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Diversificação de espécies e da morfologia em serpentes da família Viperidae: padrões e processos

Species and morphological diversification in snakes of the family Viperidae: patterns and processes

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Prof. Dr. Marcio Roberto Costa Martins
Orientador

“There ought to be something very
special about the boundary
conditions of the universe - and
what can be more special than that
there is no boundary?”
Stephen Hawking

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SUMÁRIO

RESUMO

A diversidade de espécies e fenotípica pode variar consideravelmente entre grupos taxonômicos e ao longo do tempo em uma mesma linhagem. O estudo de tais variações tornou-se um dos principais objetivos da biologia evolutiva fornecendo informações importantes a respeito dos possíveis mecanismos que regulam a biodiversidade. Dessa forma, o objetivo geral da presente tese foi investigar os padrões da diversificação de espécies e da morfologia em um grupo cosmopolita de serpentes, a família Viperidae, e os potenciais processos subjacentes. Primeiramente, (1) reconstruímos as relações filogenéticas e estimamos os tempos de divergência entre as linhagens da família Viperidae utilizando uma abordagem Bayesiana. (2) Aplicando um método recentemente desenvolvido (BAMM), exploramos como as taxas de especiação e extinção variaram ao longo da radiação do grupo inferindo os possíveis processos reguladores. Por fim, (3) analisamos se a evolução do tamanho do corpo e as taxas de especiação variam nos diferentes habitats ocupados pelos viperídeos (terrestres vs arborícola). Nesta tese geramos a filogenia molecular de viperídeos mais completa até o momento utilizando sequências para 11 genes mitocondriais e nucleares abrangendo 79% das espécies viventes (264 terminais) e todos com exceção de um gênero. De maneira geral, foi possível obter relações filogenéticas robustas para o grupo com a maioria dos gêneros sendo monofilética. Os tempos de divergência obtidos indicam que os viperídeos começaram a diversificar em meados do Paleoceno tardio/meio do Eoceno inferindo idades um pouco mais tardias que o encontrado em estudos anteriores. Durante a radiação do grupo, um aumento nas taxas de especiação parece ter ocorrido durante a diversificação dos crotalíneos (pit vipers) em decorrência não só da evolução das fossetas loreais mas também como resultado de mudanças geológicas e climáticas na Ásia e da invasão do novo mundo. Após este rápido aumento inicial, as taxas de especiação desaceleraram em direção ao presente. Por fim, os resultados aqui apresentados indicam que apesar dos habitats arborícolas limitarem a evolução morfológica nos viperídeos, a evolução da arborealidade parece não afetar as taxas de especiação que permanecem similares entre linhagens arborícolas e terrestres. Isto sugere dois cenários: (1) a especiação acontece de forma independente das mudanças morfológicas nos viperídeos; ou (2) o isolamento geográfico seria um mecanismo importante na diversificação de linhagens arborícolas contrabalançando decréscimos nas oportunidades de especiação possivelmente relacionados às pressões seletivas impostas pelo ambiente arborícola. A presente tese contribui para entendermos mais sobre como evoluíram os viperídeos ao longo dos seus ~50 milhões de anos. Além de propor cenários e hipóteses a serem futuramente explorados com os viperídeos, elaboramos uma discussão ampla e conceitual a respeito dos possíveis mecanismos por trás da diversificação de espécies e da morfologia que poderiam também ser contemplados para outros grupos de organismos. Portanto, a

presente tese contribui não só para entendermos os mecanismos que geram e mantém a diversidade de serpentes, mas também para enriquecer a discussão dos mecanismos que geram e mantém a biodiversidade como um todo.

ABSTRACT

Species and phenotypic diversity may vary considerably between taxonomic groups and through time for a given lineage. The study of such variation became one of the main goals of evolutionary biology and provides important information related to the possible mechanisms regulating biodiversity. The general goal of the present thesis was to investigate the patterns of species and morphological diversification in a cosmopolitan group of snakes, the family Viperidae, and the potential underlying processes. First, (1) we estimated the phylogenetic relationships and divergence times between lineages of the family Viperidae using a Bayesian approach; then we (2) applyed a recently developed method (BAMM) to explore how speciation and extinction rates varied during the radiation of the group suggesting possible underlying processes. Finally, (3) we analyzed if body size evolution and speciation rates showed distinct patterns among vipers occurring in different habitats (terrestrial vs arboreal). Herein we generated the most complete molecular phylogeny for vipers until this moment using sequences from 11 mitochondrial and nuclear genes comprising 79% of extant species (264 terminals) and all except one genus. In general, we were able to recover well supported phylogenetic relationships with most genera being monophyletic. Divergence time estimates suggested that vipers started to diversify around the late Paleocene/middle Eocene finding older ages than previous studies. During the group radiation, an increase in speciation rates seems to have occurred during the diversification of crotalines (pit vipers) not only due to the evolution of loreal pits but also as a result of climatic and geological changes in Asia and the invasion of the New World. After this rapid initial increase, speciation rates decelerated toward the present. Lastly, the results presented here suggest that although arboreal habitats constrain morphological evolution in vipers the evolution of arboreality does not seem to affect speciation rates, which remain similar among arboreal and terrestrial lineages. Our results suggest two distinct scenarios: (1) speciation could be independent of morphological evolution in vipers; or (2) geographic isolation would be an important mechanism underlying species diversification in arboreal lineages offsetting decreases in speciation opportunities potentially related to the selective pressures imposed by the arboreal environment. The present thesis contribute to increase our understanding about how vipers evolved during their ~50 million years. In addition to providing scenarios and hypotheses to be further explored with vipers, we elaborated a broad and conceptual discussion about the possible mechanisms underlying species and morphological diversification that might apply to other groups of organisms. Therefore, this thesis comprises a contribution that goes beyond the understanding of mechanisms generating and maintaining the diversity of snakes, but will hopefuly enrich the discussion of mechanisms that generate and maintain biodiversity as a whole.

O contraste no número de espécies entre os diferentes grupos de organismos constitui um dos padrões mais notáveis e intrigantes da diversidade biológica (Alfaro et al. 2009, Rabosky et al., 2013). Tais diferenças podem, dentre outros fatores, ser explicadas pelas diferentes taxas de diversificação (especiação menos extinção) associadas a cada grupo (Alfaro et al., 2009, Rabosky, 2014). Apesar de diferenças nas taxas médias de diversificação estarem por muitas vezes associadas a riqueza de espécies nos grupos atuais, tanto as taxas de especiação quanto as de extinção podem variar consideravelmente ao longo do tempo (e.g. Rabosky et al., 2014, Silvestro et al., 2015). Diversos são os mecanismos que poderiam explicar tais variações temporais (e.g. Glor, 2010, Losos and Mahler, 2010, Moen and Morlon, 2014). A invasão de novas áreas e o surgimento de inovações chave (Simpson, 1953), a competição por recursos (Silvestro et al., 2015) ou alterações bruscas no clima (Moen and Morlon, 2014), são citados como fatores que poderiam alterar as taxas de especiação e extinção de um dado grupo.

Assim como a diversidade de espécies, a diversidade fenotípica também pode variar consideravelmente entre grupos e ao longo do tempo. Entretanto, um grupo rico em espécies não necessariamente é também diverso fenotipicamente (e.g. *Plethodon*, Kozak et al., 2006) e vice-versa. De fato, a diversificação fenotípica e de espécies podem fornecer informações importantes a respeito dos possíveis mecanismos geradores de biodiversidade. Por exemplo, as famosas "radiações adaptativas" (see Glor, 2010, Losos and Mahler, 2010) estão frequentemente associadas a uma "explosão" tanto de espécies quanto de morfologia após a colonização de novas áreas ou zonas adaptativas (Simpson, 1953). Esta "explosão" é comumente relacionada à hipótese das oportunidades ecológicas (Schluter, 2000), onde as populações ocupam diferentes nichos culminando na formação de diversas espécies. Entretanto, existem situações em que a invasão de uma nova zona adaptativa (Simpson, 1953) limita ao invés de impulsionar a diversificação morfológica (e.g. Collar et al., 2009). Por fim, certos grupos podem ser considerados como radiações "não adaptativas" onde sugere-se que eventos de vicariância sucessivos podem ter culminado na formação de espécies com pouca variação morfológica (Kozak et al., 2006, Rundell and Price, 2009).

Para enterdemos como evolui a biodiversidade é muito importante conhecer e entender a história filogenética das diferentes linhagens que a compõe. As árvores filogenéticas permitem que pesquisadores explorem mais profundamente a origem da diversidade biológica vivente e extinta, bem como os seus possíveis mecanismos reguladores. A partir da década de 80, o "pensamento filogenético" ("phylogenetic tree thinking", Losos, 2011) tornou evidente a importância de incorporar as relações filogenéticas das espécies em inúmeras linhas de pesquisa. A filogenética deixou de ser área exclusiva da sistemática passando também a ser interesse de outras áreas como a ecologia. Desde então, inúmeros foram os trabalhos descrevendo métodos visando incorporar uma perspectiva filogenética nas análises evolutivas, como o clássico trabalho de Felsenstein (1985). Atualmente é praticamente impossível publicar um trabalho comparativo (e.g. comparações entre espécies) sem utilizar uma abordagem filogenética (Losos, 2011). Essa crescente necessidade de se obter filogenias levou ao aumento tanto na disponibilidade de sequências moleculares como de métodos filogenéticos e comparativos. Hoje, é possível baixar sequências diretamente de bases de dados públicas (e.g. GenBank) e gerar árvores filogenéticas rapidamente para muitos grupos de estudos (e.g. Pearse and Purvis, 2013). Além disso, existem métodos que prometem inserir na filogenia táxons sem sequências moleculares usando apenas informações de hierarquia taxonômica (e.g. Jetz et al. 2012, mas ver crítica em Rabosky, 2015).

Apesar de toda a sua importância, as árvores filogenéticas muitas vezes são mal-interpretadas (Baum, 2014, Omland, 2014) e nos últimos anos diversos trabalhos foram publicados com o intuito de esclarecer alguns dos problemas conceituais (e.g. Baum et al., 2005, Baum e Smith, 2013, ver referências em Baum, 2014). Como frisado por Omland (2014) “Most biologists need not know how various phylogenetic algorithms work, but all biologists should know what trees represent and how trees inform our understanding of the process of evolution”. Apesar disso, dada a aparente rapidez com que filogenias podem ser geradas, muitas vezes estas deixam de ser o foco principal de um estudo tornando-se item da sua metodologia. No entanto, a utilização das filogenias apenas como uma ferramenta metodológica não pode esconder o seu principal objetivo e significado, além da real complexidade que existe na sua elaboração, nas decisões e nos cuidados que devem ser tomados por parte dos pesquisadores. Acima de tudo a filogenia é uma hipótese, e todo o trabalho que baseia-se nesta hipótese pode conter grandes deficiências se a árvore filogenética também as tiverem ou se não for bem interpretada. Assim, torna-se extremamente importante dedicar especial atenção à filogenia que está sendo usada no trabalho, cuidado esse por muitas vezes ignorado.

Entender os processos que moldam a biodiversidade depende também da habilidade em explorar as possíveis relações entre a evolução biológica e os padrões históricos (Sauquet et al., 2012). Para tal, integrar o uso de fósseis com filogenias moleculares incorporando dessa forma informações sobre a idade de um ou mais nós da filogenia, representa um passo importante nessa direção. Como resultado, datar uma filogenia significa converter seus comprimentos de ramo para o tempo geológico absoluto (em milhões de anos, e.g. Hedges and Kumar, 2009), sendo possível conhecer os tempos de divergência entre as linhagens. Trabalhos recentemente publicados elaboram estratégias para a escolha dos pontos de calibração (e.g. fósseis, eventos biogeográficos) como forma de diminuir o risco de incluir erros nas datações das filogenias (e.g. Parham et al., 2012, Sauquet et al., 2012). Além das calibrações em si, a escolha dos *priors* ou idades mínimas e máximas das calibrações são fonte de erros potenciais e também devem ser investigadas com cuidado (Ho and Phillips 2009). Dessa forma, calibrar a filogenia em relação ao tempo é também passo crucial em trabalhos com perspectivas evolutivas (Sauquet et al., 2012).

Sendo o objetivo geral desta tese explorar os padrões por trás da diversificação de espécies e da morfologia em serpentes da família Viperidae e os possíveis processos que geram estes padrões, dedicamos o primeiro capítulo a reconstruir relações filogenéticas robustas entre as linhagens do grupo. Para tal, usamos um método filogenético Bayesiano integrando sequências moleculares de bases públicas e inéditas para 79% das espécies que compõe a família. Cabe ressaltar que uma curadoria cuidadosa foi realizada após a obtenção dos dados moleculares. Esta curadoria se mostrou importante devido à erros inerentes a grandes bases de dados. Além disso, utilizando fósseis, datamos tais relações filogenéticas o que permitiu também investigarmos as divergências entre as linhagens do grupo ao longo do tempo geológico (Capítulo I). De posse da filogenia datada, exploramos a dinâmica de diversificação que caracterizou a radiação evolutiva dos viperídeos (Capítulo II). Especificamente, o segundo capítulo foca em como e por que as taxas de especiação e extinção variaram ao longo do tempo nesta família de serpentes. Por fim, dado que o fenótipo pode sugerir possíveis paisagens adaptativas nas quais as linhagens diversificaram, analisamos se a evolução do fenótipo e as taxas de especiação dos viperídeos apresentam padrões similares em linhagens ocupando um mesmo habitat porém distintos entre eles (Capítulo III).

Modelo de estudo

A família Viperidae representa um dos grupos mais notáveis de serpentes do mundo, por sua alta diversidade de espécies (329 espécies), sua ampla distribuição (Uetz e Hosek, 2014), seu mecanismo complexo de inoculação de veneno (Kardong e Lavin-Murcio, 1993) e por sua grande importância médica (Wüster et al., 2008). Fazem parte dessa família serpentes conhecidas pelo público em geral como as cascavéis e jararacas. Acredita-se que o grupo tenha se originado na Ásia ou na África e atualmente ocorre tanto no Velho quanto no Novo Mundo (Greene, 1997, Wüster et al., 2008). Os viperídeos apresentam grande variação no número de espécies que compõe suas diferentes linhagens. A subfamília Viperinae, restrita ao Velho Mundo, possui em torno de 98 espécies, enquanto Crotalinae, amplamente distribuída no Velho e Novo Mundo, possui em torno de 232 espécies. Mais dramática ainda é a diferença na riqueza de espécies entre crotalíneos e sua subfamília irmã, Azemiopinae, a qual compreende apenas duas espécies restritas ao sudeste asiático. A alta riqueza da subfamília Crotalinae é frequentemente associada a eventos que potencialmente levaram a um aumento da formação de novas espécies como o surgimento da fosseta loreal, característica exclusiva deste grupo, e a invasão do Novo Mundo (Greene, 1997, Burbrink et al., 2012).

Além da grande variação na riqueza de espécies, os viperídeos também impressionam pela diversidade em sua ecologia. Espécies deste grupo ocorrem nas maiores latitudes e altitudes já registradas para qualquer outra serpente: acima de 65° N em *Vipera berus* e 47° S em *Bothrops ammodytoides*, e até 4800 m de altitude em *Gloydius himalayanus* no Nepal e 4570 m em *Crotalus triseriatus* no México; Maritz et al., *in review*). Abrangem espécies tanto ovíparas quanto vivíparas (Fenwick et al., 2012) e possuem uma dieta extremamente ampla incluindo, por exemplo, mamíferos, répteis, anfíbios, aves e invertebrados, sendo algumas espécies mais generalistas que outras (Greene, 1997, Martins et al., 2002). A maior parte das espécies é principalmente terrestre, mas linhagens associadas ao ambiente arborícola surgiram em pelo menos oito gêneros (ver Capítulo III). Por ser um grupo excepcionalmente heterogêneo tanto no número de espécies que compõe cada linhagem quanto em relação à ecologia, a família Viperidae é um ótimo modelo para explorar os padrões e processes envolvidos na diversificação de espécies e da morfologia em serpentes.

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MOLECULAR PHYLOGENY AND DIVERGENCE TIME ESTIMATES OF VIPERS (VIPERIDAE): A BAYESIAN APPROACH

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Abstract. Snakes of the cosmopolitan family Viperidae comprise around 329 venomous species distributed in three subfamilies, Azemiopinae, Viperinae, and Crotalinae. Phylogenetics of vipers have greatly improved in the past decades due to the access to new DNA sequences. Here, we present the most complete molecular phylogeny for the Viperidae to date comprising sequences from nuclear and mitochondrial genes representing 79% of all living vipers and all except one genus. We also investigated the time of divergence between lineages using six fossils to time-calibrate the tree. Our phylogenetic analyses retrieved high support values for the monophyletism of the family and subfamilies, and 22 out of 27 genera for which we could test for monophyly, as well as well-supported intergeneric relationships throughout the family. In contrast to other studies, we were able to recover a strongly supported sister clade to the New World crotalines that comprises *Gloydius*, *Ovophis*, *Protobothrops* and *Trimeresurus gracilis*. Time of divergence estimates suggested that crown vipers started to radiate around the late Paleocene to middle Eocene with subfamilies crown group ages most likely dating back to the Eocene. The invasion of the New World might have taken place sometime close to the Oligocene/Miocene boundary. Although some phylogenetic aspects still require further investigation (e.g. relationships among New World vipers), the present study represents an important addition to the knowledge of the phylogenetic relationships and tempo of diversification in vipers.

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 some aspects of their phylogenetic relationships are still poorly understood. Species are currently arranged in 34 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. Crotalinae, or the "pit vipers", is the most diverse and widely distributed lineage of vipers, comprising about 229 species (~70% of all viperid species) (Campbell and Lamar, 2004; Phelps, 2010; Uetz and Hosek, 2014) occurring both in the Old and New World.

A robust understanding of the phylogenetic affinities of vipers is important not only for systematics itself but also for ecology and macroevolution, and even for other fields not directly related to biology such as medicine, pharmacology, and toxicology (Castoe and Parkinson, 2006). The systematics of vipers has greatly improved in the past decades, especially regarding the phylogenetic position and monophyletism of the three subfamilies. Although previous studies based on both morphological and molecular evidence suggest that *Causus* should comprise a separated subfamily (e.g. Groombridge, 1986, Herrmann and Joger, 1995), more recent studies now agree in the placement of *Causus* inside Viperines (although its position within the subfamily remains under debate, see Herrmann et al., 1999 and Wüster et al., 2008, Pyron et al., 2013). The genus *Azemiops* has been considered as part of viperines but is now undoubtedly considered as a distinct subfamily closely related to the crotalines (Liem et al., 1971, Orlov et al., 2013, Pyron et al., 2013). However, crotalines relationships closer to the root have been a controversial issue, in particular with respect to whether *Hypnale*, *Garthius*, *Deinagkistrodon*, *Calloselasma* and *Tropidolaemus* form a monophyletic clade (Malhortra and Thorpe, 2004, Malhortra et al., 2010) or a paraphyletic configuration (Parkinson et al., 2002, Castoe and Parkinson, 2006). Morphological studies suggested a polyphyletic origin of NW pit vipers (e.g. Brattstrom, 1964, Burger, 1971) but molecular works strongly support the monophyletism of the clade suggesting a single invasion of the New World via the Beringian Land Bridge, which seems to be the predominant view (Parkinson et al., 2002, Castoe and Parkinson, 2006, Wüster et al., 2008). However, neither morphological nor molecular studies were able to confidently recover the intergeneric relationships among most NW genera (e.g. Gutberlet and Harvey, 2002, Castoe and Parkinson, 2006).

In the past years, the access to new Viperidae DNA sequences has greatly improved the limited phylogenetic inference done solely based on morphological data (see Castoe and Parkinson, 2006) but few studies (Fenwick et al., 2012; Pyron et al., 2013; Wüster et al., 2008) that have used molecular data have investigated the phylogenetic relationships of vipers in a broader phylogenetic context. The pioneering study by Wüster et al. (2008) included all except one Viperidae genera in their molecular analysis but was limited to 85 species and therefore explored the phylogenetic relationships among higher taxa. Although recent works by Fenwick et al. (2012) and Pyron et al. (2013) included 218 and 209 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) 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 (e.g. Viperidae crown group), 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 is possible to use additional fossils for conducting dating analysis of vipers (see Material and Methods) avoiding biogeographic events, which have been suggested to be problematic on 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 vipers' diversification.

Herein we assembled the most complete time-calibrated molecular dataset of the family Viperidae to investigate the phylogenetic relationships and time of divergence among vipers using a Bayesian approach.

MATERIAL AND METHODS

Taxon sampling and data acquisition

The Reptile Database (Uetz and Hosek 2014) currently recognizes 329 species in the family Viperidae. From those, we included 261 species as terminal taxa in the ingroup of our phylogenetic tree. As in Pyron et al. (2013), we also included *Bothrops colombiensis*, *B. isabelae* and *Crotalus tortugensis* as distinct species although some researchers (e. g., Campbell and Lamar, 2004; Castoe and Parkinson, 2006; Uetz and Hosek 2014) consider the first two as synonyms of *B. atrox* and the third as synonym of *C. atrox* (but see Grismer, 1999). Thus, our sampling for Viperidae encompasses 264 taxa corresponding to 79.5% 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 (72/98), 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).

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 all 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 165 new sequences for vipers. 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 the most recently published viper phylogenies. We include 46 more species than Fenwick et al. (2012) and 55 more than Pyron et al. (2013). Moreover, our molecular dataset comprises 11 genes and 1192 sequences whereas Fenwick et al. (2012) and Pyron et al. (2013) comprise 785 and 854 sequences for 4 and 10 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 10712 base pairs (bp) and dataset 2 contained 4419 bp. 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). 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. Fossils details and justifications on the choice of calibration schemes are described below.

1. Stem-Alethinophidia

Haasiophis terrasanctus Tchernov, Rieppel, Zaher, Polcyn, Jacobs 2000. Set as the most recent common ancestor of *Indotyphlops braminus* and Alethinophidia. A complete, articulated specimen recovered from the Ein Yabrud quarries, near Ramallah, West Bank Palestinian Territories (Hebrew University of Jerusalem, Paleontological Collections, HUJ-Pal. EJ 695). The position of the Ein Yabrud quarries within the Cenomanian is unclear, and may fall either in the Early Cenomanian Bet-Meir Formation (Chalifa and Tchernov, 1982) or the Late Cenomanian Amminadav Formation (Chalifa, 1985). Root minimum age constrained to 93.9 Ma. We follow Head (2015) in using an age range that reflects the overall uncertainty of the position of Ein Yabrud, with the minimum age set for the Cenomanian (Ogg and Hinnov, 2012). Hsiang et al. (2015) concluded that *Haasiophis terrasanctus* is positioned as a stem-Alethinophidia instead of a crown-Alethinophidia in their combined, molecular and morphological, unconstrained analysis (their figure 3). Hsiang et al.'s unconstrained topology represents a more inclusive hypothesis for the position of *Haasiophis terrasanctus*. Because Hsiang et al.'s analysis incorporates the known phylogenetic uncertainties related to the position of this fossil, and it is based on the largest set of unconstrained evidence, we prefer to use *Haasiophis* as a stem-althinophidian instead of a crown-taxa as traditionally used.

2. Stem-Colubroidea

Procerophis sahnii Rage, Folie, Rana, Singh, Rose, Smith 2008. Set as the most recent common ancestor of Colubroidea and the remaining snakes. Minimum age constrained to 54 Ma. *Procerophis sahnii* is known from the early Eocene (early Ypresian) Cambay Formation of Vastan Lignite Mine, Gujarat, India (Sahni et al., 2006; Rana et al., 2008; Rage et al., 2008). A single well-preserved posterior trunk vertebra (Rana Collection from Vastan, VAS 1014). The shape of the vertebra, prezygapophyseal processes and paracotylar foramina suggest the fossil belongs to the Colubroidea (Rage et al., 2008).

3. Stem-Boinae

Titanoboa cerrejonensis Head, Bloch, Hastings, Bourque, Cadena, Herrera, Polly, Jaramillo 2009. Set as the most recent common ancestor of Boinae and the remaining snakes. Minimum age constrained to 58 Ma. (Jaramillo et al., 2007; Head, 2015). *Titanoboa cerrejonensis* is known from the middle-late Paleocene (Palynological zone Cu-02) Cerrejón Formation of La Puente Pit, Cerrejón Coal Mine, Guajira Peninsula, Colombia (Jaramillo et al., 2005, 2007). A single precloacal vertebra (Florida Museum of Natural History, UF/IGM 1). Paracotylar foramina morphology and a convex anterior zygosphene margin suggests this fossil belongs to the Boinae (Head et al., 2009, 2015; Albino and Brizuela, 2014). Additionally, a preliminary analysis of cranial elements by Head et al. (2013) places it in the stem lineage of Boinae.

4. Stem-Elapidae

Elapidae gen. & sp. indet. Kuch, Müller, Mödden, Mebs 2006. Set as the most common recent ancestor of Elapidae and the remaining snakes. Minimum age constrained to 19.5 Ma. (set as the minimum age estimate for the MN2 unit of the European Land Mammal Age) (Kuch et al., 2006; Hilgen et al., 2012). Several tubular fangs deposited in the collection of the Institut Für Geowissenschaften der Universität Mainz, Germany, and referred to an early elapid species from the Early Miocene (Aquitanian) of the Oppenheim/Nierstein quarry, Mainz basin, Rhineland-

Palatinate, Germany. Kuch et al. (2006) considered the material to belong to a member of the family Elapidae based on confident similarities in the curvature and suture present between the fossil material and present-day elapids.

5. Stem-Viperidae

Viperidae. gen. & sp. indet. Kuch, Müller, Mödden, Mebs 2006. Set as the most common recent ancestor of Viperidae and the remaining snakes. Minimum age constrained to 19.5 Ma. (set as the minimum age estimate for the MN2 unit of the European Land Mammal Age; Kuch et al., 2006; Hilgen et al., 2012). Several fangs deposited in the collection of the Institut Für Geowissenschaften der Universität Mainz, Germany, and referred to a viperid species from the Early Miocene (Aquitanian) of the Oppenheim/Nierstein quarry, Mainz basin, Rhineland-Palatinate, Germany. Kuch et al. (2006) referred the fangs to a member of the family Viperidae based on details of the curvature, length, presence of prominent anterior blade ridges, and shape of the venom discharge orifice.

6. Stem-*Sistrurus*

Sistrurus sp. indet. Parmley and Holman 2007. Set as the most common recent ancestor of *Sistrurus catenatus* and the remaining snakes. Minimum age constrained to 10.3 Ma. (set as the minimum age estimate for the Clarendonian NALMA; Hilgen et al., 2012). A well preserved trunk vertebra (University of Nabraska State Museum, UNSM 100251) from the late Miocene of the Ash Hollow formation (Clarendonian), recovered from from fluvial channel fill of the Pratt Slide site, located near Johnstown, Brown County, Nebraska, USA. The fossil is referred to the extant genus *Sistrurus* Parmley and Holman (2007) based on the presence of zygosphenal spine considered a derived trait unique to the genus.

We used lognormal prior distributions to incorporate uncertainty surrounding fossil calibration. Lognormal is often the most appropriate distribution for calibrations using paleontological data assigning the highest point probability for the calibration point to be somewhat older than the oldest fossil (Ho and Phillips, 2009). In all cases, hard minimum bounds were set accordingly to the minimum age of the geological interval that the fossil belongs. The establishment of maximum bounds to calibrate prior distributions is regarded as critical (Ho and Phillips, 2009) especially when dealing with groups with a relatively poor fossil record like vipers. We set the maximum 95% in three different manners: around 61.5 mya, when the calibration point located at the divergence between the Viperidae and their sister group, 47.4 mya, when calibration point was located in the ingroup, and around 145 mya in the other cases. 61.5 mya is the age of divergence between Viperidae and the outgroup estimated by Wüster et al. (2008), 47.4 mya is the age of the most recent common ancestor (MRCA) of Viperidae estimated by Wüster et al. (2008) and 145 mya refers to the age of the oldest snake fossil known (Colston et al., 2013; Hofstetter, 1960).

For each concatenated dataset we ran four replicates of our phylogenetic analyses with different starting trees or random seed 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.

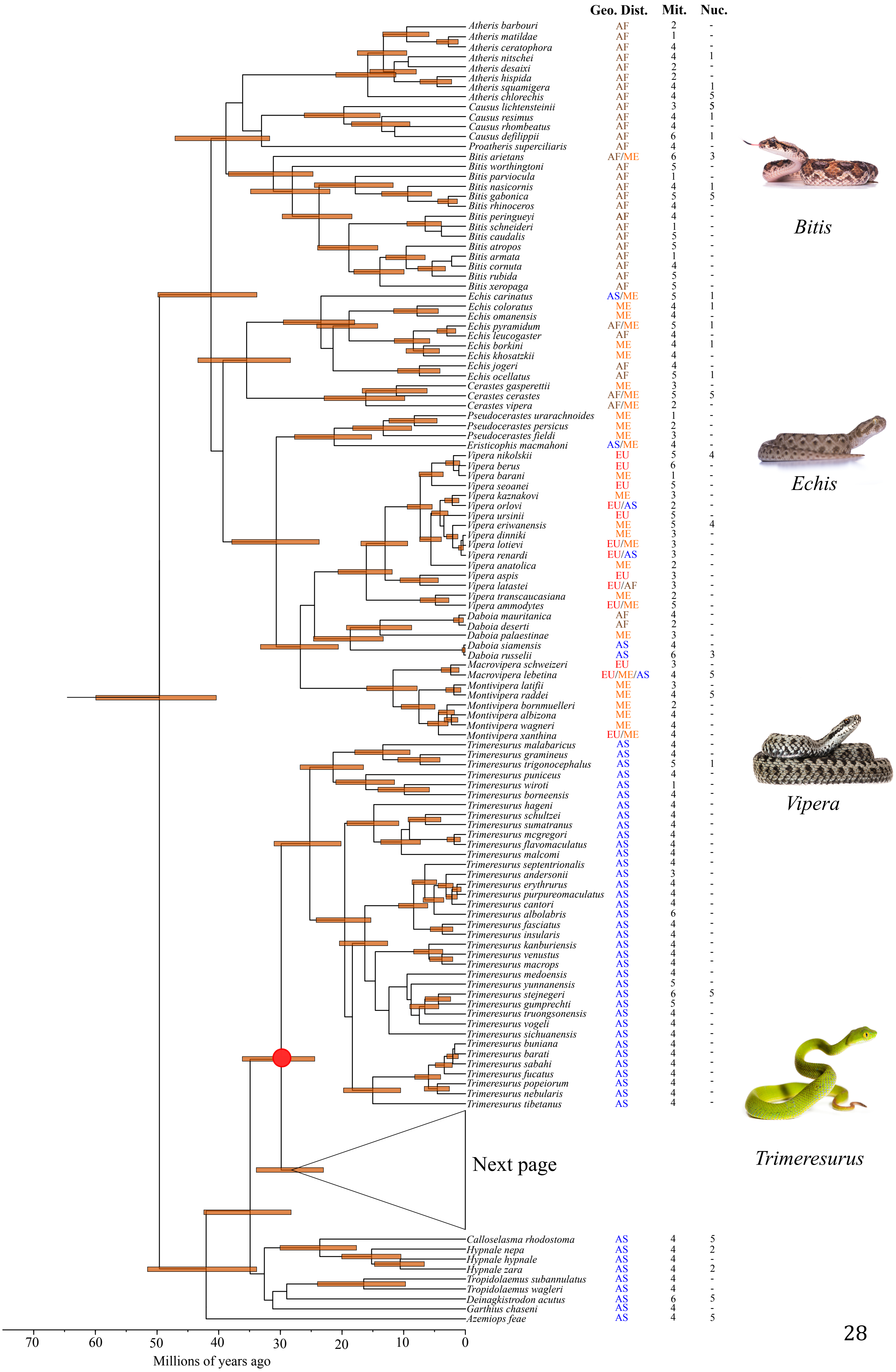
RESULTS

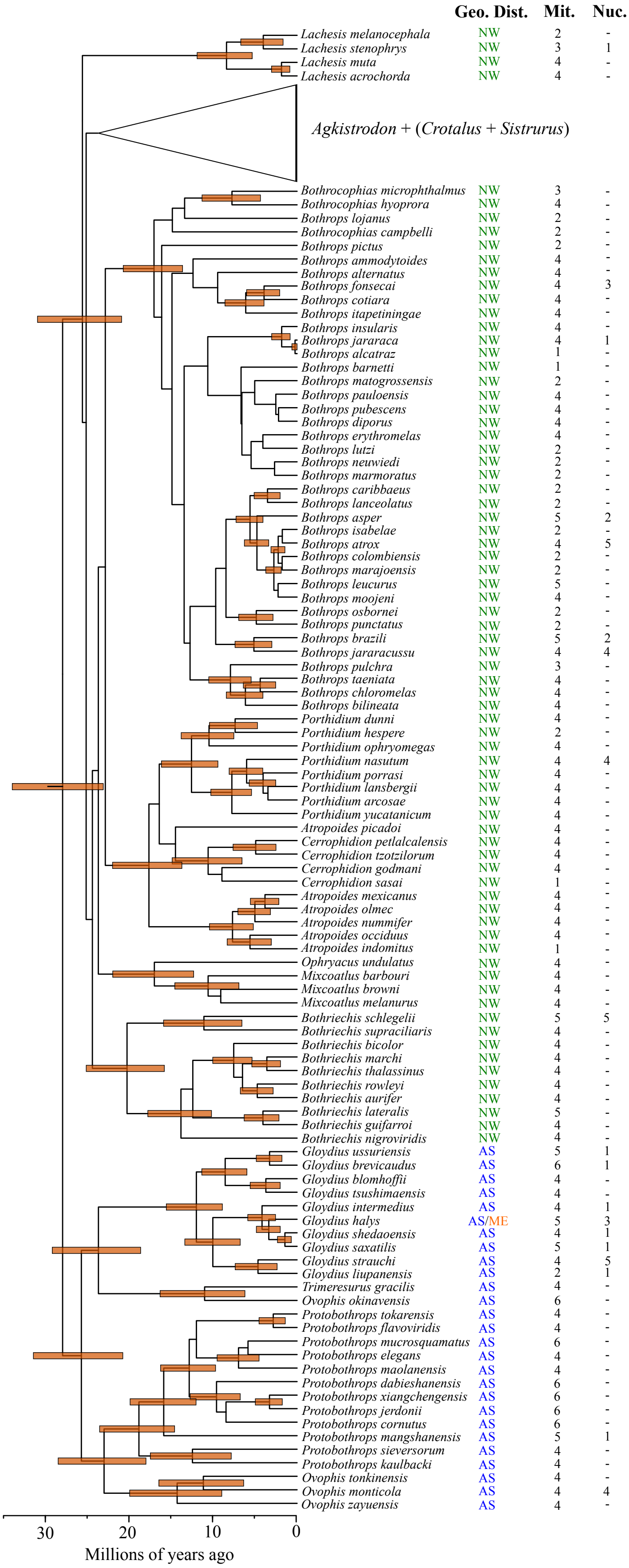
We generated maximum credibility trees after discarding burn-in with a posterior probability limit of 95% (Figure 1, Figure S1, Figure S2). Both datasets yielded similar topologies and divergent times estimates for the family Viperidae (Figures 1 and S2, Table 1). Thus, results will focus on our main 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 1, Figure 1 and Figure S2). The complete maximum credibility tree for dataset 1 including the outgroup and calibration points can be visualized through Figure 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 Figure 1) confirming the monophyly of the family and subfamilies (Viperinae, Azemiopinae 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, 22 presented high posterior probabilities in favor of their monophyletism (see Discussion 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 (Figure 1). Among the remaining lineages of the subfamily Viperinae, *Echis* and *Cerastes* also showed a robust sister relationship (Figure 1). Regarding the affinities in the subfamily Crotalinae, *Hypnale*, *Garthius*, *Deinagkistrodon*, *Calloselasma* and *Tropidolaemus* formed a weak supported monophyletic clade sister to the remaining crotalines but *Hypnale* and *Calloselasma* formed a well-supported clade (Figure 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 (Figure 1). Among the 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* (Figure 1). However, more inclusive relationships and some higher-level relationships were recovered with low support among New World pit vipers (see Figure 1).

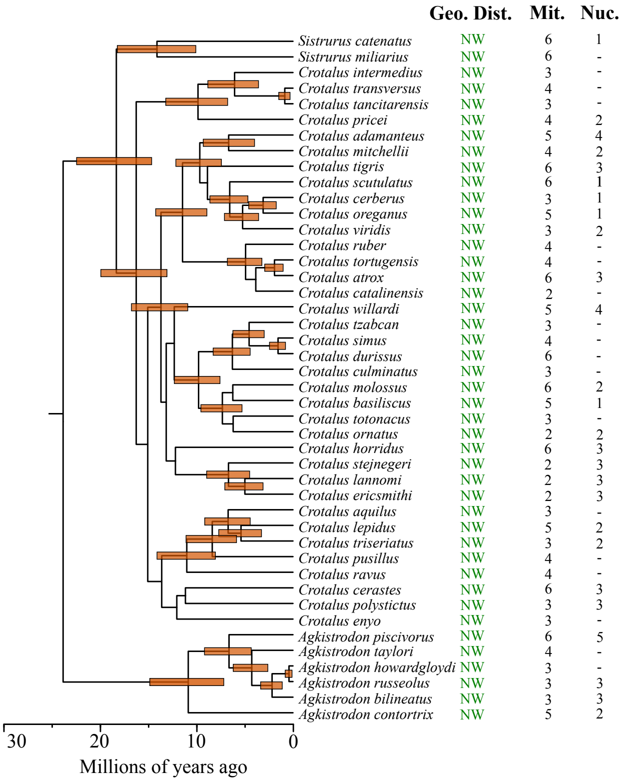
Table 1. Comparison of age divergence estimates (in millions of years ago; mean and 95% high posterior density interval) between datasets analyzed in the present study, Fenwick et al. (2012), and Wüster et al. (2008). tmrca = time of maximum recent common ancestor, *approximate age.

	Dataset 1 (this study)		**Dataset 2 (this study)**		**Wüster et al. 2008**		**Fenwick et al. (2012)***	
Root (*Indotyphlops braminus* vs others)	111.73	95.3 - 134.03	108.52	94.87 - 129.27	-	-	-	-
tmrca Viperidae	49.67	40.44 - 59.98	52.41	43.17 - 62.58	47.4	38.1 - 57.4	25.5	-
tmrca Viperinae	41.3	33.86 - 49.91	44.86	36.87 - 53.75	39.7	31.9 - 48.1	22.5	-
tmrca Crotalinae	34.96	28.31 - 42.45	37.65	30.71 - 45.27	31.2	25.4 - 37.8	17.5	-
tmrca *Trimeresurus*	25.27	20.21 - 31.07	27.37	22.16 - 32.97	-	-	-	-
tmrca NW Crotalinae	25.68	20.99 - 31.05	27.86	23.18 - 33.53	22.1	17.9 - 26.9	12.5	-
tmrca *Lachesis*	8.44	5.37 - 11.96	9.26	5.93 - 12.95	6.5	3.5 - 9.8	-	-
tmrca *Sistrurus*	14.1	10.07 - 18.21	14.29	10.16 - 18.45	-	-	-	-


Geo. Dist. Mit. Nuc.
Atheris barbouri AF 2 -
Atheris matildae AF 1 -
Atheris ceratophora AF 4 -
Atheris nitschei AF 4 1
Atheris desaixi AF 2 -
Atheris hispida AF 2 -
Atheris squamigera AF 4 1
Atheris chlorechis AF 4 5
Causus lichtensteinii AF 3 5
Causus resimus AF 4 1
Causus rhombeatus AF 4 -
Causus defilippii AF 6 1
Proatheris superciliaris AF 4 -
Bitis arietans AF/ME 6 3
Bitis worthingtoni AF 5 -
Bitis parviocula AF 1 -
Bitis nasicornis AF 4 1
Bitis gabonica AF 5 5
Bitis rhinoceros AF 4 -
Bitis peringueyi AF 4 -
Bitis schneideri AF 1 -
Bitis caudalis AF 5 -
Bitis atropos AF 5 -
Bitis armata AF 1 -
Bitis cornuta AF 4 -
Bitis rubida AF 5 -
Bitis xeropaga AF 5 -
Echis carinatus AS/ME 5 1
Echis coloratus ME 4 1
Echis omanensis ME 4 -
Echis pyramidum AF/ME 5 1
Echis leucogaster AF 4 -
Echis borkini ME 4 1
Echis khosatzkii ME 4 -
Echis jogeri AF 4 -
Echis ocellatus AF 5 1
Cerastes gasperettii ME 3 -
Cerastes cerastes AF/ME 5 5
Cerastes vipera AF/ME 2 -
Pseudocerastes urarachnoides ME 1 -
Pseudocerastes persicus ME 2 -
Pseudocerastes fieldi ME 3 -
Eristicophis macmahoni AS/ME 4 -
Vipera nikolskii EU 5 4
Vipera berus EU 6 -
Vipera barani ME 1 -
Vipera seoanei EU 5 -
Vipera kaznakovi ME 3 -
Vipera orlovi EU/AS 2 -
Vipera ursinii EU 5 -
Vipera eriwanensis ME 5 4
Vipera dinniki ME 3 -
Vipera lotievi EU/ME 3 -
Vipera renardi EU/AS 3 -
Vipera anatolica ME 2 -
Vipera aspis EU 3 -
Vipera latastei EU/AF 3 -
Vipera transcaucasiana ME 2 -
Vipera ammodytes EU/ME 5 -
Daboia mauritanica AF 4 -
Daboia deserti AF 2 -
Daboia palaestinae ME 3 -
Daboia siamensis AS 4 -
Daboia russelii AS 6 3
Macrovipera schweizeri EU 3 -
Macrovipera lebetina EU/ME/AS 4 5
Montivipera latifii ME 3 -
Montivipera raddei ME 4 5
Montivipera bornmuelleri ME 2 -
Montivipera albizona ME 4 -
Montivipera wagneri ME 4 -
Montivipera xanthina EU/ME 4 -
Trimeresurus malabaricus AS 4 -
Trimeresurus gramineus AS 4 -
Trimeresurus trigonocephalus AS 5 1
Trimeresurus puniceus AS 4 -
Trimeresurus wiroti AS 1 -
Trimeresurus borneensis AS 4 -
Trimeresurus hageni AS 4 -
Trimeresurus schultzei AS 4 -
Trimeresurus sumatranus AS 4 -
Trimeresurus mcgregori AS 4 -
Trimeresurus flavomaculatus AS 4 -
Trimeresurus malcomi AS 4 -
Trimeresurus septentrionalis AS 4 -
Trimeresurus andersonii AS 3 -
Trimeresurus erythrurus AS 4 -
Trimeresurus purpureomaculatus AS 4 -
Trimeresurus cantori AS 4 -
Trimeresurus albolabris AS 6 -
Trimeresurus fasciatus AS 4 -
Trimeresurus insularis AS 4 -
Trimeresurus kanburiensis AS 4 -
Trimeresurus venustus AS 4 -
Trimeresurus macrops AS 4 -
Trimeresurus medoensis AS 4 -
Trimeresurus yunnanensis AS 5 -
Trimeresurus stejnegeri AS 6 5
Trimeresurus gumprechti AS 5 -
Trimeresurus truongsonensis AS 4 -
Trimeresurus vogeli AS 4 -
Trimeresurus sichuanensis AS 4 -
Trimeresurus buniana AS 4 -
Trimeresurus barati AS 4 -
Trimeresurus sabahi AS 4 -
Trimeresurus fucatus AS 4 -
Trimeresurus popeiorum AS 4 -
Trimeresurus nebularis AS 4 -
Trimeresurus tibetanus AS 4 -
Next page
Calloselasma rhodostoma AS 4 5
Hypnale nepa AS 4 2
Hypnale hypnale AS 4 -
Hypnale zara AS 4 2
Tropidolaemus subannulatus AS 4 -
Tropidolaemus wagleri AS 4 -
Deinagkistrodon acutus AS 6 5
Garthius chaseni AS 4 -
Azemiops feae AS 4 5
Bitis
Echis
Vipera
Trimeresurus
70
60
50
40
30
20
10
0
Millions of years ago



	Geo. Dist.	Mit.	Nuc.
Lachesis melanocephala	NW	2	-
Lachesis stenophrys	NW	3	1
Lachesis muta	NW	4	-
Lachesis acrochorda	NW	4	-
Bothrocophias microphthalmus	NW	3	-
Bothrocophias hyoprora	NW	4	-
Bothrops lojanus	NW	2	-
Bothrocophias campbelli	NW	2	-
Bothrops pictus	NW	2	-
Bothrops ammodytoides	NW	4	-
Bothrops alternatus	NW	4	-
Bothrops fonsecai	NW	4	3
Bothrops cotiara	NW	4	-
Bothrops itapetiningae	NW	4	-
Bothrops insularis	NW	4	-
Bothrops jararaca	NW	4	1
Bothrops alcatraz	NW	1	-
Bothrops barnetti	NW	1	-
Bothrops matogrossensis	NW	2	-
Bothrops pauloensis	NW	4	-
Bothrops pubescens	NW	4	-
Bothrops diporus	NW	4	-
Bothrops erythromelas	NW	4	-
Bothrops lutzi	NW	2	-
Bothrops neuwiedi	NW	2	-
Bothrops marmoratus	NW	2	-
Bothrops caribbaeus	NW	2	-
Bothrops lanceolatus	NW	2	-
Bothrops asper	NW	5	2
Bothrops isabelae	NW	2	-
Bothrops atrox	NW	4	5
Bothrops colombiensis	NW	2	-
Bothrops marajoensis	NW	2	-
Bothrops leucurus	NW	5	-
Bothrops moojeni	NW	4	-
Bothrops osbornei	NW	2	-
Bothrops punctatus	NW	2	-
Bothrops brazili	NW	5	2
Bothrops jararacussu	NW	4	4
Bothrops pulchra	NW	3	-
Bothrops taeniata	NW	4	-
Bothrops chloromelas	NW	4	-
Bothrops bilineata	NW	4	-
Porthidium dunni	NW	4	-
Porthidium hespere	NW	2	-
Porthidium ophryomegas	NW	4	-
Porthidium nasutum	NW	4	4
Porthidium porrasi	NW	4	-
Porthidium lansbergii	NW	4	-
Porthidium arcosae	NW	4	-
Porthidium yucatanicum	NW	4	-
Atropoides picadoi	NW	4	-
Cerrophidion petlalcalensis	NW	4	-
Cerrophidion tzotzilorum	NW	4	-
Cerrophidion godmani	NW	4	-
Cerrophidion sasai	NW	1	-
Atropoides mexicanus	NW	4	-
Atropoides olmec	NW	4	-
Atropoides nummifer	NW	4	-
Atropoides occiduus	NW	4	-
Atropoides indomitus	NW	1	-
Ophryacus undulatus	NW	4	-
Mixcoatlus barbouri	NW	4	-
Mixcoatlus browni	NW	4	-
Mixcoatlus melanurus	NW	4	-
Bothriechis schlegelii	NW	5	5
Bothriechis supraciliaris	NW	4	-
Bothriechis bicolor	NW	4	-
Bothriechis marchi	NW	4	-
Bothriechis thalassinus	NW	4	-
Bothriechis rowleyi	NW	4	-
Bothriechis aurifer	NW	4	-
Bothriechis lateralis	NW	5	-
Bothriechis guifarroi	NW	4	-
Bothriechis nigroviridis	NW	4	-
Gloydius ussuriensis	AS	5	1
Gloydius brevicaudus	AS	6	1
Gloydius blomhoffii	AS	4	-
Gloydius tsushimaensis	AS	4	-
Gloydius intermedius	AS	4	1
Gloydius halys	AS/ME	5	3
Gloydius shedaoensis	AS	4	1
Gloydius saxatilis	AS	5	1
Gloydius strauchi	AS	4	5
Gloydius liupanensis	AS	2	1
Trimeresurus gracilis	AS	4	-
Ovophis okinavensis	AS	6	-
Protobothrops tokarensis	AS	4	-
Protobothrops flavoviridis	AS	4	-
Protobothrops mucrosquamatus	AS	6	-
Protobothrops elegans	AS	4	-
Protobothrops maolanensis	AS	4	-
Protobothrops dabieshanensis	AS	6	-
Protobothrops xiangchengensis	AS	6	-
Protobothrops jerdonii	AS	6	-
Protobothrops cornutus	AS	6	-
Protobothrops mangshanensis	AS	5	1
Protobothrops sieversorum	AS	4	-
Protobothrops kaulbacki	AS	4	-
Ovophis tonkinensis	AS	4	-
Ovophis monticola	AS	4	4
Ovophis zayuensis	AS	4	-



	Geo. Dist.	Mit.	Nuc.
Sistrurus catenatus	NW	6	1
Sistrurus miliarius	NW	6	-
Crotalus intermedius	NW	3	-
Crotalus transversus	NW	4	-
Crotalus tancitarensis	NW	3	-
Crotalus pricei	NW	4	2
Crotalus adamanteus	NW	5	4
Crotalus mitchellii	NW	4	2
Crotalus tigris	NW	6	3
Crotalus scutulatus	NW	6	1
Crotalus cerberus	NW	3	1
Crotalus oreganus	NW	5	1
Crotalus viridis	NW	3	2
Crotalus ruber	NW	4	-
Crotalus tortugensis	NW	4	-
Crotalus atrox	NW	6	3
Crotalus catalinensis	NW	2	-
Crotalus willardi	NW	5	4
Crotalus tzabcan	NW	3	-
Crotalus simus	NW	4	-
Crotalus durissus	NW	6	-
Crotalus culminatus	NW	3	-
Crotalus molossus	NW	6	2
Crotalus basiliscus	NW	5	1
Crotalus totonacus	NW	3	-
Crotalus ornatus	NW	2	2
Crotalus horridus	NW	6	3
Crotalus stejnegeri	NW	2	3
Crotalus lannomi	NW	2	3
Crotalus ericsmithi	NW	2	3
Crotalus aquilus	NW	3	-
Crotalus lepidus	NW	5	2
Crotalus triseriatus	NW	3	2
Crotalus pusillus	NW	4	-
Crotalus ravus	NW	4	-
Crotalus cerastes	NW	6	3
Crotalus polystictus	NW	3	3
Crotalus enyo	NW	3	-
Agkistrodon piscivorus	NW	6	5
Agkistrodon taylori	NW	4	-
Agkistrodon howardgloydi	NW	3	-
Agkistrodon russeolus	NW	3	3
Agkistrodon bilineatus	NW	3	3
Agkistrodon contortrix	NW	5	2

Bothrops

Crotalus

Figure 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 (Geo. Dist.) and the number of mitochondrial (Mit.) and nuclear (Nuc.) gene sequences for each taxon are also shown. AF (brown) = Africa, ME (orange) = Middle East, EU (red) = Europe, AS (blue) = Asia, NW (green) = New World. Red circle indicates the position of the speciation rate shift (see Chapter II). Images of vipers by Thor Hakonsen (http://thorhakonsen.com/).

Divergence time estimates suggest that the ancestral of vipers diverged from its sister group in the Paleocene (around 64.5 Mya) and that crown vipers started to diversify around the late Paleocene to middle Eocene (59.9 - 40.4 Mya) (95% high posterior density interval of the main dataset). During the Eocene, around 49.91 - 33.86 Mya, the Viperinae subfamily started to diversify. The crown group of crotalines is placed around 42.45 - 28.31 Mya in the middle Eocene to late Oligocene. 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 Figure 1 and Table 1.

DISCUSSION

Phylogenetic inference

Phylogenetic relationships among vipers recovered in the present study agree in many aspects with those from previously published works (e.g. Pyron et al., 2013; Wüster et al., 2008). Although all subfamilies and the majority of genera were considered monophyletic we could not recover monophyly for the following genera: *Bothrops* and *Bothrocophias*, *Atropoides*, *Trimeresurus* and *Ovophis*.

The Andean *Bothrops lojanus* have 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) have been recovered with great confidence in both morphological and molecular analyses (Carrasco et al., 2012; Pyron et al., 2013; 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 and Parkinson, 2006; Castoe et al., 2009; 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 monophyletism of the genus (Castoe and Parkinson, 2006; Castoe et al., 2009). 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 seems to be insufficient to elucidate the relationship between *A. picadoi* and the remaining *Atropoides*.

Like previous studies (e.g. Malhotra et al., 2010; Pyron et al., 2013; Wüster et al., 2008), 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; Pyron et al., 2013; Wüster et al., 2008). Here, we not just found a monophyletic clade comprising "*Ovophis*" *okinavensis*, "*Trimeresurus*" *gracilis* and the genus *Gloydius* but we recovered this relationship with a very high support value (>0.99) contrasting with the results from other studies (e.g. Pyron et al., 2013). 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* (Malhortra and Thorpe, 2004; Malhortra et al., 2010). However, more studies are needed in order to make a reliable decision.

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 (see 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, Figure 1), *Cerastes* (Wüster et al., 2008; Figure S2) or *Echis* (Pyron et al., 2013). Interestingly, molecular studies have found weak support for these more inclusive positions of *Causus* among viperines. Additionally, the present study corroborates Wüster et al. (2008) and Pyron et al. (2013) in recovering well-supported phylogenetic relationships between Eurasian vipers but differs from these studies as we found a strongly supported relationship 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 we found this clade to be monophyletic agreeing with Malhotra et al. (2010) who also analyzed nuclear sequences, both studies recovered a weakly supported configuration. Paraphyletic configurations were frequently recovered in studies using only mitochondrial genes (Castoe and Parkinson, 2006; Wüster et al., 2008) or including nuclear genes for only one genus (Pyron et al., 2013) but also with weak support. Despite our dataset comprising only mitochondrial genes (dataset 2) also suggested a weakly supported monophyletic configuration, posterior probability values doubled when we included nuclear sequences. That is, including nuclear sequences for other species belonging to this crotaline clade (e.g. *Tropidolaemus* species, *Garthius chaseni*) might be the key to obtain higher support values and confidently estimate the relationship between these genera connected by long-branches. Irrespective to the true configuration of *Tropidolaemus*, *Deinagkistrodon*, *Garthius*, *Hypnale* and *Calloselasma*, a strongly supported relationship between *Calloselasma* and *Hypnale* have been recovered here and in other studies (Pyron et al., 2013; Wüster et al., 2008).

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; 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 viper phylogenetics (e.g. Gutberlet and Harvey, 2002; Pyron et al., 2013; Wüster et al., 2008), 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 Figure 1). However, differently from morphological analyses (e.g. Gutberlet and Harvey, 2002), molecular studies recovered a well-supported clade comprising *Porthidium*, *Cerrophidion* and *Atropoides* (Pyron et al., 2013; Wüster et al., 2008; present study), and our results also agree with Pyron et al. (2013) and Wüster et al. (2008) in recovering highly supported clades comprising the genera *Crotalus* + *Sistrurus*, and *Mixcoatlus* + *Ophryacus*.

Divergence time estimates

Our dating analyses suggest slightly older ages for Viperidae than those proposed by Wüster et al. (2008) (Table 1). However, our date estimates are much older than those suggested by Fenwick et al. (2012) (Table 1). 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.

Although the oldest fossil known for vipers dates back to 19.5 Mya (early Miocene, Kuch et al., 2006), our date estimates agree with Wüster et al. (2008) that the ancestral of vipers diverged from its sister group during the Paleocene. Age estimates for the crown group 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 crown group ages most likely dating back to the Eocene. Additionally, close to the Oligocene/Miocene boundary the subfamily Crotalinae invaded the New World. The invasion of the New World by crotalines has been proposed to have occurred as a single event via the Beringian Land Bridge (e.g. Wüster et al., 2002; Wuster et al., 2008). The Beringian Land Bridge was an important corridor for vertebrate dispersal but since its formation around 100 Mya, dispersal by many warm adapted taxa was only possible during the increased temperatures that maintained a Boreotropical forest belt throughout the Eocene during the Eocene climatic optimum (Wolfe, 1975, Towsend et al., 2011). Given the uncertainty in our date estimates, crotalines could have started to diversify both during the Eocene or the Oligocene not providing confidence on whether this lineage have dispersed to North America through the Boreotropical forest belt (Eocene) or during a cooler time period (Oligocene).

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). It is generally accepted that the emergence of the Isthmus occurred at about 3.5 Mya (but see Bacon et al., 2013, 2015), making the dispersion explanation for older lineages of South American pit vipers like *Bothrops* and *Bothrocophias*, more elusive. These older pit viper clades may have invaded South America by stepping-stone dispersal over the Central American archipelago, as suggested for other lineages (e.g. Jansa et al., 2013). In fact, very recently, studies have suggested that migration through the

Isthmus of Panama (not necessarily a fully continuous landmass) might in fact have occurred much earlier (Bacon et al., 2013, 2015; Farris et al., 2011; Montes et al., 2012) at some point between early Oligocene and early Miocene (31 - 16.1 Mya). Our date estimates for *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.

CONCLUSIONS

The phylogenetic relationships among vipers recovered well-supported clades with most genera being monophyletic. However, like 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 also 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. On the other hand, we could not recover well supported relationships for some deeper nodes in NW pit vipers indicating that the clade still needs further investigation. Our date estimates suggested that vipers started to diversify around the end of the middle Paleocene/middle Eocene epochs recovering older ages than previous studies. Until the early Miocene pit vipers emerged and rapidly colonized the NW. Moreover, date estimates for South American clades are in accordance with an earlier migration than the usually accepted date for the emergence of the Isthmus of Panama.

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SUPPLEMENTARY FIGURES

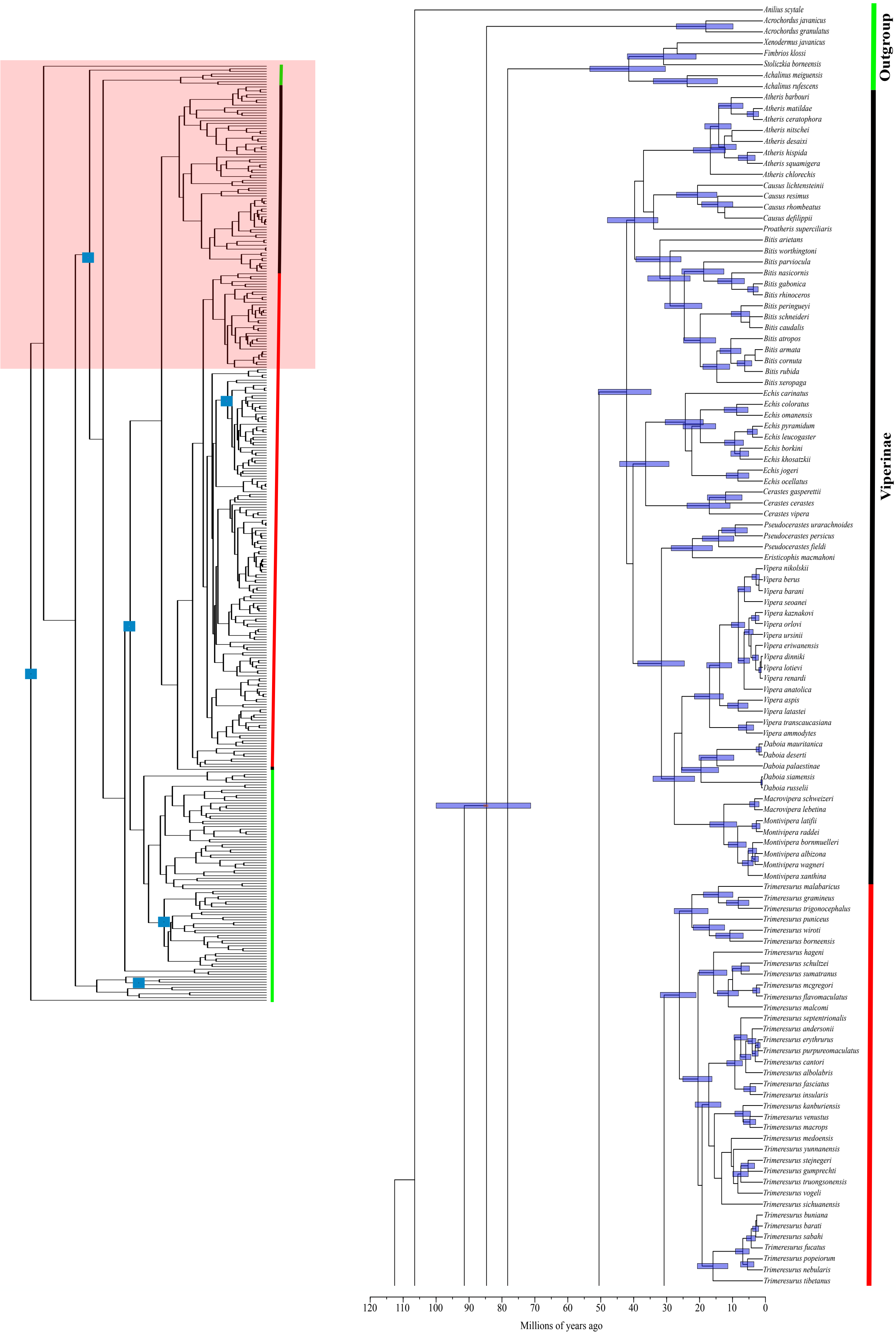
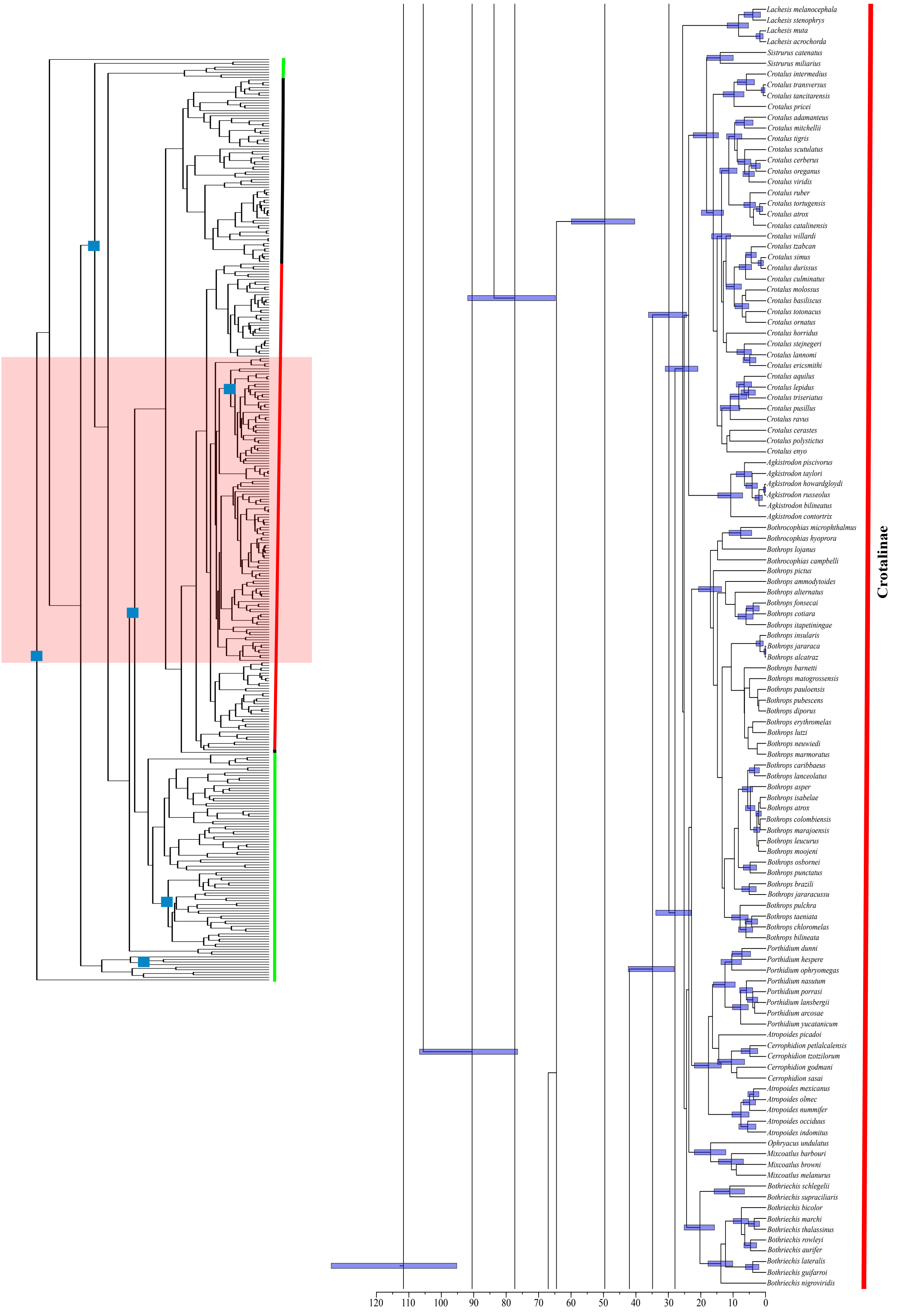


Figure S1. part1



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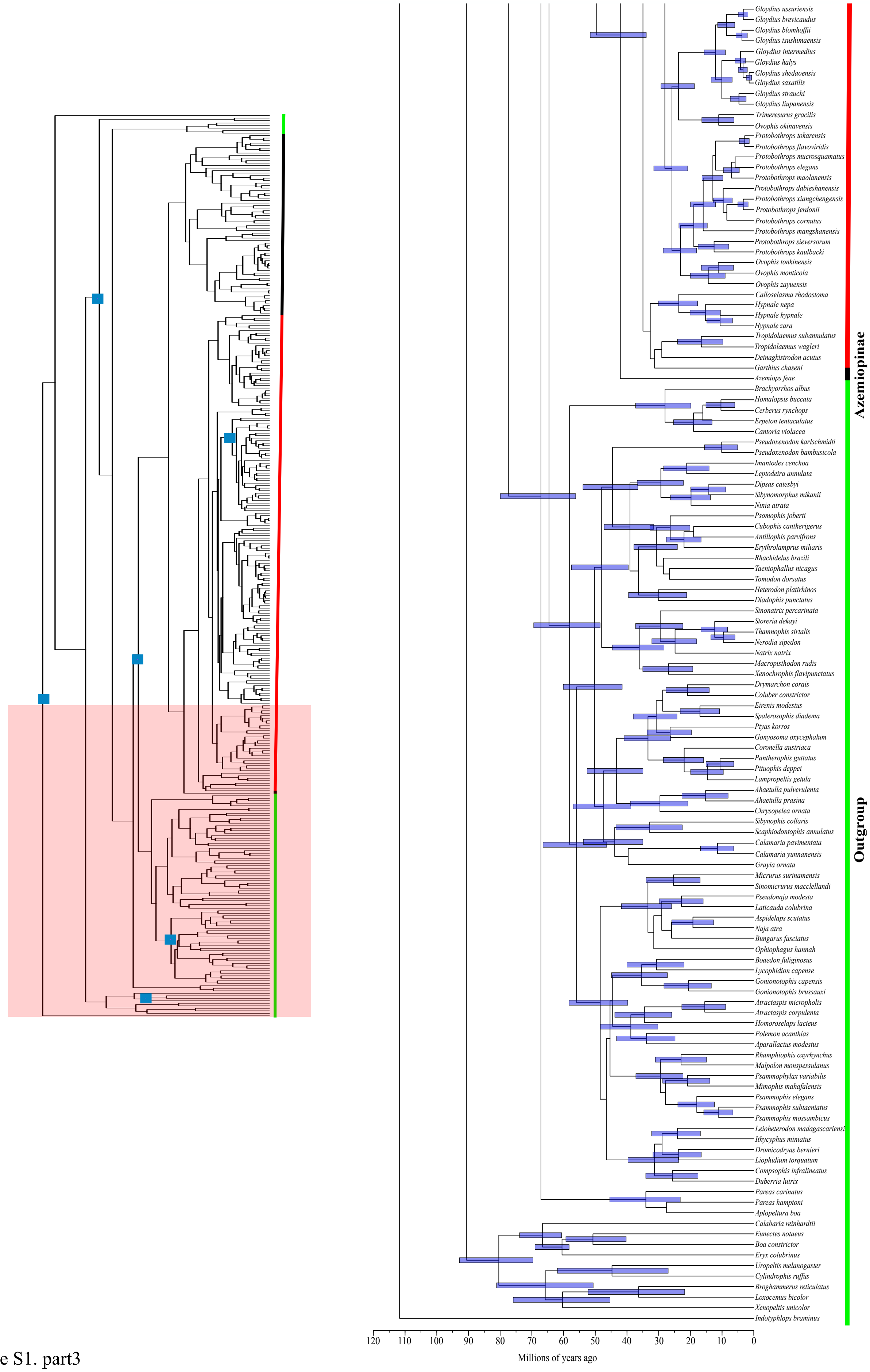

Figure S1. part3

Figure S1. Full version (including the outgroup) of the maximum credibility tree generated for the family Viperidae using the dataset comprising sequences from all 11 genes. Blue squares denote calibration points. 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. Large red squares denote the position zoomed.

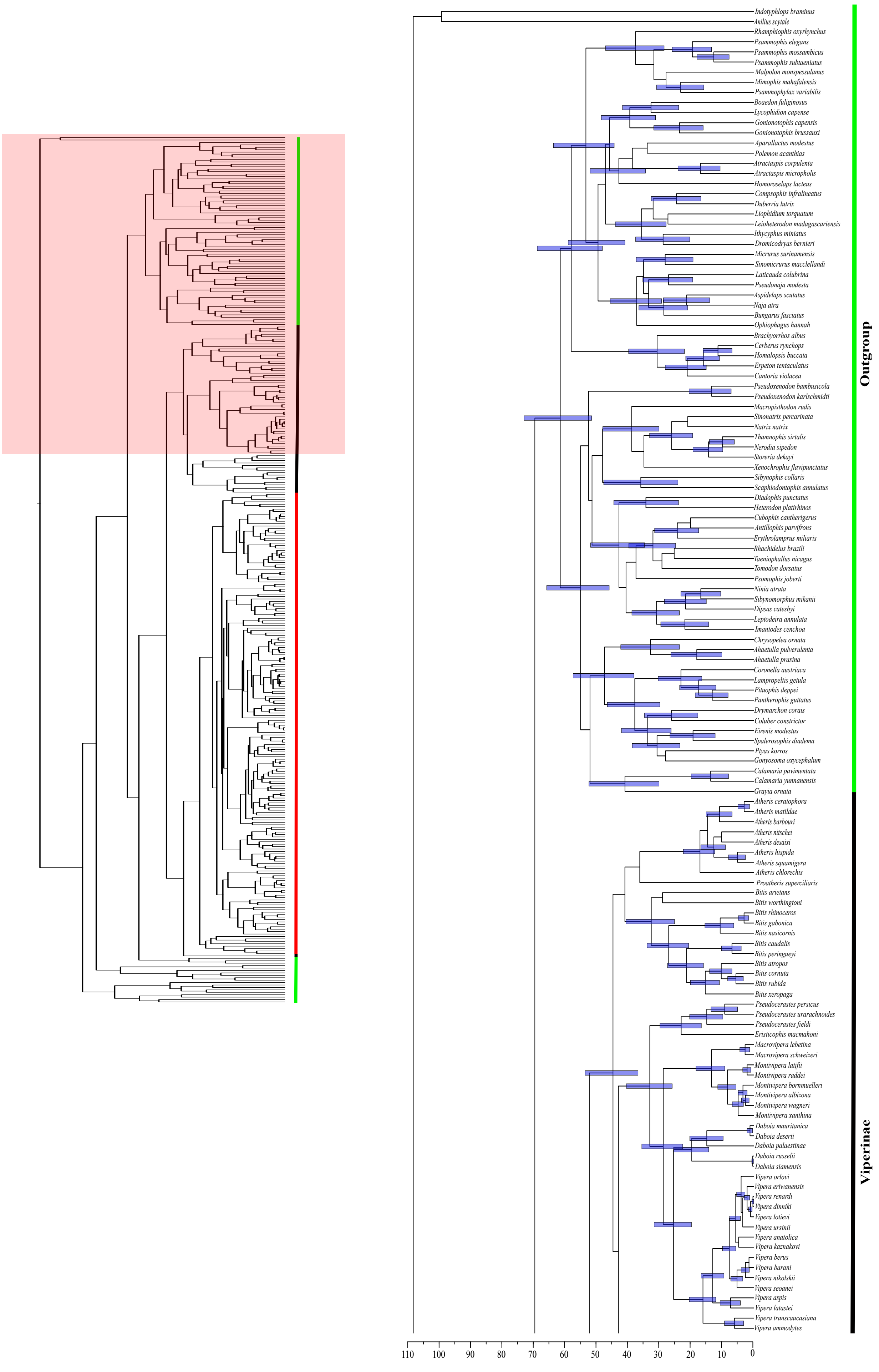

Figure S2. part1

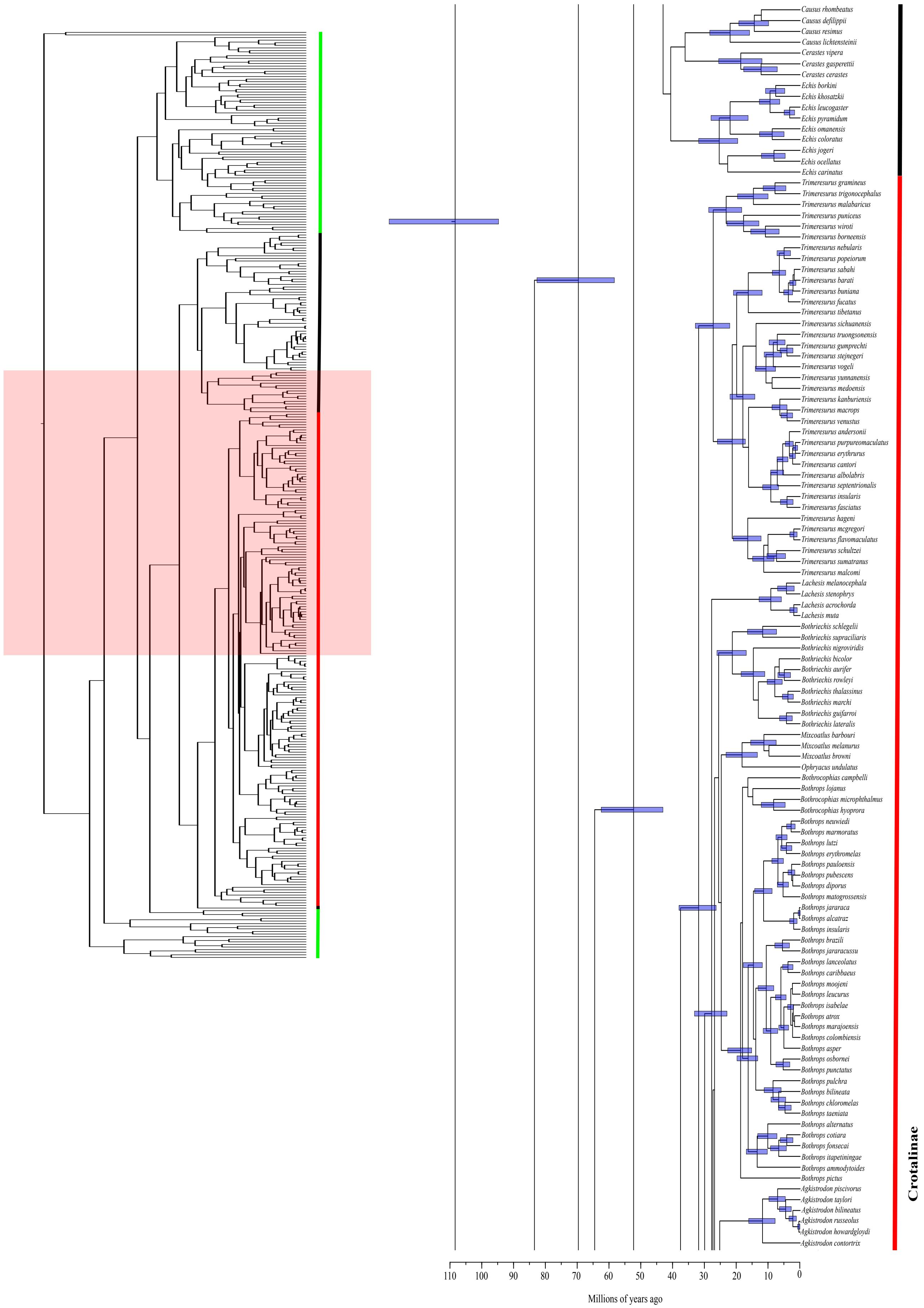

Figure S2. part2

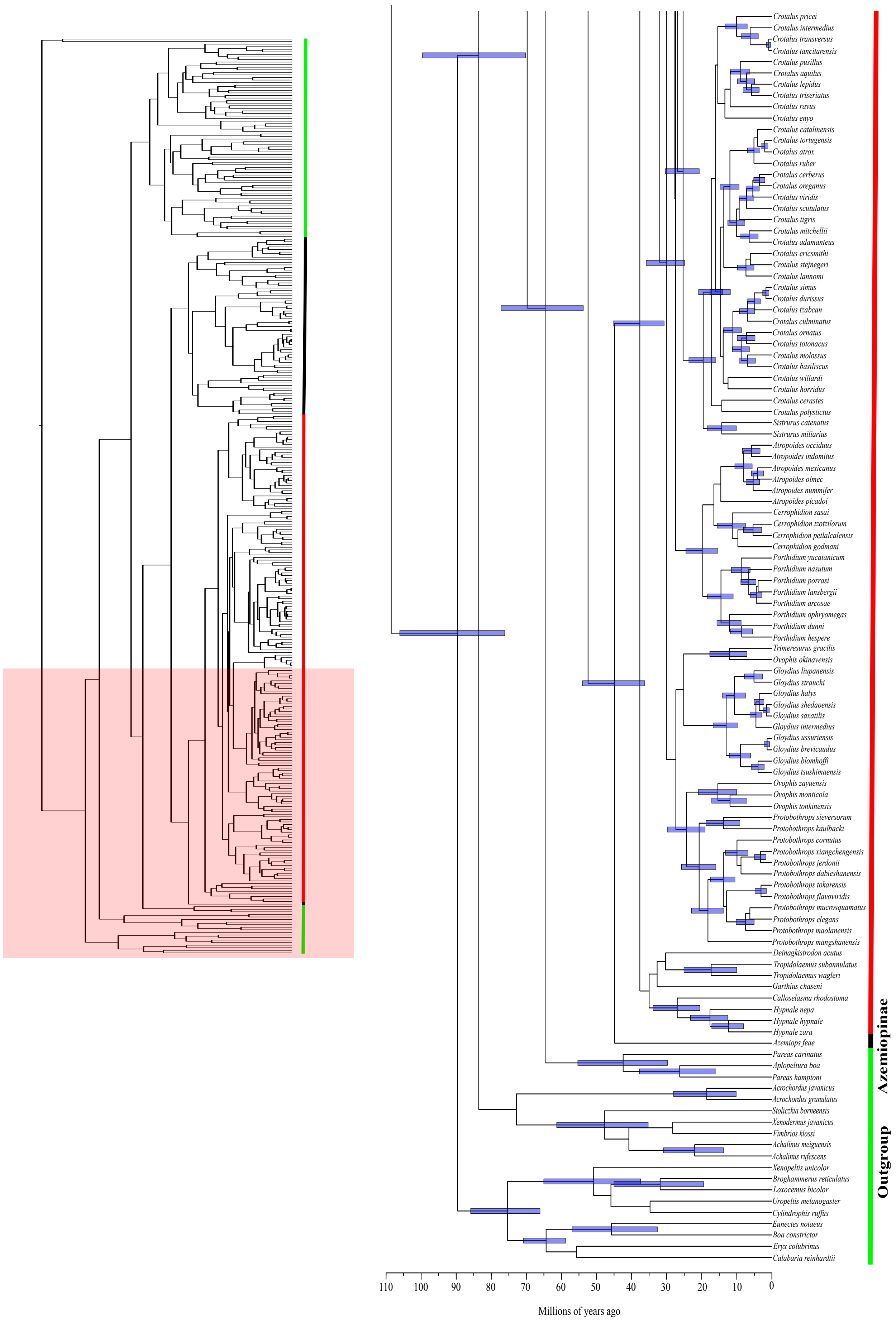

Figure S2. part3

Figure S2. Maximum credibility tree generated for the family Viperidae using the concatenated dataset comprising sequences from 12S, 16S, Cytb and NAD4 genes. These four mitochondrial genes comprise sequences representing the majority of the species included in the phylogenetic analysis. 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. Large red squares denote the position zoomed.

SUPPLEMENTARY TABLES

Table S1. GenBank and Bold accession numbers of sequences analyzed. Accession numbers in bold correspond to new sequences to be deposited in GenBank. * denotes sequences obtained from the Bold systems database. YPX = State Key Laboratory of Genetic resources and Evolution, Kunming Institute of Zoology. Murphy et al. (2011): Murphy, J.C., Mumpuni, Sanders, K.L., 2011. First molecular evidence for the phylogenetic placement of the enigmatic snake genus *Brachyorrhos* (Serpentes: Caenophidia). Molecular Phylogenetics and Evolution 61(3), 953-957.

Species	**Mitochondrial genes**						**Nuclear genes**				
	12S	16S	Cytb	ND4	ND2	Cox1	Cmos	Rag1	NT3	Bndf	Jun
Ingroup											
Agkistrodon_bilineatus	AF156591	AF156570	-	AF156583	-	-	-	-	-	-	-
Agkistrodon_contortrix	AF259224	AF259117	EU483263	AF156576	-	EANAH587-12*	-	EU402833	-	EU402623	-
Agkistrodon_howardgloydi	AF156592	AF156571	-	AF156584	-	-	-	-	-	-	-
Agkistrodon_piscivorus	NC_009768-**YPX802**	EF669477-**YPX802**	DQ523161	DQ523161	DQ523161	NC_009768	**YPX802**	**YPX802**	**YPX802**	**YPX802**	**YPX802**
Agkistrodon_russeolus	L01766.1	AF156573	-	AF156586	-	-	-	-	-	-	-
Agkistrodon_taylori	AF156590	AF156569	EU483477	AF156581	-	-	-	-	-	-	-
Atheris_barbouri	-	AJ275739	AJ275686	-	-	-	-	-	-	-	-
Atheris_ceratophora	DQ305410	DQ305433	DQ305456	DQ305474	-	-	-	-	-	-	-
Atheris_chlorechis	**YPX797**	**YPX797**	**YPX797**	EU624211	-	-	**YPX797**	**YPX797**	**YPX797**	**YPX797**	**YPX797**
Atheris_desaixi	-	AJ275733	AJ275680	-	-	-	-	-	-	-	-
Atheris_hispida	-	AJ275734	AJ275681	-	-	-	-	-	-	-	-
Atheris_matildae	-	-	JF825389	-	-	-	-	-	-	-	-
Atheris_nitschei	AY223650	AY223663	AF471070	AY223618	-	-	AF471125	-	-	-	-
Atheris_squamigera	AF544762	EU624279	EU624303	EU624212	-	-	AF544734	-	-	-	-
Atropoides_indomitus	-	-	-	KC354700	-	-	-	-	-	-	-
Atropoides_mexicanus	KC847268	KC847255	KC847271	KC847289	-	-	-	-	-	-	-
Atropoides_nummifer	DQ305422	DQ305445	DQ061195	DQ061220	-	-	-	-	-	-	-
Atropoides_occiduus	DQ305423	DQ305446	AY220315	AY220338	-	-	-	-	-	-	-
Atropoides_olmec	AY223656	AY223669	AY220321	AY220344	-	-	-	-	-	-	-
Atropoides_picadoi	AF057208	AF057255	AY223583	U41872	-	-	-	-	-	-	-
Azemiops_feae	**YPX807**	AF512748-**YPX807**	AY352747-**YPX807**	AY352808	-	-	**YPX807**	EU402836	**YPX807**	EU402628	**YPX807**
Bitis_arietans	**YPX408**	**YPX408**	AY235728-**YPX408**	EU852304	JX073288	EU852310	-	**YPX408**	**YPX408**	-	**YPX408**
Bitis_armata	-	-	-	-	JX073291	-	-	-	-	-	-
Bitis_atropos	EU624246	EU624281	AJ275691	EU624214	JX073287	-	-	-	-	-	-
Bitis_caudalis	EU624247	EU624282	AJ275693	EU624215	JX073293	-	-	-	-	-	-
Bitis_cornuta	EU624248	EU624283	EU624305	EU624216	-	-	-	-	-	-	-
Bitis_gabonica	**YPX816**	**YPX816**	AJ275695-**YPX816**	EU624217	JX073296	-	**YPX816**	**YPX816**	**YPX816**	**YPX816**	**YPX816**
Bitis_nasicornis	DQ305411	DQ305434	DQ305457	DQ305475	-	-	AY187970	-	-	-	-
Bitis_parviocula	-	-	-	-	JX073292	-	-	-	-	-	-
Bitis_peringueyi	DQ305412	DQ305435	DQ305458	DQ305476	-	-	-	-	-	-	-

Bitis_rhinoceros	EU624250	EU624285	AJ275696	EU624218	-	-	-	-	-	-	-
Bitis_rubida	EU624251	EU624286	EU624306	EU624219	JX073290	-	-	-	-	-	-
Bitis_schneideri	-	-	-	-	JX073297	-	-	-	-	-	-
Bitis_worthingtoni	EU624252	AJ275745	AJ275692	EU624220	JX073295	-	-	-	-	-	-
Bitis_xeropaga	EU624253	EU624287	EU624307	EU624221	JX073294	-	-	-	-	-	-
Bothriechis_aurifer	DQ305425	DQ305448	DQ305466	DQ305483	-	-	-	-	-	-	-
Bothriechis_bicolor	DQ305426	DQ305449	DQ305467	DQ305484	-	-	-	-	-	-	-
Bothriechis_guifarroi	KC847265	KC847264	KC847274	KC847287	-	-	-	-	-	-	-
Bothriechis_lateralis	AF057211	AF057258	AY223588	U41873	-	EANAH450-12*	-	-	-	-	-
Bothriechis_marchi	DQ305428	DQ305451	DQ305469	DQ305486	-	-	-	-	-	-	-
Bothriechis_nigroviridis	AF057212	AF057259	AY223589	AY223635	-	-	-	-	-	-	-
Bothriechis_rowleyi	DQ305427	DQ305450	DQ305468	DQ305485	-	-	-	-	-	-	-
Bothriechis_schlegelii	AF057213	AF057260	AY223590	AF292611	-	EANAH419-12*	AF544680	AY487374	FJ434084	FJ433983	-
Bothriechis_supercilliaris	DQ305429	DQ305452	DQ305470	DQ305487	-	-	-	-	-	-	-
Bothriechis_thalassinus	DQ305424	DQ305447	DQ305465	DQ305482	-	-	-	-	-	-	-
Bothrocophias_campbelli	-	-	AF191582	AF292622	-	-	-	-	-	-	-
Bothrocophias_hyoprora	AF057206	AF057253	AY223593	AF292614	-	-	-	-	-	-	-
Bothrocophias_microphthalmus	AY223657	AY223670	-	AY223638	-	-	-	-	-	-	-
Bothrops_alcatraz	-	-	AY865822	-	-	-	-	-	-	-	-
Bothrops_alternatus	AY223660	AY223673	AY223601	AY223642	-	-	-	-	-	-	-
Bothrops_ammodytoides	AY223658	AY223671	AY223595	AY223639	-	-	-	-	-	-	-
Bothrops_asper	AF057218	AF057265	AY223599	U41876	-	EANAH434-12*	-	EU402838	-	EU402630	-
Bothrops_atrox	**YPX813**	**YPX813**	AF246267-**YPX813**	AF292642	-	-	**YPX813**	**YPX813**	**YPX813**	**YPX813**	**YPX813**
Bothrops_barnetti	-	-	-	-	-	EANAH423-12*	-	-	-	-	-
Bothrops_bilineatus	AF057214	AF057261	AY223591	U41875	-	-	-	-	-	-	-
Bothrops_brazili	EU867252	EU867264	AF292597	AF292635	-	EANAH874-12*	**YPX812**	**YPX812**	-	-	-
Bothrops_caribbaeus	-		AF292598	AF292636	-	-	-	-	-	-	-
Bothrops_chloromelas	DQ305430	DQ305453	DQ305471	DQ305488	-	-	-	-	-	-	-
Bothrops_colombiensis	-	-	AF292602	AF292640	-	-	-	-	-	-	-
Bothrops_cotiara	AF057217	AF057264	AY223597	AY223640	-	-	-	-	-	-	-
Bothrops_diporus	DQ305431	DQ305454	DQ305472	DQ305489	-	-	-	-	-	-	-
Bothrops_erythromelas	AF057219	AF057266	AY223600	U41877	-	-	-	-	-	-	-
Bothrops_fonsecai	**YPX411**	**YPX411**	**YPX411**	AF292618	-	-	**YPX411**	**YPX411**	-	-	**YPX411**
Bothrops_insularis	AF057216	AF057263	AY223596	AF292628	-	-	-	-	-	-	-
Bothrops_isabelae	-	-	AF292603	AF292641	-	-	-	-	-	-	-
Bothrops_itapetiningae	EU867253	EU867265	EU867277	EU867289	-	-	-	-	-	-	-
Bothrops_jararaca	**YPX412**	EU867266	**YPX412**	AY122860	-	-	-	-	**YPX412**	-	-
Bothrops_jararacussu	**YPX413**	**YPX413**	AY223602-**YPX413**	AF292634	-	-	**YPX413**	**YPX413**	**YPX413**	-	**YPX413**
Bothrops_lanceolatus	-	-	AF292599	AF292637	-	-	-	-	-	-	-
Bothrops_leucurus	EU867255	EU867267	EU867279	EU867291	-	EANAH422-12*	-	-	-	-	-
Bothrops_lojanus	-	-	FR691566	FR691536	-	-	-	-	-	-	-
Bothrops_lutzi	-	-	KF801131	KF801261	-	-	-	-	-	-	-
Bothrops_marajoensis	-	-	AF292605	AF292643	-	-	-	-	-	-	-
Bothrops_marmoratus	-	-	KF801137	KF801265	-	-	-	-	-	-	-
Bothrops_mattogrossensis	-	-	KF801150	KF801278	-	-	-	-	-	-	-
Bothrops_moojeni	EU867256	EU867268	EU867282	EU867293	-	-	-	-	-	-	-
Bothrops_neuwiedi	-	-	AF292585	AF292623	-	-	-	-	-	-	-
Bothrops_osbornei	-	-	AF292595	AF292633	-	-	-	-	-	-	-

Bothrops_pauloensis	EU867260	EU867272	EU867284	EU867296	-	-	-	-	-	-	-
Bothrops_pictus	-	-	AF292583	AF292621	-	-	-	-	-	-	-
Bothrops_pubescens	JN870180	JN870192	JN870200	JN870206	-	-	-	-	-	-	-
Bothrops_pulchra	JN870179	-	AF292593	AF292631	-	-	-	-	-	-	-
Bothrops_punctatus	-	-	AF292594	AF292632	-	-	-	-	-	-	-
Bothrops_taeniata	**YPX414**	**YPX414**	AY223592-**YPX414**	AF292629	-	-	-	-	-	-	-
Calloselasma_rhodostoma	**YPX806**	**YPX806**	AF292569	AY352813	-	-	**YPX806**	**YPX806**	**YPX806**	**YPX806**	**YPX806**
Causus_defilippii	AF057186	AF057233	AY223556	NC13479	NC13479	NC13479	-	-	-	EU402633	-
Causus_lichtensteini	**YPX796**	**YPX796**	**YPX796**	-	-	-	**YPX796**	**YPX796**	**YPX796**	**YPX796**	**YPX796**
Causus_resimus	AY223649	AY223662	AY223555	AY223616	-	-	AF544696	-	-	-	-
Causus_rhombeatus	DQ305409	DQ305432	DQ305455	U41866	-	-	-	-	-	-	-
Cerastes_cerastes	**YPX800**	**YPX800**	AF471028	EU624222	-	EU852311	AF471131-AF544679-**YPX800**	EU852329	**YPX800**	**YPX800**	**YPX800**
Cerastes_gasperettii	JN870181	AJ275756	AJ275704	-	-	-	-	-	-	-	-
Cerastes_vipera	-	AJ275757	AJ275705	-	-	-	-	-	-	-	-
Mixcoatlus_barbouri	HM363639	HM363640	HM363641	HM363642	-	-	-	-	-	-	-
Cerrophidion_godmani	DQ305419	DQ305442	AY220325	AY220348	-	-	-	-	-	-	-
Cerrophidion_petlalcalensis	DQ305420	DQ305443	DQ061202	DQ061227	-	-	-	-	-	-	-
Cerrophidion_sasai	-	-	-	DQ061225	-	-	-	-	-	-	-
Cerrophidion_tzotzilorum	JN870182	JN870193	DQ061203	DQ061228	-	-	-	-	-	-	-
Crotalus_adamanteus	AF259255-**YPX793**	**YPX793**	AF259185	U41880	-	KC750807	**YPX793**	JN621000	**YPX793**	-	**YPX793**
Crotalus_aquilus	AF259232	AF259125	AF259162	HQ257782	-	-	-	-	-	-	-
Crotalus_atrox	AF259256	AF259148	AY223608	DQ679873	AY016239	EANAH725-12*	JN620892	JN621002	KF410312	-	-
Crotalus_basiliscus	AF259244	AF259136	AY704845	AY704894	AY704796	-	-	-	KF410313	-	-
Crotalus_catalinensis	AF259259	AF259151	-	-	-	-	-	-	-	-	-
Crotalus_cerastes	AF259235	AF259128	U69773	JN620962	AY016245	EANAH642-12*	JN620894	JN621003	KF410314	-	-
Crotalus_cerberus	-	-	JN620813	JN620963	AY016225	-	JN620895	-	-	-	-
Crotalus_culminatus	-	-	AY704830	AY704880	AY704785	-	-	-	-	-	-
Crotalus_durissus	AF259248	AF259140	AF200223	AF292608	AY704789	JQ627372	-	-	-	-	-
Crotalus_enyo	AF259245	AF259137	-	-	AY016246	-	-	-	-	-	-
Crotalus_ericsmithi	-	-	KF410284	KF410290	-	-	KF410301	KF410330	KF410315	-	-
Crotalus_horridus	AF259252	AF259144	AF337057	NC14400	NC14400	NC14400	KF410302	JN621004	KF410316	-	-
Crotalus_intermedius	AF259238	AF259131	-	JN870208	-	-	-	-	-	-	-
Crotalus_lannomi	-	-	KF410280	KF410292	-	-	KF410303	KF410331	KF410318	-	-
Crotalus_lepidus	AF259230	AF259123	AF259160	U41881	-	EANAH735-12*	JN620898	JN621005	-	-	-
Crotalus_mitchellii	AF259250	AF259142	-	JN620967	AY016241	-	JN620899	JN621006	-	-	-
Crotalus_molossus	AF259243	AF259135	JN620845	JN620995	AY704797	EANAH740-12*	JN620924	JN621030	-	-	-
Crotalus_oreganus	AF259253	AF259145	AF147857	AF194161	AY016221	-	AF471135	-	-	-	-
Crotalus_ornatus	-	-	JN620820	JN620970	-	-	JN620902	JN621009	-	-	-
Crotalus_polystictus	AF259236	AF512747	-	KF410294	-	-	KF410305	KF410332	KF410320	-	-
Crotalus_pricei	AF259237	AF259130	-	JN022878	-	EANAH897-12*	KF410306	-	KF410321	-	-
Crotalus_pusillus	AF259229	AF259122	AF259159	HQ257774	-	-	-	-	-	-	-
Crotalus_ravus	AF259228	AF259121	AY223609	AY223647	-	-	-	-	-	-	-
Crotalus_ruber	AF259261	AF259153	-	DQ679838	-	EANAH1090-13*	-	-	-	-	-
Crotalus_scutulatus	AF259254	AF259146	AF292571	AF194167	AY016237	EANAH766-12*	-	-	KF410322	-	-
Crotalus_simus	EU624240	EU624274	EU624302	AY704885	-	-	-	-	-	-	-
Crotalus_stejnegeri	-	-	KF410283	KF410296	-	-	KF410308	KF410333	KF410323	-	-
Crotalus_tancitarensis	JN022897	JN022897	-	JN022851	-	-	-	-	-	-	-

Crotalus_tigris	AF259249	AF259141	AY223606	AF156574	AY016240	EANAH747-12*	JN620900	JN621007	GQ334665	-	-
Crotalus_tortugensis	AF259257	AF259149	AF259187	DQ679839	-	-	-	-	-	-	-
Crotalus_totonacus	-	-	AY704839	AY704889	AY704795	-	-	-	-	-	-
Crotalus_transversus	AF259239	JN022895	AF259169	JN022840	-	-	-	-	-	-	-
Crotalus_triseriatus	HQ257625	HQ257625	-	HQ257870	-	-	KF410310	-	KF410325	-	-
Crotalus_tzcaban	-	-	AY704806	AY704856	AY704791	-	-	-	-	-	-
Crotalus_viridis	-	-	JN620819	AF194160	AY016235	-	JN620901	JN621008	-	-	-
Crotalus_willardi	AF259242-**YPX794**	AF259133-**YPX794**	AF259170-**YPX794**	JN870209	-	EANAH756-12*	**YPX794**	**YPX794**	**YPX794**	**YPX794**	-
Daboia_deserti	-	AJ275765	AJ275712	-	-	-	-	-	-	-	-
Daboia_mauritanica	EU624261	EU624295	EU624313	EU624229	-	-	-	-	-	-	-
Daboia_palaestinae	JN870183	AJ275775	AJ275722	-	-	-	-	-	-	-	-
Daboia_russellii	EU913478	NC011391	NC11391	EU913478	NC11391	NC011391	AF471156	EU402843	-	EU402636	-
Daboia_siamensis	DQ305413	DQ305436	DQ305459	DQ305477	-	-	-	-	-	-	-
Deinagkistrodon_acutus	DQ343647-**YPX799**	NC010223-**YPX799**	EU913476-**YPX799**	DQ343647	DQ343647	NC010223	**YPX799**	**YPX799**	**YPX799**	**YPX799**	**YPX799**
Echis_borkini	GQ359644	GQ359730	GQ359486	GQ359563	-	-	-	-	-	-	-
Echis_carinatus	EU852313	EU852319	EU852295	GQ359524	-	EU852307	-	EU852325	-	-	-
Echis_coloratus	EU852315	EU852321	EU852297	EU624224	-	EU852309	-	EU852327	-	-	-
Echis_jogeri	GQ359641	GQ359732	GQ359476	GQ359560	-	-	-	-	-	-	-
Echis_khosatzkii	GQ359615	EU642575	EU642584	GQ359536	-	-	-	-	-	-	-
Echis_leucogaster	GQ359622	GQ359705	GQ359456	GQ359541	-	-	-	-	-	-	-
Echis_ocellatus	EU852312	EU852318	EU642592	GQ359510	-	EU852306	-	EU852324	-	-	-
Echis_omanensis	GQ359631	GQ359715	GQ359466	GQ359550	-	-	-	-	-	-	-
Echis_pyramidum	EU852314	EU852320	EU852296	EU624226	-	EU852308	-	EU852326	-	-	-
Eristicophis_macmahoni	EU624259	EU624293	AJ275711	EU624227	-	-	-	-	-	-	-
Garthius_chaseni	AY352791	AY352729	AY352760	AY352825	-	-	-	-	-	-	-
Gloydius_blomhoffi	AY352780	AY352719	AY352751	AY352814	-	-	-	-	-	-	-
Gloydius_brevicaudus	AY352781	AY352720	AY352752	AY352815	NC_011390	NC_011390	JQ687505	-	-	-	-
Gloydius_halys	**YPX792**	AF057238	AY223564	AY223621	AY662540	-	-	AY662614_**YPX782**	**YPX792**	**YPX792**	-
Gloydius_intermedius	EF012806	JN870194	JN870201	U41869	-	-	JQ687507	-	-	-	-
Gloydius_liupanensis	-	-	JQ687491	JQ687472	-	-	JQ687510	-	-	-	-
Gloydius_saxatilis	JN870185	JN870195	JN870202	JN870210	-	JQ798882	JQ687508	-	-	-	-
Gloydius_shedaoensis	AF057194	AF057241	AY223566	AY223623	-	-	AF435019	-	-	-	-
Gloydius_strauchi	EF012813-**YPX798**	AF057239	AY223563-**YPX798**	EF012795	-	-	**YPX798**	**YPX798**	**YPX798**	**YPX798**	**YPX798**
Gloydius_tsushimaensis	JN870186	JN870196	JN870203	JN870211	-	-	-	-	-	-	-
Gloydius_ussuriensis	AF057193	AF057240	AY223565	AY223622	-	JQ798886	JQ687520	-	-	-	-
Hypnale_hypnale	AY352778	AY352717	AY352750	AY352812	-	-	-	-	-	-	-
Hypnale_nepa	KC347325	KC347362	KC347463	KC347491	-	-	KC347401	KC347439	-	-	-
Hypnale_zara	KC347326	KC347363	KC347485	KC347513	-	-	KC347402	KC347440	-	-	-
Lachesis_acrochorda	JN870187	JN870197	JN870204	JN870212	-	-	-	-	-	-	-
Lachesis_melanocephala	-	-	U96018	U96028	-	-	-	-	-	-	-
Lachesis_muta	AF057221	AF057268	AY223604	AY223644	-	-	-	-	-	-	-
Lachesis_stenophrys	AF057220	AF057267	AY223603	EU402644	-	-	-	-	-	-	-
Macrovipera_lebetina	**YPX817**	**YPX817**	AJ275713	DQ897729	-	-	**YPX817**	**YPX817**	**YPX817**	**YPX817**	**YPX817**
Macrovipera_schweizeri	EU624262	AJ275768	AJ275715	-	-	-	-	-	-	-	-
Mixcoatlus_browni	HM363650	HM363651	HM363652	HM363653	-	-	-	-	-	-	-
Montivipera_albizona	EU624265	AJ275780	AJ275727	DQ897731	-	-	-	-	-	-	-
Montivipera_bornmuelleri	-	AJ275779	AJ275726	-	-	-	-	-	-	-	-

Montivipera_latifii	JN870191	JN870199	JN870205	-	-	-	-	-	-	-	-
Montivipera_raddei	**YPX818**	AJ275784	AJ275730-**YPX818**	-	-	EANAH446-12*	**YPX818**	**YPX818**	**YPX818**	**YPX818**	**YPX818**
Montivipera_wagneri	JN870188	AJ275778	AJ275725	JN870213	-	-	-	-	-	-	-
Montivipera_xanthina	EU624268	AJ275777	AJ275724	EU624234	-	-	-	-	-	-	-
Mixcoatlus_melanurus	AF057210	AF057257	AY223587	AY223634	-	-	-	-	-	-	-
Ophryacus_undulatus	AF057209	AF057256	AY223586	AY223633	-	-	-	-	-	-	-
Ovophis_monticola	AY763191-**YPX808**	**YPX808**	AY352748-AF171907	AY352809	-	-	**YPX808**	**YPX808**	**YPX808**	**YPX808**	-
Ovophis_okinavensis	AB175670	AB175670	NC_007397	NC_007397	NC_007397	NC_007397	-	-	-	-	-
Ovophis_tonkinensis	HQ325308	HQ325092	HQ325132	HQ325209	-	-	-	-	-	-	-
Ovophis_zayuensis	HQ325273	HQ325089	HQ325150	HQ325208	-	-	-	-	-	-	-
Porthidium_arcosae	EU624241	EU624275	AF292575	AF292613	-	-	-	-	-	-	-
Porthidium_dunni	AY223654	AY223667	AY223581	DQ061242	-	-	-	-	-	-	-
Porthidium_hespere	-	-	EU017535	EU016099	-	-	-	-	-	-	-
Porthidium_lansbergii	EU624242	EU624276	AY713375	AF393623	-	-	-	-	-	-	-
Porthidium_nasutum	**YPX810**	**YPX810**	DQ061210-**YPX810**	DQ061232	-	-	**YPX810**	-	**YPX810**	**YPX810**	**YPX810**
Porthidium_ophryomegas	AF057205	AF057252	AY223580	U41888	-	-	-	-	-	-	-
Porthidium_porrasi	DQ305421	DQ305444	DQ061214	DQ061237	-	-	-	-	-	-	-
Porthidium_yucatanicum	JN870189	JN870198	DQ061215	DQ061244	-	-	-	-	-	-	-
Proatheris_superciliaris	EU624263	EU624296	AJ275685	EU624230	-	-	-	-	-	-	-
Protobothrops_cornutus	AY294276	AY294267	AY294272	AY294262	NC_022695	NC_022695	-	-	-	-	-
Protobothrops_dabieshanensis	NC_022473	NC_022473	NC_022473	NC_022473	NC_022473	NC_022473	-	-	-	-	-
Protobothrops_elegans	AF057201	AF057248	AY223575	U41893	-	-	-	-	-	-	-
Protobothrops_flavoviridis	AY352792	AY352730	AY223574	AY352826	-	-	-	-	-	-	-
Protobothrops_jerdonii	EF012807	AY763193	AY294274	AY294264	NC_021402	NC_021402	-	-	-	-	-
Protobothrops_kaulbacki	DQ666056	DQ666055	DQ666060	DQ666057	-		-	-	-	-	-
Protobothrops_mangshanensis	AY352787	AY352726	AY352758	AY352821	-	JX233620	JQ687524	-	-	-	-
Protobothrops_maolanensis	JN799405	JN799398	JN799401	JN799409	-	-	-	-	-	-	-
Protobothrops_mucrosquamatus	AY223653	AY223666	AY223577	AY223629	NC_021412	NC_021412	-	-	-	-	-
Protobothrops_sieversorum	DQ305414	DQ305437	AY352753	DQ305478	-	-	-	-	-	-	-
Protobothrops tokarensis	AF057202	AF057249	AY223576	AY223628	-	-	-	-	-	-	-
Protobothrops_xiangchengensis	AY763189	AY763207	DQ666062	DQ666058	KF460436	KF460436	-	-	-	-	-
Pseudocerastes_persicus	-	AJ275770	AJ275717	-	-	-	-	-	-	-	-
Pseudocerastes urarachnoides	-	-	KF314714	-	-	-	-	-	-	-	-
Pseudocerastes_fieldi	EU624264	AJ275769	AJ275716	-	-	-	-	-	-	-	-
Sistrurus_catenatus	DQ464268	AF259119	AY223610	HQ257759	GQ359800	EANAH863-12*	-	-	GQ334686	-	-
Sistrurus miliarius	**YPX795**	AF259120_**YPX795**	EU483385	HQ257760	GQ359815	EANAH768-12*	-	-	-	-	-
Trimeresurus_albolabris	AF057195	AF057242	AY223567	U41890	KF311102	KF311102	-	-	-	-	-
Trimeresurus_andersonii	AY352801	AY352740	-	AY352835	-	-	-	-	-	-	-
Trimeresurus barati	AY371753	AY371769	AY371801	AY371837	-	-	-	-	-	-	-
Trimeresurus_borneensis	AY352783	AY352722	AY352754	AY352817	-	-	-	-	-	-	-
Trimeresurus_buniana	AY371752	AY371778	AY371818	AY371853	-	-	-	-	-	-	-
Trimeresurus cantori	AY352802	AY352741	AF171889	AY352836	-	-	-	-	-	-	-
Trimeresurus_erythrurus	AF517161	AF517174	AF171900	AF517217	-	-	-	-	-	-	-
Trimeresurus_fasciatus	GQ428492	GQ428466	GQ428475	GQ428482	-	-	-	-	-	-	-
Trimeresurus flavomaculatus	AY059535	AY059551	AF171916	AY059584	-	-	-	-	-	-	-
Trimeresurus_fucatus	AY059537	AY059553	AY371796	AY059588	-	-	-	-	-	-	-

Trimeresurus_gracilis	DQ305415	DQ305438	AF171913	AY352823	-	-	-	-	-	-	-
Trimeresurus_gramineus	AY352793	AY352731	AY352762	AY352827	-	-	-	-	-	-	-
Trimeresurus_gumprechti	EU443791	EU443792	AY321489	EU443787	-	KC171172	-	-	-	-	-
Trimeresurus_hageni	AY059536	AY059552	AY059567	AY059585	-	-	-	-	-	-	-
Trimeresurus_insularis	AY059534	AY352738	AY059568	AY352833	-	-	-	-	-	-	-
Trimeresurus_kanburiensis	AY289219	AY352737	AY289225	AY289231	-	-	-	-	-	-	-
Trimeresurus_macrops	AF517163	AF517176	AF517184	AF517219	-	-	-	-	-	-	-
Trimeresurus_malabaricus	AY059548	AY059564	AY059569	AY059587	-	-	-	-	-	-	-
Trimeresurus_malcolmi	AY371758	AY371793	AY371832	AY371861	-	-	-	-	-	-	-
Trimeresurus_mcgregori	AY371756	AY371795	AY371831	AY371858	-	-	-	-	-	-	-
Trimeresurus_medoensis	AY352797	AY352735	AY352765	AY352831	-	-	-	-	-	-	-
Trimeresurus_nebularis	AY371737	AY371774	AY371814	AY371839	-	-	-	-	-	-	-
Trimeresurus_popeiorum	AY059538	AY059554	AY059571	AY059590	-	-	-	-	-	-	-
Trimeresurus_puniceus	AF517164	AF517177	AF517192	AF517220	-	-	-	-	-	-	-
Trimeresurus_purpureomaculatus	AF517162	AF517175	AF517188	AF517218	-	-	-	-	-	-	-
Trimeresurus_sabahi	AY371736	AY371771	AY371815	AY371842	-	-	-	-	-	-	-
Trimeresurus_schultzei	AY352785	AY352725	AY352756	AY352819	-	-	-	-	-	-	-
Trimeresurus_septentrionalis	AY059543	AY352724	AY352755	AY352818	-	-	-	-	-	-	-
Trimeresurus_sichuanensis	HQ850445	HQ850446	HQ850448	HQ850450	-	-	-	-	-	-	-
Trimeresurus_stejnegeri	NC012146-**YPX805**	FJ752492_**YPX805**	**YPX805**	FJ752492	NC_012146	NC_012146	**YPX805**	**YPX805**	**YPX805**	**YPX805**	**YPX805**
Trimeresurus_sumatranus	AY371759	AY371788	AY371828	AY371866	-	-	-	-	-	-	-
Trimeresurus_tibetanus	AY352776	AY352715	AY352749	AY352810	-	-	-	-	-	-	-
Trimeresurus_trigonocephalus	AY059549	AY059565	AF171890	AY059597	-	-	KC347412	-	-	-	-
Trimeresurus_truongsonensis	EU443817	EU443818	EU443815	EU443816	-	-	-	-	-	-	-
Trimeresurus_venustus	AY293931	AY352723	AF171914	AY289228	-	-	-	-	-	-	-
Trimeresurus_vogeli	AF517170	AF517183	AY059574	EU443808	-	-	-	-	-	-	-
Trimeresurus_wiroti	-	-	DQ646788	-	-	-	-	-	-	-	-
Trimeresurus_yunnanensis	EU443813	EU443814	EF597523	EF597527	-	KC171173	-	-	-	-	-
Tropidolaemus_subannulatus	AF057198	AF057245	AY223571	AY223625	-	-	-	-	-	-	-
Tropidolaemus_wagleri	AF517167	AF517180	AF517191	AF517223	-	-	-	-	-	-	-
Vipera_ammodytes	EU624266	EU624297	AM944799	EU624232	AM944799	-	-	-	-	-	-
Vipera_anatolica	-	-	KC316113	-	-	KC122762	-	-	-	-	-
Vipera_aspis	JN870190	-	AM944742	-	AM944744	-	-	-	-	-	-
Vipera_barani	-	-	AY321092	-	-	-	-	-	-	-	-
Vipera_berus	EU543221	DQ186081	DQ186032	DQ897728	EU625373	KC122718	-	-	-	-	
Vipera_dinniki	-	AJ275773	AJ275720	-	-	KC122721	-	-	-	-	-
Vipera_eriwanensis	**YPX803**	**YPX803**	**YPX803**	FR727021	-	KC122733	**YPX803**	-	**YPX803**	**YPX803**	**YPX803**
Vipera_kaznakovi	-	-	AY321093	FR727034	-	KC122724	-	-	-	-	-
Vipera_latastei	-	-	AY321094	JX649572	AY321074	-	-	-	-	-	-
Vipera_lotievi	-	-	JN204717	FR727030		KC122732	-	-	-	-	-
Vipera_nikolskii	**YPX814**	**YPX814**	**YPX814**	-	EU625372	EANAH876-12*	**YPX814**	**YPX814**	-	**YPX814**	**YPX814**
Vipera_orlovi	-	-	KC176746	-	-	KC122734	-	-	-	-	-
Vipera_renardi	-	-	HQ845740	FR727033	-	KC122761		-	-	-	-
Vipera_seoanei	-	AJ275782	DQ186030	FR727035	AY321071	KC122736	-	-	-	-	-
Vipera_transcaucasiana	-	-	DQ186479	-	AY321070	-	-	-	-	-	-
Vipera_ursinii	AF236687	-	AY311383	FR726981	AY321069	KC122737	-	-	-	-	-
Outgroup											

Achalinus_meiguensis	FJ424614	FJ424614	FJ424614	FJ424614	FJ424614	NC_011576	-	-	-	-	-
Achalinus_rufescens	**YPX105**	**YPX105**	**YPX105**	U49319	-	-	-	**YPX105**	**YPX105**	**YPX105**	**YPX105**
Acrochordus_granulatus	AB177879	NC007400	-	NC_007400	-	-	**YPX464**	AY487388_EU402831	-	-	EF144042
Acrochordus_javanicus	**YPX001**	**YPX001**	**YPX001**	HM234055	-	-	-	**YPX001**	**YPX001**	**YPX001**	**YPX001**
Ahaetulla_prasina	**YPX543**	**YPX543**	-	-	-	-	**YPX543**	-	**YPX543**	**YPX543**	**YPX543**
Ahaetulla_pulverulenta	KC347304	-	KC347454	KC347512	-	-	KC347378	KC347416	-	-	-
Anilius_scytale	FJ755180	-	FJ755180	FJ755180	NC_014343	NC_014343	AF544722_AY099965	AY988072_AY487382	AY988055	FJ433961	FJ434012
Antillophis_parvifrons	**YPX108**	**YPX108**	FJ416740_**YPX108**	FJ416814	FJ416778	-	-	**YPX108**	**YPX108**	**YPX108**	**YPX108**
Aparallactus_modestus	FJ404130	AY611824	AY612007	FJ404332	-	-	AY611916_FJ404235	-	-	-	-
Aplopeltura_boa	AF544761	AF544787		U49312	-	-	AF544715	-	FJ434085	FJ433984	EF144044
Aspidelaps_scutatus	**YPX589**	**YPX589**	AY188007_**YPX589**	AY058969	-	-	**YPX589**	**YPX589**	**YPX589**	**YPX589**	**YPX589**
Atractaspis_corpulenta	Z46597	AY611837	AY612020	FJ404335	-	-	AY611929_FJ404238	DQ993174	-	-	-
Atractaspis_micropholis	AF544740	AY611823	AY612006	FJ404336	-	-	AY611915_AF544677	-	FJ434095	FJ433994	EF144053
Boa_constrictor	NC_007398	NC_007398	AF471036	NC_007398	NC_007398	NC_007398	AF471115_AF544676_AF471115	AY487351_EU402837	AY988047	AY988030	-
Boaedon_fuliginosus	AY122681	AY188079	AF471060	HQ207151	-	AY122663	DQ486163_AF544686	AY487378_EU402849	FJ434094	-	EF144052
Brachyorrhos_albus	Murphy et al. (2011)	Murphy et al. (2011)	Murphy et al. (2011)	-	-	-	-	-	-	-	-
Broghammerus_reticulatus	Z46448	-	U69860_FJ717481	-	-	-	AF544675	EU624119_AY487396	FJ434074	FJ433969	FJ434020
Bungarus_fasciatus	NC_011393	NC_011393	EU579523	EU579523	NC_011393	NC_011393	AF544732_AY058924_**YPX591**	AY487389	**YPX591**	**YPX591**	**YPX591**
Calabaria_reinhardtii	Z46464	-	AY099985	AF302943	-	-	AF544682_AY099978	EU402839_AY487391	AY988058	AY988041	FJ434023
Calamaria_pavimentata	**YPX548**	**YPX548**	**YPX548**	-	-	-	**YPX548**	EF144092	**YPX548**	**YPX548**	**YPX548**
Calamaria_yunnanensis	**YPX503**	**YPX503**	**YPX503**	-	-	-	**YPX503**	**YPX503**	**YPX503**	**YPX503**	**YPX503**
Cantoria_violacea	AF499292_**YPX087**	**YPX087**	EF395897_**YPX087**	-	-	-	EF395922	**YPX087**	**YPX087**	**YPX087**	**YPX087**
Cerberus_rynchops	AF499289_**YPX500**	**YPX500**	**YPX500**	U49327	-	-	**YPX500**	**YPX500**	**YPX500**	**YPX500**	**YPX500**
Chrysopelea_ornata	**YPX063**	**YPX063**	**YPX063**	KC347496	-	-	KC347393	**YPX063**	**YPX063**	**YPX063**	**YPX063**
Coluber_constrictor	U96794_AY122819_**YPX528**	**YPX528**	EU180486	AF138746	AY487002	AY122735	**YPX528**	**YPX528**	**YPX528**	**YPX528**	**YPX528**
Compsophis_infralineatus	FJ404157_**YPX97**	**YPX097**	EF203990	FJ404359	-	-	EF203996_EF204000_FJ404259	**YPX097**	**YPX097**	**YPX097**	-
Coronella_austriaca	AY122836_**YPX537**	**YPX537**	AY486930_**YPX537**	AY487065	**AY487026**	AY122752	AY486954	**YPX537**	**YPX537**	**YPX537**	-
Cubophis_cantherigerus	AF158405	AF158475	AF544669	FJ416818	FJ416782	-	AF544694	AY487376	FJ434100	FJ433999	EF144057
Cylindrophis_ruffus	NC_007401	-	NC_007401	NC_007401	NC_007401	NC_007401	AF471133_AF544698	AY662613	AY988054	EU402635	**YPX157**
Diadophis punctatus	AY577015	**YPX089**	EU193843	EU194025	-	-	AF471122_AF544705_YPX089	AY487403	**YPX089**	EU402637	**YPX089**
Dipsas_catesbyi	Z46459	**YPX119**	EF078537_**YPX119**	EF078585	-	-	JQ598977	**YPX119**	**YPX119**	**YPX119**	**YPX119**
Dromicodryas_bernieri	**YPX096**	**YPX096**	DQ979987	FJ404353	-	-	AY187975	**YPX096**	**YPX096**	**YPX096**	**YPX096**
Drymarchon corais	HM565758	**YPX068**	AF471064_**YPX68**	DQ902314	DQ902207	-	AF471137	**YPX068**	**YPX068**	**YPX068**	**YPX068**
Duberria_lutrix	FJ404154_**YPX090**	**YPX090**	DQ486413_**YPX90**	DQ486308	-	-	FJ387207_DQ486161_AF471138	**YPX090**	**YPX090**	**YPX090**	**YPX090**
Eirenis_modestus	AY039143	**YPX533**	**YPX533**	AY487072	AY487033	AY039181	**YPX533**	**YPX533**	**YPX533**	**YPX533**	-
Erpeton tentaculatus	AF499286_**YPX527**	**YPX527**	EF395913_**YPX527**	-	-	-	AF544700_EF395936_**YPX527**	**YPX527**	**YPX527**	**YPX527**	**YPX527**
Erythrolamprus_miliaris	AF158409_**YPX129**	**YPX129**	**YPX129**	-	-	-	JQ598982	**YPX129**	**YPX129**	**YPX129**	**YPX129**
Eryx_colubrinus	AF544747	-	U69811	-	-	-	AF544716_DQ465568	EU402845_AY487392	FJ434078	DQ465570	FJ434027
Eunectes notaeus	AM236347	-	AM236347	AM236347	AM236347	AM236347	**YPX491**	AY988063	AY988046	AY988029	**YPX491**
Fimbrios_klossi	-	-	**YPX545**	-	-	-	-	**YPX545**	-	**YPX545**	-
Gonionotophis_brussauxi	FJ404156	**YPX054**	AY612043	FJ404358	-	-	AY611952_FJ404258	**YPX054**	**YPX054**	**YPX054**	**YPX054**
Gonionotophis capensis	AF544769	**YPX060**	HQ207116	AF544665	-	-	AF544703	AY487379	FJ434096	FJ433995	EF144054
Gonyosoma_oxycephalum	AY122678_YPX558	**YPX558**	AF471084_**YPX558**	DQ902309	DQ902241	AY122660	**YPX558**	**YPX558**	**YPX558**	**YPX558**	**YPX558**
Grayia_ornata	AF158434	AF158503	**YPX055**	AF544663	-	-	**YPX055**	EF144091	**YPX055**	**YPX055**	EF144061
Heterodon platirhinos	AY577019	**YPX568**	**YPX568**	AF402659	FJ416750	-	**YPX568**	**YPX568**	-	**YPX568**	-
Homalopsis_buccata	AF499288_**YPX560**	EF395868_YPX560	EF395917	-	-	-	EF395940_AF544701_**YPX560**	-	**YPX560**	**YPX560**	**YPX560**

Homoroselaps_lacteus	FJ404135_**YPX158**	AY611809_**YPX158**	AY612026_**YPX158**	AY058976	-	-	FJ404241_AY611935	-	**YPX158**	**YPX158**	**YPX158**
Imantodes_cenchoa	EU728586_**YPX127**	EU728586_**YPX127**	EU728586_**YPX127**	NC_013988	NC_013988	NC_013988	GQ457865	**YPX127**	**YPX127**	**YPX127**	-
Ithycyphus_miniatus	**YPX098**	**YPX098**	AY188019_**YPX98**	-	-	-	AY187980	**YPX098**	**YPX098**	**YPX098**	**YPX127**
Lampropeltis_getula	AY122821_**YPX511**	**YPX511**	FJ997820_**YPX511**	AF138759	-	AY122738	**YPX511**	EU402848_**YPX511**	**YPX511**	**YPX511**	**YPX511**
Laticauda_colubrina	U96799	EU547138	EU547040	AY058977	-	-	AY058932_EU366446	AY487404_EU402850	FJ434091	EU402647	EF144050
Leioheterodon_madagascariensis	AF544768_**YPX502**	AY188061	AY188022_**YPX502**	FJ404371	-	-	**YPX502**_AF544685_AY187983	AY487377	**YPX502**	**YPX502**	**YPX502**
Leptodeira_annulata	GQ457806	GQ457746	EF078516	FJ416787	FJ416749	-	AF544690_GQ457866	AY487375	FJ434099	FJ433998	EF144056
Liophidium_torquatum	**YPX100**	**YPX100**	DQ979984_**YPX100**	-	-	-	AY187984	**YPX100**	**YPX100**	**YPX100**	**YPX100**
Loxocemus_bicolor	Z46456	-	AY099993	-	-	-	AF544730_AY444035	EU402854_AY487406	FJ434072	FJ433967	FJ434018
Lycophidion_capense	FJ404178	AY611893	AY612075	FJ404376	-	-	DQ486168_FJ404279	EU402855	-	EU402652	-
Macropisthodon_rudis	**YPX091**	**YPX091**	**YPX091**	U49326	JQ687458	-	JQ687452	**YPX091**	**YPX091**	**YPX091**	**YPX091**
Malpolon_monspessulanus	**YPX570**	**YPX570**	AY058965_**YPX570**	FJ404320	-	-	**YPX570**	**YPX570**	**YPX570**	**YPX570**	**YPX570**
Micrurus_surinamensis	AF544770	AF544799	EF137415	EF137407	-	-	EF137422_AF544708	AY487411	FJ434092	FJ433991	EF144051
Mimophis_mahafalensis	**CTMZ-04140**	-	**CTMZ-04140**	DQ486202	-	-	AY187993	**CTMZ-04140**	**CTMZ-04140**	**CTMZ-04140**	**CTMZ-04140**
Naja_atra	**YPX829**	EU913475_**YPX829**	EU913475_**YPX829**	EU921898	EU921898	NC_011389	**YPX829**	-	**YPX829**	**YPX829**	**YPX829**
Natrix_natrix	**YPX538**	-	AY866541_**YPX538**	AY873716	AY870624	AY122664	AF471121_AF544697_**YPX539**	EU402858	-	EU402655	**YPX538**
Nerodia_sipedon	AF402630_**YPX525**	**YPX525**	AF402913_**YPX052**	-	DQ915150	GQ278935	**YPX052**	**YPX052**	**YPX052**	**YPX052**	**YPX052**
Ninia_atrata	GQ457814	**YPX131**	**YPX131**	GQ334659	-	-	GQ457874	**YPX131**	**YPX131**	**YPX131**	**YPX131**
Ophiophagus_hannah	EU921899_**YPX830**	EU921899_**YPX830**	EU921899_**YPX830**	EU921899	NC_011394	NC_011394	**YPX830**	**YPX830**	**YPX830**	**YPX830**	**YPX830**
Pantherophis_guttatus	AY122814	AM236349_**YPX071**	AM236349	AM236349	DQ902218	AY122730	**YPX071**	-	-	**YPX071**	**YPX071**
Pareas_carinatus	AF544773	AF544802	JQ598940	-	-	-	AF544692	-	FJ434086	FJ433985	EF144045
Pareas_hamptoni	**YPX562**	**YPX562**	AY425809_**YPX562**	-	-	-	**YPX562**	EU402860_**YPX562**	**YPX562**	**YPX562**	**YPX562**
Pituophis_deppei	**YPX515**	**YPX515**	FJ627818_**YPX515**	AF138765	FJ627848	AY122741	**YPX515**	**YPX515**	**YPX515**	**YPX515**	**YPX515**
Polemon_acanthias	FJ404138	AY611848	AY612031	FJ404341	-	-	FJ404243_AY611940	-	-	-	-
Psammophis_elegans	**YPX530**	**YPX530**	-	EU526862	-	-	**YPX530**	-	**YPX530**	**YPX530**	**YPX530**
Psammophis_mossambicus	**YPX094**	**YPX094**	FJ404314_**YPX094**	DQ486283	-	-	DQ486185_FJ404224	**YPX094**	-	**YPX094**	**YPX094**
Psammophis_subtaeniatus	-	**YPX095**	DQ486358	DQ486253	-	-	-	**YPX095**	**YPX095**	**YPX095**	**YPX095**
Psammophylax_variabilis	AF544774	AY611864	AY612046	EU526859	-	-	AF544709_AY611955	AY487380	FJ434097	FJ433996	EF144055
Pseudonaja_modesta	EU547098	**YPX588**	EU547049	DQ098490	-	-	**YPX588**	-	**YPX588**	**YPX588**	**YPX588**
Pseudoxenodon_bambusicola	**YPX551**	**YPX551**	-	-	-	-	**YPX551**	EF144090	**YPX551**	**YPX551**	**YPX551**
Pseudoxenodon_karlschmidti	**YPX564**	**YPX564**	**YPX564**	-	-	-	**YPX564**	**YPX564**	**YPX564**	**YPX564**	**YPX564**
Psomophis_joberti	GQ457829	GQ457768	**YPX137**	-	-	-	GQ457889	**YPX137**	**YPX137**	**YPX137**	**YPX137**
Ptyas_korros	AF236680_**YPX542**	**YPX542**	AY486929_**YPX542**	AY487062	AY487023	AY122652	**YPX542**	-	**YPX542**	**YPX542**	**YPX542**
Indotyphlops_braminus	DQ343649	-	DQ343649	NC_010196	NC_010196	DQ343649	AF544717_AY099980	AY444062_AY487410	GU902556	FJ433959	FJ434010
Rhachidelus_brazili	JQ598837	JQ598897	**YPX139**	-	-	-	-	**YPX139**	**YPX139**	**YPX139**	**YPX139**
Rhamphiophis oxyrhynchus	**YPX523**	**YPX523**	-	-	-	-	FJ387213_AF544710_**YPX523**	-	**YPX523**	**YPX523**	**YPX523**
Scaphiodontophis_annulatus	-	-	GQ927323	-	-	-	GQ927318	**YPX080**	**YPX080**	**YPX080**	**YPX080**
Sibynomorphus_mikanii	GQ457832	GQ457771	**YPX141**	-	-	-	GQ457892	**YPX141**	**YPX141**	**YPX141**	**YPX141**
Sibynophis collaris	**YPX535**	**YPX535**	KC000121	JN211315	KC000133	-	**YPX535**	**YPX535**	-	**YPX535**	**YPX535**
Sinomicrurus_macclellandi	**YPX590**	**YPX590**	**YPX590**	EF137410	-	-	**YPX590**	**YPX590**	**YPX590**	**YPX590**	**YPX590**
Sinonatrix_percarinata	**YPX552**	**YPX552**	**YPX552**	JQ687414	JQ687455	-	**YPX552**	**YPX552**	**YPX552**	**YPX552**	**YPX552**
Spalerosophis diadema	AY039148_**YPX526**	**YPX526**	AY486926_**YPX526**	AY487059	AY487020	AY039186	**YPX526**	**YPX526**	**YPX526**	**YPX526**	**YPX526**
Stoliczkia_borneensis	AF544779	AF544808	-	-	-	-	AF544721	AY487398	FJ434083	FJ433982	EF144043
Storeria_dekayi	AF402639_**YPX567**	**YPX567**	AF471050_**YPX567**	EF417365	EF417460	EF417389	**YPX567**	-	**YPX567**	**YPX567**	-
Taeniophallus nicagus	**YPX146**	**YPX146**	**YPX146**	-	-	-	**CTMZ0483**	**YPX146**	**YPX146**	**YPX146**	-
Thamnophis_sirtalis	**YPX539**	**YPX539**	AF420193	AF420196	DQ995365	-	DQ902094	**YPX539**	**YPX539**	**YPX539**	**YPX539**

Tomodon_dorsatus	GQ457838	GQ457777	**YPX148**	-	-	-	GQ457897	**YPX148**	**YPX148**	**YPX148**	**YPX148**
Uropeltis_melanogaster	AF512739	-	-	-	-	-	-	AY487399	FJ434070	FJ433965	FJ434016
Xenochrophis_flavipunctatus	AF544780	AF544809	-	-	FJ416748	-	AF544714	-	FJ434102	FJ434001	EF144060
Xenodermus_javanicus	AF544781	AF544810	AY425810	U49320	-	-	AF544711	-	-	EU402667	-
Xenopeltis_unicolor	NC_007402	-	NC_007402	NC_007402	NC_007402	NC_007402	AF544689_AY099977	EU402870_AY487400	DQ465562	EU402668	FJ434019

Table S2. Partition schemes and nucleotide substitution models suggested by PartitionFinder for each dataset. Dataset 1 corresponds to our main dataset and dataset 2 corresponds to the sequences from 16S, 12S, cytb and nd4 genes (see text for details).

Scenarios	**Partition**	**Best Model**
Dataset 1	12S, 16S	GTR+I+G
	Codon 1st and 2nd pos of bndf and 2nd pos of rag1	HKY+I+G
	Codon 3rd pos of bndf and jun	K80+G
	Codon 1st pos of cox1	SYM+I+G
	Codon 2nd pos of cox1	GTR+I+G
	Codon 3rd pos of cox1 and cytb	GTR+I+G
	Codon 1st pos of cmos, jun and rag1, and 2nd pos of cmos and jun	HKY+I+G
	Codon 3rd pos of cmos and rag	GTR+G
	Codon 1st pos of Cytb and ND4	GTR+I+G
	Codon 2nd pos of Cytb, ND2, and ND4	GTR+I+G
	Codon 1st pos of nd2	GTR+I+G
	Codon 3rd pos of nd2 and nd4	GTR+G
	Codon of 1st and 2nd pos of Nt3	SYM+I+G
	Codon 3rd position of Nt3	GTR+G
Dataset 2	12S, 16S	GTR+I+G
	Codon 1st pos of cytb and nd4	GTR+I+G
	Codon 2nd pos of cytb and nd4	GTR+I+G
	Codon 3rd pos of cytb	GTR+G
	Codon 3rd pos of nd4	GTR+G

DIVERSIFICATION DYNAMICS IN VIPERS

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Abstract. Snakes of the family Viperidae comprise around 329 venomous species distributed worldwide with the three sub-families showing striking heterogeneity in species richness. While the subfamily Azemiopinae comprises only two species, 70% of all viper species are arranged in the subfamily Crotalinae, also known as 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. Given the disparity in species number among the lineages of vipers and the unique events that characterized the evolution of pit vipers, we explored the hypothesis that crotalines have undergone an explosive radiation. 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. Our results suggest that 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.

INTRODUCTION

The disparity in species richness across the tree of life is an intriguing large-scale pattern. For example, the order Squamata comprise around 9.900 species whereas its sister lineage, the Rhynchocephalia (tuataras), comprise only two (Hedges and Vidal, 2009, Uetz and Hosek, 2014). In order to explain such patterns scientists need to understand the underlying mechanisms driving species richness among lineages (Rabosky et al., 2012). Present-day heterogeneities in species richness can reflect different temporal dynamics of species diversification during the evolutionary history of lineages. While groups with higher species richness could be associated with higher diversification rates, low diversification rates could characterize the poor species clades like the

Tuatara (Alfaro et al., 2009). However, the fossil record indicates that species richness dramatically changed through time and lineages comprising few species in the present used to be much more diverse in the past (e.g. Quental and Marshall, 2010). This in turn suggests that diversification rates might also have changed through time. Thus, exploring the temporal patterns of species diversification can yield significant insights into the processes that might regulate diversity.

Although paleontological data are ideal to explore patterns and processes regulating diversity through time (Quental and Marshall, 2010, Silvestro et al., 2015), most groups of organisms exhibit a poor fossil record. However, in the past years several methods have been proposed to investigate the dynamics in species diversification using solely extant species by inferring their history through molecular phylogenies (e.g. Magallon and Sanderson, 2001, Alfaro et al., 2009). Although those methods were heavily critizized (Quental and Marshall, 2010, Rabosky, 2010), newer methods promise to account for speciation and extinction rate variation among lineages and along time (Morlon et al., 2011, Rabosky et al., 2013, Rabosky, 2014) and seem to better describe diversification dynamics (e.g. Morlon et al., 2011). These methods together with an increase in the availability of time-calibrated molecular phylogenies for several groups opened numerous possibilities to understand the diversification dynamics across the tree of life.

The cosmopolitan group of vipers (Serpentes: Viperidae) shows a striking heterogeneity in diversity among lineages, and a number of hypothesis have been proposed to explain differential success within the group. 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 in each side of their heads between the eye and the nostril ("loreal pits") (Goris, 2011; Roelke and Childress, 2007), which have been suggested as a key innovation (e.g. Greene, 1997) even though not directly tested. Crotalines also invaded the New World, an event frequently associated to an 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 towards 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 diversification dynamics of vipers. We also explicitly explored the hypothesis that crotalines, the most diverse subfamily, undergone an explosive radiation (Greene 1997; Wüster et al., 2008) by assessing if diversification rates significantly increased during their radiation comparing with those from the remaining vipers.

MATERIAL AND METHODS

To perform diversification analyses while incorporating phylogenetic uncertainty we randomly sampled 100 trees from the posterior distribution of dataset 1 (our main dataset, see Chapter I). 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 under-sampling (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 264 terminals in our sampling scheme) as well as for Viperinae (98 known species and 72 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 a novel approach named BAMM (Bayesian Analysis of Macroevolution Mixtures, Rabosky, 2014; Rabosky et al., 2013; Rabosky et al., 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 Figure 1 in Rabosky, 2014). This 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; Rabosky, 2012). 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 in Chapter I. 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 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 S1). 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).

RESULTS

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 (Figure 1). 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 (Figure 1). 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 (Figure 1). The gamma statistics test after correcting for incompleteness did not show any evidence for slowdowns among viperines (Figure 1).

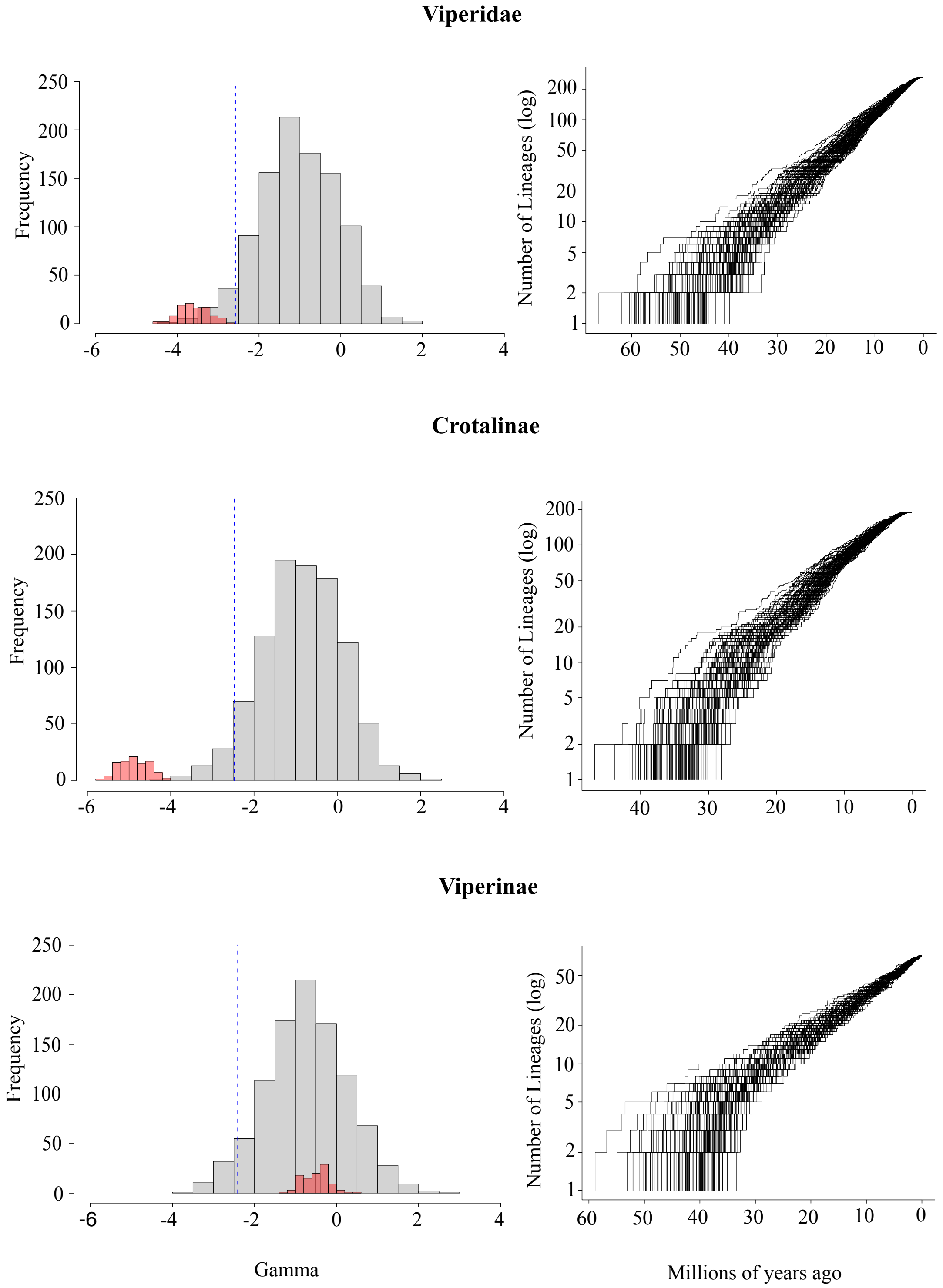

Figure 1. Simulated (shaded bars) gamma values under the null model of constant diversification rates vs empirical (red bars) gamma values, and lineage through time plots estimated for all Viperidae and subfamilies Crotalinae and Viperinae separately. The gamma values for empirical trees were calculated for 100 trees from the posterior distribution of trees (see Chapter I) and therefore incorporate phylogenetic uncertainity. Blue dashed lines represent the threshold where the null hypothesis of constant diversification rates is rejected at a 5% level.

When looking for dynamic heterogeneity, BAMM indicated that models with one or two shifts in macroevolutionary rate regimes were chosen more frequently during MCMC analyses (Figure 2). However, after discounting the shifts likely to be attributed to the prior alone, the most frequently sampled shift configuration estimated in BAMM for 83 trees (out of 100) comprised only one shift (Figure 3). Fourteen trees showed no shifts as the most frequent shift configuration, and three trees showed two shifts (Figure 4A and B). Configurations with one shift (Figure 3) suggest an increase in speciation rates after the first divergence event among crotalines, thus comprising part of the OW crotalines and the whole NW crotaline clade, with decay in speciation rates towards the present. Configurations with two shifts (Figure 4B) include the shift described above plus a second shift representing an increase in speciation rates that varies in the exact position but always among the genera *Vipera*, *Macrovipera*, *Montivipera*, *Daboia*, *Eristicophis* and *Pseudocerastes*.

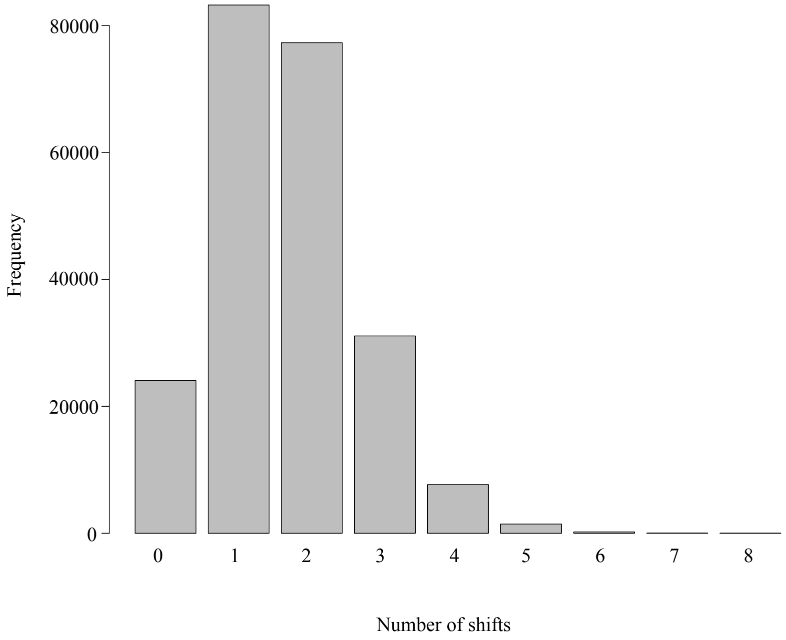

Figure 2. Number of shifts in macroevolutionary regimes found by BAMM during MCMC for the 100 posterior trees analyzed. A model with one shift was visited more frequently. Note the histogram shows the number of shifts before eliminating those shifts that were probable given the prior alone.

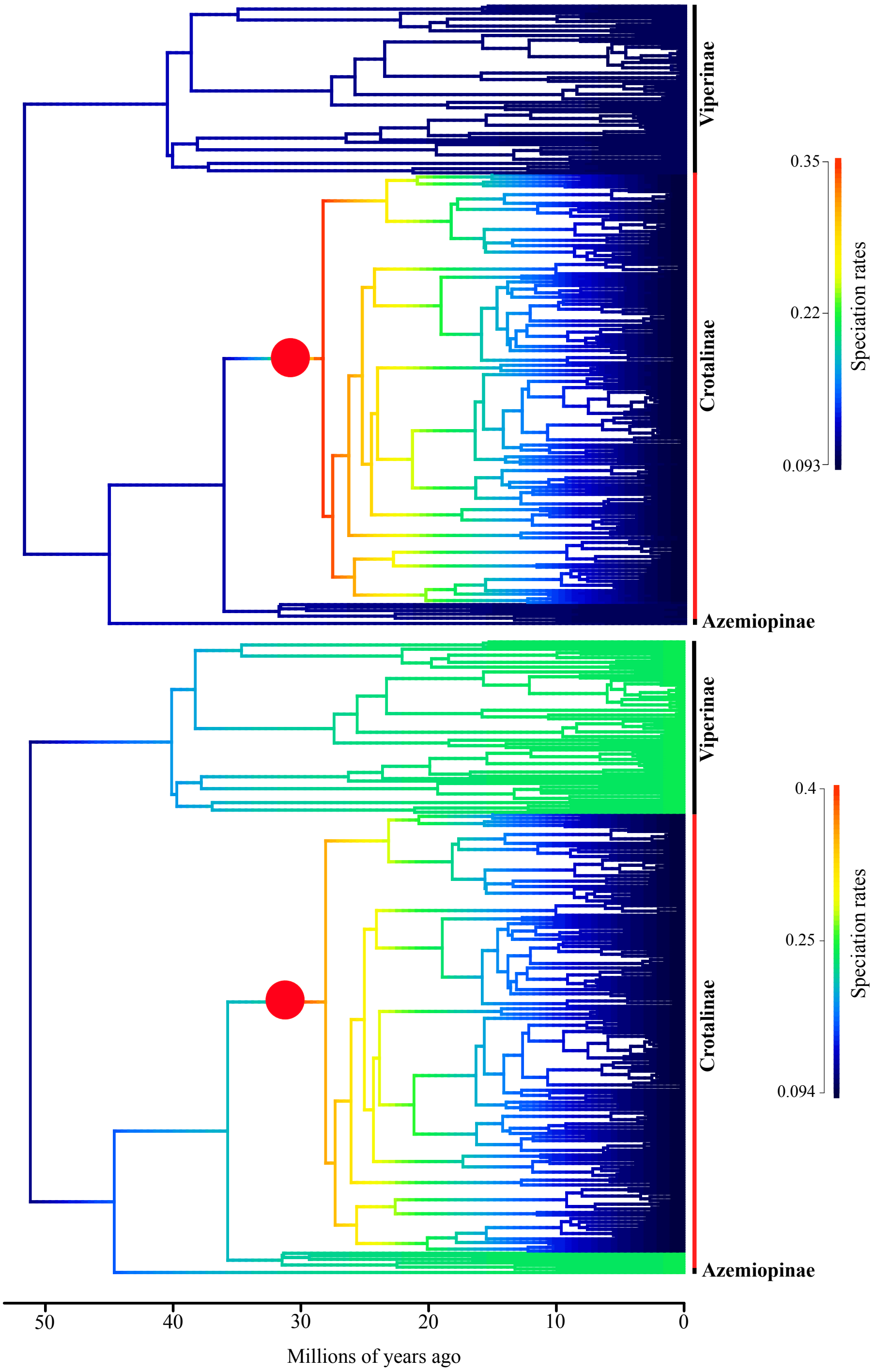

Figure 3. Best shift configuration sampled by BAMM for 83 phylogenetic trees. For each tree, speciation rates are calculated as follow: in the upper figure instantaneous rates for each posterior sample are calculated along each branch, and these instantaneous rates are then averaged. In the bottom figure 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.

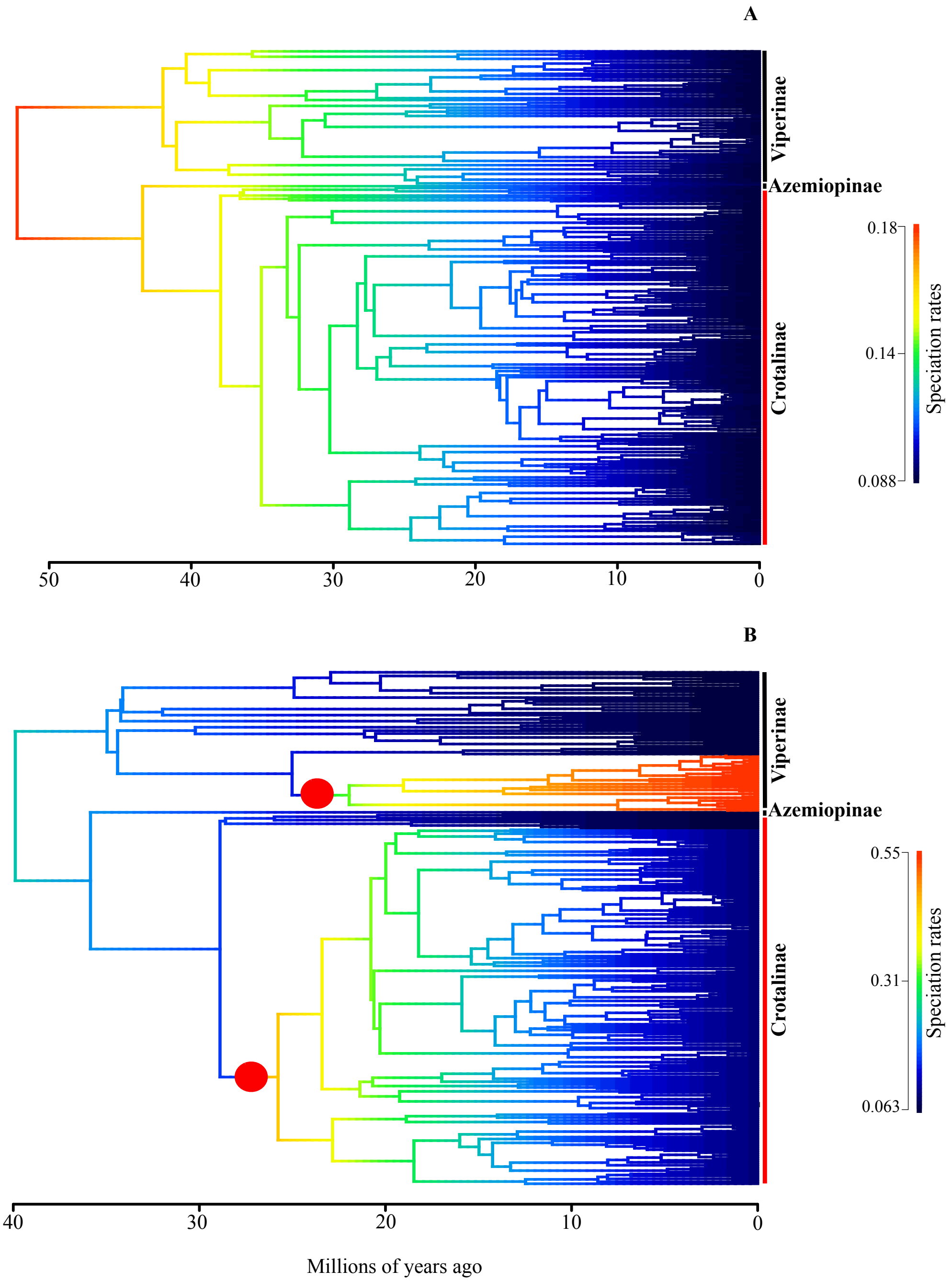

Figure 4. A) Best shift configuration sampled by BAMM for 14 phylogenetic trees. B) Best shift configuration sampled by BAMM for three phylogenetic trees. Red circles indicate the place where rate shifts might have occurred.

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) (Figure

3), 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 rates associated with a slightly decay on it and another with small initial values of speciation and a gentle increase on it (Figure 5A). This suggests an increase or a decrease in speciation rates and even constant speciation rates during the background diversification.

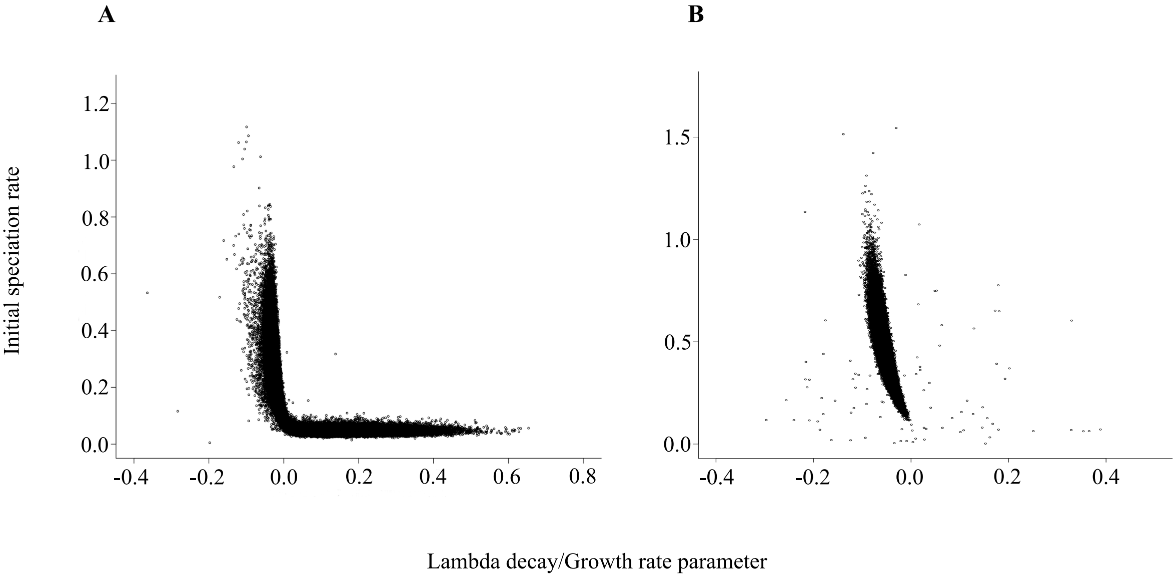

Figure 5. A) Two optimum parameter combinations estimated by BAMM for the background regime leading to uncertainty in rate estimates. B) Parameter combination estimated by BAMM for the pos-shift regime indicating confidence for a scenario where rates slow down toward the present.

Rate through time plots show that initially speciation rates slightly decreases but after a given amount of time (Figure 6A) experience a considerable increase. After this burst, speciation starts to decrease. On the other hand, extinction rates seem to remain roughly constant for most of the time (Figure 6B). Speciation through time plots generated for the Crotalinae subfamily and the remaining vipers separately (Figure 6C) 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 (Figure 6D).

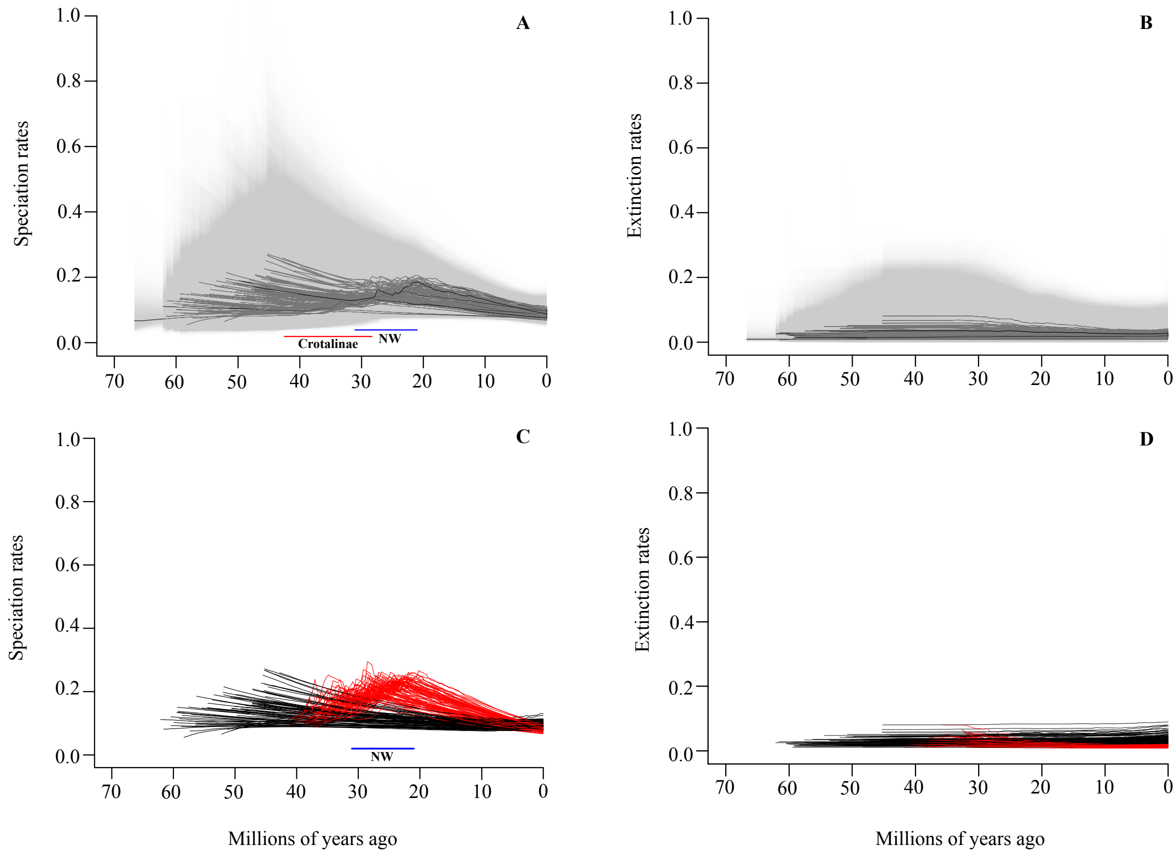

Figure 6. A) Speciation and B) extinction rates through time estimated for 100 phylogenetic trees for all vipers. Shaded regions denote the 95% credible interval of the posterior distribution of rates at a given point in time. C) Speciation and D) extinction rates through time estimated for the 83 phylogenetic trees showing the one shift configuration as the most frequent diversification scenario (see Results 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 figures 6C and 6D for better visualization. The red and blue bars indicate the posterior density interval (dataset 1) of the crown group ages of the Crotalinae subfamily and the NW crotalines respectively.

DISCUSSION

Two distinct diversification regimes characterized the radiation of vipers. The diversification regime suggested by BAMM for the background lineages are inconclusive and prevent us from discussing which possible scenario took 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 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). Regardless of the real scenario underlying the early diversification of vipers and of the background lineages as a whole, it becomes evident that a different diversification regime took place during the diversification of crotalines when speciation rates started to rapidly increase (Figure 3 and 6 A, C). Surprisingly, this shift in speciation rates took place after the first divergence event among crotalines and not at the crown group of pit vipers as expected. The shift did not comprise the five very species-poor genera *Calloselasma*, *Deinagkistrodon*, *Garthius*, *Hypnale* and *Tropidolaemus* which are connected by long branches in our phylogenetic trees (see Figure 1 in Chapter I), suggesting infrequent speciation and/or frequent extinction events during their radiation. One could argue that a species undersampling effect on this clade could have biased the shift position in BAMM analyses. However, besides the fact that BAMM takes into account possible species undersampling effects (see Material and Methods), 73% of the clade species were included in our analyses. Thus, the distinct diversification regime found comprises only part of the OW crotalines and the whole NW clade (see Figure 1 in Chapter I and Figure 3 this chapter). Our results suggest that the emergence of the loreal pits in crotalines did not immediately trigger 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. 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 Figure 1 in Chapter I) 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). Despite the uncertainty regarding the true time period of the collision between India and Asia (70-50 Mya), the effects of this collision are thought to have propagated throughout the Oligocene and Miocene comprising the uplift of the Tibetan plateau and of several mountain ranges in central Asia (Favre et al., 2015; Wang, 2004). These deformations together with the subsequent Asia-Australia collision 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).

These climate and geological changes described may be linked to the shifts in speciation rates found among crotalines. 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). Forest expansion may have also favored arboreality in Asian pit vipers with the subsequent diversification of arboreal lineages contributing to the increase in speciation rates. Interestingly, the first lineage split after the speciation shift gave rise to the arboreal and species rich genus *Trimeresurus*. We think that forest expansion in the Oligocene and Miocene might have provided a significant increase in the availability of niches and opportunities for OW crotalines to speciate.

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

The ecological opportunity hypothesis is usually evoked to explain explosive radiations in spatially limited systems such as islands or lakes (e.g. Darwin's finches, Schluter, 2000; Cichlids fishes, Seehausen, 2006). When colonizing these closed systems, lineages rapidly speciate in sympatry with significant phenotypic change reflecting niche differentiation (e.g. different beaks associated with different diets among Darwin's Finches, Grant, 1981). Continental radiations may be more complex because geographical limits are much broader compared to islands (Derryberry et al., 2011; Price et al., 2014). Instead of diversifying in sympatry, the recently arrived continental lineages can disperse and occupy a wider variety of environments. The first NW pit viper populations could have easily spread into different environments due to the availability of new adequate potential habitats and the scarcity of competitors/predators. As populations reached a very large area, distinct selective pressures imposed by each environment would bring populations closer to distinct and spatially distributed adaptive optima increasing genetic and ecological divergence between them (see Cornell, 2013; Nosil, 2012, Sacks et al., 2008). Under this scenario, divergent natural selection would pull populations apart turning them into new species increasing speciation rates during the early diversification of NW pit vipers. Differently from the processes underlying radiations on islands, here, ecological opportunities were spatially distributed and sympatric speciation might have had a secondary role in generating new lineages. The fast speciation events after the invasion of the NW by pit vipers could thus be related to rapid dispersion followed by diversification into different environments characterized by distinct abiotic/biotic conditions (see Sacks et al., 2008).

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). We suspect that in the case of vipers, the relevant driver is a geographic space that becomes saturated in a short time period. For example, 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. Speciation rates would thus slowdown because the presence of a recently adapted lineage in an area might inhibit the invasion of another (niche pre-emption, see Wiens, 2011), and a large set of geographically spaced and distinct environments were no longer available all at the same time as they once were. At this time, inter-specific interactions could have started to play a major role in shaping the diversification dynamics of NW pit vipers, contributing to the decline in speciation rates. We should keep in mind, however, that most snakes, especially vipers, seem to have a very low-cost lifestyle compared to endotherms (e.g., Greene, 1983, 1986;

Nagy et al., 1999) and given the low food intake by vipers (Fitch, 1982; Greene, 1986; Martins et al. 2002), competition for food might not necessarily be the main reason why one viper species would avoid an environment already occupied by another.

A similar scenario could explain speciation slowdowns among OW crotalines with new opportunities emerging with the forest expansion in Asia. Dispersion of OW pit vipers would have been restricted to the new forested areas because differently from the NW, competitors and predators were already established in other areas. 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 longer time intervals and/or in much more local scales compared to previous time periods. Although speculative, the scenarios above provide several predictions that can be easily tested in future studies. For such scenarios to produce a burst in speciation followed by a deceleration, the time to fully occupy a new environment or continent would have to be a lot faster than the time it takes for a new species to be formed.

A slowdown in speciation, however, does not imply an association with niche divergence (Moen and Morlon, 2014). The increase in 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.

CONCLUSIONS

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 frequently 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 after the first split event among crotalines, pit 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. Our results evoke a series of interesting specific hypothesis, but directly testing those ideas is beyond the scope of the present chapter. Additionally, further investigation is needed to fully understand the role of adaptation to spatially distributed environments and/or vicariance events during the radiation of pit vipers, which will certainly improve our understanding of the different potential regulators of biodiversity in vipers.

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SUPPLEMENTARY TABLE

Table S1. Clade specific sampling probabilities used in BAMM

speciesName	cladeName	samplingFraction
Agkistrodon_bilineatus	Agkistrodon	1
Agkistrodon_contortrix	Agkistrodon	1
Agkistrodon_howardgloydi	Agkistrodon	1
Agkistrodon_piscivorus	Agkistrodon	1
Agkistrodon_russeolus	Agkistrodon	1
Agkistrodon_taylori	Agkistrodon	1
Atheris_barbouri	Viperinae	0.73
Atheris_ceratophora	Viperinae	0.73
Atheris_chlorechis	Viperinae	0.73
Atheris_desaixi	Viperinae	0.73
Atheris_hispida	Viperinae	0.73
Atheris_matildae	Viperinae	0.73
Atheris_nitschei	Viperinae	0.73
Atheris_squamigera	Viperinae	0.73
Atropoides_indomitus	Atro_cerro_porth	0.95
Atropoides_mexicanus	Atro_cerro_porth	0.95
Atropoides_nummifer	Atro_cerro_porth	0.95
Atropoides_occiduus	Atro_cerro_porth	0.95
Atropoides_olmec	Atro_cerro_porth	0.95
Atropoides_picadoi	Atro_cerro_porth	0.95
Azemiops_feae	Azemiopinae	0.5
Bitis_arietans	Viperinae	0.73
Bitis_armata	Viperinae	0.73
Bitis_atropos	Viperinae	0.73
Bitis_caudalis	Viperinae	0.73
Bitis_cornuta	Viperinae	0.73
Bitis_gabonica	Viperinae	0.73
Bitis_nasicornis	Viperinae	0.73
Bitis_parviocula	Viperinae	0.73
Bitis_peringueyi	Viperinae	0.73
Bitis_rhinoceros	Viperinae	0.73
Bitis_rubida	Viperinae	0.73
Bitis_schneideri	Viperinae	0.73
Bitis_worthingtoni	Viperinae	0.73
Bitis_xeropaga	Viperinae	0.73
Bothriechis_aurifer	Bothriechis	1
Bothriechis_bicolor	Bothriechis	1
Bothriechis_guifarroi	Bothriechis	1
Bothriechis_lateralis	Bothriechis	1
Bothriechis_marchi	Bothriechis	1
Bothriechis_nigroviridis	Bothriechis	1

Bothriechis_rowleyi	Bothriechis	1
Bothriechis_schlegelii	Bothriechis	1
Bothriechis_supercilliaris	Bothriechis	1
Bothriechis_thalassinus	Bothriechis	1
Bothrocophias_campbelli	Bothrocophias_Bothrops	0.75
Bothrocophias_hyoprora	Bothrocophias_Bothrops	0.75
Bothrocophias_microphthalmus	Bothrocophias_Bothrops	0.75
Bothrops_alcatraz	Bothrocophias_Bothrops	0.75
Bothrops_alternatus	Bothrocophias_Bothrops	0.75
Bothrops_ammodytoides	Bothrocophias_Bothrops	0.75
Bothrops_asper	Bothrocophias_Bothrops	0.75
Bothrops_atrox	Bothrocophias_Bothrops	0.75
Bothrops_barnettii	Bothrocophias_Bothrops	0.75
Bothrops_bilineatus	Bothrocophias_Bothrops	0.75
Bothrops_brazili	Bothrocophias_Bothrops	0.75
Bothrops_caribbaeus	Bothrocophias_Bothrops	0.75
Bothrops_chloromelas	Bothrocophias_Bothrops	0.75
Bothrops_colombiensis	Bothrocophias_Bothrops	0.75
Bothrops_cotiara	Bothrocophias_Bothrops	0.75
Bothrops_diporus	Bothrocophias_Bothrops	0.75
Bothrops_erythromelas	Bothrocophias_Bothrops	0.75
Bothrops_fonsecai	Bothrocophias_Bothrops	0.75
Bothrops_insularis	Bothrocophias_Bothrops	0.75
Bothrops_isabelae	Bothrocophias_Bothrops	0.75
Bothrops_itapetiningae	Bothrocophias_Bothrops	0.75
Bothrops_jararaca	Bothrocophias_Bothrops	0.75
Bothrops_jararacussu	Bothrocophias_Bothrops	0.75
Bothrops_lanceolatus	Bothrocophias_Bothrops	0.75
Bothrops_leucurus	Bothrocophias_Bothrops	0.75
Bothrops_lojanus	Bothrocophias_Bothrops	0.75
Bothrops_lutzi	Bothrocophias_Bothrops	0.75
Bothrops_marajoensis	Bothrocophias_Bothrops	0.75
Bothrops_marmoratus	Bothrocophias_Bothrops	0.75
Bothrops_mattogrossensis	Bothrocophias_Bothrops	0.75
Bothrops_moojeni	Bothrocophias_Bothrops	0.75
Bothrops_neuwiedi	Bothrocophias_Bothrops	0.75
Bothrops_osbornei	Bothrocophias_Bothrops	0.75
Bothrops_pauloensis	Bothrocophias_Bothrops	0.75
Bothrops_pictus	Bothrocophias_Bothrops	0.75
Bothrops_pubescens	Bothrocophias_Bothrops	0.75
Bothrops_pulchra	Bothrocophias_Bothrops	0.75
Bothrops_punctatus	Bothrocophias_Bothrops	0.75
Bothrops_taeniata	Bothrocophias_Bothrops	0.75
Calloselasma_rhodostoma	Calloselasma	1
Causus_defilippii	Viperinae	0.73
Causus_lichtensteini	Viperinae	0.73
Causus_resimus	Viperinae	0.73
Causus_rhombeatus	Viperinae	0.73

Cerastes_cerastes	Viperinae	0.73
Cerastes_gasperettii	Viperinae	0.73
Cerastes_vipera	Viperinae	0.73
Cerrophidion_barbouri	Atro_cerro_porth	0.95
Cerrophidion_godmani	Atro_cerro_porth	0.95
Cerrophidion_petlalcalensis	Atro_cerro_porth	0.95
Cerrophidion_sasai	Atro_cerro_porth	0.95
Cerrophidion_tzotzilorum	Atro_cerro_porth	0.95
Crotalus_adamanteus	crot_sist	0.90
Crotalus_aquilus	crot_sist	0.90
Crotalus_atrox	crot_sist	0.90
Crotalus_basiliscus	crot_sist	0.90
Crotalus_catalinensis	crot_sist	0.90
Crotalus_cerastes	crot_sist	0.90
Crotalus_cerberus	crot_sist	0.90
Crotalus_culminatus	crot_sist	0.90
Crotalus_durissus	crot_sist	0.90
Crotalus_enyo	crot_sist	0.90
Crotalus_ericsmithi	crot_sist	0.90
Crotalus_horridus	crot_sist	0.90
Crotalus_intermedius	crot_sist	0.90
Crotalus_lannomi	crot_sist	0.90
Crotalus_lepidus	crot_sist	0.90
Crotalus_mitchellii	crot_sist	0.90
Crotalus_molossus	crot_sist	0.90
Crotalus_oreganus	crot_sist	0.90
Crotalus_ornatus	crot_sist	0.90
Crotalus_polystictus	crot_sist	0.90
Crotalus_pricei	crot_sist	0.90
Crotalus_pusillus	crot_sist	0.90
Crotalus_ravus	crot_sist	0.90
Crotalus_ruber	crot_sist	0.90
Crotalus_scutulatus	crot_sist	0.90
Crotalus_simus	crot_sist	0.90
Crotalus_stejnegeri	crot_sist	0.90
Crotalus_tancitarensis	crot_sist	0.90
Crotalus_tigris	crot_sist	0.90
Crotalus_tortugensis	crot_sist	0.90
Crotalus_totonacus	crot_sist	0.90
Crotalus_transversus	crot_sist	0.90
Crotalus_triseriatus	crot_sist	0.90
Crotalus_tzcaban	crot_sist	0.90
Crotalus_viridis	crot_sist	0.90
Crotalus_willardi	crot_sist	0.90
Daboia_deserti	Viperinae	0.73
Daboia_mauritanica	Viperinae	0.73
Daboia_palaestinae	Viperinae	0.73
Daboia_russellii	Viperinae	0.73

Daboia_siamensis	Viperinae	0.73
Deinagkistrodon_acutus	Deinagkistrodon	1
Echis_borkini	Viperinae	0.73
Echis_carinatus	Viperinae	0.73
Echis_coloratus	Viperinae	0.73
Echis_jogeri	Viperinae	0.73
Echis_khosatzkii	Viperinae	0.73
Echis_leucogaster	Viperinae	0.73
Echis_ocellatus	Viperinae	0.73
Echis_omanensis	Viperinae	0.73
Echis_pyramidum	Viperinae	0.73
Eristicophis_macmahoni	Viperinae	0.73
Garthius_chaseni	Garthius	1
Gloydius_blomhoffi	Gloydius	0.8
Gloydius_brevicaudus	Gloydius	0.8
Gloydius_halys	Gloydius	0.8
Gloydius_intermedius	Gloydius	0.8
Gloydius_liupanensis	Gloydius	0.8
Gloydius_saxatilis	Gloydius	0.8
Gloydius_shedaoensis	Gloydius	0.8
Gloydius_strauchi	Gloydius	0.8
Gloydius_tsushimaensis	Gloydius	0.8
Gloydius_ussuriensis	Gloydius	0.8
Hypnale_hypnale	Hypnale	1
Hypnale_nepa	Hypnale	1
Hypnale_zara	Hypnale	1
Lachesis_acrochorda	Lachesis	1
Lachesis_melanocephala	Lachesis	1
Lachesis_muta	Lachesis	1
Lachesis_stenophrys	Lachesis	1
Macrovipera_lebetina	Viperinae	0.73
Macrovipera_schweizeri	Viperinae	0.73
Mixcoatlus_browni	Oph_mix	0.75
Montivipera_albizona	Viperinae	0.73
Montivipera_bornmuelleri	Viperinae	0.73
Montivipera_latifii	Viperinae	0.73
Montivipera_raddei	Viperinae	0.73
Montivipera_wagneri	Viperinae	0.73
Montivipera_xanthina	Viperinae	0.73
Ophryacus_melanurus	Oph_mix	0.75
Ophryacus_undulatus	Oph_mix	0.75
Ovophis_monticola	Ovophis	0.6
Ovophis_okinavensis	Gloydius	0.8
Ovophis_tonkinensis	Ovophis	0.6
Ovophis_zayuensis	Ovophis	0.6
Porthidium_arcosae	Atro_cerro_porth	0.95
Porthidium_dunni	Atro_cerro_porth	0.95
Porthidium_hespere	Atro_cerro_porth	0.95

Porthidium_lansbergii	Atro_cerro_porth	0.95
Porthidium_nasutum	Atro_cerro_porth	0.95
Porthidium_ophryomegas	Atro_cerro_porth	0.95
Porthidium_porrasi	Atro_cerro_porth	0.95
Porthidium_yucatanicum	Atro_cerro_porth	0.95
Proatheris_superciliaris	Viperinae	0.73
Protobothrops_cornutus	Protobothrops	0.85
Protobothrops_dabieshanensis	Protobothrops	0.85
Protobothrops_elegans	Protobothrops	0.85
Protobothrops_flavoviridis	Protobothrops	0.85
Protobothrops_jerdonii	Protobothrops	0.85
Protobothrops_kaulbacki	Protobothrops	0.85
Protobothrops_mangshanensis	Protobothrops	0.85
Protobothrops_maolanensis	Protobothrops	0.85
Protobothrops_mucrosquamatus	Protobothrops	0.85
Protobothrops_sieversorum	Protobothrops	0.85
Protobothrops_tokarensis	Protobothrops	0.85
Protobothrops_xiangchengensis	Protobothrops	0.85
Pseudocerastes_persicus	Viperinae	0.73
Pseudocerastes_urarachnoides	Viperinae	0.73
Pseudocerastesfieldi	Viperinae	0.73
Sistrurus_catenatus	crot_sist	0.90
Sistrurus_miliarius	crot_sist	0.90
Trimeresurus_albolabris	Trimeresurus	0.75
Trimeresurus_andersonii	Trimeresurus	0.75
Trimeresurus_barati	Trimeresurus	0.75
Trimeresurus_borneensis	Trimeresurus	0.75
Trimeresurus_buniana	Trimeresurus	0.75
Trimeresurus_cantori	Trimeresurus	0.75
Trimeresurus_erythrurus	Trimeresurus	0.75
Trimeresurus_fasciatus	Trimeresurus	0.75
Trimeresurus_flavomaculatus	Trimeresurus	0.75
Trimeresurus_fucatus	Trimeresurus	0.75
Trimeresurus_gracilis	Gloydius	0.8
Trimeresurus_gramineus	Trimeresurus	0.75
Trimeresurus_gumprechti	Trimeresurus	0.75
Trimeresurus_hageni	Trimeresurus	0.75
Trimeresurus_insularis	Trimeresurus	0.75
Trimeresurus_kanburiensis	Trimeresurus	0.75
Trimeresurus_macrops	Trimeresurus	0.75
Trimeresurus_malabaricus	Trimeresurus	0.75
Trimeresurus_malcolmi	Trimeresurus	0.75
Trimeresurus_mcgregori	Trimeresurus	0.75
Trimeresurus_medoensis	Trimeresurus	0.75
Trimeresurus_nebularis	Trimeresurus	0.75
Trimeresurus_popeiorum	Trimeresurus	0.75
Trimeresurus_puniceus	Trimeresurus	0.75
Trimeresurus_purpureomaculatus	Trimeresurus	0.75

Trimeresurus_sabahi	Trimeresurus	0.75
Trimeresurus_schultzei	Trimeresurus	0.75
Trimeresurus_septentrionalis	Trimeresurus	0.75
Trimeresurus_sichuanensis	Trimeresurus	0.75
Trimeresurus_stejnegeri	Trimeresurus	0.75
Trimeresurus_sumatranus	Trimeresurus	0.75
Trimeresurus_tibetanus	Trimeresurus	0.75
Trimeresurus_trigonocephalus	Trimeresurus	0.75
Trimeresurus_truongsonensis	Trimeresurus	0.75
Trimeresurus_venustus	Trimeresurus	0.75
Trimeresurus_vogeli	Trimeresurus	0.75
Trimeresurus_wiroti	Trimeresurus	0.75
Trimeresurus_yunnanensis	Trimeresurus	0.75
Tropidolaemus_subannulatus	Tropidolaemus	0.4
Tropidolaemus_wagleri	Tropidolaemus	0.4
Vipera_ammodytes	Viperinae	0.73
Vipera_anatolica	Viperinae	0.73
Vipera_aspis	Viperinae	0.73
Vipera_barani	Viperinae	0.73
Vipera_berus	Viperinae	0.73
Vipera_dinniki	Viperinae	0.73
Vipera_eriwanensis	Viperinae	0.73
Vipera_kaznakovi	Viperinae	0.73
Vipera_latastei	Viperinae	0.73
Vipera_lotievi	Viperinae	0.73
Vipera_nikolskii	Viperinae	0.73
Vipera_orlovi	Viperinae	0.73
Vipera_renardi	Viperinae	0.73
Vipera_seoanei	Viperinae	0.73
Vipera_transcaucasiana	Viperinae	0.73
Vipera_ursinii	Viperinae	0.73

ARBOREAL HABITATS CONSTRAIN MORPHOLOGICAL BUT NOT SPECIES DIVERSIFICATION IN VIPERS

Content of this chapter will be submitted to the journal Proceedings of the Royal Society of London B

Abstract. A shift into novel environmental conditions faced by a lineage is frequently suggested to be associated with an increase in ecological opportunities, which consequently will spur both species and morphological diversification. Although most adaptive radiation studies have relied on this tenet, it is indeed feasible that lineages might sometimes face the opposite situation, where changing environmental conditions will prevent lineages to diversify. Here we investigate the potential association between habitat and diversification regime in vipers. We hypothesize that arboreal habitats impose stronger limitations in morphological evolution consequently decreasing the speciation opportunities among arboreal compared to terrestrial vipers. Using recently developed trait-evolution models and a Bayesian species diversification method while incorporating phylogenetic and habitat reconstruction uncertainty, we show that although arboreal habitats strongly limit morphological evolution in vipers, speciation rates remain similar between habitats. We suggest two distinct scenarios underlying our results: (1) speciation could be independent of morphological evolution in vipers; or (2) geographic isolation would be an important mechanism underlying species diversification in arboreal lineages offsetting decreases in speciation opportunities potentially related to the selective pressures imposed by the arboreal environment.

INTRODUCTION

The exceptional diversity found in many groups of organisms has long been associated with factors that promote an increase in the ecological opportunities faced by a lineage (Schluter, 2000, Simpson, 1949, 1953, Yoder et al., 2010). In its simplest form, ecological opportunity can be defined as “a wealth of evolutionary accessible resources little used by competing taxa” (Schluter 2000). The invasion of new habitats, the emergence of a key innovation, the extinction of a predator or competitor are all considered as possible triggers of a rapid increase in ecological opportunities (Simpson, 1949, Yoder et al., 2010). The emergence of new ecological conditions can therefore trigger divergent selection, leading populations to adapt into the several new niches and ultimately promote speciation (Lapiedra et al., 2013, Nosil, 2012, Schluter, 2001). Given that the morphology of an organism reflects its ecology (Wainwright and Reilly, 1994), the emergence of ecological opportunities is, under this scenario, expected to generate an increase in both species and morphological diversification of a lineage experiencing such opportunities.

In fact ecological opportunity is frequently suggested as the main cause of adaptive radiations (Glor, 2010, Yoder et al., 2010), which are usually characterized by a higher rate of speciation and morphological evolution. Classic adaptive radiations such as Darwin's Finches (e.g. Grant and Grant, 2008), Caribbean *Anolis* lizards (Losos, 2009), and coral reef fishes (Alfaro et al., 2007, Price et al., 2011), all comprise examples of such scenario, where the invasion of previously unoccupied regions (e.g. islands) rapidly generated new morpho-types, presumably reflecting niche partitioning, (e.g. diet, microhabitat), ultimately leading to the rapid formation of new species (Schluter, 2000). Although adaptive radiations constitute remarkable examples of how shifts in ecological conditions led to a great increase in both species and morphological diversification of a lineage, adaptive radiations are considered an exception in nature (see Figure 15.4 in Losos and Mahler, 2010).

Several processes underlying the diversification patterns of different groups of organisms might not end up generating an impressive number of species or morphological variation (e.g. Kozak et al., 2006). In fact, it is easy to imagine a scenario where the invasion of a new habitat would constrain, instead of spur, both species and morphological diversification. Certain habitats might impose strong selective pressures, and evolutionary forces reducing trait variance like stabilizing selection (see Hansen, 1997) might allow only lineages with a specific combination of phenotypes to persist favoring evolutionary stasis and niche conservatism (Lapiedra et al., 2013). In an adaptive landscape framework (i.e. mapping fitness onto trait combinations, Laughlin and Messier, 2015), this specific combination of traits would represent a sharp adaptive peak comprising the higher fitness values in those habitats. Here, divergent selection would have a limited role in speciation, and diversification would be the result of mechanisms that generate species without necessarily generating significant morphological variation (e.g. geographic isolation followed by drift, Coyne and Orr, 2004, Nosil, 2012). In contrast, a scenario conductive to an adaptive radiation (i.e. those comprising ecological opportunities) might be better represented by multiple adaptive peaks favoring lineages with different morphological combinations to persist. In this case, divergent selection and speciation rate are assumed to be intertwined.

Snakes comprise a cosmopolitan group known to have radiated into a diverse array of habitats facing different ecological conditions during their evolutionary history (Greene, 1997, Uetz and Hosek, 2014). Although diversification rates vary considerably among snakes (e.g. Pyron and Burbrink, 2012), we do not know if this variation results from the interaction with different environments. Of particular interest is the invasion of the arboreal habitat, which took place multiple times during the evolutionary history of snakes (Lillywhite and Henderson, 1993). Although many species are terrestrial, some are considered to be fully arboreal (see Greene, 1997). Given its inherent limitations of being elongated and limbless organisms, arboreal snakes are known to possess a suite of behavioral, morphological and physiological adaptations allowing them to deal with the challenges of living in a structurally complex environment such as the arboreal habitats (e.g. Martins et al., 2001, Pizzatto et al., 2007, Sheehy et al., 2016). Moreover, distantly related arboreal lineages tend to show similar phenotypes and arboreal life style in snakes is usually considered to result in "morphological syndromes" (Greene, 1997, Lillywhite and Henderson, 1993). Thus, arboreal snake lineages comprise a striking example of evolutionary convergence (*sensu* Losos, 2011). In contrast, terrestrial snakes are known to have a much more generalized morphology (Greene, 1997). The similar phenotype among arboreal snakes and a much more varied one among terrestrial ones suggest that arboreal and terrestrial habitats might represent distinct adaptive landscape scenarios. In this sense, the invasion of the arboreal habitat by a terrestrial lineage of snakes might be accompanied by strong constrains in their morphology which could potentially affect species diversification dynamics.

Vipers (family Viperidae) are ideal models to explore the effect that habitat might impose in morphological and species diversification. Currently there are 329 viper species (Uetz and Hosek, 2014) and arboreality independently evolved multiple times, perhaps as many as 13 times in eight different genera (Figure 1). Additionally, arboreal lineages are not restricted to a single continent and instead are distributed in Central and South America, Africa and Asia (Campbell and Lamar, 2004, Orlov et al., 2002, Phelps, 2010, Figure 1). Differently from other snake groups, non-arboreal vipers do not comprise fossorial or aquatic species and are for the most part strictly terrestrial (Campbell and Lamar, 2004, Phelps, 2010 but see Material and Methods). Moreover, Hendry et al. (2014) recently suggested that changes in habitat use might be associated with changes in speciation rates in New World vipers. Thus, vipers constitute an ideal group to investigate how the invasion of a new habitat, and its new selective pressures, might affect diversification of both morphological and species diversification.

In the present study, our goal was to investigate the potential association between habitat and diversification regimes in vipers. Specifically, we explored if the invasion of arboreal habitats led to changes in morphological and species diversification. We first investigated the tempo and mode of morphological evolution associated with the different habitats. Given the potential limitations that arboreal life might impose on a snake (Lilliwhite and Henderson, 1997, Sheehy et al., 2016) we hence expected arboreal vipers to have a more constrained morphology when compared to terrestrial ones. We also expected that lineages switching from habitats comprising multiple adaptive peaks (terrestrial) to others characterized by only one main peak (arboreal) would be associated with smaller speciation rates given the decrease in speciation opportunities related to the phenotypic constraint that might be associated with these new environments.

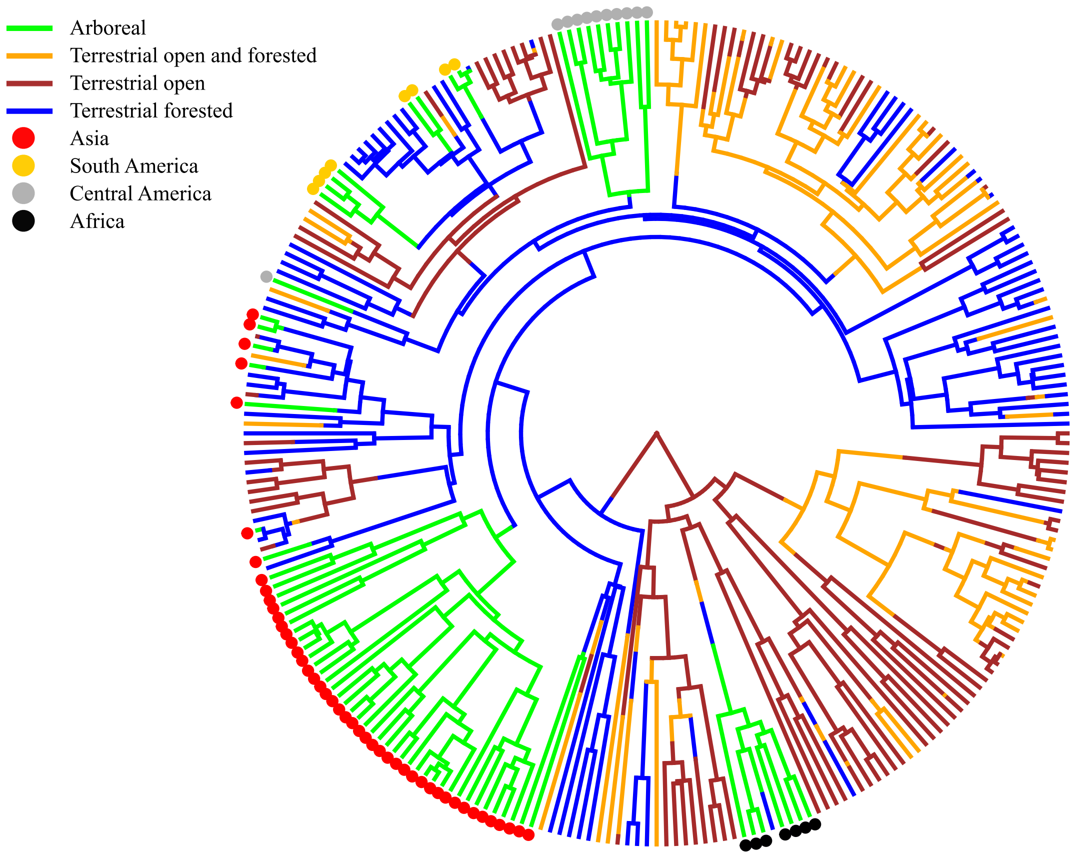

Figure 1. Example of a stochastic character mapping (simmap) on a viper phylogeny from the 1000 analyzed. Colors on branches denote the evolution of different habitats in vipers. Circles at tips indicate the predominant geographical occurrence of arboreal lineages.

MATERIAL AND METHODS

Phylogenies

In the present study, we used 100 comprehensive time-calibrated phylogenetic trees of extant vipers randomly obtained from the posterior distribution of BEAST analyses performed elsewhere (Chapter I). Phylogenetic reconstructions comprised 264 viper species and combined newly collected sequences and others previously available in GenBank and BOLD databases obtained for six mitochondrial (12S, 16S, cytb, nad4, nad2, cox1) and five nuclear (bndf, c-mos, jun, nt3, rag1) genes (for detailed information regarding phylogenetic reconstruction see Chapter I).

Thus, the phylogenetic scenario used here represents the most complete phylogenetic dataset for vipers to date, and includes almost 80% of all described species and all except one genus of vipers (Uetz and Hosek, 2014).

Habitat information

We characterized the habitat of each viper species present in the phylogeny using published literature and observations of researchers with recognized expertise in this snake group (Table S1). We categorized vipers as being: 1- arboreal; 2 - terrestrial restricted to open habitats; 3 - terrestrial restricted to forests; 4 - terrestrial occurring in both open and forested habitats. We chose to subdivide terrestrial vipers into three categories because while arboreal vipers are predominantly restricted to forests, terrestrial vipers occur not only in forested but also in open habitats (e.g. Campbell and Lamar, 2004, Phelps, 2010) and it is reasonable to expect that such different habitats (close forested vs open habitat) might impose very different selective regimes. By using such subdivision we are also being more conservative from the perspective of our proposed hypothesis given that using a single terrestrial category including both species restricted to forested and open habitats would more easily render evidence in favor of a more restricted morphology and lower speciation rates in arboreal species.

Some viper species occurring in arboreal habitats can also be frequently found on the ground and thus could be considered as “semi-arboreal”. However, arboreal and semi-arboreal vipers seem to be closely related in their morphospace (Figure S1, 11 semi-arboreal species shown) suggesting they share the same morphological syndromes, and thus, that potential selective pressures imposed by the arboreal environment might also affect semi-arboreal lineages. Moreover, semi-arboreal vipers comprise only 17 species in the phylogeny and would represent a very species poor habitat category, which would negatively affect the performance of methodological approaches to study the phenotypic and species diversification between different ecological regimes (e.g. Davis et al., 2013). Therefore, we decided to include “semi-arboreal” vipers into the “arboreal” category. Lastly, *Agkistrodon piscivorus* is sometimes considered as “semi-aquatic” (e.g. Vincent et al., 2005), but given that it is only one species we chose to include it in the terrestrial category.

Ancestral state reconstructions

We used stochastic character mapping (Simmap, R package Phytools, Revell 2012) to reconstruct the evolutionary history of habitat in vipers assigning the habitat categories to the internal branches of phylogenetic trees. We first fitted three different continuous-time Markov models of trait evolution (“equal rates”, “symmetrical rates” or “all rates different”, Harmon et al., 2008) across our 100 posterior randomly chosen trees and species habitat information to evaluate which one best explain our dataset (Table S2). We used the function “make.simmap” to estimate the habitat evolutionary history across our 100 trees. For each one of the posterior trees we set the transition rates matrix (Q) to be empirically estimated: first the previously chosen continuous-time Markov model (“equal rates”, “symmetrical rates” or “all rates different”) is fitted to our datasets (phylogenetic trees and habitat information), a single value of Q is estimated and used to simulate character histories (Revell, 2012). In each of the 100 posterior trees, we simulated 10 maps to incorporate uncertainty in the ancestral state reconstruction. However, for seven out of a 100 trees the “make.simmap” function could not estimate character histories under the best Markov model chosen (“all rates different”). Excluding these seven trees could remove part of the phylogenetic uncertainty incorporated in our analyses, and thus, in those cases we chose to use a simpler Markov model (“symmetrical rates”, Table S2) instead. In total we generated 1000 SIMMAP trees to be used in trait evolution analyses incorporating phylogenetic and ancestral estimates uncertainty.

Morphological data

We used snout-vent length (distance from the tip of the snout to the cloaca) as a proxy for body size, which is considered to be a relevant trait to many physiological and ecological adaptations across different animals (Bonner, 2011, Feldman et al., 2015). We chose to use body size instead of body mass because the later cannot be measured with accuracy in preserved specimens. In snakes, body size is frequently related to different life-history aspects (see Greene, 1997, Lillywhite and Henderson, 1993), including thermoregulation, fecundity, and diet. Apart from correlating allometrically with numerous other morphological traits body size explains a greater proportion of trait variance in vipers (83%, present study, results not shown, see França et al. 2008 for another example with snakes). Additionally, previous studies using independent contrasts (Felseinstein, 1985) either found an association between arboreality and a decrease in body size (e.g. Sheehy et al., 2016) or no association between this trait and the evolution of arboreal habits in snakes (e.g. Martins et al., 2001, Pizzatto et al., 2007). Hendry et al. (2014) suggested that arboreality is related to a decrease in body size in New World vipers but using total length as a proxy for body size. However, none of these studies investigated if the evolution of arboreality could impose constraining evolutionary force on morphological evolution.

Body size measurements were taken from several preserved specimens of vipers deposited in the National Museum of Rio de Janeiro (MNRJ), Butantan Institute (IBSP), Museum of Zoology of the University of São Paulo (MZUSP), Natural History Museum of London (BMNH), National

Museum of Natural History of Paris (MNHNP), Raffles Museum of Biodiversity Research (ZRC), University of Texas at Arlington (UTA), California Academy of Sciences (CAS), American Museum of Natural History (AMNH), Field Museum of Natural History (FMNH), and Smithsonian National Museum of Natural History (USNM) (Appendix I). We were able to get measurements for 236 viper species and to complement our morphological database, we also added literature records for 13 species not represented in the scientific collections visited (Table S1). In total we were able to get body size measurements for 94% of the species included in the phylogeny representing 75% of all viper species. Table S1 provides a summary of body size data gathered for the present study.

In the following analyses we used mostly measurements of adult males. However, for 16 out of 249 species we only had access to morphological measurements for adult females (Table S1). We chose to include female body size data instead of excluding these 16 species from our analyses and are confident that including these data have not biased our results (see Appendix II). We attempted to measure several individuals for each species but because snakes and especially vipers are frequently damaged when killed, we were not able to include measurements for several preserved specimens. Thus, the number of specimens varies among species. When more than one individual was measured, we used the average body size of each species. All measurements were log transformed (base 10) prior to analyses.

Models of trait evolution

To explore if the invasion of arboreal habitats is associated with changes in the morphological diversification of vipers we used different models of character evolution implemented in a model-fitting framework, namely Brownian Motion “BM” and Ornstein-Uhlenbeck “OU” models (Beaulieu et al., 2012, Price and Hopkins 2015). The OU process is ideal to model changes in selection regimes, such as differences in the phenotypic optima (Θ), phenotypic rate (σ^2, also known as stochastic motion parameter) or strength of selection (α) between habitats (Ho and Ané, 2014). The phenotypic optima describes a hypothetical phenotype toward which populations are evolving when under an OU process; the phenotypic rate (stochastic motion parameter) measures the intensity of stochastic fluctuations in the evolutionary process, in other words, the rate of stochastic evolution away from the phenotypic optima; the strength of selection indicates the attraction toward the phenotypic optima measuring how fast a trait evolves to the associated phenotypic optima (Butler and King, 2004, Beaulieu et al., 2012, Hansen, 1997). When $\alpha = 0$ constraint to stay near the optima disappears and the OU process reduces to Brownian motion (see Butler and King, 2004, Beaulieu et al., 2012, Ho and Ané, 2014). We expect that the phenotypic rate will be smaller and/or the strength of selection will be larger in arboreal habitats compared to terrestrial ones suggesting that arboreal vipers might be evolving under a distinct and possibly more constrained selection regime.

We fitted seven different models to the body size of vipers and simmap trees using the R package OUwie (Beaulieu et al., 2012), after pruning species according to body size data. The models used were: (1) BM1, a single rate Brownian motion model; (2) OU1, a single optimum model; both (1) and (2) suggest that phenotype is evolving independently of habitat; (3) BMS, a multi-rate Brownian motion model that allows phenotypic rate to differ among habitats; (4) OUM, assumes distinct phenotypic optima for each habitat while having a single phenotypic rate and strength of selection for all habitats; (5) OUMV, allows a different phenotypic rate related to different phenotypic optima for each habitat; (6) OUMA, allows a different strength of selection and a different phenotypic optima for each habitat; (7) OUMVA, allows both phenotypic rate and strength of selection, as well as different phenotypic optima for each habitat.

After performing OUwie analyses, we followed Price and Hopkins (2015) in first checking if the eigenvalues of the Hessian matrix were positive, which indicates that parameters were reliably estimated (Beaulieu et al., 2012, Price and Hopkins, 2015). We also checked if the phenotypic optima estimated were biologically feasible. We considered as biologically feasible optimum values between one and five (log10) (values within or close to the interval of the empirical log10 values for body size, see Figure 2). Using this interval we excluded analyses returning very unrealistic optima (optimum values equal or higher than five would represent lineages with body size evolving to 100 meters or more). When we found negative eigenvalues and unreliable phenotypic optima, we excluded the results for the corresponding model and tree combination. We calculated the Akaike criterion for small sample size (AICc) (Burnham and Anderson, 2002) followed by the ΔAICc to investigate which model best explains body size evolution in each simmap tree. A model was considered significantly better when the ΔAICc difference between the second and the best model was higher than two. Quantifying model fitting for each simmap tree combination separately allowed us to assess the sensitivity of model selection to alternative habitat evolutionary histories and phylogenetic scenarios combinations (e.g. Collar et al., 2011).

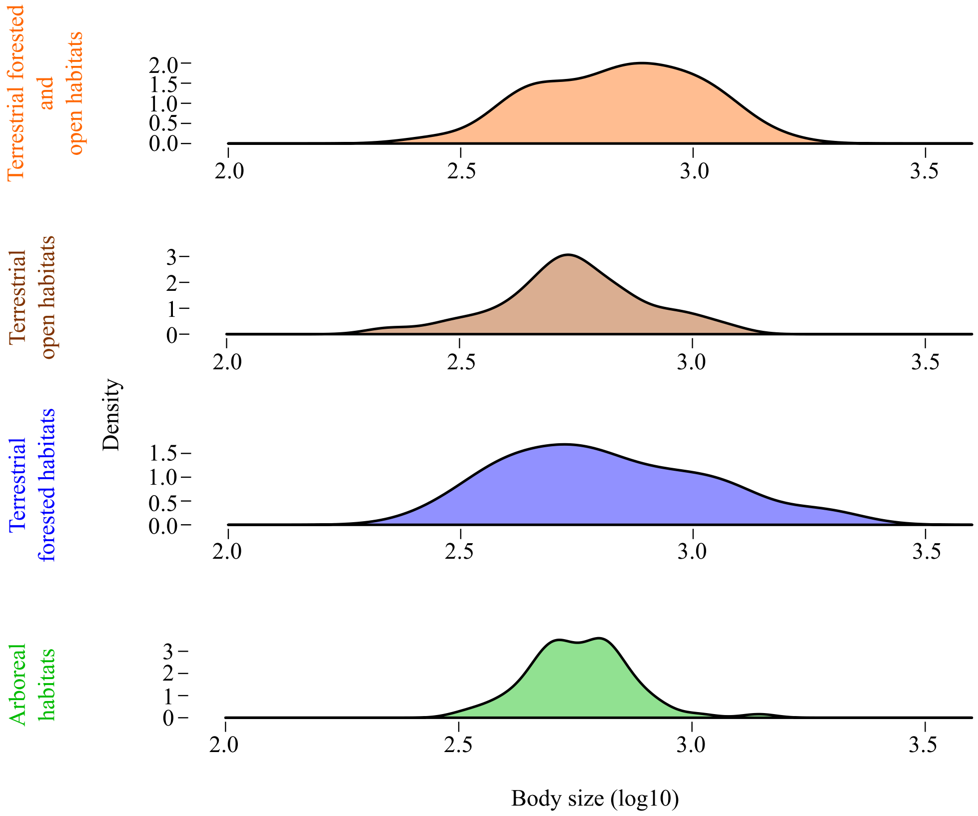

Figure 2. Body size distribution in vipers across different habitats.

Fitting all seven models for a given simmap tree was rarely possible (26% of the simmaps analyzed in OUwie were able to fit all seven models; see Results) mainly because fitting different α for each habitat (OUMA and OUMVA) frequently resulted on inaccurate estimates. These inaccurate fittings were frequently associated with negative eigenvalues (parameters not reliably estimated) or more occasionally with unrealistic estimated optima (e.g. values falling far beyond the empirical body size values). Failing to adequately fit α varying models in certain simmaps have been reported before (Moen et al., 2015, Price and Hopkins, 2015) and are likely to be related to very complex estimated character histories, which renders OUwie with not enough data to infer body size evolution under these models (e.g. limited number of tips in the phylogenetic tree for very complex histories).

Previous studies (Moen et al. 2015, Price and Hopkins 2015) have simply removed complex models from the analysis. But because estimating the α parameter would greatly improve the evaluation of our trait evolution hypothesis, we decided to keep these complex models in our analyses whenever possible. We note that different simmap tree combinations allowed different models to be fit and that at least one complex model (e.g. OUMA, OUMV or OUMVA) could always be fit. Thus we expected to be able to at least discern if OUMA, OUMV or OUMVA were best models over BM, BMS, OU1 and OUM. By not restricting ourselves to the very simple models we expect to be able to investigate the possibility of a more constrain evolution in the phenotype of arboreal when compared to terrestrial vipers. A detailed investigation regarding the limitations of OUwie in fitting complex models is beyond the scope of this study. However, information regarding how many simmaps were able to fit each of the seven trait evolution models is available in Table 1.

Finally, we performed additional analyses to test for model adequacy. First, we randomly selected 300 simmap trees used in OUwie analyses for which the best model was one of the three more complex models (100 simmap trees for OUMVA, 100 for OUMV and 100 for OUMA). Collectively those explain the vast majority of our estimates. We then simulated 100 continuous traits for each of the 300 simmap trees using the corresponding empirical parameter values (Θ, σ^2 and α) estimated by OUwie under the best model chosen for each tree (OUMVA, OUMV or OUMA) and compared the resulting body size distribution to the empirical one. By doing this we explored if simulated values (estimated based on empirical parameters) recovered empirical body size values and hence allowing us to evaluate the adequacy of the best chosen models.

Species diversification

In order to investigate if habitat evolution has an impact on species diversification in vipers we used the Multiple State Speciation Extinction (MuSSE) model (FitzJohn, 2012) fitted to 100 randomly chosen phylogenetic trees obtained from BEAST posterior distribution (Chapter I). The MuSSE models were implemented in a Bayesian MCMC framework accounting for phylogenetic and rate value uncertainties to be naturally incorporated in the analysis. We performed MuSSE analyses in the R environment using the diversitree package (FitzJohn, 2012) and a script implementing the MCMC approach (available at https://github.com/dsilvestro/mcmc-diversitree), allowing all parameters to be independently estimated (no constrains). We ran MuSSE for 2,000,000 steps to achieve convergence of parameters discarding estimates from 13 trees that failed to converge.

We combined the posterior distributions of parameters from the 87 phylogenetic trees analyzed into one posterior distribution for each parameter estimated by MuSSE (speciation and extinction in each habitat and transition rates among habitats). We calculated speciation minus extinction for each iteration of the MCMC to build the posterior distribution for net diversification rates in each habitat. To explore if arboreal lineages have smaller speciation rates compared to terrestrial ones, we calculated the differences between speciation rates estimated for arboreal lineages and those estimated for the three terrestrial categories for each MCMC step. By doing this we built posterior distributions of differences and considered speciation rates of arboreal lineages to be smaller and distinct from the remaining when values were negative and 0 was not included inside the 95% highest posterior density (HPD) calculated for each posterior difference. Although our original hypothesis relates to differences in speciation rates between arboreal and terrestrial vipers, we also present results for net diversification, extinction and transitions rates.

Recently, the performance of the state-dependent speciation extinction models (xxSSE models) has been criticized (Maddison and FitzJohn, 2014, Rabosky and Goldberg, 2015). The major concerns of xxSSE models are related to these models showing high type I error. Even though our results do not show any associations between diversification rates and habitat (see Results), our parameter estimates could still be compromised by model inadequacy (Rabosky and Goldberg, 2015). Following Price et al. (2012) and Burin et al. (*accepted*) we performed posterior predictive simulations in order to evaluate how reliable our results are. We simulated 1000 trees using the empirical number of states (four) and the median of the posterior distribution of each parameter estimated in MuSSE analyses using the trait-dependent simulation function tree.musse (diversitree R package, FitzJohn, 2012). We set the root states in the simulations proportionally to the empirical proportion of each state (i.e. each habitat) observed in the empirical dataset. We then calculated the proportion of states (arboreal and the three terrestrial categories) recovered in each simulated tree and compared it to the empirical distribution. By doing this we could check if the proportions of empirical states lie within the values delimited by the simulated proportions, i.e. if simulated proportions can generate realistic predictions about trait state proportions.

RESULTS

Trait evolution

We were able to successfully fit all seven trait evolution models in 261 simmaps from the 1000 analyzed (26% of all simmaps). Among these 261, 243 (93%) suggested OUMVA as the best model (ΔAICc difference between the second and the best model was higher than two). Although most simmaps were not able to fit all seven models (returning negative eigenvalues or unrealistic phenotypic optima) when taking all of the 1000 analyzed, 89% suggested OU models with multiple θ and different α and/or σ^2 parameters as best models (OUMVA: 436 simmaps, OUMV: 347 simmaps, OUMA: 111 simmaps, Table 1). Looking to the fittings of each model separately (each line in Table 1), OUMVA was the best model in 93% of the simmaps it was fitted (436 from 465). Considering the simmaps in which OUMV was fitted (983), 35% suggested OUMV as the best model (347 from 983) but 44% suggested OUMVA (434 from 983). However, it is worth mentioning that from the 983 simmaps fitting OUMV, only 465 were also able to fit OUMVA, probably underestimating the real proportion of simmaps suggesting OUMVA as the best model (Table 1). Among the simmaps in which OUMA was fitted (493), 22% suggested it as the best model (111 from 493) whereas 49% suggested OUMVA (245 from 493). Similar to OUMV fittings, from the 493 simmaps that fitted OUMA, only 263 also fitted OUMVA, also probably underestimating the real proportion of simmaps suggesting OUMVA as the best model (Table 1). In general, our results suggest that OUMVA, the most complex model, is chosen as the best model in most of the simmaps it was fitted with OUMV and OUMA being chosen when OUMVA could not be fitted (Table 1).

Table 1. Number of OUwie fittings for each model (number at the top, total of 1000 simmaps) in trait evolution analyses, and the respective number each model was chosen as the best model (number at the bottom). Each line represents fitting and best model frequency relative to the sample of simmaps fitted for each model separately (shaded). For example, in the first line 465 simmaps analyzed by OUwie fitted OUMVA and 436 suggested it as the best model. From these 465 simmaps, 463 fitted both OUMVA and OUMV, but only 11 suggested OUMV as the best model. Best model frequency in each line does not sum the number of fittings of each reference model (in the first line example, 465) because some simmaps analyzed did not suggest a best model (ΔAICc difference between the second and best model smaller or equal to 2).

	BM1	BMS	OU1	OUM	OUMA	OUMV	**OUMVA**
Fittings	465	464	465	465	263	463	**465**
Best model	0	0	0	0	2	11	**436**
	BM1	BMS	OU1	OUM	OUMA	**OUMV**	OUMVA
Fittings	983	982	983	982	490	**983**	463
Best model	0	0	12	1	110	**347**	434
	BM1	BMS	OU1	OUM	**OUMA**	OUMV	OUMVA
Fittings	493	493	493	493	**493**	490	263
Best model	0	0	1	0	**111**	96	245
	BM1	BMS	OU1	**OUM**	OUMA	OUMV	OUMVA
Fittings	999	998	999	**999**	493	982	465
Best model	0	0	12	**15**	111	347	436
	BM1	BMS	**OU1**	OUM	OUMA	OUMV	OUMVA
Fittings	1000	999	**1000**	999	493	983	465
Best model	0	0	**12**	15	111	347	436
	BM1	**BMS**	OU1	OUM	OUMA	OUMV	OUMVA
Fittings	999	**999**	999	998	493	982	464
Best model	0	**0**	12	15	111	347	435
	BM1	BMS	OU1	OUM	OUMA	OUMV	OUMVA
Fittings	**1000**	999	1000	999	493	983	465
Best model	**0**	0	0	15	111	347	436

Because OUMVA could not be fitted in somewhat more than half of the simmaps, we also present parameter estimates for the other two frequently chosen models, OUMV and OUMA (Figure 3, Figure S2). When looking at the parameter estimates it is clear that a very similar scenario is depicted independently of the best model chosen (OUMVA, OUMV or OUMA, Figure 3 and Figure S2). Parameter estimates usually show similar values among the different three best models (exceptions are the large σ^2 values in Figure 3E). Independently of the best model, body size optima (Θ) tend to show larger values for terrestrial vipers occurring in both forested and open habitats or restricted to forested habitats compared to terrestrial vipers restricted to open habitats or occurring in arboreal habitats (Figures 3A, D, G). Interestingly, body size optima estimates show less variation in arboreal compared to terrestrial habitats (Figure 3, Figure S2).

When α was allowed to vary between categories (models OUMVA and OUMA), arboreal habitats show higher α suggesting that after a lineage invades the arboreal environment, body size might evolve faster toward the optima compared to terrestrial habitats (Figures 3B, H, and Figure S2). Some analyses even suggested α values very close to zero for terrestrial categories indicating that body size could be evolving under a Brownian motion instead of an Ornstein-Uhlenbeck process in those habitats (Figure 3B, H, Figure S2). In contrast, when σ^2 was allowed to vary between habitats (models OUMVA and OUMV), arboreal habitats showed smaller σ^2 compared to terrestrial categories (Figures 3C, E, F, Figure S2), with OUMVA analyses suggesting even smaller σ^2 in arboreal habitats, frequently approaching zero (Figure 3C, Figure S2). Smaller σ^2 suggest that body size evolves under a much smaller rate of stochastic fluctuation in arboreal habitats compared to terrestrial ones. It is important to note that although σ^2 estimates under the OUMV model comprise two sets of values with different magnitudes, each one represent estimates from a distinct sample of simmaps that is the same across habitats. Thus, both σ^2 estimates still suggest smaller σ^2 in arboreal relative to terrestrial habitats.

Simulations performed using empirical parameters estimated under the most frequently chosen OUwie models (OUMA, OUMV, OUMVA) recovered body size values similar to empirical ones (Figure S3). Thus, simulations suggest that parameters were reliably estimated in our OUwie analyses.

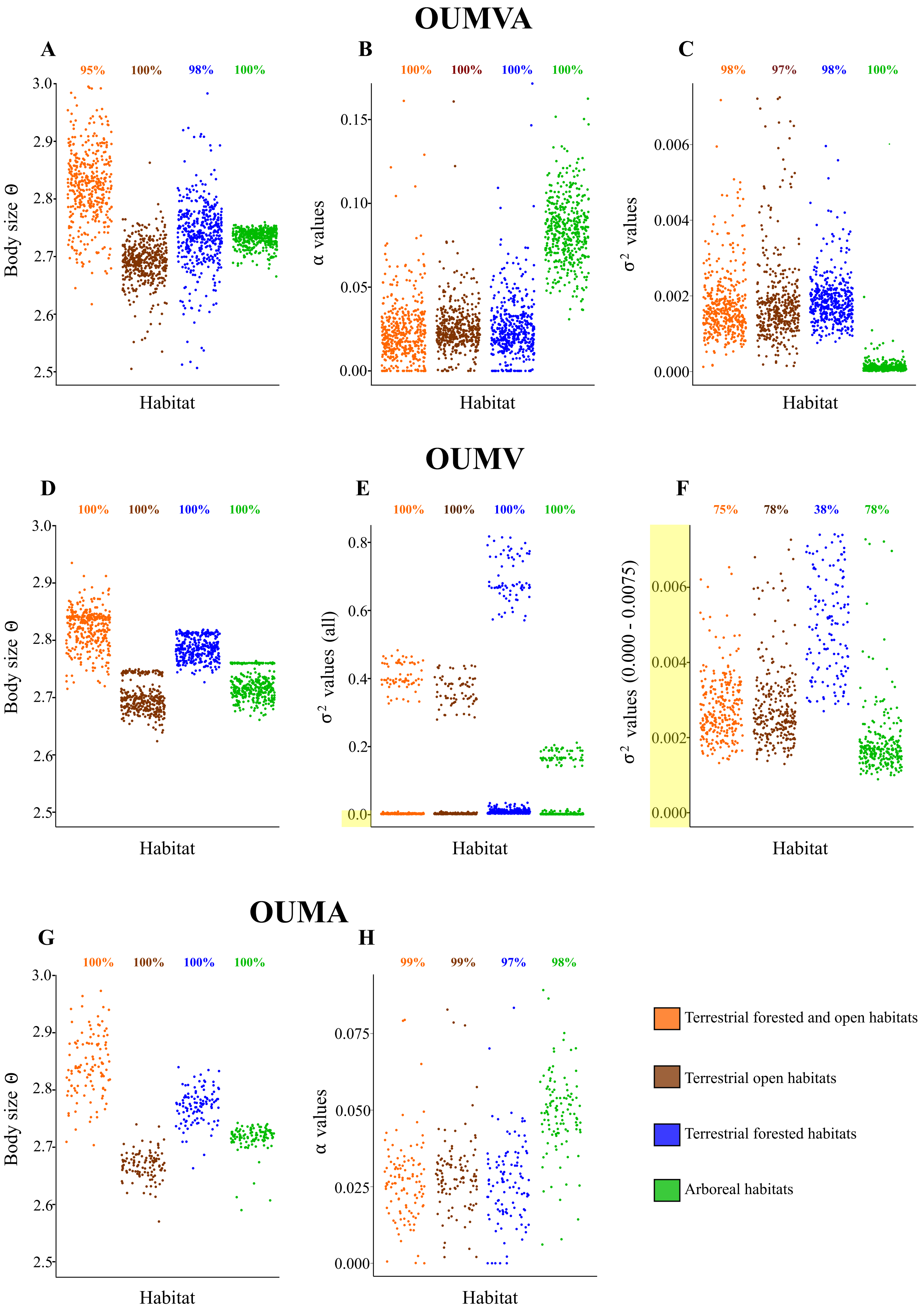
OUMVA
A
95%
100%
98%
100%
Body size Θ
3.0
2.9
2.8
2.7
2.6
2.5
Habitat
B
100%
100%
100%
100%
α values
0.15
0.10
0.05
0.00
Habitat
C
98%
97%
98%
100%
σ2 values
0.006
0.004
0.002
0.000
Habitat
OUMV
D
100%
100%
100%
100%
Body size Θ
3.0
2.9
2.8
2.7
2.6
2.5
Habitat
E
100%
100%
100%
100%
σ2 values (all)
0.8
0.6
0.4
0.2
0.0
Habitat
F
75%
78%
38%
78%
σ2 values (0.000 - 0.0075)
0.006
0.004
0.002
0.000
Habitat
OUMA
G
100%
100%
100%
100%
Body size Θ
3.0
2.9
2.8
2.7
2.6
2.5
Habitat
H
99%
99%
97%
98%
α values
0.075
0.050
0.025
0.000
Habitat
Terrestrial forested and open habitats
Terrestrial open habitats
Terrestrial forested habitats
Arboreal habitats

Figure 3. Parameters estimates under OUMVA (A-C), OUMV (D-F), and OUMA (G, H) when chosen as best models. Dots represent OUwie analyses performed in distinct simmaps. For better visualization we excluded extreme values and numbers on the top represent the proportion of values included. Because sigma values estimated under OUMV model have two orders of magnitude, we presented the smaller values separately in graph F. However, it is important to point out that these two orders of magnitude estimated for σ^2 represent distinct sample of simmaps and thus both still recovered the same pattern of σ^2 estimated under OUMVA with arboreal vipers associated with smaller sigma values.

Species diversification

Contrary to our expectations, speciation rates do not differ among habitats (Figure 4). If speciation rates of arboreal lineages were smaller than terrestrial ones, we would expect the 95% HPD intervals of posterior distributions of differences (arboreal minus terrestrial) to comprise only negative values. However, although medians fall either in positive or negative values depending on the terrestrial category being compared, the 95% HPD intervals include zero in all comparisons (Figure 4B). Similarly, extinction and net diversification rates do not differ among habitats (Figure S4 and S5).

Transition rates estimated by MuSSE suggest that during the diversification of vipers, transitions between habitats predominantly occurred among terrestrial ones (Figure 4C). Higher transition rates occurred between lineages from open habitats and those occurring in both open and forested habitats, with higher rates into open habitats (Figure 4C). Interestingly, the open and forested habitats seems to act as an “intermediate stage” for shifts between more restricted terrestrial categories (terrestrial forested habitats and terrestrial open habitats, Figure 4C). Transitions into arboreal habitats seem to have been mostly limited to occur from terrestrial lineages restricted to forested habitats. Finally, transition estimates suggest that once a lineage becomes arboreal, it will rarely become terrestrial again (Figure 4C).

Our posterior predictive simulations indicate that the rate values obtained in MuSSE analyses represent a plausible and reliable evolutionary scenario, since the distribution of trait state proportions from simulated trees encompass the trait state proportions observed in our data (Figure S6).

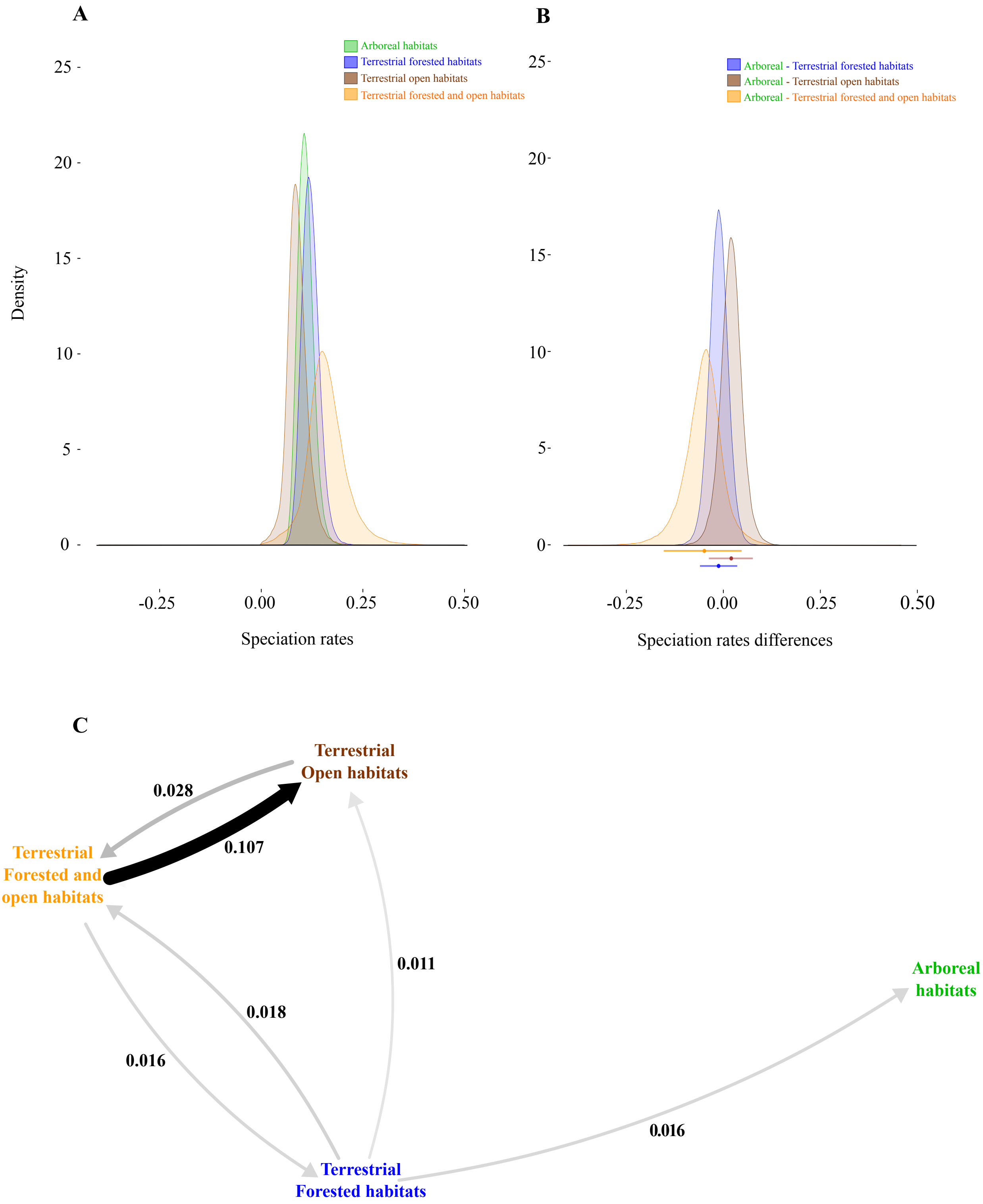

Figure 4. (A) Posterior distributions of speciation rates estimated for arboreal and terrestrial lineages. (B) Posterior distributions of differences between speciation rates of arboreal and terrestrial lineages. Bars represent 95% HPD interval and dots represent medians. (C) Median of the estimated transition rates between lineages from distinct habitats. Transitions rates less than or equal to 0.01 are not shown.

DISCUSSION

Body size evolves differently between arboreal and terrestrial habitats in vipers. Trait evolution analyses suggest that habitats are associated with distinct body size optima, and in accordance with our expectation, arboreal habitats seem to impose stronger evolutionary constrains in phenotypic diversification. Surprisingly, MuSSE analyses did not find any substantial difference between speciation rates among habitats. That is, phenotypic and species diversification seem to be decoupled, suggesting that phenotypic constrains associated to the arboreal habitat do not affect species diversification in vipers.

Being elongated and limbless organisms, snakes face several challenges in arboreal habitats, such as moving through the discontinuous physical environment and the gravitational effects on blood circulation (see Lillywhite and Henderson, 1993, Pizzatto et al., 2007, Sheehy et al., 2016). As a consequence, living in arboreal habitats demand a much more specialized morphology compared to other environments (as suggested in the literature by the "morphological syndromes", Greene, 1997, Lillywhite and Henderson, 1993), potentially preventing further phenotypic diversification, which might be more relaxed in terrestrial habitats. Although other studies explored potential associations between arboreal habitats and body size evolution among snakes (e.g. Martins et al., 2001, Pizzatto et al., 2007, Sheehy et al., 2016), these studies solely investigated correlational trends (i.e. evolution of arboreal habits associated with a decrease in body size) finding contrasting results. Despite extensively suggested in the literature (e.g. Greene, 1997, Lillywhite and Henderson, 1993, Lillywhite et al., 2012), this is the first study to find evidence for distinct morphological evolutionary regimes among habitats in snakes with arboreal ones imposing stronger limits on morphological evolution. Interesting, limitations on morphological diversification imposed by arboreal habitats are not exclusive among vipers but also seem to strongly constrain morphology among pigeons and doves (Lapiedra et al., 2013). Given that a change to arboriality might in fact impose novel enviromental challenges, the evolution of arboreality might frequently characterize a remarkable event during the radiation of a lineage where shifts into this functional demanding ecological regime might promote a strong effect in phenotypic diversification.

The distribution of body sizes in arboreal vipers tends to be intermediate and more clumped when compared to terrestrial lineages (Figure 2). Indeed trait evolution models suggested that arboreal vipers might be evolving toward an intermediate body size optima compared to terrestrial habitats. In arboreal habitats, having larger body sizes could increase gravitational disturbance in blood circulation, especially in elongate organisms assuming vertically oriented postures such as snakes (e.g. Lillywhite and Henderson, 1993, Sheehy et al., 2016). This could be especially true for vipers because differently from other arboreal snakes, some physiological adaptations to arboreality suggested to potentially overcome gravitational impacts on blood circulation (e.g. anterior heart position), did not evolve among arboreal vipers (Lillywhite et al., 2012). Thus, longer body sizes would be even more problematic for arboreal vipers. Moreover, although arboreal snakes have a smaller mass/body size ratio compared to terrestrial ones (e.g. Feldman and Meiri, 2013, Guyer and Donnely, 1990), there might be a limit in decreasing mass relative to an increase in body size. Therefore, an increase in body size would also increase mass bringing difficulties for more heavy bodied snakes in moving along small and fragile tree branches (Ray, 2012, Sheehy et al., 2016). In contrast, limits to the evolution of smaller body sizes in arboreal snakes have not been extensively explored in the literature. Studying NW vipers, Hendry et al. (2014) suggest that despite the overall decrease in body size in arboreal lineages there may be a lower bound on female size possibly imposed by fecundity selection. Our results indicate however that a lower bound in body size evolution in fact exists among arboreal vipers, at least in males which were the commonly measured sex in this study. We thus argue that being too small may also prevent snakes in spanning gaps between branches leading to an inefficient locomotion along trees. Thus, it seems completely feasible that neither a body size that is too large nor too small characterizes the optimum for vipers leaving in arboreal habitats. Different body size optima associated with arboreal relative to other habitats have also been suggested for lizards (Collar et al., 2011) and pigeons and doves (Lapiedra et al., 2013).

Trait evolution analyses also show that body size optimum in arboreal habitats seems to be more confidently estimated than for terrestrial habitats (Figure 3). This greater variation found among the optima estimated for terrestrial habitats might reflect its greater environmental heterogeneity comprising several opportunities for terrestrial lineages to adapt driving them to distinct adaptive peaks. We interpret the observed variation on the model selection analysis seen for terrestrial habitats as a result of models with different estimated optima equally explaining body size evolution in terrestrial vipers. We note thought that the suggestion of terrestrial habitats comprising multiple adaptive peaks relative to arboreal ones has also been made in studies focusing in other animal groups (e.g. lizards, Collar et al., 2011, Birds, Lapiedra et al., 2013). This idea is, in fact, in accordance with the differences in α and σ^2 estimates found between habitats.

Alpha and σ^2 are very important to the interpretations of our results because they describe the possible phenotypic evolutionary trajectories found among habitats. The three most frequently chosen trait evolution models suggested the same general pattern regarding α and σ^2 : lineages evolving in arboreal habitats tend to be associated with higher α and smaller σ^2 compared to those evolving in the three terrestrial habitats. Taken together, higher α and smaller σ^2 mean weaker phylogenetic correlation, that is, ancient adaptations are less important relative to the adaptation to new conditions (Hansen, 1997), faster evolution toward the optima and weaker stochastic noise (or variance accumulation rate) (Butler and King, 2004, Hansen, 1997, Ho and Ané, 2014). Thus, these two parameter estimates are in accordance with a scenario where vipers might be evolving toward a broader range of morphological variants in terrestrial habitats. On the other hand, limited morphotypes are able to persist in arboreal habitats and once a lineage invades the arboreal environment, strong selective pressures rapidly drive morphology toward this optima. It is interesting to note that arboreal snakes tend to evolve from lineages that inhabit terrestrial forested habitats (Figure 4C), which depending on the model are characterized by either a slightly larger body size optima then arboreal habitats (Figure 3D and Figure 3G), or in the case of the most complex model, to have a broader distribution of body size that encompasses larger and smaller species (Figure 3A). This is indeed in accordance with a scenario that suggests a very strong selection towards evolving smaller or larger body size (the higher alpha value) and a strong phenotypic constraint once the arboreal optima is attained (smaller sigma).

One could argue that α values recovered in the present study might be too small compared to other studies (e.g. Lapiedra et al., 2013) possibly suggesting that processes described here do not differ from a Brownian-like process (see Cooper et al., 2015) and that the differences found between arboreal and terrestrial habitats would be negligible. However, as suggested by Cressler et al. (2015), interpretation and comparison of α and σ^2 between studies are complicated because of their dependence on time and trait being studied. Snakes have much less varied morphologies compared to other animals and thus, magnitude and directions that their body size have to evolve will undoubtedly be much more limited. To get an idea if the estimated α correspond to meaningful values, it is more interesting to interpret α values relative to the height of the phylogenetic tree used in the study by estimating the phylogenetic half-life ($t_{1/2}$): $\ln(2)/\alpha$ (Cooper et al., 2015, Ho and Ané, 2014). Phylogenetic half-life measures the time an ancestral phenotype takes to evolve halfway toward the optimum after a lineage invade a new selective regime (in our case, a different habitat). Thus, if $t_{1/2}$ is extremely large relative to the tree height it suggests that the OU process may be extremely weak (Cooper et al., 2015). Among vipers, $t_{1/2}$ of body size in arboreal habitats comprise much shorter times (8.23 or 14.25 mya, Table 2) than the stem age of most arboreal lineages (~25-35 mya, see Chapter I) and thus, are even shorter compared to the tree height of vipers (~50 mya crown group, see Chapter I). Phylogenetic half-lives in terrestrial habitats are comparatively larger suggesting that body size evolves at least two times faster toward the optimum in arboreal habitats than in terrestrial ones (Table 2). This difference is even more pronounced for phylogenetic half-lives estimated when OUMVA was the best model (Table 2). That is, α values in the present study seem to comprise plausible estimates undoubtedly showing evidence that different evolutionary processes are associated to the evolution of body size in arboreal when compared to terrestrial vipers.

Table 2. Median of phylogenetic half lives ($\ln(2)/\alpha$) estimated for each habitat category across simmaps suggesting OUMVA or OUMA as the best trait evolution models. Mya: million of years ago.

	Terrestrial forested and open habitats	**Terrestrial open habitats**	**Terrestrial forested habitats**	**Arboreal habitats**
OUMVA	32.69 mya	29.56 mya	30.56 mya	8.23 mya
OUMA	26.51 mya	24.65 mya	26.07 mya	14.05 mya

Because morphology usually reflects the ecology of an organism (Wainwright and Reilly, 1994) and selection to different niches is known to underlie species diversification in several groups (e.g. Grant and Grant, 2008, Losos, 2009), speciation is frequently coupled with morphological changes (see Nosil, 2012). Thus, an ecological regime imposing strong constrains in the morphological evolution of a lineage is expected to comprise limited speciation opportunities for that lineage to diversify. However, this was not found in vipers. Although trait evolution analyses suggest that morphological evolution is more constrained among lineages evolving in arboreal habitats, differently from what we expected, this limited adaptive landscape does not seem to have affected the speciation opportunities faced by arboreal relative to terrestrial vipers; in other words, speciation rates remain similar between both types of habitats. In fact, the present study is in accordance with a decoupling between morphological and species diversification in vipers, bringing light into the speciation dynamics that are driving the diversity of the group.

A decoupling of morphological and taxonomic diversity has been found in the fossil record (Benton, 2015, Ruta et al., 2013) indicating that, over geological time scales, patterns of constraints in morphology might change and species diversity can accumulate without increasing morphological diversity (Foote, 1993). In fact, although underappreciated, there are several mechanisms of morphologically static cladogenesis (i.e. speciation not accompanied by significantly morphological change) (Bickford et al., 2006). For example, after two populations become geographically isolated random changes in gene frequencies might take place due to genetic drift or bottleneck effects ultimately causing hybrid sterility or inviability (Coyne and Orr, 2004, Nosil, 2012). Geographically isolated populations could also give rise to new species through processes involving selection and still do not show significant morphological differences. As an example, in processes like mutation-order speciation, different but incompatible mutations are fixed in separated populations adapting to similar selective regimes (Butlin et al., 2012, Nosil and Flaxman, 2011, Nosil, 2012). Similary to drift, reproductive isolation would emerge, for example, due to genetic conflicts between populations (e.g. Coyne and Orr, 2004). Moreover, mating preferences divergence on chemical signals between isolated populations could also underlie species diversification without leading to morphological changes. Indeed, differential recognition with subsequently preference for different chemical signals is suggested to play a significant role during the speciation process in different organisms (Review in Smadja and Butlin, 2009), especially among those relying more upon chemical senses than vision (Bickford et al., 2006), like snakes (Fornasiero et al., 2011, Shine et al., 2002). It is worth mentioning that mutation-driven or sexual chemical selection do not only occur in the presence of geographic isolation, but these processes might be significantly enhanced when populations are geographically isolated (e.g. Fornasiero et al., 2011, Nosil and Flexman, 2011).

At first glance, our results could suggest that mechanisms underlying species diversification in vipers might agree with a scenario where speciation events are not coupled with morphological changes. That is, selective pressures imposed on body size, even if constraining it, might not have affected the probability of speciation among habitats rendering speciation rates similar. The morphological diversity observed among terrestrial lineages would have arrived due to natural selection mostly after speciation is completed and not as drivers of reproductive isolation (Rundell and Price, 2009, Svensson, 2012). Interesting, the formation of cryptic species, usually related to species diversification without morphological change, has been described among both terrestrial and arboreal vipers (e.g. Jadin et al., 2012, Sanders et al., 2006). Alternatively, if morphological differentiation indeed occur during the speciation process in vipers, the similar speciation rates found among habitats showing distinct morphological regimes require an additional explanation. In fact, we could argue that after populations are geographically isolated, completion of the speciation process might occur more frequently in arboreal compared to terrestrial habitats potentially offsetting any decrease in speciation opportunities mediated by divergent selection in the arboreal environment.

The evolutionary transitions rates found here suggest that once arboreality evolved in vipers, reversals to terrestrial habitats are rare (Figure 4C). In fact this pattern seems to predominate among arboreal lineages in general (e.g. Lapiedra et al., 2013). In contrast, our results show that transitions between terrestrial habitats are more common (Figure 4C). Vipers comprise several genera including solely arboreal species (and thus, without any reversions to terrestriality, see Figure 1), such as the species-rich *Trimeresurus* comprising around 50 species and estimated to have ~35 million years (Uetz and Hosek, 2014, Chapter I). As proposed by Wiens (2004) the tendency of lineages to retain their niches over long evolutionary time scales (i.e. phylogenetic niche conservatism), and thus, failing to adapt to new ecological conditions, is a key factor during speciation following geographic isolation (see also Kozak and Wiens, 2006). Under this scenario it is possible that individuals of arboreal lineages would be unable to persist in new environmental conditions rendering their geographic range to become fragmented (Wiens, 2004). Assuming such mechanism in our case would suggest that speciation rates would remain similar between habitats because the decrease in speciation opportunities due to divergent selection in arboreal habitats would be offset by the higher frequency of speciation mediated by geographic isolation in lineages inhabiting those environments.

A scenario where an increase in the opportunities to speciate via geographic isolation offset the decrease in divergent selection opportunities in arboreal habitats might indeed leave a detectable signal in the geographical ranges of lineages. If geographical isolation is the predominant process underlying species divergence among arboreal lineages, terrestrial ones are expected to show higher spatial overlap. However, if no differences are detected, geographic isolation might be a common pattern among vipers despite of their habitat. If this is the case, the different morphological trajectories but similar speciation rates found among habitats would possibly suggest that speciation mechanisms among vipers might not be followed by morphological differentiation.

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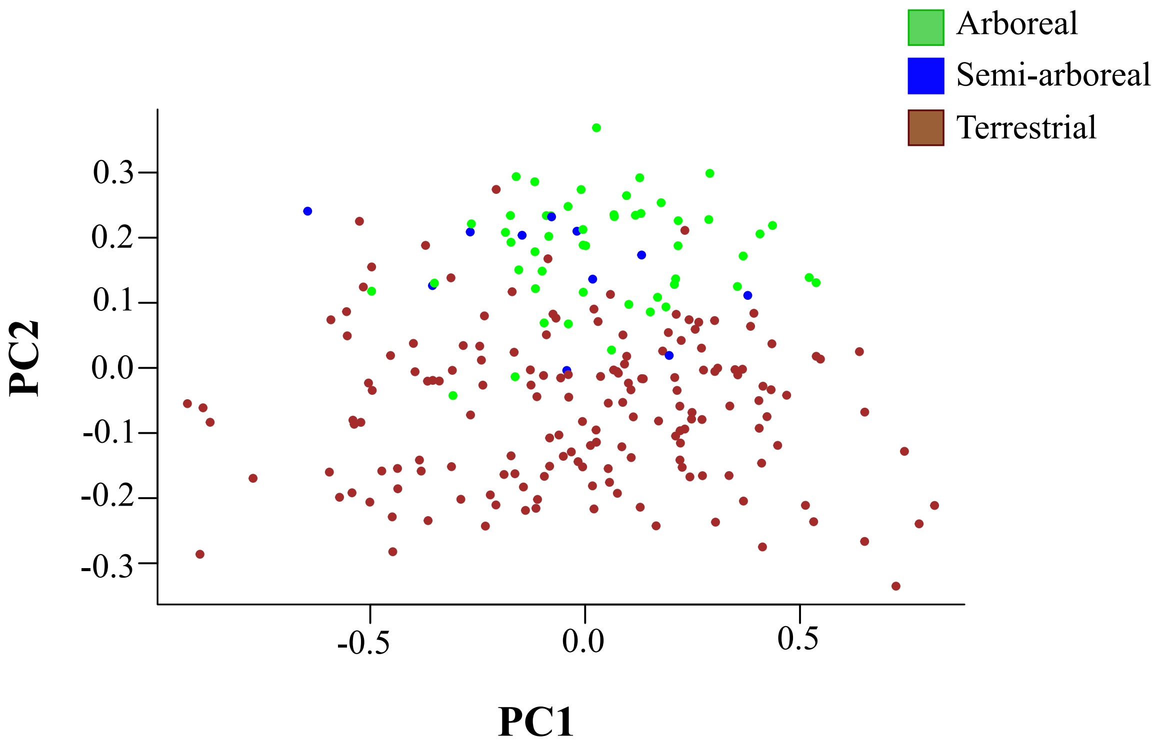

Figure S1. Morphospace of arboreal, semi-arboreal and terrestrial vipers.

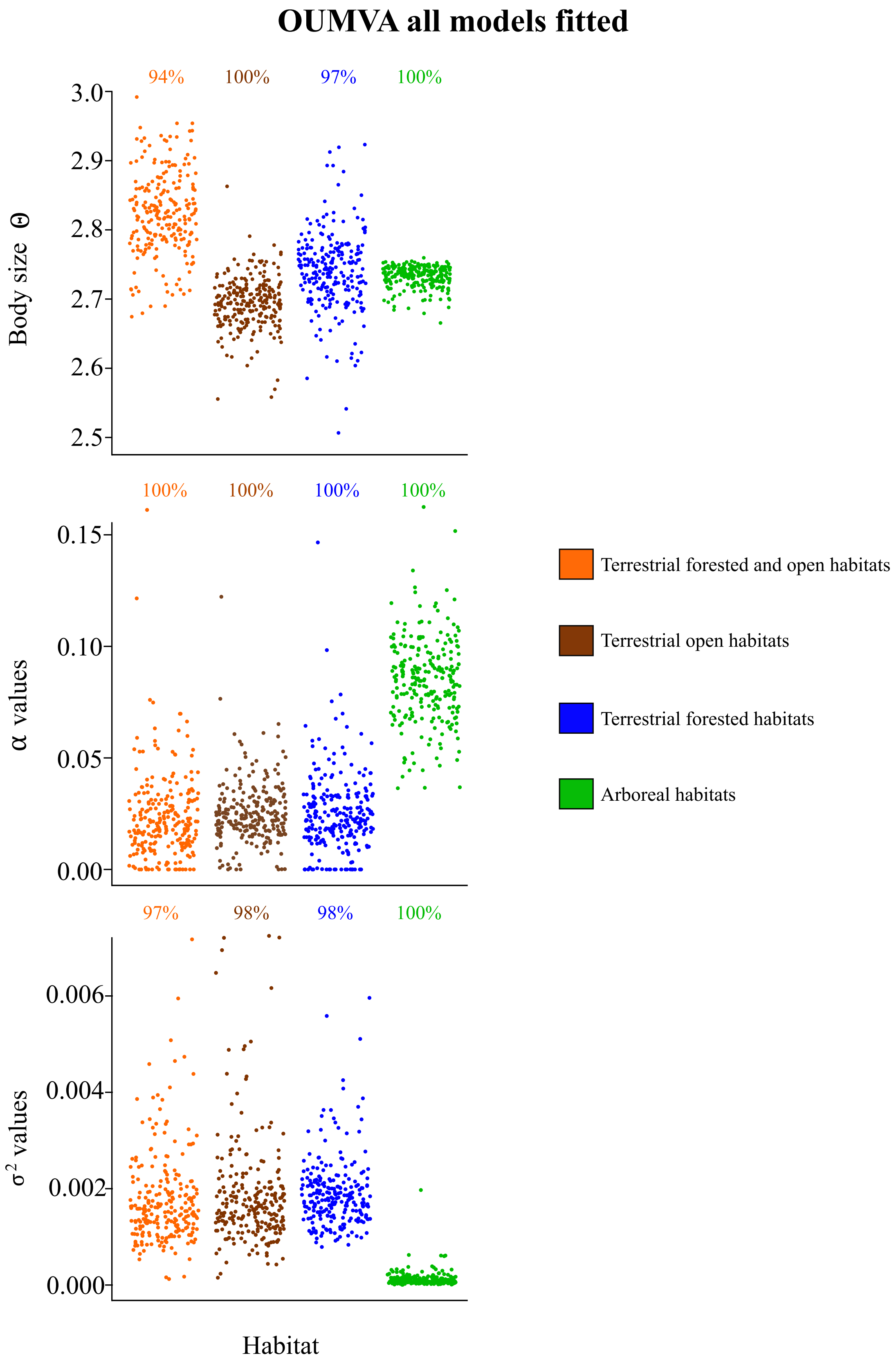

Figure S2. Parameters estimates under OUMVA when chosen as the best model in OUwie analyses where all models could be fitted (261 simmaps). Dots represent OUwie analyses performed in distinct simmaps. For better visualization we excluded extreme values and numbers on the top represent the proportion of values included.

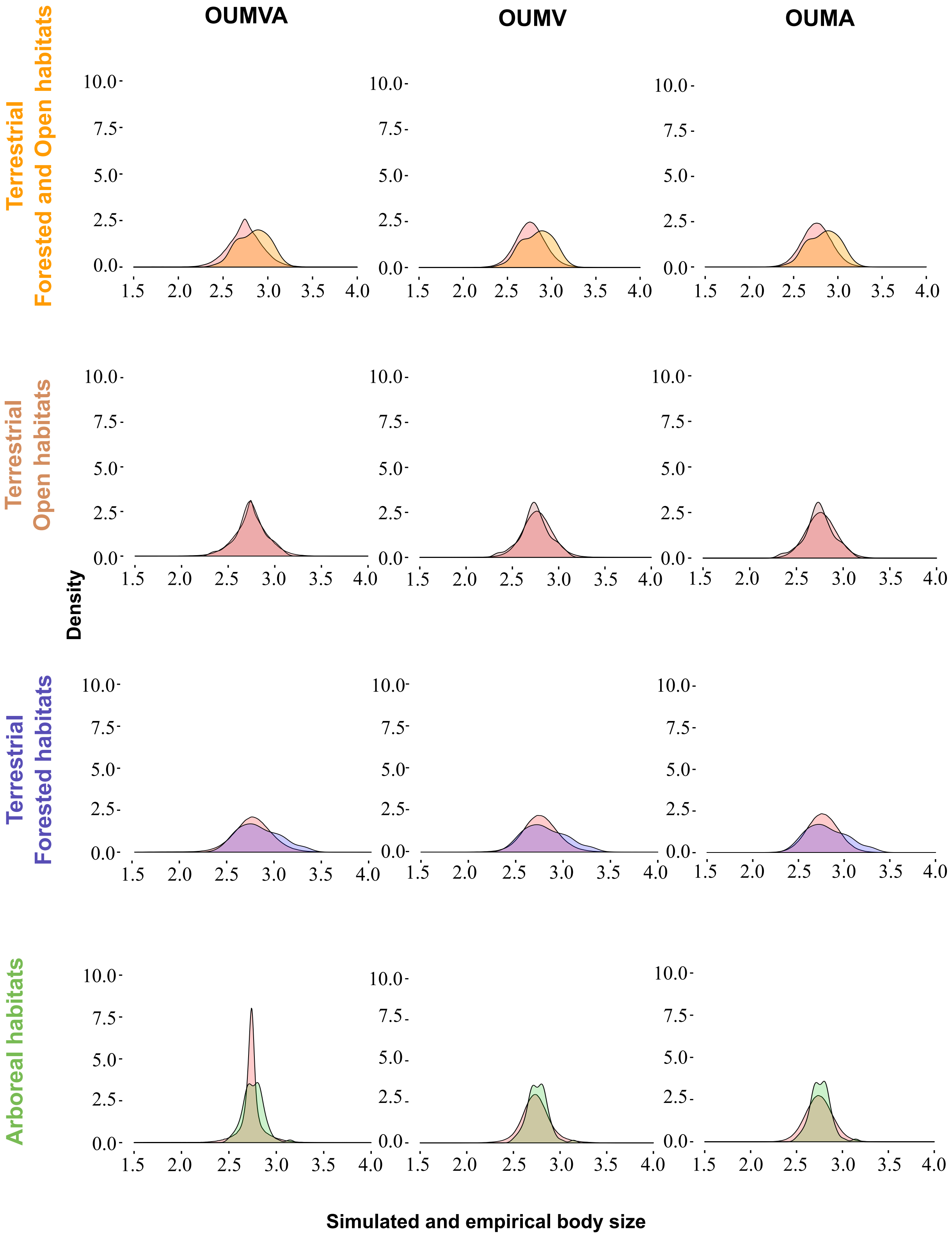

Figure S3. Simulated (light red curves) body size values using parameters estimated under each of the most frequently chosen models (OUMVA, OUMV and OUMA) (see methods for details on simulations) compared to empirical body size values in each habitat category.

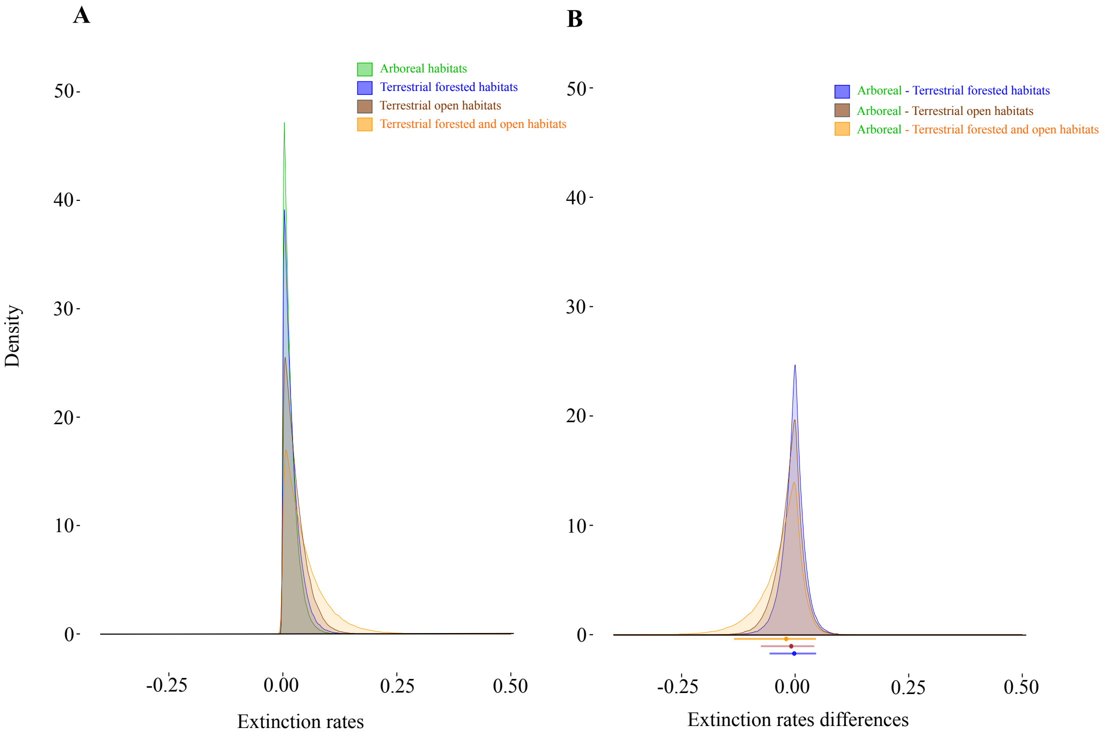

Figure S4. (A) Posterior distributions of extinction rates estimated for arboreal and terrestrial lineages. (B) Posterior distributions of differences between extinction rates of arboreal and terrestrial lineages. Bars represent 95% HPD interval and dots represent medians.

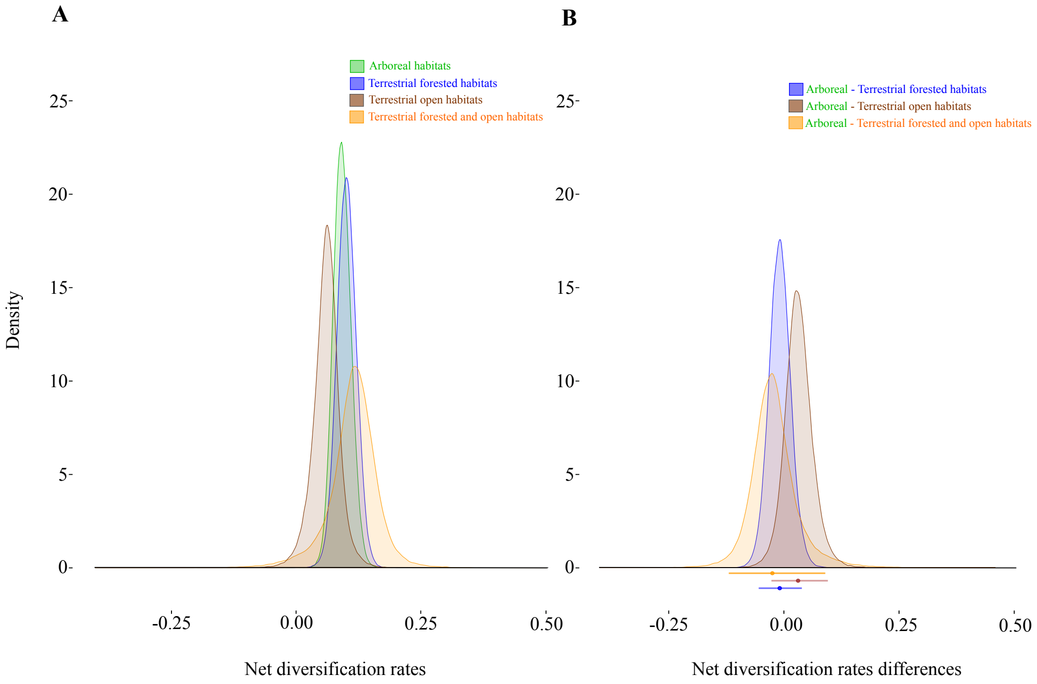

Figure S5. (A) Posterior distributions of net diversification rates estimated for arboreal and terrestrial lineages. (B) Posterior distributions of differences between net diversification rates of arboreal and terrestrial lineages. Bars represent 95% HPD interval and dots represent medians.

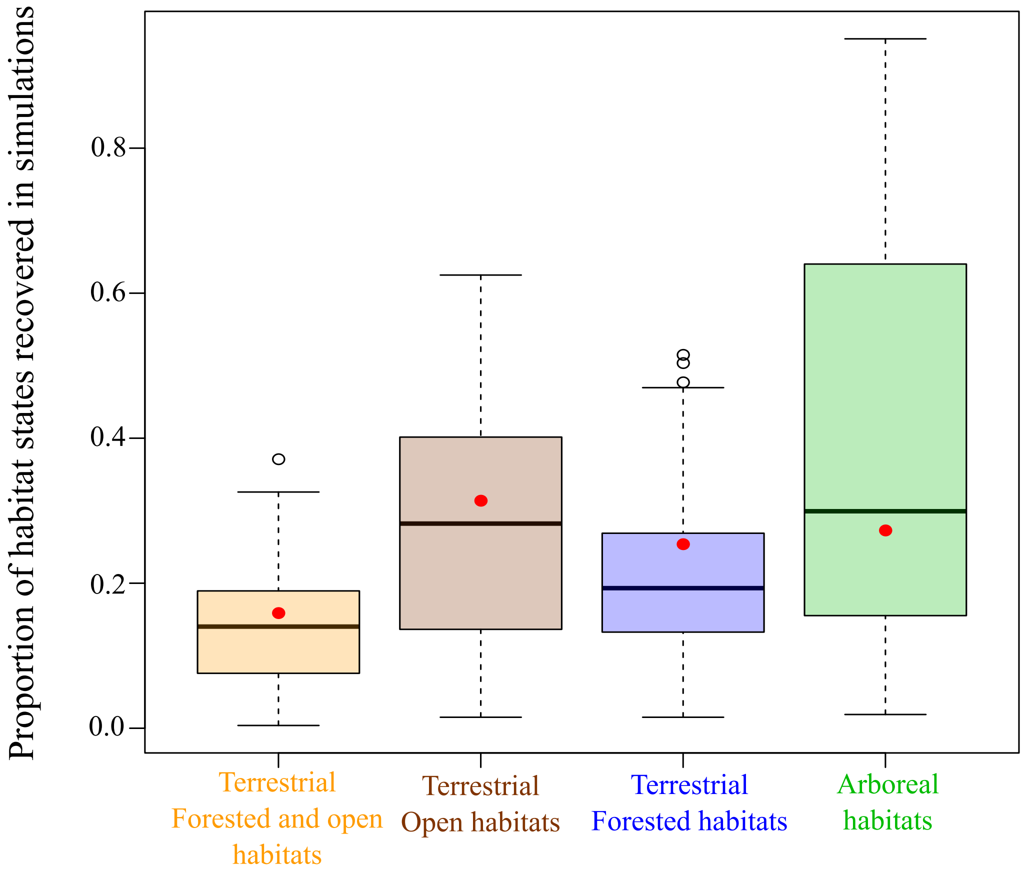

Figure S6. Posterior predictive simulations showing the proportion of each habitat state recovered by simulated trees and the empirical proportion found in vipers (red dot).

SUPPLEMENTARY TABLES

Table S1. Average body size (mm) and habitat use for viper species included in the present study. N = number of individuals measured for each species, 1 = terrestrial open and forested habitats, 2 = terrestrial open habitats, 3 = terrestrial forested habitats, 4 = arboreal habitats. * denotes species for which females measurements were used.

Species	Mean adult body size	N	Source	Habitat	Source
Agkistrodon bilineatus	855.7	4	*This study*	1	Gloyd and Conant (1990), Campbell and Lamar (2004)
Agkistrodon contortrix	958.2	5	*This study*	1	Gloyd and Conant (1990), Campbell and Lamar (2004)
Agkistrodon howardgloydi	750	1	*This study*	1	Solórzano et al. (1999), Campbell and Lamar (2004)
Agkistrodon piscivorus	889.5	6	*This study*	1	Gloyd and Conant (1990), Campbell and Lamar (2004)
Agkistrodon russeolus	-	-	-	1	Campbell and Lamar (2004)
Agkistrodon taylori	648	3	*This study*	1	Deufel and Cundall (2006), Lavin et al. (2007)
Atheris barbouri	282*	2	*This study*	3	Mallow et al. (2003), Phelps (2010), Menegon et al. (2011)
Atheris ceratophora	365	3	*This study*	4	Branch (2005), Phelps (2010)
Atheris chlorechis	459.5	2	*This study*	4	Mallow et al. (2003), Phelps (2010)
Atheris desaixi	473.5	4	*This study*	4	Mallow et al. (2003), Branch (2005)
Atheris hispida	474.6	5	*This study*	4	Branch (2005)
Atheris matildae	539.5	2	Menegon et al. (2011)	4	Menegon et al. (2011)
Atheris nitschei	522,7	6	*This study*	4	Mallow et al. (2003), Branch (2005), Phelps (2010)
Atheris squamigera	427.3	8	*This study*	4	Mallow et al. (2003), Branch (2005), Phelps (2010)
Atropoides indomitus	577	1	*This study*	3	Smith and Ferrari-Castro (2008),
Atropoides mexicanus	743.3	9	*This study*	1	Campbell and Lamar (2004)

Atropoides nummifer	589.5	4	*This study*	3	Campbell and Lamar (2004), Lopez-Luna and Canseco-Márquez (2007)
Atropoides occiduus	665.8	4	*This study*	3	Campbell and Lamar (2004), Hampton (2011)
Atropoides olmec	583.3	3	*This study*	3	Hampton (2011), Calderón Mandujano and Lopez-Luna (2014)
Atropoides picadoi	1078	5	*This study*	3	Campbell and Lamar (2004), Hampton (2011)
Azemiops feae	571	6	*This study*	1	Orlov et al. (2002), Leviton et al. (2003), Mallow et al. (2003), Phelps (2010)
Bitis arietans	821.8	9	*This study*	1	Branch (2005), Deufel and Cundall (2006), Phelps (2010)
Bitis armata	223	1	*This study*	2	Phelps (2010)
Bitis atropos	492.8	5	*This study*	2	Mallow et al. (2003)
Bitis caudalis	295	5	*This study*	2	Branch (2001), Mallow et al. (2003), Phelps (2010)
Bitis cornuta	436.5	4	*This study*	2	Branch (2001), Mallow et al. (2003), Phelps (2010)
Bitis gabonica	1143.8	6	*This study*	3	Branch (2001) and (2005), Mallow et al. (2003), Phelps (2010)
Bitis nasicornis	824.2	6	*This study*	3	Branch (2005), Deufel and Cundall (2006), Phelps (2010)
Bitis parviocula	-	-	-	1	Largen and Rasmussen (1993), Mallow et al. (2003)
Bitis peringueyi	259.7	6	*This study*	2	Branch (2001), Mallow et al. (2003), Phelps (2010)
Bitis rhinoceros	1352	1	*This study*	3	Phelps (2010)
Bitis rubida	-	-	-	2	Phelps (2010)
Bitis schneideri	217	2	*This study*	2	Branch (2001), Phelps (2010)
Bitis worthingtoni	357.3	3	*This study*	2	Mallow et al. (2003), Phelps (2010)
Bitis xeropaga	390	2	*This study*	2	Branch (2001), Phelps (2010)
Bothriechis aurifer	736.7	7	*This study*	4	Campbell and Lamar (2004)
Bothriechis bicolor	647.8	4	*This study*	4	Campbell and Lamar (2004)
Bothriechis guifarroi	653	1	*This study*	4	Towsend et al. (2013)

Bothriechis lateralis	679.4	9	*This study*	4	Campbell and Lamar (2004)
Bothriechis marchi	656.5	11	*This study*	4	McCranie and Castañeda (2006)
Bothriechis nigroviridis	404.8	6	*This study*	4	Campbell and Lamar (2004), Deufel and Cundall (2006)
Bothriechis rowleyi	738.1	9	*This study*	4	Campbell and Lamar (2004)
Bothriechis schlegelii	516	5	*This study*	4	Campbell and Lamar (2004), Deufel and Cundall (2006)
Bothriechis supraciliaris	320	1	*This study*	4	Solórzano (1998)
Bothriechis thalassinus	674.3	3	*This study*	4	Campbell and Smith (2000)
Bothrocophias campbelli	675*	1	*This study*	3	Gutberlet and Campbell (2001), Campbell and Lamar (2004), Cisneros-Heredia et al. (2006), Valencia et al. (2008)
Bothrocophias hyoprora	405.9	9	*This study*	3	Campbell and Lamar (2004), Cisneros-Heredia et al. (2006)
Bothrocophias microphthalmus	446	2	*This study*	3	Campbell and Lamar (2004), Cisneros-Heredia et al. (2006)
Bothrops alcatraz	462.3	3	*This study*	4	Campbell and Lamar (2004)
Bothrops alternatus	775.6	9	*This study*	2	Martins et al. (2001), Fenwick et al. (2009)
Bothrops ammodytoides	451	4	*This study*	2	Campbell and Lamar (2004), Fenwick et al. (2009)
Bothrops asper	1241.5	4	*This study*	3	Campbell and Lamar (2004), Fenwick et al. (2009)
Bothrops atrox	913.5	6	*This study*	3	Campbell and Lamar (2004), Fenwick et al. (2009)
Bothrops barnetti	600.8	4	*This study*	2	Schmith and Walker (1943), Campbell and Lamar (2004), Kwiatkowski and Burt (2011)
Bothrops bilineata	538.3	3	*This study*	4	Martins et al. (2001), Campbell and Lamar (2004), Harvey et al. (2005)
Bothrops brazili	842	2	*This study*	3	Martins et al. (2001), Campbell and Lamar (2004), Fenwick et al. (2009)
Bothrops caribbaeus	1096.3	4	*This study*	3	Campbell and Lamar, 2004, Henderson (2004), Deufel and Cundall (2006)
Bothrops chloromelas	571*	1	*This study*	4	Campbell and Lamar (2004), Hampton (2011)
Bothrops colombiensis	-	-	-	3	Silva et al. (1985), Rivas et al. (2012)
Bothrops cotiara	728.7	7	*This study*	1	Campbell and Lamar (2004), Fenwick et al. (2009)

Bothrops diporus	503	1	*This study*	2	Silva and Rodrigues (2008), Fenwick et al. (2009)
Bothrops erythromelas	479.3	6	*This study*	2	Campbell and Lamar (2004), Fenwick et al. (2009)
Bothrops fonsecai	775	6	*This study*	1	Martins et al. (2001), Fenwick et al. (2009)
Bothrops insularis	660.5	8	*This study*	4	Amaral (1921a,b)
Bothrops isabelae	-	-	-	3	Campbell and Lamar (2004), Fenwick et al. (2009), Rivas et al. (2012)
Bothrops itapetiningae	392	2	*This study*	2	Martins et al. (2001), Campbell and Lamar (2004)
Bothrops jararaca	833	26	*This study*	3	Schmith and Walker (1943), Campbell and Lamar (2004)
Bothrops jararacussu	995	6	*This study*	3	Martins et al. (2001), Campbell and Lamar (2004), Fenwick et al. (2009)
Bothrops lanceolatus	1189.8	4	*This study*	3	Gosner (1987), Campbell and Lamar (2004), Henderson (2004)
Bothrops leucurus	772.5	8	*This study*	3	Martins et al. (2001), Campbell and Lamar (2004), Fenwick et al. (2009)
Bothrops lojanus	514.4	5	*This study*	3	Campbell and Lamar (2004), Harvey et al. (2005)
Bothrops lutzi	550.5	2	*This study*	2	Campbell and Lamar (2004), Silva and Rodrigues (2008), Fenwick et al. (2009)
Bothrops marajoensis	-	-	-	3	Campbell and Lamar (2004), Fenwick et al. (2009), M. Martins pers. comm.
Bothrops marmoratus	521.5	2	*This study*	2	Silva and Rodrigues (2008), Carrasco et al. (2012)
Bothrops matogrossensis	636	4	*This study*	2	Martins et al. (2001), Silva and Rodrigues (2008), Fenwick et al. (2009)
Bothrops moojeni	863.3	20	*This study*	3	Campbell and Lamar (2004), Fenwick et al. (2009), M. Martins pers. comm.
Bothrops neuwiedi	681.9	8	*This study*	2	Martins et al. (2001), Campbell and Lamar (2004), Silva and Rodrigues (2008)
Bothrops osbornei	-	-	-	4	Campbell and Lamar (2004), Fenwick et al. (2009)
Bothrops pauloensis	661.8	4	*This study*	2	Martins et al. (2001), Campbell and Lamar (2004), Silva and Rodrigues (2008), Fenwick et al. (2009)
Bothrops pictus	691.8	4	*This study*	2	Campbell and Lamar (2004), Kwiatkowski and Burt (2011)
Bothrops pubescens	698	2	*This study*	3	Martins et al. (2001), Fenwick et al. (2009), M. Hartmann pers. comm.
Bothrops pulchra	589	1	*This study*	4	Gutberlet and Campbell (2001)

Bothrops punctatus	770	2	*This study*	4	Campbell and Lamar (2004)
Bothrops taeniata	706*	1	*This study*	4	Martins et al. (2001), Campbell and Lamar (2004)
Calloselasma rhodostoma	644.7	3	*This study*	3	Orlov et al. (2002)
Causus defilippii	297.6	9	*This study*	2	Branch (2001), Mallow et al. (2003), Phelps (2010)
Causus lichtensteinii	413.6	7	*This study*	3	Branch (2005), Mallow et al. (2003), Phelps (2010)
Causus resimus	532.3	8	*This study*	2	Branch (2005), Mallow et al. (2003), Phelps (2010)
Causus rhombeatus	559.6	7	*This study*	2	Branch (2005), Mallow et al. (2003), Phelps (2010)
Cerastes cerastes	534.2	5	*This study*	2	Mallow et al. (2003), Phelps (2010)
Cerastes gasperettii	542	5	*This study*	2	Mallow et al. (2003), Phelps (2010)
Cerastes vipera	343.2	6	*This study*	2	Mallow et al. (2003), Phelps (2010)
Cerrophidion godmani	643.5	6	*This study*	1	Campbell and Lamar (2004), Jadin et al. (2012)
Cerrophidion petlalcalensis	345	1	López-luna et al. (1999)	3	López-Luna et al. (1999), Campbell and Lamar (2004)
Cerrophidion sasai	583	3	*This study*	3	Jadin et al. (2012)
Cerrophidion tzotzilorum	370	2	*This study*	3	Campbell and Lamar (2004)
Crotalus adamanteus	1494.7	6	*This study*	1	Campbell and Lamar (2004)
Crotalus aquilus	655	7	*This study*	2	Campbell and Lamar (2004), Mociño-Deloya et al. (2008)
Crotalus atrox	1184.3	6	*This study*	2	Armstrong and Murphy (1979), Campbell and Lamar (2004)
Crotalus basiliscus	1265.4	5	*This study*	3	Campbell and Lamar (2004)
Crotalus catalinensis	996	1	*This study*	2	Campbell and Lamar (2004), Arnaud et al., 2008, Martins et al., 2008
Crotalus cerastes	517.4	8	*This study*	2	Campbell and Lamar (2004)
Crotalus cerberus	916	3	*This study*	2	Campbell and Lamar (2004)
Crotalus culminatus	1223.3	3	*This study*	2	Campbell and Lamar (2004)

Crotalus durissus	962	14	*This study*	2	Campbell and Lamar (2004)
Crotalus enyo	705.8	8	*This study*	2	Taylor and Price (2001), Campbell and Lamar (2004)
Crotalus ericsmithi	505	1	*This study*	3	Campbell and Flores-Villela (2008), J. Reyes-Velasco pers. comm.
Crotalus horridus	1247.7	6	*This study*	3	Campbell and Lamar (2004)
Crotalus intermedius	521.7	9	*This study*	3	Campbell and Lamar (2004)
Crotalus lannomi	493	2	Reyes-Velasco et al. (2010)	3	Campbell and Lamar (2004), Reyes-Velasco et al. (2010)
Crotalus lepidus	659.3	7	*This study*	1	Campbell and Lamar (2004)
Crotalus mitchellii	882.7	6	*This study*	2	Campbell and Lamar (2004)
Crotalus molossus	1130.8	6	*This study*	1	Campbell and Lamar (2004)
Crotalus oreganus	817.4	5	*This study*	1	Campbell and Lamar (2004)
Crotalus ornatus	1135	1	*This study*	1	Campbell and Lamar (2004)
Crotalus polystictus	778.3	8	*This study*	2	Campbell and Lamar (2004), Meik et al. (2012)
Crotalus pricei	496.6	8	*This study*	1	Campbell and Lamar (2004)
Crotalus pusillus	564.9	7	*This study*	2	Campbell and Lamar (2004)
Crotalus ravus	686.3	6	*This study*	1	Campbell and Lamar (2004)
Crotalus ruber	1064.1	8	*This study*	2	Klauber (1956), Douglas et al. (2006),
Crotalus scutulatus	740.6	5	*This study*	2	Campbell and Lamar (2004)
Crotalus simus	1174.6	5	*This study*	1	Campbell and Lamar (2004)
Crotalus stejnegeri	592	1	*This study*	3	Campbell and Lamar (2004), J. Reyes-Velasco pers. comm.
Crotalus tancitarensis	406.5*	2	*This study*	3	Campbell (2007)
Crotalus tigris	666.6	10	*This study*	2	Campbell and Lamar (2004), Douglas et al. (2006)
Crotalus tortugensis	1078	1	*This study*	2	Campbell and Lamar (2004)

Crotalus totonacus	1096	3	*This study*	3	Campbell and Lamar (2004)
Crotalus transversus	309	1	*This study*	2	Campbell and Lamar (2004)
Crotalus triseriatus	532.2	8	*This study*	1	Campbell and Lamar (2004)
Crotalus tzcaban	1082.7	3	*This study*	1	Campbell and Lamar (2004)
Crotalus viridis	950.8	5	*This study*	1	Campbell and Lamar (2004)
Crotalus willardi	625	8	*This study*	1	Campbell and Lamar (2004)
Daboia deserti	897	5	*This study*	2	Mallow et al. (2003), Phelps (2010)
Daboia mauritanica	851.8	4	*This study*	2	Mallow et al. (2003), Phelps (2010)
Daboia palaestinae	771.8	5	*This study*	3	Phelps (2010)
Daboia russelii	1076.8	5	*This study*	1	Mallow et al. (2003), Phelps (2010)
Daboia siamensis	921.4	5	*This study*	1	Phelps (2010), Wogan (2012)
Deinagkistrodon acutus	978	8	*This study*	1	Orlov et al. (2002)
Echis borkini	-	-	-	2	G. Vogel pers. comm.
Echis carinatus	512.8	5	*This study*	2	Mallow et al. (2003), Phelps (2010)
Echis coloratus	571.6	8	*This study*	2	Mallow et al. (2003), Phelps (2010)
Echis jogeri	280*	1	*This study*	1	Phelps (2010)
Echis khosatzkii	422	1	*This study*	2	Mallow et al. (2003), Els and Rasbi (2012)
Echis leucogaster	470.4	7	*This study*	2	Mallow et al. (2003), Phelps (2010)
Echis ocellatus	473.2	7	*This study*	1	Mallow et al. (2003), Phelps (2010)
Echis omanensis	519	5	*This study*	2	Phelps (2010)
Echis pyramidum	461.7	6	*This study*	2	Mallow et al. (2003), Phelps (2010)
Eristicophis macmahoni	534.2	5	*This study*	2	Mallow et al. (2003), Phelps (2010)

Garthius chaseni	420	1	*This study*	3	Das (2012)
Gloydius blomhoffii	475.6	8	*This study*	2	Gloyd and Conant (1990), Hampton (2011), O'Shea (2011)
Gloydius brevicaudus	532.3	7	*This study*	2	Gloyd and Conant (1990)
Gloydius halys	555.7	3	*This study*	2	Orlov et al. (2002)
Gloydius intermedius	569.8	4	*This study*	3	Orlov et al. (2002)
Gloydius liupanensis	-	-	-	2	Gloyd and Conant (1990), P. David pers. comm.
Gloydius saxatilis	635.4	5	*This study*	3	Gloyd and Conant (1990), Deufel and Cundall (2006)
Gloydius shedaoensis	639.8	4	Shine et al. (2002), (2003)	4	Shine et al. (2002)
Gloydius strauchi	416.8	4	*This study*	2	Gloyd and Conant (1990), Hampton (2011), P. David pers. comm.
Gloydius tsushimaensis	-	-	-	3	Gloyd and Conant (1990)
Gloydius ussuriensis	453.6	5	*This study*	2	Orlov et al. (2002)
Hypnale hypnale	308.4	5	*This study*	3	Maduwage et al. (2009), Sawant et al. (2010)
Hypnale nepa	264.7	3	*This study*	3	Maduwage et al. (2009)
Hypnale zara	325	1	*This study*	3	Maduwage et al. (2009)
Lachesis acrochorda	1800.5	2	*This study*	3	Campbell and Lamar (2004)
Lachesis melanocephala	1925.5*	2	*This study*	3	Campbell and Lamar (2004), Pla et al. (2013)
Lachesis muta	1932.3	9	*This study*	3	Martins and Oliveira (1998), Campbell and Lamar (2004)
Lachesis stenophrys	1929.8	5	*This study*	3	Campbell and Lamar (2004), Deufel and Cundall (2006)
Macrovipera lebetina	956.8	6	*This study*	2	Mallow et al. (2003), Phelps (2010)
Macrovipera schweizeri	557.5	2	*This study*	2	Mallow et al. (2003), Phelps (2010)
Mixcoatlus barbouri	441.7	3	*This study*	3	Campbell and Lamar (2004), Jadin et al. (2011)
Mixcoatlus browni	351	1	*This study*	3	Campbell and Lamar (2004), Jadin et al. (2011)

Mixcoatlus melanurus	442.3	6	*This study*	1	Campbell and Lamar (2004), Jadin et al. (2011)
Montivipera albizona	644.7	6	Mulder (1994)	2	Phelps (2010), G. Nilson pers. comm., P. David pers. comm.
Montivipera bornmuelleri	505.7	3	*This study*	2	Mallow et al. (2003), Phelps (2010)
Montivipera latifii	622	4	*This study*	2	Mallow et al. (2003), Phelps (2010)
Montivipera raddei	730.3	4	*This study*	2	Phelps (2010), Hampton (2011)
Montivipera wagneri	575	1	*This study*	2	Phelps (2010), G. Nilson pers. comm., P. David pers. comm.
Montivipera xanthina	800.1	7	*This study*	2	Böhme et al. (2009), Wilson and Grillitsch (2009), Phelps (2010), Hampton (2011)
Ophryacus undulatus	639.4	9	*This study*	4	Campbell and Lamar (2004), Hampton (2011), Jardin et al. (2011)
Ovophis monticola	535.3	6	*This study*	2	Orlov et al. (2002), Leviton et al. (2003)
Ovophis okinavensis	544.8	5	*This study*	3	Kadota (2011)
Ovophis tonkinensis	529	2	*This study*	3	Orlov et al. (2002)
Ovophis zayuensis	626	1	*This study*	3	Orlov et al. (2002), Guo et al. (2012)
Porthidium arcosae	466*	1	*This study*	3	Campbell and Lamar (2004), Valencia et al. (2010)
Porthidium dunni	374.6	18	*This study*	3	Campbell and Lamar (2004)
Porthidium hespere	605*	1	*This study*	3	Campbell (1976), Campbell and Lamar (2004)
Porthidium lansbergii	375.8	8	*This study*	3	Campbell and Lamar (2004)
Porthidium nasutum	359.2	5	*This study*	3	Campbell and Lamar (2004)
Porthidium ophryomegas	485.1	9	*This study*	3	Campbell and Lamar (2004)
Porthidium porrasi	402.3*	3	*This study*	3	Lamar and Sasa (2003), Campbell and Lamar (2004)
Porthidium yucatanicum	423.4	5	*This study*	3	Campbell and Lamar (2004)
Proatheris superciliaris	458.5	2	*This study*	2	Branch (2001), Phelps (2010)
Protobothrops cornutus	349	1	*This study*	4	Orlov et al. (2002)

Protobothrops dabieshanensis	696*	1	Huang et al. (2012)	1	Huang et al. (2012)
Protobothrops elegans	927.7	6	*This study*	3	G. Vogel pers. comm.
Protobothrops flavoviridis	1391.3	6	*This study*	4	Aird et al. (2013), P. David pers. comm.
Protobothrops jerdonii	865.3	7	*This study*	4	Orlov et al. (2002), Leviton et al. (2003)
Protobothrops kaulbacki	1219.8	4	*This study*	1	Leviton et al. (2003), Guo et al. (2012), P. David pers. comm.
Protobothrops mangshanensis	-	-	-	4	Zhou (2012), Gong et al. (2013)
Protobothrops maolanensis	588	3	Yang et al. (2011)	2	Yang et al. (2011), Wang and Lau (2012)
Protobothrops mucrosquamatus	815.1	8	*This study*	3	Orlov et al. (2002)
Protobothrops sieversorum	1060	1	Herrmann et al. (2002)	3	Herrmann et al. (2002)
Protobothrops tokarensis	818.5	2	*This study*	4	P. David pers. comm.
Protobothrops xiangchengensis	-	-	-	2	Guo and Jiang (2012)
Pseudocerastes persicus	735.8	4	*This study*	2	Mallow et al. (2003), Phelps (2010)
Pseudocerastes urarachnoides	582*	1	*This study*	2	Anderson and Papenfuss (2009), Phelps (2010)
Pseudocerastes fieldi	673.8	5	*This study*	2	Phelps (2010)
Sistrurus catenatus	663.3	9	*This study*	2	Holycross and Mackessy (2002), Campbell and Lamar (2004)
Sistrurus miliarius	525.3	6	*This study*	1	May et al. (1996), Campbell and Lamar (2004)
Trimeresurus albolabris	526.4	5	*This study*	4	Vijayakumar and David (2006), Whitaker (2008)
Trimeresurus andersonii	502.3	3	*This study*	4	Whitaker (2008)
Trimeresurus barati	511.5	2	*This study*	4	Iskandar et al. (2012)
Trimeresurus borneensis	472.5	2	*This study*	4	David et al. (2006), Deufel and Cundall (2006)
Trimeresurus buniana	649	1	*This study*	4	Grismer (2012)
Trimeresurus cantori	582.3	4	*This study*	4	Vijayakumar and David (2006), Whitaker (2008)

Trimeresurus erythrurus	534.2	6	*This study*	4	Orlov et al. (2002)
Trimeresurus fasciatus	437	1	*This study*	4	Deufel and Cundall (2006), P. David pers. comm.
Trimeresurus flavomaculatus	709.3	3	*This study*	4	Sanders et al. (2004), Deufel and Cundall (2006)
Trimeresurus fucatus	616.3	3	*This study*	4	Chanhome et al. (2011), Wogan et al. (2012)
Trimeresurus gracilis	397	1	Lin and Tu (2008)	4	Zhao (2006), Jiang and Lau (2012)
Trimeresurus gramineus	531.7	7	*This study*	4	Whitaker (2008), Sawant and Jadhav (2013)
Trimeresurus gumprechti	574.8	4	*This study*	4	Chanhome et al. (2011), Stuart et al. (2012)
Trimeresurus hageni	650.8	6	*This study*	4	Ryabov et al. (2002), Sanders et al. (2004)
Trimeresurus insularis	513.3	4	*This study*	4	Auliya (2010), G. Vogel pers. comm.
Trimeresurus kanburiensis	428	1	*This study*	4	Chanhome et al. (2011)
Trimeresurus macrops	471.9	7	*This study*	4	Stuart et al. (2012)
Trimeresurus malabaricus	455.8	5	*This study*	4	Whitaker (2008), Sawant and Jadhav (2013)
Trimeresurus malcolmi	1030.5*	2	*This study*	4	Sanders et al. (2004)
Trimeresurus mcgregori	588.8	5	*This study*	4	Sanders et al. (2004)
Trimeresurus medoensis	493.2	9	David et al. (2001)	4	Leviton et al. (2003), Whitaker (2008)
Trimeresurus nebularis	782	1	*This study*	4	Grismer (2012)
Trimeresurus popeiorum	584.2	6	*This study*	4	Orlov et al. (2002)
Trimeresurus puniceus	501.5	6	*This study*	4	Orlov et al. (2002)
Trimeresurus purpureomaculatus	655.2	6	*This study*	4	Leviton et al. (2003), Vogel et al. (2012)
Trimeresurus sabahi	654	2	*This study*	4	Orlov et al. (2002)
Trimeresurus schultzei	746	2	*This study*	4	Sanders et al. (2004)
Trimeresurus septentrionalis	634*	3	*This study*	4	Orlov et al. (2002)

Trimeresurus sichuanensis	-	-	-	4	Guo and Wang (2011), P. Guo pers. comm.
Trimeresurus stejnegeri	512	9	*This study*	4	Gumpretchi (1997), Creer et al. (2002), Lin et al. (2006)
Trimeresurus sumatranus	862.5	2	*This study*	4	Ryabov et al. (2002), Sanders et al. (2004)
Trimeresurus tibetanus	528	1	*This study*	4	Orlov et al. (2002)
Trimeresurus trigonocephalus	594	6	*This study*	4	Gumpretchi (1999), Deufel and Cundall (2006)
Trimeresurus truongsonensis	498.5	4	Orlov et al. (2004)	4	Orlov et al. (2004), Ziegler et al. (2006)
Trimeresurus venustus	390	1	*This study*	4	Chanhome et al. (2011)
Trimeresurus vogeli	722.6	5	*This study*	4	Chanhome et al. (2011), Stuart and Nguyen (2012)
Trimeresurus wiroti	485	1	*This study*	4	Chanhome et al. (2011), Grismer and Chan-Ard (2012), G. Vogel pers. comm.
Trimeresurus yunnanensis	660.8	4	*This study*	4	Leviton et al. (2003), P. David pers. comm.
Tropidolaemus subannulatus	613.7*	6	*This study*	4	Auliya et al. (2012)
Tropidolaemus wagleri	587.7	3	*This study*	4	Orlov et al. (2002), Grismer and Chan-Ard (2012)
Vipera ammodytes	608.8	11	*This study*	2	Phelps (2010)
Vipera anatolica	-	-	-	1	Tok et al. (2009), Phelps (2010)
Vipera aspis	541.5	12	*This study*	2	Luiselli et al. (2007), Phelps (2010), Hampton (2011)
Vipera barani	449.3	3	Franzes and Heckes (2000), Avci et al. (2004)	1	Mallow et al. (2003), Tok et al. (2009), Phelps (2010), Hampton (2011)
Vipera berus	456.8	12	*This study*	2	Phelps (2010), Hampton (2011)
Vipera dinniki	367*	1	*This study*	2	Mallow et al. (2003), Phelps (2010)
Vipera eriwanensis	327.5	4	*This study*	2	Tuniyev et al. (2009), Phelps (2010)
Vipera kaznakovi	409.5	4	*This study*	1	Mallow et al. (2003), Tuniyev et al. (2009), Phelps (2010)
Vipera latastei	453.4	5	*This study*	1	Brito (2003), Phelps (2010)
Vipera lotievi	-	-	-	1	Mallow et al. (2003), Tuniyev et al. (2009), Phelps (2010)

Vipera nikolskii	410	1	*This study*	1	Zinenko (2006), Phelps (2010)
Vipera orlovi	380	1	Tuniyev and Ostrovskikh (2001)	1	Tuniyev and Ostrovskikh (2001), Tuniyev et al. (2012), Tuniyev pers. comm.
Vipera renardi	484.44	9	*This study*	2	Höggren et al. (1993), Phelps (2010)
Vipera seoanei	428.6	7	*This study*	1	Mallow et al. (2003), Phelps (2010), Brito pers. comm.
Vipera transcaucasiana	571	1	*This study*	2	Mallow et al. (2003), Phelps (2010), Hampton (2011)
Vipera ursinii	388.2	5	*This study*	2	Filippi and Luiselli (2004), Luiselli et al. (2006), Phelps (2010)

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Table S2. ΔAICc calculated for continuous-time Markov models of trait evolution fitted to each simmap used in the analyses. ER = equal rates model, SYM = symmetrical rates model, ARD = all rates different model. * denotes simmaps for which we used the second best Markov model fitted.

		ΔAICc	
SIMMAP	**ER**	**SYM**	**ARD**
1	35.84	5.75	0.00
2	35.88	6.96	0.00
3	34.54	5.70	0.00
4	36.94	4.89	0.00
5	32.50	3.85	0.00
6	38.08	5.37	0.00
7	37.51	5.71	0.00
8	35.54	4.32	0.00
9	35.39	5.20	0.00
10	32.97	6.07	0.00
11	31.24	3.76	0.00
12	36.32	4.79	0.00
13	35.07	4.26	0.00
14	34.37	5.75	0.00
15	33.27	4.68	0.00
16	33.74	4.23	0.00
17	36.64	4.32	0.00
18	33.25	5.47	0.00
19*	30.96	4.75	0.00
20	31.51	3.23	0.00
21	30.36	4.48	0.00
22	37.38	5.34	0.00
23	33.19	5.74	0.00
24	35.51	5.99	0.00
25	34.27	4.55	0.00
26	35.31	7.63	0.00
27	32.93	5.22	0.00
28	30.46	5.27	0.00
29	28.56	3.42	0.00
30	34.83	5.08	0.00
31	36.20	4.86	0.00
32	34.64	5.19	0.00
33	31.87	4.00	0.00
34	39.14	6.38	0.00
35	32.18	5.10	0.00
36	34.97	4.71	0.00
37	38.46	6.48	0.00
38	35.48	5.35	0.00
39*	36.69	5.36	0.00
40	32.59	4.01	0.00

41	32.19	4.24	0.00
42	35.28	6.07	0.00
43	35.50	5.23	0.00
44*	32.08	4.10	0.00
45	33.78	4.31	0.00
46	38.02	6.03	0.00
47	32.40	4.02	0.00
48*	34.42	5.33	0.00
49	35.87	4.11	0.00
50	32.16	3.44	0.00
51	35.86	7.67	0.00
52	35.49	4.86	0.00
53	31.02	4.35	0.00
54	36.59	6.17	0.00
55	36.75	5.59	0.00
56	32.62	4.84	0.00
57	32.18	4.05	0.00
58	32.48	4.91	0.00
59	33.28	5.33	0.00
60	30.05	5.32	0.00
61	26.22	1.92	0.00
62	30.40	4.03	0.00
63	36.64	5.36	0.00
64	28.69	0.88	0.00
65*	36.90	6.63	0.00
66	31.50	5.22	0.00
67	29.70	3.64	0.00
68	33.41	3.55	0.00
69	35.29	4.67	0.00
70	32.68	4.67	0.00
71	27.30	0.00	4.23
72	31.83	3.82	0.00
73	33.22	4.19	0.00
74	35.18	6.16	0.00
75	34.64	4.86	0.00
76	34.89	5.12	0.00
77	32.75	4.85	0.00
78	34.26	5.33	0.00
79*	34.50	5.03	0.00
80	31.76	2.43	0.00
81	33.27	3.47	0.00
82	32.40	4.38	0.00
83	30.90	4.61	0.00
84	36.51	6.45	0.00
85	33.29	2.97	0.00
86	31.96	4.63	0.00
87	36.45	6.88	0.00
88	35.63	6.00	0.00

89	35.78	5.06	0.00
90	36.21	6.69	0.00
91	37.63	6.19	0.00
92	36.93	7.05	0.00
93	31.62	4.46	0.00
94	33.70	5.44	0.00
95	38.00	4.49	0.00
96*	38.35	6.63	0.00
97	35.61	3.61	0.00
98	34.90	5.88	0.00
99	32.05	4.50	0.00
100	32.74	4.77	0.00

APPENDIX I

Museum specimens analyzed in the present study. UTA = University of Texas at Arlington; CAS = California Academy of Sciences; FMNH = Field Museum of Natural History; AMNH = American Museum of National History; USNM = Smithsonian National Museum of Natural History; BMNH = Natural History Museum of London; MNHN = Muséum national d'Histoire naturelle; FMNH = Florida Museum of Natural History; MNRJ = Museu Nacional do Rio de Janeiro; IBSP = Instituto Butantan; MZUSP = Museu de Zoologia da Universidade de São Paulo; ZRC = The Raffles Museum of Biodiversity Research of the National University of Singapore.

Species	Museum	Catalog number
Agkistrodon_bilineatus	UTA	R6359
Agkistrodon_bilineatus	UTA	R2449
Agkistrodon_bilineatus	CAS	204153
Agkistrodon_bilineatus	FMNH	39093
Agkistrodon_contortrix	AMNH	128682
Agkistrodon_contortrix	AMNH	128678
Agkistrodon_contortrix	AMNH	95950
Agkistrodon_contortrix	AMNH	134673
Agkistrodon_contortrix	AMNH	146527
Agkistrodon_howardgloydi	UTA	R59855
Agkistrodon_piscivorus	USNM	203553
Agkistrodon_piscivorus	USNM	203565
Agkistrodon_piscivorus	USNM	203566
Agkistrodon_piscivorus	AMNH	107946
Agkistrodon_piscivorus	AMNH	90128
Agkistrodon_piscivorus	AMNH	161861
Agkistrodon_taylori	UTA	R8117
Agkistrodon_taylori	FMNH	28794
Agkistrodon_taylori	AMNH	124948
Atheris_ceratophora	BMNH	1960.1.6.54
Atheris_ceratophora	CAS	173812
Atheris_ceratophora	CAS	162615
Atheris_chlorechis	BMNH	1907.4.18.6
Atheris_chlorechis	UTA	R58204
Atheris_desaixi	BMNH	1969. 2419
Atheris_desaixi	BMNH	979
Atheris_desaixi	BMNH	980
Atheris_desaixi	UTA	R50162
Atheris_hispida	CAS	141750
Atheris_hispida	CAS	122747
Atheris_hispida	CAS	147906
Atheris_hispida	FMNH	154900
Atheris_hispida	AMNH	11873

Atheris_nitschei	BMNH	1954.1.12.65-66
Atheris_nitschei	MNHParis	1980.1278
Atheris_nitschei	MNHParis	1981.476
Atheris_nitschei	MNHParis	1934.14
Atheris_nitschei	CAS	201654
Atheris_nitschei	CAS	201655
Atheris_squamigera	BMNH	1934.12.1.35
Atheris_squamigera	BMNH	1901.6.24.65-66
Atheris_squamigera	BMNH	1908.5.30.35
Atheris_squamigera	BMNH	1908.5.30.33
Atheris_squamigera	BMNH	1969.2554-2556
Atheris_squamigera	BMNH	1908.10.20.25
Atheris_squamigera	BMNH	1907.5.22.66
Atheris_squamigera	BMNH	1906.5.28.84
Atropoides_indomitus	UTA	R52952
Atropoides_mexicanus	UTA	R6738
Atropoides_mexicanus	UTA	R12943
Atropoides_mexicanus	UTA	R43610
Atropoides_mexicanus	UTA	R45500
Atropoides_mexicanus	USNM	578902
Atropoides_mexicanus	FLMNH	119441
Atropoides_mexicanus	FLMNH	141977
Atropoides_mexicanus	FLMNH	142074
Atropoides_mexicanus	FLMNH	144757
Atropoides_nummifer	UTA	R24842
Atropoides_nummifer	UTA	R6206
Atropoides_nummifer	UTA	R12562
Atropoides_nummifer	UTA	R51731
Atropoides_occiduus	UTA	R34158
Atropoides_occiduus	UTA	R12785
Atropoides_occiduus	UTA	R9089
Atropoides_occiduus	UTA	R31979
Atropoides_olmec	UTA	R16964
Atropoides_olmec	UTA	R25113
Atropoides_olmec	UTA	R18691
Atropoides_picadoi	BMNH	1913.7.19.1.47
Atropoides_picadoi	UTA	R18215
Atropoides_picadoi	UTA	R24834
Atropoides_picadoi	UTA	R24835
Atropoides_picadoi	UTA	R32080
Azemiops_feae	MNHParis	1953.463
Azemiops_feae	UTA	R35599
Azemiops_feae	UTA	R32069
Azemiops_feae	UTA	R18701
Azemiops_feae	FMNH	218627
Azemiops_feae	FMNH	152987
Bitis_arietans	BMNH	1936.7.3.39
Bitis_arietans	BMNH	1916.3.20.1

Bitis_arietans	BMNH	1906.8.25.3
Bitis_arietans	BMNH	90.2.26.15-17
Bitis_arietans	BMNH	1916.3.20.1
Bitis_arietans	BMNH	1975.1113
Bitis_arietans	BMNH	1975.1097
Bitis_arietans	BMNH	1948.1.2.35
Bitis_arietans	MNHParis	1904.205
Bitis_armata	FMNH	206280
Bitis_atropos	BMNH	1908.3.26.2
Bitis_atropos	MNHParis	323
Bitis_atropos	UTA	R46188
Bitis_atropos	UTA	R7325
Bitis_atropos	CAS	135749
Bitis_caudalis	BMNH	1969.2639
Bitis_caudalis	BMNH	1988.583
Bitis_caudalis	BMNH	65.5.4.153A
Bitis_caudalis	BMNH	65.5.4.153B
Bitis_caudalis	CAS	214702
Bitis_cornuta	UTA	R34491
Bitis_cornuta	UTA	R38536
Bitis_cornuta	UTA	R34492
Bitis_cornuta	UTA	R26613
Bitis_gabonica	BMNH	1969.526
Bitis_gabonica	BMNH	1908.3.4.2
Bitis_gabonica	MNHParis	1988.2441
Bitis_gabonica	MNHParis	1988.50
Bitis_gabonica	FMNH	216460
Bitis_gabonica	FMNH	30874
Bitis_nasicornis	S/N	
Bitis_nasicornis	BMNH	1955.1.11.68-69
Bitis_nasicornis	BMNH	1932.2.3.1
Bitis_nasicornis	MNHParis	1899
Bitis_nasicornis	MNHParis	1988.3925
Bitis_nasicornis	MNHParis	1988.3923
Bitis_peringueyi	BMNH	1976.263
Bitis_peringueyi	UTA	R25696
Bitis_peringueyi	UTA	R25700
Bitis_peringueyi	UTA	R25697
Bitis_peringueyi	UTA	R39081
Bitis_peringueyi	CAS	111963
Bitis_rhinoceros	MNHParis	1986.105
Bitis_schneideri	BMNH	1967.594
Bitis_schneideri	UTA	R25701
Bitis_xeropaga	BMNH	1976.87
Bitis_xeropaga	FMNH	233126
Bitis_worthingtoni	BMNH	1952.1.2.30-31
Bitis_worthingtoni	BMNH	1952.1.2.30-31
Bitis_worthingtoni	FMNH	58141

Bothriechis_aurifer	UTA	R39070
Bothriechis_aurifer	UTA	R50585
Bothriechis_aurifer	UTA	R51728
Bothriechis_aurifer	UTA	R6525
Bothriechis_aurifer	UTA	R10434
Bothriechis_aurifer	UTA	R26574
Bothriechis_aurifer	UTA	R36211
Bothriechis_bicolor	UTA	R24759
Bothriechis_bicolor	UTA	R34156
Bothriechis_bicolor	UTA	R2274
Bothriechis_bicolor	FMNH	20162
Bothriechis_guifarroi	UTA	R60303
Bothriechis_lateralis	BMNH	96.10.8.18-19
Bothriechis_lateralis	UTA	R51155
Bothriechis_lateralis	UTA	R51154
Bothriechis_lateralis	UTA	R7634
Bothriechis_lateralis	UTA	R16351
Bothriechis_lateralis	FLMNH	54148
Bothriechis_lateralis	FLMNH	39820
Bothriechis_lateralis	FLMNH	70572
Bothriechis_lateralis	FLMNH	85322
Bothriechis_marchi	UTA	R13034
Bothriechis_marchi	UTA	R7159
Bothriechis_marchi	UTA	R13617
Bothriechis_marchi	UTA	R32068
Bothriechis_marchi	UTA	R50571
Bothriechis_marchi	FMNH	37217
Bothriechis_marchi	FMNH	21892
Bothriechis_marchi	AMNH	46957
Bothriechis_marchi	FLMNH	144679
Bothriechis_marchi	FLMNH	52555
Bothriechis_marchi	FLMNH	52554
Bothriechis_nigroviridis	UTA	R10433
Bothriechis_nigroviridis	UTA	R24841
Bothriechis_nigroviridis	FMNH	154530
Bothriechis_nigroviridis	FMNH	154515
Bothriechis_nigroviridis	USNM	32580
Bothriechis_nigroviridis	AMNH	142203
Bothriechis_rowleyi	UTA	R23954
Bothriechis_rowleyi	UTA	R6207
Bothriechis_rowleyi	UTA	R22243
Bothriechis_rowleyi	UTA	R13619
Bothriechis_rowleyi	UTA	R25703
Bothriechis_rowleyi	UTA	R6636
Bothriechis_rowleyi	AMNH	102895
Bothriechis_rowleyi	AMNH	102894
Bothriechis_rowleyi	FLMNH	52553
Bothriechis_schlegelii	MNRJ	8794

Bothriechis_schlegelii	UTA	R12957
Bothriechis_schlegelii	USNM	562913
Bothriechis_schlegelii	AMNH	35780
Bothriechis_schlegelii	FLMNH	157577
Bothriechis_supercilliaris	UTA	R35246
Bothriechis_thalassinus	UTA	R42259
Bothriechis_thalassinus	UTA	R38891
Bothriechis_thalassinus	UTA	R44438
Bothrocophias_campbelli	AMNH	22094
Bothrocophias_hyoprora	FMNH	165849
Bothrocophias_hyoprora	FMNH	56171
Bothrocophias_hyoprora	USNM	S/N
Bothrocophias_hyoprora	AMNH	54601
Bothrocophias_hyoprora	AMNH	53369
Bothrocophias_hyoprora	AMNH	52808
Bothrocophias_hyoprora	AMNH	52265
Bothrocophias_hyoprora	AMNH	52098
Bothrocophias_hyoprora	AMNH	113642
Bothrocophias_microphthalmus	BMNH	58.7.25.13
Bothrocophias_microphthalmus	AMNH	52470
Bothrops_alcatraz	IBSP	85710
Bothrops_alcatraz	IBSP	57105
Bothrops_alcatraz	IBSP	55791
Bothrops_alternatus	MNRJ	8608
Bothrops_alternatus	MNRJ	22192
Bothrops_alternatus	MNRJ	8602
Bothrops_alternatus	MNRJ	22094
Bothrops_alternatus	MNRJ/AL	5553
Bothrops_alternatus	IBSP	85656
Bothrops_alternatus	IBSP	85659
Bothrops_alternatus	IBSP	84796
Bothrops_alternatus	IBSP	84797
Bothrops_ammodytoides	BMNH	72.11.4.2
Bothrops_ammodytoides	FMNH	10832
Bothrops_ammodytoides	USNM	73421
Bothrops_ammodytoides	AMNH	110143
Bothrops_asper	MNRJ	8757
Bothrops_asper	MNRJ	8758
Bothrops_asper	MNRJ	8759
Bothrops_asper	UTA	R50466
Bothrops_atrox	MNRJ	15003
Bothrops_atrox	MNRJ	16821
Bothrops_atrox	MNRJ	16825
Bothrops_atrox	MNRJ	230
Bothrops_atrox	MNRJ	8577
Bothrops_atrox	MNRJ	974
Bothrops_barnetti	BMNH	1946.1.18.81
Bothrops_barnetti	BMNH	1946.1.18.71

Bothrops_barnetti	FMNH	9787
Bothrops_barnetti	FMNH	41603
Bothrops_bilineata	MNRJ	3434
Bothrops_bilineata	MNRJ	3424
Bothrops_bilineata	MNRJ	3044
Bothrops_brazili	USNM	316671
Bothrops_brazili	USNM	345184
Bothrops_caribbaeus	UTA	R16311
Bothrops_caribbaeus	USNM	15082
Bothrops_caribbaeus	AMNH	134681
Bothrops_caribbaeus	AMNH	134683
Bothrops_chloromelas	AMNH	104298
Bothrops_cotiara	MNRJ	10078
Bothrops_cotiara	MNRJ/AL	5401A
Bothrops_cotiara	IBSP	1100
Bothrops_cotiara	IBSP	811
Bothrops_cotiara	IBSP	1143
Bothrops_cotiara	IBSP	1031
Bothrops_cotiara	IBSP	1034
Bothrops_diporus	IBSP	84180
Bothrops_erythromelas	MNRJ	3068
Bothrops_erythromelas	MNRJ	7528
Bothrops_erythromelas	MNRJ	3067
Bothrops_erythromelas	MNRJ	7527
Bothrops_erythromelas	MNRJ	16925
Bothrops_erythromelas	MNRJ	9074
Bothrops_lutzi	IBSP	552
Bothrops_lutzi	IBSP	563
Bothrops_fonsecai	MNRJ	19280
Bothrops_fonsecai	MNRJ	8601
Bothrops_fonsecai	MNRJ	19280
Bothrops_fonsecai	MNRJ	8333
Bothrops_fonsecai	IBSP	31019
Bothrops_fonsecai	IBSP	53962
Bothrops_insularis	IBSP	18491
Bothrops_insularis	IBSP	1969
Bothrops_insularis	IBSP	1882
Bothrops_insularis	IBSP	1965
Bothrops_insularis	IBSP	1972
Bothrops_insularis	IBSP	1960
Bothrops_insularis	IBSP	1951
Bothrops_insularis	IBSP	18484
Bothrops_leucurus	MNRJ	22731
Bothrops_leucurus	MNRJ	3943
Bothrops_leucurus	MNRJ	9801
Bothrops_leucurus	MNRJ	2516
Bothrops_leucurus	IBSP	20666
Bothrops_leucurus	IBSP	50505

Bothrops_leucurus	IBSP	20663
Bothrops_leucurus	IBSP	20659
Bothrops_itapetiningae	MNRJ	19345
Bothrops_itapetiningae	IBSP	S/N
Bothrops_moojeni	MNRJ	5396
Bothrops_moojeni	MNRJ	7169
Bothrops_moojeni	MNRJ	7120
Bothrops_moojeni	MNRJ	15302
Bothrops_moojeni	MNRJ	7113
Bothrops_moojeni	MNRJ	14029
Bothrops_moojeni	MNRJ	9020
Bothrops_moojeni	MNRJ	8226
Bothrops_moojeni	MNRJ	21876
Bothrops_moojeni	MNRJ	14994
Bothrops_moojeni	MNRJ	7109
Bothrops_moojeni	MNRJ	7122
Bothrops_moojeni	MNRJ	5396
Bothrops_moojeni	MNRJ	10046
Bothrops_moojeni	MNRJ	7499
Bothrops_moojeni	MNRJ	20280
Bothrops_moojeni	MNRJ	7118
Bothrops_moojeni	IBSP	44905
Bothrops_moojeni	IBSP	20249
Bothrops_moojeni	IBSP	44920
Bothrops_jararacussu	MZUSP	3100
Bothrops_jararacussu	MNRJ	17830
Bothrops_jararacussu	MNRJ	21859
Bothrops_jararacussu	MNRJ	20918
Bothrops_jararacussu	MNRJ	14041
Bothrops_jararacussu	IBSP	11559
Bothrops_jararaca	MZUSP	4496
Bothrops_jararaca	MZUSP	2255
Bothrops_jararaca	MZUSP	9479
Bothrops_jararaca	MZUSP	9436
Bothrops_jararaca	MZUSP	8064
Bothrops_jararaca	MZUSP	7572
Bothrops_jararaca	MNRJ	13108
Bothrops_jararaca	MNRJ	20347
Bothrops_jararaca	MNRJ	21094
Bothrops_jararaca	MNRJ	12754
Bothrops_jararaca	MNRJ	10260
Bothrops_jararaca	MNRJ	10592
Bothrops_jararaca	MNRJ	7929
Bothrops_jararaca	MNRJ	932
Bothrops_jararaca	MNRJ	18921
Bothrops_jararaca	MNRJ	17834
Bothrops_jararaca	MNRJ	10077
Bothrops_jararaca	MNRJ	20347

Bothrops_jararaca	MNRJ	7900
Bothrops_jararaca	MNRJ	19948
Bothrops_jararaca	IBSP	82274
Bothrops_jararaca	IBSP	77469
Bothrops_jararaca	IBSP	85415
Bothrops_jararaca	IBSP	85414
Bothrops_jararaca	IBSP	85418
Bothrops_jararaca	IBSP	85417
Bothrops_pauloensis	MNRJ	22694
Bothrops_pauloensis	MNRJ	22697
Bothrops_pauloensis	MNRJ	18557
Bothrops_pauloensis	IBSP	63402
Bothrops_neuwiedi	MNRJ	20696
Bothrops_neuwiedi	MNRJ	19957
Bothrops_neuwiedi	MNRJ	19958
Bothrops_neuwiedi	MNRJ	18955
Bothrops_neuwiedi	MNRJ	16950
Bothrops_neuwiedi	MNRJ	17860
Bothrops_neuwiedi	MNRJ	19398
Bothrops_neuwiedi	IBSP	7990
Bothrops_lanceolatus	MNHParis	1988.104
Bothrops_lanceolatus	MNHParis	1988.108
Bothrops_lanceolatus	MNHParis	1988.110
Bothrops_lanceolatus	MNHParis	1988.106
Bothrops_lojanus	BMNH	1930.10.12.26-27
Bothrops_lojanus	BMNH	1930.10.12.26-27
Bothrops_lojanus	BMNH	1931.11.3.12-14
Bothrops_lojanus	BMNH	1935.11.3.115-119
Bothrops_lojanus	USNM	98935
Bothrops_marmoratus	IBSP	57153
Bothrops_marmoratus	IBSP	57155
Bothrops_matogrossensis	MNRJ	20961
Bothrops_matogrossensis	MNRJ	20963
Bothrops_matogrossensis	MNRJ	20960
Bothrops_matogrossensis	MNRJ	8227
Bothrops_pictus	BMNH	19.11.12.20.12
Bothrops_pictus	MNHParis	6410
Bothrops_pictus	MNHParis	1543
Bothrops_pictus	FMNH	5662
Bothrops_pubescens	MNRJ	12138
Bothrops_pubescens	MNRJ	10079
Bothrops_pulchra	BMNH	78.1.25.54
Bothrops_punctatus	BMNH	1914.5.21.71
Bothrops_punctatus	AMNH	107914
Bothrops_taeniata	MNRJ	10836
Calloselasma_rhodostoma	MNHParis	1988.69
Calloselasma_rhodostoma	MNHParis	1988.70
Calloselasma_rhodostoma	MNHParis	1990.3824

Causus_defilippii	BMNH	1975.1120
Causus_defilippii	BMNH	1952.1.10.81
Causus_defilippii	BMNH	1952.1.10.85
Causus_defilippii	BMNH	1952.1.10.86
Causus_defilippii	BMNH	93.10.26.71-72
Causus_defilippii	BMNH	1983.1089
Causus_defilippii	BMNH	95.2.26.3
Causus_defilippii	MNHParis	1896.596
Causus_defilippii	MNHParis	1896.595
Causus_lichtensteini	BMNH	1919.8.16.99
Causus_lichtensteini	BMNH	1950.1.2.11-12
Causus_lichtensteini	BMNH	1950.1.2.11-12
Causus_lichtensteini	BMNH	1934.12.1.34
Causus_lichtensteini	BMNH	1907.5.22.63-5
Causus_lichtensteini	BMNH	1907.5.22.63-5
Causus_lichtensteini	MNHParis	1973.1245
Causus_resimus	BMNH	1924.2.19.7
Causus_resimus	BMNH	1907.12.2.29
Causus_resimus	BMNH	1912.10.14.7
Causus_resimus	BMNH	1924.5.21.23
Causus_resimus	BMNH	1911.7.8.19
Causus_resimus	MNHParis	1989.39
Causus_resimus	MNHParis	1904.204
Causus_resimus	MNHParis	1988.2115
Causus_rhombeatus	BMNH	48.3.13.3-4
Causus_rhombeatus	BMNH	1929.4.14.16-18
Causus_rhombeatus	BMNH	1954.1.12.54
Causus_rhombeatus	BMNH	95.5.3.53-5
Causus_rhombeatus	BMNH	58.4.11.4
Causus_rhombeatus	MNHParis	1912.382
Causus_rhombeatus	MNHParis	1921.491
Cerastes_cerastes	BMNH	1970.1760
Cerastes_cerastes	BMNH	1912.11.30.130-138
Cerastes_cerastes	BMNH	97.10.26.633
Cerastes_cerastes	MNHParis	4022
Cerastes_cerastes	AMNH	116016
Cerastes_gasperettii	BMNH	1986.351
Cerastes_gasperettii	BMNH	1985.654
Cerastes_gasperettii	BMNH	1994.133
Cerastes_gasperettii	USNM	129454
Cerastes_gasperettii	USNM	129881
Cerastes_vipera	BMNH	1912.11.9.135
Cerastes_vipera	BMNH	1987.2362
Cerastes_vipera	MNHParis	4014
Cerastes_vipera	CAS	138623
Cerastes_vipera	FMNH	81217
Cerastes_vipera	FMNH	22927
Mixcoatlus_barbouri	UTA	R15558

Mixcoatlus_barbouri	UTA	R4450
Mixcoatlus_barbouri	FMNH	38503
Cerrophidion_godmani	UTA	R37293
Cerrophidion_godmani	UTA	R7143
Cerrophidion_godmani	UTA	R42230
Cerrophidion_godmani	UTA	R38272
Cerrophidion_godmani	UTA	R24765
Cerrophidion_godmani	USNM	319985
Cerrophidion_sasai	UTA	R51399
Cerrophidion_sasai	UTA	R51403
Cerrophidion_sasai	UTA	R51402
Cerrophidion_tzotzilorum	UTA	R9641
Cerrophidion_tzotzilorum	AMNH	103133
Crotalus_adamanteus	USNM	250
Crotalus_adamanteus	USNM	104562
Crotalus_adamanteus	AMNH	81997
Crotalus_adamanteus	AMNH	63830
Crotalus_adamanteus	AMNH	160660
Crotalus_adamanteus	AMNH	815537
Crotalus_aquilus	UTA	R12595
Crotalus_aquilus	UTA	R22525
Crotalus_aquilus	UTA	R17904
Crotalus_aquilus	UTA	R6180
Crotalus_aquilus	UTA	R18341
Crotalus_aquilus	UTA	R25407
Crotalus_aquilus	AMNH	64604
Crotalus_atrox	CAS	65086
Crotalus_atrox	CAS	65085
Crotalus_atrox	CAS	33656
Crotalus_atrox	CAS	63879
Crotalus_atrox	CAS	14366
Crotalus_atrox	CAS	65698
Crotalus_basiliscus	CAS	159399
Crotalus_basiliscus	CAS	24095
Crotalus_basiliscus	CAS	74403
Crotalus_basiliscus	AMNH	64254
Crotalus_basiliscus	AMNH	31886
Crotalus_catalinensis	UTA	R32129
Crotalus_cerastes	CAS	183159
Crotalus_cerastes	CAS	192596
Crotalus_cerastes	CAS	223518
Crotalus_cerastes	CAS	182489
Crotalus_cerastes	CAS	182478
Crotalus_cerastes	CAS	101442
Crotalus_cerastes	CAS	12779
Crotalus_cerastes	USNM	205522
Crotalus_cerberus	CAS	17539
Crotalus_cerberus	FMNH	51766

Crotalus_cerberus	FMNH	18423
Crotalus_culminatus	UTA	R41028
Crotalus_culminatus	CAS	71761
Crotalus_culminatus	FMNH	38500
Crotalus_durissus	MNRJ	7133
Crotalus_durissus	MNRJ	1362
Crotalus_durissus	MNRJ	8597
Crotalus_durissus	MNRJ	22949
Crotalus_durissus	MNRJ	8753
Crotalus_durissus	MNRJ	15353
Crotalus_durissus	MNRJ	14995
Crotalus_durissus	MNRJ	21744
Crotalus_durissus	MNRJ	14996
Crotalus_durissus	MNRJ	21850
Crotalus_durissus	MNRJ	21873
Crotalus_durissus	IBSP	83928
Crotalus_durissus	UTA	R21912
Crotalus_durissus	UTA	R12673
Crotalus_enyo	UTA	R8082
Crotalus_enyo	UTA	R7470
Crotalus_enyo	UTA	R7500
Crotalus_enyo	CAS	143853
Crotalus_enyo	CAS	14022
Crotalus_enyo	CAS	103469
Crotalus_enyo	CAS	90317
Crotalus_enyo	CAS	143987
Crotalus_ericsmithi	UTA	R55372
Crotalus_horridus	AMNH	46392
Crotalus_horridus	AMNH	128165
Crotalus_horridus	AMNH	129137
Crotalus_horridus	AMNH	4082
Crotalus_horridus	AMNH	129420
Crotalus_horridus	AMNH	146519
Crotalus_intermedius	UTA	R6232
Crotalus_intermedius	UTA	R4539
Crotalus_intermedius	UTA	R4538
Crotalus_intermedius	UTA	R9354
Crotalus_intermedius	CAS	93777
Crotalus_intermedius	AMNH	102477
Crotalus_intermedius	AMNH	102909
Crotalus_intermedius	AMNH	90799
Crotalus_intermedius	AMNH	72476
Crotalus_lepidus	UTA	R8343
Crotalus_lepidus	UTA	R8331
Crotalus_lepidus	UTA	R17836
Crotalus_lepidus	UTA	R32144
Crotalus_lepidus	UTA	R16285
Crotalus_lepidus	UTA	R7434

Crotalus_lepidus	AMNH	138254
Crotalus_mitchellii	CAS	52842
Crotalus_mitchellii	CAS	103470
Crotalus_mitchellii	CAS	182495
Crotalus_mitchellii	CAS	40092
Crotalus_mitchellii	CAS	85698
Crotalus_mitchellii	CAS	45887
Crotalus_molossus	CAS	48048
Crotalus_molossus	CAS	48047
Crotalus_molossus	CAS	48042
Crotalus_molossus	AMNH	67305
Crotalus_molossus	AMNH	26025
Crotalus_molossus	AMNH	138246
Crotalus_oreganus	CAS	238534
Crotalus_oreganus	CAS	223732
Crotalus_oreganus	CAS	209200
Crotalus_oreganus	USNM	209457
Crotalus_oreganus	AMNH	66076
Crotalus_ornatus	USNM	103738
Crotalus_polystictus	MNHParis	1975.145
Crotalus_polystictus	MNHParis	5092
Crotalus_polystictus	MNHParis	1888.284
Crotalus_polystictus	UTA	R4499
Crotalus_polystictus	UTA	R6927
Crotalus_polystictus	AMNH	161898
Crotalus_polystictus	AMNH	19861
Crotalus_polystictus	AMNH	19858
Crotalus_pricei	UTA	R6251
Crotalus_pricei	UTA	R45019
Crotalus_pricei	UTA	R57636
Crotalus_pricei	CAS	169788
Crotalus_pricei	CAS	48037
Crotalus_pricei	CAS	121003
Crotalus_pricei	CAS	48036
Crotalus_pricei	CAS	48034
Crotalus_pusillus	UTA	R4530
Crotalus_pusillus	UTA	R9358
Crotalus_pusillus	UTA	R6119
Crotalus_pusillus	FMNH	37048
Crotalus_pusillus	FMNH	39120
Crotalus_pusillus	FMNH	39113
Crotalus_pusillus	FMNH	39112
Crotalus_ravus	UTA	R12610
Crotalus_ravus	UTA	R12607
Crotalus_ravus	UTA	R12608
Crotalus_ravus	UTA	R12625
Crotalus_ravus	UTA	R25859
Crotalus_ravus	UTA	R13633

Crotalus_ruber	CAS	223537
Crotalus_ruber	CAS	169526
Crotalus_ruber	CAS	195596
Crotalus_ruber	CAS	103467
Crotalus_ruber	CAS	53717
Crotalus_ruber	CAS	51258
Crotalus_ruber	CAS	146940
Crotalus_ruber	CAS	104358
Crotalus_scutulatus	AMNH	69936
Crotalus_scutulatus	AMNH	68345
Crotalus_scutulatus	AMNH	68321
Crotalus_scutulatus	AMNH	68958
Crotalus_scutulatus	AMNH	88819
Crotalus_simus	UTA	R5252
Crotalus_simus	UTA	R21911
Crotalus_simus	UTA	R33133
Crotalus_simus	UTA	R52497
Crotalus_simus	UTA	R7701
Crotalus_stejnegeri	UTA	R6234
Crotalus_tancitarensis	UTA	R52401
Crotalus_tancitarensis	FMNH	39115
Crotalus_tigris	UTA	R31060
Crotalus_tigris	UTA	R32248
Crotalus_tigris	CAS	92265
Crotalus_tigris	CAS	92266
Crotalus_tigris	CAS	192769
Crotalus_tigris	CAS	102913
Crotalus_tigris	USNM	156808
Crotalus_tigris	AMNH	64273
Crotalus_tigris	AMNH	26024
Crotalus_tigris	AMNH	02537
Crotalus_tortugensis	FMNH	18426
Crotalus_totonacus	UTA	R7222
Crotalus_totonacus	UTA	R7224
Crotalus_totonacus	USNM	209856
Crotalus_transversus	UTA	R3988
Crotalus_triseriatus	MNHParis	1898.254
Crotalus_triseriatus	MNHParis	1641
Crotalus_triseriatus	UTA	R14516
Crotalus_triseriatus	UTA	R12601
Crotalus_triseriatus	UTA	R59041
Crotalus_triseriatus	UTA	R32788
Crotalus_triseriatus	FMNH	39110
Crotalus_triseriatus	AMNH	106588
Crotalus_tzcaban	UTA	R6802
Crotalus_tzcaban	UTA	R6732
Crotalus_tzcaban	USNM	46400
Crotalus_viridis	CAS	10419

Crotalus_viridis	CAS	93802
Crotalus_viridis	CAS	93803
Crotalus_viridis	USNM	313396
Crotalus_viridis	AMNH	121442
Crotalus_willardi	UTA	R9356
Crotalus_willardi	UTA	R17849
Crotalus_willardi	UTA	R32081
Crotalus_willardi	UTA	R22526
Crotalus_willardi	UTA	R6942
Crotalus_willardi	UTA	R17855
Crotalus_willardi	UTA	R21922
Crotalus_willardi	UTA	R18229
Daboia_deserti	MNHParis	1988.27
Daboia_deserti	MNHParis	1988.2497
Daboia_deserti	MNHParis	1988.4000
Daboia_deserti	MNHParis	1990.4197
Daboia_deserti	UTA	R8226
Daboia_mauritanica	BMNH	1906.10.31.11
Daboia_mauritanica	BMNH	1906.10.31.13
Daboia_mauritanica	MNHParis	1989.99
Daboia_mauritanica	MNHParis	1961.327
Daboia_palaestinae	BMNH	1937.9.4.1
Daboia_palaestinae	BMNH	1984.1220
Daboia_palaestinae	MNHParis	1989.70
Daboia_palaestinae	MNHParis	1986.123A
Daboia_palaestinae	MNHParis	1986.123B
Daboia_russelii	MNHParis	1962.259
Daboia_russelii	MNHParis	1962.258
Daboia_russelii	MNHParis	3187A
Daboia_russelii	AMNH	84442
Daboia_russelii	AMNH	161845
Daboia_siamensis	BMNH	1929.10.10.1-2
Daboia_siamensis	BMNH	1987.456
Daboia_siamensis	BMNH	1987.454
Daboia_siamensis	BMNH	1987.451
Daboia_siamensis	MNHParis	4020
Deinagkistrodon_acutus	MNHParis	1993.3464
Deinagkistrodon_acutus	MNHParis	1969.8
Deinagkistrodon_acutus	UTA	R32133
Deinagkistrodon_acutus	UTA	R8190
Deinagkistrodon_acutus	CAS	18765
Deinagkistrodon_acutus	CAS	121007
Deinagkistrodon_acutus	FMNH	127212
Deinagkistrodon_acutus	AMNH	83994
Echis_carinatus	BMNH	1983.716
Echis_carinatus	BMNH	1903.3.6.54
Echis_carinatus	BMNH	1964.259
Echis_carinatus	MNHParis	1988.3993

Echis_carinatus	AMNH	88472
Echis_coloratus	BMNH	1986.451
Echis_coloratus	BMNH	1986.456
Echis_coloratus	BMNH	1985.732
Echis_coloratus	MNHParis	1988.97
Echis_coloratus	MNHParis	1997.3446
Echis_coloratus	MNHParis	1990.4194
Echis_coloratus	MNHParis	1990.4192
Echis_coloratus	CAS	139767
Echis_jogeri	MNHParis	1993.0144
Echis_khosatzkii	BMNH	97.3.11.117
Echis_leucogaster	BMNH	1961.1849
Echis_leucogaster	BMNH	1961.1850
Echis_leucogaster	BMNH	1906.5.7.8
Echis_leucogaster	MNHParis	2002.0779
Echis_leucogaster	MNHParis	1985.454
Echis_leucogaster	CAS	136088
Echis_leucogaster	USNM	161948
Echis_ocellatus	BMNH	1976.688
Echis_ocellatus	BMNH	1976.725
Echis_ocellatus	BMNH	1975.704
Echis_ocellatus	BMNH	1975.706
Echis_ocellatus	MNHParis	1977.602
Echis_ocellatus	MNHParis	1977.596
Echis_ocellatus	MNHParis	1977.603
Echis_omanensis	BMNH	1977.84
Echis_omanensis	BMNH	1972.712
Echis_omanensis	BMNH	1971.127
Echis_omanensis	BMNH	1978.353
Echis_omanensis	CAS	250912
Echis_pyramidum	BMNH	1954.1.15.53-54
Echis_pyramidum	BMNH	1938.8.4.85-87
Echis_pyramidum	BMNH	51.7.17.157
Echis_pyramidum	BMNH	11.1.3a
Echis_pyramidum	MNHParis	1981.482
Echis_pyramidum	MNHParis	1990.4203
Eristicophis_macmahoni	BMNH	S/N
Eristicophis_macmahoni	UTA	R16392
Eristicophis_macmahoni	UTA	R16391
Eristicophis_macmahoni	UTA	R15665
Eristicophis_macmahoni	UTA	R16390
Garthius_chaseni	FMNH	71860
Gloydius_blomhoffi	BMNH	1870.1.14.2
Gloydius_blomhoffi	BMNH	1980.6.25.10
Gloydius_blomhoffi	BMNH	1893.4.20.28
Gloydius_blomhoffi	MNHParis	1999.7969
Gloydius_blomhoffi	MNHParis	1999.7970
Gloydius_blomhoffi	MNHParis	1888.37

Gloydius_blomhoffi	MNHParis	1888.36
Gloydius_blomhoffi	CAS	26732
Gloydius_brevicaudus	MNHParis	1902.74
Gloydius_brevicaudus	MNHParis	831
Gloydius_brevicaudus	FMNH	188988
Gloydius_brevicaudus	FMNH	188975
Gloydius_brevicaudus	AMNH	108495
Gloydius_brevicaudus	AMNH	108496
Gloydius_brevicaudus	AMNH	108498
Gloydius_halys	MNHParis	865
Gloydius_halys	AMNH	119011
Gloydius_halys	AMNH	123300
Gloydius_intermedius	MNHParis	1896.206
Gloydius_intermedius	CAS	121001
Gloydius_intermedius	USNM	203374
Gloydius_intermedius	USNM	203380
Gloydius_saxatilis	CAS	31540
Gloydius_saxatilis	CAS	31543
Gloydius_saxatilis	CAS	31528
Gloydius_saxatilis	AMNH	108502
Gloydius_saxatilis	AMNH	108505
Gloydius_strauchi	FMNH	15171
Gloydius_strauchi	FMNH	15134
Gloydius_strauchi	USNM	81980
Gloydius_strauchi	AMNH	58080
Gloydius_ussuriensis	CAS	14586
Gloydius_ussuriensis	FMNH	188999
Gloydius_ussuriensis	FMNH	S/N
Gloydius_ussuriensis	FMNH	11480
Gloydius_ussuriensis	FMNH	11481
Hypnale_hypnale	BMNH	1931.5.13.98
Hypnale_hypnale	CAS	17270
Hypnale_hypnale	AMNH	102462
Hypnale_hypnale	AMNH	99384
Hypnale_hypnale	AMNH	132250
Hypnale_nepa	BMNH	1931.5.13.100
Hypnale_nepa	BMNH	1937.8.1.1
Hypnale_nepa	BMNH	1894.9.11.39
Hypnale_zara	BMNH	1946.1.19.96
Lachesis_acrochorda	UTA	R56349
Lachesis_acrochorda	UTA	R7418
Lachesis_melanocephala	UTA	R7417
Lachesis_melanocephala	UTA	R10347
Lachesis_muta	MNRJ	13256
Lachesis_muta	MNRJ	3039
Lachesis_muta	MNRJ	241
Lachesis_muta	MNRJ	18048
Lachesis_muta	UTA	25601

Lachesis_muta	UTA	R25600
Lachesis_muta	MNRJ	3544
Lachesis_muta	MNRJ	4760
Lachesis_muta	MNRJ	8028
Lachesis_stenophrys	UTA	R9323
Lachesis_stenophrys	UTA	R25597
Lachesis_stenophrys	UTA	R18211
Lachesis_stenophrys	USNM	165008
Lachesis_stenophrys	AMNH	172358
Macrovipera_lebetina	BMNH	1900.1.19.3
Macrovipera_lebetina	BMNH	79.8.15.34
Macrovipera_lebetina	MNHParis	1229
Macrovipera_lebetina	MNHParis	7737
Macrovipera_lebetina	CAS	218070
Macrovipera_lebetina	CAS	179736
Macrovipera_schweizeri	BMNH	1936.1.4.2-6
Macrovipera_schweizeri	BMNH	1936.1.4.2-7
Mixcoatlus_browni	CAS	135274
Montivipera_bornmuelleri	BMNH	1957.1.13.80
Montivipera_bornmuelleri	BMNH	1957.1.13.72
Montivipera_bornmuelleri	BMNH	1984.1227
Montivipera_latifii	BMNH	1976.555
Montivipera_latifii	BMNH	1976.554
Montivipera_latifii	BMNH	1976.552
Montivipera_latifii	AMNH	109617
Montivipera_raddei	BMNH	1976.556
Montivipera_raddei	BMNH	1976.557
Montivipera_raddei	UTA	R7165
Montivipera_raddei	CAS	135746
Montivipera_wagneri	MNHParis	1988.9011
Montivipera_xanthina	BMNH	1984.1226
Montivipera_xanthina	MNHParis	1989.128
Montivipera_xanthina	MNHParis	1989.71
Montivipera_xanthina	MNHParis	1988.3867
Montivipera_xanthina	MNHParis	1988.22
Montivipera_xanthina	MNHParis	1988.26
Montivipera_xanthina	CAS	135748
Mixcoatlus_melanurus	UTA	R6818
Mixcoatlus_melanurus	UTA	R9610
Mixcoatlus_melanurus	UTA	R6817
Mixcoatlus_melanurus	UTA	R22451
Mixcoatlus_melanurus	UTA	R16309
Mixcoatlus_melanurus	UTA	R12559
Ophryacus_undulatus	UTA	R4518
Ophryacus_undulatus	UTA	R4642
Ophryacus_undulatus	UTA	R4108
Ophryacus_undulatus	UTA	R4640
Ophryacus_undulatus	UTA	R25115

Ophryacus_undulatus	UTA	R4835
Ophryacus_undulatus	UTA	R4832
Ophryacus_undulatus	UTA	R32426
Ophryacus_undulatus	UTA	R4858
Ovophis_monticola	BMNH	1930.11.16.13
Ovophis_monticola	BMNH	1930.11.16.14
Ovophis_monticola	MNHParis	2011.02.49
Ovophis_monticola	MNHParis	1935.115
Ovophis_monticola	FMNH	232827
Ovophis_monticola	FMNH	258632
Ovophis_okinavensis	BMNH	1906.8.16.45
Ovophis_okinavensis	MNHParis	1986.103
Ovophis_okinavensis	UTA	R22391
Ovophis_okinavensis	UTA	R28192
Ovophis_okinavensis	CAS	21930
Ovophis_tonkinensis	MNHParis	1948.105
Ovophis_tonkinensis	MNHParis	2011.0246
Ovophis_zayuensis	CAS	233203
Porthidium_arcosae	UTA	R55938
Porthidium_dunni	UTA	R17905
Porthidium_dunni	UTA	R4367
Porthidium_dunni	FMNH	104957
Porthidium_dunni	AMNH	91108
Porthidium_dunni	AMNH	19874
Porthidium_dunni	AMNH	66859
Porthidium_dunni	AMNH	66868
Porthidium_dunni	AMNH	66866
Porthidium_dunni	AMNH	66862
Porthidium_dunni	AMNH	66854
Porthidium_dunni	AMNH	66870
Porthidium_dunni	AMNH	66867
Porthidium_dunni	AMNH	65171
Porthidium_dunni	AMNH	65167
Porthidium_dunni	AMNH	65169
Porthidium_dunni	AMNH	65166
Porthidium_dunni	AMNH	68076
Porthidium_dunni	AMNH	65874
Porthidium_hespere	UTA	R4443
Porthidium_lansbergii	BMNH	71.2.7.4
Porthidium_lansbergii	BMNH	S/N
Porthidium_lansbergii	UTA	R3677
Porthidium_lansbergii	CAS	116159
Porthidium_lansbergii	FMNH	166032
Porthidium_lansbergii	FMNH	166080
Porthidium_lansbergii	FMNH	166097
Porthidium_lansbergii	FMNH	166073
Porthidium_nasutum	UTA	R31057
Porthidium_nasutum	UTA	R14181

Porthidium_nasutum	UTA	R23065
Porthidium_nasutum	UTA	R26408
Porthidium_nasutum	UTA	R39207
Porthidium_ophryomegas	UTA	R39755
Porthidium_ophryomegas	UTA	R28565
Porthidium_ophryomegas	UTA	R39217
Porthidium_ophryomegas	UTA	R28563
Porthidium_ophryomegas	UTA	R45885
Porthidium_ophryomegas	UTA	R45887
Porthidium_ophryomegas	UTA	R7722
Porthidium_ophryomegas	AMNH	161915
Porthidium_ophryomegas	AMNH	64407
Porthidium_porrasi	UTA	R59119
Porthidium_porrasi	UTA	R52898
Porthidium_porrasi	UTA	R30830
Porthidium_yucatanicum	UTA	R16960
Porthidium_yucatanicum	FMNH	36185
Porthidium_yucatanicum	FMNH	36176
Porthidium_yucatanicum	FMNH	36187
Porthidium_yucatanicum	FMNH	36182
Proatheris_superciliaris	BMNH	1934.4.6.36
Proatheris_superciliaris	BMNH	1929.11.2.7
Protobothrops_cornutus	MNHParis	1937.35
Protobothrops_elegans	CAS	21958
Protobothrops_elegans	CAS	21954
Protobothrops_elegans	CAS	21962
Protobothrops_elegans	CAS	21967
Protobothrops_elegans	CAS	21947
Protobothrops_elegans	CAS	21948
Protobothrops_flavoviridis	BMNH	87.10.5.1-2
Protobothrops_flavoviridis	BMNH	1906.8.16.35-36
Protobothrops_flavoviridis	BMNH	1906.8.16.35-36
Protobothrops_flavoviridis	MNHParis	1893.11
Protobothrops_flavoviridis	MNHParis	1895.142
Protobothrops_flavoviridis	USNM	121469
Protobothrops_jerdonii	BMNH	1974.910
Protobothrops_jerdonii	BMNH	91.6.13.1
Protobothrops_jerdonii	BMNH	1940.6.5.96
Protobothrops_jerdonii	BMNH	1940.6.5.95
Protobothrops_jerdonii	BMNH	1940.6.5.94
Protobothrops_jerdonii	MNHParis	1935.0114
Protobothrops_jerdonii	MNHParis	1938.0151
Protobothrops_kaulbacki	BMNH	1936.7.4.42
Protobothrops_kaulbacki	BMNH	1946.1.19.23
Protobothrops_kaulbacki	BMNH	1946.1.19.24
Protobothrops_kaulbacki	CAS	22430
Protobothrops_mucrosquamatus	MNHParis	1935.118
Protobothrops_mucrosquamatus	MNHParis	1897.100

Protobothrops_mucrosquamatus	MNHParis	1948.108
Protobothrops_mucrosquamatus	CAS	224365
Protobothrops_mucrosquamatus	CAS	238906
Protobothrops_mucrosquamatus	CAS	244954
Protobothrops_mucrosquamatus	CAS	18920
Protobothrops_mucrosquamatus	CAS	18923
Protobothrops_tokarensis	MNHParis	1988.2106
Protobothrops_tokarensis	USNM	348518
Pseudocerastes_persicus	MNHParis	1990.4.156
Pseudocerastes_persicus	MNHParis	1990.4.155
Pseudocerastes_persicus	MNHParis	1990.4.151
Pseudocerastes_persicus	MNHParis	1990.4.157
Pseudocerastes_urarachnoides	FMNH	170929
Pseudocerastes_fieldi	UTA	R8178
Pseudocerastes_fieldi	FMNH	11061
Pseudocerastes_fieldi	AMNH	94418
Pseudocerastes_fieldi	AMNH	107688
Pseudocerastes_fieldi	AMNH	94419
Sistrurus_catenatus	UTA	R6809
Sistrurus_catenatus	UTA	R1335
Sistrurus_catenatus	UTA	R11290
Sistrurus_catenatus	UTA	R54126
Sistrurus_catenatus	UTA	R53622
Sistrurus_catenatus	UTA	R30852
Sistrurus_catenatus	FMNH	135026
Sistrurus_catenatus	FMNH	95447
Sistrurus_catenatus	AMNH	8700
Sistrurus_miliarius	UTA	R25121
Sistrurus_miliarius	UTA	R22378
Sistrurus_miliarius	UTA	R56016
Sistrurus_miliarius	USNM	234410
Sistrurus_miliarius	USNM	234405
Sistrurus_miliarius	USNM	491
Trimeresurus_albolabris	MNHParis	1999.9474
Trimeresurus_albolabris	UTA	ENS 14106
Trimeresurus_albolabris	USNM	163960
Trimeresurus_albolabris	USNM	163965
Trimeresurus_albolabris	USNM	72077
Trimeresurus_andersonii	BMNH	1937.3.1.23
Trimeresurus_andersonii	BMNH	1937.3.1.23
Trimeresurus_andersonii	BMNH	1940.3.9.37
Trimeresurus_barati	BMNH	1915.12.2.42
Trimeresurus_barati	UTA	ENS 15785
Trimeresurus_borneensis	BMNH	94.8.37
Trimeresurus_borneensis	BMNH	94.6.30.67
Trimeresurus_buniana	BMNH	2005.1609
Trimeresurus_cantori	BMNH	1940.3.9.41
Trimeresurus_cantori	BMNH	1940.3.9.42

Trimeresurus_cantori	BMNH	1940.3.9.40
Trimeresurus_cantori	USNM	29445
Trimeresurus_erythrurus	BMNH	61.10.2.6
Trimeresurus_erythrurus	UTA	R29684
Trimeresurus_erythrurus	CAS	243175
Trimeresurus_erythrurus	CAS	239502
Trimeresurus_erythrurus	CAS	240036
Trimeresurus_erythrurus	AMNH	63897
Trimeresurus_fasciatus	MNHParis	2000.401
Trimeresurus_flavomaculatus	BMNH	2002.18
Trimeresurus_flavomaculatus	MNHParis	1900.426
Trimeresurus_flavomaculatus	FMNH	270194
Trimeresurus_fucatus	MNHParis	1900.4283
Trimeresurus_fucatus	MNHParis	1900.4284
Trimeresurus_fucatus	MNHParis	1991.295
Trimeresurus_gramineus	MNRJ	13269
Trimeresurus_gramineus	MNHParis	4058
Trimeresurus_gramineus	CAS	14979
Trimeresurus_gramineus	CAS	18751
Trimeresurus_gramineus	CAS	18748
Trimeresurus_gramineus	CAS	18752
Trimeresurus_gramineus	CAS	18747
Trimeresurus_gumprechti	BMNH	2002.53
Trimeresurus_gumprechti	MNHParis	1987.3835
Trimeresurus_gumprechti	MNHParis	1999.9072
Trimeresurus_gumprechti	CAS	234873
Trimeresurus_hageni	BMNH	1988.859
Trimeresurus_hageni	BMNH	1988.861
Trimeresurus_hageni	ZRC	22932
Trimeresurus_hageni	ZRC	25397
Trimeresurus_hageni	ZRC	22935
Trimeresurus_hageni	UTA	R55257
Trimeresurus_insularis	MNHParis	4057
Trimeresurus_insularis	USNM	579386
Trimeresurus_insularis	USNM	579784
Trimeresurus_insularis	USNM	573677
Trimeresurus_kanburiensis	BMNH	1992.535
Trimeresurus_macrops	BMNH	1988.876
Trimeresurus_macrops	BMNH	1988.1877
Trimeresurus_macrops	BMNH	1988.1057
Trimeresurus_macrops	BMNH	1988.1053
Trimeresurus_macrops	BMNH	1988.1052
Trimeresurus_macrops	BMNH	1988.865
Trimeresurus_macrops	MNHParis	1885.389
Trimeresurus_malabaricus	BMNH	74.4.29.136-139.1027
Trimeresurus_malabaricus	MNHParis	1913.5
Trimeresurus_malabaricus	CAS	17273
Trimeresurus_malabaricus	FMNH	217680

Trimeresurus_malabaricus	FMNH	30763
Trimeresurus_malcolmi	BMNH	1929.12.22.116
Trimeresurus_malcolmi	FMNH	239948
Trimeresurus_mcgregori	BMNH	2002.06
Trimeresurus_mcgregori	BMNH	2002.4
Trimeresurus_mcgregori	BMNH	2002.9
Trimeresurus_mcgregori	BMNH	2002.8
Trimeresurus_mcgregori	MNHParis	1900.427
Trimeresurus_nebularis	BMNH	2003.1
Trimeresurus_popeiorum	BMNH	1924.5.20.38
Trimeresurus_popeiorum	BMNH	60.3.19.1300B
Trimeresurus_popeiorum	BMNH	72.4.17.137
Trimeresurus_popeiorum	CAS	240640
Trimeresurus_popeiorum	FMNH	178656
Trimeresurus_popeiorum	FMNH	265805
Trimeresurus_puniceus	BMNH	85.12.31.32-33
Trimeresurus_puniceus	BMNH	85.12.31.32-34
Trimeresurus_puniceus	MNHParis	1991.3286
Trimeresurus_puniceus	FMNH	183785
Trimeresurus_puniceus	FMNH	138662
Trimeresurus_puniceus	FMNH	131846
Trimeresurus_purpureomaculatus	BMNH	1988.397
Trimeresurus_purpureomaculatus	MNHParis	1990.4282
Trimeresurus_purpureomaculatus	MNHParis	1990.3880
Trimeresurus_purpureomaculatus	MNHParis	1999.6588
Trimeresurus_purpureomaculatus	AMNH	63899
Trimeresurus_purpureomaculatus	AMNH	63900
Trimeresurus_sabahi	FMNH	251048
Trimeresurus_sabahi	USNM	130253
Trimeresurus_schultzei	FMNH	53561
Trimeresurus_schultzei	USNM	37871
Trimeresurus_septentrionalis	BMNH	1950.1.5.64
Trimeresurus_septentrionalis	BMNH	1955.1.13.82
Trimeresurus_septentrionalis	CAS	135750
Trimeresurus_stejnegeri	BMNH	1953.1.2.88-92
Trimeresurus_stejnegeri	BMNH	S/N
Trimeresurus_stejnegeri	BMNH	S/N
Trimeresurus_stejnegeri	BMNH	S/N
Trimeresurus_stejnegeri	MNHParis	1912.353
Trimeresurus_stejnegeri	MNHParis	1912.352
Trimeresurus_stejnegeri	MNHParis	1912.354
Trimeresurus_stejnegeri	MNHParis	1999.9064
Trimeresurus_stejnegeri	MNHParis	1999.9055
Trimeresurus_sumatranus	BMNH	84.1.8.47
Trimeresurus_sumatranus	USNM	134125
Trimeresurus_tibetanus	CAS	177677
Trimeresurus_trigonocephalus	MNHParis	245
Trimeresurus_trigonocephalus	UTA	R40461

Trimeresurus_trigonocephalus	UTA	R8191
Trimeresurus_trigonocephalus	UTA	R34536
Trimeresurus_trigonocephalus	UTA	R32527
Trimeresurus_trigonocephalus	USNM	254789
Trimeresurus_venustus	BMNH	1988.384
Trimeresurus_vogeli	FMNH	180274
Trimeresurus_vogeli	FMNH	180273
Trimeresurus_vogeli	FMNH	180272
Trimeresurus_vogeli	FMNH	180260
Trimeresurus_vogeli	FMNH	180256
Trimeresurus_wiroti	UTA	R38540
Trimeresurus_yunnanensis	CAS	215141
Trimeresurus_yunnanensis	CAS	234261
Trimeresurus_yunnanensis	FMNH	7065
Trimeresurus_yunnanensis	AMNH	62255
Tropidolaemus_subannulatus	BMNH	1946.1.19.33
Tropidolaemus_subannulatus	MNHParis	2011.02.52
Tropidolaemus_subannulatus	MNHParis	5821
Tropidolaemus_subannulatus	MNHParis	1891.0082
Tropidolaemus_subannulatus	CAS	8679
Tropidolaemus_subannulatus	FMNH	3566
Tropidolaemus_wagleri	BMNH	97.12.28.58
Tropidolaemus_wagleri	BMNH	1.2.9.0
Tropidolaemus_wagleri	BMNH	86.12.28.24
Vipera_ammodytes	MNRJ	21214
Vipera_ammodytes	BMNH	1931.12.15.3
Vipera_ammodytes	BMNH	1987.1848
Vipera_ammodytes	BMNH	1988.52
Vipera_ammodytes	BMNH	1920.1.2.2828
Vipera_ammodytes	MNHParis	7732
Vipera_ammodytes	MNHParis	1918.138
Vipera_ammodytes	MNHParis	1918.136
Vipera_ammodytes	MNHParis	1904-
Vipera_ammodytes	MNHParis	1904.595
Vipera_ammodytes	MNHParis	1904.593
Vipera_aspis	BMNH	1920.1.20.250
Vipera_aspis	BMNH	90.5.17.17-18
Vipera_aspis	BMNH	809
Vipera_aspis	MNHParis	1905.411
Vipera_aspis	MNHParis	1905.453
Vipera_aspis	MNHParis	1905.452
Vipera_aspis	MNHParis	8485
Vipera_aspis	MNHParis	1939.25
Vipera_aspis	MNHParis	1922.296
Vipera_aspis	MNHParis	9861
Vipera_aspis	MNHParis	9858
Vipera_aspis	MNHParis	1993.1776
Vipera_berus	MNRJ	20774

Vipera_berus	MNRJ	237
Vipera_berus	MNHParis	1904.581
Vipera_berus	MNHParis	1904.582
Vipera_berus	MNHParis	1904.586
Vipera_berus	MNHParis	1898.407
Vipera_berus	MNHParis	1900.178
Vipera_berus	MNHParis	3999A
Vipera_berus	MNHParis	1904.578
Vipera_berus	MNHParis	1884.579
Vipera_berus	CAS	14579
Vipera_berus	CAS	26101
Vipera_dinniki	FMNH	236002
Vipera_eriwanensis	MNHParis	1989.54
Vipera_eriwanensis	MNHParis	1989.52
Vipera_eriwanensis	MNHParis	1989.58
Vipera_eriwanensis	MNHParis	1989.57
Vipera_kaznakovi	MNHParis	1997.3302
Vipera_kaznakovi	FMNH	223694
Vipera_kaznakovi	FMNH	223695
Vipera_kaznakovi	AMNH	161837
Vipera_latastei	BMNH	1904.2.12.1-4
Vipera_latastei	BMNH	92.11.21.1
Vipera_latastei	BMNH	94.6.8.2 / 94.11.10.1
Vipera_latastei	MNHParis	5758
Vipera_latastei	FMNH	168015
Vipera_nikolskii	FMNH	257149
Vipera_renardi	BMNH	91.2.23.1
Vipera_renardi	BMNH	79.11.14.270
Vipera_renardi	BMNH	95.3.1.4-5
Vipera_renardi	BMNH	95.3.1.4-5
Vipera_renardi	BMNH	93.12.30.7
Vipera_renardi	MNHParis	1919.124
Vipera_renardi	FMNH	83965
Vipera_renardi	AMNH	84028
Vipera_renardi	AMNH	160760
Vipera_seoanei	BMNH	1914.5.26.35
Vipera_seoanei	BMNH	1920.1.20.2827
Vipera_seoanei	BMNH	95.4.30.35-37
Vipera_seoanei	MNHParis	1884.266
Vipera_seoanei	MNHParis	1889.584
Vipera_seoanei	MNHParis	1889.585
Vipera_seoanei	MNHParis	1971.278
Vipera_transcaucasiana	UTA	R18219
Vipera_ursinii	BMNH	92.4.4.4 / 92.9.13.12-13 / 93.2.10.1-3
Vipera_ursinii	MNHParis	1898.334
Vipera_ursinii	MNHParis	1966.1069
Vipera_ursinii	AMNH	161847
Vipera_ursinii	AMNH	90453

APPENDIX II

In the present study we used body size measurements mostly taken from male specimens. However, for 16 species out of 249 we only had access to female specimens, and we choose to keep their body size data in trait evolution analysis. We argue that including these female measurements did not biased our results for several reasons.

First, these 16 species are not restricted to a single habitat but are distributed across all habitat categories: two are terrestrial occurring in both forested and open habitats, two are terrestrial restricted to open habitats, seven are terrestrial restricted to forests and five are arboreal species. Additionally, we estimated sexual size dimorphism for body size in vipers we had information for at least two males and two females of each species using mean snout-vent length as a proxy for body size (calculation done following Hendry et al., 2014).

Although male-biased sexual dimorphism (MBSSD) occur among vipers, differences between the sexes are concentrated mostly below 10% meaning that for most species females are at best only 10% smaller than males (Figure S7). Additionally, higher degrees of MBSSD (> 0.1) mostly concentrate in the genus *Crotalus* (Figure S7, also suggested by Hendry et al. 2014 for total length) which represents only one species in the female sample used in trait evolution analyses. In contrast, female-biased sexual size dimorphism (FBSSD) is more widespread in vipers and suggested to predominantly occur among arboreal NW vipers (Hendry et al., 2014), but most values are concentrated below 0.2. Additionally, extreme values (> 0.2) seem to occur among species attaining female body sizes larger than 500 mm (Figure S8). Six of the 16 species we included in our analyses are small in size (smaller than 500 mm, see Table S1) and probably would not show extreme values of female-biased sexual size dimorphism. Although 10 species comprise female body sizes larger than 500 mm they are not restricted to a single habitat category: one is terrestrial occurring in both forested and open habitats, one is terrestrial and restricted to open habitats, three are terrestrials restricted to forests and five are arboreals.

Finally, using a two-sample Wilcoxon test we evaluated if body size significantly differs between the sexes in species phylogenetically related to the 16 for which we used female measurements based on the phylogenetic tree presented in Chapter I. From these 16 species, 10 are phylogenetic related to species not presenting significant body size differences between the sexes ($p = 1.00 - 0.15$). Although six are phylogenetically related to species presenting significant intersexual differences in body size ($p = 0.030 - 0.002$), all being female-biased, three are terrestrials restricted to forests and the other three are arboreals and thus are not restricted to a single habitat category.

Given the above considerations we suggest that is very unlikely that using female measurements for these 16 species would have biased our results toward a more constrained body size in arboreal species.

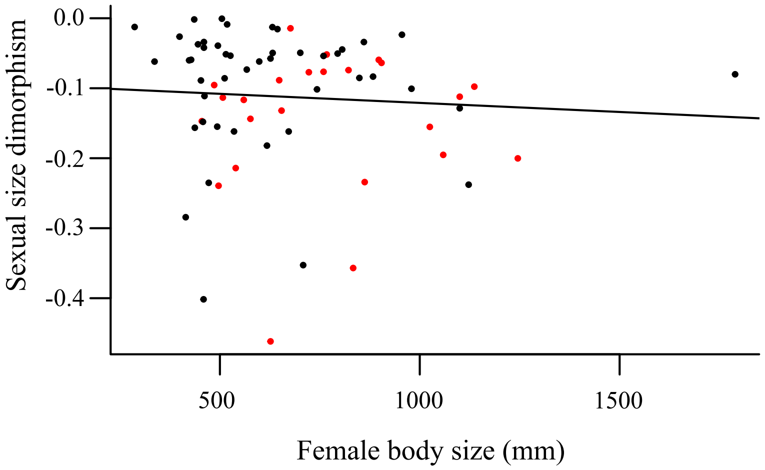

Figure S7. Male-biased sexual size dimorphism among vipers (MBSSD = -[(larger sex/ smaller sex) - 1]) relative to female body size (R^2 = 0.004, p = 0.56). Red circles indicate species belonging to the genus *Crotalus*. Variables were log10 transformed prior to the analysis. This plot was constructed using only species where males are larger than females.

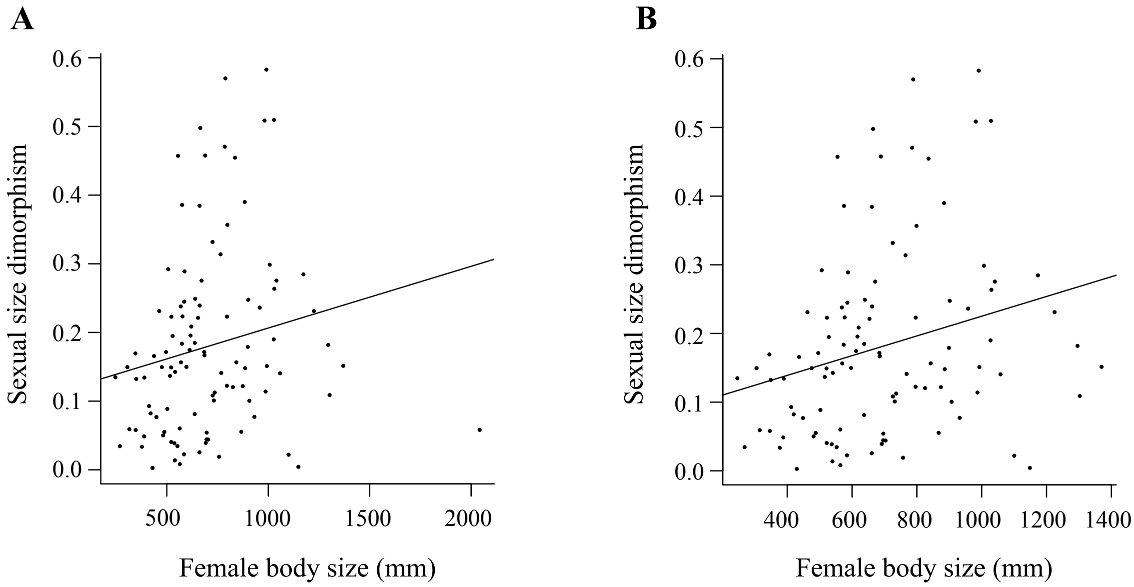

Figure S8. Female-biased sexual size dimorphism among vipers (FBSSD = (larger sex/smaller sex)-1) relative to female body size. A) Including the larger species in our sample *Lachesis stenophrys* (R^2 = 0.03, p = 0.04). B) Excluding *Lachesis stenophrys* (R^2 = 0.04, p = 0.01).

Variables were log10 transformed prior to the analysis. This plot was constructed using only species where female are larger than males.

References

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). Journal of Evolutionary Biology 27(4), 760-771.

Usando informações das espécies viventes, os capítulos que compõe a presente tese visaram explorar quais os padrões que caracterizaram a radiação dos viperídeos, buscando entender os possíveis processos responsáveis por moldar tais padrões ao longo dos seus ~50 milhões de anos:

- Utilizando sequências de 11 genes nucleares e mitocondriais para 264 espécies de viperídeos (79% de todas as espécies viventes), foi possível reconstruir relações filogenéticas robustas para diferentes clados que compõe o grupo. Os tempos de divergência obtidos indicam que o grupo deve ter começado a diversificar em meados do Paleoceno tardio/meio do Eoceno inferindo idades pouco mais tardias que o encontrado em estudos anteriores. Além disso, as idades estimadas permitiram estabelecer possíveis conexões entre a diversificação do grupo e determinadas mudanças geológicas e climáticas que ocorreram na Terra há milhões de anos atrás.

- A radiação dos viperídeos foi marcada por eventos importantes como, por exemplo, o surgimento das fossetas loreais na subfamília Crotalinae. Os resultados obtidos nesta tese indicam que um aumento nas taxas de especiação envolvendo parte do clado dos crotalíneos ocorreu provavelmente em decorrência não só da evolução das fossetas loreais mas também como resultado da expansão das florestas do sudeste asiático e da invasão do novo mundo. Após este rápido aumento nas taxas de especiação, porém, estas passam a desacelerar em direção ao presente. Com base em nossos resultados propomos um cenário para explicar como deve ter acontecido esta rápida multiplicação de linhagens e o porquê da sua desaceleração.

- A interação com diferentes habitats também pode afetar a dinâmica de diversificação de um grupo. Durante sua evolução, os viperídeos ocuparam diversos habitats incluindo o habitat arborícola. O ambiente arborícola parece impor pressões seletivas importantes nas linhagens de serpentes em geral e, os resultados aqui sugerem que nos viperídeos, a evolução morfológica foi de fato diferente nas linhagens arborícolas quando comparadas àquelas ocorrendo em habitats terrestres. Entretanto, a conquista do habitat arborícola que parece ter limitado a evolução morfológica dos viperídeos não parece ter afetado as suas taxas de especiação. Com esses resultados, relacionamos os padrões macroevolutivos encontrados com diferentes e plausíveis mecanismos de especiação que seriam importantes em gerar espécies nos diferentes habitats, concluindo que: (1) a especiação pode acontecer independente de mudanças morfológicas nos viperídeos, ou (2) o isolamento geográfico pode ter sido o mecanismo de especiação predominante entre os viperídeos arborícolas.

O presente trabalho contribuiu para desvendar como se deu a evolução de um dos grupos de serpentes mais notáveis do mundo, os viperídeos. Indo além, ressaltamos a importância de entendermos mais sobre árvores filogenéticas para podermos integrar o uso destas em trabalhos de ecologia evolutiva. Nossa abordagem nos permitiu recuperar padrões e inferir processos que moldaram a radiação dos viperídeos e que poderiam possivelmente ter moldado a diversificação de outros grupos de organismos. Além disso, nossos resultados sugerem cenários e hipóteses a serem futuramente explorados e que deverão contribuir ainda mais para melhor entendermos os padrões e mecanismos que geram e mantém a biodiversidade.