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Phylogenetic Relationships and Evolution of Snakes

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Phylogenetic Relationships and Evolution of Snakes

A Dissertation

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Conservation Biology

by

Alex Figueroa

B.S. Rutgers University, 2001
M.S. Loma Linda University, 2006

August, 2016

Dedicated to my family and friends

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Abstract

Snakes represent an impressive evolutionary radiation of over 3,500 widely-distributed species, categorized into 515 genera, encompassing a diverse range of morphologies and ecologies. This diversity is likely attributable to their distinctive morphology, which has allowed them to populate a wide range of habitat types within most major ecosystems. In my first chapter, I provide the largest-yet estimate of the snake tree of life using maximum likelihood on a supermatrix of 1745 taxa (1652 snake species + 7 outgroup taxa) and 9,523 base pairs from 10 loci (5 nuclear, 5 mitochondrial), including previously unsequenced genera (2) and species (61). I then use this phylogeny to test hypotheses regarding heterogeneity in diversification rates and how this shaped overall patterns of snake diversity in Chapter 2. I also used the species-level phylogeny to test the evolution of habitat use in snakes, morphological variation, and whether distantly-related species exhibit morphological convergence in Chapter 3. Finally, in Chapter 4 I investigate how prehensile tails effect striking performance in arboreal snakes.

Convergence; Diversification; Ecomorphology; Evolution; Habitat use; Performance; Phylogeny; Snakes; Species-level; Striking

Chapter 1. A Species-level Phylogeny of Extant Snakes with Description of a New Colubrid Subfamily and Genus

Abstract

With over 3,500 species encompassing a diverse range of morphologies and ecologies, snakes make up 36% of squamate diversity. Despite several attempts at estimating higher-level snake relationships and numerous assessments of generic- or species-level phylogenies, a large-scale species-level phylogeny solely focusing on snakes has not been completed. Here, we provide the largest-yet estimate of the snake tree of life using maximum likelihood on a supermatrix of 1745 taxa (1652 snake species + 7 outgroup taxa) and 9,523 base pairs from 10 loci (5 nuclear, 5 mitochondrial), including previously unsequenced genera (2) and species (61). Increased taxon sampling resulted in a phylogeny with a new higher-level topology and corroborate many lower-level relationships, strengthened by high nodal support values (> 85%) down to the species level (73.69% of nodes). Although the majority of families and subfamilies were strongly supported as monophyletic with > 88% support values, some families and numerous genera were paraphyletic, primarily due to limited taxon and loci sampling leading to a sparse supermatrix and minimal sequence overlap between some closely-related taxa. With all rogue taxa and *incertae sedis* species eliminated, higher-level relationships and support values remained relatively unchanged, except in five problematic clades. Our analyses resulted in new topologies at higher- and lower-levels; resolved several previous topological issues; established

novel paraphyletic affiliations; designated a new subfamily, Ahaetuliinae, for the genera *Ahaetulla*, *Chrysopelea*, *Dendrelaphis*, and *Dryophiops*; and appointed *Hemerophis* (*Coluber*) *zebrinus* to a new genus, *Mopanveldophis*. Although we provide insight into some distinguished problematic nodes, at the deeper phylogenetic scale, resolution of these nodes may require sampling of more slowly-evolving nuclear genes.

Introduction

Phylogenies form the cornerstone of our understanding of evolutionary relationships between organisms and provide a historical basis for testing and inferring ecological and evolutionary processes (Harvey and Pagel, 1991; Huelsenbeck and Rannala, 1997; Pagel, 1999; Whelan et al., 2001). Although phylogenetic methodologies have witnessed an explosion of advancements, estimating large trees remains costly, time-intensive, and computationally difficult. Thus, most analyses have concentrated on resolving the relationships of smaller taxonomic groups, culminating in the accumulation of published sequences available for compiling into larger datasets, or "super-matrices" (Driskell et al., 2004; McMahon and Sanderson, 2006). Coalescent-based species-trees methods are currently favored over concatenated approaches owing to their greater accuracy, but their use for large datasets is still impractical (Edwards, 2009; Lambert et al., 2015). Consequently, many researchers rely on the supermatrix approach (de Queiroz and Gatesy, 2007) or on shortcut coalescence methods (Gatesy and Springer, 2014). The supermatrix uses concatenated sequences to estimate large-scale phylogenies with branch lengths (Burleigh et al., 2015; McCormack et al., 2013; Pyron and Wiens, 2011; Piwczyński et al., 2014; Pyron et al., 2013a; Rabosky et al., 2013; Soltis et al., 2013). This technique has earned criticism because large amounts of missing data may obscure phylogenetic signal, leading to uncertainty in topology and branch lengths (Lemmon, 2009; Lemmon and Lemmon, 2013; Sanderson et al., 2010; Thomson and Shaffer, 2009), but shortcut coalescence methods are also prone to these same shortcomings (Gatesy and Springer, 2014). However, several studies have shown that concatenated procedures may nonetheless produce similar results to species-trees (Pyron et al., 2014b; Lambert et al., 2015), particularly when there

is no agreement among gene trees, and between gene and species trees (Edwards, 2009). This is also the case for deep divergences because shortcut coalescence has difficulty integrating gene-tree incongruity at this level (Gatesy and Springer, 2014). Our goal for this study was to estimate a species-level phylogeny for snakes using the supermatrix technique.

To date, only two studies have estimated a species-level phylogeny of snakes (Pyron et al., 2013a; Zheng and Wiens, 2016), with the latter adding more independent loci to the dataset of the former. These studies featured 1262 known snake species, integrated as part of a larger phylogeny focusing on Squamata, accounting for merely 39% of the total snake diversity at the time. At greater than 3,500 species (Uetz and Hošek, 2015), over a thousand more than the estimate provided by Heise et al. (1995) two decades earlier, and with the recent recognition of new families and subfamilies (Adalsteinsson et al., 2009; Chen et al., 2013; Kelly et al., 2009; Pyron and Wallach, 2014; Pyron et al., 2014a; Vidal et al., 2010a), phylogenetic estimates of the snake tree of life are markedly underrepresented. Indeed, the first phylogenetic analysis including all families and subfamilies was only recently completed (Pyron and Burbrink, 2012), and only included one representative from each rank. Over the years, researchers have emphasized resolving higher-level snake relationships (Cadle, 1988; Chen et al., 2013; Dowling et al., 1996; Gower et al., 2005; Heise et al., 1995; Hsiang et al., 2015; Kelly et al., 2003; Lawson et al., 2004; Lawson et al., 2005; Pyron et al., 2011; Pyron and Burbrink, 2012; Pyron et al., 2013a; Pyron et al., 2013b; Pyron et al., 2014b; Reeder et al., 2015; Slowinski and Lawson, 2002; Vidal and Hedges, 2002a; Vidal et al., 2007; Vidal et al., 2009; Wiens et al., 2008; Wiens et al., 2012; Zaher et al., 2009; Zheng and Wiens, 2016), and topology within families: typhlopids (Adalsteinsson et al., 2009; Hedges et al., 2014; Pyron and Wallach, 2014; Vidal et al., 2010b); boids (Noonan and Chippindale, 2006; Pyron et al., 2014a; Rawlings et al., 2008;

Reynolds et al., 2014); acrochordids (Sanders et al., 2011); xenodermatids (Teynie et al., 2015); homalopsids (Alfaro et al., 2008; Murphy et al., 2011); pareatids (You et al., 2015); viperids (Castoe and Parkinson, 2006; Lenk et al., 2001b; Malhotra et al., 2010); elapids and lamprophiids (Kelly et al., 2008; Kelly et al., 2009; Sanders et al, 2013; Vidal et al., 2008); dipsads (Grazziotin et al., 2012; Vidal et al., 2010a); pseudoxendontids (Zhang and Huang, 2013); natricines (McVay et al., 2015); sibynophiids (Chen et al., 2013); and colubrids (Lawson et al., 2005; Pyron et al., 2011). Despite these efforts, many unresolved nodes remain scattered throughout the entire snake tree, such as the monophyly of Scolecophidia (Pyron et al., 2013a), topology of Typhlopinae (Pyron and Wallach, 2014), monophyly of Cylindrophiidae and Anomochilidae (Gower et al., 2005), topology of Booidea (Pyron et al., 2014a; Reynolds et al., 2014), placement of Xenophidiidae and Bolyeridae (Reynolds et al., 2014), and several issues within Caenophidia (Lawson et al., 2005; Pyron et al., 2011; Pyron et al., 2014b). With higher-level relationships of snakes still not settled, our understanding of the snake tree of life remains incomplete.

Although snakes have received a great deal of attention from biologists (Mullin and Seigel, 2009; Seigel and Collins, 1993; Seigel et al., 1987), studies of snake biology from comparative and evolutionary perspectives are scarce relative to other reptile taxa such as lizards, in part because of the lack of comprehensive and well-supported snake phylogenies. Estimating a clade-wide species-level phylogeny for snakes with utility for testing evolutionary hypotheses will greatly augment our knowledge of snake biology. Here, we present an updated hypothesis on extant snake phylogeny with increased sampling using the supermatrix approach comprising 1745 taxa (1652 snake species + 7 outgroup taxa), representing 46.33% of the currently known snake species from all known families and subfamilies (Table 1.1), an increase of 7.24% from

Pyron et al. (2013a) and Zheng and Wiens (2016. Accepting this tree, we discuss higher-level relationships and highlight taxonomic issues at the genus-level.

Table 1.1. Number of taxa sampled per family or subfamily. Families are listed in order according to Figure 1.1. For the taxonomy of families and subfamilies, we use Adalsteinsson et al. (2009) for Anomalepididae and Leptotyphlopidae, Pyron and Wallach (2014) for Gerrhopilidae, Typhlopidae, and Xenotyphlopidae, Pyron et al. (2014a) for Booidea, and Pyron et al. (2013a) for Alethinophidia. The number of species per clade was taken from The Reptile Database (http://www.reptile-database.org/) on 10/01/2015. Percentages of the number of species sampled do not include taxa not assigned to species status. Paraphyletic taxa are included under their traditional family and/or subfamily. In the Total cell for total number of species, the number not in parentheses equals the sum of the values in the table and the number in the parentheses equals the number returned when a search for Serpentes is conducted in The Reptile Database. Percentage for total number of species sampled is based on 3566 species.

Clade	Number of Species Sampled (% Sampled)	Total Number of Species
Scolecophidia		
Anomalepididae	2 (11%)	18
Leptotyphlopidae	--	--
Epictinae	17 (23%) – 2 sp.	64
Leptotyphlopinae	18 (36%)	50
Gerrhopilidae	2 (11%)	18
Xenotyphlopidae	2 (100%) – 1 sp.	1
Typhlopidae		
Typhlopinae	52 (52%) – 19 sp.	64
Afrotyphlopinae	19 (26%) – 3 sp.	61
Madatyphlopinae	2 (15%)	13
Asiatyphlopinae*	49 (33%) – 8 sp.	124
Alethinophidia		
Aniliidae	1 (100%)	1
Tropidophiidae	10 (29%)	34
Calabariidae	1 (100%)	1
Candoiidae	3 (60%)	5
Sanziniidae	3 (75%)	4
Charinidae		
Charininae	3 (75%)	4
Ungaliophiinae	3 (100%)	3
Erycidae	9 (75%)	12
Boidae	24 (80%)	30
Cylindrophiidae	2 (15%)	13
Anomochilidae	1 (33%)	3

Table 1.1 Continued.

Uropeltidae	15 (28%) – 1 sp.	54
Xenopeltidae	1 (50%)	2
Loxocemidae	1 (100%)	1
Pythonidae	32 (80%)	40
Bolyeridae	1 (50%)	2
Xenophidiidae	1 (50%)	2
Acrochordidae	3 (100%)	3
Xenodermatidae	4 (22%)	18
Pareatidae	16 (80%)	20
Viperidae		
Viperinae	66 (67%)	98
Azemiopinae	1 (50%)	2
Crotalinae	190 (82%) – 1 sp.	231
Homalopsidae	26 (47%) – 1 sp.	53
Lamprophiidae		
Psammophiinae	45 (87%) – 3 sp.	52
Prosymninae	5 (31%)	16
Pseudaspidinae	2 (100%)	2
Atractaspidinae	7 (30%)	23
Aparallactinae	11 (23%)	47
Lamprophiinae	31 (43%)	72
Pseudoxyrhophiinae	61 (64%) – 4 sp.	89
Elapidae	195 (54%) – 1 sp.	358
Colubridae		
Sibynophiinae	6 (55%)	11
Natricinae	110 (47%) – 3 sp.	226
Pseudoxenodontinae	5 (36%) – 1 sp.	11
Dipsadinae	242 (32%) – 2 sp.	754
Grayiinae	3 (75%)	4
Calamariinae	4 (5%)	87
Ahaetullinae **subfam**. **nov**.	27 (48%)	56
Colubrinae	315 (47%) – 3 sp.	670
Incertae Sedis	4[†]	22
TOTAL	1652 (46.33%)	3549 (3566)

*Number of species of *Xerotyphlops* is included in Asiatyphlopinae.

[†]*Buhoma depressiceps*, *Buhoma procterae*, and *Oxyrhabdium leporinum* are all listed as *incertae sedis* on The Reptile Database, but *Micrelaps bicoloratus* is not. We list these four species as *incertae sedis* because of their variable topological history (see Fig. 1.1).

Materials and Methods

Tissue data collection and sequence acquisition

We constructed a dataset of 1745 taxa (1659 species), of which the following seven species represent outgroups: *Calotes versicolor*, *Chamaeleo calyptratus*, *Elgaria multicarinata*, *Heloderma suspectum*, *Liolaemus darwinii*, *Plica plica*, and *Varanus salvator*. The dataset consisted of 9,523 bp from the following 10 genes: three mitochondrial protein-coding genes, cytochrome b (cyt-b; 1,107 bp; 1,398 taxa), NADH subunit 2 (ND2; 1,042 bp; 334 taxa), and NADH subunit 4 (ND4; 802 bp; 986 taxa); two non-coding ribosomal genes (12S; 790 bp; 1,023 taxa) and (16S; 649 bp; 1,167 taxa); and five nuclear protein-coding genes, brain-derived neurotrophic factor precursor (BDNF; 675 bp; 314 taxa), neurotrophin-3 (NT3; 669 bp; 449 taxa), oocyte maturation factor Mos (c-mos; 753 bp; 957 taxa), and two recombination-activating genes (RAG-1.1; 926 bp; 209 taxa, RAG-1.2; 880 bp; 166 taxa; RAG-1.3; 517 bp; 153 taxa), and (RAG-2; 716 bp; 153 taxa). We split RAG-1 into three separate alignments because the majority of sequences did not overlap, but instead formed three separate segments of overlapping sequences. Sequences for seven outgroups and 1591 snake species were downloaded from GenBank (S1.1 Table). To maximize gene coverage for each species, we combined sequences from multiple individuals of the same species. We sequenced an additional 150 tissue samples from 88 species, of which 61 were not previously sequenced (S1.2 Table). Eighteen we field collected and 132 we obtained from museum vouchers. For field collected samples, we obtained tissue from tail clips or ventral scale clips using sterilized scissors, from snakes collected in Costa Rica and Singapore. We placed all tissue samples in 90% ethanol under the Alexander D.

McKelvy Field Series (ADM). Methods for tissue collection were approved by the University of New Orleans Animal Welfare Committee and by both permitting agencies for each country: Costa Rica, Ministerio del Ambiente y Energía Sistema Nacional de Areas de Conservación, permit ACTo-GASP-PIN-023-2010, and; Singapore, NParks, permit NP/RP11-030. Museum tissue samples represent a combination of liver, muscle, and heart tissue and were gathered from the following museums: AMNH, CAS, FMNH, KU, LSUHC, LSUMNS, MVZ, and YPM (refer to S1.2 Table for museum codes). Species we sequenced are identified by species name and voucher number (S1.2 Table). For taxonomic classification, we consulted The Reptile Database (http://www.reptile-database.org/). As of October 2015, the database recognizes 3566 species of snakes. Our dataset accounted for approximately 46.33% of currently recognized snake species.

DNA extraction, amplification, sequencing, and alignment

We extracted genomic DNA from tissue samples following the standard protocol provided for Qiagen® DNeasy kits. We sequenced six genes: 16S, c-mos, cyt-b, ND4, NT3, and RAG-1. A list of the primers used, their source, and annealing temperatures are provided in S1.3 Table. We aliquoted a 2 µl portion of each purified DNA extract and combined it with GoTaq Green MasterMix (Promega Corp.), primers from respective gene, and deionized water to create a 10 µl reaction to be used in the Polymerase Chain Reaction (PCR). We placed all PCR reactions on a thermal cycler under the following protocol: 95 °C for 2 min; 95 °C for 30 s; 50 °C for 30 s for 40 cycles; 72 °C for 1:15 min; 72 °C for 3-5 min; and chilled at 4 °C until taken off cycler. Next, we cleaned the PCR products using 1 µL of ExoSap-IT (USB Corp.) per 10 µL of PCR product. We performed cycle sequencing on purified PCR products using 1 µL primer

(10 µM), 2 µL template, and 5 µL deionized water along with a Big Dye Terminator 3.1 (Amersham Pharmacia Biotech) reaction premix for 50 cycles of 96 °C for 10 s; 45 °C for 5 s; and 60 °C for 4 min and purified using a Sephdex column, then used an ABI 3130XL Genetic Analyzer to determine nucleotide sequences of each sample.

We aligned all sequences using the default parameters of the Geneious alignment, and refined alignments using the default parameters of the MUSCLE alignment (Edgar, 2004) in the program Geneious v4.8.4 (http://www.genious.com; Kearse et al., 2012). We then edited alignments by eye and trimmed ambiguous end regions. For some genes, a few species had identical sequences with other taxa so we retained the first taxon in alphabetical order (Pyron et al., 2013a; S1.1 Table). Finally, we used Geneious to concatenate all genes to create a supermatrix. This matrix contained 71.41% of missing data; however, previous studies have shown that missing data does not negatively influence topology, branch length estimates, and node support (Pyron et al., 2011; Pyron et al., 2013a; Pyron et al., 2013b; Zheng and Wiens, 2016). We deposited all sequences generated from this study in GenBank (S1.2 Table). The final alignment is available at the DataDryad repository (http://datadryad.org/).

Phylogenetic inference

We performed phylogenetic analyses on the 10-gene concatenated matrix using the maximum likelihood (ML) criterion in the program RAxML HpC-2 v8 (Stamatakis, 2014) on the CIPRES portal (http://www.phylo.org; Miller et al., 2010). First, we analyzed each gene separately to check topological congruence by performing rapid bootstrap analyses and pruned misplaced taxa with suspect placement out of the alignment, before concatenating them into the

final alignment. The following five species were removed from the alignment due to poor placement for all genes: *Boiga siamensis* FMNH267726, *Chrysopelea ornata* LSUHC7158, *Dipsadoboa werneri*, *Emydocephalus ijmae*, and *Psammodynastes pictus* FMNH267940. We conducted analyses by generating starting trees under the default parsimony model and obtained node support from 100 non-parametric bootstrap replicates using the GTRGAMMA model for all genes and codon partitions since the GTRGAMMA model is recommended over GTR + Γ + I as the 25 rate categories implemented with GTRGAMMA accounts for potentially invariant sites (Stamatakis, 2006). After concatenating the genes, we performed a rapid bootstrap analysis on the data partitioned by gene and codon position and obtained node support from 1000 non-parametric bootstrap replicates using the GTRGAMMA model.

Rogue taxa can present themselves in phylogenetic estimates due to ambiguous or insufficient phylogenetic signal (Sanderson and Shaffer, 2002). These taxa decrease resolution and support in any best tree estimate because they cannot be placed with any confidence anywhere in the tree due to occupying numerous different phylogenetic positions in a set of trees (Wilkinson, 1996). Thus to produce a more informative best tree estimate with improved clade support, we identified and eliminated rogue taxa with the webserver version of RogueNaRok at http://rnr.h-its.org/submit (Aberer et al., 2013) using the support on best tree estimate threshold, optimizing support, and maximum dropset size of 1. To avoid pruning a large number of taxa, we only pruned 22 taxa that had a random improvement score (i.e., fraction of improvement in bootstrap support values throughout the tree when the selected taxon is pruned and all rogue taxa above it are also pruned) above 0.8 (S1.4 Table). We acknowledge that excluding additional rogue taxa will improve clade support values, but we wanted to include a maximum number of taxa to estimate a more comprehensive phylogeny. After pruning rogue taxa, the final dataset

resulted in 1745 taxa (1659 species). We then performed 10 ML searches on 10 random stepwise addition parsimony-based starting trees using the GTRGAMMA model. Next, we executed a final topology optimization on the best scoring ML tree to produce a nearest-neighbor interchange (NNI)-optimized estimate of the ML tree also using the GTRGAMMA model. Finally, we assessed node support using the non-parametric Shimodaira-Hasegawa-Like (SHL) implementation of the approximate likelihood-ratio test (aLRT; Anisimova and Gascuel, 2006) based on several advantages over other support methods and considered SHL values of 85% or greater as strong support (Pyron et al., 2013a). We also estimated the tree with all rogue taxa from the first analysis and species classified as *incertae sedis*, all within the family Lamprophiidae (*Buhoma depressiceps*, *Buhoma procterae*, *Micrelaps bicoloratus*, and *Oxyrhabdium leporinum*), eliminated to scrutinize their influence on higher-level relationships.

Nomenclatural Acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub: 3966804E-D532-4C52-92AC-BECAE776E434. The electronic edition of this work was published in a

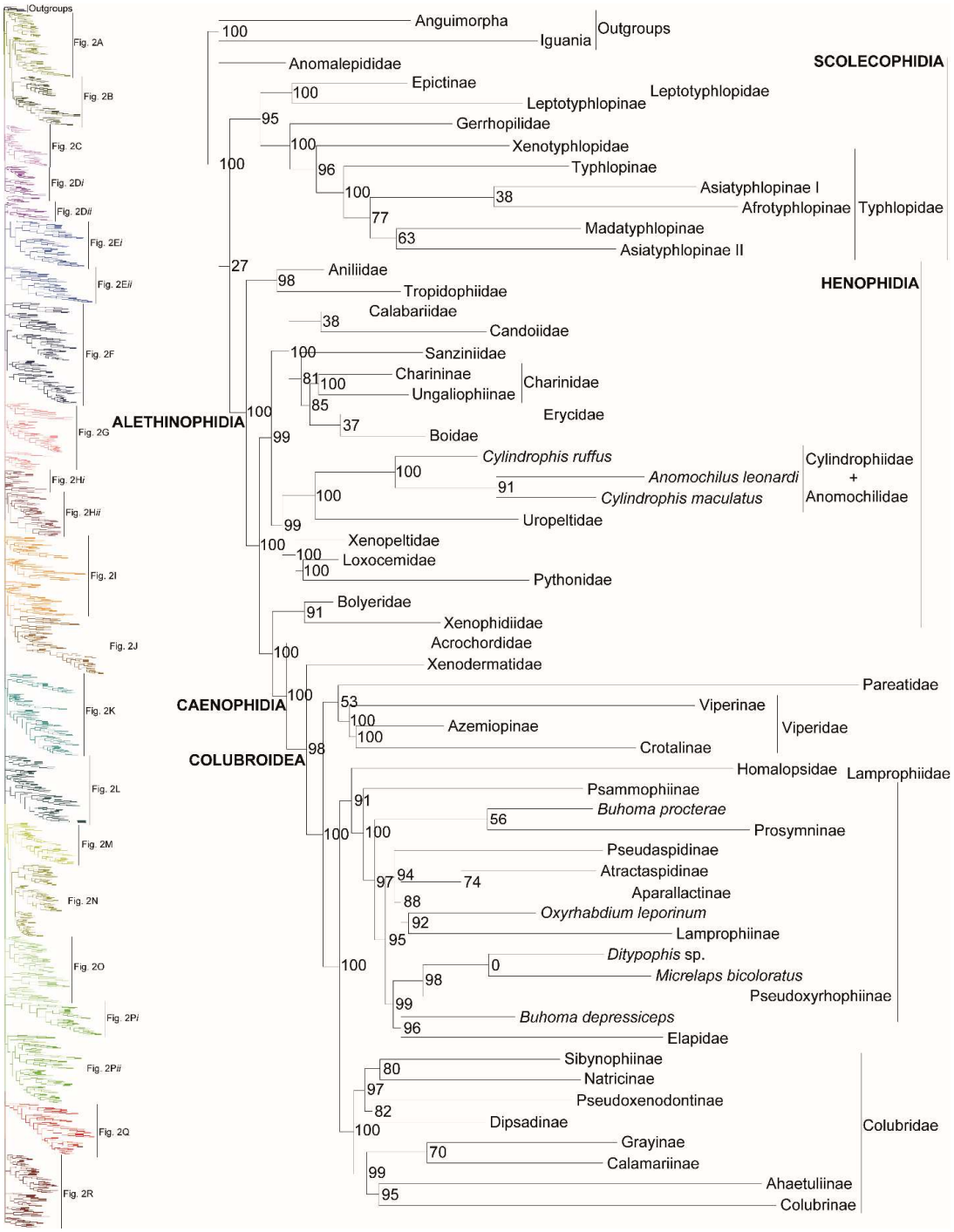
journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS.

Results and Discussion

Higher-level phylogeny

As in previous studies, we find very strong support (SHL = 100) for the clade Serpentes (Hsiang et al., 2015; Pyron et al., 2013a; Reeder et al., 2015; Townsend et al., 2004; Wiens et al., 2012; Zheng and Wiens, 2016). In Fig. 1.1 we display a summary of the full ML tree (lnL = -919390.188) to exhibit relationships above the genus-level and present the full species-level tree in Fig. 1.2, made available in Newick format in S1 File and on the DataDryad repository (http://datadryad.org/). Overall, more than half of the nodes in the full species-tree received strong support (73.45% of nodes with SHL values > 85). In the following section we largely compare our tree to Pyron et al., (2013a), since they provide a recent detailed comparison to preceding publications and because theirs is the only other clade-wide species-level tree (but see Zheng and Wiens, 2016). In general, we substantiate many of the higher-level relationships reported in Pyron et al., (2013a); however, several differences also exist. Support for monophyly for each family and subfamily was above 88%, except for Gerrhopilidae (SHL = 48), and Cylindrophiidae was paraphyletic with Anomochilidae (Gower et al., 2005; Reynolds et al., 2014; Zheng and Wiens, 2016).

Figure 1.1. Abridged phylogeny on final dataset of 1652 snake species and seven outgroup taxa displaying higher-level relationships. Maximum-likelihood phylogenetic estimate based on 10 concatenated genes. Tips represent families and sub-families. Commonly recognized higher-level clades are labeled in all caps and bold. Species classified as Lamprophiidae *incertae sedis* are also shown since they did not place within a subfamily. Node values represent SHL support values. Skeleton of the species tree is displayed on the left, colored and labeled as they appear in Figure 1.2.



Scolecophidia

Similar to many prior examinations, we find relationships within Scolecophidia unresolved (Burbrink and Crother, 2011; Heise et al., 1995; Pyron and Burbrink, 2012; Pyron et al., 2013a; Pyron et al., 2013b; Reeder et al., 2015; Rieppel, 1988; Scanlon and Lee, 2011; Underwood, 1967; Vidal et al., 2009; Vidal et al., 2010b; Wiens et al., 2008; Wiens et al., 2012; Zheng and Wiens, 2016), with studies showing either Scolecophidia 85,86] (Heise et al., 1995; Vidal et al., 2010b), Anomalepididae (Pyron et al., 2013a; Pyron et al., 2013b) or Leptotyphlopidae + Typhlopoidea (Reeder et al., 2015; Vidal et al., 2009; Wiens et al., 2008; Wiens et al., 2012; Zheng and Wiens, 2016) as sister to all snakes. Morphology also reveals uncertainty surrounding Scolecophidia (reviewed in Burbrink and Crother, 2011), but based on the presence of vestigial supratemporal and ectopterygoid bones, absent in other scolecophidians, Anomalepididae may be the most basal scolecophidian (Scanlon and Lee, 2011). We believe future work will lead to a reclassification of Scolecophidia, but until then relationships within the infraorder remain problematic. In addition, we find weak support for the placement of Asiatyphlopinae, Afrotyphlopinae, and Madatyphlopinae within Typhlopidae as in previous studies (Hedges et al., 2014; Kornilios et al., 2013; Pyron and Wallach, 2014; Pyron et al., 2013a; Vidal et al., 2010b; Zheng and Wiens, 2016). The issue appears to lie primarily with the placement of *Argyrophis* (Hedges et al., 2014) and *Xerotyphlops* (Hedges et al., 2014; Pyron et al., 2013a; Zheng and Wiens, 2016), which together formed Asiatyphlopinae I. *Xerotyphlops* is represented by two species, one occurring in the eastern Mediterranean and the other on Socotra Island (Kornilios et al., 2013), and *Argyrophis* is distributed from western Asia to Southeast Asia (Kornilios et al., 2013; Pyron and Wallach, 2014). Discordance in topology therefore appears

associated with these two genera being intermediate in distribution between African and Asian typhlopids, which may show affinities to clades from both regions.

Henophidia

As mentioned above, Cylindrophiidae is paraphyletic with Anomochilidae. Difficulty in resolving this relationship is likely due to the representation of *Anomochilus* by one species and two genes (12S and 16S), and *Cylindrophis* by two species with greater gene coverage. Both of these families were formerly shown as part of or paraphyletic with Uropeltidae (Pyron et al., 2013b; Reeder et al., 2015; Wiens et al., 2008; Wiens et al., 2012. Based on the history of paraphyly between these families, Burbrink and Crother (2011) recommended synonymizing Cylindrophiidae and Anomochilidae with Uropeltidae to resolve these families. However, we recommend retaining the current classification until more species are sampled (Table 1.1) on the grounds that Cylindrophiidae + Anomochilidae share morphological features not present in Uropeltidae (Burbrink and Crother, 2011; Gower et al., 2005) and since strong support has been shown distinguishing them from Uropeltidae (Pyron and Burbrink, 2012; Pyron et al., 2013a; Pyron et al., 2013b; Zheng and Wiens, 2016; this study). For boids, our analysis validates the taxonomic changes made in Pyron et al., (2014a), but differs in topology from previous assessments in the placement of Calabariidae, Candoiidae, and Sanziniidae (Pyron et al., 2013a; Reynolds et al., 2014; Zheng and Wiens, 2016). Although the relationship Erycidae + Boidae is recovered in all studies (Pyron et al., 2013a; Zheng and Wiens, 2016; this study), except one (Reynolds et al., 2014), support for this relationship is low. Thus, the only node we can have

confidence in is the one joining Charininae and Ungaliophiinae (Pyron et al., 2013a; Reynolds et al., 2014; Zheng and Wiens, 2016; this study).

Xenophidiidae and Bolyeridae

Perhaps the most notable difference from the topology of Pyron et al. (2013a) was the placement we recovered for Xenophidiidae + Bolyeridae (SHL = 91). Earlier studies showed them as sister to various clades within Henophidia (Lawson et al., 2004; Pyron and Burbrink, 2012; Pyron et al., 2013b; Reeder et al., 2015; Zheng and Wiens, 2016), but we found very strong support (SHL = 100) for them as sister to Caenophidia (SHL = 100), as also shown in other studies (Reynolds et al., 2014; Scanlon and Lee, 2011). In addition, these snakes possess morphological characters, particularly within the palate, bolstering their close relationship with Caenophidia and not to Henophidia (Scanlon and Lee, 2011). Pyron et al. (2013a) is the only study showing a disassociation between these families placing Xenophidiidae as sister to Alethinophidia, with the exception for Aniliidae + Tropidophiidae, and Bolyeridae as sister to Booidea. Currently, both clades are represented by one species and Xenophidiidae by only one gene (cyt-b). Both clades contain two species; for Xenophidion, both species are known only from one specimen each, and for Bolyeridae, *Bolyeria* is extinct, and *Casarea* is rare (Lawson et al., 2004), so obtaining additional sequences for either clade is unlikely. If this placement is retained, then Caenophidia should be redefined to include Xenophidiidae and Bolyeridae, or they should be given their own taxonomic grouping.

Caenophidia

Pyron et al. (2014b) recently reviewed and attempted to resolve several problematic issues within Caenophidia. The major problems hindering resolution of this clade are 1) placement of Xenodermatidae inside or outside of Colubroidea; 2) placement of Homalopsidae; 3) topology of Lamprophiidae; and 4) topology of Colubridae. Previous studies have placed Xenodermatidae as sister to Acrochordidae (Kelly et al., 2003; Pyron et al., 2013a) or as basal in Colubroidea (Chen et al., 2013; Pyron et al., 2011; Reeder et al., 2015; Vidal and Hedges, 2002b; Wiens et al., 2008; Zheng and Wiens, 2016), have placed Homalopsidae as sister to Lamprophiidae + Elapidae (Chen et al., 2013; Pyron et al., 2011; Pyron et al., 2013a) or as sister to (Lamprophiidae + Elapidae) + Colubridae (Lawson et al., 2005; Pyron and Burbrink, 2012; Reeder et al., 2015; Vidal et al. 2007; Wiens et al., 2008; Zheng and Wiens, 2016), and have shown conflicting topologies for the subfamilies within Lamprophiidae and Colubridae (Chen et al. 2013; Kelly et al., 2003; Kelly et al., 2009; Pyron et al., 2011; Pyron et al., 2013a; Vidal et al. 2007; Wiens et al., 2008; Zheng and Wiens, 2016). Pyron et al. (2014b) used seven methods to examine these relationships showing Xenodermatidae as basal in Colubroidea with varying support and Homalopsidae as sister to (Lamprophiidae + Elapidae) + Colubridae with strong support. However, they expressed little confidence in resolving the topology within Lamprophiidae and Colubridae since several divergences were defined by low support. We confirm their findings that Xenodermatidae is sister to the rest of Colubroidea (SHL = 100) and that relationships within Lamprophiidae and Colubridae remain unresolved, but our findings for the placement of Homalopsidae contradicted theirs, as we recovered strong support (SHL = 91) for Homalopsidae + Lamprophiidae, and found Elapidae to be nested within Lamprophiidae.

Typically, Lamprophiidae and Elapidae are recovered as distinct clades (Kelly et al., 2009; Lawson et al., 2005; Pyron et al., 2011; Pyron et al., 2013a; Pyron et al., 2013b; Pyron et al., 2014b; Vidal et al., 2008), but we found strong support (SHL = 96) for Elapidae + *Buhoma depressiceps* as sister to Pseudoxyrhophiinae (SHL = 99), shown previously only in Pyron and Burbrink (2012). The topology of Lamprophiidae is complicated by the presence of several *incertae sedis* taxa (see Lamprophiidae; Kelly et al., 2009; Lawson et al., 2005; Pyron and Burbrink, 2012; Pyron et al., 2013b), but Elapidae remains nested within Lamprophiidae even when these taxa are removed (S1.1 Fig.). In addition, we found the placement of Pareatidae and Viperidae within Colubroidea unresolved. Pareatidae is consistently placed as sister to Viperidae, which is sister to Colubridae, Elapidae, Homalopsidae, and Lamprophiidae (Chen et al. 2013; Pyron and Burbrink, 2012; Pyron et al., 2011; Pyron et al., 2013a; Pyron et al., 2013b; Reeder et al., 2015; Zheng and Wiens, 2016). A possible explanation for this is that our dataset includes the greatest sampling of pareatids, adding seven additional species previously not included in higher-level relationships, two we sequenced and five from You et al. (2015).

Lamprophiidae

Part of the issue with resolving the topologies within Lamprophiidae, and within Colubridae, is that they exemplify rapid radiations manifested by the presence of short internodes (Pyron et al., 2011). Yet another major issue hindering progress within Lamprophiidae is the presence of several *incertae sedis* taxa, not identified as rogue taxa by RogueNaRok. These taxa constantly show contrasting phylogenetic placement between studies (Kelly et al., 2009; Lawson et al., 2005; Pyron et al., 2011; Pyron et al., 2013a; Vidal and Hedges, 2002a; Vidal et al., 2008;

Zheng and Wiens, 2016). We are reluctant in placing any confidence in the topology between subfamilies recovered for Lamprophiidae, despite high support values. However, the topology after all rogues and *incertae sedis* taxa were pruned remained essentially the same (S1.1 Fig.) adding supplementary support for this topology. Nonetheless, our topology differs from earlier studies. Previous studies have consistently recovered the sister relationship between Aparallactinae + Atractaspidinae (Kelly et al., 2009; Lawson et al., 2005; Pyron and Burbrink, 2012; Pyron et al., 2011; Pyron et al., 2013a; Pyron et al., 2013b; Pyron et al., 2014b; Vidal et al., 2008); however, we found this relationship unresolved, likely due to the strong placement (SHL = 95) of *Atractaspis irregularis* as sister to these two clades, and this taxon is represented by only one gene. The topology recovered here was Psammophiinae + ((*B. procterae* + Prosymninae) + (Pseudaspidinae + (Atractaspidinae + Aparallactinae) + (*O. leporinum* + Lamprophiinae)) + (((*Ditypophis* sp. + *M. bicoloratus*) + Pseudoxyrhophiinae) + (*B. depressiceps* + Elapidae)))). All nodes received strong support (SHL > 88), except for subclades *B. procterae* + Prosymninae and *Ditypophis* sp. + *M. bicoloratus*. Pyron et al. (2013a) had augmented the definition of Pseudaspidinae to include *Buhoma* and *Psammodynastes*. With added sampling of *Psammodynastes*, we recovered this genus as paraphyletic with *Rhamphiophis oxyrhynchus* (SHL = 100) within Psammophiinae, making *Rhamphiophis* paraphyletic (Fig. 1.2G). *Buhoma*, on the other hand, was split with *B. procterae* sister to Prosymninae and *B. depressiceps* sister to Elapidae. *Oxyrhabdium leporinum* was sister to Lamprophiinae and *Micrelaps bicoloratus* was placed within Pseudoxyrhophiinae. In all preliminary and final analyses, *Psammodynastes* constantly occupied the same phylogenetic position; however, placement of the other four species was erratic and always differed. Therefore, we tentatively include *Psammodynastes* as part of Psammophiinae. Due to their perpetual variable placement,

we continue recognizing *Buhoma*, *M. bicoloratus*, and *O. leporinum* as Lamprophiidae *incertae sedis*.

Colubridae

For Colubridae, we recovered the following four subclades: i) Sibynophiinae + Natricinae (SHL = 80); ii) Pseudoxenodontinae + Dipsadinae (SHL = 82); iii) Grayiinae + Calamariinae (SHL = 70); and iv) Ahaetuliinae **subfam. nov.** + Colubrinae (SHL = 95). The nodes between these subclades all received very strong support (SHL > 97). The only consistently recovered clade among these is subclade ii (Chen et al. 2013; Pyron and Burbrink, 2012; Pyron et al., 2011; Pyron et al., 2013b; Pyron et al., 2014b); although other studies do not recover this subclade (Grazziotin et al., 2012; Pyron et al., 2013a; Zheng and Wiens, 2016). Several studies also regularly recovered the subclade Natricinae + (Pseudoxenodontinae + Dipsadinae) (Chen et al. 2013; Pyron and Burbrink, 2012; Pyron et al., 2011; Pyron et al., 2014b), but we do not uncover that relationship here. Instead, Natricinae formed a subclade with Sibynophiinae, also reported in (Pyron et al., 2013b). The subfamily Sibynophiinae was only recently included in molecular analyses, originally grouped with Calamariinae (Chen et al. 2013), then subsequently placed as sister to Grayiinae + Colubrinae (Pyron et al., 2013a; Zheng and Wiens, 2016), and to Calamariinae + (Colubrinae + Grayiinae) (Pyron et al., 2014b). The subfamily Grayiinae was also recently described (Vidal et al. 2007) and grouped with Calamariinae in that study, also recovered in Pyron and Burbrink (2012). However, Grayiinae has most frequently been grouped with Colubrinae (Chen et al. 2013; Lawson et al., 2005; Pyron et al., 2011; Pyron et al., 2013a; Pyron et al., 2013b; Pyron et al., 2014b; Zheng and Wiens, 2016). Dipsadinae is exclusively a

New World family, but recent placement of *Stichophanes* and *Thermophis* as sister to Dipsadinae (Peng et al., 2014; Pyron et al., 2013a; Wang et al., 2014) expanded its distribution into the Old World. Pyron et al. (2013a) did not include *Stichophanes*, and they mentioned that *Thermophis* may even warrant its own subfamily. However, our results do not uphold this view since we show *Stichophanes* + *Thermophis* (SHL = 96; Fig. 1.2L) as placed within Dipsadinae. Wang et al. (2014), on the other hand, supported *Stichophanes* + *Thermophis* as sister to Dipsadinae, but their dataset was not as extensive and did not include *T. zhaoermii*. Until now, the basal node of Colubrinae has remained ambiguous. Pyron et al. (2013a) suggested that monophyly of *Ahaetulla*, *Chrysopelea*, and *Dendrelaphis* at the base of Colubrinae, may warrant recognition as a distinct subfamily, but support for division of these taxa in their study was low. Due to increased sampling, and the inclusion of *Dryophiops*, we established strong support for recognizing these taxa as a new subfamily, using the name proposed by Pyron et al. (2013a), Ahaetuliinae **subfam. nov.**

Higher-level phylogeny with all rogue taxa eliminated

With all rogue taxa (101) and *incertae sedis* species (4) eliminated, higher-level relationships and support values remained relatively unchanged (S1.1 Fig.). Where changes in topology or support values occurred, it was in the problematic clades discussed above, specifically Typhlopidae, Booidea, Pareatidae + Viperidae, Lamprophiidae, and Colubridae. For Typhlopidae, *Xerotyphlops* formed a clade by itself, sister to all other typhlopids. Madatyphlopinae formed a moderately supported (SHL = 87) clade with Typhlopinae. However, the placements of Afrotyphlopinae and Asiatyphlopinae remained unresolved. In Booidea, the

placement of Calabariidae + Candoiidae swapped with Sanziniidae, greatly altering support values throughout Booidea, except in Charininae + Ungaliophiinae. Within Colubroidea, the placement of Pareatidae and Viperidae remains unresolved. Interestingly, with *incertae sedis* species removed from Lamprophiidae, topology of the subfamilies and of Elapidae within Lamprophiidae remained the same and the relationship between Atractaspidinae and Aparallactinae was strongly resolved, providing compelling support for the topology recovered. However, the node joining Prosymninae to all other lamprophiids became ambiguous. Relationships within Colubridae remained stable, except that Pseudoxenodontinae placed as sister to all other colubrids. In addition, we note that the sister relationship of Xenopeltidae to Loxocemidae + Pythonidae became ambiguous, and that with the exclusion of Xenophidiidae as a rogue taxon, Bolyeridae still placed as sister to Caenophidia with high support (SHL = 99), upholding its position outside of Henophidia.

Figure 1.2. Species-level phylogeny on final dataset of 1652 snake species. Maximum-likelihood phylogenetic estimate based on 10 concatenated genes. Node values represent SHL support values. Seven outgroup taxa are not shown. Colors of clades indicate their position in the overall tree, shown at left. Newly sequenced taxa are highlighted in bold. Skeleton of the species tree is displayed on the left with displayed subfamilies/families highlighted. Letters denoted by i and ii represent parts of the tree where external branches do not connect to the part of the tree immediately preceding it. A) Anomalepididae, Epictinae, Leptotyphlopinae, Gerrhopilidae, Xenotyphlopidae, and Typhlopinae B) Asiatyphlopinae I, Afrotyphlopinae; Madatyphlopinae, and Asiatyphlopinae II; C) Aniliidae, Tropidophiidae, Calabariidae, Candoiidae, Sanziniidae, Charininae, Ungaliophiinae, Erycidae, and Boidae; D*i*) Cylindrophiidae + Anomochilidae, Uropeltidae, Xenopeltidae, Loxocemidae, and Pythonidae, D*ii*) Bolyeridae, Xenophidiidae, Acrochordidae, Xenodermatidae, and Pareatidae; E*i*) Viperinae; E*ii*) Azemiopinae, and Crotalinae; F) Crotalinae cont.; G) Homalopsidae, Psammophiinae, *Buhoma procterae*, Prosymninae, Pseudaspidinae, Atractaspidinae, and Aparallactinae; H*i*) *Oxyrhabdium leporinum*, Lamprophiinae, H*ii*) *Ditypophis* sp. + *Micrelaps bicoloratus*, and Pseudoxyrhophiinae; I) *Buhoma depressiceps* and Elapidae; J) Elapidae cont.; K) Sibynophiinae and Natricinae; L) Pseudoxenodontinae and Dipsadinae; M) Dipsadinae cont.; N) Dipsadinae cont.; O), Grayiinae, Calamariinae, Ahaetullinae **subfam. nov.**, and Colubrinae; P*i*) Colubrinae cont.; P*ii*) Colubrinae cont.; Q) Colubrinae cont.; R) Colubrinae cont.


Fig. 2A
Fig. 2B
Fig. 2C
Anomalepididae
Leptotyphlopidae
Epictinae
Leptotyphlopinae
Gerrhopilidae
Xenotyphlopidae
Typhlopinae
Asiatyphlopinae
Afrotyphlopinae
Madatyphlopinae
Outgroups
Liotyphlops albirostris
Typhlophis squamosus
Rhinoleptus koniagui
Trilepida macrolepis
Rena humilis
Rena sp.
Rena dissecta
Rena dulcis
Siagonodon septemstriatus
Epictia tenella
Epictia goudotii
Epictia columbi
Epictia magnamaculata
Tetracheilostoma breuili
Tetracheilostoma carlae
Mitophis sp.
Mitophis asbolepis
Mitophis pyrites
Mitophis leptipileptus
Myriopholis longicauda
Myriopholis algeriensis
Myriopholis boueti
Myriopholis rouxestevae
Myriopholis blanfordi
Myriopholis macrorhyncha
Myriopholis adleri
Namibiana occidentalis
Leptotyphlops pitmani
Leptotyphlops merkeri
Leptotyphlops sp
Leptotyphlops nigroterminus
Leptotyphlops kafubi
Leptotyphlops distanti
Leptotyphlops scutifrons
Leptotyphlops nigricans
Leptotyphlops conjunctus
Leptotyphlops sylvicolus
Gerrhopilus mirus
Gerrhopilus hedraeus
Xenotyphlops sp.
Xenotyphlops grandidieri
Amerotyphlops reticulatus
Amerotyphlops tasymicris
Amerotyphlops brongersmianus
Typhlops caymanensis
Typhlops arator
Typhlops perimychus
Typhlops anousius
Typhlops anchaurus
Typhlops contorhinus
Typhlops biminiensis
Typhlops notorachius
Typhlops geotomus
Typhlops monastus
Typhlops dominicanus
Typhlops guadeloupensis
Typhlops granti
Typhlops catapontus
Typhlops hypomethes
Typhlops richardi
Typhlops platycephalus
Typhlops naugus
Typhlops syntherus
Typhlops sulcatus
Typhlops agoralionis
Typhlops hectus
Typhlops sylleptor
Typhlops capitulatus
Typhlops jamaicensis
Typhlops rostellatus
Typhlops sp. 19
Typhlops sp. 20
Typhlops pusillus
Typhlops sp. 16
Typhlops sp. 18
Typhlops sp. 17
Typhlops schwartzi
Typhlops eperopeus
Typhlops titanops
Typhlops sp. 11
Typhlops sp. 12
Typhlops sp. 13
Typhlops sp. 14
Typhlops sp. 15
Typhlops sp. 8
Typhlops sp. 9
Typhlops sp. 2
Typhlops sp. 1
Typhlops sp. 6
Typhlops sp. 7
Typhlops sp. 5
Typhlops sp. 4
Typhlops sp. 3
Xerotyphlops vermicularis
Xerotyphlops socotranus
Argyrophis muelleri
Argyrhophis sp. 5
Argyrophis diardii
Argyrhophis sp. 1
Argyrhophis sp. 3
Argyrhophis sp. 4
Argyrhophis sp. 2
Rhinotyphlops lalandei
Rhinotyphlops unitaeniatus
Letheobia feae
Letheobia newtoni
Letheobia simonii
Letheobia episcopus
Afrotyphlops obtusus
Afrotyphlops sp. 1
Afrotyphlops sp. 2
Afrotyphlops angolensis
Afrotyphlops elegans
Afrotyphlops mucruso
Afrotyphlops schlegelii
Afrotyphlops bibronii
Afrotyphlops fornasinii
Afrotyphlops sp. 3
Afrotyphlops lineolatus
Afrotyphlops congestus
Afrotyphlops punctatus
Madatyphlops andasibensis
Madatyphlops arenarius
Malayotyphlops ruber
Malayotyphlops luzonensis
Indotyphlops pammeces
Indotyphlops braminus
Indotyphlops sp.
Indotyphlops albiceps
Ramphotyphlops lineatus
Ramphotyphlops sp.
Ramphotyphlops acuticaudus
Acutotyphlops kunuaensis
Acutotyphlops subocularis
Acutotyphlops sp.
Anilios affinis
Anilios bituberculatus
Anilios grypus
Anilios leptosomus
Anilios longissimus
Anilios howi
Anilios unguirostris
Anilios proximus
Anilios wiedii
Anilios nigrescens
Anilios silvia
Anilios polygrammicus
Anilios ganei
Anilios ligatus
Anilios troglodytes
Anilios kimberleyensis
Anilios guentheri
Anilios ammodytes
Anilios diversus
Anilios bicolor
Anilios splendidus
Anilios pinguis
Anilios waitii
Anilios centralis
Anilios australis
Anilios hamatus
Anilios pilbarensis
Anilios endoterus

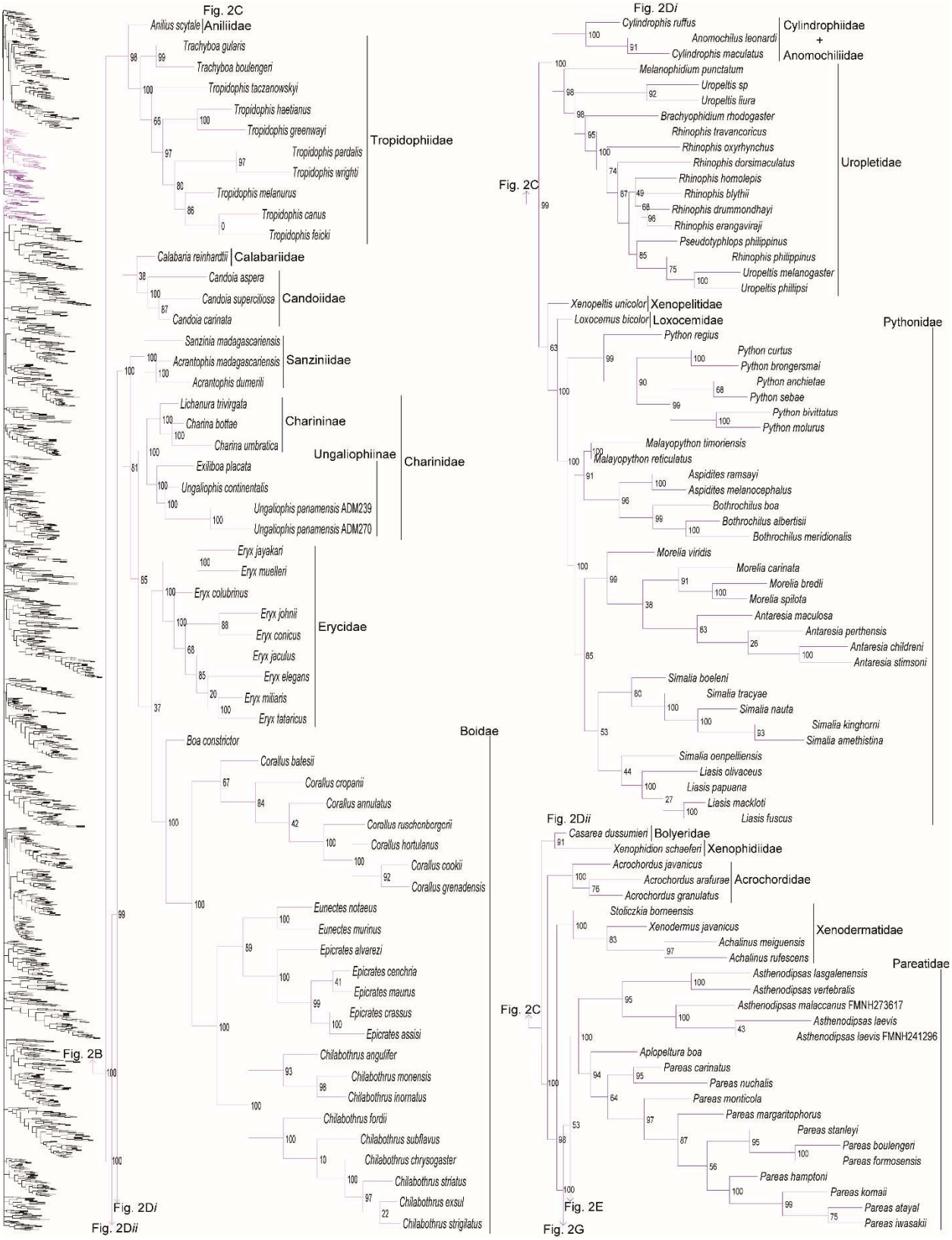


Fig. 2C
Anilius scytale
Aniliidae
Trachyboa gularis
Trachyboa boulengeri
Tropidophis taczanowskyi
Tropidophis haetianus
Tropidophis greenwayi
Tropidophis pardalis
Tropidophis wrighti
Tropidophis melanurus
Tropidophis canus
Tropidophis feicki
Tropidophiidae
Calabaria reinhardtii
Calabariidae
Candoia aspera
Candoia superciliosa
Candoia carinata
Candoiidae
Sanzinia madagascariensis
Acrantophis madagascariensis
Acrantophis dumerili
Sanziniidae
Lichanura trivirgata
Charina bottae
Charina umbratica
Charininae
Exiliboa placata
Ungaliophis continentalis
Ungaliophis panamensis ADM239
Ungaliophis panamensis ADM270
Ungaliophiinae
Charinidae
Eryx jayakari
Eryx muelleri
Eryx colubrinus
Eryx johnii
Eryx conicus
Eryx jaculus
Eryx elegans
Eryx miliaris
Eryx tataricus
Erycidae
Boa constrictor
Corallus batesii
Corallus cropanii
Corallus annulatus
Corallus ruschenbergerii
Corallus hortulanus
Corallus cookii
Corallus grenadensis
Eunectes notaeus
Eunectes murinus
Epicrates alvarezi
Epicrates cenchria
Epicrates maurus
Epicrates crassus
Epicrates assisi
Chilabothrus angulifer
Chilabothrus monensis
Chilabothrus inornatus
Chilabothrus fordii
Chilabothrus subflavus
Chilabothrus chrysogaster
Chilabothrus striatus
Chilabothrus exsul
Chilabothrus strigilatus
Boidae
Fig. 2B
Fig. 2Di
Fig. 2Dii
Fig. 2Di
Cylindrophis ruffus
Anomochilus leonardi
Cylindrophis maculatus
Cylindrophiidae
+
Anomochiliidae
Melanophidium punctatum
Uropeltis sp
Uropeltis liura
Brachyophidium rhodogaster
Rhinophis travancoricus
Rhinophis oxyrhynchus
Rhinophis dorsimaculatus
Rhinophis homolepis
Rhinophis blythii
Rhinophis drummondhayi
Rhinophis erangaviraji
Pseudotyphlops philippinus
Rhinophis philippinus
Uropeltis melanogaster
Uropeltis phillipsi
Uropeltidae
Fig. 2C
Xenopeltis unicolor
Xenopeltidae
Loxocemus bicolor
Loxocemidae
Python regius
Python curtus
Python brongersmai
Python anchietae
Python sebae
Python bivittatus
Python molurus
Pythonidae
Malayopython timoriensis
Malayopython reticulatus
Aspidites ramsayi
Aspidites melanocephalus
Bothrochilus boa
Bothrochilus albertisii
Bothrochilus meridionalis
Morelia viridis
Morelia carinata
Morelia bredli
Morelia spilota
Antaresia maculosa
Antaresia perthensis
Antaresia childreni
Antaresia stimsoni
Simalia boeleni
Simalia tracyae
Simalia nauta
Simalia kinghorni
Simalia amethistina
Simalia oenpelliensis
Liasis olivaceus
Liasis papuana
Liasis mackloti
Liasis fuscus
Fig. 2Dii
Casarea dussumieri
Bolyeridae
Xenophidion schaeferi
Xenophidiidae
Acrochordus javanicus
Acrochordus arafurae
Acrochordus granulatus
Acrochordidae
Stoliczkia borneensis
Xenodermus javanicus
Achalinus meiguensis
Achalinus rufescens
Xenodermatidae
Pareatidae
Asthenodipsas lasgalenensis
Asthenodipsas vertebralis
Asthenodipsas malaccanus FMNH273617
Asthenodipsas laevis
Asthenodipsas laevis FMNH241296
Fig. 2C
Aplopeltura boa
Pareas carinatus
Pareas nuchalis
Pareas monticola
Pareas margaritophorus
Pareas stanleyi
Pareas boulengeri
Pareas formosensis
Pareas hamptoni
Pareas komaii
Pareas atayal
Pareas iwasakii
Fig. 2E
Fig. 2G

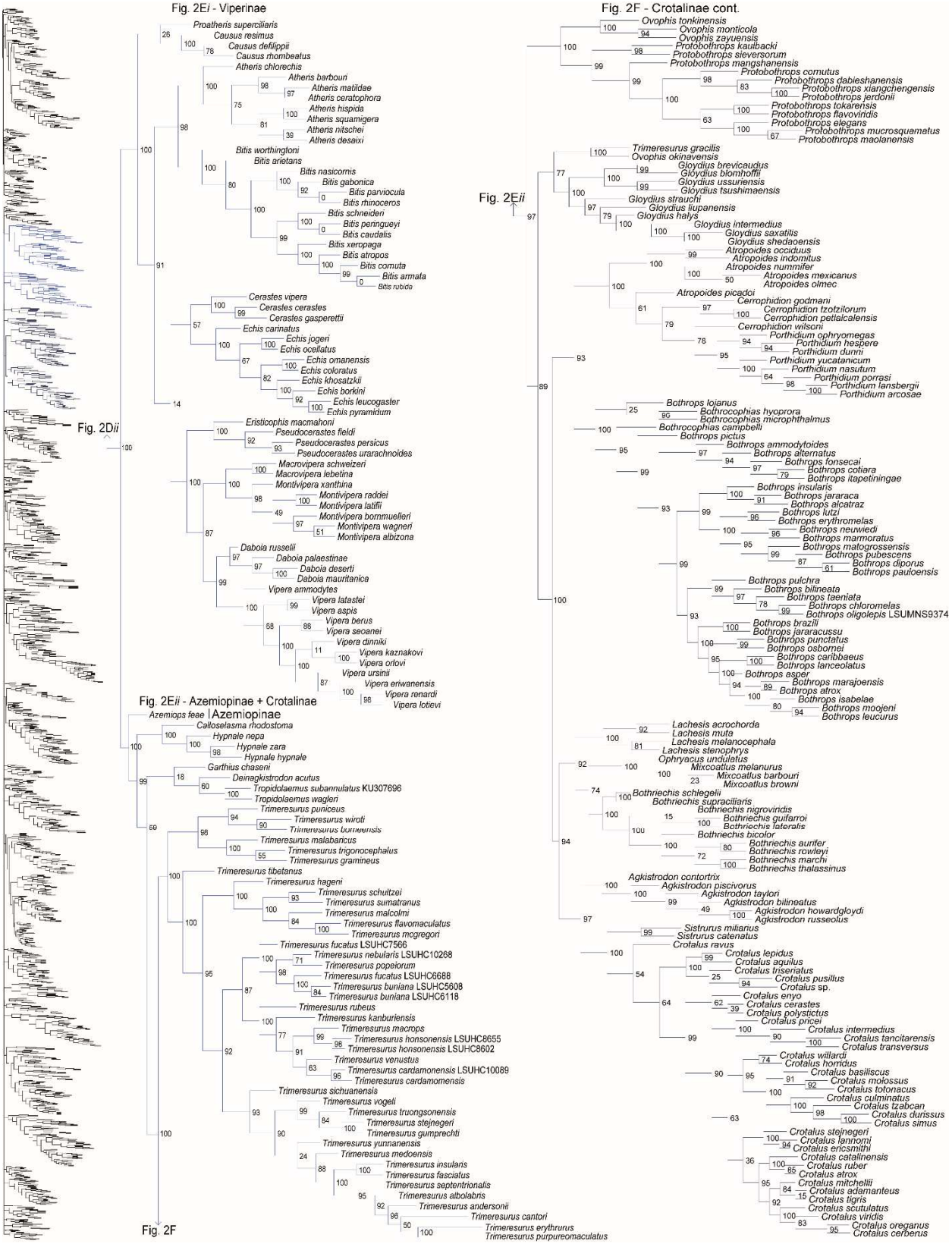


Fig. 2Ei - Viperinae
Proatheris superciliaris
Causus resimus
Causus defilippii
Causus rhombeatus
Atheris chlorechis
Atheris barbouri
Atheris matildae
Atheris ceratophora
Atheris hispida
Atheris squamigera
Atheris nitschei
Atheris desaixi
Bitis worthingtoni
Bitis arietans
Bitis nasicornis
Bitis gabonica
Bitis parviocula
Bitis rhinoceros
Bitis schneideri
Bitis peringueyi
Bitis caudalis
Bitis xeropaga
Bitis atropos
Bitis cornuta
Bitis armata
Bitis rubida
Cerastes vipera
Cerastes cerastes
Cerastes gasperettii
Echis carinatus
Echis jogeri
Echis ocellatus
Echis omanensis
Echis coloratus
Echis khosatzkii
Echis borkini
Echis leucogaster
Echis pyramidum
Fig. 2Dii
Eristicophis macmahoni
Pseudocerastes fieldi
Pseudocerastes persicus
Pseudocerastes urarachnoides
Macrovipera schweizeri
Macrovipera lebetina
Montivipera xanthina
Montivipera raddei
Montivipera latifii
Montivipera bornmuelleri
Montivipera wagneri
Montivipera albizona
Daboia russelii
Daboia palaestinae
Daboia deserti
Daboia mauritanica
Vipera ammodytes
Vipera latastei
Vipera aspis
Vipera berus
Vipera seoanei
Vipera dinniki
Vipera kaznakovi
Vipera orlovi
Vipera ursinii
Vipera eriwanensis
Vipera renardi
Vipera lotievi
Fig. 2Eii - Azemiopinae + Crotalinae
Azemiops feae | Azemiopinae
Calloselasma rhodostoma
Hypnale nepa
Hypnale zara
Hypnale hypnale
Garthius chaseni
Deinagkistrodon acutus
Tropidolaemus subannulatus KU307696
Tropidolaemus wagleri
Trimeresurus puniceus
Trimeresurus wiroti
Trimeresurus borneensis
Trimeresurus malabaricus
Trimeresurus trigonocephalus
Trimeresurus gramineus
Trimeresurus tibetanus
Trimeresurus hageni
Trimeresurus schultzei
Trimeresurus sumatranus
Trimeresurus malcolmi
Trimeresurus flavomaculatus
Trimeresurus mcgregori
Trimeresurus fucatus LSUHC7566
Trimeresurus nebularis LSUHC10268
Trimeresurus popeiorum
Trimeresurus fucatus LSUHC6688
Trimeresurus buniana LSUHC5608
Trimeresurus buniana LSUHC6118
Trimeresurus rubeus
Trimeresurus kanburiensis
Trimeresurus macrops
Trimeresurus honsonensis LSUHC8655
Trimeresurus honsonensis LSUHC8602
Trimeresurus venustus
Trimeresurus cardamonensis LSUHC10089
Trimeresurus cardamomensis
Trimeresurus sichuanensis
Trimeresurus vogeli
Trimeresurus truongsonensis
Trimeresurus stejnegeri
Trimeresurus gumprechti
Trimeresurus yunnanensis
Trimeresurus medoensis
Trimeresurus insularis
Trimeresurus fasciatus
Trimeresurus septentrionalis
Trimeresurus albolabris
Trimeresurus andersonii
Trimeresurus cantori
Trimeresurus erythrurus
Trimeresurus purpureomaculatus
Fig. 2F
Fig. 2F - Crotalinae cont.
Ovophis tonkinensis
Ovophis monticola
Ovophis zayuensis
Protobothrops kaulbacki
Protobothrops sieversorum
Protobothrops mangshanensis
Protobothrops cornutus
Protobothrops dabieshanensis
Protobothrops xiangchengensis
Protobothrops jerdonii
Protobothrops tokarensis
Protobothrops flavoviridis
Protobothrops elegans
Protobothrops mucrosquamatus
Protobothrops maolanensis
Fig. 2Eii
Trimeresurus gracilis
Ovophis okinavensis
Gloydius brevicaudus
Gloydius blomhoffii
Gloydius ussuriensis
Gloydius tsushimaensis
Gloydius strauchi
Gloydius liupanensis
Gloydius halys
Gloydius intermedius
Gloydius saxatilis
Gloydius shedaoensis
Atropoides occiduus
Atropoides indomitus
Atropoides nummifer
Atropoides mexicanus
Atropoides olmec
Atropoides picadoi
Cerrophidion godmani
Cerrophidion tzotzilorum
Cerrophidion petlalcalensis
Cerrophidion wilsoni
Porthidium ophryomegas
Porthidium hespere
Porthidium dunni
Porthidium yucatanicum
Porthidium nasutum
Porthidium porrasi
Porthidium lansbergii
Porthidium arcosae
Bothrops lojanus
Bothrocophias hyoprora
Bothrocophias microphthalmus
Bothrocophias campbelli
Bothrops pictus
Bothrops ammodytoides
Bothrops alternatus
Bothrops fonsecai
Bothrops cotiara
Bothrops itapetiningae
Bothrops insularis
Bothrops jararaca
Bothrops alcatraz
Bothrops lutzi
Bothrops erythromelas
Bothrops neuwiedi
Bothrops marmoratus
Bothrops matogrossensis
Bothrops pubescens
Bothrops diporus
Bothrops pauloensis
Bothrops pulchra
Bothrops bilineata
Bothrops taeniata
Bothrops chloromelas
Bothrops oligolepis LSUMNS9374
Bothrops brazili
Bothrops jararacussu
Bothrops punctatus
Bothrops osbornei
Bothrops caribbaeus
Bothrops lanceolatus
Bothrops asper
Bothrops marajoensis
Bothrops atrox
Bothrops isabelae
Bothrops moojeni
Bothrops leucurus
Lachesis acrochorda
Lachesis muta
Lachesis melanocephala
Lachesis stenophrys
Ophryacus undulatus
Mixcoatlus melanurus
Mixcoatlus barbouri
Mixcoatlus browni
Bothriechis schlegelii
Bothriechis supraciliaris
Bothriechis nigroviridis
Bothriechis guifarroi
Bothriechis lateralis
Bothriechis bicolor
Bothriechis aurifer
Bothriechis rowleyi
Bothriechis marchi
Bothriechis thalassinus
Agkistrodon contortrix
Agkistrodon piscivorus
Agkistrodon taylori
Agkistrodon bilineatus
Agkistrodon howardgloydi
Agkistrodon russeolus
Sistrurus miliarius
Sistrurus catenatus
Crotalus ravus
Crotalus lepidus
Crotalus aquilus
Crotalus triseriatus
Crotalus pusillus
Crotalus sp.
Crotalus enyo
Crotalus cerastes
Crotalus polystictus
Crotalus pricei
Crotalus intermedius
Crotalus tancitarensis
Crotalus transversus
Crotalus willardi
Crotalus horridus
Crotalus basiliscus
Crotalus molossus
Crotalus totonacus
Crotalus culminatus
Crotalus tzabcan
Crotalus durissus
Crotalus simus
Crotalus stejnegeri
Crotalus lannomi
Crotalus ericsmithi
Crotalus catalinensis
Crotalus ruber
Crotalus atrox
Crotalus mitchellii
Crotalus ademanteus
Crotalus tigris
Crotalus scutulatus
Crotalus viridis
Crotalus oreganus
Crotalus cerberus

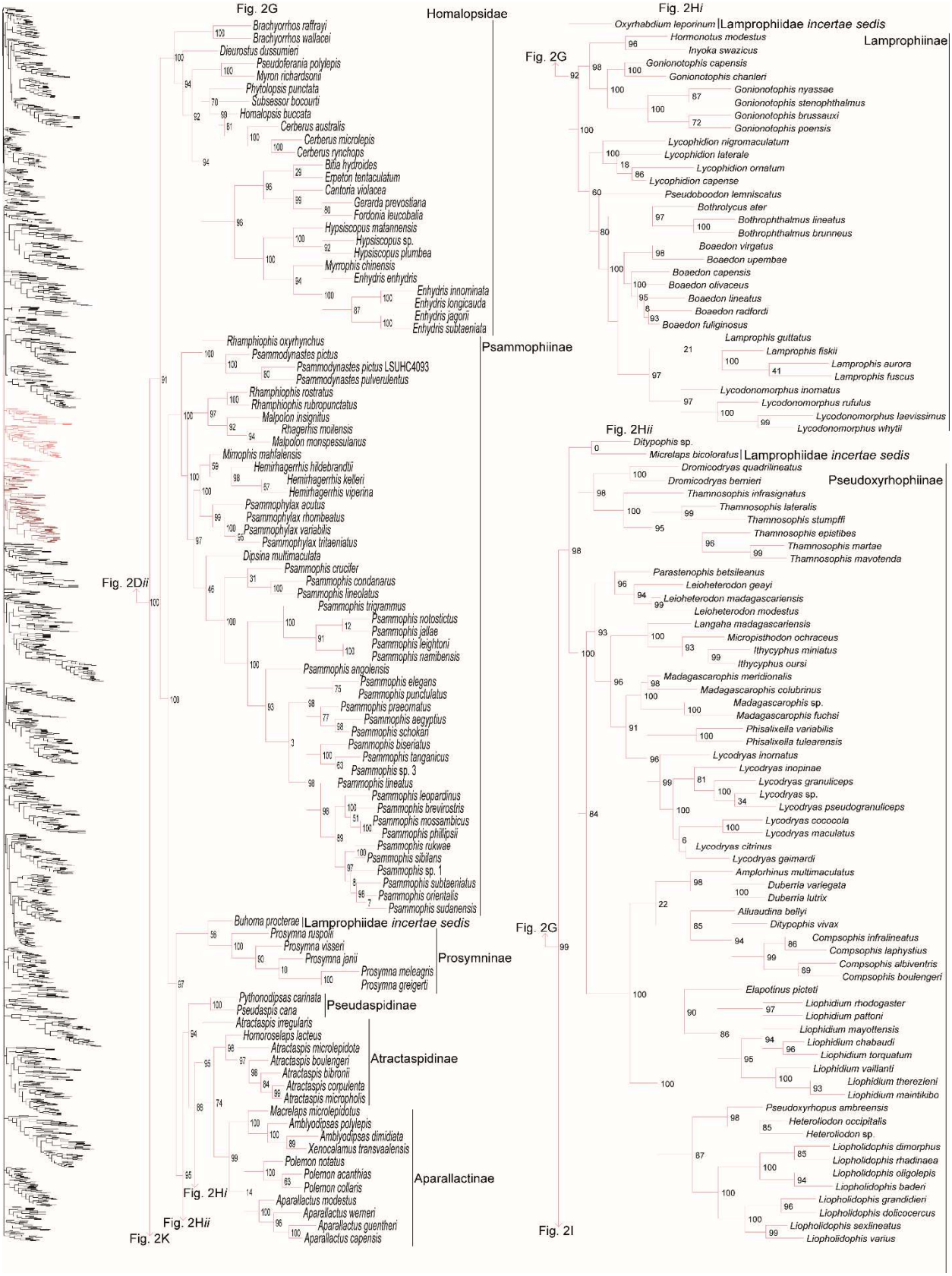


Fig. 2G
Homalopsidae
Brachyorrhos raffrayi
Brachyorrhos wallacei
Dieurostus dussumieri
Pseudoferania polylepis
Myron richardsonii
Phytolopsis punctata
Subsessor bocourti
Homalopsis buccata
Cerberus australis
Cerberus microlepis
Cerberus rynchops
Bitia hydroides
Erpeton tentaculatum
Cantoria violacea
Gerarda prevostiana
Fordonia leucobalia
Hypsiscopus matannensis
Hypsiscopus sp.
Hypsiscopus plumbea
Myrrophis chinensis
Enhydris enhydris
Enhydris innominata
Enhydris longicauda
Enhydris jagorii
Enhydris subtaeniata
Psammophiinae
Rhamphiophis oxyrhynchus
Psammodynastes pictus
Psammodynastes pictus LSUHC4093
Psammodynastes pulverulentus
Rhamphiophis rostratus
Rhamphiophis rubropunctatus
Malpolon insignitus
Rhagerhis moilensis
Malpolon monspessulanus
Mimophis mahfalensis
Hemirhagerrhis hildebrandtii
Hemirhagerrhis kelleri
Hemirhagerrhis viperina
Psammophylax acutus
Psammophylax rhombeatus
Psammophylax variabilis
Psammophylax tritaeniatus
Dipsina multimaculata
Psammophis crucifer
Psammophis condanarus
Psammophis lineolatus
Psammophis trigrammus
Psammophis notostictus
Psammophis jallae
Psammophis leightoni
Psammophis namibensis
Psammophis angolensis
Psammophis elegans
Psammophis punctulatus
Psammophis praeornatus
Psammophis aegyptius
Psammophis schokari
Psammophis biseriatus
Psammophis tanganicus
Psammophis sp. 3
Psammophis lineatus
Psammophis leopardinus
Psammophis brevirostris
Psammophis mossambicus
Psammophis phillipsii
Psammophis rukwae
Psammophis sibilans
Psammophis sp. 1
Psammophis subtaeniatus
Psammophis orientalis
Psammophis sudanensis
Fig. 2Dii
Buhoma procterae
Lamprophiidae incertae sedis
Prosymna ruspolii
Prosymna visseri
Prosymna janii
Prosymna meleagris
Prosymna greigerti
Prosymninae
Pythonodipsas carinata
Pseudaspis cana
Pseudaspidinae
Atractaspis irregularis
Homoroselaps lacteus
Atractaspis microlepidota
Atractaspis boulengeri
Atractaspis bibronii
Atractaspis corpulenta
Atractaspis micropholis
Atractaspidinae
Macrelaps microlepidotus
Amblyodipsas polylepis
Amblyodipsas dimidiata
Xenocalamus transvaalensis
Polemon notatus
Polemon acanthias
Polemon collaris
Aparallactus modestus
Aparallactus werneri
Aparallactus guentheri
Aparallactus capensis
Aparallactinae
Fig. 2Hi
Fig. 2Hii
Fig. 2K
Fig. 2Hi
Oxyrhabdium leporinum
Lamprophiidae incertae sedis
Hormonotus modestus
Inyoka swazicus
Gonionotophis capensis
Gonionotophis chanleri
Gonionotophis nyassae
Gonionotophis stenophthalmus
Gonionotophis brussauxi
Gonionotophis poensis
Lycophidion nigromaculatum
Lycophidion laterale
Lycophidion ornatum
Lycophidion capense
Pseudoboodon lemniscatus
Bothrolycus ater
Bothrophthalmus lineatus
Bothrophthalmus brunneus
Boaedon virgatus
Boaedon upembae
Boaedon capensis
Boaedon olivaceus
Boaedon lineatus
Boaedon radfordi
Boaedon fuliginosus
Lamprophis guttatus
Lamprophis fiskii
Lamprophis aurora
Lamprophis fuscus
Lycodonomorphus inornatus
Lycodonomorphus rufulus
Lycodonomorphus laevissimus
Lycodonomorphus whytii
Lamprophiinae
Fig. 2G
Fig. 2Hii
Ditypophis sp.
Micrelaps bicoloratus
Lamprophiidae incertae sedis
Dromicodryas quadrilineatus
Dromicodryas bernieri
Thamnosophis infrasignatus
Thamnosophis lateralis
Thamnosophis stumpffi
Thamnosophis epistibes
Thamnosophis martae
Thamnosophis mavotenda
Pseudoxyrhophiinae
Parastenophis betsileanus
Leioheterodon geayi
Leioheterodon madagascariensis
Leioheterodon modestus
Langaha madagascariensis
Micropisthodon ochraceus
Ithycyphus miniatus
Ithycyphus oursi
Madagascarophis meridionalis
Madagascarophis colubrinus
Madagascarophis sp.
Madagascarophis fuchsi
Phisalixella variabilis
Phisalixella tulearensis
Lycodryas inornatus
Lycodryas inopinae
Lycodryas granuliceps
Lycodryas sp.
Lycodryas pseudogranuliceps
Lycodryas cococola
Lycodryas maculatus
Lycodryas citrinus
Lycodryas gaimardi
Amplorhinus multimaculatus
Duberria variegata
Duberria lutrix
Alluaudina bellyi
Ditypophis vivax
Compsophis infralineatus
Compsophis laphystius
Compsophis albiventris
Compsophis boulengeri
Elapotinus picteti
Liophidium rhodogaster
Liophidium pattoni
Liophidium mayottensis
Liophidium chabaudi
Liophidium torquatum
Liophidium vaillanti
Liophidium therezieni
Liophidium maintikibo
Pseudoxyrhopus ambreensis
Heteroliodon occipitalis
Heteroliodon sp.
Liopholidophis dimorphus
Liopholidophis rhadinaea
Liopholidophis oligolepis
Liopholidophis baderi
Liopholidophis grandidieri
Liopholidophis dolicocercus
Liopholidophis sexlineatus
Liopholidophis varius
Fig. 2G
Fig. 2I

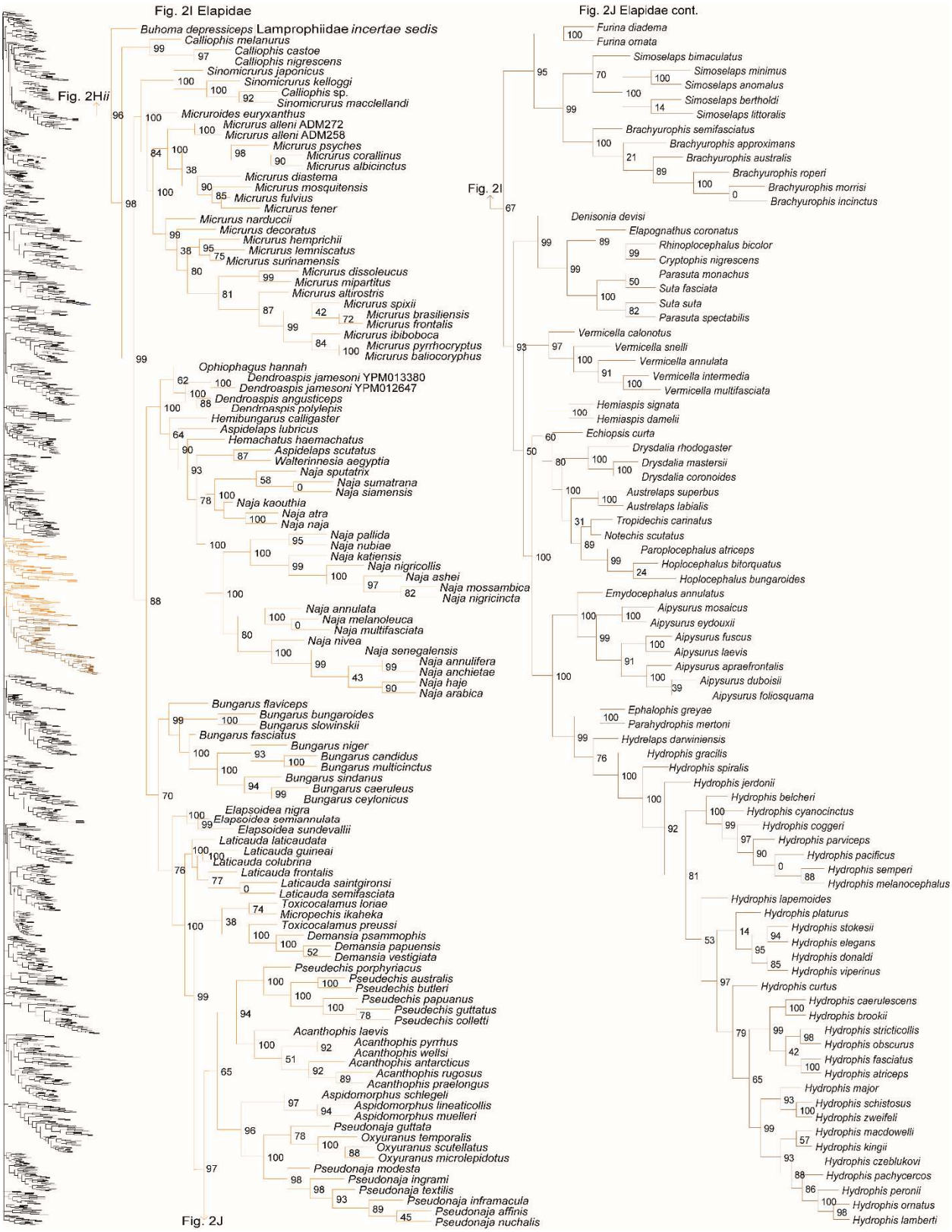


Fig. 2I Elapidae
Fig. 2J Elapidae cont.
Fig. 2Hii
Fig. 2I
Fig. 2J
Buhoma depressiceps Lamprophiidae incertae sedis
Calliophis melanurus
Calliophis castoe
Calliophis nigrescens
Sinomicrurus japonicus
Sinomicrurus kelloggi
Calliophis sp.
Sinomicrurus macclellandi
Micruroides euryxanthus
Micrurus alleni ADM272
Micrurus alleni ADM258
Micrurus psyches
Micrurus corallinus
Micrurus albicinctus
Micrurus diastema
Micrurus mosquitensis
Micrurus fulvius
Micrurus tener
Micrurus narduccii
Micrurus decoratus
Micrurus hemprichii
Micrurus lemniscatus
Micrurus surinamensis
Micrurus dissoleucus
Micrurus mipartitus
Micrurus altirostris
Micrurus spixii
Micrurus brasiliensis
Micrurus frontalis
Micrurus ibiboboca
Micrurus pyrrhocryptus
Micrurus baliocoryphus
Ophiophagus hannah
Dendroaspis jamesoni YPM013380
Dendroaspis jamesoni YPM012647
Dendroaspis angusticeps
Dendroaspis polylepis
Hemibungarus calligaster
Aspidelaps lubricus
Hemachatus haemachatus
Aspidelaps scutatus
Walterinnesia aegyptia
Naja sputatrix
Naja sumatrana
Naja siamensis
Naja kaouthia
Naja atra
Naja naja
Naja pallida
Naja nubiae
Naja katiensis
Naja nigricollis
Naja ashei
Naja mossambica
Naja nigricincta
Naja annulata
Naja melanoleuca
Naja multifasciata
Naja nivea
Naja senegalensis
Naja annulifera
Naja anchietae
Naja haje
Naja arabica
Bungarus flaviceps
Bungarus bungaroides
Bungarus slowinskii
Bungarus fasciatus
Bungarus niger
Bungarus candidus
Bungarus multicinctus
Bungarus sindanus
Bungarus caeruleus
Bungarus ceylonicus
Elapsoidea nigra
Elapsoidea semiannulata
Elapsoidea sundevallii
Laticauda laticaudata
Laticauda guineai
Laticauda colubrina
Laticauda frontalis
Laticauda saintgironsi
Laticauda semifasciata
Toxicocalamus loriae
Micropechis ikaheka
Toxicocalamus preussi
Demansia psammophis
Demansia papuensis
Demansia vestigiata
Pseudechis porphyriacus
Pseudechis australis
Pseudechis butleri
Pseudechis papuanus
Pseudechis guttatus
Pseudechis colletti
Acanthophis laevis
Acanthophis pyrrhus
Acanthophis wellsi
Acanthophis antarcticus
Acanthophis rugosus
Acanthophis praelongus
Aspidomorphus schlegeli
Aspidomorphus lineaticollis
Aspidomorphus muelleri
Pseudonaja guttata
Oxyuranus temporalis
Oxyuranus scutellatus
Oxyuranus microlepidotus
Pseudonaja modesta
Pseudonaja ingrami
Pseudonaja textilis
Pseudonaja inframacula
Pseudonaja affinis
Pseudonaja nuchalis
Furina diadema
Furina ornata
Simoselaps bimaculatus
Simoselaps minimus
Simoselaps anomalus
Simoselaps bertholdi
Simoselaps littoralis
Brachyurophis semifasciatus
Brachyurophis approximans
Brachyurophis australis
Brachyurophis roperi
Brachyurophis morrisi
Brachyurophis incinctus
Denisonia devisi
Elapognathus coronatus
Rhinoplocephalus bicolor
Cryptophis nigrescens
Parasuta monachus
Suta fasciata
Suta suta
Parasuta spectabilis
Vermicella calonotus
Vermicella snelli
Vermicella annulata
Vermicella intermedia
Vermicella multifasciata
Hemiaspis signata
Hemiaspis damelii
Echiopsis curta
Drysdalia rhodogaster
Drysdalia mastersii
Drysdalia coronoides
Austrelaps superbus
Austrelaps labialis
Tropidechis carinatus
Notechis scutatus
Paroplocephalus atriceps
Hoplocephalus bitorquatus
Hoplocephalus bungaroides
Emydocephalus annulatus
Aipysurus mosaicus
Aipysurus eydouxii
Aipysurus fuscus
Aipysurus laevis
Aipysurus apraefrontalis
Aipysurus duboisii
Aipysurus foliosquama
Ephalophis greyae
Parahydrophis mertoni
Hydrelaps darwiniensis
Hydrophis gracilis
Hydrophis spiralis
Hydrophis jerdonii
Hydrophis belcheri
Hydrophis cyanocinctus
Hydrophis coggeri
Hydrophis parviceps
Hydrophis pacificus
Hydrophis semperi
Hydrophis melanocephalus
Hydrophis lapemoides
Hydrophis platurus
Hydrophis stokesii
Hydrophis elegans
Hydrophis donaldi
Hydrophis viperinus
Hydrophis curtus
Hydrophis caerulescens
Hydrophis brookii
Hydrophis stricticollis
Hydrophis obscurus
Hydrophis fasciatus
Hydrophis atriceps
Hydrophis major
Hydrophis schistosus
Hydrophis zweifeli
Hydrophis macdowelli
Hydrophis kingii
Hydrophis czeblukovi
Hydrophis pachycercos
Hydrophis peronii
Hydrophis ornatus
Hydrophis lamberti


Fig. 2K
Scaphiodontophis annulatus
Sibynophis bistrigatus
Sibynophis subpunctatus
Sibynophis chinensis
Sibynophis collaris
Sibynophis triangularis
Sibynophiinae
Fig. 2K - Natricinae
Macropisthodon rudis
Amphiesma stolatum
Hebius sauteri
Herpetoreas burbrinki
Herpetoreas platyceps
Trachischium monticola
Hebius deschauenseei
Hebius modestum
Hebius venningi
Hebius boulengeri
Hebius khasiense
Hebius parallelum
Hebius popei
Hebius optatum
Hebius atemporale
Hebius vibakari
Hebius sp.
Hebius octolineatum
Hebius bitaeniatum
Hebius metusium
Hebius craspedogaster
Hebius johannis
Natriciteres olivacea
Afronatrix anoscopus
Xenochrophis schnurrenbergeri
Atretium schistosum
Xenochrophis asperrimus
Atretium yunnanensis
Xenochrophis piscator
Xenochrophis punctulatus
Xenochrophis flavipunctatus
Xenochrophis vittatus
Rhabdophis sp.
Rhabdophis chrysargos
Rhabdophis himalayanus
Balanophis ceylonensis
Macropisthodon rhodolemas ADM0003
Rhabdophis tigrinus
Rhabdophis subminiatus
Rhabdophis swinhonis
Rhabdophis nuchalis
Rhabdophis guangdongensis
Rhabdophis leonardi
Rhabdophis nigrocinctus
Rhabdophis adleri
Aspidura trachyprocta
Aspidura sp.
Aspidura ceylonensis
Aspidura guentheri
Aspidura drummondhayi
Opisthotropis guangxiensis
Opisthotropis lateralis
Opisthotropis cheni
Opisthotropis latouchii
Sinonatrix aequifasciata
Sinonatrix percarinata
Sinonatrix annularis
Natrix maura
Natrix tessellata
Natrix natrix
Liodytes pygaea
Liodytes rigida
Liodytes alleni
Clonophis kirtlandii
Virginia valeriae
Haldea striatula
Storeria dekayi
Storeria storerioides
Storeria occipitomaculata
Tropidoclonion lineatum
Regina grahamii
Nerodia cyclopion
Nerodia floridana
Regina septemvittata
Nerodia erythrogaster
Nerodia taxispilota
Nerodia rhombifer
Nerodia fasciata
Nerodia clarkii
Nerodia sipedon
Nerodia harteri
Nerodia paucimaculata
Thamnophis sirtalis
Thamnophis proximus
Thamnophis sauritus
Thamnophis nigronuchalis
Thamnophis rufipunctatus
Thamnophis elegans
Adelophis foxi
Thamnophis melanogaster
Thamnophis valida
Thamnophis exsul
Thamnophis godmani
Thamnophis scaliger
Thamnophis sumichrasti
Thamnophis scalaris
Thamnophis fulvus
Thamnophis chrysocephalus
Thamnophis pulchrilatus
Thamnophis cyrtopsis
Thamnophis eques
Thamnophis marcianus
Thamnophis hammondii
Thamnophis brachystoma
Thamnophis butleri
Thamnophis radix
Thamnophis ordinoides
Thamnophis atratus
Thamnophis gigas
Thamnophis couchii
Fig. 2G
Fig. 2L
Fig. 2O
Fig. 2L
Plagiopholis styani
Pseudoxenodon bambusicola
Pseudoxenodon sp.
Pseudoxenodon karlschmidti
Pseudoxenodon macrops
Pseudoxenodontinae
Diadophis punctatus
Fig. 2L - Dipsadinae
Heterodon platirhinos
Heterodon simus
Heterodon nasicus
Farancia erytrogramma
Farancia abacura
Carphophis vermis
Carphophis amoenus
Contia tenuis
Contia longicaudae
Stichophanes ningshaanensis
Thermophis baileyi
Thermophis shangrila
Thermophis zhaoermii
Fig. 2K
Enulius sp.
Amastridium veliferum
Tantalophis discolor
Rhadinaea flavilata
Rhadinaea fulvivittis
Coniophanes fissidens
Pliocercus euryzonus ADM241
Pliocercus euryzonus ADM240
Imantodes inornatus
Imantodes cenchoa
Imantodes gemmistratus
Imantodes lentiferus
Imantodes chocoensis
Leptodeira nigrofasciata
Leptodeira frenata
Leptodeira uribei
Leptodeira punctata
Leptodeira splendida
Leptodeira polysticta
Leptodeira septentrionalis
Leptodeira bakeri
Leptodeira annulata
Leptodeira maculata
Leptodeira rubricata
Pseudoleptodeira latifasciata
Hypsiglena tanzeri
Hypsiglena jani
Hypsiglena slevini
Hypsiglena unaocularus
Hypsiglena chlorophaea
Hypsiglena ochrorhyncha
Hypsiglena affinis
Hypsiglena torquata
Adelphicos quadrivirgatum
Hydromorphus concolor
Tretanorhinus nigroluteus
Cryophis hallbergi
Geophis turbidus
Geophis dubius
Geophis juarezi
Geophis carinosus
Geophis occabus
Geophis latifrontalis
Geophis godmani
Atractus wagleri
Atractus reticulatus
Atractus badius
Atractus zidoki
Atractus schach
Atractus elaps
Atractus albuquerquei
Atractus flammigerus
Atractus trihedrurus
Atractus zebrinus
Ninia atrata
Sibon noalamina
Tropidodipsas fischeri MVZ143527
Tropidodipsas sartorii
Sibon dimidiatus LSUMNS6689
Sibon nebulatus
Sibon annulatus ADM242
Sibon annulatus ADM0007
Dipsas articulata
Dipsas indica
Dipsas pratti
Dipsas peruana LSUMNS1532
Sibynomorphus vagus KU219121
Dipsas pavonina LSUMNS14372
Sibynomorphus turgidus
Sibynomorphus mikanii
Sibynomorphus turgidus LSUMNS6459
Thamnodynastes pallidus
Dipsas catesbyi
Dipsas pavonina LSUMNS13989
Dipsas albifrons
Sibynomorphus ventrimaculatus
Sibynomorphus neuwiedi
Dipsas neivai
Dipsas variegata
Fig. 2M

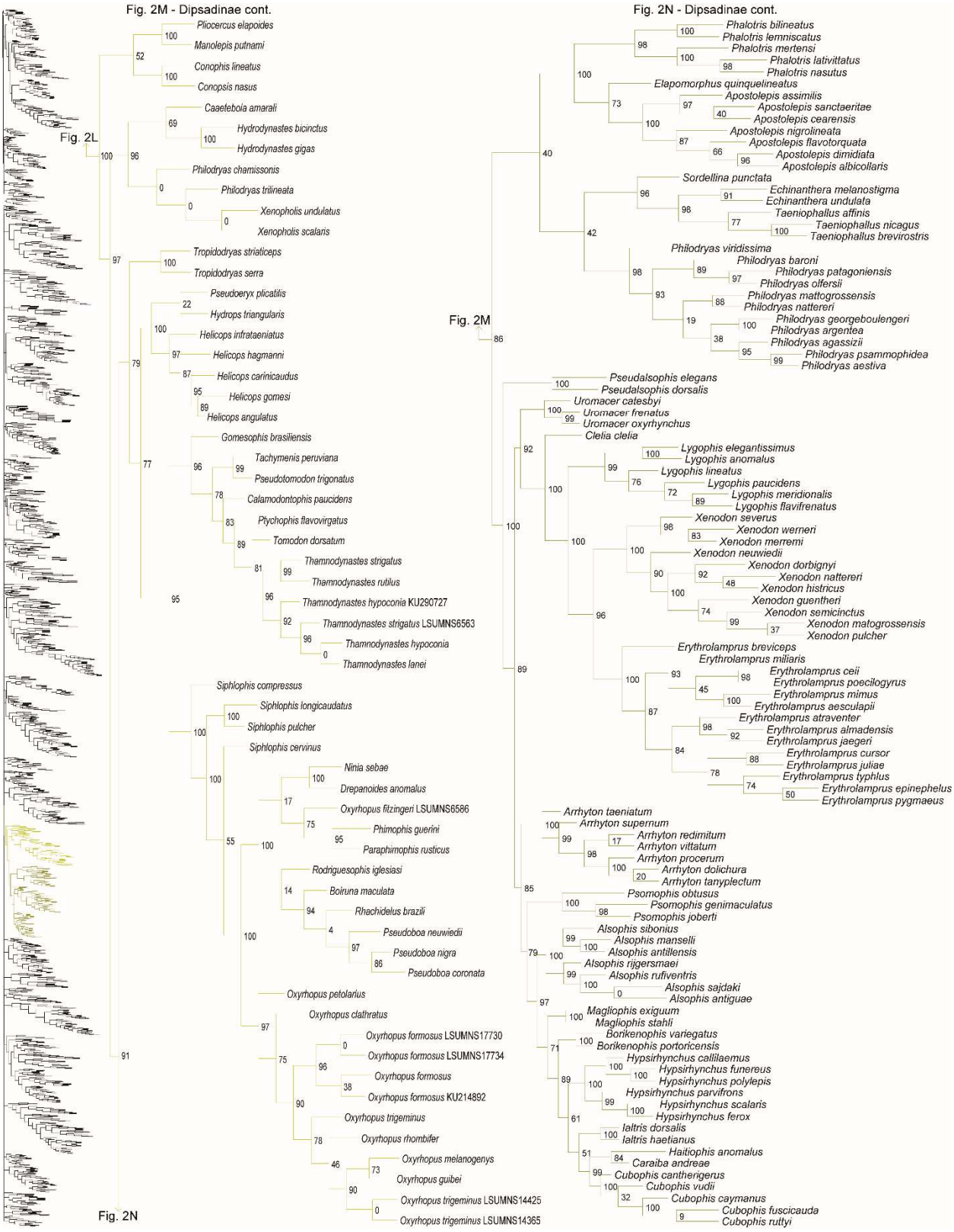


Fig. 2M - Dipsadinae cont.
Fig. 2N - Dipsadinae cont.
Fig. 2L
Fig. 2M
Fig. 2N
Pliocercus elapoides
Manolepis putnami
Conophis lineatus
Conopsis nasus
Caaetebola amarali
Hydrodynastes bicinctus
Hydrodynastes gigas
Philodryas chamissonis
Philodryas trilineata
Xenopholis undulatus
Xenopholis scalaris
Tropidodryas striaticeps
Tropidodryas serra
Pseudoeryx plicatilis
Hydrops triangularis
Helicops infrataeniatus
Helicops hagmanni
Helicops carinicaudus
Helicops gomesi
Helicops angulatus
Gomesophis brasiliensis
Tachymenis peruviana
Pseudotomodon trigonatus
Calamodontophis paucidens
Ptychophis flavovirgatus
Tomodon dorsatum
Thamnodynastes strigatus
Thamnodynastes rutilus
Thamnodynastes hypoconia KU290727
Thamnodynastes strigatus LSUMNS6563
Thamnodynastes hypoconia
Thamnodynastes lanei
Siphlophis compressus
Siphlophis longicaudatus
Siphlophis pulcher
Siphlophis cervinus
Ninia sebae
Drepanoides anomalus
Oxyrhopus fitzingeri LSUMNS6586
Phimophis guerini
Paraphimophis rustica
Rodriguesophis iglesiasi
Boiruna maculata
Rhachidelus brazili
Pseudoboa neuwiedii
Pseudoboa nigra
Pseudoboa coronata
Oxyrhopus petolarius
Oxyrhopus clathratus
Oxyrhopus formosus LSUMNS17730
Oxyrhopus formosus LSUMNS17734
Oxyrhopus formosus
Oxyrhopus formosus KU214892
Oxyrhopus trigeminus
Oxyrhopus rhombifer
Oxyrhopus melanogenys
Oxyrhopus guibei
Oxyrhopus trigeminus LSUMNS14425
Oxyrhopus trigeminus LSUMNS14365
Phalotris bilineatus
Phalotris lemniscatus
Phalotris mertensi
Phalotris lativittatus
Phalotris nasutus
Elapomorphus quinquelineatus
Apostolepis assimilis
Apostolepis sanctaeritae
Apostolepis cearensis
Apostolepis nigrolineata
Apostolepis flavotorquata
Apostolepis dimidiata
Apostolepis albicollaris
Sordellina punctata
Echinanthera melanostigma
Echinanthera undulata
Taeniophallus affinis
Taeniophallus nicagus
Taeniophallus brevirostris
Philodryas viridissima
Philodryas baroni
Philodryas patagoniensis
Philodryas olfersii
Philodryas mattogrossensis
Philodryas nattereri
Philodryas georgeboulengeri
Philodryas argentea
Philodryas agassizii
Philodryas psammophidea
Philodryas aestiva
Pseudalsophis elegans
Pseudalsophis dorsalis
Uromacer catesbyi
Uromacer frenatus
Uromacer oxyrhynchus
Clelia clelia
Lygophis elegantissimus
Lygophis anomalus
Lygophis lineatus
Lygophis paucidens
Lygophis meridionalis
Lygophis flavifrenatus
Xenodon severus
Xenodon werneri
Xenodon merremi
Xenodon neuwiedii
Xenodon dorbignyi
Xenodon nattereri
Xenodon histricus
Xenodon guentheri
Xenodon semicinctus
Xenodon matogrossensis
Xenodon pulcher
Erythrolamprus breviceps
Erythrolamprus miliaris
Erythrolamprus ceii
Erythrolamprus poecilogyrus
Erythrolamprus mimus
Erythrolamprus aesculapii
Erythrolamprus atraventer
Erythrolamprus almadensis
Erythrolamprus jaegeri
Erythrolamprus cursor
Erythrolamprus juliae
Erythrolamprus typhlus
Erythrolamprus epinephelus
Erythrolamprus pygmaeus
Arrhyton taeniatum
Arrhyton supernum
Arrhyton redimitum
Arrhyton vittatum
Arrhyton procerum
Arrhyton dolichura
Arrhyton tanyplectum
Psomophis obtusus
Psomophis genimaculatus
Psomophis joberti
Alsophis sibonius
Alsophis manselli
Alsophis antillensis
Alsophis rijgersmaei
Alsophis rufiventris
Alsophis sajdaki
Alsophis antiguae
Magliophis exiguum
Magliophis stahli
Borikenophis variegatus
Borikenophis portoricensis
Hypsirhynchus callilaemus
Hypsirhynchus funereus
Hypsirhynchus polylepis
Hypsirhynchus parvifrons
Hypsirhynchus scalaris
Hypsirhynchus ferox
Ialtris dorsalis
Ialtris haetianus
Haitiophis anomalus
Caraiba andreae
Cubophis cantherigerus
Cubophis vudii
Cubophis caymanus
Cubophis fuscicauda
Cubophis ruttyi

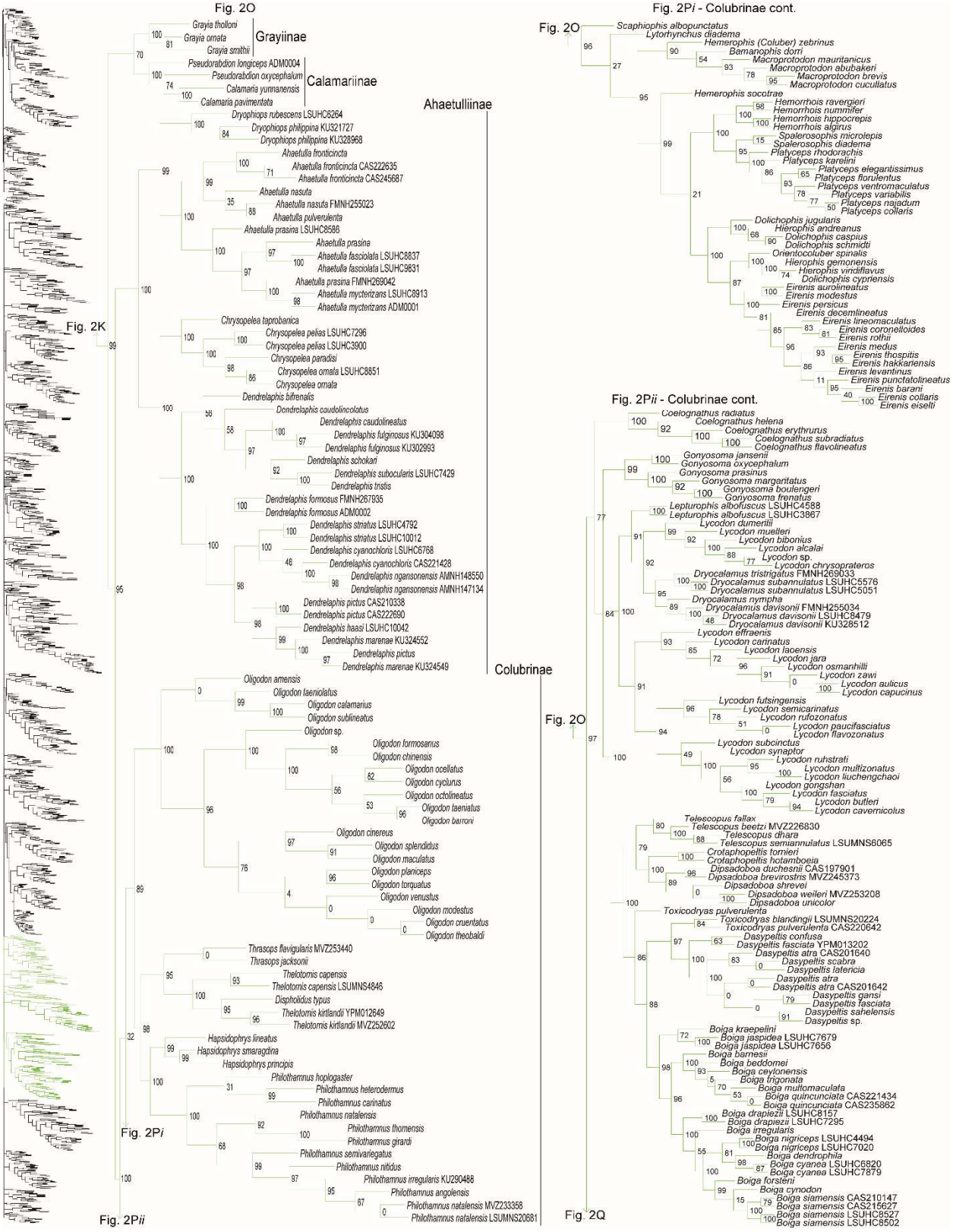


Fig. 2O
Grayiinae
Calamariinae
Ahaetulliinae
Colubrinae
Fig. 2K
Fig. 2Pi
Fig. 2Pii
Fig. 2Pi - Colubrinae cont.
Fig. 2Pii - Colubrinae cont.
Fig. 2O
Fig. 2Q


Fig. 2Q - Colubrinae cont.
Euprepiophis mandarinus
Euprepiophis conspicillata
Euprepiophis perlacea
Oreocryptophis porphyraceus
Archelaphe bella
Elaphe zoigeensis
Elaphe quatuorlineata
Elaphe sauromates
Elaphe davidi
Elaphe dione
Elaphe bimaculata
Elaphe climacophora
Elaphe carinata
Elaphe quadrivirgata
Elaphe anomala
Elaphe schrenckii
Orthriophis hodgsoni
Orthriophis cantoris
Orthriophis taeniurus
Orthriophis moellendorffi
Zamenis hohenackeri
Zamenis persicus
Rhinechis scalaris
Zamenis situla
Zamenis lineatus
Zamenis longissimus
Ptyas korros
Oocatochus rufodorsatus
Coronella austriaca
Coronella girondica
Senticolis triaspis
Rhinocheilus lecontei
Pseudelaphe flavirufa
Arizona elegans
Pantherophis bairdi
Pantherophis alleghaniensis
Pantherophis spiloides
Pantherophis obsoletus
Pantherophis vulpinus
Pantherophis ramspotti
Pantherophis guttatus
Pantherophis slowinskii
Pantherophis emoryi
Pituophis lineaticollis
Pituophis deppei
Pituophis vertebralis
Pituophis catenifer
Pituophis ruthveni
Pituophis melanoleucus
Bogertophis subocularis
Bogertophis rosaliae
Cemophora coccinea
Lampropeltis calligaster
Lampropeltis zonata
Lampropeltis elapsoides
Lampropeltis webbi
Lampropeltis knoblochi
Lampropeltis pyromelana
Lampropeltis mexicana
Lampropeltis ruthveni
Lampropeltis micropholis
Lampropeltis polyzona
Lampropeltis abnorma
Lampropeltis triangulum
Lampropeltis alterna
Lampropeltis gentilis
Lampropeltis annulata
Lampropeltis extenuata
Lampropeltis splendida
Lampropeltis californiae
Lampropeltis getula
Lampropeltis holbrooki
Lampropeltis nigra
Fig. 2Pii
Fig. 2R
Fig. 2R - Colubrinae cont.
Fig. 2Q
Liopeltis frenatus CAS225548
Ptyas mucosa
Ptyas carinata LSUHC10004
Ptyas carinata LSUHC7600
Cyclophiops major
Ptyas dhumnades
Ptyas nigromarginata CAS236202
Ptyas nigromarginata CAS241947
Cyclophiops multicinctus FMNH255569
Cyclophiops multicinctus AMNH153707
Cyclophiops multicinctus
Cyclophiops multicinctus KU291939
Ptyas fusca ADM0006
Ptyas fusca FMNH269019
Ptyas luzonensis
Ptyas luzonensis KU330134
Ptyas luzonensis KU329295
Dendrophidion percarinatum
Drymobius rhombifer
Dendrophidion dendrophis
Dendrophidion percarinatum MVZ204098
Dendrophidion percarinatum MVZ204099
Oxybelis fulgidus
Oxybelis aeneus
Leptophis depressirostris YPM016868
Leptophis depressirostris LSUMNS146385
Leptophis diplotropis LSUMNS6328
Leptophis ahaetulla
Opheodrys aestivus
Opheodrys vernalis
Chironius fuscus KU214847
Chironius fuscus LSUMNS13875
Chironius laevicollis
Chironius scurrulus LSUMNS9368
Chironius scurrulus
Chironius scurrulus LSUMNS8940
Chironius scurrulus AMNH152263
Chironius grandisquamis
Chironius flavolineatus
Chironius monticola AMNH150692
Chironius quadricarinatus
Chironius monticola
Chironius bicarinatus
Chironius fuscus
Chironius exoletus AMNH152259
Chironius exoletus
Chironius carinatus
Chironius multiventris YPM016079
Chironius multiventris YPM016080
Chironius laurenti
Chironius multiventris LSUMNS12556
Chironius multiventris
Chironius foveatus
Chironius exoletus LSUMNS16518
Chironius multiventris LSUMNS17665
Drymarchon corais
Drymarchon couperi
Conopsis biserialis
Conophis vittatus
Pseudoficimia frontalis
Symphiolis lippiens
Tantilla relicta
Tantilla coronata
Tantilla gracilis
Tantilla hobartsmithi
Tantilla nigriceps
Tantilla planiceps
Stenorrhina freminvillei
Chionactis occipitalis
Sonora semiannulata
Sonora aemula
Chilomeniscus stramineus
Sonora michoacanensis
Sonora mutabilis
Tantilla melanocephala
Coluber schotti
Coluber taeniatus
Coluber lateralis
Coluber flagellum
Coluber bilineatus
Coluber constrictor
Rhinobothryum lentiginosum
Salvadora mexicana
Salvadora grahamiae
Drymoluber dichrous
Drymoluber brazili
Mastigodryas bifossatus
Mastigodryas melanolomus
Mastigodryas boddaerti
Phyllorhynchus decurtatus
Phyllorhynchus browni
Salvadora hexalepis
Trimorphodon biscutatus
Spilotes pullatus
Spilotes pullatus ADM259
Spilotes sulphureus
Phrynonax shropshirei LSUMNS7806
Spilotes sulphureus LSUMNS14023
Spilotes sulphureus LSUMNS8937
Phrynonax poecilonotus
Phrynonax poecilonotus ADM0005
Phrynonax shropshirei LSUMNS7085
Phrynonax polylepis
Phrynonax poecilonotus AMNH152285

Genus- and species-level phylogeny

Of the 147 samples we sequenced, two genera (*Dryophiops*, and *Liopeltis*) and 61 species were not previously incorporated in any phylogenetic analyses. *Dryophiops* placed within Ahaetullinae **subfam. nov.** as sister to *Ahaetulla* (SHL = 99), and *Liopeltis* fell within Colubrinae as sister taxon (SHL = 97) to *Ptyas* + *Cyclophiops*. We recovered strong support for the phylogenetic placement of 105 of our samples (SHL > 85). For taxa where our sequences resulted in multiple terminals of the same species, the following species were not monophyletic: *Ahaetulla nasuta*, *A. prasina*, *Chironius exoletus*, *C. fuscus*, *C. monticola*, *C. multiventris*, *Dasypeltis fasciata*, *Dendrelaphis cyanochloris*, *D. marenae*, *Dendrophidion percarinatum*, *Philothamnus natalensis*, *Phrynonax poecilonotus*, *P. shropshirei*, *Psammodynastes pictus*, *Sibynomorphus turgidus*, *Spilotes sulphureus*, and *Trimeresurus fucata*. Throughout the entire tree, most genera were monophyletic with varied node support. Space does not allow for exhaustive scrutiny at the generic and species level of our tree with previous publications, although a cursory examination reveals consistency with previous publications. Instead, we focus on assessing the placement of paraphyletic genera, most of which require greater sampling of species and genes, or perhaps individuals, to provide an improved appraisal of their phylogenetic positions.

Paraphyly at the lower-level of the tree emerged due to various reasons. For some clades paraphyly is well-established and confirmed here, more notably in *Brachyophidium*, *Pseudotyphlops*, *Rhinophis*, and *Uropeltis* in Uropeltidae (Fig. 1.2D*i*) (Bossuyt et al., 2004; Pyron et al., 2013a; Pyron et al., 2013b; Reynolds et al., 2014); *Ovophis* and *Trimeresurus* in respect to *Ovophis okinavensis* + *Trimeresurus gracilis* as basal to *Gloydius* (Fig. 1.2F)

(Malhotra and Thorpe, 2004; Malhotra et al., 2010); *Adelophis*, *Amphiesma*, *Atretium*, *Nerodia*, *Regina*, *Thamnophis*, *Tropidoclonion*, and *Xenochrophis* in Natricinae (Fig. 1.2K) (Alfaro and Arnold, 2001; Guo et al., 2014; McVay et al., 2015; Pyron et al., 2013a); and *Dipsas*, *Geophis*, and *Sibynomorphus* in Dipsadinae (Fig. 1.2L) (Grazziotin et al., 2012; Pyron et al., 2013a; Vidal et al., 2010a; Zaher et al., 2009). Additional taxa include: variable placement of *Morelia viridis* (Fig. 1.2D*i*) (Kluge, 1993; Lawson et al., 2004; Pyron et al., 2013a; Rawlings et al., 2008) and *Bothrocophias campbelli* (Fig. 1.2F) (Fenwick et al., 2009); and *Suta* with *Parasuta* (Fig. 1.2J) (Pyron et al., 2013a; Sanders et al., 2008). Clearly, these clades require further inspection. On the other hand, we were able to rectify other paraphyletic taxa with strong support, specifically within Colubrinae: *Boiga*, *Chironius*, *Coronella*, *Crotaphopeltis*, *Dasypeltis*, *Dipsadoboa*, *Hapsidophrys*, and *Philothamnus*, *Rhinechis,* and *Scaphiophis*.

In some taxa, such as *Cerrophidion wilsoni* (Fig. 1.2F), *Atractus irregularis* (Fig. 1.2G), *Ditypophis* sp. (Fig. 1.2H*ii*), *Aspidelaps irregularis* (Fig. 1.2I), *Pseudonaja guttata* (Fig. 1.2I), *Geophis* with *Atractus* (Fig. 1.2L), *Sibon noalamina* (Fig. 1.2L), *Philodryas chamissonis* and *P. trilineata* (Fig. 1.2M), *Conophis* and *Conopsis* (Fig. 1.2M & Fig. 1.2R), *Ptyas korros* (Fig. 1.2Q), *Tantilla melanocephala* (Fig. 1.2R), and *Salvadora hexalepis* (Fig. 1.2R), sequence overlap with related taxa was zero or minimal. Whereas for the following taxa, their placement were unresolved: Typhlopidae, *Rhinotyphlops unitaeniata* (Fig. 1.2B); Uropeltidae, *Rhinophis philippinus* (Fig. 1.2D); Pythonidae, *Simalia oenpelliensis* (Fig. 1.2D); Viperidae, *Atropoides picadoi* and *Bothrops lojanus* (Fig. 1.2F); Elapidae, *Toxicocalamus loriae* (Fig. 1.2I); Natricinae, *Macropisthodon rhodolemas* ADM0003 (Fig. 1.2K); Dipsadinae, *Oxyrhopus fitzingeri* LSUMNS6586 and *Siphlophis cervinus* (Fig. 1.2M); Calamariinae, *Pseudorabdion oxycephalum* (Fig. 1.2O); and Colubrinae, *Hierophis andreanus* and *Dolichophis cypriensis* (Fig. 1.2P*i*),

Pantherophis and *Pituophis* (Fig. 1.2Q), *Drymobius rhombifer, Dendrophidion dendrophis, Chilomeniscus stramineus, Tantilla melanocephala,* and *Salvadora hexalepis* (Fig. 1.2R).We do not classify *Calliophis* and *Sinomicrurus* as paraphyletic until the identity of *Calliophis* sp. is known.

For some clades, paraphyly was strongly supported allowing us to synonymize these taxa. Within Psammophiinae, we synonymize *Rhagerhis moilensis with Malpolon*. This species consistently forms a monophyletic clade with *Malpolon* (Carranza et al., 2006; Kelly et al., 2008; Kelly et al., 2009; Pyron et al., 2013a) (Fig. 1.2G), but two studies Böhme and De Pury, 2011; Vidal et al., 2008), inaccurately cite Kelly et al. (2008) as providing evidence for their separation. In Aparallactinae, we synonymize *Xenocalamus* with *Amblyodipsas* (Fig. 1.2G), also recovered in Pyron et al. (2013a), the only other study including these taxa. Within Colubrinae we synonymize several clades. First, we synonymize *Lepturophis* and *Dryocalamus* with *Lycodon*, which forms a strong clade (SHL = 100) with these taxa strongly embedded within (Grismer et al., 2014; Pyron et al., 2013a) (Fig. 1.2P*ii*). Next, we synonymize *Rhinechis scalaris*, a species with an erratic phylogenetic history (Lenk et al., 2001; Utiger et al., 2002), with *Zamenis*, but the addition of more genes shows it related to *Zamenis* (Burbrink and Lawson, 2007; Pyron et al., 2013a) (Fig. 1.2Q), with which it has morphological affinities to (Schulz, 1995). Finally, we also synonymize *Cyclophiops* with *Ptyas*. Previously recovered as sister clades (Chen et al., 2014; Pyron et al., 2013a), our increased sampling for both genera shows that *Ptyas* forms a strong clade (SHL = 95) with the two species of *Cyclophiops* strongly nested within two separate subclades (Fig. 1.2R). Conversely, in other clades paraphyly was strong, but we do not propose taxonomic changes, specifically in *Hebius sauteri* placing with *Amphiesma* (Fig. 1.2K), *Balanophis ceylonensis* within *Rhabdophis* (Fig. 1.2K), *Thamnodynastes pallidus*

placing with *Sibynomorphus* (Fig. 1.2L), *Pliocercus* split (Fig. 1.2L & 1.2M), *Ninia* split (Fig. 1.2L & 1.2M), *Dispholidus typus* within *Thelotornis* (Fig. 1.2O), *Chionactis occipitalis* placing with *Sonora* (Fig. 1.2R), and *P. shropshirei* LSUMNS7806 within *Spilotes* (Fig. 1.2R), mainly because these taxa, or taxa they placed with, are presented for the first time in a phylogenetic analysis.

In the case of *Hemerophis*, after the genus *Bamanophis* was erected for *Coluber dorri* (Schätti and Trape, 2008), *H. zebrinus* remained as the only Old World *Coluber* representative, until it was recently recognized as *Hemerophis* without justification (Uetz and Hošek, 2015; Wallach et al., 2014). Yet, the two are distantly-related within a clade of Old World racers (Nagy et al., 2003; Nagy et al., 2004; Pyron et al., 2011; Pyron et al., 2013a). *H. zebrinus* is typically placed in a clade sister to *Bamanophis* and *Macroprotodon*, but a very recent study incorporating new sequence data for *Rhynchocalamus*, not included here, places *H. zebrinus* as the basal lineage within this clade sister to (*Bamanophis* + *Macroprotodon*) and all other Old World racers Šmíd et al. (2015); while *H. socotrae*, occupies a branch away from this clade. Nagy et al. (2004) shows weak support for a sister relationship between the two using maximum parsimony, but shows them separated with greater support using Bayesian inference and ML. Therefore, we create a new genus for *H. zebrinus*, *Mopanveldophis* **gen. nov.**

Supermatrix approach

Despite the utility of the supermatrix approach, this method is also potentially responsible for uncertainty in some nodes. Compiling available molecular data from numerous studies leads to a sparse data matrix with a substantial portion of missing data unequally scattered throughout

the alignment due to sampling differences between studies (Burleigh et al., 2015). Our dataset consisted of 71.41% of missing data with several taxa represented by a single gene to taxa with data spanning all loci. Heterogeneity in sparse data matrices can alter topological relationships and negatively impact tree support by increasing the presence of rogue taxa (Wilkinson, 1996). Rogue taxa typically are characterized by little character data that do not overlap with closely-related taxa (Thomson and Shaffer, 2009). We identified and removed 22 rogue taxa from our data matrix, 12 of which were delineated by one gene and eight by two genes. The genes 12S, 16S, c-mos, and ND4 were most associated with rogue taxa. These genes evolve more slowly and are not adequate for delimiting species-level relationships (see methods), and several families in our tree are only represented by one or two individuals with few sequenced loci (i.e., Anomalepididae, Anomochilidae, Bolyeridae, Cylindrophiidae, and Xenophidiidae; Table 1.1). Many taxa in the tree with low support were also represented by a single gene. Furthermore, lack of sequence overlap between closely-related species can also lead to misplacement of taxa in the tree, sometimes with high support as mentioned above. However, many taxa with extensive missing data were placed correctly in the tree (e.g., *Chironius multiventris*, *Pseudocerastes urarachnoides*, *Rhabdophis chrysargos*, *Trimeresurus wiroti*), grouping with closely-related taxa with high support, confirming that increased taxon sampling is a favorable choice for improving phylogenetic accuracy (Hedtke et al., 2006), even with a high percentage of missing data (Wiens and Tiu, 2012). This can occur when the overall number of characters in the data matrix is high (Driskell et al., 2004; Philippe et al., 2004; Roure et al., 2013; Wiens and Moen, 2008; Wiens and Morrill, 2011), especially for SHL support values since they are not negatively affected by the amount of missing data in the data matrix (Pyron et al., 2011).

In many cases, denser sampling influenced phylogenetic relationships and node support (Nabhan and Sarkar, 2011). For example, adding 30 samples of 18 species (14 never before sequenced) to Ahaetuliinae, resolved the basal Colubrinae node and distinguished Ahaetuliinae as a new subfamily. Increased taxon sampling also resolved several paraphyletic issues at the generic level, identified new associations of paraphyly, mostly due to poor gene sampling, resulted in new phylogenetic hypotheses for some taxa such as *Scaphiophis*, *Stichophanes* + *Thermophis*, and *Xerotyphlops*, and prompted us to make some taxonomic changes. Moreover, our sequencing contribution resulted in complete or nearly complete taxonomic coverage of several genera, including *Ahaetulla*, *Asthenodipsas*, *Chrysopelea*, *Dendroaspis*, *Dryocalamus*, *Dryophiops*, *Phrynonax*, *Ptyas*, and *Ungaliophis*, and greatly increased representation of species of the speciose genera *Boiga* and *Dendrelaphis*. Nonetheless, many challenges exist to estimating the snake tree of life.

Taxonomic descriptions

Subfamily Ahaetuliinae subfam. nov. urn:lsid:zoobank.org:act: 22C47597-1DEF-45A4-ABAC-11C4911557AD

Type genus. *Ahaetulla* Link (1807)

Content. Four genera containing 56 species. *Ahaetulla* (8 species), *Chrysopelea* (5 species), *Dendrelaphis* (41 species), and *Dryophiops* (2 species).

Etymology. From the Sri Lankan language Sinhala, ahaetulla/ahata gulla/as gulla, meaning “eye plucker” or “eye picker” for belief that they pluck out the eyes of humans

as accounted by the Portuguese traveler João Ribeiro in 1685 (as cited in Weinstein et al., 2011).

Diagnosis and Definition. Snakes of this subfamily are arboreal and are diagnosed by keeled ventral and subcaudal scales (laterally notched in some species), and enlarged posterior grooved fangs lacking in some *Dendrelaphis*. Support for monophyly of this clade is very strong (SHL = 100) as also reported in Pyron et al. (2013a). Ahaetuliinae is further split into two monophyletic groups: 1) *Dryophiops* and *Ahaetulla* (SHL = 96) and; 2) *Chrysopelea* and *Dendrelaphis* (SHL = 100). Diagnostic characteristics of the first group include, elongate and laterally-compressed bodies, elongate heads, 15 smooth mid-body dorsal scale rows, and large eyes with horizontal pupils and well-developed canthus rostralis outfitting these snakes with binocular vision (Walls, 1942). Features diagnostic of the second group include, slender body, rectangular slightly compressed heads, large eyes with round pupils, 13–17 smooth to weakly-keeled mid-body dorsal scale rows. *Chrysopelea* are celebrated for their unique gliding behavior, whereas *Dendrelaphis* are capable of jumping (Socha, 2011).

Sister taxon. Previously placed within Colubrinae, Ahaetuliinae forms a strong (SHL = 95) sister relationship with Colubrinae, also weakly supported by Pyron et al. (2013a).

Distribution. Members of this subfamily inhabit various habitats, but are mostly associated with forests distributed from Pakistan, Sri Lanka and India, north to Nepal and Bangladesh, eastwards all throughout Southeast Asia to southern China, Philippines, Papua New Guinea, and northeast Australia.

Remarks. The name *Ahaetulla* has suffered from a tumultuous nomenclatural history (Savage and Oliver, 1956). In addition, members of these genera have historically been

grouped with unrelated taxa based on absence or presence of hypapophyses (Boulenger, 1896; Brongersma, 1938).

Genus *Mopanveldophis* gen. nov. urn:lsid:zoobank.org:act: 3B0CB6A0-1EEC-4512-9E77-B105C22ACABB

Type species. *Mopanveldophis zebrinus*.

Content. The genus is monotypic containing only the species, *Mopanveldophis zebrinus*.

Etymology. The generic nomen *Mopanveldophis* is derived from the word "mopanveld", the name of the type of habitat the specimens were found in, and the Greek adjective *ophis*, meaning "snake". This name refers to veld habitat distributed in Southern Africa, from the Afrikaans word "field", that is dominated by the mopane tree, *Colophospermum mopane*, from the Sechuana word "mopani".

Diagnosis and Definition. As described in Broadley and Schätti (1997) and Bauer et al. (2001), a snake with pale grey dorsal coloration and irregular broad, dark crossbands becoming faint in coloration posteriorly and on tail. Ventrals are uniform white with irregular lateral black spots, and subcaudals are also white with lateral grey stippling. Dorsal portion of head is uniform grey-brown with yellowish orange snout and labials, and dark markings on supralabials 2-6. Dorsal scales with two apical pits, 23 scale rows near neck, 23 at midbody, and 17–19 anterior to the vent. Approximately 195 ventrals, 90 paired subcaudals, and divided anal scute. Nine supralabials with the fifth and sixth entering the orbit, one anterior subocular smaller than the loreal shield and situated above the fourth and anterior part of the fifth supralabials, and two preoculars and two postoculars. Also, diagnosed by a single large lower anterior temporal shield above the

7th and 8th supralabials, two upper anterior temporal, three posterior temporal, and maxillary with 17 + 2 teeth separated by a diastema. Its banded pattern was suggested as Batesian mimicry of the sympatric spitting cobra, *Naja nigricollis*. *Bamanophis* differs by having 25–27 scale rows near neck, 29–33 at midbody, and 17 near vent, 229-265 ventral scale and 75–95 paired subcaudals, lacking an anterior subocular, having one posterior subocular, 10 supralabials, and 15–19 maxillary teeth with diastema (Schätti and Trape, 2008).

Sister taxa. *M. zebrinus* is basal lineage to a clade including *Bamanophis* + *Macroprotodon*, placed within a larger clade of Old World racers (Nagy et al., 2003; Nagy et al., 2004; Pyron et al., 2011; Pyron et al., 2013a).

Distribution. Currently recognized as endemic to northern Namibia, Africa (Herrmann and Branch, 2013), but its range may extend into Angola, Africa (Bauer et al., 2001).

Remarks. First described from a dead specimen collected in 1991 (Broadley and Schätti, 1997), the species is currently known from only three specimens (Bauer et al., 2001). Upon its description it was assigned to the genus *Coluber*, presumably on basis of similar morphology, but then switched to *Hemerophis* (Uetz and Hošek, 2015; Wallach et al., 2014) with no published reasoning. Schätti and Trape (2008) provide an account detailing the differences of *Bamanophis* to other racer species, including *M. zebrinus*.

Conclusions

At less than half (46.33%) of the total snake diversity sampled, we provide the most comprehensive sampling effort to date, but remain far from fully estimating the snake tree of life.

This sampling effort pales in comparison to larger clades such as birds that have approximately 70% of more than 10,000 species sequenced (Burleigh et al., 2015). Although our results provide resolution for several higher-level nodes, these nodes may continue to prove problematic. Collectively, future analyses should target or pay special attention to the following ten issues: 1) resolving topology of Scolecophidia; 2) resolving topology of Typhlopinae; 3) resolving paraphyly of Cylindrophiidae with Anomochilidae; 4) placement of Xenophidiidae and Bolyeridae; 5) resolving topology of Booidea; 6) placement of Xenodermatidae; 7) placement of Pareatidae; 8) placement of Homalopsidae; 9) resolving topology of Lamprophiidae + Elapidae; and 10) resolving topology of Colubridae. Clearly, greater taxon and gene sampling will help better formulate a picture of snake relationships and resolve ambiguous nodes in the tree (Hedtke et al., 2006; Nabhan and Sarkar, 2011). Taxa most lacking in representation are fossorial clades, mainly Afrotyphlopinae, Anomalepididae, Aparallactinae, Calamariinae, Cylindrophiidae, Epictinae, Gerrhopilidae, Madatyphlopinae, Uropeltidae, and Xenodermatidae at below 30% (Table 1.1). Similar deficiencies occur at the genus level, but are not listed here. The genes most frequently sampled for snakes are 12S, 16S, c-mos, cyt-b, and ND4, and should be considered as candidate genes in future studies. Sampling more nuclear genes will also be crucial in resolving deeper nodes (Zheng and Wiens, 2016). Where coalescence-based methods are practiced, researchers should place emphasis on short and weakly supported branches since they are more prone to incomplete lineage sorting and thus, conflict most often with branches on species-trees (Lambert et al., 2015). This phylogeny has major implications on snake evolution such as on the evolution of gape size and the evolution of venom-delivery systems (Scanlon and Lee, 2011; Vidal and Hedges, 2002b; Vidal et al., 2009), and serves as a resource for formulating future studies on snake phylogenetics.

Acknowledgements

We are indebted to the many researchers who donated tissue samples to museum collections and uploaded their sequences on GenBank. For supporting this research in the way of loaning tissue samples, We would like to acknowledge curators and staff at the Ambrose Monell Cryo Collection (AMCC) at the American Museum of Natural History, New York (AMNH), the California Academy of Sciences (CAS), the Field Museum of Natural History (FMNH), the Natural History Museum & Biodiversity Research Center at the University of Kansas (KU), and the LSU Museum of Natural Science Collection of Genetic Resources (LSUMNS) and L.J. Vitt and his NSF grants DEB-9200779 and DEB-9505518, the Museum of Vertebrate Zoology (MVZ), and the Peabody Museum of Natural History Yale University (YPM).

We thank Robin Rowe at the University of New Orleans W.M. Keck Conservation and Molecular Genetics Laboratory for sequencing molecular samples, and Caño Palma Biological Research Station in Costa Rica and David Bickford at The National University of Singapore for providing lab space during field research. We also like to thank several anonymous reviewers for providing useful comments that helped improve the manuscript.

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Supporting Information

Table S1.1. List of GenBank accession numbers for 7 outgroup taxa and 1615 snake species. Two sequences were deleted during preliminary tree searches and 21 were identified as rogue taxa and pruned from the dataset leaving 1592 snake species from GenBank in the tree. Names represent species names as listed on The Reptile Database (http://www.reptile-database.org/) as of October 2015. Refer to S1.4 Table for list of rogue taxa. Taxa deleted during preliminary tree searches are highlighted in red, rogue taxa are highlighted in yellow, and sequences that were deleted because they were identical to other sequences are highlighted in green.

Table S1.2. List of taxa, institutional voucher numbers, and GenBank accession numbers for tissue samples extracted and sequenced in this study. Tissue samples for *Boiga siamensis* FMNH267726, *Chrysopelea ornata* LSUHC7158, and *Psammodynastes pictus* FMNH267940 were represented by clear chromatograms, but placed poorly in preliminary phylogenetic trees, so they were not included in the final data matrix. Therefore, we did not deposit these taxa in GenBank. *Tropidolaemus subannulatus* KU327425 was identified as a rogue taxon by RogueNaRok and was pruned from the dataset and thus, is not represented in the phylogeny.

Table S1.3. Six loci, gene type, gene length, primer name, PCR annealing temperature and primer source.

Table S1.4. Rogue taxa as identified by RogueNaRok Web-Server (http://rnr.h-its.org/submit). Each taxon is associated with a raw improvement score (R.I.S.), which represents the fraction of improvement in bootstrap support values throughout the tree when the selected taxon is pruned and all rogue taxa above it are also pruned. We performed one run and chose to sacrifice relatively lower node support values to maximize the number of taxa represented in the phylogeny. Thus we elected to only prune taxa with R.I.S. greater than 0.8, resulting in a total of 22 pruned taxa (highlighted in bold).

Figure S1.1. Abridged phylogeny displaying higher-level relationships with all rogue taxa and incertae sedis species eliminated. Maximum-likelihood phylogenetic estimate based on 10 concatenated genes. Tips represent families and sub-families. Commonly recognized higher-level clades are labeled in all caps and bold. Node values represent SHL support values. Skeleton of the species tree is displayed on the left, colored and labeled as they appear in Fig. 1.2.

File S1.1. Newick format maximum-likelihood phylogeny for 1745 taxa representing 1652 snake species and 7 outgroup taxa displayed in Fig. 1.2.

Chapter 2. Patterns of Lineage Diversification in Snakes: Testing Venom-Delivery as a Key Innovation

Abstract

Snakes represent an impressive evolutionary radiation of over 3,500 widely-distributed species encompassing a diverse range of morphologies and ecologies. This diversity is likely attributable to their distinctive morphology, which has allowed them to populate a wide range of habitat types. Species richness among snake families also varies considerably, from monotypic families such as Xenotyphlopidae and Aniliidae, to Dipsadinae which comprises 754 species. We used 14 fossil calibrations to date a recently published snake phylogeny comprising 1625 snake species delineating every extant family and subfamily to investigate snake macroevolutionary speciation dynamics. We also test if the clades Alethinophidia, Caenophidia, Viperidae, Lamprophiidae, Elapidae, Colubridae, Natricinae, Dipsadinae, and Colubrinae are characterized by increased speciation rates and if the evolution of venom-delivery in Colubroidea and front-fanged venom-delivery in Viperidae and Elapidae are distinguished by shifts in diversification rate. Our dates indicate snakes split from lizards approximately 120.74 mya with extant snakes originating 113 mya. Divergence between the two infraorders, Scolecophidia and Alethinophidia, occurred 112.96 mya, and the most diverse clade of snakes, the Caenophidia, arose 70.86 mya. Snake diversification carries the signature of ecological opportunity, with speciation rates declining over time. However, a small spike in speciation rates appears roughly

100 mya corresponding to the origin of Alethinophidia, and a more considerable increase accompanies the rise of Caenophidia. Heterogeneity in snake diversification rates is largely shaped by two slowdowns and five increases and is strongly supported by greater than 95% cumulative probability. Deceleration in speciation rates occurred within Scolecophidia and Henophidia; whereas the five gains developed over a relatively short period of time within clades of Caenophidia. The most notable of these increases happened within Viperidae and Elapidae, which both independently evolved extremely developed front-fanged venom-delivery systems and which accumulated 331 and 358 species, respectively. However, STRAPP analysis demonstrated that venom-delivery is not associated with increased diversification in these clades.

Introduction

Species richness is a testament to evolution's capacity to generate biodiversity. However, species diversity is unequally distributed and varies by orders of magnitude at all phylogenetic levels (Hutchinson, 1959; Hunt et al., 2007; Butlin et al., 2009). Disparity in species richness is most commonly attributed to differences in net diversification rates (i.e., differences in speciation and extinction rates) among clades (Kirkpatrick and Slatkin 1993; Mooers and Heard, 1997; Barraclough and Nee 2001) and is expected to leave a signature on phylogenetic trees (Ree, 2005). Where rate differences are large, this is manifest as shifts in diversification rates (Simpson, 1944; Sanderson and Donoghue, 1996). Researchers often aim to correlate these rate shifts with certain species traits (Slowinski and Guyer, 1993; Barraclough et al., 1998) or aspects of the environment (Davies et al., 2004; Weir and Schluter, 2007; Day et al., 2008) that may favor diversification (Heard and Hauser, 1995; Ree, 2005). Extinction is likely the main cause of depauperate lineages (Pyron and Burbrink, 2012), but ecological opportunity associated with the appearance of key innovations, transitions to new environments, or ecological release can also cause imbalance in species richness (Simpson 1953; Heard and Hauser, 1995; Donoghue, 2005; Yoder et al. 2010), although the tempo of diversification ultimately slows as ecomorphological niches fill (Nee et al., 1992; Schluter, 2000; Gavrilets and Losos 2009).

Key innovations play a crucial role in diversification because they contribute to ecological divergence by way of morphological and ecological specialization, leading to increases in diversification rates (Mitter et al., 1988; Heard and Hauser, 1995; Hodges and Arnold, 2005). As such, the appearance and influence of key innovations constitutes one of several evolutionary process that ultimately shape phylogenetic trees (Rabosky, 2014).

Phylogenetic evidence for an association between key innovations and increased diversification generally comes from comparisons of clade size (Heard and Hauser, 1995; Ree, 2005) or from state-dependent analyses (Maddison et al., 2007; Rabosky and Huang, 2015), but state-dependent analyses generally do not account for unmeasured traits that may act in conjunction with focal traits or may even explain more of the variation in rate shifts (Beaulieu and O'Meara, 2015). While key innovations have received a great deal of attention within the context of adaptive radiations (Yoder et al., 2010; Losos, 2010; Givnish, 2015), relatively few studies have examined how the evolution of such traits might affect diversity at the family- or genus-level. To accurately identify the lineages responsible for diversification rate shifts, it is important to have a well-resolved tree with fine phylogenetic resolution because rate shifts may be induced by multiple subclades as opposed to the clade as a whole (Alfaro et al., 2013).

Here, we use the species-level phylogeny of Chapter 1 comprising 1652 species to investigate how diversification rate heterogeneity shapes overall patterns of snake diversity, and to identify rate shifts. Snakes constitute an evolutionary radiation of over 3,500 extant species within Squamata (Uetz & Hošek, 2015), encompassing a diverse range of morphologies and habits (Gans, 1961; Greene, 1997). Two major features stand out as hallmarks of snake diversity making them an ideal model system for testing hypotheses regarding diversification and ecological opportunity: 1) species richness is extremely imbalanced from the genus-level upwards (see Chapter 1) and 2) the majority of snake diversity is concentrated in Alethinophidia, specifically within the superfamily Caenophidia and the family Colubridae. Snakes are divided into two infraorders; the basal Scolecophidia (‘blindsnakes’ and ‘threadsnakes’) and the Alethinophidia (‘typical snakes’). Scolecophidia accounts for only 417 snake species (11.7% of known snake diversity), of which all members are small, fossorial, possess short tails and

reduced eyes, have a small gape size, and feed frequently on ants and termites. Alethinophidia, on the other hand, hosts the remaining diversity of snakes, spanning all extremes of morphologies, habitats, diet, and habits. Species diversity within Alethinophidia is extremely disproportionate, with most species (ca. > 2900; > 82%) appointed to Caenophidia ('advanced snakes') disseminated across eight families. Yet, nearly all of the species diversity is contained within the following four families: 1) Viperidae; 2) Lamprophiidae; 3) Elapidae; and 4) Colubridae. Of these, Colubridae has by far the most species (> 1800) currently spread throughout eight subfamilies, accounting for 62% of the species in Caenophidia. More specifically, 1650 of these species reside within Natricinae, Dipsadinae, and Colubrinae. Reasons for this massive inequality in species richness remains unknown, but has been suggested to be related to the evolution of venom-delivery, with the presence of supralabial secretory serous cells early in the superfamily Colubroidea (Vidal, 2002; Jackson, 2003), which is a clade within Caenophidia composed of all the families except for Acrochordidae.

To date, the only other study examining clade-wide diversification rates in snakes is based on sampling performed at the genus-level (Pyron and Burbrink, 2012). This study showed a shift at the most recent common ancestor of Viperidae, Homalopsidae, Colubridae, Elapidae, and Lamprophiidae, and proposed that the key innovation of venom delivery systems along with the colonization of new areas, particularly of the New World, provided ecological opportunity that helped spur the increase in diversification rates in Colubroidea (Caenophidia minus Acrochordidae). However, the location of this shift does not provide evidence for venom as a key innovation, and since not all members of Colubroidea are venomous (Jackson, 2003), an increase in diversification rates should therefore be seen either at the base of Colubroidea or in clades with high species richness whose members are all venomous. Additional examinations

into snake diversification rates focused on smaller, individual clades such as crotalids, lampropeltines, thamnophiines (Burbrink and Pyron, 2010; Burbrink et al., 2012a; McVay et al., 2015), alsophiines (Burbrink et al., 2012b), elapids (Lee et al., 2016), 2010), the sea snake genus *Hydrophis* (Lukoschek and Keogh, 2006; Sanders et al., 2010), rattlesnakes (Blair and Sánchez-Ramírez, 2016), and viperids (Lynch, 2009), but lacked the comparative, macroevolutionary approach required to detect higher-level shifts in diversification rates (Rabosky, 2014). In this paper, we consider how and why species-richness in snakes is imbalanced by first time-calibrating the phylogeny of Chapter 1 and estimating divergence times. We then test the following three hypotheses: 1) Speciation rates increase in Alethinophidia, Caenophidia, Viperidae, Lamprophiidae, Elapidae, Colubridae, Natricinae, Dipsadinae, and Colubrinae; 2) Diversification shift occurs at the base of Colubroidea in association with the evolution of venom; 3) Diversification shifts are associated with the independent evolution of specialized front-fanged venom-delivery systems in Viperidae and Elapidae, and their resultant high species richness.

Materials and Methods

Phylogeny and Divergence Time Estimation

The phylogeny of Chapter 1 is the largest-yet estimate of the snake tree of life and used a maximum likelihood approach on a supermatrix of 1745 taxa (1652 snake species + 7 outgroup taxa) and 9,523 base pairs, representing all recognized families and subfamilies. Owing to the size of their dataset, we used treePL to date the tree (Smith and O'Meara, 2012), which

implements the penalized likelihood optimality criterion to penalize rate differences across the tree by allowing for different rates on different branches (Sanderson, 2002). This program combines the standard derivative-based "greedy hill-climbing" optimization with a stochastic partial simulated annealing algorithm to overcome optimization challenges of local minima in estimating divergence times in large phylogenetic trees (Smith and O'Meara, 2012). In total, we constrained the ages of 14 nodes using minimum and maximum ages from fossil specimens as constraints (Table S2.1). Selection of fossil calibration points for snakes has a controversial history based on taxonomic uncertainty, making dates from some previous studies unreliable (for details see Sanders et al., 2010; Head, 2015). Since tree shape is fashioned by how node ages are distributed over time, leading to asymmetry among lineages across the entire tree, increased taxon sampling is considered to improve the accuracy of divergence estimates by minimizing overrepresentation of older nodes in rate variation (Nee et al., 1994; Heath et al., 2008). We ran treePL using a two-step process. First, we ran a random subsample and replicate cross-validation (RSRCV) analysis from 0.001 to 100,000, increasing in increments of 0.1, to determine the optimal smoothing value. We selected the optimal smoothing value with the lowest Chi-square value (0.1) used during the penalty procedure process of penalized likelihood. RSRCV randomly samples with replacement multiple terminals and is much faster and produces similar results to standard cross-validation where each terminal taxon is iteratively removed (Smith and O'Meara, 2012). Second, we ran a thorough analysis under the additive penalty function (untransformed rates), applicable when root nodes are calibrated (Sanderson, 2002), set with the following parameters: gradient-based, auto-differentiation based, and auto-differentiation cross-validation-based optimizers were all set to 1; penalized likelihood replicates = 200,000; cross validation simulated annealing iterations = 50,000. Prior to comparative analyses we removed outgroup

taxa and pruned the following *incertae sedis* taxa and species responsible for paraphyly: *Aspidelaps lubricus*, *Atractaspis irregularis*, *Atropoides picadoi*, *Apostolepis sanctaeritae*, *Bothrocophias campbelli*, *Bothrops lojanus*, *Bothrops isabelae*, *Buhoma depressiceps*, *Buhoma procterae*, *Cerrophidion wilsoni*, *Conophis lineatus*, *Conophis vittatus*, *Conopsis biserialis*, *Conopsis nasus*, *Geophis godmani*, *Morelia viridis*, *Micrelaps bicoloratus*, *Oxyrhopus fitzingeri*LSUMNS6586, *Oxyrhabdium leporinum*, *Philodryas chamissonis*, *Philodryas trilineata*, *Ptyas korros*, *Pseudonaja guttata*, *Salvadora hexalepis*, *Sibon noalamina*, *Simalia oenpelliensis*, *Tantilla melanocephala*.

Lineage Diversification

We tested heterogeneity in species richness by modelling macroevolutionary dynamics of diversification using Bayesian analysis of macroevolutionary mixtures (BAMM; Rabosky et al., 2013; Rabosky, 2014; Rabosky et al., 2014a). BAMM uses reversible-jump Metropolis-coupled Markov Chain Monte Carlo (MC3) to detect and quantify heterogeneity in evolutionary rates and to detect subclades sharing a common macroevolutionary rate dynamic by mapping distinct sets of rate shifts and identifying their location on the tree. Importantly, BAMM does not identify a single set of independent rate shifts within a given dataset, but instead classifies configurations of rate shifts (i.e., sets of shifts that are sample together; Rabosky et al., 2014a). We applied BAMM to the time-calibrated tree running MC3 for 10 million generations and sampling from the posterior distribution every 1000 generations. The first 10% of samples we discarded as burn-in, then we checked for convergence of parameter estimates (i.e., log-likelihoods, numbers of processes, and evolutionary rate parameters) by evaluating means of effective sample sizes

using the R package CODA (Plummer et al., 2006). We summarized and visualized the tree with mapped macroevolutionary rate parameters using the R package BAMMtools (Rabosky et al., 2014b). Since all our shift configurations had low probabilities, we were unable to extract the single shift configuration with the highest posterior probability. Instead, we used the maximum shift credibility to extract the shift configuration that maximizes the marginal probability of rate shifts along individual branches (Rabosky, 2014). For comparison, we also calculated model-averaged diversification rates and shifts. For clades experiencing shifts in diversification patterns, we estimated rate-through-time curves from the joint posterior density of parameters. We also calculated the 95% cumulative shift probability that a diversification shift occurred on each branch. Finally, we identified different macroevolutionary cohort regimes (i.e., shared, potentially dynamic diversification process shared by all lineages downstream from the location of a rate shift; Shi and Rabosky, 2015). For these analyses, we incorporated incomplete taxon sampling (Shi and Rabosky, 2015) at the genus level (Table 2.1). There is a total of 515 snake genera (Uetz & Hošek, 2015), of these, we sampled 402 genera, leaving 113 not sampled, and accounted for 46.33% of the total extant snake diversity (Table S2.2).

Table 2.1. Number of taxa sampled per family or subfamily. Families are listed in order according to Figure 2.1. For the taxonomy of families and subfamilies, we use Adalsteinsson et al., (2009) for Anomalepididae and Leptotyphlopidae, Pyron and Wallach (2014) for Gerrhopilidae, Typhlopidae, and Xenotyphlopidae, Pyron et al. (2014b) for Booidea, and Pyron et al. (2013a) for Alethinophidia. The number of species per clade was taken from The Reptile Database (http://www.reptile-database.org/) on 10/01/2015. Percentages of the number of species sampled do not include taxa not assigned to species status. Paraphyletic taxa are included under their traditional family and/or subfamily. In the Total cell for total number of species, the number not in parentheses equals the sum of the values in the table and the number in the parentheses equals the number returned when a search for Serpentes is conducted in The Reptile Database. Percentage for total number of species sampled is based on 3566 species. Total sampled snake diversity is 46.33%. For those clades represented by one species, we used the date of their divergence from their sister clade. Dates for the following papers represent divergence dates from sister clade, not age of clade: Burbrink and Pyron (2008), Vidal et al. (2009); Scanlon and

Lee (2011), and; Pyron and Burbrink (2012). Pan-Serpentes (total-group) = fossil stem snakes + crown snakes. Serpentes (crown-group) = extant snakes + extinct taxa.

Taxon	Species Richness	Clade Age (millions of years)					
		Burbrink & Pyron, 2008	Vidal et al., 2009	Scanlon & Lee, 2011	Pyron & Burbrink, 2012	Zheng & Wiens, 2015	This Study
Pan-Serpentes (Total)	--	N/A	166.0	162.0*	N/A	128.10	120.74
Serpentes (Crown)	--	144.2	159.9	113.5	140.80	122.73	113.00
Scolecophidia	--	144.2	159.9	108.58	140.80	122.73	113.00
Anomalepididae	18 (11%)	N/A	N/A	108.58	134.59	40.72	78.84
Leptotyphlopidae	--	109.3	151.9	92.79	131.27	89.60	103.68
Epictinae	64 (23%)	N/A	N/A	N/A	N/A	75.94	94.80
Leptotyphlopinae	50 (36%)	N/A	N/A	N/A	N/A	81.61	83.11
Gerrhopilidae	18 (11%)	N/A	N/A	N/A	74.37	80.45	57.18
Xenotyphlopidae	1 (100%)	N/A	N/A	N/A	59.41	85.41	105.1
Typhlopidae	--	109.3	151.9	92.79	59.41	70.66	102.78
Typhlopinae	64 (52%)	N/A	N/A	N/A	N/A	40.40	98.62
Afrotyphlopinae	61 (26%)	N/A	N/A	N/A	N/A	34.76	60.97
Madatyphlopinae	13 (15%)	N/A	N/A	N/A	N/A	68.08	41.25
Asiatyphlopinae	124 (33%)	N/A	N/A	N/A	N/A	62.91	61.56 + 90.77
Alethinophidia	--	132.9	105.8	92.83	101.50	92.70	100.50
Aniliidae	1 (100%)	63.1	89.1	79.54	91.80	79.81	84.89
Tropidophiidae	34 (29%)	63.1	89.1	18.18	91.80	24.60	73.49
Calabariidae	1 (100%)	50.2	N/A	72.84	55.65	45.36	76.80
Candoiidae	5 (60%)	N/A	N/A	62.43	N/A	16.60	62.57
Sanziniidae	4 (75%)	N/A	N/A	N/A	N/A	16.92	73.66
Charinidae	--	N/A	N/A	60.27	N/A	35.47	98.64
Charininae	4 (75%)	N/A	N/A	60.27	N/A	19.82	71.01
Ungaliophiinae	3 (100%)	44.5	N/A	39.41	N/A	26.66	97.05
Erycidae	12 (75%)	N/A	N/A	64.47	45.02	31.47	72.93
Boidae	30 (80%)	44.5	86.3	57.55	45.02	32.09	64.00
Cylindrophiidae	13 (15%)	41.0	N/A	47.39	44.45	29.20	60.20
Anomochilidae	3 (33%)	N/A	N/A	39.94	44.45	24.72	33.22
Uropeltidae	54 (28%)	41.0	92.0	39.94	56.84	36.59	81.15
Xenopeltidae	2 (50%)	51.3	70.1	72.28	77.00	52.41	99.86
Loxocemidae	1 (100%)	37.1	43.7	44.83	47.12	33.42	99.48
Pythonidae	40 (80%)	37.1	43.7	23.93	47.12	22.67	99.34
Bolyeridae	2 (50%)	N/A	96.9	70.98	68.40	48.48	50.48
Xenophidiidae	2 (50%)	N/A	N/A	67.41	68.40	48.48	50.48
Caenophidia	--	N/A	90.7	53.91	N/A	80.59	70.00
Acrochordidae	3 (100%)	N/A	90.7	53.91	84.66	30.77	25.00
Colubroidea	--	N/A	82.2	43.09	84.70	75.20	69.99
Xenodermatidae	18 (22%)	N/A	82.2	43.09	76.08	38.57	60.10
Pareatidae	20 (80%)	46.6	64.0	N/A	65.39	40.65	64.61
Viperidae	--	32.9	54.3	28.65	N/A	42.83	45.00
Viperinae	98 (67%)	N/A	N/A	N/A	30.89	36.26	41.84

Table 2.1 Continued.

Azemiopinae	2 (50%)	N/A	N/A	N/A	30.39	37.22	44.99
Crotalinae	231 (82%)	N/A	N/A	N/A	35.66	31.75	44.98
Homalopsidae	53 (47%)	41.6	49.2	N/A	53.38	27.79	65.05
Lamprophiidae	--	N/A	41.5	N/A	N/A	47.86	69.95
Psammophiinae	52 (77%)	27.4	N/A	N/A	34.87	29.89	66.76
Prosymninae	16 (31%)	N/A	N/A	N/A	44.51	36.73	39.59
Pseudaspidinae	2 (100%)	27.4	N/A	N/A	28.90	28.36	37.51
Atractaspidinae	23 (30%)	32.8	N/A	N/A	30.29	32.02	38.95
Aparallactinae	47 (23%)	N/A	N/A	N/A	30.29	32.58	42.74
Lamprophiinae	72 (43%)	N/A	N/A	N/A	28.90	35.38	69.72
Pseudoxyrhophiinae	89 (64%)	30.3	N/A	N/A	34.86	35.63	65.03
Elapidae	358 (54%)	25.6	41.5	N/A	34.86	38.96	35.00
Colubridae	--	N/A	N/A	28.65	N/A	48.69	69.97
Sibynophiinae	11 (55%)	N/A	N/A	N/A	N/A	37.34	54.32
Natricinae	226 (47%)	N/A	39.8	N/A	38.28	36.99	68.22
Pseudoxenodontinae	11 (36%)	38.2	32.9	N/A	N/A	21.78	54.34
Dipsadinae	754 (32%)	34.6	32.9	N/A	33.65	41.52	69.95
Grayiinae	4 (75%)	N/A	N/A	N/A	30.42	25.10	41.87
Calamariinae	87 (5%)	34.6	N/A	N/A	30.42	33.45	43.40
Ahaetullinae	56 (48%)	N/A	N/A	N/A	N/A	31.38	62.42
Colubrinae	670 (47%)	38.2	36.6	N/A	35.63	40.34	69.95

*The authors report the date of the Anguimorpha - Pan-Serpentes divergence at 162 in their figure 3.2 and as 172 in the text. In this table we record the date provided in the figure.

Recently, several papers have demonstrated that state-dependent diversification analyses suffer from high Type I errors (FitzJohn, 2012; Machac, 2014; Rabosky and Goldberg, 2015) partially due to phylogenetic pseudoreplication (Maddison and FitzJohn, 2014) and additional unknown factors (Rabosky and Goldberg, 2015). Based on these and additional criticisms, Rabosky and Huang (2015) declare that "it seems likely that many trait-dependent diversification relationships reported in the literature are not real". At the forefront of these analyses is BiSSE, which does not test if independent shifts in character state correlate with shifts in diversification (Maddison and FitzJohn, 2014) and assumes all variation in diversification rates can be explained by the proposed two character states (Maddison et al., 2007). Two recently introduced methods, the hidden state speciation and extinction model (HiSSE; Beaulieu and O'Meara, 2015) and structured rate permutations on phylogenies (STRAPP; Rabosky and Huang, 2015), attempt

to alleviate this issue. HiSSE accounts for variation in diversification rates which may be attributable to a non-specified, unobserved discrete ("hidden state") trait, which may display diversification dynamics and transition rates distinct from the focal trait. However, HiSSE also does not account for phylogenetic pseudoreplication (Rabosky and Huang, 2015). STRAPP, on the other hand, tests association between trait and diversification rates against a null distribution generated by taxon-block permutations that randomly reshuffles diversification rates throughout the tree preserving the covariances in rate regimes among taxa, making it robust to phylogenetic pseudoreplication (Rabosky and Huang, 2015). The main advantages of STRAPP is that it unambiguously accounts for the number of independent diversification rate shifts in the phylogeny and does not require that variation in diversification rates be explained by specified character states. Therefore, to test if venom-delivery or goo-eating is associated with shifts in diversification rates, we used STRAPP, as implemented in BAMMtools, to test the correlation between the trait and the BAMM estimated diversification rates. We ran 10000 permutations and used the Mann-Whitney *U*-test statistic to check for significance between diversification rates and created to files with binary traits coded as front-fanged venom-delivery (non-venomous or venomous).

Results

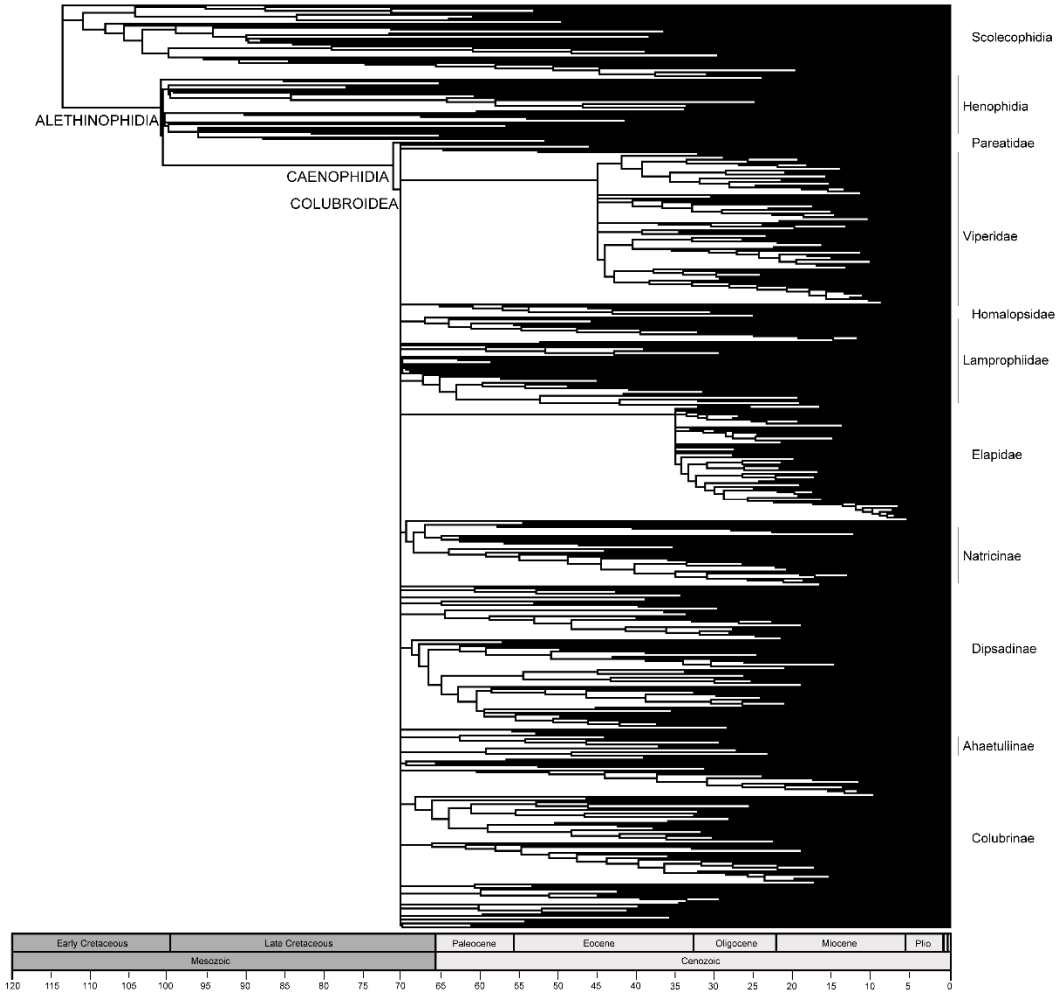
Time-calibrated phylogeny

A summary of the time-calibrated tree is presented in Fig. 2.1 and the full time-calibrated tree is available in Newick format in File S2.1. Inferring the date of origin for any given clade

requires the ancestral node having at least two descendant species; if not, then only the date of divergence from the most recent common ancestor (MRCA) with the sister clade is provided. Estimated dates for the major snake clades vary considerably among studies (Table 2.1; see Hsiang et al., 2015 for approximate dates) and from studies providing divergence estimates for smaller clades: blindsnakes (Vidal et al., 2010); Henophidia (Noonan and Chippindale, 2006; Sanders et al., 2010); elapids (Sanders et al., 2008; Sanders et al., 2013a); and Natricinae (McVay et al., 2015). However, dates for Viperidae were similar to those reported in a previous study (Wüster et al., 2008). These discrepancies are likely due to differences in fossil calibrations chosen and divergence estimate methods. Similar to other studies using treePL, some of our calibrated nodes remained stuck on the minimum or maximum calibration date (Shi & Rabosky, 2015), probably due to very short internal branches leading to little correspondence between the model of autocorrelated rates and the rates in the tree (S. Smith personal communication). Zheng and Wiens (2015) also used treePL, but they estimated divergence dates for all of Squamata, and only specified one calibration point (at the root) for snakes.

Based on our estimates, snakes split from Anguimorpha + Iguania approximately 120.74 million years ago (mya). Extant snakes originated about 113 mya with the divergence between the two Infraorders, Scolecophidia and Alethinophidia, occurring 112.96 mya. By 25 mya all the currently recognized families and subfamilies have evolved. Acrochordidae contains the youngest genera originating 25 mya and *Macrovipera* is the youngest genus appearing at 9.29 mya (Table S2.2). Divergence among the major clades within Scolecophidia and Henophidia proceeded slowly. The quickest splits within these clades were between Leptotyphlopidae and Typhlopidae, and between Xenopeltidae, Loxocemidae, and Pythonidae. On the other hand, all the families within Caenophidia, except for Acrochordidae, Viperidae, and Elapidae, evolved

Figure 2.1. Time-calibrated tree of 1625 snake species. Representative clades are labeled for reference. Axis is in millions of years and shows the geologic time scale. The first three epochs beginning from the present are not labeled and represent the Holocene, Pleistocene, and Pliocene.



immediately after Caenophidia (ca. within 10 my) split from Bolyeridae + Xenophidiidae (ca. 70.86 mya), suggesting rapid radiation within this clade (Fig. 2.1; Table 2.1). Viperidae and Elapidae are also suggestive of explosive species diversification as they are relatively young clades that radiated into a large number of species. Within the families of Lamprophiidae and Colubridae, the subfamilies also diverged rapidly (Fig. 2.1), some at (e.g. Dipsadinae and Colubrinae at 69.95 mya) or about the same time (e.g., Sibynophiinae at 54.32 mya and

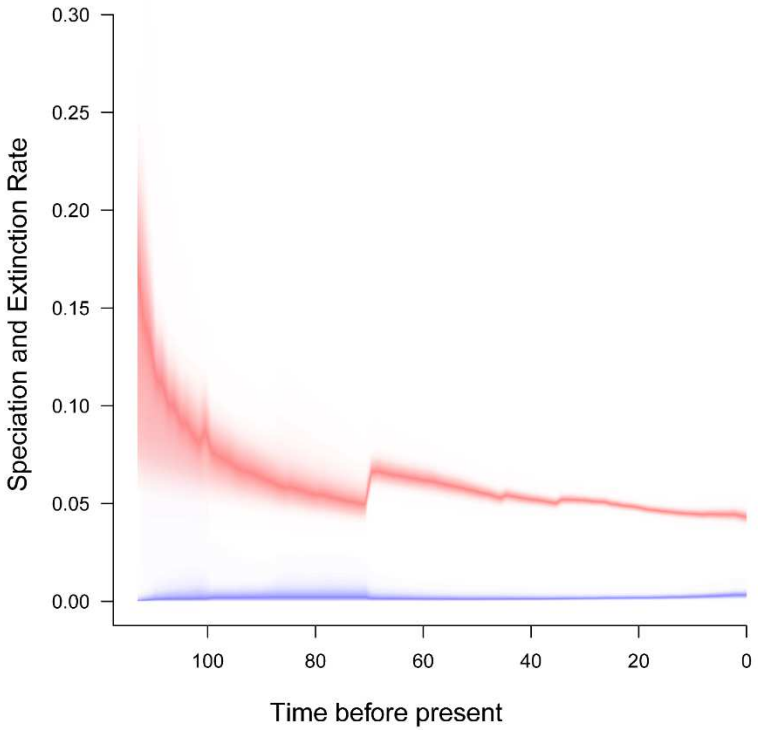
Pseudoxenodontinae at 54.32 mya), likely leading to the difficulties in resolving relationships within those families (Pyron et al., 2014a; see Chapter 1).

Lineage Diversification

All BAMM parameters had effective sample sizes > 200. Snakes diversified with a mean background speciation rate of 0.049 and a mean extinction rate of 0.002 (Fig. 2.2). Diversification rates in snakes carry the signature of ecological opportunity, starting out high when they split off from lizards and declining towards the present. However, this decline is punctuated by a small increase in speciation rate approximately 100 mya identifying the origin of Alethinophidia and higher level divisions within Henophidia, and by a larger spike at 70 mya, marking the origin of Caenophidia and rapid evolution of all its families and subfamilies (Fig. 2.2). We also find elevated speciation rates in Viperidae, Lamprophiidae, Elapidae, Colubridae, Natricinae, Dipsadinae, and Colubrinae (Fig. 2.3; Fig. 2.4). All these clades are characterized by decreasing rates over their history, with rates in Natricinae falling below the background speciation rate, except in Lamprophiidae, which shows a spike in diversification rates just below 40 mya that remains elevated towards the present, and in Elapidae which experiences an increase later in its history delineated by the exceptional speciation rate in the young, derived *Hydrophis* clade (Fig. 2.4E).

BAMM identified 7 diversification shifts distributed throughout the entire phylogeny under the maximum shift credibility, beginning with a slowdown in the branch leading to the 'core' Scolecophidia, which excludes Anomalepididae, and a slowdown in Henophidia that includes the clades Booidea, Uropeltoidea, and Pythonoidea (Fig. 2.4; Fig. 2.5). Speciation rates

Figure 2.2. Speciation (red) and extinction (blue) rates with credibility intervals for snakes over time. Mean speciation background rate is 0.049 and mean extinction background rate is 0.002.



in these two clades always remain below the background speciation rate, likely because of the elevated speciation rate of Caenophidia. The remaining 5 shifts all occurred within Caenophidia and represent increases in diversification rates (Fig. 2.4): 1) Viperidae (ca. 331 species; Table 2.1); 2) Elapidae (ca. 358 species; Table 2.1), including *Buhoma depressiceps*; 3) *Hydrophis*, excluding *H. gracilis*, *H. jerdonii*, and *H. spiralis* (ca. 43 species; Table S2.2) - our results do not place the diversification shift at the base of *Hydrophis*, as in Sanders et al. (2010), but instead excludes *H. gracilis*, *H. jerdonii*, and *H. spiralis*, which was not included in their analysis and placed outside of the focal *Hydrophis* clade (see Chapter 1); 4) the Neotropical "goo-eating" clade within Dipsadinae that includes the genera *Geophis*, *Atractus*, *Sibon*, *Tropidodipsas*, *Dipsas*, *Sibynomorphus*, *Ninia atrata* (ca. 266 species; Table S2.2), and *Thamnodynastes pallidus*, which may not belong to this clade (see Chapter 1), and; 5) a clade of Old World racers

within Colubrinae that include the genera *Dolichophis*, *Hierophis*, *Orientocoluber*, and *Eirenis* (ca. 27 species; Table S2.2).

The set of distinct shift configurations that account for 95% of probability of the data, and which was used to compute the maximum shift credibility and mean phylorate plots, is shown in Fig. S2.1. Each of these clades evolved a large number of species in a relatively short period of time (Fig. 2.1; Fig. 2.4), and their shift in increased diversification rates is strongly supported by cumulative shift probability analysis (Fig. 2.6). Each of these clades also experience decreasing speciation towards the present except for Elapidae and the "goo-eaters" (Fig. 2.4D & Fig. 2.4F). Macroevolutionary cohort analysis indicates strong heterogeneity in diversification rates with speciation rates in each of the delineated clades with shifts being decoupled from other clades in the tree (Fig. 2.7). The mean phylorate plot was highly congruent with the maximum shift credibility plot, but identified four additional diversification shifts with a slight increase in diversification rates for Erycidae + Boidae, larger increases in diversification rates in *Trachischium monticola* + *Hebius* within Natricinae, in *Erythrolamprus* within Colubrinae and in the same clade of Old World racers as above, but also including the genera *Hemorrhois*, *Spalerosophis*, and *Platyceps*, and a slight shift in one of the basal clades within Psammophiinae (Fig. 2.5B). In testing for state-dependent diversification, the STRAPP analysis found no support for front-fanged venom-delivery increasing rates of diversification in Viperidae and Elapidae ($P = 0.245$).

Figure 2.3. Rate-variation-through-time plots showing increased diversification rates for various snake lineages. The red line signifies speciation rate for each clade and the black line shows the speciation rate for all snakes. Shading intensity for speciation rate represents 90% Bayesian credible interval on the distribution of rates through time. A) Alethinophidia. B) Caenophidia. C) Lamprophiidae. D) Colubridae. E) Natricinae. F) Dipsadinae. G) Colubrinae.

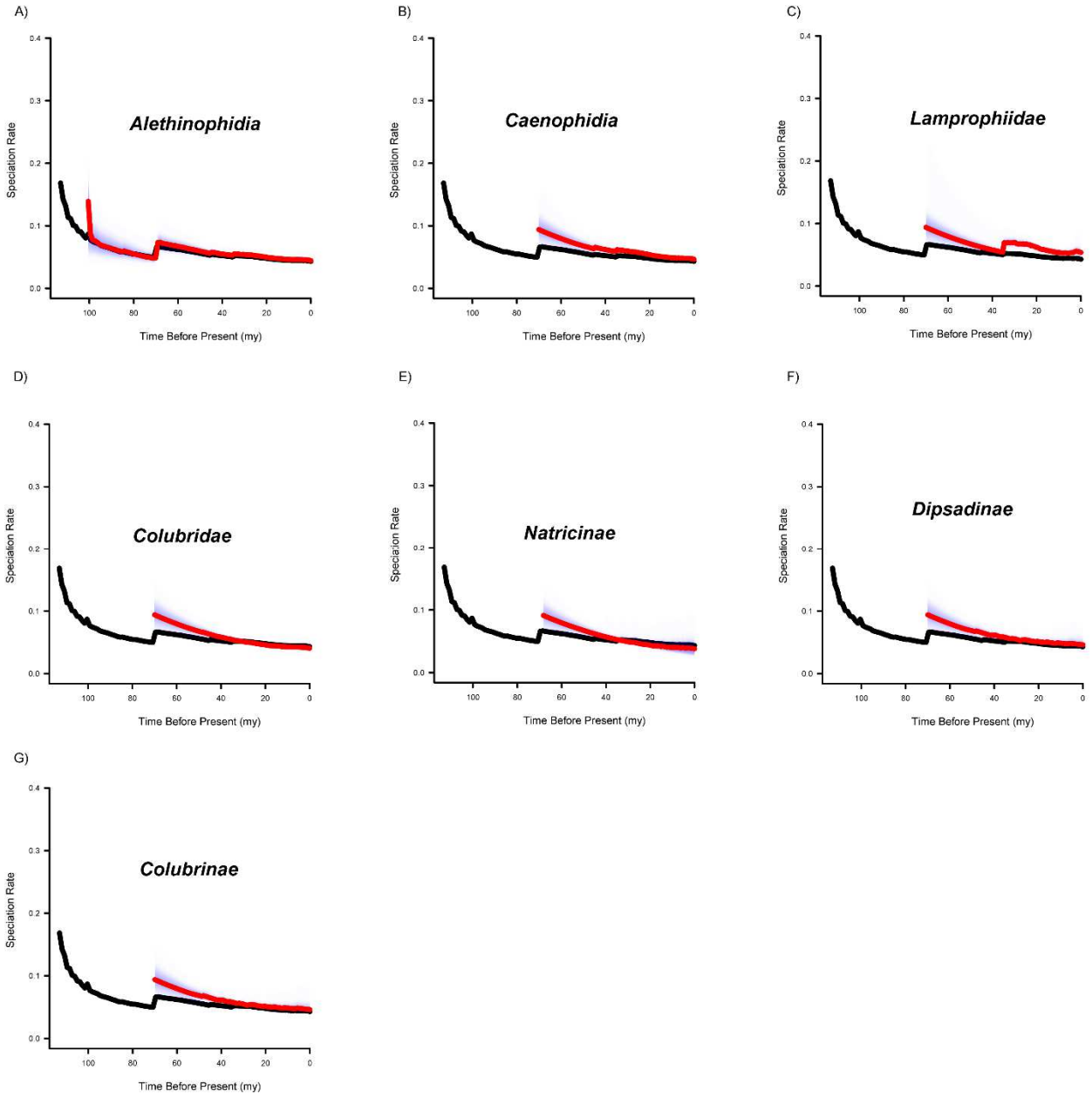
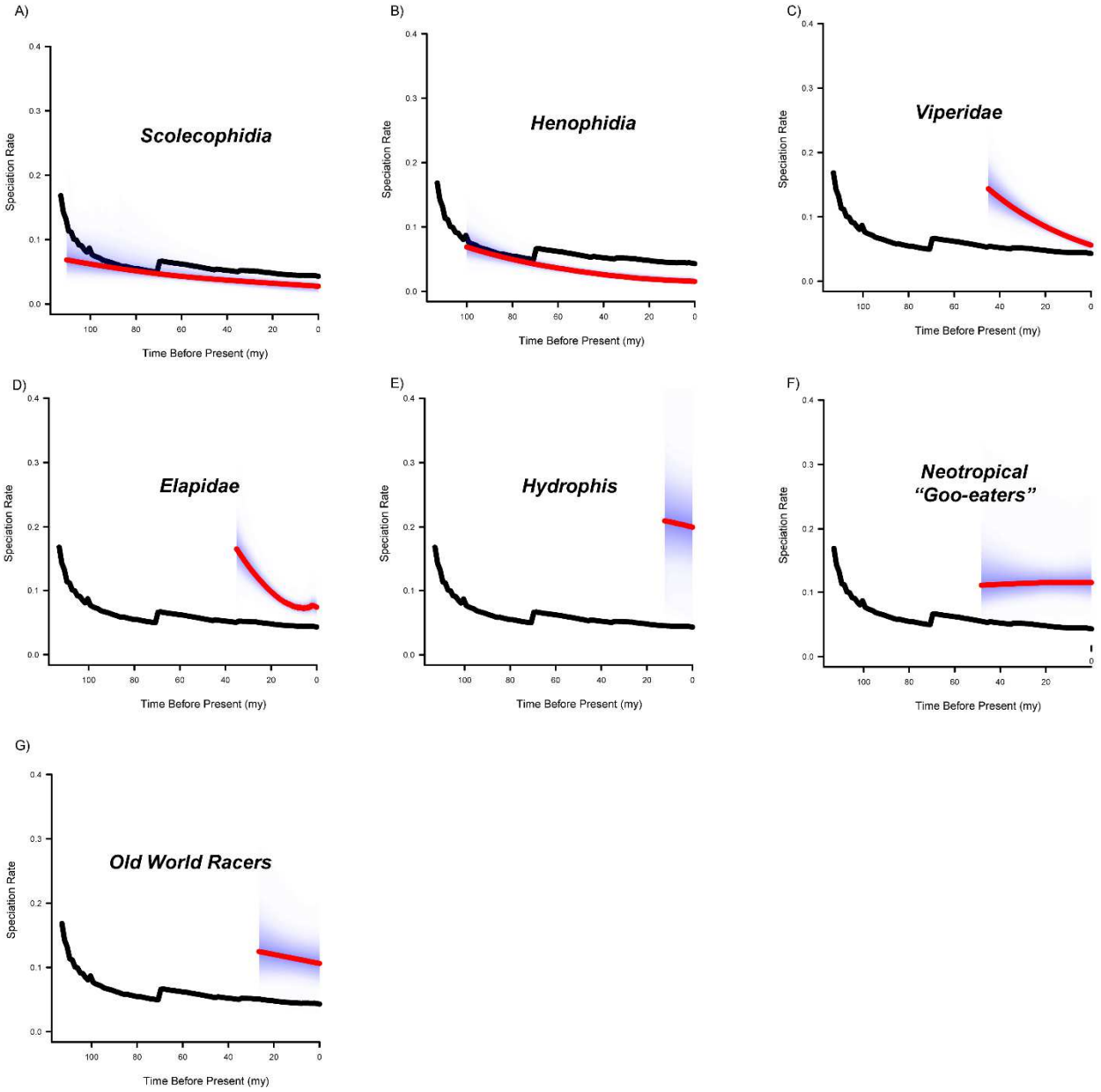


Figure 2.4. Rate-variation-through-time plots for each of the 7 clades from Fig. 2.3 that experienced shifts in diversification rates. The red line signifies speciation rate for each clade and the black line shows the speciation rate for all snakes. Shading intensity for speciation rate represents 90% Bayesian credible interval on the distribution of rates through time. A) Scolecophidia, with the exception of Anomalepididae. B) Henophidia, with the exception of Aniliidae + Tropidophiidae and Bolyeridae + Xenophidiidae. C) Viperidae. D) Elapidae, including *Buhoma depressiceps*. D) *Hydrophis*, excluding *H. gracilis*, *H. jerdonii*, and *H. spiralis*. F) The "goo-eating" dipsads, *Geophis*, *Atractus*, *Sibon*, *Tropidodipsas*, *Dipsas*, *Sibynomorphus*, *Ninia atrata*, and *Thamnodynastes pallidus*. G) The Old World racers, *Dolichophis*, *Hierophis*, *Orientocoluber*, and *Eirenis* within Colubrinae. Graphs A and B represent decreases in speciation rate and C-G represent increases in speciation rate.



Discussion

Shifts in diversification rates amongst clades can lead to unbalanced patterns of species richness across phylogenetic trees, but the drivers of rate shifts are not always readily apparent. Using a species-level phylogeny comprising all known snake families and subfamilies, we estimated divergence times, calculated diversification rates, identified rate shifts, and tested hypotheses regarding differences in speciation rates and whether shifts in diversification rates are associated with key innovations in the form of the evolution of venom in Colubroidea and in front-fanged venom delivery systems in Viperidae and Elapidae. Although much discrepancy surrounds the age of the major snake clades (Table 2.1), snakes most likely arose during the Early Cretaceous, no later than 172 mya (Scanlon and Lee, 2011). Most estimates place the date of origin for Pan-Serpentes between 166.0-120.74 mya and between 159.9-113 mya for Serpentes (Wiens et al., 2006; Burbrink and Pyron, 2008; Scanlon and Lee, 2011; Pyron and Burbrink, 2012; Hsiang et al., 2015; Zheng and Wiens, 2015). The principal divisions in the major clades of snakes, and within clades of Scolecophidia and Henophidia took place late in the Late Cretaceous, but most snake genera and species arose within the Cenozoic (Fig. 2.1). Anomalepididae is the basalmost clade of snakes (see Chapter 1) and appeared shortly after the origin of Serpentes. The core Scolecophidia clade and Alethinophidia split from one another quickly thereafter, 112.95 mya, with the core Scolecophidia clade originating 110.39 mya. Alethinophidia, however, did not appear until 100.5 mya, and immediately following, rapid divergence of the major Henophidia clades ensued (Scanlon and Lee, 2011; Hsiang et al., 2015). These time periods corresponding with the Cretaceous Terrestrial Revolution when multiple higher-level taxa experienced rapid speciation (Lloyd et al., 2008). Caenophidia also was

subjected to accelerated, but more pronounced, splitting into its major lineages after its origin 70 mya, as also previously suggested (Greene, 1997). Shortly thereafter, several caenophidian clades, specifically within Lamprophiidae and Colubridae, rapidly diversified (Fig. 2.3; Fig. 2.4) coinciding with the Cretaceous-Paleogene mass extinction, resulting in the loss of over 75% in biodiversity, including large-bodied squamates, but triggered diversification in small-bodied squamates (Longrich et al., 2012). However, many caenophidian genera and species originate in the Eocene when the Earth undergoes global warming, and after the recovery and radiation of mammals (Longrich et al., 2012), a major prey source. The increased basal diversification of Alethinophidia and Caenophidia, as evidenced by their short internal branches (Fig. 2.1), may explain high levels of homoplasy in molecular characters (Kelly et al., 2003) and why there is difficulty in resolving some problematic nodes, specifically for Boidae (Pyron et al., 2014b; Reynolds et al., 2014; Scanlon and Lee, 2011) and major lineages within Caenophidia (Kelly et al., 2009; Pyron et al., 2014a; see Chapter 1).

As implied by the basal short branches characterizing Alethinophidia and Caenophidia, these two clades are characterized by elevated speciation rates at the time of their origin, providing support for our first hypothesis that speciation rates are higher in these two clades. Despite the noticeable spike in background speciation rates coinciding with the origin of these two clades (Fig. 2.4), there were no rate shifts associated with either clade, but rates remained above the background rate throughout the history of Caenophidia. Why these two clades quickly radiated into their constituent families and subfamilies remains unknown and warrants investigation. The background speciation rate in snakes tends to slow over time (Fig. 2.2) exemplify niche-filling processes characteristic of ecological opportunity and diversity dependence (Yoder et al., 2010; Rabosky et al., 2012), but nonetheless remained high on interior

branches throughout the evolution of snakes with only two significant slowdowns occurring within clades of Scolecophidia and Henophidia. In contrast, Pyron and Burbrink (2012) showed an increase in diversification rates in Typhlopidae. Although Pyron and Burbrink (2012) offered no explanation, the rate shift in Typhlopidae likely occurs because this clade contains most of the species diversity in Scolecophidia, possibly due to their habit of consuming prey whole compared to other scolecophidians (Cundall and Greene, 2000). Together, Scolecophidia and Henophidia consist of 624 species, merely 5% of the total snake diversity (Table 2.1), and therefore were diagnosed with slowing of diversification rates when compared to Caenophidia, which accounts for the remaining 2900+ species. When considering Alethinophidia, which also comprises Henophidia, snake diversity exceeds 3000 species, constituting the only clade of squamates with unusually great species richness (Ricklefs et al., 2007), but the factors driving this immense diversity remain unknown. Since the majority of species richness is within Caenophidia, venom-delivery is frequently regarded as a key innovation because it allowed for the transition over from constriction in capturing and digesting a wider range of prey and prey sizes (Savitzky, 1980; Vidal, 2002; Jackson, 2003; Pyron and Burbrink, 2012). Yet, the evolution of venom-delivery is complicated with several, rear-fanged clades not radiating to any exceptional degree (e.g., Homalopsidae), independently losing the venom gland, and by different clades evolving different types of toxins (Fry et al., 2008). Clearly, increased speciation rates in Alethinophidia and Caenophidia serve as one explanation, but since we found no support for our second hypothesis that Colubroidea underwent a shift in diversification rate, this suggests that processes acting in clades with high lineage accumulation largely overshadow the evolution of venom early in Colubroidea.

Figure 2.5. Phylorate plots displaying speciation rates through time among snake lineages plotted on the time-calibrated tree. A) Maximum shift credibility tree showing 7 diversification shifts. Diversification shifts are in the following clades: 1) Scolecophidia, with the exception of Anomalepididae; 2) Henophidia, with the exception of Aniliidae + Tropidophiidae and Bolyeridae + Xenophidiidae; 3) Viperidae; 4) Elapidae, including *Buhoma depressiceps*; 5) *Hydrophis*, excluding *H. gracilis*, *H. jerdonii*, and *H. spiralis*; 6) the "goo-eating" dipsads, *Geophis*, *Atractus*, *Sibon*, *Tropidodipsas*, *Dipsas*, *Sibynomorphus*, *Ninia atrata*, and *Thamnodynastes pallidus*; and 7) the Old World racers, *Dolichophis*, *Hierophis*, *Orientocoluber*, and *Eirenis* within Colubrinae. B) Mean evolutionary rate plot showing 11 diversification shifts. Branches are colored by estimated net diversification rates (blue = slower speciation and red = faster speciation). Diversification shifts are in the following clades: 1) Scolecophidia, with the exception of Anomalepididae; 2) Henophidia, with the exception of Aniliidae + Tropidophiidae and Bolyeridae + Xenophidiidae; 3) Erycidae + Boidae; 4) Viperidae; 5) slight shift within Psammophiinae that is difficult to locate; 6) Elapidae, including *Buhoma depressiceps*; 7) *Hydrophis*, excluding *H. gracilis*, *H. jerdonii*, and *H. spiralis*; 8) *Trachischium monticola* + *Hebius* within Natricinae; 9) the "goo-eating" dipsads, *Geophis*, *Atractus*, *Sibon*, *Tropidodipsas*, *Dipsas*, *Sibynomorphus*, *Ninia atrata*, and *Thamnodynastes pallidus*; 10) *Erythrolamprus* within Colubrinae; and 11) the same clade of Old World racers as above, but also including the genera *Hemorrhois*, *Spalerosophis*, and *Platyceps*.

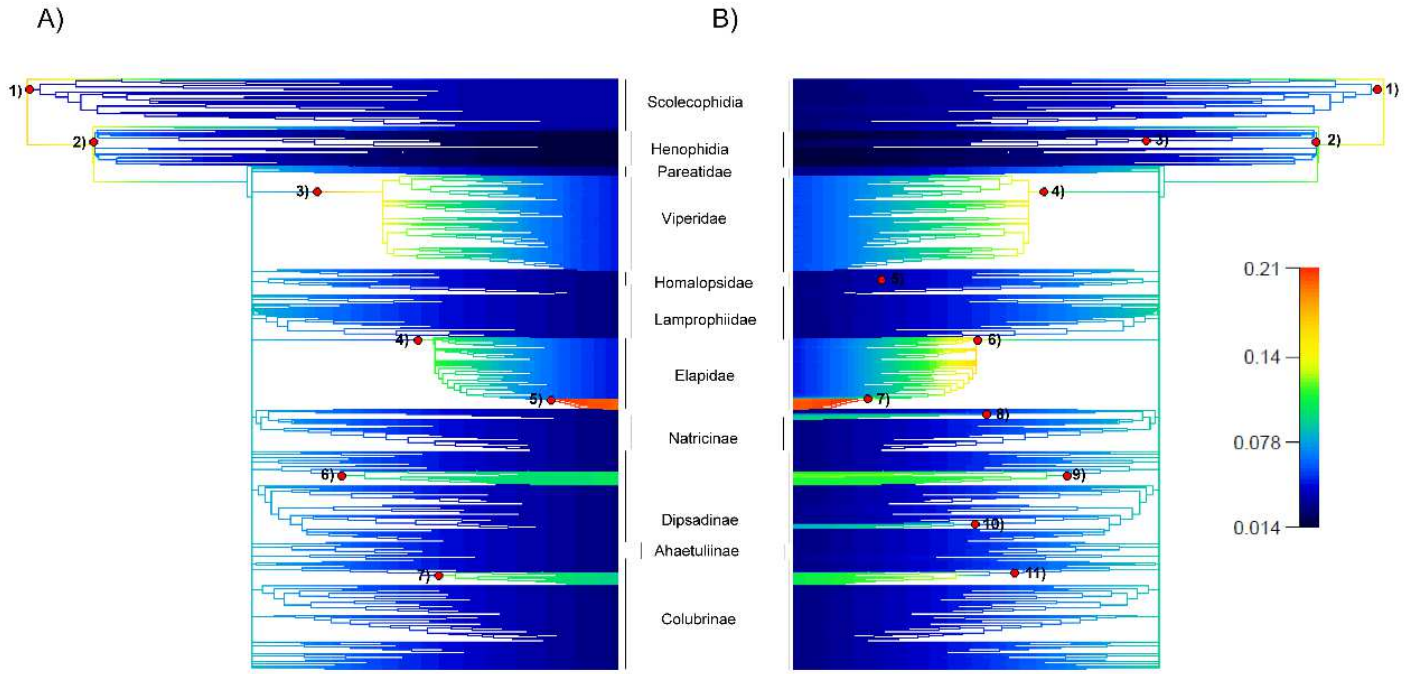
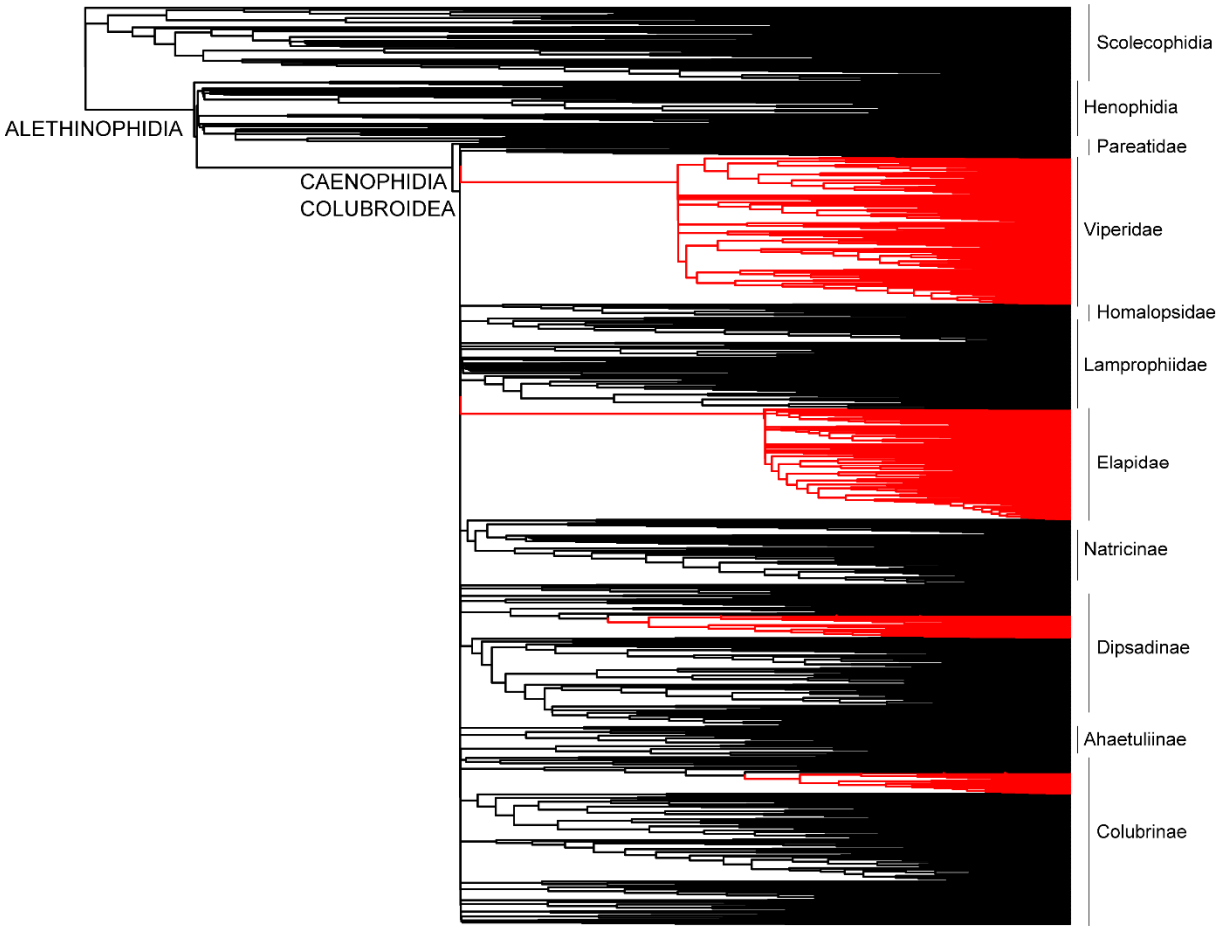
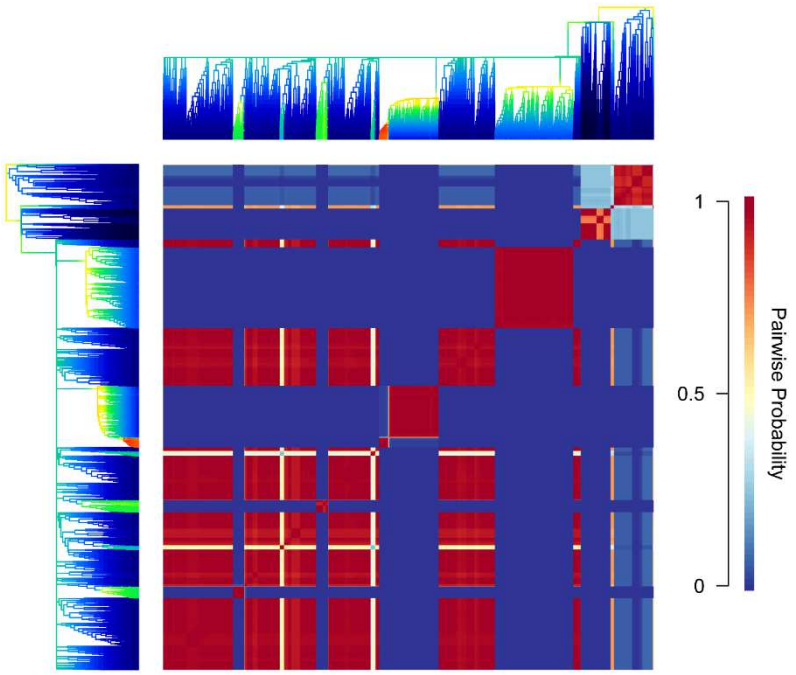


Figure 2.6. Cumulative shift probability plot showing the cumulative probability on each branch that a shift occurred. Occurrence of a shift implies that macroevolutionary dynamics on focal branch are decoupled from background diversification rate. Branches colored in red denote cumulative shift probability of 0.95 or higher.



We also find support for our first hypothesis that the species-rich clades Viperidae, Lamprophiidae, Elapidae, Colubridae, Natricinae, Dipsadinae, and Colubrinae are typified by high speciation rates. For Viperidae, Lamprophiidae, Elapidae, Dipsadinae, and Colubrinae, rates remain above the background speciation rate. Thus, high species richness in Caenophidia is due to increased diversification within several of its constituent clades, and not by an overall rate shift in Colubroidea. Despite high species richness in all these clades, shifts in diversification rates were only detected in Viperidae and Elapidae, partially supporting our third hypothesis that

Figure 2.7. Macroevolutionary cohort matrix displaying pairwise probability that clades share common macroevolutionary rate parameters. Red identifies those taxa that share similar diversification rates and blue identifies taxa whose rates are decoupled from the rest. The matrix illustrates strong heterogeneity in diversification rates, particularly in each of the clades from Fig. 2.4 and Fig. 2.5A that experienced shifts in diversification rates.



these clades underwent a shift in diversification rate, as evident by their much greater speciation rates (Fig. 2.4). Thus, based on this method we have support for an association of front-fanged venom-delivery and increased diversification in these two clades; however, based on our STRAPP analysis this association is not realized, failing to support the second part of our fourth hypothesis and showing that simply proposing a key innovation where a rate shift occurs does not demonstrate a causal link (Cracraft, 1990; Heard and Hauser, 1995). For instance, similar to Viperidae and Elapidae, Atractaspidinae (ca. 23 species; Table 2.1) also independently evolved a front-fanged venom system, but did not radiate to the extent of viperids and elapids (Jackson, 2003), suggesting other factors were likely responsible. Two notable differences among these clades are the restricted distribution and conserved morphology and habits of Atractaspidinae

compared to the global distribution and widely variable morphology and ecology of viperids and elapids. As suggested by some authors colonization of new areas, especially of rodent-rich habitats of temperate areas by vipers (Ineich et al., 2006), and of arid habitats (Byrne et al., 2008), or more specifically, the Australo-Melanesian region (Keogh, 1998; Scanlon and Lee, 2004; Sanders et al., 2008) by elapids may better explain diversification shifts in these two clades. Likewise, independent colonization of the New World by these two clades and also by Natricinae, Dipsadinae, and twice in Colubrinae (Chen et al., 2013) may help explain high speciation rates and high lineage accumulation in these clades as suggested by Pyron and Burbrink (2012).

In addition to the shifts in Viperidae and Elapidae, we detected increased shifts in *Hydrophis*, Neotropical "goo-eaters", and in a clade of Old World racers. *Hydrophis* was previously shown to represent an adaptive radiation within Elapidae (Voris and Voris, 1983; Lukoschek and Keogh, 2006). *Hydrophis* is characterized by exceptionally elevated speciation rates generated by differences in trophic ecology, where generalists and specialized macro- and microcephalic forms partition the dietary and habitat niche in species-dense assemblages (Sanders et al., 2013b). Such high local-diversity (i.e., species packing) arises due to effective niche partitioning (Schoener, 1974; Connell, 1978), which is a strong driver of diversification, as also shown in hummingbirds (McGuire et al., 2014), and increases the likelihood of species creating their own ecological opportunity (Erwin, 2008; Losos, 2010; Ricklefs, 2010). "Goo-eating" snakes (i.e., snakes that specialize feeding on soft-bodied invertebrates (Cadle and Greene, 1993) possess a seromucous infralabial gland that functions in controlling mucus and in transporting highly viscous prey, which independently evolved in Neotropical "goo-eaters" and in Pareatidae (Zaher et al., 2014). However, based on current phylogenetic hypotheses, this trait

may have independently evolved twice within Dipsadinae due to the removed phylogenetic position of the genus *Adelphicos* (Zaher et al., 2014; see Chapter 1) from the rest of the Neotropical "goo-eaters" (i.e., *Atractus*, *Dipsas*, *Geophis*, *Ninia*, *Sibon*, *Sibynomorphus*, *Tropidodipsas*) a highly diversified clade of greater than 250 species, including the most species rich snake genera *Atractus* (ca. 138 species; Table S2.2), that evolved from the less-diverse (~31 species), vertebrate-consuming, rear-fanged Leptodeirini (Mulcahy, 2007). The seromucous infralabial gland has been proposed as the cause of high species richness in Neotropical "goo-eaters" (Zaher et al., 2014); however, *Adelphicos* (ca. 6 species) and Pareatidae (ca. 20 species) are relatively species-poor, suggesting some other factor drove the Neotropical "goo-eating" radiation. The shift in the clade of Old World racers was unexpected and is likely linked with the radiation of *Eirenis* (ca. 20 species; Table S2.2), an ecologically-derived group with distinctive morphological characters, most notably dwarfism, that are associated with a cryptic lifestyle and led to rapid radiation over a short period (Nagy et al., 2004; Mahlow et al., 2013; Rajabizadeh et al., 2015).

Understanding factors leading to extraordinary lineage accumulation within these clades will further expand our knowledge of the macroevolutionary processes that produced the great caenophidian radiation. In comparison to other vertebrates which possess limbs capable of evolutionary and structural modification to meet functional demands, snakes appear at a disadvantage due to their morphologically-constrained body plan. Yet, the lack of limbs and body elongation are most likely the driving force behind snake evolution and diversity, and probably allowed for ecological opportunity early in their evolutionary history, providing them access to available resources, novel habitats, and prey not available to other predators (Gans, 1975; Pough, 1983). Even though the snake-like body shape (i.e., highly elongate body with

reduced or absent limbs) has independently evolved several times in squamates, only snakes radiated to an exceptional degree (Greer, 1991; Wiens et al., 2006; Shine and Wall, 2008). Shine and Wall (2008) postulate that trunk elongation associated with burrowing locomotion provided the structural foundation permitting snakes to shift to ingesting large meals because of an increase in gut volume. However, this hypothesis remains to be tested, along with other proposed key innovations for snakes, that mainly involve structural modifications associated with feeding biology (Pough, 1983). These key innovations include kinetic skull and jaw disarticulation in early macrostomates, which provided large gapes for consuming prey whole (Gans, 1961; Greene, 1983; Vincent et al., 2006; Longrich et al., 2012); constriction (Greene and Burghardt, 1978; Boback et al., 2012); associated venom-delivery adaptations (Jackson, 2003; Fry et al., 2008); and the seromucous infralabial gland and asymmetrical dentition of "goo-eaters" (Hoso et al., 2007; Zaher et al., 2014). So far, front-fanged venom-delivery does not appear to be a key innovation that increased diversification rates in viperids and elapids suggesting we should look beyond key innovations and consider synergistic factors as well.

Acknowledgements

We would like to thank Stephen A. Smith for his help with treePL and Liam Revell for his assistance with phytools.

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Supporting Information

Table S2.1. Nodes and fossil ages used for estimating divergence times. Age is given as a range (minimum and maximum ages) or as a minimum age given in millions of years (Myr). Refer to references for justification for each fossil calibration selected. Our nodes Boidae, Charininae, *Charina* correspond to Boinae, Charinidae, and Charininae, respectively, in Head (2015).

Table S2.2. List of 402 genera sampled and 113 genera not sampled in Chapter 1. For genera sampled, the total number of species, the number of species sampled, and the percentage of species sampled is provided for each genus. For genera not sampled, the total number of species, family or subfamily designation, and distribution is provided. *i.s.* = *incertae sedis*.

Figure S2.1. Phylorate plots for each of the shift configurations sets that account for 95% of probability of the data. Values above each plot represents posterior probability for each set of shift configurations. Black circles indicate locality of occurrence for a shift in diversification rates.

File S2.1. Newick file for time-calibrated phylogeny for 1745 taxa representing 1652 snake species and 7 outgroup taxa displayed in Fig. 2.1.

Chapter 3. The Evolution of Habitat Use in Snakes: A Specialized Body Shape Suitable for Diverse Habitat Associations

Abstract

Over 3,500 species of snakes presently inhabit diverse habitats in all major non-Arctic biomes, from soils, caves, and forest canopies, to numerous types of aquatic ecosystems. Morphology often relates to functional performance, and species occupying different habitat types are considered to show morphological adaptations suited for their habitats. We use the most recent and comprehensive snake phylogeny to examine habitat use and body shape variation in snakes to test three hypotheses: 1) Individual habitat use categories independently evolved numerous times in snakes; 2) Species from different habitat associations form morphological clusters by occupying distinct regions of multivariate morphospace; and 3) Species from similar habitat associations show convergence in both morphology and adaptive regimes. Stochastic character mapping inferred snakes as having evolved from an ancestrally fossorial lifestyle and that throughout their evolutionary history, have undergone multiple expansions into new habitats as they colonized and transitioned, often repeatedly, between a multitude of habitat types. Species associated with different habitats widely overlapped in body shape morphospace, and did not form clearly defined morphological groups. Hierarchical clustering showed that distantly-related species of similar habitat use formed several morphological subclusters, but these

subclusters were part of larger clusters that included species from different habitat associations. Snakes converged on morphology based on phenotypic distance, but did not converge in morphospace due to the invasion of divergent taxa into their phylomorphospace. We estimated between 39 adaptive regimes, of which 11 were convergent using the AICc criterion, which is criticized for over-fitting models, and six adaptive regimes using the pBIC criterion.

Introduction

The study of functional traits that allow individuals to interact with their environment in ecologically-relevant ways forms a cornerstone of our understanding of adaptation. An important finding in this regard is that species have in many cases independently evolved similar functional solutions to the same ecological challenges. This convergent evolution of analogous functional traits in distantly-related taxa usually occurs in response to species occupying similar ecological niches (see Stayton 2015), modeled as a peak in the adaptive landscape (Mahler, et al. 2013; Arbuckle, et al. 2014). Thus, we can distinguish between pattern-based convergence, where lineages independently evolved similarity (Stayton 2015), and process-based convergence, where convergence arises due to some evolutionary process (Stayton 2015), most commonly by convergent lineages entering equivalent adaptive regimes (Schluter, 2000; Mahler, et al. 2013). General examples of convergence include fins for swimming in fish and mammals (Donley, et al. 2004) or wings for flying in insects, pterodactyls, birds, and bats (Maina 2000). As these examples illustrate, convergence can occur both over broad taxonomic scales, and at finer taxonomic levels. Perhaps the most well-known example of repeated morphological convergence is that of Caribbean *Anolis* lizards, which are grouped into 6 classes of habitat specialists known as "ecomorphs" (i.e., lineages similar in morphology and behavior that occupy the same structural habitat niche, but not sharing a recent common ancestor; (Williams 1972; Losos 2009). Convergence provides strong support for repeatability in evolution (Losos, et al. 1998; Mahler, et al. 2013) and for adaptation (Harvey and Pagel 1991) as distantly-related species adapt to inhabit all or parts of the same multidimensional niches (Harmon, et al. 2005). Morphology most often reflects phylogeny, and morphological divergence occurs in response to ecological factors such

as habitat use and diet (Arnold 1983). Because morphology is highly correlated with both behavior and ecology, collectively known as ecomorphology (Williams 1972), variation in morphology serves as a predictor of differences in resource use (Williams 1972; Arnold 1983; Losos 1990).

Unlike anoles and other vertebrates that have limbs capable of evolutionary and structural change, snakes lack limbs with which to interact with their environment, resulting in a specialized and highly conserved body plan, capable of comparatively limited modifications and adaptations. The evolutionary loss of limbs in snakes therefore required structural innovations to overcome functional challenges associated with locomotion and prey handling. In spite of this clear morphological constraint and originating from a fossorial lifestyle (Bellairs and Underwood 1951; Shine and Wall 2008; Yi and Norell 2015), snakes exploded into a radiation of over 3,500 extant species (Uetz and Hošek 2014), encompassing a diverse range of morphologies and habits (Gans 1961; Pough 1983; Greene, et al. 1997). Yet despite this diversity, and perhaps due to the number of limited external morphological characters to quantify, variation in habitat transitions and snake morphology has received little attention, much less within an appropriate phylogenetic framework. In this study we trace the evolution of habitat use and quantify morphological variation in snakes to test hypotheses regarding morphological convergence.

We currently lack an understanding of how snake lineages filled and transitioned between available habitats, or even how habitat use is distributed amongst snakes. Most evidence points to a terrestrial, more specifically, a subterranean origin (Shine and Wall 2008; Hsiang, et al. 2015; Yi and Norell 2015). Thus, from a fossorial origin, snakes went on to invade all major non-Arctic biomes and occupy nearly every habitat stratum of terrestrial ecosystems, from deep soils to high forest canopies, as well as aquatic ecosystems, both freshwater and marine (Greene, et al.

1997). Some of these habitat associations are known to have evolved independently several times (Lillywhite and Henderson 1993; Colston, et al. 2010; Brischoux and Shine 2011). Snakes are often used as models of niche partitioning (Toft 1985; Luiselli 2006), a necessary component in maintaining community structure by limiting competitive species-interactions (Pianka 1974; Schoener 1974), particularly between closely-related and ecologically-similar species to promote sympatry (Richman and Price 1992), and also in taxa, like snakes, that form species-dense communities. Such communities will benefit from species partitioning broad habitat use categories (e.g., fossorial, aquatic, terrestrial, or arboreal) based on differential structural habitat use (see methods; Rand 1964). For most snakes, habitat association is based solely on qualitative observations (Reinert 1992), mainly owing to their secretive lifestyle. The term 'secretive' is often used interchangeably with cryptozoic (i.e., fossorial/subterranean), but many snakes spend a large part of their time concealed in some type of substrate (i.e., burrows, holes, rocks, vegetation).

To distinguish between conserved morphologies due to phylogeny and morphological shifts associated with an ecological origin, we use the time-calibrated phylogenetic tree of Chapter 2 consisting of 1652 species, the most recent and comprehensive phylogeny to date. To trace the evolutionary history of habitat use from ancestral species to descendant taxa, we mapped habitat use categories onto the phylogenetic tree and used stochastic character mapping to estimate ancestral habitat states and infer historical transitions between habitat types. Sympatric snakes tend to show the greatest ecological divergence in habitat use and the use of dietary resources (Reinert 1993; Luiselli 2006); therefore, we predict that the majority of variation in morphology lies in head shape. We specifically tested the following hypotheses concerning niche-based divergence: 1) Individual habitat use categories independently evolved

numerous times in snakes; 2) Species from different habitat associations form morphological clusters by occupying distinct regions of multivariate morphospace; and 3) Species from similar habitat associations show converge in morphology and converge in similar adaptive regimes.

Materials and Methods

Ancestral Character State Estimation and Habitat Use Transitions

Habitat use in snakes is usually classified into broad categories (e.g., fossorial, aquatic, terrestrial, or arboreal). Further dividing these categories by identifying physical features of the habitat and morphological adaptations associated with habitat use (sensu Rand, 1964) can provide slightly more specific and/or biologically accurate descriptions of habitat use and identify microhabitat specializations. Therefore, to infer the history of habitat use in snakes, we defined 9 general habitat categories (9-state model; fossorial, semifossorial, semiaquatic, freshwater, marine, aquatic-mixed, terrestrial, semiarboreal, and arboreal) and 28 specific habitat categories (28-state model) of snake habitat association (Table 3.1) by surveying the literature to identify habitat associations for all species represented on our phylogeny (Table S3.1). For many species, insufficient qualitative information is available to categorize their specific habitat use, in which case we simply kept their broad habitat association. We used the phytools package (Revell 2012) in R to perform stochastic character mapping (SCM) on the 9- and 28-state habitat use models, which uses a Markov chain Monte Carlo (MCMC) approach to sample character histories from their posterior distribution to obtain a sample of unambiguous histories on the tree (Huelsenbeck, et al. 2003). Because phytools has difficulty estimating ancestral states on a time-

calibrated phylogeny, we converted the time-calibrated tree into an ultrametric tree using penalized likelihood, which retained a highly congruent branching structure to the time-calibrated tree. Next, we excluded outgroups and assessed the fit of the following three discrete trait maximum likelihood (ML) models using the R package geiger (Harmon, et al. 2008): (i) transition rates between states are equal (ER); (ii) forward and reverse transition rates between states are equal, but differ between different trait combinations (SYM); and (iii) all transition rates are different (ARD). Afterwards, we compared model fit using corrected Akaike information criterion (AICc) and selected the model with the lowest value. We then implemented SCM using the model with the lowest AICc value. Since SCM samples character states at nodes and changes in character state along edges, we replicated SCM 1000 times on the time-calibrated tree to estimate the number of character changes, the proportion of time spent in each character state, and the posterior probabilities that each internal node is in each character state.

Table 3.1. List and definitions for 28 types of specific habitat associations used to characterize habitat use of snakes.

Habitat Association	Definition
Subterranean	
Subterranean-Burrower	Specialized burrowers most active in the soil or in nests/mounds of social insects (i.e., ants and termites)
Soil-Burrower	Specialized burrowers most active below the surface in soil
Sand-Burrower	Specialized burrowers occupying sabulicole (sand) environments
Subterranean-Debris	Most active under various cover items (leaf litter, logs, rocks, etc.)
Subterranean-Rocks	Most active under rocky cover items (scree, cap rocks, rock crevices, etc.) in rupicolous (rocky) environments
Aquatic	
Lentic	Exclusively aquatic in slow-moving freshwater environments (lakes and ponds)
Aquatic-Freshwater	Most active in freshwater environments (rivers, lakes, ponds, etc.)
Freshwater-Burrower	Burrows into aquatic soil, aquatic vegetation or use mud tunnels
Aquatic-Mixed	Active in marine, brackish, or freshwater environments
Aquatic-Mixed-Burrower	Burrows into aquatic vegetation or mudflats in marine, brackish, or freshwater environments
Riverine	Exclusively aquatic in riverine ecosystems

Table 3.1 Continued.

Amphibious	Marine snakes that come ashore for various activities (basking, reproduction, digestion, shelter, etc.)
Intertidal	Mangroves, mud flats, tidal rivers, estuaries, and marshes
Intertidal-Burrower	Burrows into mudflats or use intertidal burrow systems in marine, brackish, or freshwater environments
Coastal	Marine snake inhabiting river mouths, estuaries, shoals, seas along coasts (preferences for turbidity exists between species
Reef-Flats	Most active along sandy bottoms from shore to coral reefs
Coral-Reefs	Most active among coral reefs
Deep-Water	Deep water near land or coral reefs
Pelagic	Open seas far from land
Terrestrial	
Terrestrial	Activity occurs predominately above the ground surface
Terrestrial-Fossorial	Equal use of terrestrial and fossorial environments
Terrestrial-Aquatic	Equal use of terrestrial and aquatic environments
Terrestrial-Scansorial	Predominately terrestrial snakes, adept at climbing (some species are troglodytic - active in caves)
Terrestrial-Arboreal	Equal use of terrestrial and arboreal environments
GeneralistI	Found in terrestrial, aquatic, and arboreal environments
GeneralistII	Found in terrestrial, fossorial, and aquatic environments
GeneralistIII	Found in terrestrial, fossorial, and arboreal environments
Arboreal	
Arboreal*	Specialized climbers most active in arboreal environments

*Arboreal snakes likely partition arboreal habitats, but very little data exists to make any discriminations.

Taxon Sampling and Morphological Measurements

To quantify variation in morphology and character evolution, we sampled a maximum of 15 specimens per species, resulting in a dataset of 1715 specimens for 284 species. To provide more phylogenetic coverage, we included numerous species from a recently published dataset (Grundler and Rabosky 2014) to incorporate more non-arboreal tips on the phylogeny since our data consisted principally of arboreal species, resulting in a dataset of 405 species. Specific habitat use in our morphological dataset is distributed as follows: Amphibious = 1; Aquatic-Freshwater = 10; Aquatic-Mixed = 2; Aquatic-Mixed-Burrower = 2; Arboreal = 124; Freshwater-Burrower = 5; Generalist-I = 6; Generalist-II = 3; Intertidal = 2; Intertidal-Burrower

= 4; Sand-Burrower = 9; Soil-Burrower = 7; Subterranean-Debris = 42; Subterranean-Rock = 1; Terrestrial = 79; Terrestrial-Aquatic = 9; Terrestrial-Arboreal = 61; Terrestrial-Fossorial = 16; and Terrestrial-Scansorial = 22. Due to limited availability of museum specimens for some species, we obtained measurements from both sexes since variation among species outweigh variation within species. For each specimen we measured nine external morphological characters to account for variation in body shape: (snout-vent length [SVL], tail length (distance from the cloaca to the tip of the tail [TL]), mid-body width [MBW]; and head shape: head length (tip of the snout to the end of the quadrate [HL]), jaw length (tip of the snout to the end of the bottom quadrate [JL]), head width (at the widest part of the head [HW]), head depth (at the tallest part of the head [HD]), interocular distance (shortest distance between the edges of the eyes [IO]), and eye diameter (ED). For SVL, TL, we used dental floss to measure the size of each character, then measured the dental floss to the nearest 0.1 cm on a meter stick. The remaining characters were measured to the nearest 0.1 mm using Mitutoyo digital calipers. Variables measured in cm were converted to mm for statistical analyses.

Morphological Variation

We performed all statistical analyses in R (Team 2011). We analyzed morphological variation by conducting a suite of non-phylogenetic and phylogenetic tests on the mean for each variable. Since body size accounts for the majority of the observed morphological variation, we analyzed variation based on shape by calculating the residuals for each $\log_{10}$-transformed character using linear regressions against SVL to correct for body size. Biological shape is a composite of multiple traits making morphometric data essentially a multivariate test requiring

dimension reduction to test the hypothesis that species form discrete clusters in multivariate morphospace. To analyze morphological variation and to test if species from different habitat associations form morphological clusters, we performed principal components analysis (PCA) and hierarchical clustering. We performed PCA, using the 'psych' and 'GPArotation' packages, on the covariance matrix of the size-corrected variables to reduce the dimensionality of the data, retaining PCs with eigenvalues > 1 for further analyses (Ricklefs and Travis 1980). PCA allows comparison of species distributions within multivariate morphological space and identification of patterns of correlation among morphological variables. Next, we used hierarchical clustering to identify morphological subclusters. We performed hierarchical clustering using the package 'stats', by calculating a Euclidean distance matrix on the size-corrected variables and by using Ward's clustering method to minimize within-cluster variance. To visualize morphology associated with each cluster, we created boxplots using the PC loadings for each species and their cluster affiliation. Finally, to examine if morphology is related to morphological cluster, we carried out a multivariate analysis of variance (MANOVA). If the MANOVA reveals any significant differences in morphology due to morphological cluster, we will use analysis of variance (ANOVA) to identify where those differences lie.

Test for Morphological Convergence

To identify morphological convergence, we performed two separate analyses on the PC scores. The first is a pattern-based approach, which requires known or putative convergent taxa be specified a priori, and the second is a process-based approach that does not require convergent taxa be known a priori. Since several species from the morphological dataset are not included in

the phylogeny, we substituted their names for the names of closely-related species in the tree (Pennell, et al. 2016). Using this procedure, we managed to manually replace 37 species, but omitted 23 species due to the lack of available tips on the phylogeny of closely-related species (Supplement 3). For the pattern-based approach, we used the package 'convevol' to quantify the amount of independently evolved similarity within our PC scores (Stayton, 2015). This procedure takes into account morphological similarity, but does not require a certain level of similarity, and incorporates two approaches. First is a distance-based procedure, which calculates between two lineages as a proportion of the distance between both species tips and the largest distance between those taxa throughout their evolutionary history (anywhere between the species tips and their most recent common ancestor [MRCA]) (C_1-C_4): C_1, the proportion of the maximum distance from the MRCA in morphospace between focal taxa that has been reduced by phenotypic evolution; C_2, similar to C_1, but accounts for the amount of morphological change; C_3, the proportion of evolution attributable to convergence between focal taxa; and C_4, the proportion of evolution attributable to convergence to the smallest clade containing the focal taxa. Second, is a frequency-based measure (C_5), which quantifies the number of lineages that have evolved into a certain region of morphospace and counts the number of lineages entering the region of the morphospace occupied by the hypothesized convergent taxa (C_5). For C_1, values of 0 correspond to no convergence and values of 1 equal "complete" convergence, and for all other values of C, the greater the value, the greater convergence is. We performed 1000 simulations of evolution along the phylogeny using BM, calculating convergence measures for each simulation in convevol to determine if the observed C value is greater than would be expected by chance (P-value).

For our process-based analysis, we tested for convergence by detecting the phylogenetic

placement and magnitude of evolutionary trait shifts, known as regimes, and identify whether distantly-related taxa share the same regime. To test for shared evolutionary trait regimes, we used the package 'l1ou', which fits Ornstein-Uhlenbeck (OU) models in a stepwise fashion to estimate species placement within a multidimensional adaptive landscape of trait space without the a priori designation of ecomorphs or selective regimes (Khabbazian, et al. 2016). All clades in the tree are assumed to evolve around different optima (i.e., adaptive regimes) until independent lineages sharing a common optimum are identified and convergence is achieved. This procedure applies a phylogenetic lasso method, which considerably speeds up analyses, on OU models and selects the best-fit model using the phylogenetic Bayesian information criterion (pBIC), a new test that accounts for the phylogenetic correlation between species for approximating the number of shifts in the marginal probability. This procedure is designed to reduce the detection of false shifts from those that overfit models, like the corrected Akaike Information Criterion (AICc) (Ho and Ané 2014). However, for comparison we re-ran the analysis using the AICc, which produces results similar to SURFACE (Ingram, et al. 2013), the first method designed to test for shared evolutionary trait regimes. We performed these methods on the PC scores with a maximum of 50 shifts, and calculated support for each shift by running 1000 bootstrap iterations.

Results

Hypothesis 1 - Multiple independent origins of habitat use

Under both models, more time was spent in a terrestrial state, nearly double the time of

any other state, and each habitat state independently evolved multiple times, with terrestriality, fossorialism, and arboreality evolving more frequently, under both models (Table 3.2 & Table 3.3). The best-fit model selected for broad habitat use ($p < 0.001$, AICc = 2760.54) and for specific habitat use ($p < 0.001$, AICc = 3770.376) was ARD. Based on the posterior distribution of 1000 simulated trees, there were approximately 398.20 state changes for the 9-state model and 480.91 state changes for the 28-state model. The phylogeny used here comprised 552 terrestrial snakes, 406 arboreal snakes, 432 subterranean snakes, and 182 aquatic snakes, which culminated in branches in the simulated stochastic mapped trees spending more time in terrestrial, arboreal, and subterranean character states than in aquatic states. As expected, habitat state changes were most frequent between closely-associated habitat states such as terrestrial to fossorial and arboreal, and rare between inaccessible habitat states, such as from fossorial to most other states, and amongst aquatic, arboreal, and fossorial states. Stochastic character mapping estimated the MRCA of extant snakes as 99.7% fossorial in broad habitat use (Fig. 3.1), and as 99.8% subterranean-burrower in specific habitat use (Fig. 3.2). For Alethinophidia, the MRCA was estimated as 99.7% and 100% terrestrial in broad and specific habitat use, respectively. In general, character states for the majority of nodes were unambiguous for both models (i.e., characterized by one character state).

Hypothesis 2 - Morphospace variation based on habitat association

When plotting morphological variation based on specific habitat use (Fig. 3.3), species of different habitat associations widely overlapped in morphospace, and did not form clearly defined groups, indicating that habitat associations as defined in this paper, are not identified by

Table 3.2. Summary of character state changes for 9 categories of general habitat use based on the all-rates-different model ($p < 0.001$, AICc = 2760.54) replicated over 1000 stochastically mapped trees. There were approximately 398.20 character changes. Percentages exemplify the amount of time spent in each character state. Transitions are read on the horizontal, not the vertical. Posterior probabilities estimated the root node of snakes as being 99.7% fossorial.

	Habitat Use								
Habitat Use	Fossorial (15.77%)	Semifossorial (14.15%)	Semiaquatic (2.58%)	Freshwater (3.05%)	Marine (1.61%)	Aquatic-Mixed (2.06%)	Terrestrial (36.29%)	Semiarboreal (4.81%)	Arboreal (19.68%)
Fossorial	--	1.929	0	0	0	0	1.030	0	0
Semifossorial	9.162	--	0.037	0	2.028	2.093	34.609	1.305	0
Semiaquatic	0.270	0	--	7.227	0	0.222	2.993	0	0
Freshwater	0	7.562	9.603	--	0	8.546	5.489	0	0
Marine	0	0	0	1.022	--	0	0	0	0
Aquatic-Mixed	0.315	0	0	9.353	0	--	0	0	0
Terrestrial	0.860	70.004	24.606	2.544	0	4.723	--	17.361	83.623
Semiarboreal	0	7.072	3.174	1.071	0	0	27.754	--	2.307
Arboreal	0	0.905	2.780	0	0	0	33.294	11.545	--
Total	10.607	87.472	40.200	21.217	2.028	15.584	105.169	30.211	85.93

Table 3.3. Summary of character state changes for 28 categories of specific habitat use based on the all-rates-different model ($p< 0.001$, AICc = 3770.376) replicated over 1000 stochastically mapped trees. There were approximately 480.91 character changes. Percentages exemplify the amount of time spent in each character state. Transitions are read on the horizontal, not the vertical. Posterior probabilities estimated the root node of snakes as being 99.8% subterranean-burrower in habit.

	Habitat Use													
Habitat Use	Subterranean-Burrower (12.41%)	Soil-Burrower (3.29%)	Sand-Burrower (2.16%)	Subterranean-Debris (7.70%)	Subterranean-Rock (0.42%)	Lentic (0.02%)	Aquatic-Freshwater (3.50%)	Freshwater-Burrower (0.49%)	Aquatic-Mixed (0.60%)	Aquatic-Mixed-Burrower (0.44%)	Riverine (0.00%)	Amphibious (0.36%)	Intertidal (0.55%)	Intertidal-Burrower (0.23%)
Subterranean-Burrower	--	0	0	0	0	0	0	0	0	0	0	0	0	0
Soil-Burrower	0	--	0	2.221	0	0	0	0	0	0.214	0	0	0	0
Sand-Burrower	0	0	--	0.160	0	0	0	0	0	0.030	0	0	0	0
Subterranean-Debris	0	3.573	0	--	0	0	0	0	0	0	0	1.082	1.365	0
Subterranean-Rock	0.001	0.033	0.042	4.046	--	0.026	0.048	0.015	0.032	0.024	0.011	0.004	0.016	0.009
Lentic	0.002	0.003	0.001	0.022	0.003	--	0.012	0.006	0.007	0.008	0.002	0.003	0.009	0.028
Aquatic-Freshwater	0	0	0	6.356	0	0	--	5.308	9.718	0	0	0	0.955	1.105
Freshwater-Burrower	0	0.014	0.009	0.172	0.018	0.015	0.059	--	0.060	1.050	0.012	0.008	0.032	0.031
Aquatic-Mixed	0	0.018	0.022	0.829	0.035	0.019	0.261	0.097	--	0.027	0.007	0	0.249	0.028
Aquatic-Mixed-Burrower	0	0.667	0.065	0.111	0.021	0.016	0.056	1.179	0.033	--	0.013	0.034	0.035	0.010
Riverine	0	0.006	0	0.013	0.003	0.004	0.003	0.007	0.004	0.010	--	0.001	0.008	0.001
Amphibious	0	0.016	0.005	0.011	0.006	0.004	0.015	0.008	0.004	0.008	0.002	--	0.014	0.005
Intertidal	0	0.015	0.018	0.047	0.014	0.020	0	0.030	0.090	0.015	0.015	0.015	--	0.021
Intertidal-Burrower	0	0.004	0.006	0.015	0.007	0.967	0.030	0.023	0.034	0.012	0.006	0.005	0.021	--
Coastal	0	0.002	0.001	0.006	0.004	0.022	0.001	0.015	0.011	0.012	1.024	0.001	0.052	0.012
Reef-Flat	0	0.001	0.002	0.009	0.008	0.010	0.002	0.006	0.008	0.012	0.010	0.008	0.015	0.012
Coral-Reefs	0	0.007	0.006	0.013	0.003	0.007	0.004	0.009	0.004	0.004	0.006	0.005	0.008	0.004
Deep-Water	0	0.007	0.003	0.023	0.004	0.006	0.016	0.012	0.005	0.008	0.007	0	0.006	0.005
Pelagic	0	0.006	0.003	0.025	0.002	0.003	0.006	0.003	0.004	0.009	0.001	0.005	0.009	0.004
Terrestrial	0	0.518	13.109	28.840	0	0	6.585	0	0.836	1.887	0	0	0.470	0
Terrestrial-Fossorial	0	4.352	4.230	1.305	2.844	0	0	0	0	0	0	0.024	0	0
Terrestrial-Aquatic	0	0	0.031	0.610	0.115	0	0.888	0	0.106	0.048	0	0	0.033	0
Terrestrial-Scansorial	0	0	0	0	1.417	0	1.009	0	0	0	0	0	0	0
Terrestrial-Arboreal	0	0	0	0	0	0	1.027	0	0	0	0	0	0	0
GeneralistI	0	0.071	0.084	0.183	0.075	0.026	0.133	0.040	0.113	0.067	0.016	0	0.106	0.023

Table 3.3 Continued.

GeneralistII	0.001	0.023	0.013	0.082	0.014	0.011	0.054	0.009	0.031	0.026	0.005	0	0.028	0.006
GeneralistIII	0.002	0.050	0.033	0.078	0.044	0.004	0.052	0.021	0.041	0.045	0.010	0.002	0.081	0.006
Arboreal	0	0	0	0.555	0	0	0	0	0.974	0	0	0	0	0
Total	0.006	9.386	17.683	45.732	4.637	1.160	10.261	6.788	12.115	3.516	1.147	1.197	3.512	1.310

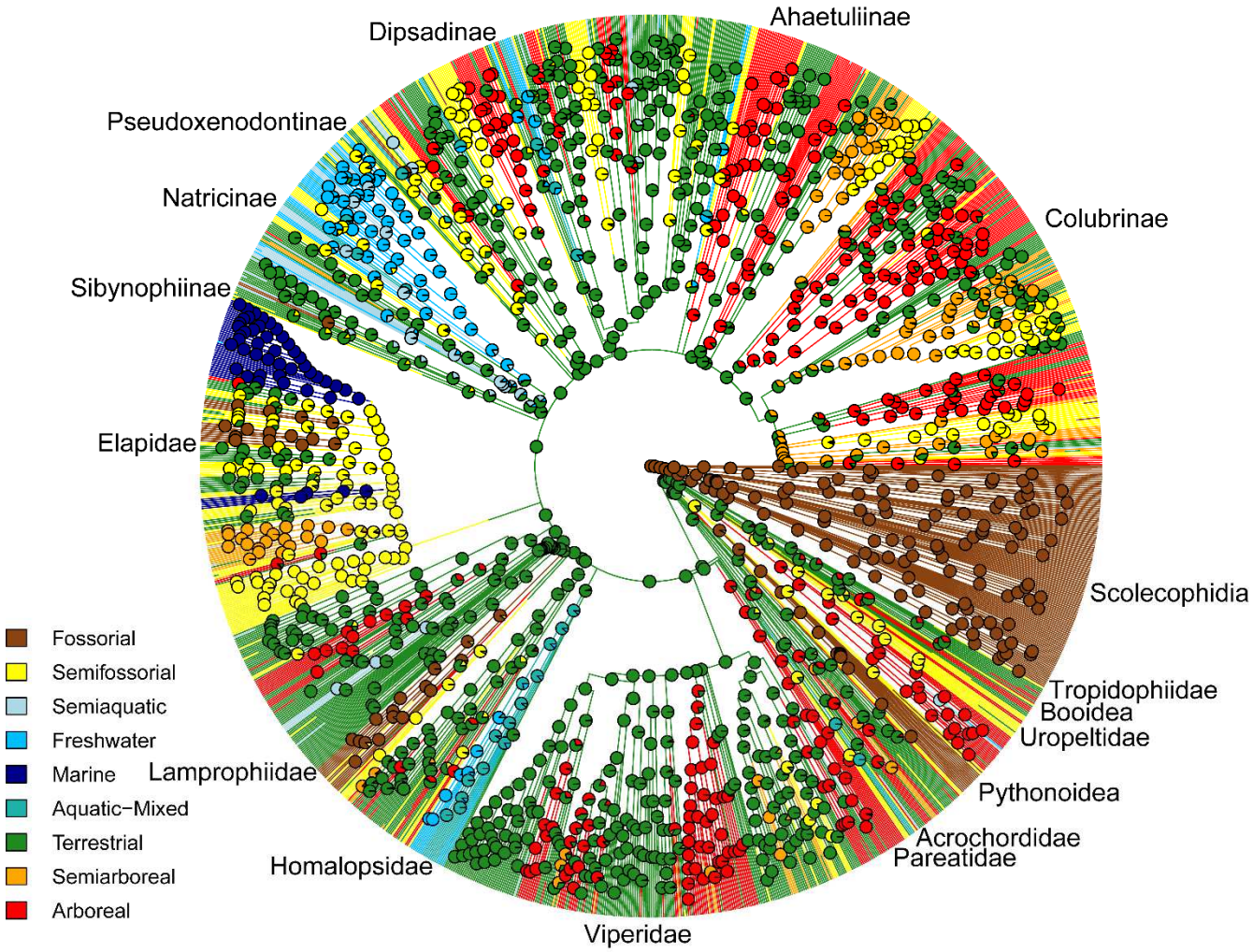
Table 3.3. Continued.

	Habitat Use													
Habitat Use	Coastal (0.69%)	Reef-Flat (0.22%)	Coral-Reefs (0.04%)	Deep-Water (0.10%)	Pelagic (0.02%)	Terrestrial (36.73%)	Terrestrial-Fossorial (3.50%)	Terrestrial-Aquatic (2.02%)	Terrestrial-Scansorial (3.84%)	Terrestrial-Arboreal (7.44%)	GeneralistI (0.38%)	GeneralistII (0.19%)	GeneralistIII (0.17%)	Arboreal (12.47%)
Subterranean-Burrower	0	0	0	0	0	1.000	0	0	0	0	0	0	0	0
Soil-Burrower	0	0	0	0	0	0	0.117	0	0	0	0	0	0	0
Sand-Burrower	0	0	0	0	0	1.445	0.066	0.004	0.052	0.363	0.047	0	0	0.075
Subterranean-Debris	0	0	0	0	0	16.816	6.498	0.691	0	0	0	4.066	0	1.489
Subterranean-Rock	0.010	0.002	0.010	0.015	0.020	1.085	0.069	0.032	0.039	0.027	0.036	0.044	0.016	0.020
Lentic	0.006	0.008	0.011	0.013	0.001	0.009	0.007	0.005	0.002	0.002	0.010	0.011	0.002	0.003
Aquatic-Freshwater	0	0	0	0	0	6.488	0.399	12.235	0	0	0	0	0	0
Freshwater-Burrower	0.019	0.017	0.012	0.018	0.011	0.038	0.765	0.068	0.012	0.009	0.022	0.026	0.014	0.007
Aquatic-Mixed	0.029	0.010	0.017	0.026	0.018	0.086	0.088	0.167	0.019	0.025	0.048	0.037	0.019	0.02
Aquatic-Mixed-Burrower	0.012	0.006	0.018	0.025	0.014	0.044	0.051	0.059	0.022	0.027	0.043	0.035	0.024	0.010
Riverine	0.005	0	0.016	0.029	0.008	0.006	0.006	0.004	0	0.001	0.009	0.009	0.002	0
Amphibious	0.005	0.009	0.008	0.006	0.004	0.007	0.014	0.004	0.003	0.003	0.009	0.016	0.001	0.012
Intertidal	1.003	0.987	0.021	0.273	0.013	0.037	0.046	0.034	0.009	0.010	0.021	0.035	0.018	0.014
Intertidal-Burrower	0.007	0.007	0.020	0.013	0.005	0.022	0.022	0.025	0.001	0	0.011	0.014	0.005	0.005
Coastal	--	1.132	2.979	6.586	0.985	0.008	0.011	0.006	0.004	0.003	0.019	0.021	0.013	0.002
Reef-Flat	0.938	--	0.985	0.024	0.016	0.002	0.005	0.003	0.003	0.003	0.011	0.004	0.012	0.002
Coral-Reefs	0.014	0.019	--	0.034	0.009	0.009	0.008	0.008	0.003	0.005	0.005	0.016	0.002	0.009
Deep-Water	0.126	0.009	0.033	--	0.016	0.005	0.016	0.005	0.003	0.001	0.003	0.012	0.002	0.001
Pelagic	0.009	0.011	0.011	0.026	--	0.007	0.011	0.008	0.003	0.002	0.003	0.010	0	0.003
Terrestrial	0	0	0	0	0	--	21.772	21.159	20.664	58.566	3.841	0	2.580	29.009

Table. 3.3 Continued.

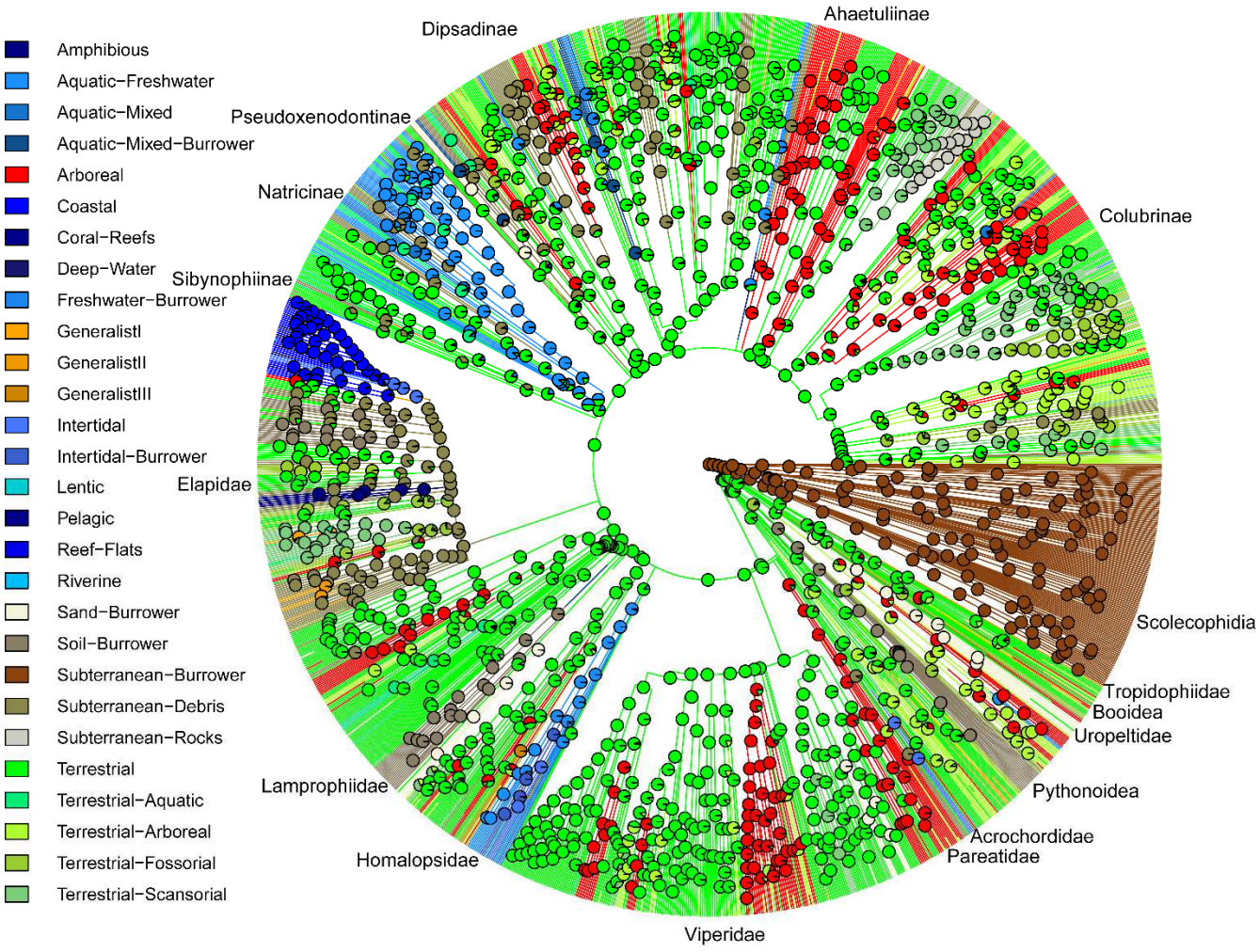
Terrestrial-Fossorial	0	0	0	0	0	10.357	--	0	1.299	0	0.424	0	0	0
Terrestrial-Aquatic	0	0	0	0	0	1.369	0.245	--	0	0	0.627	0.028	0.025	0
Terrestrial-Scansorial	0	0	0	0	0	16.912	2.642	2.985	--	1.135	1.248	0	0	0
Terrestrial-Arboreal	0	0	0	0	0	19.680	1.373	0	8.254	--	2.936	0	0	15.873
GeneralistI	0.023	0.017	0.039	0.024	0.029	0.244	0.738	0.244	0.098	0.126	--	0.124	0.063	0.128
GeneralistII	0.010	0.019	0.008	0.012	0.010	0.060	0.053	0.035	0.013	0.015	0.028	--	0.015	0.031
GeneralistIII	0.015	0.007	0.021	0.014	0.014	0.065	0.067	0.045	0.038	0.033	0.059	0.046	--	0.028
Arboreal	0	0	0	0	0	10.089	0	0	1.967	27.895	2.304	0	0	--
Total	2.231	2.260	4.209	7.138	1.173	85.890	35.089	37.826	32.508	88.251	11.764	4.554	2.813	46.741

Figure 3.1. Stochastic character mapping of 9-state snake habitat use. Ancestral state estimation on 9-states of snake habitat use based on the all-rates-different model ($p < 0.001$, AICc = 2760.54) replicated over 1000 trees. Posterior probabilities estimated the root node of snakes as being 99.7% fossorial. Outgroups are not included.



specific, unique morphologies. Arboreal and terrestrial snakes occupied the largest area of morphospace, with most arboreal species loading on the bottom right hemisphere of the plot and most terrestrial species loading on the middle of the plot. Aquatic and subterranean snakes occupied the bottom left hemisphere and the upper right portion. The first two components of the PCA explained 85.73% of the variation in morphology, with the first PC summarizing variation in body width and head shape, and PC2 explaining variation primarily in tail length and eye diameter (Table 3.4). Ward's hierarchical cluster analysis resulted in two major clusters demonstrating broad overlap in morphospace

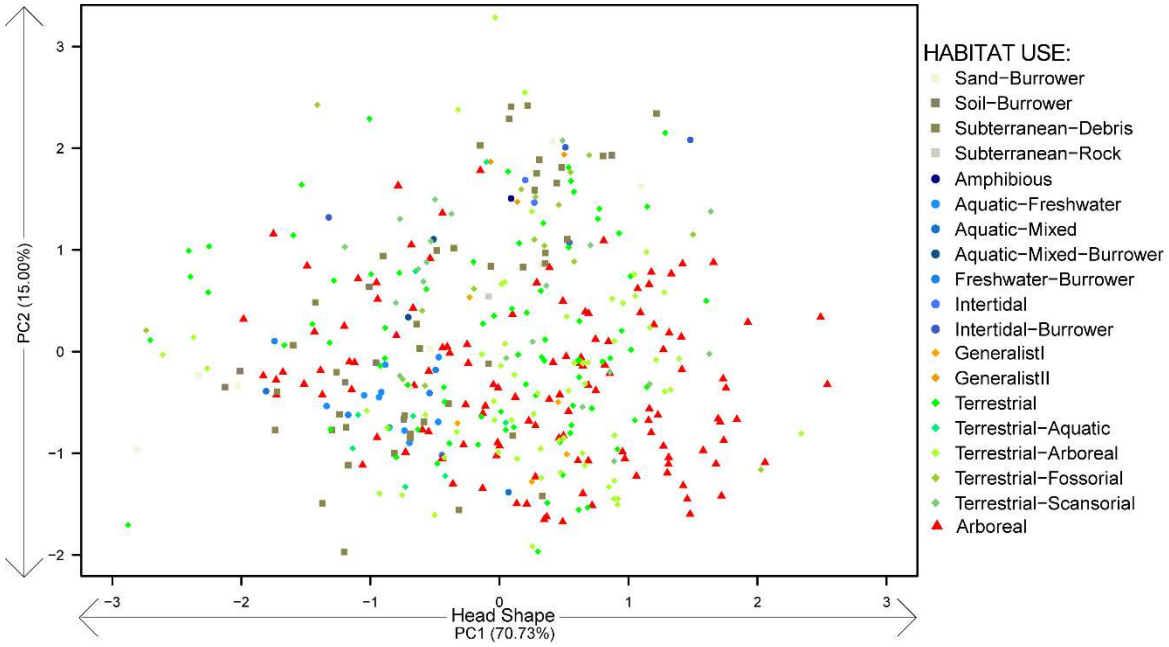
Figure 3.2. Stochastic character mapping of 28-state snake habitat use. Ancestral state estimation on 9-states of snake habitat use based on the all-rates-different model ($p < 0.001$, AICc = 3770.376) replicated over 1000 trees. Posterior probabilities estimated the root node of snakes as being 99.8% subterranean-burrower in specific habitat. Outgroups are not included.



and supporting the PCA analysis (Fig. 3.4). The two clusters differ significantly in morphology (Wilks' $\lambda = 0.445$, $F_{1, 403} = 250.89$, $p < 0.001$) in both PCs (PC1: $F_{1, 403} = 330.15$, $p < 0.001$; PC2: $F_{1, 403} = 47.22$, $p < 0.001$). Cluster 1 represents thicker species with large heads and eyes, and longer tails. Since hierarchical clustering results in a dendrogram with numerous branches, to recognize subclusters of morphologically similar species, we arbitrarily extracted 19 subclusters corresponding to the number of habitat associations in the PCA (Fig. 3.3) using the command 'rect.hclust'. The subclusters did not

equate to habitat associations, but distantly-related species from similar habitat associations did form several clades within different subclusters suggesting morphological convergence. The 19 subclusters also differ significantly in morphology (Wilks' $\lambda = 0.734$, $F_{1, 403} = 72.81$, $p < 0.001$), but only in PC1 ($F_{1, 403} = 145.95$, $p < 0.001$), with species from the second cluster loading lower on PC1.

Figure 3.3. PCA plot showing morphospace for first two PCs of size-corrected traits for 405 snakes colored by specific habitat use. Habitat associations are according to Table 3.1.



Hypothesis 3 – Morphological convergence in snakes

Pattern-based convergence analysis on the morphological clusters from the hierarchical analysis resulted in C_1 values that ranged from 0.487 to 0.707, indicating that for all subclusters except one, species were morphologically similar and that taxa within those subclusters were able to close over 50% of the evolutionary distance separating them, and all were significantly convergent (Table 3.5), supporting cluster assignment from the hierarchical cluster analysis. Each subcluster was also defined by high C_2 values, over 1.0 in many cases, suggesting the magnitude of evolutionary change was high.

C_3 and C_4 values were essentially the same for each subcluster, with convergence only accounting for less than 0.60% of the total evolution in each subcluster from their recent common ancestor, and for less than 0.70% of the total evolution in the smallest clade containing the taxa represented in each subcluster (Table 3.5). On the other hand, C_5 values varied considerably between subclusters, ranging from 12 to 43, but none was significant, specifying that numerous lineages cross over into the morphological space of the subclusters (Table 3.5), as demonstrated by the broad overlap in morphospace (Fig. 3.3).

The best-fitting pBIC model (pBIC = 3064.004) identified six adaptive regime shifts, of which all were unique and none were convergent (Table 3.6). Both PCs supported each shift, but not each shift received high bootstrap support (Fig. 3.5A). The six adaptive shifts occurred in three major clades, Viperidae, Elapidae, and Dipsadinae, with four shifts occurring in Elapidae. Most of these taxa are subterranean or terrestrial in habits, suggesting they are utilizing the morphological adaptive landscape differently and are diverging in morphology. In comparison, the AICc model (AICc = 2872.048) identified 39 adaptive regime shifts, of which five were unique and 11 were convergent (Table 3.6). Similar to the pBIC model, shifts were supported by both PCs and not all shifts received high bootstrap support (Fig. 3.5B). Adaptive and convergent shifts occurred in species from almost every major clade and from various habitat associations. Convergent shifts even occurred between species from different habitat associations, supporting the findings from the PCA and hierarchical cluster analysis that species from different habitat associations overlap and cluster together. Again, a large number of shifts occurred within Elapidae, but not all taxa were subterranean or terrestrial. As demonstrated in Khabbazian et al. (2016), the pBIC model was more conservative in identifying shifts than the AICc model used in SURFACE.

Table 3.4. Principal component loadings on snout-vent-length corrected residuals of nine external morphological characters. The first two principal components accounted for 85.73% of the total variation. PC1 represents body width and head shape, and PC2 represents tail and eye shape.

Variable	PC1	PC2
Tail Length		**0.937**
Mid-Body Width	**0.825**	0.157
Head Length	**0.724**	0.543
Jaw Length	**0.740**	0.529
Head Width	**0.956**	0.176
Head Depth	**0.939**	0.197
Interocular Distance	**0.890**	0.279
Eye Diameter	0.436	**0.836**
Eigenvalue	5.66	1.20
% Variation Explained	70.73%	15.00%

Figure 3.4. Hierarchical clustering dendrogram of 405 snakes and boxplots of first two principal components for 19 subclusters. Hierarchical clustering analysis resulted in two major clusters, labeled on the plot.

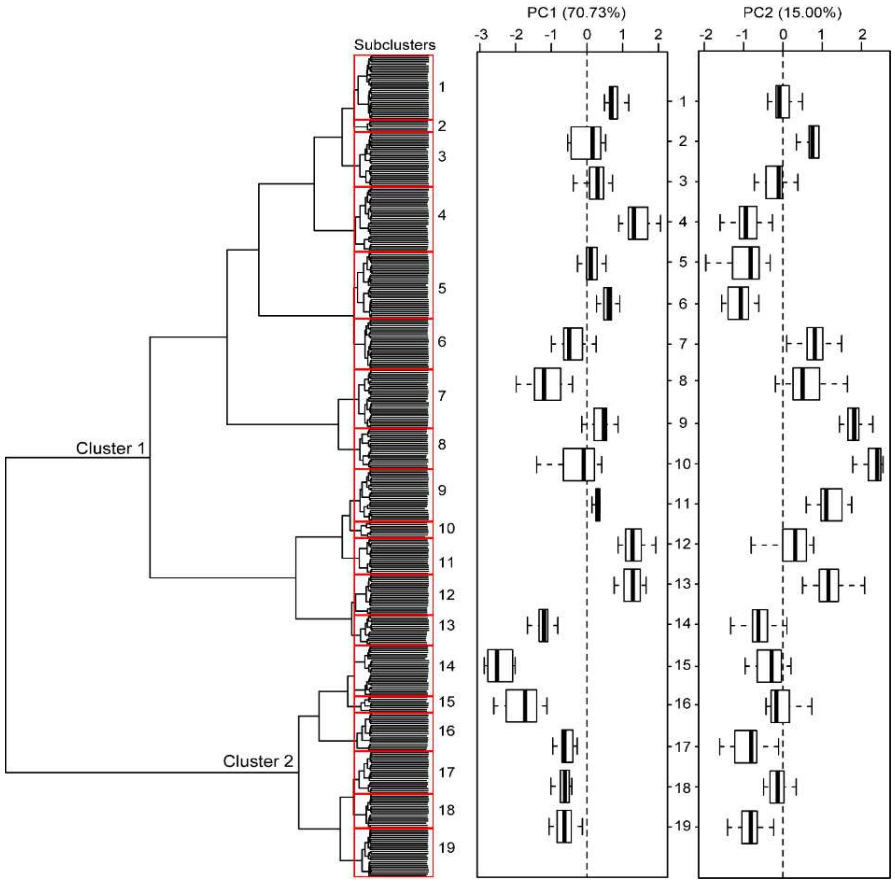


Table 3.5. Similarity- (C_1-C_4) and frequency-based (C_5) convergence measures for snakes. C_1 = the proportion of the maximum distance between focal taxa that has been closed by evolution; C_2 = C_1 while accounting for the magnitude of change; C_3 = the amount of evolution attributable to convergence between focal taxa; C_4 = the amount of evolution attributable to convergence to the smallest clade containing these focal taxa; and C_5 = quantifies the number of lineages that have evolved into a certain region of morphospace and counts the number of lineages entering the region of the morphospace occupied by the hypothesized convergent taxa. Higher C values correspond to greater convergence. Significance tests are for C_1 and C_5, and significant *P*-values are displayed in bold.

Subclusters	**Convergence values**						
	C1	***p***	**C2**	**C3**	**C4**	**C5**	***p***
Subcluster 1	0.613	**< 0.001**	0.699	0.002	0.002	31	0.551
Subcluster 2	0.529	**< 0.001**	0.894	0.003	0.003	22	0.651
Subcluster 3	0.487	**< 0.001**	0.532	0.002	0.002	27	0.752
Subcluster 4	0.626	**< 0.001**	1.116	0.003	0.003	25	0.200
Subcluster 5	0.537	**< 0.001**	0.741	0.002	0.002	29	0.597
Subcluster 6	0.702	**< 0.001**	1.062	0.003	0.003	26	0.165
Subcluster 7	0.506	**< 0.001**	0.729	0.002	0.002	34	0.492
Subcluster 8	0.528	**< 0.001**	0.729	0.002	0.002	32	0.502
Subcluster 9	0.654	**< 0.001**	1.198	0.003	0.003	32	0.136
Subcluster 10	0.606	**< 0.001**	1.617	0.005	0.005	12	0.173
Subcluster 11	0.576	**< 0.001**	0.848	0.002	0.003	23	0.319
Subcluster 12	0.628	**< 0.001**	1.238	0.004	0.004	29	0.266
Subcluster 13	0.618	**< 0.001**	1.329	0.004	0.004	22	0.271
Subcluster 14	0.546	**< 0.001**	0.983	0.003	0.003	39	0.208
Subcluster 15	0.707	**< 0.001**	2.054	0.006	0.007	12	0.095
Subcluster 16	0.552	**< 0.001**	1.163	0.003	0.003	29	0.181
Subcluster 17	0.570	**< 0.001**	0.879	0.003	0.003	38	0.341
Subcluster 18	0.598	**< 0.001**	0.770	0.002	0.003	41	0.199
Subcluster 19	0.593	**< 0.001**	0.890	0.003	0.003	43	0.269

Table 3.6. List of adaptive and convergent regimes and their associated taxa as identified by l1ou for pBIC and AICc model. The pBIC model resulted in 6 adaptive regimes, of which 0 were convergent. The AICc model resulted in 39 adaptive regimes, of which 11 were convergent. Convergent regimes are highlighted in bold.

Adaptive Peak	Taxa	Convergent Regime
pBIC		
1)	*Trimeresurus popeiorum*	1
2)	*Hemachatus haemachatus*	2
3)	*Acanthophis antarcticus*	3
4)	*Furina diadema, F. ornata, Simoselaps anomalus, S. bertholdi, Brachyurophis semifasciatus, Denisonia devisi, Elapognathus coronatus, Cryptophis nigrescens, Suta monachus, S. fasciata, S. suta, Vermicella calonotus, Hemiaspis signata, H. damelii, Echiopsis curta, Drysdalia mastersii, D. coronoides, Austrelaps superbus, Tropidechis carinatus, Notechis scutatus, Hoplocephalus stephensii, H. bitorquatus, H. bungaroides*	5
5)	*Vermicella intermedia*	6
6)	*Sibynomorphus neuwiedi*	4
AICc		
1)	*Ungaliophis continentalis, U. panamensis*	**1**
2)	*Corallus ruschenbergerii, C. hortulanus, C. cookii*	**7**
3)	*Chilabothrus angulifer, C. fordii, C. chrysogaster, C. striatus*	**15**
4)	*Xenopeltis unicolor, Morelia viridis*	**1**
5)	*Pareas boulengeri*	**13**
6)	*Trimeresurus gramineus*	**11**
7)	*Trimeresurus hageni, T. nebularis, T. fucatus, T. stejnegeri, T. purpureomaculatus*	**9**
8)	*Trimeresurus popeiorum*	4
9)	*Trimeresurus insularis*	**2**
10)	*Ophryacus undulatus*	**10**
11)	*Bitia hydroides, Cantoria violacea, Fordonia leucobalia*	**8**
12)	*Gerarda prevostiana*	**6**
13)	*Compsophis infralineatus, C. laphystius*	**13**
14)	*Micrurus corallinus*	**13**
15)	*Ophiophagus hannah, D. jamesoni, D. angusticeps, D. polylepis*	**9**
16)	*Hemachatus haemachatus*	5
17)	*Pseudohaje goldii*	**2**
18)	*Naja mossambica*	**2**
19)	*Naja haje*	**2**
20)	*Demansia psammophis, D. vestigiata*	**15**
21)	*Demansia papuensis*	**11**
22)	*Acanthophis antarcticus*	**6**
23)	*Pseudonaja modesta*	**2**

Table 3.6 Continued.

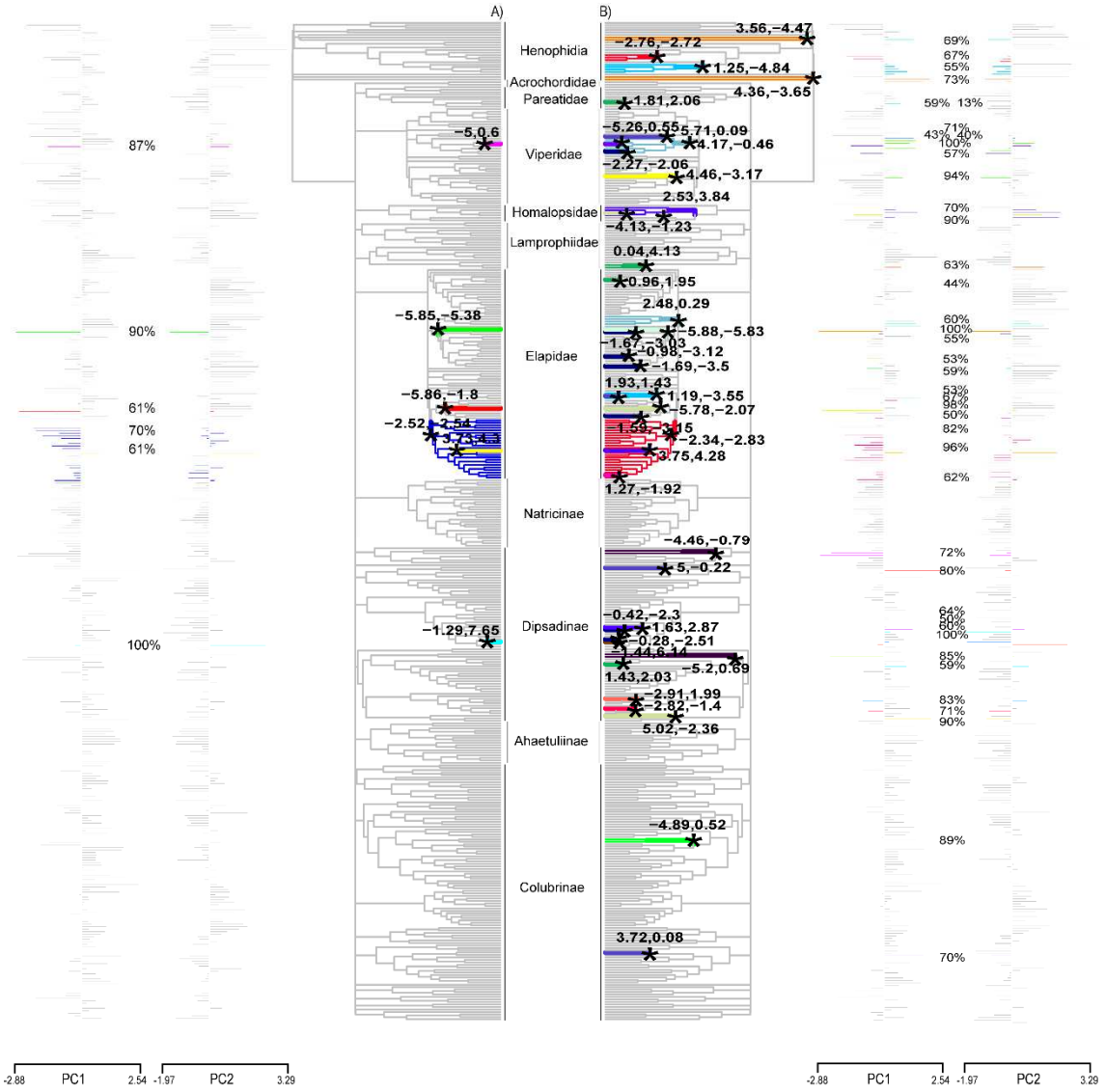
24)	*Furina diadema, F. ornata, Simoselaps anomalus, S. bertholdi, Brachyurophis semifasciatus, Denisonia devisi, Elapognathus coronatus, Cryptophis nigrescens, Suta monachus, S. fasciata, S. suta, Vermicella calonotus, Hemiaspis signata, H. damelii, Echiopsis curta, Drysdalia mastersii, D. coronoides, Austrelaps superbus, Tropidechis carinatus, Notechis scutatus, Hoplocephalus stephensii, H. bungaroides*	**7**
25)	*Vermicella intermedia*	**8**
26)	*Hoplocephalus bitorquatus*	16
27)	*Heterodon platirhinos, H. simus*	**12**
28)	*Imantodes inornatus*	**11**
29)	*Sibynomorphus mikanii*	**8**
30)	*Sibynomorphus turgidus*	**2**
31)	*Sibynomorphus ventrimaculatus*	**2**
32)	*Sibynomorphus neuwiedi*	3
33)	*Tropidodryas striaticeps, T. serra*	**12**
34)	*Thamnodynastes pallidus*	**13**
35)	*Philodryas baroni*	14
36)	*Philodryas argentea*	**6**
37)	*Uromacer catesbyi*	**10**
38)	*Crotaphopeltis tornieri, C. hotamboeia*	**12**
39)	*Oxybelis fulgidus*	**11**

Discussion

Habitat Use

Habitat use is an important source of biological variation because shifts into novel habitats sets the stage for morphological and cladogenic diversification as species are challenged by new selective regimes (Schluter 2000; Yoder, et al. 2010). Since morphology is highly correlated with ecology (Williams 1972; Arnold 1983; Losos 1990), similarities in habitat use acts as a predictor of convergence in morphology and other life-history traits. Stochastic character mapping supports earlier studies (Bellairs and Underwood 1951; Shine and Wall 2008; Yi and Norell 2015), strongly pointing to a subterranean origin, heavily influenced by the

Figure 3.5. Morphological convergent adaptive regimes identified by l1ou for 405 snakes. Pruned time-constrained phylogeny and bar graphs showing evolutionary shift configurations for first two principal components. A) pBIC model. B) AICc model. Colored branches illustrate taxa undergoing shift in adaptive regime and black/grey branches depicting non-adaptive regimes. Shifts are marked by a star and shift magnitude in the optimum trait value for each PC.



phylogenetic position of Scolecophidia. Indeed, a burrowing origin is often implicated as the catalyst for many of the features that characterize snakes (reviewed in Shine and Wall 2008). From this fossorial condition, snakes transitioned into terrestrial habits at the base of Alethinophidia, and encountered ecological opportunity providing them with new and more plentiful resources, especially since the morphology of snakes allow them to exploit prey not readily accessible to other predators (Gans 1975; Pough 1983). As Alethinophidia diversified, snakes continued expanding into other habitats, even recolonizing subterranean habitats. Thus, diversification of habitat use, as shown by extant snakes, occurred after the rise of Alethinophidia coinciding with an explosion of snake diversity when snakes also diverged in all aspects of morphology, ecology, and behavior (see Chapter 2). Thus, as they radiated and filled niches, and communities continued to grow, more opportunities arose for species to adapt and diversify (Losos 2010). Furthermore, since snakes form species-dense communities, they may have created their own ecological opportunity (Erwin 2008; Losos 2010; Ricklefs 2010) by partitioning niches to limit competition (Toft 1985; Luiselli 2006).

Nearly all habitat associations independently evolved multiple times, setting the stage for convergent evolution. Repeated evolution of certain habitat categories, such as arboreality, from comparatively accessible habitats, suggests that shifts into these habitat states may not require drastic changes in morphology. Since most transitions transpired to or away from terrestrial habitats, terrestrial snakes may maintain a generalized morphology, favorable for adapting to changes in habitat state. This could partially explain why snakes of different habitat associations exhibited considerable overlap in morphospace.

Morphological Shape Variation

The broad overlap in morphospace shows that snakes from different habitat associations share similar regions of the morphological landscape, indicating that habitat use is not directly tied to morphology. Variation in morphology derives from differences in functional performance in ecologically-relevant traits, and is expected to match the species' environment (Arnold, 1983; Irschick and Garland, 2001). In other taxa, where species have been shown to form distinct morphological clusters related to habitat use, these taxa vary in their appendages such as length of antennae and pereopods in cave amphipods (Trontelj et al., 2012) and limb shape in various lizards (Losos, 1990 Revell et al., 2007; Goodman et al., 2008; Collar et al., 2010). Thus, it may be that the conserved body plan of snakes (i.e., elongate, cylindrical body with no limbs), which can only be altered in length, width, height, and head shape, is capable of performing within different functional environments, which is advantageous given that most species move across multiple environments. In this case, species evolve to either function effectively in multiple environments or specialize for a specific environment, potentially incurring tradeoffs (Bonnet et al., 2005). Perhaps the most functionally demanding activities for snakes are burrowing, climbing, and swimming, with some species possessing specialized traits such as small, solid heads in burrowers (Shine and Wall, 2008) and paddle tails in sea snakes (Aubret and Shine, 2008), which we do not examine here. However, most snakes are capable of at least climbing and swimming (Greene, 1997), and snake species generally have little need to burrow since they feed on prey not found in soils or use burrows from other animals for shelter. For instance, snakes traversing arboreal environments can cross gaps in the canopy by using the posterior portion of their elongate bodies to form loops or coils around branches for support, and using the anterior

portion of their body to extend and grasp substrate, instead of jumping like other wingless animals (Lillywhite and Henderson, 1993). Arboreal snakes have an advantage by having lighter bodies relative to snakes from other habitat associations (Pizzatto et al., 2007), precluding branches bending under their weight. Elongation and limblessness provides organisms with the advantage of moving more efficiently by using lateral undulation, the main type of locomotion in snakes, which they use to move in various contexts, including climbing and swimming (Gans, 1975; Astley and Jayne, 2009), eliminating the need for specialized locomotor modes, although snakes do use alternative modes of locomotion, including specialized modes, but these are not accompanied by major external morphological adaptations other than variations in scale shape or number (Gans, 1986; Greene, 1997). This may partially explain why variation in morphological shape was more prevalent in head shape. Given that the lack of limbs greatly reduces the number of quantitative characters related to locomotor performance, and traits associated with locomotion have not been clearly identified, it could be that the characters we measured are not functionally-relevant traits, nor do they adequately capture variation associated with locomotion. However, the role the lack of limbs and body elongation played early in snake evolution is undeniable (Gans, 1975; see Chapter 2), and is presumed to have provided snakes with the structural foundation to ingest large meals (Shine and Wall, 2008), which was the precursor for evolutionary changes in head shape.

The lack of limbs constrains prey capture and handling, requiring snakes to swallow prey whole. As such, snakes are gape-limited predators, restricted in the size and shape of prey they can eat, and selection acts to decrease the time needed to swallow prey (Vincent et al., 2006). Accordingly, head shape is a strong determinant of diet (Savitzky, 1983). In other gape-limited organisms, such as fish, variation in morphology is also predominately concentrated at the

trophic level (Frédérich and Vandewalle, 2011; Mushick et al., 2012; Lobato et al., 2014). In those studies, morphological types are quite distinct, but morphology and trophic ecology are also associated with habitat use. Although, we did not examine diet, but previous studies examining head shape in snakes have demonstrated variation in head shape in relation to diet (Hampton, 2011) and convergence in species consuming similar prey (Fabre et al., 2016). However, we do not have an understanding of feeding functional diversity since performance studies of feeding in snakes is limited to aquatic species (Herrel et al., 2008). In aquatic taxa, head shape differs based on prey type and whether species use laterally- or frontally-directed strikes (Drummond, 1983; Young, 1991; Herrel et al., 2008) and distantly-related taxa have converged in head shape and strike type (Bilcke et al., 2006; Herrel et al., 2008). Yet, head shape, to a lesser degree, is also associated with habitat use, particularly for burrowing species (Fabre et al., 2016), and in other burrowing organisms (Navas et al., 2004; Barros et al., 2011), activity patterns (Fabre et al., 2016), sexual dimorphism (Vincent and Herrel, 2007), and predator defense (Dalbosco et al., 2012). Therefore, it is important to differentiate between conflicting signals of selection on head shape. A detailed comparative study of head shape in snakes has yet to be conducted, but in general, dietary generalists have wider and taller heads (Fabre et al., 2016), piscivorus species have longer and narrower heads (Herrel et al., 2008; Fabre et al., 2016), and those eating crustaceans have wide and tall heads with modified skulls and teeth, and small gapes for crushing prey (Fabre et al., 2016). Ultimately, selection on feeding biology culminated in many key innovations and shaped the diversity we witness today (see Chapter 2).

Morphological Convergence in Snakes

Convergence in morphology develops when the number of ways to perform a certain function is limited (Herrel et al., 2008). In snakes, selection could favor divergence in morphological traits associated with head shape, due to variation in diet, rather than in characters related to locomotion. This distinction is important for two reasons: 1) niches are multidimensional, and species may converge in different aspects of a particular niche (Harmon et al., 2005); and 2) individual traits can evolve at different rates, such as head shape evolving faster than body shape in cichlids (Young et al., 2009). If diet indeed outweighs the importance of locomotion in establishing niche placement in snakes, morphological traits associated with diet may diverge more rapidly for reasons mentioned above.

Convergence is often demonstrated when species cluster together in trait space (Harmon et al., 2005; Trontelj et al., 2012; Stayton, 2015). Our cluster analyses revealed that distantly-related species clustered in trait space, but clustering did not orient with habitat use. By testing convergence using two methods, a pattern-based and a process-based approach, we reached different conclusions regarding morphological convergence in snakes. The pattern-based approach tests that similar phenotypes evolved independently in multiple lineages (Stayton, 2015). Within each subcluster of the hierarchical analysis species were morphologically similar to each other, and this was supported by the distance-based measure of convergence. However, the phylomorphospace analysis as conducted under the frequency-based measure of convergence showed that numerous taxa invaded the morphospace of each subcluster, making convergence nonsignificant. This is likely due to the large number of species constituting each subcluster, making the region of morphospace for each subcluster large enough for more taxa to invade.

Recently, more focus has been placed on testing the process of adaptation in producing convergent evolution (Ingram and Mahler, 2013; Uyeda and Harmon, 2014; Bastide et al., 2015; Khabbazian et al., 2016). These methods test for evolved similarity due to adaptation by using patterns of morphological variation to estimate shifts in adaptive regimes on a phylogeny (Ingram and Mahler, 2013). Specifically, they test that taxa independently underwent similar adaptive shifts in trait evolution, and associate shifts to variation in ecology. Due to problems of model overfitting using the AIC criterion (Ho and Ané 2014), we tested shifts in adaptive regimes associated with habitat use using AIC and the more conservative, pBIC criterion. Model fitting under the AIC criterion resulted in 39 adaptive regime shifts, with 11 being convergent, and the pBIC resulted in only six adaptive regime shifts, with none being convergent. Regime shifts appear throughout the entire phylogeny under the AIC criterion, but is focused primarily in subterranean elapids under the pBIC criterion. Inspection of hierarchical subclusters show that taxa considered convergent using the AIC model do not all cluster together. Lack of convergent shifts indicate that taxa with similar ecologies may have not yet reached the same adaptive peaks (Friedman et al. 2016) or multiple dimensions of niche have not been captured in our dataset (Harmon et al., 2005).

Conclusions

Snakes arose from a fossorial origin and diversification in habitat use ensued after the rise of Alethinophidia, with several habitat categories evolving numerous times. Variation in morphological shape overlaps broadly in snakes making it difficult to classify species habitat use based on morphology. Therefore, an appropriate understanding of habitat use requires adequate

field studies quantifying a species spatial ecology. Morphological variation is largely associated with head shape, most likely related to divergence in diet than in habitat use. Snakes feed on various types of prey that encompass different sizes and shapes (Colston et al., 2010), and incorporate different prey-handling mechanisms (Cundall and Greene, 2000), yet it is unknown how these relate to head shape. Geometric morphometrics is superior at capturing more of the functionally important variation (Adams et al., 2004) in head features and should be adopted to more adequately provide insight into the diversity of feeding systems in snakes. Furthermore, since snakes form species-dense communities, it would be interesting to examine body shape and ecological functional diversity within communities to see how snakes partition these axes because convergent evolution seems prominent in species-dense communities where species exceed the number of available niches (terHorst et al., 2010).

Acknowledgements

We would like to acknowledge the curators and staff at the following museums for providing us with lab space: American Museum of Natural History, New York (AMNH), Academy of Natural Sciences (ANSP), California Academy of Sciences (CAS), Field Museum of Natural History (FMNH), Los Angeles County Natural History Museum (LACM), Lee Kong Chian Natural History Museum (LKCNHM), LSU Museum of Natural Science (LSUMNS), Harvard University Museum of Comparative Zoology (MCZ). We also want to thank Liam Revell (phytools), Cécile Ané and Mohammad Khabbazian ('l1ou), and Tristan Stayton (convevol) for providing help with their respective R packages. This research was funded by the following grants to A.F.: Jessup Fellowship from ANSP, Charles Stearns Memorial Grant-In-Aid

from CAS, FMNH Collections Study Grant, and National Science Foundation East Asia & Pacific Summer Institute (OISE-1107819).

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Supporting Information

Table S3.1. Habitat use of sampled snakes. Taxonomic nomenclature follows the current classification indexed in the Reptile Database (http://www.reptile-database.org/). For some species, references may reflect outdated taxonomic status. Individual species are coded for habitat association according to Table 3.1. References for this table are listed below. Habitat use for species without a reference were inferred from sister taxa.

File 3.1. Description of substituted taxa.

Chapter 4. Striking from a Limb: Context, Morphology, and Strike Performance in a Prehensile-tailed Arboreal Snake, *Corallus hortulanus*

Abstract

Whole-organism performance varies with ecological and behavioural context and arboreal environments place unique functional demands on organisms, whereby animals must remain stable while negotiating complex, precarious surfaces at often considerable heights. We measured strike performance under two behavioural contexts, predatory and defensive, in an arboreal snake, *Corallus hortulanus*, to determine if acceleration, velocity, and target distance differ between individuals that use their prehensile tails to perch compared with those that have their tails constrained. We test the hypothesis that prehensile tails provide arboreal snakes with an anchor for support from which they can launch fast strikes and incorporate their entire trunk in striking such that more distance is covered between the snake and its target. Furthermore, we posit that predatory and defensive strike kinematics is affected differently by tail constraints. Prehensile tails did not allow snakes to strike with greater velocity and acceleration. However, during defensive strikes, acceleration greatly decreased in trials when their tails were constrained relative to unconstrained trials. Treeboas also launched predatory strikes at significantly shorter distances than defensive strikes, suggesting that acceleration is maximized and maintained

during predatory strikes to cover shorter distances quicker. This study demonstrates that behavioural and ecological context both contribute to observed variation in striking performance, and highlights the dynamic role of morphology in determining performance in different contexts.

Introduction

An organism's morphology is fashioned by selection to meet functional demands imposed by its environment. Structural habitat is particularly important because it varies both within and among different environments, thereby challenging an organism's performance in different contexts (Rand 1964; Irschick & Losos 1999). The three-dimensional configuration of arboreal environments is especially challenging since surfaces used by organisms for support and locomotion vary in size, mass, density, and incline, and are often interspersed with gaps and obstructions. Maneuvering and performing ecological tasks within this habitat thus requires operating with great stability. Consequently, arboreal organisms exhibit traits such as claws and toe pads (Cartmill 1974; Hanna & Barnes, 1991; Irschick et al. 1996; Wolff & Gorb 2014), modified limbs for grasping (Manzano et al. 2008; Herrel et al. 2012; Sustaita et al. 2013), and prehensile tails (Emmons & Gentry 1983) which aid them in navigating these complex environments and enable other key performance traits.

Whole-organism performance (i.e. measurements of individuals conducting dynamic, ecologically relevant behaviors such as jumping, flying, or biting; Lailvaux & Husak 2014) provides a direct and intuitive link with survival and fitness, and is therefore subject to a variety of selection pressures (Husak & Fox 2008; Irschick et al. 2008). However, the importance and utility of a particular performance trait varies depending on ecological context (Irschick and Garland, 2001). For example, *Crotaphytus collaris* lizards do not always move at their maximum sprint capacities in nature, and will modify their speed depending on whether they are foraging, escaping from a predator, or defending a territory (Husak & Fox, 2006). Given that individuals may alter the kinetic or kinematic aspects of a given performance trait depending on the scenario

at hand, ecological context is likely to be an important contributor to overall variation in the evolution and expression of whole-organism performance capacities.

Striking is a performance trait used by a variety of organisms during several important ecological contexts, most commonly predation and defense. Within snakes, for example, strikes occur either in the course of a predation attempt (Kardong 1986; Vincent et al. 2005; Cundall et al. 2007) or as a defensive mechanism against a perceived threat (Whitaker et al. 2000; LaDuc 2002; Herrel et al. 2011). Accordingly, predatory and defensive strikes have different causes and consequences and are thus likely subject to different selection pressures which may diverge or converge (Lailvaux & Kasumovic 2011). For example, venomous snakes meter and expend different quantities of venom depending on strike context (Hayes et al. 2002). Striking is used predominately by ambush foragers that feed on active, mobile prey (Huey & Pianka 1981) where a quick, unforeseen attack (but see deVries et al. 2012) is necessary to provide an element of surprise and thereby increase prey capture success. For ambush predators feeding on highly evasive prey, their predatory strikes are accompanied by fast acceleration to minimize the time prey has to escape (Higham 2007). However, some taxa have also evolved prey immobilization techniques such (i.e., constriction, envenomation, webbing, etc.) to mitigate the threat of prey retaliation and increase prey capture. Strike success may be less important in defensive strikes since it functions primarily to encourage predators to keep their distance, as evidenced by mock strikes (Whitaker et al. 2000; Figueroa personal observation). As such, distance to target is likely a key component in striking, and should differ depending on whether snakes are attempting to maximize prey capture or deter predators. While acceleration and velocity may also vary between strike types, we currently lack a proper understanding of the kinematic differences between predatory and defensive strikes.

Kinematic analyses of the strikes of terrestrial snakes show a contrast between: 1) a kinematically active region of snake morphology that exhibits changes in posture and displacement; and 2) a kinematically fixed region that experiences no displacement, but instead serves to establish a secure purchase with the ground to accelerate and generate high momentum of the active region towards strike targets (Kardong & Bels 1998; Cundall 2002). Consequently, fast strikers exhibit modifications to these two areas, specifically reduction of mass in their anterior regions that attain the highest velocity, and increase in mass of their posterior regions (Cundall 2002). Since snakes engage a considerable portion of their trunk in striking, they require a stable support from which to launch and propel their strikes. Prehensile tails were accompanied by lengthening of the tail and independently evolved in arboreal descendants of heavy-body terrestrial taxa with short tails (viz., boas, pythons, and vipers; Feldman & Meiri 2013) and likely function to provide adequate support to launch rapid strikes and also provide the advantage of freeing up the entire trunk for use in striking (Herrel et al. 2011). However, arboreal environments may hinder strike performance since many supports (i.e., thin/short/weak branches or leaves) are unstable and do not provide a reliable foundation for launching a strike. Given the potential for kinematic variation between strike types, particularly with regard to target distance, it may be that prehensile tails are used to a greater or lesser extent in predatory versus defensive strikes. Thus far, no study has explicitly tested the direct functional role of prehensile tails in affecting strike kinematics in arboreal snakes. Although previous studies have examined strike behaviour in arboreal snakes with prehensile tails (Shine et al. 2002; Herrel et al. 2011) and one on a non-arboreal snake with a prehensile tail (Smith et al. 2002), neither of these studies considered whether prehensile tails influence strike kinematics.

We tested whether predatory and defensive strike kinematics differ between *Corallus hortulanus* (Linnaeus 1758) that were able to use their prehensile tails for perching compared with those that were experimentally prevented from using their prehensile tails during the extension phase (i.e., period from initiation of forward movement to target contact; Kardong & Bels 1998). We prevented snakes from using their tails by constraining their tails with wooden dowels. We were specifically interested in addressing the following three hypotheses: 1) Prehensile tails affect general strike performance by allowing snakes to strike with greater velocity and acceleration; 2) Prehensile tail constraint affects predatory and defensive strike kinematics differently; and 3) Distance to targets are shorter in predatory strikes than defensive strikes.

Materials and methods

Snakes and husbandry

We tested strike performance in 15 male *C. hortulanus* acquired through a commercial supplier. Previous studies of snake strike kinematics achieved significant results with similar or lower sample sizes (LaDuc 2002; Vincent et al, 2005; Young 2010). Each snake was maintained within separate 10 ga. glass aquariums with bark mulch substrate, a wooden dowel perch, and a heat source (75W light bulb) on a 12L:12D photoperiod. We provided snakes with water *ad libitum* and fed them freshly-killed mice once a week. Upon acquisition, we measured each snake's snout-vent length (SVL) to the nearest 0.01 cm using digital Mitutoyo calipers (Chicago,

IL, USA), and weighed individual snakes to the nearest 0.05 g using a Pesola scale (Barr, Switzerland).

Tail Treatment

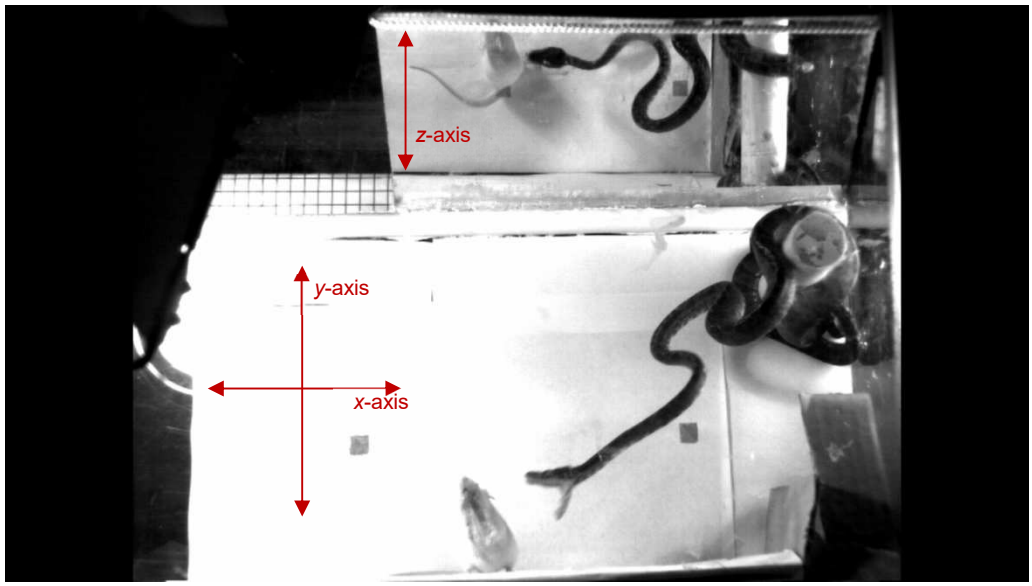
To test the effect prehensile tails have on strike performance and prey capture, we tested each individual using two treatments: 1) unconstrained (Fig. 4.1A); and 2) constrained (Fig. 4.1B). For the constrained treatment, we splinted each snake's tail by taping two, thin wooden dowels to the lateral sides of the tail from the cloaca to the tail tip. After filming the trials, the wooden dowels were removed and reattached before subsequent constrained trials.

Kinematics

The trial arena consisted of a 92x32x43 cm glass aquarium where we placed snakes on a wooden dowel perch elevated 20 cm above the aquarium floor. All strikes were oriented parallel to the length of the aquarium and along the horizontal plane. For predatory strikes, we placed a single live mouse in the aquarium and allowed it to roam freely. We chose this experimental design because previous accounts on treeboas report them as striking prey from above in the wild (Henderson 2002; Yorks et al. 2003). For defensive strikes, we waved a glove in front of the snakes. We filmed snakes for defensive strikes at least every other day with no more than five defensive strikes for each snake per day, and at least once a week for predatory strikes. The project design resulted in four treatments: 1) unconstrained-predatory strike; 2) unconstrained-defensive strike; 3) constrained-predatory strike; and 4) constrained-defensive strike.

Figure 4.1. Photographs captured from video stills showing the lateral and ventral views (mirror mounted above the snake at 45° relative to the cage floor) and the three axes to facilitate three-dimensional analyses of predatory and defensive strikes of adult male *Corallus hortulanus*. A) Photograph capturing predatory strike of a snake with tail unconstrained. Colored squares in the background were used to calibrate distance in ProAnalyst. B) Photograph captured from video still showing the lateral and ventral views (mirror mounted above the snake at 45° relative to the cage floor) of a defensive strike and showing splinted tail (labelled in photograph).

A



B

We recorded predatory and defensive strike performance using a Fastec Troubleshooter TS1000MS (San Diego, CA, USA) high-speed camera with a frame rate of 250 Hz mounted with a Computar TV 12.5-75 mm F1.2 (Commack, NY, USA) zoom lens positioned lateral to the aquarium. To illuminate the arena, we used an Impact Qualite 300W (New York, NY, USA) focusing flood light. We recorded all strikes in three dimensions (*x*, *y*, and *z*; Fig. 4.2A) by mounting a mirror at the top of the aquarium, 45 degrees to the aquarium floor. The *x* and *y* coordinates represent the horizontal and vertical dimension, respectively, along the lateral plane parallel to the camera; whereas the mirror captured the ventral view and lateral motion of the strikes providing the *z*-coordinates representing the depth dimension perpendicular to the *x*-*y* plane. We placed a sheet of white paper in the background with two squares drawn 20 cm from each other, and another sheet of white paper on the aquarium floor below the perch with two squares drawn 10 cm from each other as points of reference to facilitate converting pixel distance to a calibrated distance in centimeters when analyzing the videos.

We analyzed videos using Xcitex ProAnalyst v1.5.3.0 (Cambridge, MA, USA) by using each snake's snout (LaDuc 2002; Alfaro 2003; Bilcke et al. 2006) as a landmark and manually digitizing its displacement (i.e., distance travelled) frame by frame. We limited our analyses to the extension phase of the strike for the following two reasons: 1) maximum velocity and maximum acceleration are achieved just prior to (Kardong & Bels 1998; Alfaro 2002; Vincent et al. 2005), or after the moment of contact with the target (LaDuc 2002); and 2) due to coiling and constriction during prey capture, the snout becomes masked by the prey and thus impossible to continue tracking beyond strike contact. Digitization began at the beginning of the strike (i.e., frame preceding noticeable head movement, Alfaro 2002) and ended when the snake first made contact with its target or when the snake reached maximal forward displacement. We digitized

the displacement of the snout twice: 1) along the two-dimensional strike trajectory parallel to the camera; and 2) along the two-dimensional strike trajectory captured by the mirror. To eliminate unwanted noise that occupies the higher frequencies of the performance histograms, we smoothed the raw displacement profiles using a zero phase shift low pass Butterworth filter (Winter 2004) with a cut-off frequency set at 50 Hz. Next, we used Pythagoras' rule to merge the two 2-dimensional strike trajectories into a single 3-dimensional trajectory and calculated the three-dimensional values of velocity and acceleration from this strike profile.

Statistical Analyses

We conducted all statistical analyses on the full dataset using the `AICcmodavg` (Mazerolle 2015), `car` (Fox & Weisberg 2011), `MASS` (Venables & Ripley 2002), `nlme` (Pinheiro et al. 2013), and `psych` (Revell 2014) packages in RStudio (v.0.98.1062, R Development Core Team 2013). Prior to analyses, we Log_{10} transformed performance variables to meet the assumptions of normality and homoscedasticity. First, we compared means for treatment and strike type for each dependent variable (i.e., velocity, acceleration, and target distance) using t-tests. To test for performance differences in velocity, acceleration, and target distance between tail treatments and between strike types, we employed a stepwise multivariate linear mixed-effects model approach to account for random effects stemming from individual variation in strike performance. We entered treatment, strike type, and their interaction as fixed effects while controlling for SVL by adding it in as a covariate. Since we measured snakes repeatedly for each combination of trials, we entered individual as a random effect, and strike type as a by-individual random slope to model individual differences in strike behaviour. We

implemented this model separately on each of the three dependent variables using the maximum likelihood function and with a heterogeneous residual covariance structure to allow residual variances to differ for the two levels of strike type. Next, we performed manual backward deletion log-likelihood ratio tests to determine the minimum adequate model (i.e. the models with the lowest Akaike's information criterion [AIC] scores) by sequentially deleting one fixed effect, resulting in four possible models. We also report the AICc, which corrects for small sample sizes. We again used likelihood ratio tests to compare the fixed effects structure of the reduced models to the saturated models. We then refitted the final, reduced models using restricted maximum likelihood (REML) to obtain unbiased estimates of variance and covariance parameters. Finally, we investigated whether target distance affects strike performance in *C. hortulanus*, first by testing for Pearson correlations between target distance with velocity and acceleration, and then by performing least-squares multiple linear regression with the performance variables as the outcome variables and target distance as the predictor variable.

Results

Of the 15 snakes, we eliminated data from five due to poor strike performance (i.e., slow strikes not directed at target) or incomplete data. Videos on the 10 remaining snakes resulted in 29 digitized videos (11 unconstrained-predatory, 5 unconstrained-defensive, 7 constrained-predatory, and 6 constrained-defensive). All predatory strikes ended in prey capture. Examples of a predatory strike and a defensive strike are shown in Figs. 4.1A, B, respectively. For trials when snake tails were unconstrained, snakes exhibited higher velocity and acceleration on

average compared with when their tails were constrained for both, predatory and defensive strikes (Table 4.1). However, these differences were not significantly different.

Table 4.1. Mean values and standard deviations of non-transformed performance variables of adult male *Corallus hortulanus* for A) constrained and unconstrained treatments; B) predatory and defensive strikes; and C) each treatment for each strike type. Asterisk represents significant difference.

A

Treatment	Constrained	Unconstrained
Performance Variable		
Mean Velocity (m/s)	2.06±0.66	1.94±0.41
Mean Acceleration (m/s^2)	132.99±112.87	111.29±58.05
Mean Target Distance (cm)	12.52±7.49	14.50±5.62

B

Strike Type	Predatory	Defensive
Performance Variable		
Mean Velocity (m/s)	1.93±0.63	2.13±0.43
Mean Acceleration (m/s^2)	133.07±67.02	107.21±59.13
Mean Target Distance (cm)*	10.62±4.95	17.97±6.81

C

Strike Type	Predatory		Defensive	
Treatment	Free	Splint	Free	Splint
Performance Variable				
Mean Velocity (m/s)	1.94±0.70	1.92±0.54	2.33±0.55	1.96±0.22
Mean Acceleration (m/s^2)	132.54±82.61	133.91±36.79	134.00±30.94	84.89±70.08
Mean Target Distance (cm)	10.35±5.03	11.05±5.18	17.30±10.27	18.52±2.74

Model testing of velocity resulted in a reduced model retaining just the covariate SVL (AICc = 21.62; Table 4.2), whereas the full model was retained as the best model for acceleration (AICc = 64.63). The interaction of treatment and strike type was a significant

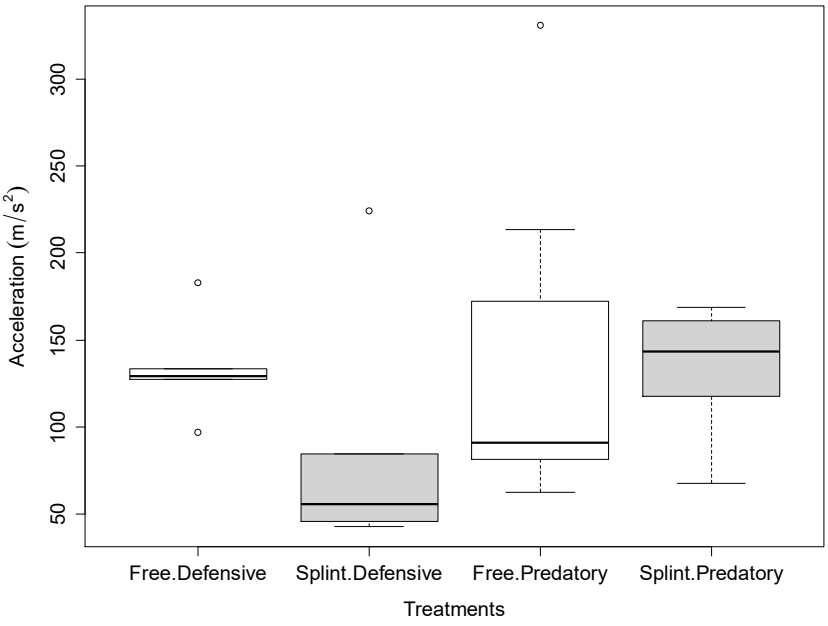
predictor of acceleration (χ^2 (9) = 4.130, p = .042; Fig. 4.2A) accounting for 26.23% of the variation in acceleration. Acceleration decreased by 0.73 m/s^2 ± 0.38 S.E. during defensive strikes when tails were splinted compared with acceleration being maintained during predatory strikes with tails unconstrained. For target distance, the best model (AICc = 68.37) was a model with strike type as a significant predictor (χ^2 (8) = 4.780, p = .029) accounting for 27.68% of the variation of target distance with distance to target decreasing by -0.66 cm ± 0.33 S.E. during predatory strikes, providing further support from the *t*-test results for defensive strikes being launched from greater distances (t (25) = 3.171, p = .004; Fig. 4.2B). We found no significant correlations between target distance with velocity or acceleration, nor did linear regression analyses reveal any significant relationships.

Discussion

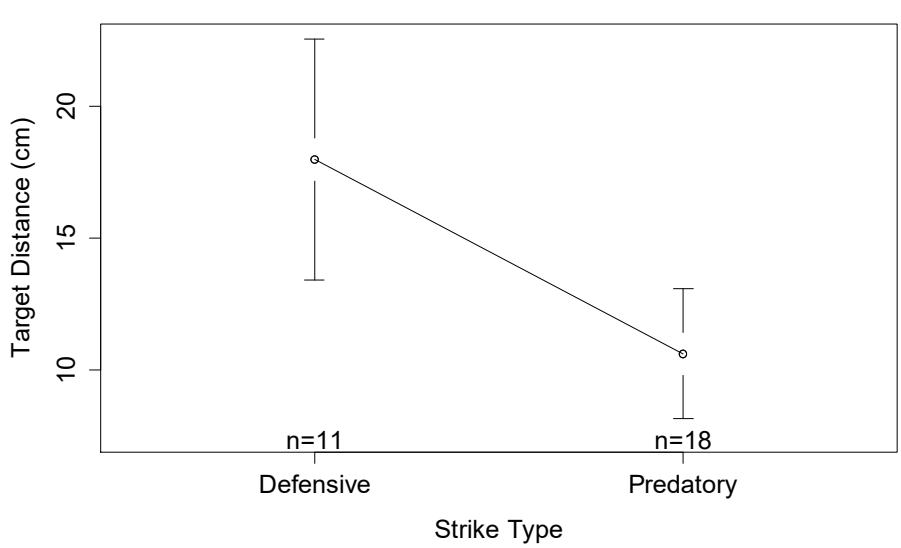
Arboreal environments present significant functional challenges to animals, and environmental complexity contributes to existing variation in performance capacities employed by individuals in different selective scenarios (Losos 1990). Previous researchers have suggested that prehensile tails play a purposeful functional role for snakes in arboreal environments, principally for support (Emmons & Gentry 1983; Lillywhite & Henderson 2001). We set out to experimentally test what influence, if any, prehensile tails have on strike kinematics in *C. hortulanus* during two behavioural strike contexts, predatory and defensive strikes. Since the snake's posterior portion needs to be anchored to launch a fast strike (Cundall 2002), we proposed that the evolutionary advantage prehensile tails offer to arboreal snakes, aside from supporting their suspended bodies from a perch, is providing a secure anchor from which they

Figure 4.2. Plots highlighting: A) the significant interaction between treatment and strike type for acceleration, and B) the influence of strike type on target distance strike type in adult male *Corallus hortulanus*. The boxplots in A) illustrate the significant difference found for the constrained treatment with acceleration in defensive strikes being significantly slower than predatory strikes. In plot B), defensive strikes were launched at significantly longer distances than predatory strikes. Values in both graphs are for the untransformed dataset. Circles in B) represent means and error bars represent 95% confidence intervals.

A



B



can launch fast strikes to capture prey and defend themselves from predators as they sit-and-wait in ambush while foraging, or while resting, as also seen in syngnathid fishes (Van Wassenbergh et al. 2011).

Table 4.2. Linear mixed model selection results testing association of velocity and acceleration with tail treatment, strike type, and their interaction, in adult male *Corallus hortulanus*. Best model based on AIC is shown in italics. AICc represents AIC corrected for small sample size.

Performance Variable	Model	Number of parameters	AIC	AICc
Velocity (m/s)	Treatment x Strike Type + SVL	4	15.91	28.14
	Treatment + Strike Type + SVL	3	14.60	24.07
	Treatment + SVL	2	14.84	22.04
	Strike Type + SVL	2	14.55	21.75
	SVL	*1*	*14.10*	*21.62*
Acceleration (m/s^2)	*Treatment x Strike Type +SVL*	*4*	*52.41*	*64.63*
	Treatment + Strike Type + SVL	3	54.54	64.02
	Treatment + SVL	2	53.58	60.78
	Strike Type + SVL	2	52.65	59.85
	SVL	1	53.58	60.78
Target Distance (cm)	Treatment x Strike Type +SVL	4	63.06	75.28
	Treatment + Strike Type + SVL	3	61.06	70.54
	Treatment + SVL	2	63.84	71.04
	Strike Type + SVL	*2*	*61.17*	*68.37*
	SVL	1	63.84	71.04

Although treeboas exhibited remarkably fast strikes during both strike contexts and during both treatments (Table 4.1), we failed to find support for our first hypothesis that prehensile tails allow snakes to strike with greater velocity and acceleration. Strike velocity and acceleration were on average higher in the unconstrained tail treatment, albeit not significantly

different. Moreover, the maximum velocity and acceleration values were observed only in unconstrained individuals. An important caveat, however, is that when individuals had their tails constrained they used a portion of their body anterior to the splint to remain attached to the perch, hinting at a possible functional role for prehensile tails. Unfortunately, to date no other study, on snakes or on other taxa, compared differences in functional performance between individuals freely able to use their prehensile tails during experimental trials with those who have their tails constrained.

In support of our second hypothesis that prehensile tail constraint varies with strike context, mixed-model analysis revealed a significant interaction between treatment and strike type for acceleration, with acceleration in constrained individuals being significantly decreased during defensive strikes compared with predatory strikes (Table 4.2; Fig. 4.1A). One possible explanation for this finding is that snakes compensated for tail constraint, allowing them to maintain high strike accelerations during predatory strikes, although the mechanism by which they might do so is not apparent from the current dataset. Existing data on snake striking provides a two-fold basis for this explanation. First, treeboas continue accelerating after striking to constrict prey (Cundall & Deufel 1999; Cundall et al. 2007). This is because after landing a strike, the force of the strike pushes the prey in the direction of the snake's head trajectory up to the point where the snake begins to coil around the prey for constriction. This is opposite to the situation with defensive strikes where snakes immediately retract their body after contact (Kardong & Bels 1998). Indeed, exploratory digitization of the contact phase of predatory strikes revealed that maximum velocity and maximum acceleration also occur after extension in *C. hortulanus* (A. Figueroa, unpublished). Secondly, rapid acceleration is favorable for capitalizing on completely surprising prey and increasing strike success, as also shown by other ambush

predators (Wainwright et al. 1991; Holzman et al. 2007; Van Wassenbergh et al. 2009). Some of these other taxa also have prehensile tails to aid in support, particularly chameleons (Zippel et al. 1999) and seahorses (Van Wassenbergh et al. 2011). Thus, acceleration is likely to be of prime importance for predatory as opposed to defensive strikes, and organisms may strive to maximize acceleration in predatory contexts.

Our third hypothesis that distance to targets are shorter in predatory strikes than defensive strikes was well supported by both *t*-test and mixed-model analysis. Treeboas launched predatory strikes from approximately 1.5 times the distance of defensive strikes (Table 4.1; Fig. 4.1B). This finding is intuitive since ambush foraging requires striking at elusive prey before they can react and remove themselves from the strike path. Snakes should therefore wait until prey are close and strike at near maximum velocity and acceleration (Frazzetta 1966; Deufel & Cundall 1999; deVries et al. 2012). By minimizing the distance of the strike, strike success and prey capture increases. LaDuc (2002) and Young (2001) demonstrated similar results in *Crotalus atrox* with defensive strikes being initiated from up to two times the distance of predatory strikes, and with significantly greater velocity. However, we did not find any association of velocity with target distance or treatment in our dataset.

An increase in the amount of the snake's trunk used in striking (i.e. strike length) will allow snakes to strike over longer distances, but also results in faster strikes (LaDuc 2002; Alfaro 2003). Target distance therefore acts as a surrogate to strike length since shorter strikes require using less of the body to strike whereas longer strikes will require using more of the body. Consequently, we urge future studies to consider it as a potential explanatory variable since it is expected to correlate with and predict strike velocity, acceleration, and target distance. LaDuc (2002) did examine strike length and reported strike length as being greater during defensive

strikes, which were also longer than predatory strikes. Thus, an added potential advantage of prehensile tails is that they free up the entire trunk to employ in striking (Herrel et al. 2011). However, when tails were constrained in this study, snakes compensated by used part of their bodies to remain attached to the perch. We nonetheless detected no significant differences in performance between constrained and unconstrained treatments.

Some snakes are capable of extraordinarily fast strikes, particularly boas, pythons, and vipers, owing to modifications to their trunk, most notably to the anterior portion of their bodies being more slender and lighter than their caudal end (Cundall & Greene 2000; Cundall 2002). In comparison to other snakes, the strikes of *C. hortulanus* are noticeably fast, even when their tails were constrained, and even faster than vipers, which are acclaimed as the fastest strikers (Table 4.3). Maximum and mean maximum velocity and acceleration in *C. hortulanus* are greater than most species previously tested except for *Erpeton tentaculatum*, where maximum acceleration is greater than treeboas for each treatment except for predatory strikes under the unconstrained treatment. Although mean maximum values in *C. hortulanus* are greater than *C. atrox* as reported by LaDuc (2002) and Young et al. (2001), maximum values in defensive strikes of *C. atrox* were greater, however these values included the contact stage for the former, but it is unclear if the contact stage is included in the latter. Unfortunately, strike kinematics of colubrids has mainly focused on comparing forward-strikes with lateral-sweeping in natricines that strike from above or below water. In general, natricines exhibit much slower acceleration due to the nature of lateral striking and striking in water, but velocity of some species is commensurate with that in boas and vipers. Interestingly, predatory and defensive strikes in *C. hortulanus* were launched at nearly twice the distances of vipers (Young et al. 2001; LaDuc et al. 2002; Vincent et al. 2005; Araújo & Martins 2007). Certainly, direct and accurate comparisons require

standardization in filming, measurement, analysis, and statistical reporting. Thus, we caution readers in interpreting comparisons. We recommend future studies strive to use standardized methods, and incorporate a larger diversity of snakes encompassing both predatory and defensive strikes.

Table 4.3. Maximum and mean maximum velocity and acceleration values for the extension stage of strikes from previous kinematic studies. For studies that reported their results as cm/s for velocity or cm/s^2 for acceleration, we converted the units into m/s or m/s^2 for easier comparison. Three-dimensional performance values are reported for this study. Aer. = Aerial strikes; Aq. = Aquatic strikes; D = Defensive; N.R. = Not Reported; P = Predatory; Terr. = Terrestrial; T.F. = Tail Free; and T.S. = Tail Splinted.

Species	Strike Type	Max. Velocity (m/s)	Avg. Max. Velocity (m/s)	Max. Acceleration (m/s^2)	Avg. Max. Acceleration (m/s^2)	Reference
Boids						
Corallus hortulanus (T.F.)	P	3.58	2.15	330.74	149.80	Present Study
Corallus hortulanus (T.S.)	P	2.60	1.89	168.62	132.38	Present Study
Corallus hortulanus (T.F.)	D	3.27	2.45	182.79	143.29	Present Study
Corallus hortulanus (T.S.)	D	2.31	2.04	224.37	124.17	Present Study
Colubrids						
Erpeton tentaculatum (Aq.)	P	N.R.	N.R.	304.42	234.44	Smith et al. 2002
Natrix maura	P	N.R	1.02	N.R	9.00	Bilcke et al. 2006
Natrix tesselata	P	N.R	0.93	N.R	8.30	Bilcke et al. 2006
Nerodia clarkia	P	0.89	N.R	N.R	N.R	Bilcke et al. 2006*
Nerodia cyclopion	P	0.24	N.R	N.R	N.R	Bilcke et al. 2006*
Nerodia fasciata	P	0.67	N.R.	N.R.	N.R.	Bilcke et al. 2006
Nerodia rhombifer	P	N.R.	0.84	N.R.	20.00	Alfaro, 2003
Pituophis catenifer	D	1.66	0.95	34.70	24.50	Greenwald, 1974
Thamnophis couchii (Aq. & Aer.)	P	1.73	1.12	N.R.	39.40	Alfaro, 2002
Thamnophis couchii	P	N.R.	0.86	N.R.	19.00	Alfaro, 2003
Thamnophis elegans	P	N.R.	0.46	N.R.	9.00	Alfaro, 2003
Thamnophis rufipunctatus (Aq.)	P	1.20	0.82	N.R.	30.05	Alfaro, 2002
Thamnophis sirtalis (Aer.)	P	N.R.	0.20	N.R.	4.00	Alfaro, 2002
Elapids						
Pseudonaja textilis	D	3.37	1.72	N.R.	N.R.	Whitaker et al. 2000

Table 4.3 Continued.

Viperids						
Agkistrodon piscivorus (Terr.)	P	1.53†	0.90†	74.70†	48.00†	Vincent, 2005
Agkistrodon piscivorus (Aq.)	P	1.62†	0.81†	75.50†	33.90†	Vincent, 2005
Bitis arietans	D	N.R.	2.60	N.R.	72.00	Young, 2010
Bothrops alternatus	D	1.31	1.23	N.R.	N.R.	Araujo & Martins, 2007
Bothrops jararaca	D	1.34	1.20	N.R.	N.R.	Araujo & Martins, 2007
Bothrops jararacussu	D	1.24	1.00	N.R.	N.R.	Araujo & Martins, 2007
Bothrops moojeni	D	1.13	1.01	N.R.	N.R.	Araujo & Martins, 2007
Bothrops pauloensis	D	1.11	1.09	N.R.	N.R.	Araujo & Martins, 2007
Crotalus atrox	P	3.48	N.R.	N.R.	N.R.	Young et al. 2001
Crotalus atrox	D	3.49	N.R.	N.R.	N.R.	Young et al. 2001
Crotalus atrox	P	2.61†	1.23	326.10†	88.94	LaDuc, 2002
Crotalus atrox	D	3.71†	2.77	333.77†	107.02	LaDuc, 2002
Gloydius shedaoensis (Adults)	D	1.71	1.32	N.R	N.R	Shine et al. 2002
Gloydius shedaoensis (Juveniles)	D	1.53	1.13	N.R	N.R	Shine et al. 2002
Trimeresurus albolabris (Males)	D	1.9	1.5	91.4	56.8	Herrel et al. 2011
Trimeresurus albolabris (Females)	D	2.4	1.6	119.0	67.7	Herrel et al. 2011
Vipera ammodytes	P	1.47	N.R	N.R.	N.R.	Janoo & Gasc, 1992

*Data cited as personal communication in Bilcke et al. (2006)
†Includes contact stage

Conclusion

Prehensile tails provide arboreal organisms with an anchor for support. We present evidence suggesting prehensile tails fulfil a similar function in arboreal snakes, allowing them to launch fast strikes and incorporate their entire trunks in striking so that more distance is covered between the snake and its target. We found that snakes accelerated significantly faster during predatory strikes than during defensive strikes in trials when their tails were constrained, possibly to compensate for that constraint. Greater acceleration during predatory strikes is likely linked to the heads of treeboas accelerating after contact up to the point where they begin to coil around prey for constriction, and to predatory strikes being launched at significantly shorter

distances than defensive strikes to maximize strike success and prey capture. Our results are consistent with those of previous studies demonstrating that behavioural and ecological context contribute to observed variation in whole-organism performance, and that these contexts should be explicitly considered in future performance studies.

Acknowledgements

We thank Lyndon Coghill, Jessica Edwards, and Robin Rowe for assistance with snake husbandry. We gratefully show appreciation to Peter Carellas, Leah Holber, and Jesse Hong from Xcitex Incorporated for their service and demonstrations on ProAnalyst. All procedures were approved by the University of New Orleans IACUC (protocol # 10-008 and 12-001).

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