.L., 2003. Molecular systematics of the Middle American jumping pitvipers (genus *Atropoides*) and phylogeography of the *Atropoides nummifer* complex. Herpetologica 59 (3), 420–431. http://dx.doi.org/10.1655/01-105.2.

Castoe, T.D., Sasa, M.M., Parkinson, C.L., 2005. Modeling nucleotide evolution at the mesoscale: the phylogeny of the Neotropical pitvipers of the Porthidium group (Viperidae: Crotalinae). Mol. Phylogenet. Evol. 37 (3), 881–898. http://dx.doi.org/10.1016/j.ympev.2005.05.013.

Castoe, T.D., Parkinson, C.L., 2006. Bayesian mixed models and the phylogeny of pitvipers (Viperidae: Serpentes). Mol. Phylogenet. Evol. 39 (1), 91–110. http://dx.doi.org/10.1016/j.ympev.2005.12.014.

Castoe, T.D., Spencer, C.L., Parkinson, C.L., 2007. Phylogeographic structure and historical demography of the western diamondback rattlesnake (Crotalus atrox): a perspective on North American desert biogeography. Mol. Phylogenet. Evol. 42 (1), 193–212. http://dx.doi.org/10.1016/j.ympev.2006.07.002.

Castoe, T.A., Daza, J.M., Smith, E.N., Sasa, M.M., Kuch, U., Campbell, J.A., Chippindale, P.T., Parkinson, C.L., 2009. Comparative phylogeography of pitvipers suggests a consensus of anciente Middle American highland biogeography. J. Biogeogr. 36 (1), 88–103. http://dx.doi.org/10.1111/j.1365-2699.2008.01991.x.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214. http://dx.doi.org/10.1186/1471-2148-7-214.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4 (5), 699–710. http://dx.doi.org/10.1371/journal.pbio.0040088.

Etienne, R.S., Haegeman, B., Stadler, T., Aze, T., Pearson, P.N., Purvis, A., Phillimore, A. B., 2012. Diversity-dependence brings molecular phylogenies closer to agreement with the fossil record. Proc. Roy. Soc. London B 279 (1815), 1300–1309. http://dx.doi.org/10.1098/rspb.2011.1439.

Farris, D.W., Jaramillo, C., Bayona, G., Restrepo-Moreno, S.A., Montes, C., Cardona, A., Mora, A., Speakman, R.J., Glascock, M.D., Valencia, V., 2011. Fracturing of the Panamanian Isthmus during initial collision with South America. Geology 39 (11), 1007–1010. http://dx.doi.org/10.1130/G32237.1.

Fenwick, A.M., Gutberlet Jr., R.L., Evans, J.A., Parkinson, C.L., 2009. Morphological and molecular evidence for phylogeny and classification of South American pitvipers, genera Bothrops, Bothriopsis, and Bothrocophias (Serpentes: Viperidae). Zool. J. Linn. Soc. 156 (3), 617–640. http://dx.doi.org/10.1111/j.1096-3642.2008.00495.x.

Fenwick, A.M., Greene, H.W., Parkinson, C.L., 2012. The serpent and the egg: unidirectional evolution of reproductive mode in vipers? J. Zool. Syst. Evol. Res. 50 (1), 59–66. http://dx.doi.org/10.1111/j.1439-0469.2011.00646.x.

Filipski, A., Murillo, O., Freydenzon, A., Tamura, K., Kumar, S., 2014. Prospects for building large timetrees using molecular data with incomplete gene coverage among species. Mol. Biol. Evol. 31 (9), 2542–2550. http://dx.doi.org/10.1093/molbev/msu200.

Glor, R.E., 2010. Phylogenetic insights on adaptive radiation. Annu. Rev. Ecol., Evol., Syst. 41, 251–270. http://dx.doi.org/10.1146/annurev.ecolsys.39.110707.173447.

Gloyd, H.K., Conant, R., 1990. Snakes of the Agkistrodon Complex: A Monographic Review. Society for the Study of Amphibians and Reptiles, St. Louis.

Goris, R.C., 2011. Infrared organs of snakes: an integral part of vision. J. Herpetol. 45 (1), 2–14. http://dx.doi.org/10.1670/10-238.1.

Grazziotin, F.G., Monzel, M., Echeverrigaray, S., Bonatto, S.L., 2006. Phylogeography of the Bothrops jararaca complex (Serpentes: Viperidae): past fragmentation and island colonization in the Brazilian Atlantic Forest. Mol. Ecol. 15, 3969–3982. http://dx.doi.org/10.1111/j.1365-294X.2006.03057.x.

Grazziotin, F.G., Zaher, H., Murphy, R.W., Scrocchi, G., Benavides, M.A., Zhang, Y.P., Bonatto, S.L., 2012. Molecular phylogeny of the New World Dipsadidae (Serpentes: Colubroidea): a reappraisal. Cladistics 28 (5), 437–459. http://dx.doi.org/10.1111/j.1096-0031.2012.00393.x.

Greene, H.W., 1997. Snakes: The Evolution of Mystery in Nature. The University of California Press, Berkeley and Los Angeles.

Greene, H.W., 2002. The ecological and behavioral context for pitviper evolution. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salta Lake City, pp. 107–117.

Grismer, L.L., 1999. An evolutionary classification of reptiles on islands in the Gulf of California, México. Herpetologica 55 (4), 446–469.

Gumprecht, A., Tillack, F., Orlov, N., Captain, A., Ryabov, S., 2004. Asian Pitvipers. GeitjeBooks, Berlin.

Gutberlet Jr., R.L., Harvey, M.B., 2002. Phylogenetic relationships of New World pit vipers as inferred from anatomical evidence. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salta Lake City, pp. 51–68.

Harmon, L.J., Schulte II, J.A., Larson, A., Losos, J.B., 2003. Tempo and mode of evolutionary radiation in Iguanian lizards. Science 15 (5635), 961–964. http://dx.doi.org/10.1126/science.1084786.

Hartmann, K., Wong, D., Stadler, T., 2010. Sampling trees from evolutionary models. Syst. Biol. 59 (4), 465–476. http://dx.doi.org/10.1093/sysbio/syq026.

Harvey, M.B., Aparicio, J., Gonzales, L., 2005. Revision of the venomous snakes of Bolivia. II: The pitvipers (Serpentes: Viperidae). Ann. Carnegie Mus. 74 (1), 1–37. http://dx.doi.org/10.2992/0097-4463(2005) 74[1:ROTVSO]2.0.CO;2.

Head, J.J., Bloch, J.I., Hastings, A.K., Bourque, J.R., Cadena, E.A., Herrera, F.A., Polly, P. D., Jaramillo, C.A., 2009. Giant boid snake from Paleocene neotropics reveals hotter past equatorial temperatures. Nature 457 (7230), 718. http://dx.doi.org/10.1038/nature07671.

Hendry, C.R., Guiher, T.J., Pyron, R.A., 2014. Ecological divergence and sexual selection drive sexual size dimorphism in new world pitvipers (Serpentes: Viperidae). J. Evol. Biol. 27, 760–771. http://dx.doi.org/10.1111/jeb.12349.

Herrmann, H.W., Joger, U., Wink, M., 1999. Morphological and molecular phylogenies of viperines: conflicting evidence? Kaupia 8, 21–30.

Ho, S.Y.W., Phillips, M.J., 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. Syst. Biol. 58, 367–380. http://dx.doi.org/10.1093/sysbio/syp035.

Holman, J.A., 2000. Fossil Snakes of North America: Origin, Evolution, Distribution, Paleoecology. Indiana University Press, Indiana.

Jadin, R.C., Gutberlet Jr., R.L., Smith, E.N., 2010. Phylogeny, evolutionary morphology, and hemipenis descriptions of the Middle American jumping pitvipers (Serpentes: Crotalinae: *Atropoides*). J. Zool. Syst. Evol. Res. 48 (4), 360–365. http://dx.doi.org/10.1111/j.1439-0469.2009.00559.x.

Jadin, R.C., Smith, E.N., Campbell, J.A., 2011. Unravelling a tangle of Mexican serpents: a systematic revision of highland pitvipers. Zool. J. Linn. Soc. 163 (3), 943–958. http://dx.doi.org/10.1111/j.1096-3642.2011.00748.x.

Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30 (14), 3059–3066. http://dx.doi.org/10.1093/nar/gkf436.

Kraus, F., Mink, D.G., Brown, W.M., 1996. Crotaline intergeneric relationships based on mitochondrial DNA sequence data. Copeia 1996 (4), 763–773.

Krochmal, A.R., Bakken, G.S., 2003. Thermoregulation is the pits: use of thermal radiation for retreat site selection by rattlesnakes. J. Exp. Biol. 206, 2539–2545. http://dx.doi.org/10.1242/jeb.00490.

Krochmal, A.R., Bakken, G.S., LaDuc, T.J., 2004. Heat in evolution's kitchen: evolutionary perspectives on the functions and origin of the facial pit of pitvipers (Viperidae: Crotalinae). J. Exp. Biol. 207, 4231–4238. http://dx.doi.org/10.1242/jeb.01278.

Kuch, U., Müller, J., Mödden, C., Mebs, D., 2006. Snake fangs from the Lower Miocene of Germany: evolutionary stability of perfect weapons. Naturwissenschaften 93 (2), 84–87. http://dx.doi.org/10.1007/00114-005-0065-y.

Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29 (6), 1695–1701. http://dx.doi.org/10.1093/molbev/mss020.

Lemmon, A.R., Brown, J.M., Stanger-Hall, K., Lemmon, E.M., 2009. The effect of ambiguous data on phylogenetic estimates obtained by maximum likelihood and Bayesian inference. Syst. Biol. 58 (1), 130–145. http://dx.doi.org/10.1093/sysbio/syp017.

Lenk, P., Kalyabina, S., Wink, M., Joger, U., 2001. Evolutionary relationships among the true vipers (Reptilia: Viperidae) Inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 19 (1), 94–104. http://dx.doi.org/10.1006/mpev.2001.0912.

Liow, L.H., Quental, T.B., Marshall, C.R., 2010. When can decreasing diversification rates be detected with molecular phylogenies and the fossil record? Syst. Biol. 59 (6), 646–659. http://dx.doi.org/10.1093/sysbio/syq052.

Losos, J.B., Mahler, D.L., 2010. Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. In: Bell, M., Futuyma, D., Eanes, W.,

Levinton, J. (Eds.), Evolution Since Darwin: The First 150 Years. Sinauer, Sunderland, pp. 381–420.
Lynch, V.J., 2009. Live-birth in vipers (Viperidae) is a key innovation and adaptation to global cooling during the Cenozoic. Evolution 63 (9), 2457–2465. http://dx.doi.org/10.1111/j.1558-5646.2009.00733.x.
Maddison, W.P., Maddison, D.R., 2009. Mesquite: A Modular System for Evolutionary Analysis v 2.71. <http://mesquiteproject.org>.
Malhotra, A., Thorpe, R.S., 2000. A phylogeny of the Trimeresurus group of pit vipers: new evidence from amitochondrial gene tree. Mol. Phylogenet. Evol. 16 (2), 199–211. http://dx.doi.org/10.1006/mpev.2000.0779.
Malhotra, A., Thorpe, R.S., 2004. A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers (*Trimeresurus* and *Ovophis*). Mol. Phylogenet. Evol. 32 (1), 83–100. http://dx.doi.org/10.1016/j.ympev.2004.02.008.
Malhotra, A., Creer, S., Pook, C.E., Thorpe, R.S., 2010. Inclusion of nuclear intron sequence data helps to identify the Asian sister group of New World pit vipers. Mol. Phylogenet. Evol. 54 (1), 172–178. http://dx.doi.org/10.1016/j.ympev.2009.09.007.
Malhotra, A., Dawson, K., Guo, P., Thorpe, R.S., 2011. Phylogenetic structure and species boundaries in the mountain pitviper *Ovophis monticola* (Serpentes: Viperidae: Crotalinae) in Asia. Mol. Phylogenet. Evol. 59 (2), 444–457. http://dx.doi.org/10.1016/j.ympev.2011.02.010.
Martins, M., Araújo, M.S., Sawaya, R.J., Nunes, R., 2001. Diversity and evolution of macrohabitat use, body size and morphology in a monophyletic group of Neotropical pitvipers (*Bothrops*). J. Zool. 254 (4), 529–538. http://dx.doi.org/10.1017/S0952836901001030.
McPeek, M.A., 2008. The ecological dynamics of clade diversification and community assembly. Am. Nat. 172 (6), e270–e284. http://dx.doi.org/10.1086/593137.
Moen, D., Morlon, H., 2014. Why does diversification slow down? Trends Ecol. Evol. 29 (4), 190–197. http://dx.doi.org/10.1016/j.tree.2014.01.010.
Montes, C., Cardona, A., McFadden, R., Morón, S.E., Silva, C.A., Restrepo-Moreno, S., Ramiréz, D.A., Hoyos, N., Farris, D., Bayona, G.A., Jaramillo, C.A., Valencia, V., Bryan, J., Flores, J.A., 2012. Evidence for middle Eocene and younger land emergence in Central Panama: implications for Isthmus closure. Geol. Soc. Am. Bull. 124 (5–6), 780–799. http://dx.doi.org/10.1130/B30528.1.
Morley, R.J., 2012. A review of the Cenozoic palaeoclimate history of Southeast Asia. In: Gower, D., Johnson, K., Richardson, J., Rosen, B., Rüber, L., Williams, S. (Eds.), Biotic Evolution and Environmental Change in Southeast Asia. Cambridge University Press, Cambridge, pp. 79–114.
Morlon, H., Potts, M.D., Plotkin, J.B., 2010. Inferring the dynamics of diversification: a coalescent approach. PLoS Biol. 8 (9), 1000493. http://dx.doi.org/10.1371/journal.pbio.1000493.
Morlon, H., Kemps, B.D., Plotkin, J.B., Brisson, D., 2012. Explosive radiation of a bacterial species group. Evolution 66 (8), 2577–2586. http://dx.doi.org/10.1111/j.1558-5646.2012.01598.x.
Murphy, R.W., Fu, J., Lathrop, A., Feltham, J.V., Kovac, V., 2002. Phylogeny of the rattlsnakes (Crotalus and Sistrurus) inferred from sequences of five mitochondrial DNA genes. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salta Lake City, pp. 69–92.
Nee, S., Mooers, A.O., Harvey, P.H., 1992. Tempo and mode of evolution revealed from molecular phylogenies. Proc. Nat. Acad. Sci. United States 89 (17), 8322–8326.
Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20 (2), 289–290. http://dx.doi.org/10.1093/bioinformatics/btg412.
Parham, J.F., Donogue, P.C.J., Bell, C.J., Calway, T.D., Head, J.J., Holroyd, P.A., Inoue, J.G., Irmis, R.B., Joyce, W.G., Ksepka, D.T., Patané, J.S.L., Smith, N.D., Tarver, J.E., Van Tuinen, M., Yang, Z., Angielczyk, K.D., Greenwood, J.M., Hipsley, C.A., Jacobs, L., Makovicky, P.J., Müller, J., Smith, K.T., Theodor, J.M., Warnock, R.C.M., Benton, M.J., 2012. Best practices for justifying fossil calibrations. Syst. Biol. 61, 346–359. http://dx.doi.org/10.1093/sysbio/syr107.
Parkinson, C.L., 1999. Molecular systematics and biogeographical history of pitvipers as determined by mitochondrial ribosomal DNA sequences. Copeia 1999 (3), 576–586.
Parkinson, C.L., Campbell, J.A., Chippindale, P.T., 2002. Multigene phylogenetic analysis of pitvipers, with comments on their biogeography. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salta Lake City, pp. 93–110.
Parmley, D., Holman, J.A., 2007. Earliest fossil record of a pigmy Rattlesnake (Viperidae: *Sistrurus* Garman). J. Herpetol. 41 (1), 141–144. http://dx.doi.org/10.1670/0022-1511(2007) 41[141:EFROAP]2.0.CO;2.
Phelps, T., 2010. Old World Vipers: A Natural History of Azemiopinae and Viperinae. Edition Chimaira, Frankfurt.
Phillimore, A.B., Price, T.D., 2008. Density-dependent cladogenesis in birds. Plos Biol. 6 (3), e71. http://dx.doi.org/10.1371/journal.pbio.0060071.
Pigot, A.L., Phillimore, A.B., Owens, I.P., Orme, D.L., 2010. The shape and temporal dynamics of phylogenetic trees arising from geographic speciation. Syst. Biol. 59 (6), 1–14. http://dx.doi.org/10.1093/sysbio/syq058.
Pook, C.E., Joger, U., Stümpel, N., Wüster, W., 2009. When continents collide: phylogeny, historical biogeography and systematics of the medically important viper genus Echis (Squamata: Serpentes: Viperidae). Mol. Phylogenet. Evol. 53 (3), 792–807. http://dx.doi.org/10.1016/j.ympev.2009.08.002.
Pybus, O.G., Harvey, P.H., 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. Proc. Roy. Soc. London B 267 (1459), 2267–2272. http://dx.doi.org/10.1098/rspb.2000.1278.
Pyron, R.A., Burbrink, F.T., 2012. Extinction, ecological opportunity, and the origins of a global snake diversity. Evolution 66 (1), 163–178. http://dx.doi.org/10.1111/j.1558-5646.2011.01437.x.
Pyron, R.A., Burbrink, F.T., Wiens, J.J., 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evol. Biol. 13, 1–53. http://dx.doi.org/10.1186/1471-2148-13-93.
Quental, T., Marshall, C.R., 2010. Diversity dynamics: molecular phylogenies need the fossil record. Trends Ecol. Evol. 25 (8), 434–441. http://dx.doi.org/10.1016/j.tree.2010.05.002.
Quental, T.B., Marshall, C.R., 2011. The molecular phylogenetic signature of clades in decline. PLoS ONE 6 (10), e25780. http://dx.doi.org/10.1371/journal.pone.0025780.
Quental, T.B., Marshall, C.R., 2013. How the red queen drives terrestrial mammals to extinction. Science 341 (6143), 290–292. http://dx.doi.org/10.1111/j.1558-5646.2009.00733.x.
Rabosky, D.L., 2010. Extinction rates should not be estimated from molecular phylogenies. Evolution 64 (6), 1816–1824. http://dx.doi.org/10.1111/j.1558-5646.2009.00926.x.
Rabosky, D.L., 2012. Testing the time-for-speciation effect in the assembly of regional biotas. Methods Ecol. Evol. 3 (2), 224–233. http://dx.doi.org/10.1111/j.2041-210X.2011.00166.x.
Rabosky, D., Lovette, I.J., 2008. Explosive evolutionary radiations: decreasing speciation or increasing extinction through time. Evolution 62 (8), 1866–1875. http://dx.doi.org/10.1111/j.1558-5646.2008.00409.x.
Rabosky, D.L., Santini, F., Eastman, J., Smith, S.A., Sidilaukas, B., Chang, J., Alfaro, M.E., 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nat. Commun. 4, 1958. http://dx.doi.org/10.1038/ncomms2958.
Rabosky, D., 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLoS ONE 9 (2), e89543. http://dx.doi.org/10.1371/journal.pone.0089543.
Rabosky, D.L., Donnellan, S.C., Grundler, M., Lovette, I.J., 2014a. Analysis and visualization of complex macroevolutionary dynamics: an example from Australian scincid lizards. Syst. Biol. 63 (4), 610–627. http://dx.doi.org/10.1093/sysbio/syu025.
Rabosky, D.L., Grundler, M., Anderson, C., Title, P., Shi, J.J., Brown, J.W., Huang, H., Larson, J.G., 2014b. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. Methods Ecol. Evol. 5 (7), 701–707. http://dx.doi.org/10.1111/2041-210X.12199.
R Development Core Team, 2013. R: A Language and Environment for Statistical Computing <http://www.R-project.org>.
Rage, J.C., Folie, A., Rana, R.S., Singh, H., Rose, K.D., Smith, T., 2008. A diverse snake fauna from the early Eocene of Vastan Lignite Mine, Gujarat, India. Acta Palaentologica Pol. 53 (3), 391–403.
Rambaut, A., Drummond, A.J., 2007. Tracer v 1.4. <http://beast.bio.ed.ac.uk/>.
Raup, D.M., 1986. Biological extinction in earth history. Science 231 (4745), 1528–1533. http://dx.doi.org/10.1126/science.11542058.
Rivas, G.A., Molina, C.R., Ugueto, G.N., Barros, T.R., Barrio-Amorós, C.L., Kok, P.J.R., 2012. Reptiles of Venezuela: an updated and commented checklist. Zootaxa 3211, 1–64.
Roelke, C.E., Childress, M.J., 2007. Defensive and infrared reception responses of true vipers, pitvipers, *Azemiops* and colubrids. J. Zool. 273 (4), 421–425. http://dx.doi.org/10.1111/j.1469-7998.2007.00346.x.
Rosenzweig, M.L., Brown, J.S., Vincent, T.L., 1987. Red Queens and ESS: the coevolution of evolutionary rates. Evol. Ecol. 1 (1), 59–94.
Rosenzweig, M.L., McCord, R.D., 1991. Incumbent replacement: evidence for longterm evolutionary progress. Paleobiology 17 (3), 202–213.
Rosenzweig, M.L., 1995. Species Diversity in Space and Time. Cambridge University Press, Cambridge.
Salomão, M.G., Wüster, W., Thorpe, R.S.BBBSP, 1999. MtDNA evolution in Neotropical pitvipers of the genus Bothrops (Squamata: Serpentes: Viperidae). Kaupia 8, 127–134.
Sanders, K.L., Mumpuni, A.H., Head, J.J., Gower, D.J., 2010. Phylogeny and divergence times of filesnakes (Acrochordus): inferences from morphology, fossils and three molecular loci. Mol. Phylogenet. Evol. 56 (3), 857–867. http://dx.doi.org/10.1016/j.ympev.2010.04.031.
Sauquet, H., Ho, S.Y.W., Gandolfo, M.A., Jordans, G.J., Wilf, P., Cantrill, D.J., Bayl, M.J., Bromham, L., Brown, G.K., Carpenter, R.J., Lee, D.M., Murphy, D.J., Sniderman, J.M.K., Udovicic, F., 2012. Testing the impact of calibration on molecular divergence times using a fossil-rich group: the case of *Nothofagus* (Fagales). Syst. Biol. 61 (2), 289–313. http://dx.doi.org/10.1093/sysbio/syr116.
Silvestro, D., Antonelli, A., Salamin, N., Quental, T.B., 2015. The role of clade competition in the diversification of North American canids. Proc. Natl. Acad. Sci. 112 (28), 8684–8689. http://dx.doi.org/10.1073/pnas.1502803112.
Stadler, T., 2009. On incomplete sampling under birth-death models and connections to the sampling-based coalescent. J. Theor. Biol. 261 (1), 58–66. http://dx.doi.org/10.1016/j.jtbi.2009.07.018.
Stadler, T., 2014. TreeSim: Simulating Trees Under the Birth-Death Model. R package v 2.1. <http://CRAN.R-project.org/package=TreeSim>.
Sun, X., Wang, P., 2005. How old is the Asian monsoon system? – Palaeobotanical records from China. Palaeogeogr. Palaeoclimatol. Palaeoecol. 222 (3–4), 181–222. http://dx.doi.org/10.1016/j.palaeo.2005.03.005.
Tchernov, E., Rieppel, O., Zaher, H., 2000. A fossil snake with limbs. Science 287 (5460), 2010–2012. http://dx.doi.org/10.1126/science.287.5460.2010.
Uetz, P., Hosek, J., 2014. The Reptile Database <http://www.reptile-database.org> (accessed November, 2014).

Vaidya, G., Lohman, D.J., Meier, R., 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27 (2), 171–180. http://dx.doi.org/10.1111/j.1096-0031.2010.00329.x.

Van Valen, L., 1973. The evolutionary law. Evol. Theor. 1, 1–30.

Vanzolini, P.E., Heyer, R.W., 1985. The American herpetofauna and the interchange. In: Stehli, F.G., Webb, S.D. (Eds.), The Great American Biotic Interchange. Plenum Press, New York, pp. 475–487.

Wang, P., 2004. Cenozoic deformation and the history of sea-land interactions in Asia. In: Clift, P., Kuhnt, W., Wang, P., Hayes, D. (Eds.), Continent-Ocean Interactions within East Asian Marginal Seas. American Geophysical Union, Washington, D.C., pp. 1–22. http://dx.doi.org/10.1029/149GM01.

Wiens, J.J., Morrill, M.C., 2011. Missing data in phylogenetic analysis: reconciling results from simulations and empirical data. Syst. Biol. 60 (5), 1–13. http://dx.doi.org/10.1093/sysbio/syr025.

Wüster, W., Peppin, L., Pook, C.E., Walker, D.E., 2008. A nesting of vipers: phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). Mol. Phylogenet. Evol. 49 (2), 445–459. http://dx.doi.org/10.1016/j.ympev.2008.08.019.

Wüster, W., Salomão, M.G., Quijada-Mascareñas, J.A., Thorpe, R.S.B.B.B.S.P., 2002. Origin and evolution of the South American pitviper fauna: evidence from mitochondrial DNA sequence analysis. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Eagle Mountain, pp. 111–128.