TRACING THE DAWN OF THE ELAPIDAE THROUGH THE MOLECULAR SYSTEMATICS AND HISTORICAL BIOGEOGRAPHY OF OLD WORLD CORALSNAKES

by

UTPAL SMART

Presented to the Faculty of the Graduate School of Biology

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS AT ARLINGTON

November 2016

Acknowledgements

Having spent over six long years as a (often helpless and bungling) graduate student in the Biology Department at UTA, I owe my gratitude to countless colleagues, faculty and staff. I would like to start by thanking my adviser Eric Smith who saw potential in me despite of my atypical academic background and gave me the opportunity to pursue my PhD under his guidance in the U.S. He has been a supportive adviser in the lab and a fun companion in the field. In the same breath, I would also like to thank all my doctoral committee members for guiding me through my dissertation research, starting with Jonathan Campbell, Esther Betran, Matt Fujita, Paul Chippindale and Todd Castoe. I would especially like to thank Matt Fujita and Todd Castoe for opening up their labs and introducing me to modern genomic techniques. Another group of people that have been vital in my student life at UTA are the office ladies, Linda Taylor, Sherri Echols, Paulette Batten and Gloria Burlingham. Without these *superwomen* I would not have made it past the first semester at UTA. I would also like to thank Zaida Alsina and Derik Austin for so generously providing their time to help me deal with incompetent or misbehaving computers. Moreover, I am grateful to Jill Castoe, Melissa Muenzler and recently Kimberly Bowles for their excellent service at the Genomic Core Facility. Carl Franklin was helpful with work in the museum and very lavish with his rather unique sense of humour.

I would additionally like to thank my funding sources namely, The Beta Phi Chapter of Phi Sigma Society for their travel and research grants and the National Science Foundation for their grant (DEB-1146324) awarded to E. N. Smith which funded my field work in Indonesia. Outside of UTA, I have several researchers and colleagues who offered me some form of assistance or support during the last six years. In this context, I thank Dr. Karthikeyan Vasudevan of LaConES and Dr. Praveen Karanth and

his lab at the Indian Institute of Science, for sequencing Indian coralsnakes, which would have otherwise never been incorporated in my research. I would also like to extend my thanks to my colleagues and friends from Indonesia, namely Pak Amir Hamidy, Pak Irvan Siddik, Pak Nia Kuriawan, Ibu Mirza Kusrini, Mbak Evy Arida, Mas Wayhoo Triloksono and Masbro Adool who made my work and stay in Indonesia fruitful and enjoyable. I am grateful to Indraneil Das, David Bickford, Lee Grismer and other researchers working in Southeast Asia for sharing their samples of rare coralsnakes with me.

The following people have played the dual role of friends and mentors in the past six years and I owe a lot to them: Jyotiska Chaudhuri, Jeff Streicher, Coleman Sheehy, Walter Schargle, Christian Cox, Heath Blackmon, Jesse Meik, Claudia Marquez amongst several other that I am sure I am forgetting. I would like to thank the Castoe and Fujita labs and also the people of my own lab namely, Panupong Arm Thammachoti, Kyle Shaney, Goutam Sarker and Elijah Wostl. I am especially indebted to close friendships that kept me going through rough times, including the corrupting influence of Jacobo Reyes Velasco and David Sanchez; the goofy and pleasurable company of Kyle Shaney, James Titus McQuillan, Corey Roelke, Debanjana Roy and Malini Kotak; the help and support of Ruben Tovar and Paul and Karen Pasichnyk; last but not the least, the love and warmth of Coridon Laws, Amrita Pai and Umilaela Arifin.

I would like to end with a profound thankyou to my family and especially my parents who are the force behind everything that I am today, all that I have achieved thus far and all that I will accomplish in the future.

Nov 3, 2016

Abstract

TRACING THE DAWN OF THE ELAPIDAE THROUGH THE MOLECULAR SYSTEMATICS AND HISTORICAL BIOGEOGRAPHY OF OLD WORLD CORALSNAKES

Utpal Smart, PhD

The University of Texas at Arlington, 2016

Supervising Professor: Eric Nelson Smith

Much debated since the early 20th century, the evolutionary history and origin of the clinically important family Elapidae is of enormous interest. A persistent lack of higher-level phylogenetic resolution however, has impeded a clear understanding of the biogeography of this charismatic group of snakes. The traditional limiting factor in studies on higher-level elapid relationships has been the availability of samples from Old World coralsnakes (genera *Sinomicrurus* and *Calliophis*). Usually small, shy and fossorial, these animals are amongst the rarest Oriental snakes and their sampling in molecular studies has been sparse. Herein, primarily using molecular data, I work out the systematic relationships and biogeography of these two elusive genera of Asian coralsnakes. I leverage multilocus datasets, employing parametric phylogenetics and multispecies coalescent methods to provide a first insight into the systematic relationships and species boundaries of *Calliophis* species from South and Southeast Asia. I further use a phylogenetic framework to investigate biogeographic drivers of diversification in the Taiwan-Ryukyu Archipelago, using the genus *Sinomicrurus*. Finally, I estimate the most

comprehensive phylogenetic relationships to-date among Old World coralsnakes and evaluate their position among the Elapidae. In doing so I make available one of the best resolved tree of higher-level elapid relationships to date. My phylogenetic analyses refute the monophyly of Old World coralsnakes and lead to a major revision of the known subfamilial relationships within Elapidae. Additionally, a fossil calibrated time-tree reveals that the genus *Calliophis* forms a distinct basal group in relation to all other elapids. My biogegoraphical analyses suggest that the Elapidae originated in Asia and the collision between the Indian and Laurasian plates may have acted as a driver of diversification in ancestral lineages. Overall, my dissertation work provides seminal information on the taxonomy, systematics and biogeography of one of the rarest and most enigmatic groups of venomous snakes in Asia. My research thus elucidates the role of major biogeographic breaks and also highlights novel patterns of evolutionary divergence across the Oriental biogeographic realm. This improved framework of phylogenetic relationships is expected to significantly benefit all future efforts of conservation and studies of toxin evolution in the Elapidae.

Table of Contents

Acknowledgements iii

Abstract v

List of Illustrations xi

List of Tables xv

Chapter 1 A brief introduction to Old World coralsnakes 1

Chapter 2 A preliminary molecular phylogeny of Indian *Calliophis* species 6

Introduction 6

Materials and Methods 8

Taxon sampling 8

Molecular methods 10

Model selection and phylogenetic analyses 11

Species tree analysis 12

Results 13

Phylogenetic analyses 13

Species tree analysis 15

Discussion 16

Chapter 3 Molecular phylogeny and species delimitation of the long-glanded Sundaland *Calliophis* 18

Introduction 18

Materials and Methods 21

Taxon sampling 21

Molecular Methods 21

Phylogenetic analyses 22

Calliophis bivirgata 22

Calliophis intestinalis 23
Species Tree and Coalescent Species Delimitation 24
Results 26
Phylogenetic relationships 26
Calliophis bivirgata 26
Calliophis intestinalis 27
Species tree and species boundaries 29
Discussion 30
Phylogenetic relationships and species boundaries in the context of current taxonomy 30
Enigmatic patterns of divergence and distribution 31
Chapter 4 34
Introduction 34
Biogeographic patterns in the Taiwan-Ryukyu archipelago 37
Methods 40
Taxon sampling 40
Molecular data 41
Supermatrix and species tree inference 42
Species delimitation 44
Divergence Time analysis 44
Geographic range evolution 45
Results 47
Supermatrix and species tree analyses 47
Species delimitation 49
Divergence time analysis 49

Geographic range evolution 52
Discussion 55
Systematics and species boundaries 55
Biogeographic implications 56
Chapter 5 Higher-level relationships and historical biogeography of the family Elapidae 60
Introduction 60
A review of higher level elapid relationships 61
A review of the biogeographic origin and history of elapids 63
Material and Methods 64
Taxon sampling 64
DNA sequencing and alignment 65
Supermatrix and coalescent phylogenetic analyses 66
Divergence dating and fossil calibrations 68
Biogeographic analyses 69
Results 71
Phylogenetic analyses 71
Divergence dating 74
Biogeographic analyses 77
Discussion 78
A well supported phylogenetic framework of higher level elapid relationships 78
Problematic taxa 82
Spatio-temporal evolution of basal elapids 83
Revised higher-level elapid relationships 86

Appendix 89

References 109

Biographical Information 120

List of Illustrations

Figure 1.1. Distribution of the three major geographical groups of Old World coralsnakes of the genera *Calliophis* and *Sinomicrurus* in the Oriental Region 2

Figure 1.2. Phylogram of the single optimal tree estimated by maximum likelihood analysis of the combined data set (mtDNA + c-mos), modified from Castoe et al. (2007) to highlight the tribe Calliophini. Support values for nodes (> 50%) are provided in grey rectangles adjacent to nodes: posterior probability (PP) based on MCMC analyses of the combined data (1st from top of rectangle, bold), bootstrap (BSS) values based on ML analysis of the combined data (2nd from top of rectangle, bold), PP based on MCMC analyses of the mtDNA data (3rd from top of rectangle, italics), and PP based on MCMC analysis of the c-mos data (bottom of rectangle, italics). A dashed line indicates support of less than 50%. 5

Figure 2.1. Geographic distribution of sampling for the construction of a matrilineal phylogeny for the Indian *Calliophis* species. The pale green polygon represents the extent of the Western Ghats mountain range. 10

Figure 2.2. Phylogeny of Indian *Calliophis* species depicted as the 50% majority rule consensus Bayesian tree. Black circles denote strong nodal support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate strong support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value 14

Figure 2.3. Species tree of Indian *Calliophis* species (with outgroups removed). Values represent posterior probabilities. 15

Figure 3.1. Strict consensus tree of the shortest trees from morphological data for coralsnakes; bootstrap values >50% shown along the interior branches (from Slowinski et al. 2001) 19

Figure 3.2. The evolutionary relationships between *Calliophis bivirgata* subspecies inferred using the UPGMA method on Cytb sequences. The optimal tree with the sum of branch length = 0.36921331 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree 26

Figure 3.3. The evolutionary relationships within *Calliophis intestinalis* populations from across the Sundashelf depicted as a maximum-likelihood consensus tree with other species of Old World coralsnakes as proximate and distant outgroups. Black circles denote strong nodal support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate full support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value. 28

Figure 4.1. The optimal parsimony and maximum likelihood tree from the cytochrome-b data. Bootstrap values >50% are shown along the interior branches (parsimony/maximum likelihood). The red square highlights the clade that was assigned the genus *Sinomicrurus* (modified from Slowinski et al al. 2001) 35

Figure 4.2. Map of the Taiwan-Ryukyu Archipelago highlighting biogeographic breaks and corresponding island groups. 38

Figure 4.3. Phylogeny of *Sinomicrurus* species based on a concatenated data set of nuclear and mitochondrial loci depicted as the 50% majority rule consensus Bayesian tree. Black circles denote strong nodal support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate strong support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value 48

Figure 4.4. bPTP species delimitation results superimposed on 50% majority rule consensus Bayesian tree of *Sinomicrurus* species. Black circles denote strong nodal

support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate strong support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value. Each candidate species is coded in a separate color with its specific epithet. While most subspecies have been raised to species status, S. hatori is sunk to *sauteri* and *S. japonicus takarai* is sunk into *S. japonicus boetgeri* 50

Figure 4.5. BEAST chronogram calibrated using a combination of the vertebrate mitochondrial clock and secondary calibrations. Major cladogenetic events are numberd 1 through 8; corresponding divergence estimates and posterior probability for each node can be found in table 4.1 51

Figure 4.6. Chronogram depicting ancestral range evolution of *Sinomicrurus* species according to the best-fitting biogeographical model (DIVALIKE+J) proposed by BioGeoBEARS. Colored squares with letters at nodes depict most likely states (ancestral ranges). Map on lower right corner depicts color coded biogeographic areas used for the analysis (excluding North America). 54

Figure 5.1. Phylogeny of Elapidae depicted as a maximum-likelihood consensus tree with Viperidae and Colubridae as distant and Lamprophiidae as proximate outgroups. Black circles denote strong nodal support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate full support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value 73

Figure 5.2. Fossil calibrated chronogram of constructed using the Fossilized Birth Death model in BEAST. Black text at nodes represent divergence time estimates for major splits in the superfamily Elapoidea (with 95% HPD values in brackets below). Posterior support for major nodes is depicted in grey text. The different shades of grayscale represent distinct epochs of the Cenozoic era that are displayed in the legend. 75

Figure 5.3. Chronogram depicting ancestral range evolution of basal elapid lineages according to the best-fitting biogeographical model (DIVALIKE+J) proposed by BioGeoBEARS. Each biogeographical realm is assigned a letter and a color and each lineage (branch) is colored according to the geographic range it occupies. Colored arrows represent dispersals out of the Oriental Region. The four major dispersal events are numbered and depicted on a corresponding maps to the left. 80

List of Tables

Table 3.1. Best clustering of samples under a multispecies coalescent model using STACEY. 'Fraction' corresponds to the posterior probability of the model and the frequency with which specific clustering solution was sampled. Clades 1-5 as defined by Figure 3.3 29

Table 4.1. Posterior probabilities and divergence times with confidence intervals at each major node for the three different calibration schemes 51

Table 4.2. Results of biogeographic model selection procedure for *Sinomicrurus* in BioGeoBEARS via Akaike Information Criterion assessment of model fit..... 53

Table 5.1. The five calibration points used in the dated phylogenetic analysis, with age and 95% HPD values in brackets (when available), their placement in the tree and the corresponding reference. 76

Table 5.2. Results of biogeographic model selection procedure for the superfamily Elapoidea in BioGeoBEARS via Akaike Information Criterion assessment of model fit... 81

Chapter 1

A brief introduction to Old World coralsnakes

Elapidae is a family of snakes that contains over 60 genera (about 300 species) of medically important snakes (Uetz, P. & Jirí Hošek eds., The Reptile Database, http://www.reptile-database.org, accessed August 13, 2015). Close to one-third of the taxonomic diversity of the Elapidae consists of coralsnakes: a radiation of usually colorful venomous snakes including five genera distributed between the Old World and the Americas (Castoe et al., 2007). The two genera of Old World coralsnakes occur broadly across most of the Indomalaya Ecozone (fig 1.1). With a total of nine species, the tropical genus *Calliophis* occurs in the Indian peninsula, Sri Lanka, Indochina, Indonesia, and the Philippines. The subtropical genus *Sinomicrurus* consists of five species distributed in India, Indochina, China, and Taiwan ranging as far east as the Ryukyu archipelago of Japan.

Chronically under-sampled in studies of molecular systematics, little is known about the evolutionary relationships or history of these enigmatic snakes (Castoe et al., 2007). Slowinski et al. (2001) were the first to provide a phylogeny for Old World coralsnakes. Based on morphological data and cytochrome-b sequence characters, they allocated these snakes into three major clades (including *Hemibungarus calligaster* which is no longer a coralsnake), erecting the genus

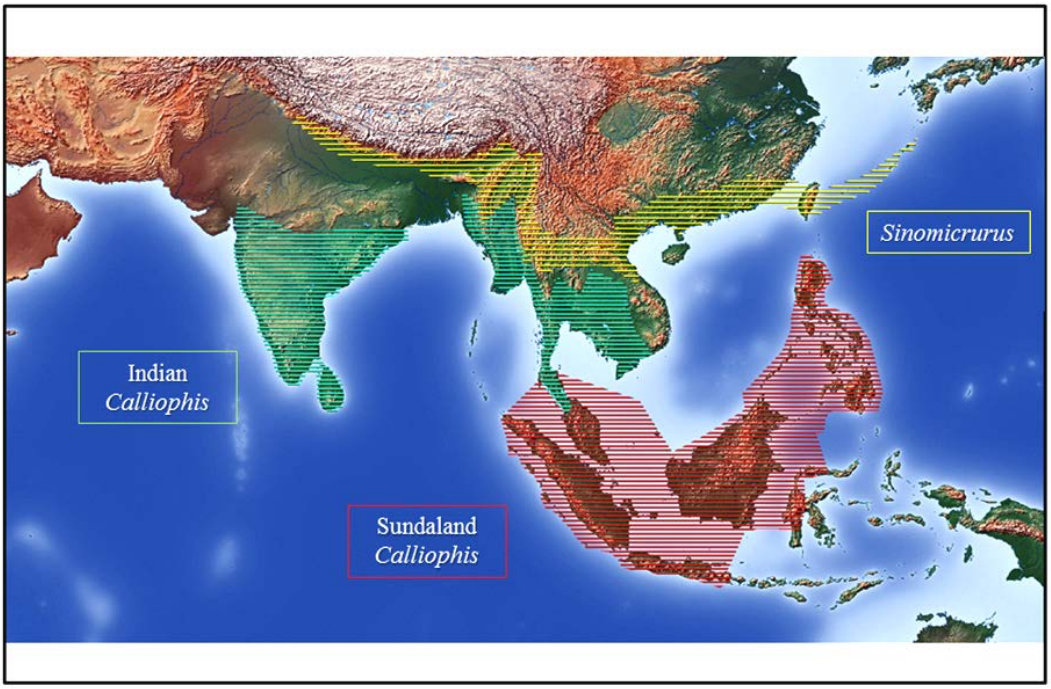


Figure1.1. Distribution of the three major geographical groups of Old World coralsnakes of the genera *Calliophis* and *Sinomicrurus* in the Oriental Region.

Sinomicrurus in the process. However since their molecular data set lacked *Calliophis* representatives they were unable to substantiate relationships between members of this genus. This study was followed by the most recent attempted at clarifying Old World coralsnake evolutionary relationships using a multilocus dataset by Castoe et al. (2007). This study recognized a monophyletic group of Old World and American coralsnakes that they named Calliophinii (fig.1.2); however since they were only able to include a single representative of the genus *Calliophis*, namely *C*. *bivirgata* from Sundaland, they assumed that all other *Calliophis* species, not included in the study (including the Indian

taxa), formed a clade with *C. bivirgata.* In their discussion, the authors underscored the need to test the assumption of monophyly in *Calliophis* when a more comprehensive sampling of these rare Asian coralsnake species became available.

On a broader note, despite several studies, the relationships between major elapid clades also remain poorly resolved and thus basal phylogenetic structure in the Elapidae has hitherto eluded systematists (Castoe et al., 2007a; Kelly et al., 2009a). These unresolved relationships and lack of a reliable temporal scale for the radiation of elapids, have also hindered adjudication between the various hypotheses of the family's spatio-temporal evolution (Slowinski and Keogh, 2000a; Slowinski et al., 2001; Kelly et al., 2009a). Given that coralsnakes remain amongst the only lineages not adequately represented in studies of higher level elapid systematics, investigating their inclusion on our current understanding of elapid phylogeny poses itself as a meaningful endeavor.

For my dissertation research, I have combined phylogenetic systematics and historical biogeographic methods to elucidate patterns of evolutionary divergence within and among the two Old World coralsnake genera. Utilizing molecular sequence analysis, morphometrics and descriptive morphology I construct for the first time a comprehensive phylogenetic backbone for Old World coralsnake systematics. Within this framework, I then discuss the dynamics of their spatio-temporal evolution. Finally, using the resultant data I clarify the basal evolutionary relationships within constituent elapid clades and shed light on the biogeographic origin of the Elapidae.

For my second chapter, I employ traditional phylogenetic and coalescent approaches, using mitochondrial DNA, to assess systematic relationships among Indian coralsnakes of the genus *Calliophis*. I complement this data with comparative hemipenial descriptions. This work is made possible by collaboration with Indian colleagues Drs. Karhtikeyan Vasudevan and Ramesh Aggarwalof the CCMB, Hyderabad, Drs. Praveen

Karanth and Deepak Veerapan of the IISC, Bangalore and Dr. Dileep Kumar at the University of Kerala.

For my third chapter I turn to the Sundaland and Philippine coralsnake species; I investigate the phylogenetic relationships and biogeography of the *Calliophis intestinalis* species complex using descriptive morphology and a mitonuclear dataset. I also use mitochondrial protein coding genes to address the systematics of the co-distributed sister species *Calliophis bivirgata*. This chapter is in collaboration with Dr. Indraneil Das of UNIMAS, Malaysia, Dr. David Bickford of NUS, Irvan Sidik of LIPI and Dr. Rafe Brown of KU. A National Science Foundation grant (DEB-1146324), awarded to E. N. Smith, funded my field work in Indonesia.

For my fourth chapter I study the systematic relationships, species boundaries and biogeographic patterns of range evolution in the genus *Sinomicrurus* using both mitochondrial and nuclear markers. This chapter is in collaboration with Dr. Hidetoshi Ota of University of Hyogo, Kobe. In the fifth and final chapter, I use multilocus data to construct the most comprehensive tree of elapid relationship to date. With the aid of fossil data and extant species, I also use this opportunity to clarify the biogeography of main clades and trace back the evolutionary origin of ancestral elapids. I finish with major revisions to the higher level taxonomic relationships of constituent elapid clades. This final chapter is in collaboration with David Sanchez and Dr. Todd *Castoe* of UTA, Drs. Karhtikeyan Vasudevan and Ramesh Aggarwal of the CCMB, Hyderabad and Dr. Hidetoshi Ota of the University of Hyogo, Kobe.

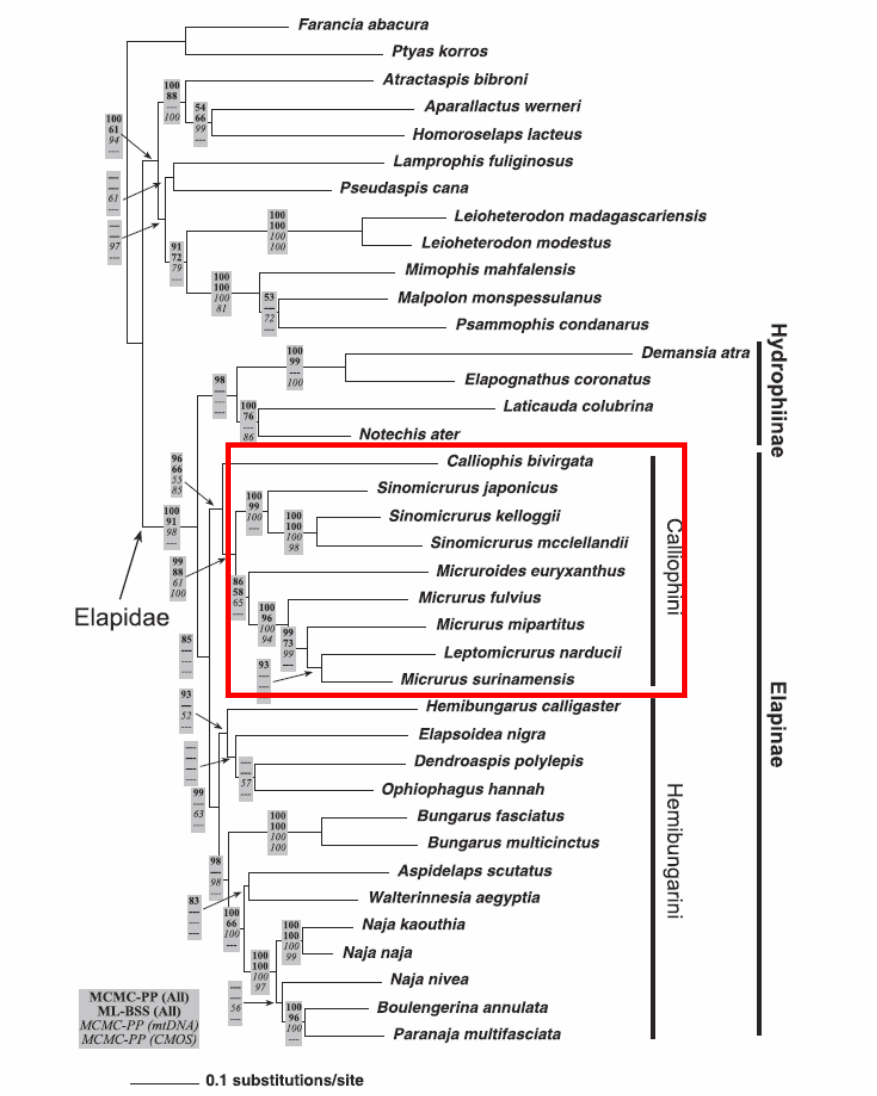


Figure 1.2. Phylogram of the single optimal tree estimated by maximum likelihood analysis of the combined data set (mtDNA + c-mos), modified from Castoe et al. (2007) to highlight the tribe Calliophini. Support values for nodes (> 50%) are provided in grey rectangles adjacent to nodes: posterior probability (PP) based on MCMC analyses of the combined data (1st from top of rectangle, bold), bootstrap (BSS) values based on ML analysis of the combined data (2nd from top of rectangle, bold), PP based on MCMC analyses of the mtDNA data (3rd from top of rectangle, italics), and PP based on MCMC analysis of the c-mos data (bottom of rectangle, italics). A dashed line indicates support of less than 50%.

Chapter 2

A preliminary molecular phylogeny of Indian *Calliophis* species

Introduction

Calliophis is a genus of Asian coralsnakes currently comprising of ten species, distributed throughout India and Southeast Asia (Whitaker et al., 2004; Das, 2010a) . Based on meristic characteristics, coloration and hemipenial structure, Indian *Calliophis* species can be broadly categorized into three major groups i.e. the Striped coralsnakes, the Blue-tailed coralsnakes and the singular *Calliophis bibroni* .

The striped coralsnakes of peninsular India, namely *Calliophis nigrescens*, *Calliophis beddomei* and *Calliophis castoe*, all have longitudinal stripes or stripes and spots and inhabit wet forests in peninsular India (Whitaker et al., 2004). These species share a small and spinous hemipenis with very little terminal bilobation, short sulcus furcation, and no associated basal pocket (Smith et al., 2012).

Calliophis nigrescens is a species complex (Ganesh and Ramanujam, 2014), endemic to the Indian subcontinent and it inhabits forested hills of the Western Ghats at an altitudinal range between 200 – 2000 m asl (Smith 1943; Das 2002; Whitaker et al., 2004). This species is intriguing in the fact that it displays striking color polymorphism across its disjunct range (Whitaker et al., 2004). Four sub-species are described on the basis of their color patterns, all of which are synonyms of *Calliophis nigrescens* after Smith (1943): *Calliophis nigrescens nigrescens* and *Calliophis nigrescens pentalineatus* are typically found south of the Palghat Gap while *Calliophis nigrescens khandalensis* and *Calliophis nigrescens concinnus* are found north of the same 30-40 kilometres wide low mountain pass (Smith, 1943; Vyas and Walmiki 2012). The level of genetic divergence between these disjunct populations and whether the Palghat gap is

segregating genetic variation remain open for investigation contingent on the availability of molecular data. Outside of the Western Ghats, *Calliophis nigrescens* is also reported from the Eastern Ghats, a series of disjunct hills running parallel to the eastern seaboard of India (Smith, 1943; Whitaker et al., 2004).

Calliophis beddomei is endemic to the hills of southern India—the Eastern and the Western Ghats (Smith, 1943; Whitaker et al., 2004). This rare snake was only recently re-discovered and re-described (Ganesh and Ramanujam, 2014) after being first collected in the late 1800s. Very little is thus known about its ecology or distribution and no molecular data is available for this striped coralsnake species yet.

And finally the recently described *Calliophis castoe* is found in the lowlands and mountains of western peninsular India (Smith et al., 2012). Comparison of the DNA sequences of *C. castoe* and the *C. nigrescens* (var. *khandallensis)* showed high genetic differentiation. Apart from this very little systematic work has been done on striped coralsnakes even though they are known to exhibit remarkable variation in color pattern and lepidosis, and warrant a more thorough analysis comparing morphology and molecules (Smith et al. 2012).

The Blue-tailed coralsnakes currently consist of three species that share melanized musculature and tissues at the base of the tail, blotching in some of the subcaudals and two of the tail bands, and a vivid red or orange venter (Smith et al., 2008). *Calliophis melanurus* is a species of Blue-tailed coralsnake distributed across peninsular India and Sri Lanka (Whitaker et al., 2004). In Sri Lanka, this species is co-distributed with another Blue-tailed coralsnake namely *Calliophis haematoetron* which is endemic to the evergreen forests of the island (Smith et al., 2008). *Calliophis maculiceps* is a third species of blue-tailed coralsnakes from Laos, southern Myanmar, Thailand, Cambodia, and northern peninsular Malaysia but absent from India (Smith et al., 2008;

Das, 2010a). No systematic study has investigated the monophyly of the blue-tailed coralsnake species to date.

Calliophis bibroni is morphologically very distinctive from both the Blue-tailed and striped coralsnakes based on the absence of a preocular scale, a single postocular scale, prefrontals in contact with the eye, a single anal shield and the presence of only one minute tooth behind the poison fang (Deepak et al., 2010). *Calliophis bibroni* also has a very unique condition not seen any other species of coralsnakes, in which individuals tend to be aposematic and disruptive overall as juveniles, and then transition into being cryptic dorsaly while remaining aposematic and disruptive ventraly after they attain a snout-vent-length of ~400ml. As with the majority of Indian coralsnakes, the phylogenetic affinity of *Calliophis bibroni* remains unknown.

Given that a clear understanding of the relationships within the Indian *Calliophis* radiation is still lacking (Slowinski 2001, Castoe et al. 2007), in this chapter we used a molecular approach to evaluate the phylogenetic relationships within and between the three aforementioned groups of Indian *Calliophis*. More specifically our goals were to use rapidly evolving mitochondrial data to: 1) determine how the individual species of Indian *Calliophis* are related to one another; 2) test whether the Palghat gap is segregating genetic variation, specifically in the case of *Calliophis nigrescens* and 3) investigate if molecular data supports the reciprocal monophyly of the three major groups of Indian coralsnakes as suggested by morphological synapomorphies.

Materials and Methods

Taxon sampling

We obtained tissues from individuals of every species comprising striped (with the exception of *Calliophis beddomei*) and blue-tailed (with the exception of *Calliophis*

haematoetron) coralsnakes and *Calliophis bibroni* for a total of 14 ingroup and two outgroup OTUs (see fig. 2.1 for locality map for collected specimens). The tissue sample for *Calliophis castoe* was obtained from a single individual from Ambe Ghat, South Goa district, India. Tissue samples for four *Calliophis nigrescens* individuals were obtained from north and south of the Palghat Gap. This included samples from one individual of *Calliophis nigrescens nigrescens* from Waynad, Kerela, one individual of *Calliophis nigrescens pentalineatus* from Megamalai, Tamil Nadu and two specimens of *Calliophis nigrescens khandalensis* from Goa and Maharashtra. Tissue for *Calliophis melanurus* were collected from an individual from Maharashtra, India and was complemented by sequence data from the same species from Sri Lanka. Data from two individuals of *Calliophis maculiceps* from the island of Langkawi, Malaysia was also included in the study to provide a complete sampling for the blue-tailed species. Tissue samples for *Calliophis bibroni* were obtained from specimens collected at Waynad, Kannur and Trivandrum, in Kerala state. We also included tissues from one individual each of the Sundaland coralsnake *Calliophis intestinalis* from Java and the American coralsnake *Leptomicrurus narduci* from Peru as outgroup taxa in order to root the phylogenetic tree.

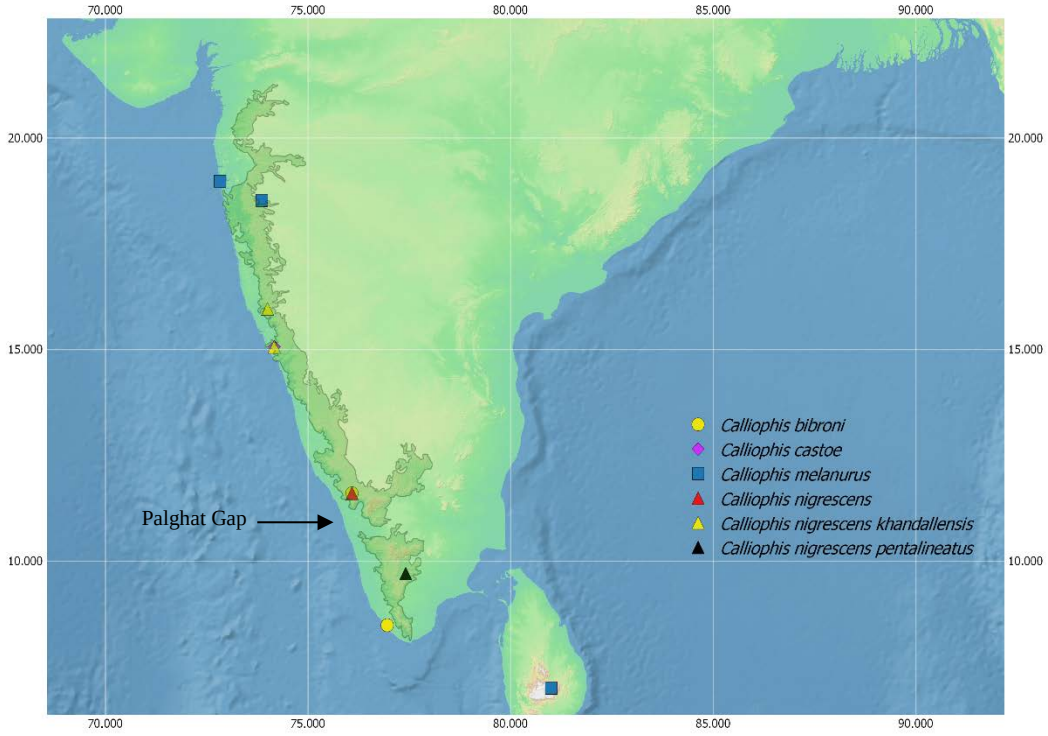


Figure 2.1. Geographic distribution of sampling for the construction of a matrilineal phylogeny for the Indian *Calliophis* species. The pale green polygon represents the extent of the Western Ghats mountain range.

Molecular methods

Since it was not possible to export tissue or DNA material out of India, the molecular work for this research was done in three different labs in India. Here I describe the general protocol that was followed. Total genomic DNA was extracted using the standard Phenyl–Chloroform–Isoamyl alcohol method and the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, California, USA) following standard protocol. A 718 base pair fragment of the mitochondrial cytochrome-b gene (cyt-b) was amplified using primers from previous studies (Arevalo *et al.*, 1994; Harvey *et al.*, 2000). The polymerase chain

reaction (PCR) thermocycling protocol consisted of an initial 2 min denaturation cycle at 95°C, followed by 30s denatur ing at 94°C, 30s annealing at 53°C and 1 min 15s extension at 72°C for 2 cycles, followed by 30s denaturing at 94°C, 30s annealing at 52°C and 1 min 15s extension at 72°C for 3 cycles, followed by 30s denaturing at 94°C, 30s annealing at 51°C and 1 min 15s extension at 72°C f or 5 cycles, followed by 30s denaturing at 94°C, 30s annealing at 50°C and 1 min 15s extension at 72°C for 30 cycles, followed by a 7 min extension at 72°C. Successful amplification was determined by gel electrophoresis of the PCR product along a 1% agarose gel, and PCR products were prepared for the sequencing reaction by using either a QIAquick PCR purification kit (Qiagen) or the ExoSAP-IT kit (United States Biochemical). All samples were sequenced at each institution's respective sequencing facilities using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) following the manufacturer's protocol.

Sequences were assembled and edited using Sequencher (Genes Code Corps., Inc.). Consensus sequences were then exported to MEGA (Tamura et al., 2011), aligned using the CLUSTAL algorithm (Larkin et al., 2007) with default parameters and manually adjusted when necessary.

Model selection and phylogenetic analyses

Optimum partitioning schemes and substitution models for the phylogenetic analyses were identified based on the Bayesian Information Criterion (BIC) using the 'greedy' search algorithm in Partition-Finder v1.1.0 (Lanfear et al., 2012). The best partitioning scheme divided the data into three unlinked partitions and provided corresponding models of evolution: codon 1: HKY+G; codon 2: HKY+I; codon 3: HKY+I.

Phylogenetic analyses were performed using a consensus of Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Gaps in alignment were treated as

missing data for all phylogenetic analyses. ML analysis employing the rapid bootstrapping algorithm was conducted using the program RAxML 8.00 (Stamatakis, 2014) on the CIPRES Science Gateway server v3.2 (Miller et al., 2010); the model GTR+G was used instead of GTR+I+G because the 25 discrete rate categories appear to better estimate invariant sites (Stamatakis, 2006). Nodal support for ML was provided by bootstrapping (1000 pseudoreplicates), with bootstrap values (BS) ≥0.70 considered strong support (Hillis and Bull, 1993).

Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analyses were conducted on a partitioned alignment using MrBayes v. 3.3(Ronquist and Huelsenbeck, 2003). Two simultaneous runs of four MCMC analyses, consisting of one cold and three incrementally heated chains, were initiated with random trees for a total of 1×10^8 generations (sampling every 10000 generations) and burn-in was set to the default values of 25%. Stationarity was examined using trace plots and ESS values (>200) on TRACER v. 1.7 (Rambaut & Drummond, 2009). A 50% majority rule consensus tree with estimates of Bayesian support was constructed using the remaining sampled trees and posterior probabilities (PP) provided nodal support for Bayesian analyses, with PP values ≥0.95 considered strong support (Alfaro et al., 2003; Huelsenbeck and Rannala, 2004; Mulcahy et al., 2011). The graphical viewer Figtree V 1.3.1 (Rambaut, 2007) was used to view and manipulate the trees from RAxML and Mr Bayes analyses.

Species tree analysis

Despite of high posterior support for groupings in the gene tree topology, incomplete lineage sorting is known to result in the species tree being different from the gene tree. Therefore, when working with single gene datasets a species tree analysis is highly recommended since it can provide a more realistic evaluation of the posterior

clade support (Drummond and Bouckaert, 2015). We thus used the hierarchical Bayesian model implemented in *BEAST v. 2.3.0 (Bouckaert et al., 2014) to estimate species trees for Indian *Calliophis* species. *BEAST estimates the species tree directly from sequence data, and incorporates uncertainty associated with gene trees (i.e. incomplete lineage sorting), nucleotide substitution model parameters and the coalescent process (Heled & Drummond 2010). The data set was split into two partitions by codon positions: {1+2} and 3. The pure birth, Yule Model was used as the tree prior and all other priors were set to default values. The HKY model of nucleotide evolution was used for both models. The analyses were run in duplicate, each for 3×10^7 generations, sampling every 3K generations, for a total of 10K sampled trees. We then used Tree Annotator v1.7.4 to discard the first10% of the samples as burn-in and to construct the Maximum-clade credibility tree from the posterior distribution of species trees. The graphical viewer Figtree V 1.3.1 (Rambaut, 2007) was used for tree visualization and manipulation.

Results

Phylogenetic analyses

Both the ML and BI analyses converged on identical topologies and members of all of five species clade with their conspecifics with high support (fig. 2.2). Moreover, each of the three groups sharing morphological synapomorphies is recovered as monophyletic. Individuals of *Calliophis nigrescens* are strongly recovered as sister taxa to individuals of *Calliophis castoe* (BS=100; PP=1). Within the *Calliophis nigrescens* species complex *Calliophis nigrescens khandallensis* is paraphyletic, with the individual from Goa emerging as sister to the *Calliophis nigrescens nigrescens* from Waynad (BS=100; PP=1) while the *Calliophis nigrescens khandallensis* from Amboli is recovered as basal to all other snakes in the complex (BS=99; PP=1) being significantly divergent from its

conspecifics (uncorrected "*p*" distance = 4.5%; table 2.1). *Calliophis nigrescens pentalineatus* is overall moderately supported as being the immediate sister taxon to the clade containing the *C. khandallensis* and *C. nigrescens* individuals (BS=100; PP=0.53).

The two Blue-tailed coralsnake species are each other's closest relatives according to both analyses but with inadequate support (BS=45; PP=0.51). *Calliophis maculiceps and Calliophis melanurus* show the deepest splits amongst members of the three groups, while *Calliophis melanurus* from India are also highly divergent from their conspecific from Sri Lanka (uncorrected "*p*" distance = 8.7%). The species *Calliophis bibroni* is recovered as basal to all other Indian *Calliophis* with low to moderate support (BS=24; PP=0.86).

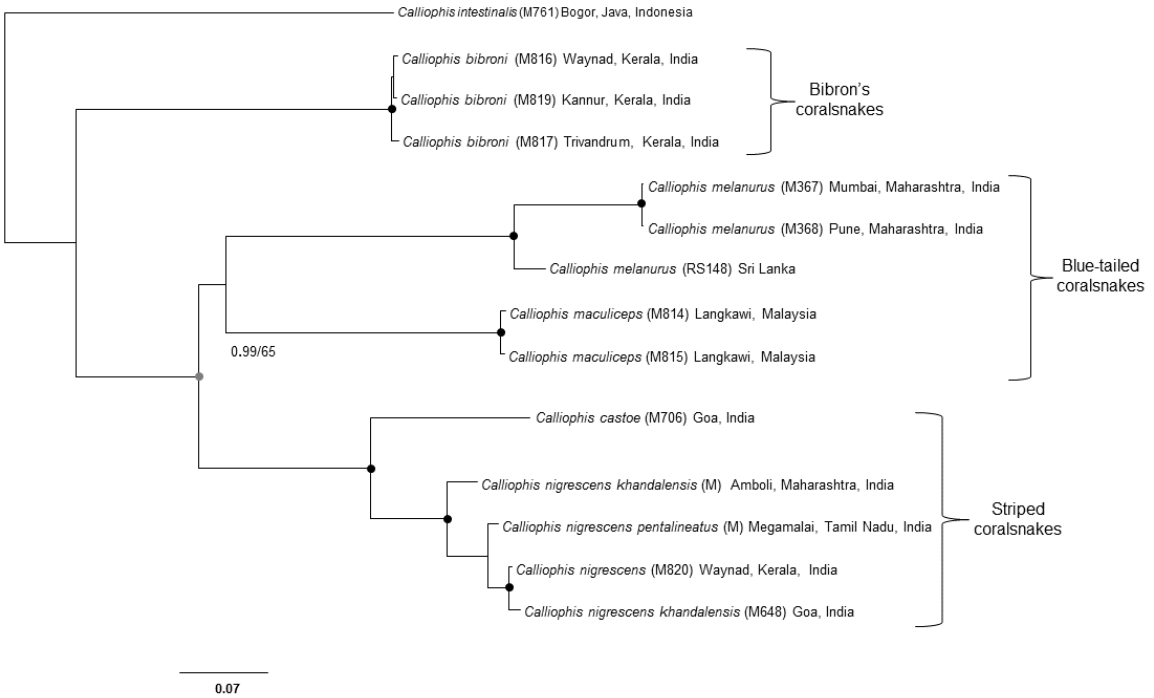


Figure 2.2. Phylogeny of Indian *Calliophis* species depicted as the 50% majority rule consensus Bayesian tree. Black circles denote strong nodal support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate strong support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value.

Species tree analysis

The *BEAST analysis broadly supports the same clades recovered in the gene tree analyses (fig. 2.3), with the exception of The Blue-tailed group which is recovered as paraphyletic; the Southeast Asian Blue-tailed species *Calliophis maculiceps* is found to be sister to *Calliophis bibroni* in contrast to the gene tree analyses where it is recovered as sister to the another Blue-tailed species *Calliophis melanurus*. However, as with the gene tree analyses, the species tree inference fails to provide adequate support (PP= 0.83) for the phylogenetic affinity of C*. maculiceps*. The Striped coralsnakes are recovered as monophyletic, with strong support (PP=0.96). *Calliophis melanurus* from India and Sri Lanka, though deeply divergent, are recovered as sister lineages with high support (PP= 0.97). The sister relationship between *Calliophis melanurus* and the Striped coralsnakes, though in agreement with the gene tree topology, fails to garner adequate posterior support (PP=0.55).

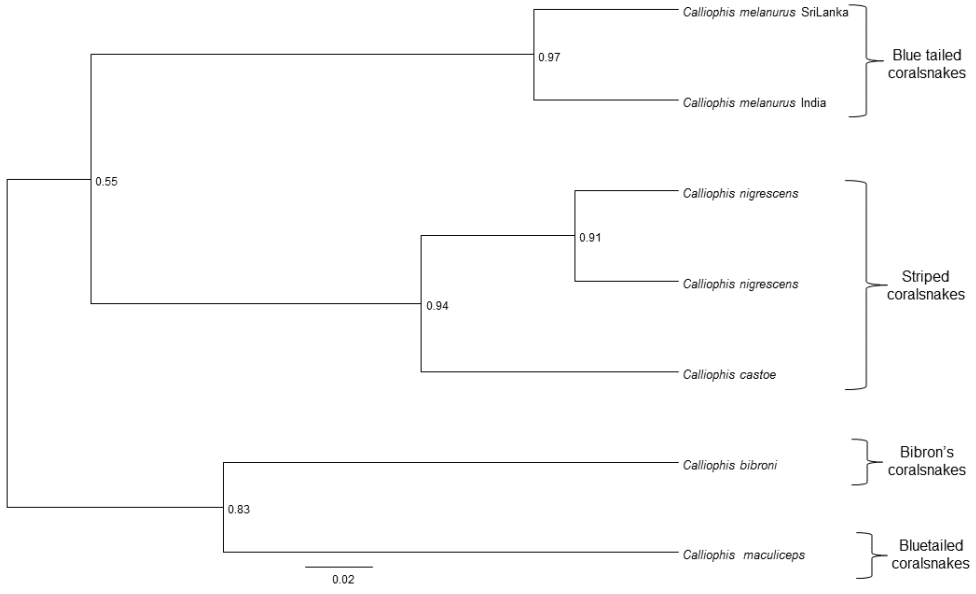


Figure 2.3. Species tree of Indian *Calliophis* species (with outgroups removed). Values represent posterior probabilities.

Discussion

The results of my study provide molecular support for the clustering of the Indian *Calliophis* species into three very divergent groups as indicated by morphology viz. Striped coralsnakes, Blue-tailed coralsnakes and Bibron's coralsnakes (Smith et al., 2008, 2012). Given the deep splits between each of these three groups, it is evident that these have been evolving independently for a long time.

There seems to be no support for the Palghat Gap acting as a biogeographic break for *Calliophis nigrescens* as made apparent by the paraphyly of *Calliophis nigrescens khandallensis.* This however, does not negate the existence of the latter as a cryptic species since the mitochondrial divergence between it and the remaining *Calliophis nigrescens* is significant. It can thus be concluded that the current delineation of different populations based on color patterns is probably misleading and does not reflect species boundaries within the complex.

The deep genetic divergence between the Indian and Sri Lankan *Calliophis melanurus* comes as a surprise and reveals the presence of a cryptic species endemic to Sri Lankan lowlands that has hitherto escaped the attention of taxonomists. Additionally *Calliophis melanurus* and its Blue-tailed congener *Calliophis maculiceps* are also separated by a large genetic distance, hinting at dispersal of a Blue-tailed species out of India and into Southeast Asia, followed by isolation and allopatric speciation of the latter. Even though the gene tree and the species tree disagree in their placement of *C. maculiceps*, there is broad consistency between the major clades supported by the two approaches.

In summary, we provide a very first insight into the systematics of Indian *Calliophis* coralsnakes. In doing so we determine that the Indian *Calliophis* radiation is

old and there has been plenty of time for different lineages to diverge along their own evolutionary trajectories. This has allowed for the formation of three clades of highly genetically differentiated Indian coralsnakes, each most likely containing multiple species. The change at the molecular level however seems to have been de-coupled from morphological evolution resulting in cryptic speciation and the underestimation of diversity across species and higher-level taxonomy. Several other species, in addition to *Calliophis nigrescens,* such as *Calliophis bibroni* and the striped coralsnake *Calliophis beddomei* are known to display significant amount of variation in color pattern across their range which could reflect cryptic or incipient speciation. Future efforts should focus on acquiring samples from *Calliophis beddomei* and procuring population level sampling of all Indian *Calliophis* species from throughout their range to get a more comprehensive picture of genetic variation and species boundaries in these elapid snakes. Moreover, lab techniques should build on our preliminary assessment of matrilineal genetic structure, by incorporating nuclear loci to better assess the phylogenetic composition of the Indian *Calliophis*.

Chapter 3

Molecular phylogeny and species delimitation of the long-glanded Sundaland *Calliophis*

Introduction

The taxonomic confusion on how to allocate major groups of Asian coralsnakes (elaborated on in Chapter 4) lead Slowinski et al. (2001) to infer the first comprehensive phylogeny for all Asian coralsnakes using both molecules and morphology. The key outcomes from their study were the erection of a new genus for subtropical Old World coralsnakes and the synonymization of the genus *Maticora* with *Calliophis* to avoid paraphyly of the latter (fig 3.1). Hence, despite a couple of major distinctive developmental and anatomical differences between the two biogeographically isolated groups (Peters, 1871; McDowell, 1986; 1987), the Sundaland coralsnake species, *M. intestinalis* and *M. bivirgata* came to be incorporated into a genus that contained mostly Indian *Calliophis*.

Restricted to the Sundaland landmass (Peninsular Malaysia and the islands of Borneo, Sumatra and Java), parts of Indochina and the Philippine Archipelago, the Sundaland coralsnakes presently comprise of just three sympatric species: The Spotted coralsnake or *Calliophis gracilis*, The Malayan Blue coralsnake or *Calliophis bivirgata* and the Malayan Striped coralsnake or *Calliophis intestinalis.*

Calliophis gracilis is known to inhabit the lowland forests and elevations up to 915m from Southern Thailand, Peninsular Malaysia, Singapore and Sumatra (Das, 2010a). Being shy and reclusive, very little is known about its ecology or evolutionary history. This is unfortunate, especially given that this is the type species for the genus *Calliophis*.

Calliophis bivirgata mainly inhabits forests, ranging from the lowland to an elevation of 1200m asl but can also be found in agriculture areas bordering forests (Das, 2010). Three subspecies are currently recognized in *C. bivirgata* based on the color and pattern of its dorsal scheme: 1) *C. b. bivirgata* is said to be restricted to the island of Java; 2) *C. b. flaviceps* recorded from parts of Indo-China, Peninsular Malaysia, Sumatra

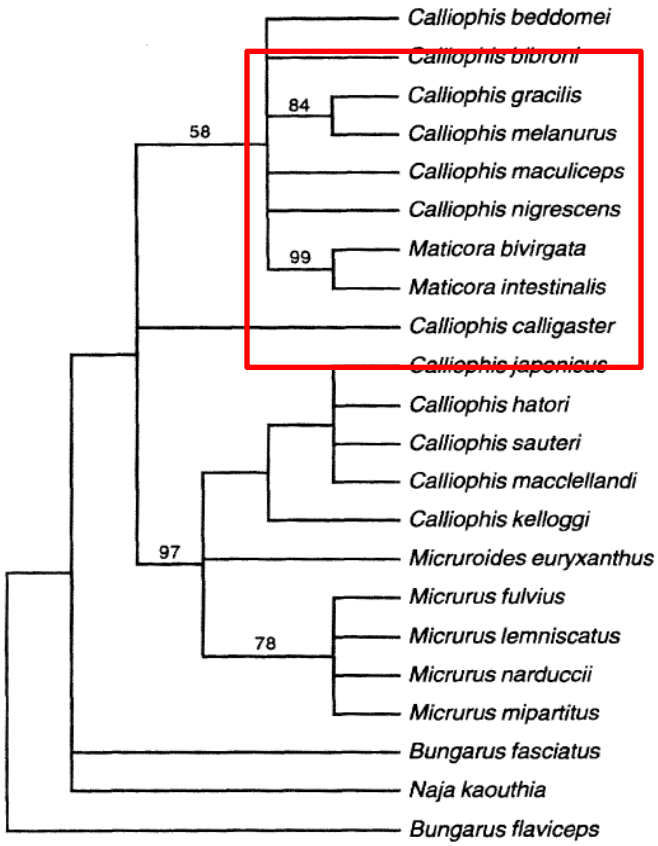


Figure 3.1. Strict consensus tree of the shortest trees from morphological data for coralsnakes displaying paraphyly of *Calliophis* with respect to *Maticora* (highlighted in red); bootstrap values >50% shown along the interior branches (from Slowinski et al. 2001).

and its smaller neighboring islands; 3) *C. b. tetrataenia* is known only from Borneo (Manthey and Grossmann, 1997; Das, 2010).

The sympatric *Calliophis intestinalis* also inhabits forests from the lowland all the way up to 1100m asl, but seems to better tolerate anthropogenic presence as its often also found in parks and gardens in urban areas (Das, 2010). In terms of intraspecific variation, as many as seven subspecies are commonly identified: 1) *C. i. intestinalis* occurs in Java and along western Sumatra*;* 2) *C. i. lineata* is found on the western coast of Sumatra as well as Peninsular Malaysia, southern Thailand and Vietnam; 3) *C. i. bilineata* is restricted to Palawan in the Philippines; 4) *C. i. philippina* is also found in the Philippines specifically in Samar and Mindanao; 5) *C. i. suluensis* is endemic to the Sulu Archipelago in southern Philippines; 6) *C. i. thepassi* occurs in Borneo and 7) *C. i. everetti* is also known from Borneo (Vogel, 2006; Das, 2010). These subspecies are differentiated based on a combination of pigmentation patterns of the dorsum and head but are not always easy to tell apart.

Though routinely included in studies on higher-level elapid phylogenetics as representatives of the *Calliophis* lineage, virtually no study has quantified the genetic underpinnings of the remarkable geographic variation in these Asian Coral species (especially *C. intestinalis*) and how it relates to their taxonomy. Here we examine, for the first time, the phylogenetic relationships and species boundaries within the Sundaland *Calliophis* species (except for *C. gracilis*, whose tissues we have not yet been able to procure) and subspecies and discuss their intriguing biogeographic implications.

Materials and Methods

Taxon sampling

A total of 15 *Calliophis intestinalis specimens* from across the greater Sundas and Peninsular Malaysia were used in this study (see Appendix, Supplementary Table 9). This included species from the highlands (>1200 m) as well as the lowlands. It is hard to ascertain whether we were able to include all subspecies in our sampling given the cryptic nature of the morphological variants in this taxon. *C. bivirgata* was used as the immediate outgroup since it is known to be the sister species to C. *intestinalis*. Additionally *C. maculiceps*, *Sinomicrurus japonicus* and *Micrurus fulvius* were also used as progressively more distant outgroup taxa to polarize the phylogenetic tree.

Molecular Methods

Tissues were collected from blood, liver, muscle, or shed skin for each individual. Genomic DNA was isolated from tissues using a Qiagen DNeasy kit (Qiagen, Valencia, California, USA) and GoTaq® Green Master Mix, 2X (Promega Corporation, Madison, Wisconsin, USA) amplification reactions was used for all samples. Thermal cycling was performed on a GeneAmp® PCR System 9700 machine (Applied BioSciences, Foster City, California, USA). The ND4 + tRNA fragments were amplified using an initial 5 min denaturation cycle at 95°C, followed by 30s denaturing at 94°C, 45s annealing at 52°C and 1 min extension at 72°C f or 38 cycles, and a final 5 min extension at 72°C. The cyt-b fragments were amplified using an initial 2 min denaturation cycle at 95°C, followed by 30s denaturing at 94°C, 30s annealing a t 53°C and 1 min 15s extension at 72°C for 2 cycles, followed by 30s denaturing at 94°C, 30s annealing at 52°C and 1 min 15s extension at 72°C for 3 cycles, followed by 30s denaturing at 94 °C, 30s annealing at 51°C and 1 min 15s extension at 72°C for 5 cycles, followed by 30s denaturing at 94°C,

30s annealing at 50°C and 1 min 15s extension at 72°C for 30 cycles, followed by a 7 min extension at 72°C. The NT3 fragment was amplified using an initial 1 min 30s denaturation cycle at 94°C, followed by 30s denaturing at 94°C, 30s annealing at 51°C and 1 min 30s extension at 72°C for 5 cycles, followed by 30s denaturing at 94°C, 30s annealing a t 50°C and 1 min 30s extension at 72°C for 5 cycles, followed by 30s denaturing at 94°C, 30s a annealing at 49°C and 1 min 30s extension at 72°C for 10 cycles, followed by 30s denaturing at 9 4°C, 30s annealing at 48°C and 1 min 30s extension at 72°C for 30 cycles, followed by a 7 mi n extension at 72°C. After quantification of PCR product was visualized on 1% agarose gel, successfully amplified PCR products were prepared for sequencing by using AMPure XP beads following the Agencourt protocol (Beckman Coulter). A BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) was used for sequencing following the manufacturer's protocol and using PCR primers. The samples were sequenced on a ABI PRISM 3100xl Genetic Analyzer in the Genomics Core Facility at the University of Texas at Arlington, USA. Alignments were constructed using the program Sequencher 4.8 (Gene Codes, Ann Arbor, Michigan, USA), and edited by eye using the program MEGA. Sequences were translated to amino acid sequences to verify the absence of stop codons and proper alignment, and edited by eye for accuracy. No internal stop codons were detected and the new sequences were deposited in GenBank.

Phylogenetic analyses

Calliophis bivirgata

Since our exploratory enquiry into *Calliophis bivirgata* did not display the marked genetic divergence between the three subspecies (Fig 3.2) we restricted our analyses of phylogenetic relationships to a simple agglomerative hierarchical clustering method.

Hence evolutionary history was inferred using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and a bootstrap test (500 replicates) with the cyt-b fragment on MEGA 5 (Tamura et al., 2011). The final tree was visualized using the same software. Uncorrected percent Pairwise distances were also generated in MEGA 5.

Calliophis intestinalis

A concatenated data set was partitioned by gene and codon position. Optimum partitioning schemes and substitution models for the phylogenetic analysis were identified based on Bayesian Information Criterion (BIC) using the 'greedy' search algorithm in Partition-Finder v1.1.0 (Lanfear et al., 2012). We used maximum likelihood (ML) and bayesian inference (BI) methods to evaluate phylogenetic relationships. Gaps in alignments were treated as missing data for all phylogenetic analyses.

ML analysis employing the rapid bootstrapping algorithm was conducted using the program RAxML 8.00 (Stamatakis, 2014) on the CIPRES Science Gateway server v3.2 (Miller et al., 2010); the model GTR+G was used instead of GTR+I+G because the 25 discrete rate categories appear to better estimate invariant sites (Stamatakis, 2006). Nodal support for ML was provided by bootstrapping (1000 pseudoreplicates), with bootstrap support (BS) values ≥0.70 considered strong support (Hillis and Bull, 1993).

Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analyses were conducted on a partitioned alignment using MrBayes v. 3.3(Ronquist and Huelsenbeck, 2003). Two simultaneous runs of four MCMC analyses, consisting of one cold and three incrementally heated chains, were initiated with random trees for a total of 1×10^8 generations (sampling every 500 generations). Burn-in was set to the default values of 25%, hence discarding the initial 50K generations. Stationarity was examined using trace plots and ESS values (>200) on TRACER v. 1.7 (Rambaut & Drummond, 2009). A 50%

majority rule consensus tree with estimates of Bayesian support was constructed using the remaining sampled trees and posterior probabilities (PP) provided nodal support for Bayesian analyses, with PP values ≥0.95 considered strong support (Alfaro et al., 2003; Huelsenbeck and Rannala, 2004; Mulcahy et al., 2011). The graphical viewer Figtree V 1.3.1 (Rambaut, 2007) was used to view and edit the trees from RAxML and Mr Bayes analyses.

Species Tree and Coalescent Species Delimitation

We used BP&P V 3.1, (a MCMC program which uses the multispecies coalescent model for analyzing DNA sequence alignments) to simultaneously estimate a species tree and delimit species (Yang and Rannala, 2014). Since BP&P requires an input topology of species relationships and pre-assigned species, we used well supported monophyletic clades from ML and Bayesian analyses as candidate species; this resulted in a six species scenario (see fig. 3.3). To assure convergence and proper mixing we first divided the data into a concatenated mitochondrial locus (Cyb +ND4) and a nuclear (NT3) locus and ran the A00 analysis (parameter estimation under the multispecies coalescent model when the phylogeny is given) (Yang, 2015) on each of them separately as well as on a single sequence file with both loci combined. These analyses were performed using different burnin and run length values until we achieved an ESS >200 for every parameter indicating stationarity of the Markov chains. We then ran analysis A11 (joint species delimitation and species tree inference). Considering that priors of θ (population sizes) and τ (age of the root) have considerable influence on model selection (Zhang et al., 2011), we used both speciation algorithms (0 and 1) and performed analyses under a variety of Γ parameter permutations (α, β) for θ and τ. In this respect we used a similar combination of priors for model parameters used by Yang (2015),

exploring the effects of small ancestral population sizes Γ (2, 2000), and deep divergence Γ (1, 100); large ancestral population sizes and deep divergences Γ (1, 100); small ancestral population sizes and shallow divergences Γ (2, 2000); large ancestral population sizes Γ (1, 10) and shallow divergences Γ (2, 2000). Heredity scalars of 0.5 and 1 were assigned for the two concatenated mitochondrial loci and the single nuclear locus respectively. Since consistency of results across multiple runs indicates good mixing (Yang, 2015), three independent runs were executed for every combination of priors, using a burnin of 8000 samples and a total run length of 8000 samples with a sample frequency of ten. Given that BPP assumes a molecular clock, we removed the outgroups, so as not to violate the clock and get spurious results (Ziheng pers. comm.) Different clades were considered distinct species if the posterior probability exceeded a threshold of 95% (Rannala and Yang, 2013).

Additionally to independently confirm our results and obtain a consensus species tree topology, we also used STACEY for BEAST2, which is based on a multispecies coalescent model that does not require a priori assignment of individuals to populations and no guide tree (Jones, 2014). The program was run 1×10^9 generations sampling every 100000 generations. The DISSECT (Jones et al., 2015) tree prior was used for the species tree. All other priors were left at their default values. We used the 6 species scheme suggested by our RAxML and Bayesian analyses and used SepciesDelimitatorAnalyses (Jones et al., 2015) to calculate minimal clusters trees. Convergence of chains was confirmed with TRACER v. 1.7 (Rambaut & Drummond, 2009) as is done for typical BEAST analyses.

Results

Phylogenetic relationships

Calliphis bivirgata

According to our UPGMA analyses *Calliophis bivirgata flaviceps* from Sumatra and Peninsular Maylasia are monophyletic with high support (BS=96). *C. b. bivirgata* is placed as sister to *C. b flaviceps* (BS=100) while the Bornean endemic C*. b. tetrataenia* is recovered as basal to both the aforementioned subspecies (BS=100) (fig. 3.2). In terms of genetic distance, substantial differentiation is seen between *Calliophis bivirgata tetrataenia* and *C. b. bivirgata* and *C.b. flaviceps* (6.2% and 7.1% respectively).

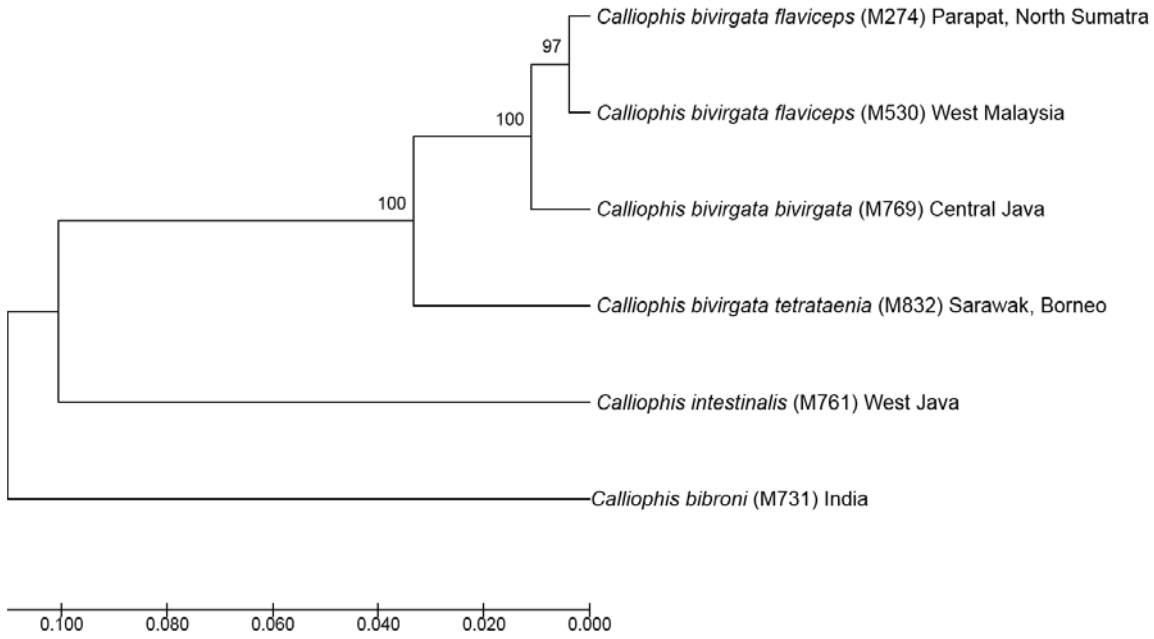


Figure 3.2.The evolutionary relationships between *Calliophis bivirgata* subspecies inferred using the UPGMA method on Cytb sequences. The optimal tree with the sum of branch length = 0.36921331 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

Calliophis intestinalis

Our ML analyses produced a single tree (lnL = -9125.10) which resulted in an identical topology to our Bayesian consensus phylogram hence we produce here one tree with both BS and PP values for every node (fig. 3.3). *Calliophis intestinalis* from across their range are recovered as monophyletic with high support (BS=100; PP=1) and display very strong phylogenetic structuring with several considerably divergent lineages. Consequently the tree of evolutionary relationships within *Calliophis intestinalis* can be divided into five distinct clades that seem to correspond strongly to elevation and weekly with contemporary geography. Clade 1 consists of individuals of from the lowlands of northern Borneo and forms a strong sister relationship (BS=100; PP=1) with Clade 2 which consists of animals from the lowlands of peninsular Sundaland (Malaysia and Singapore), Penang Island and the east coast of Sumatra (Jambi Province). Basal to these two lineages is Clade 3 (BS=100; PP=1) consisting of yet another very divergent individual from the lowland of northern Borneo. The sister group to these clades consists of three very deep lineages: Clade 4 comprises of specimens from the highlands of Borneo, Peninsular Malaysia and Sumatra (BS=100; PP=1) that surprisingly are minimally genetically differentiated despite of being populations inhabiting distant landmasses several 100 kms away from one another; Clade 5 is represented by a single

.

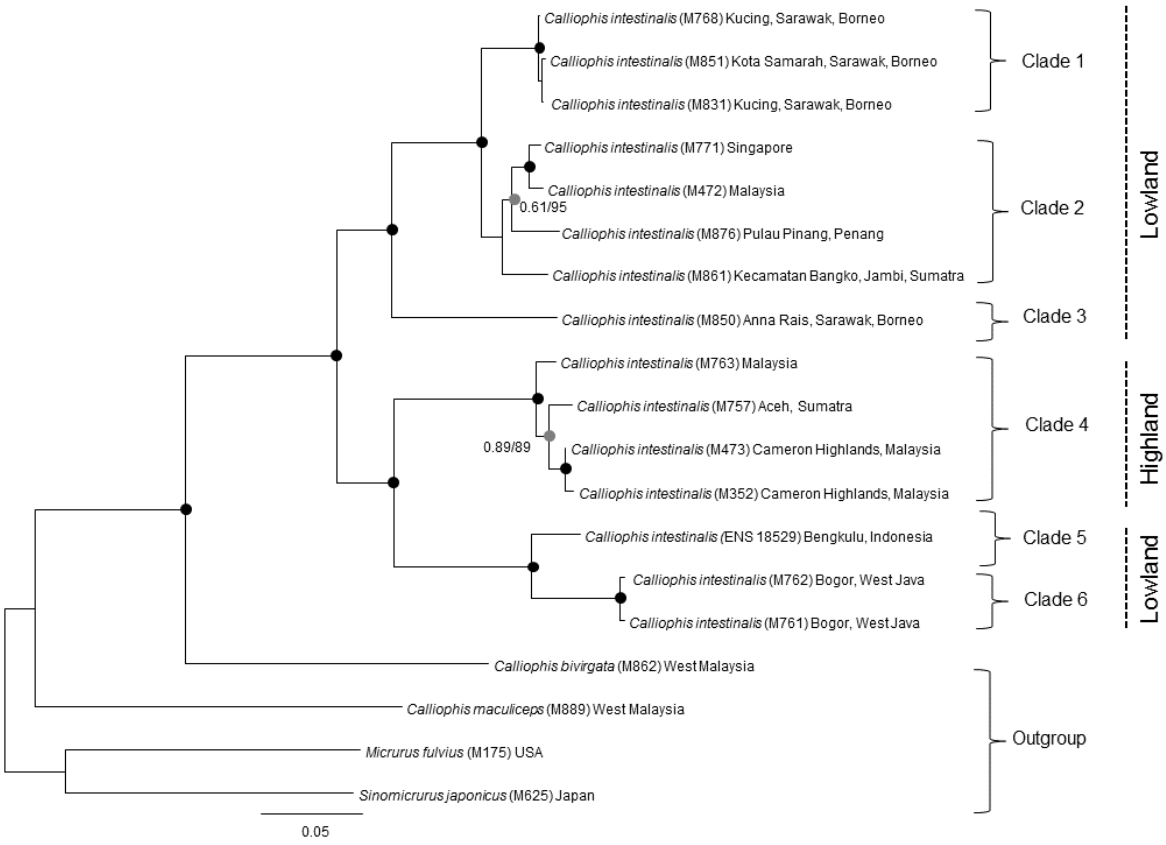


Figure 3.3. The evolutionary relationships within *Calliophis intestinalis* populations from across the Sundashelf depicted as a maximum-likelihood consensus tree with other species of Old World coralsnakes as proximate and distant outgroups. Black circles denote strong nodal support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate full support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value.

animal from the lowland of the southwest Coast of Sumatra (BS=100; PP=1) and Clade 6 inhabits the lowlands of West Java (BS=100; PP=1).

Species tree and species boundaries

All combinations of model parameters in BPP consistently furnished highest support (PP>95) for the six species model. There is strong support for Bornean lowland Clades 1 and 3 being distinct species despite of being sympatric. The species from the lowland of southern Sumatra (Clade 6) is also projected to be a distinct species even though it clades closely with the *Calliophis intestinalis* individuals from West Java. Given that different species trees might contain different delimited species, the analysis A11 did not produce a consensus tree.

The results of the STACEY species delimitation analysis were identical to the species assignments produced by BPP. SpeciesDelimitationAnalyzer produced three models: two models clustered the data into 5 taxa with mediocre support values while the model with the highest posterior support (PP = 0.99) clustered the data into 6 taxa (Table 3.1). STACEY also produced a consensus species tree that was identical to the gene tree (not shown) but with overall low nodal support.

Table 3.1. Best clustering of samples under a multispecies coalescent model using STACEY. 'Fraction' is the frequency with which specific clustering solutions are sampled and corresponds to the posterior probability of the model. Clades 1-5 as defined by Figure 3.3

Fraction	nClusters	Clade 5	Clade 4	Clade 2	Clade 6	Clade 1	Clade 3
0.9962738705170004	6	1	2	3	4	5	6
0.0034932463903120633	5	5	1	2	5	3	4
2.328830926874709E-4	5	1	2	5	3	5	4

Discussion

Phylogenetic relationships and species boundaries in the context of current taxonomy

The Sundaland *Calliophis* taxa display a broad range of morphological variation and consequently several subspecies have been described over the years. This specially holds true in the Greater Sunda populations of *Calliophis intestinalis*, which as our phylogenetic analyses indicate, are actually a species complex. The genetic divergence discovered within different populations is surprisingly deep and at least five populations merit their own species status as indicated by species delimitation analyses. Several of these lineages already have a name albeit at the subspecific level.

Clade 5 (West Javan population) is morphologically very distinctive from all other clades and the easiest to tell apart based on internal morphology and coloration. It fits accurately the description of *C. i. intestinalis* with a black dorsum and narrow yellow/white lines on the vertebral region as well as the Y shaped cream mark on the forehead. Considering the above diagnosis and the fact that *C. i. intestinalis* is only known from Java, it would be non-controversial to assign this clade the specific epithet of *C. intestinalis*. The individual that forms Clade 3 from Borneo, matches vividly the type of *C. i. thepassi*, with a rufous brown forehead, black dorsum and vertebral line extending to the tip of the tail. Thus this clade can be elevated to full species status designated as *C. thepassi*. In a similar manner, the widespread members of Clade 2 match the diagnosis and distribution of *C. i. lineata* and should be raised to species level as *C. lineata.* Assigning available names to the remaining three populations (Clades 1,4 and 5) is a trickier endeavor. A closer look at their morphological variation will be required before we can produce usable synapomorphies to delimit them as distinct species either using available names or creating new ones.

Assigning names to the two *C. bivirgata* populations is more straightforward since two of the three subspecies are restricted to their respective islands and the sampled animals don't display a lot of genetic variation among themselves. Based on our phylogenetic results the populations of Java, Sumatra and mainland Malaysia should all be assigned the senior most name amongst the two subspecies and thus elevated to species status as *C. bivirgata*. While the divergent Bornean population currently called *C. b. tetrataenia* will be elevated to the species status as *C. tetrataenia*.

Enigmatic patterns of divergence and distribution

Even though we were able to obtain a first insight into their phylogenetic relationships and diversity, we are still far from shedding ample light on the evolutionary history of these intriguing snakes. What complicates matters is that these substantially differentiated species do not associate easily with traditional biogeographic breaks. Thus, we have at least three highly divergent species just on the island of Sumatra: one from the lowlands of southern Sumatra, another from the lowlands of northern Sumatra and yet another one, with a distinct red venter, that has so far only been collected from the highlands of northern Sumatra. However, a similar looking and genetically very weakly differentiated, red-ventered population has also been found in the highlands of the Peninsular Malaysia, some 500 odd kms away. An additional member of this highland clade was collected from the highlands of Borneo (over 1000 kms from the Peninsular Malaysian locality and almost 2000 kms from the northern Sumatran site) and although very similar genetically, it purportedly does not possess a red venter (Das pers. comm.). The combination of a strictly montane distribution and the lack of direct proportionality between physical distance and genetic divergence is rather exceptional and cannot be readily explained by any known historical scenario. All that can be established, given our

present understanding, is that at some point in the not-so-distant past, these animals were not restricted to highlands and had a Sundaland-wide distribution. A subsequent change in the environment recently (geologically speaking) forced these snakes to a higher elevation across their range. The abovementioned alternations in the environment could have been geological or climatic or probably a combination of the two, given the extremely complex and dynamic paleo-history of Sundaland (Hall, 1996, 2012).

And while widely separated and elevationally isolated populations seemed to have diverged minimally, all the lowland populations in this species complex display substantial genetic divergence; often in the absence of clear geographic breaks that typically assist with the segregation of genetic variation. The island of Borneo, for example, has at least two very divergent species that occur in parapatry/sympatry. Interestingly this apparent presence of non-allopatrically derived taxa is also markedly evident in the lowland birds or the island (Sheldon et al., 2001, 2009). Ornithological studies suggest that this is a relic of a 'vicariance-dispersal dynamic' that played out during the Pleistocene glacial cycles, with the shrinking and expansion of rainforest refugia, sporadically subdividing and then bringing back into contact lowland populations (Moyle, 2005; Moyle et al., 2011). However, this mechanism of divergence seems too temporally recent to be compellingly applied to our situation, considering the large genetic distance between the two Bornean lowland species of *Calliophis*.

Based on our results, the island of Sumatra also has multiple lowland candidate species, though further inventories are necessary to see whether these overlap in distribution or not. An interesting spatial pattern of distribution has recently emerged wherein the fauna of southern Sumatra show closer phylogenetic affinity to western Javan fauna rather than northern Sumatran taxa (Smith pers. coms.; pers. obs.). In that context the sister relationship between the Southern Sumatran individual and the West

Javan taxa comes as no surprise. The hitherto undiscovered biogeographic breaks, responsible for the isolation of the remaining lowland species of *Calliophis* from further north of the island, will only be revealed once we obtain a more comprehensive sampling of these rare animals. In general, it could be surmised that the volatile geological history of Sumatra is largely responsible for the current predicament of its *Calliophis* species. Apart from widespread, violent volcanic activity that often wiped out entire ecosystems, Sumatra had also subsided and was largely inundated several times since the Eocene, with only mountains remaining above sea-level (Hall, 2012). This is expected to have isolated local biota (such as ancient *Calliophis*) to little refugia during unstable times allowing them to evolve allopatrically into the different species we see today. Java on the other hand, having arrived to its present configuration in the past two million years, is much newer than either Sumatra or Borneo and was most likely colonized by southern Sumatran species only in the recent past. This is demonstrated by the relatively shallow branch length and close phylogenetic affinity between taxa from the two areas.

One has to wonder why a similar degree of genetic proliferation is not seen in the sister taxa of *Calliophis bivirgata* which shares almost the same ecological attributes, habitat and distribution with *C. intestinalis* and yet remains virtually undifferentiated across major islands of the Sundashelf. Unfortunately, the lack of a reliable temporal scale for the radiation of Sundaland *Calliophis*, means that their discernibly complex biogeographic history and patterns of evolutionary divergence will have to remain speculative at best, in the current treatise. In this context, future work should focus on achieving a more thorough geographic sampling, systematically quantifying and describing morphological variation as well as further clarifying species boundaries.

Chapter 4

Molecular Systematics, species delimitation and range evolution of the subtropical Old World coralsnakes of the genus *Sinomicrurus*

Introduction

Based on morphological analysis (primarily focusing on the anatomy of corner of the mouth), McDowell (1986; 1987) assigned Asian coralsnakes into four groups: 1) the genus *Hemibungarus*, which was later shown to be in fact more closely allied to African elapids (Castoe et al., 2007) than any Asian coralsnakes; 2) *Calliophis*, which contained the majority of coralsnakes found in the Indian subcontinent; 3) *Maticora*, which comprised of the long-glanded coralsnakes from Sundaland as well as the Indian endemic *M. nigrescence* and the Indo-Chinese *M. maculiceps*; 4) and a group of subtropical coralsnakes that he suggested should be included in the New World genus *Micrurus* since they were anatomically very similar to the latter. However, Ota (1991) preferred instead that this subtropical clade be assimilated into the genus *Hemibungarus*. In the studies that followed, this disparity in taxonomic opinion created variability in the generic allocation of species traditional placed in *Calliophis* and *Maticora*.

The attempt to mend this impasse lead Slowinsky et al. (2001) to infer the first phylogeny for all major lineages of Oriental coralsnakes, using the mitochondrial fragment Cytb and morphology. This resulted in the exclusive nomenclatural recognition of the subtropical clade of coralsnakes (fig. 4.1); the new genus *Sinomicrurus* was thus erected for all species of Asian coralsnakes that shared a more recent common ancestor with *Sinomicrurus macclellandi* than with New World coralsnakes (Slowinski et al., 2001). The generic epithet was created to reflect a close relationship of these primarily East Asian snakes with American coralsnakes as established compellingly by the phylogeny

recovered by Slowinsky et al. (2001) and consistently confirmed by molecular studies that followed (e.g. Castoe et al., 2007; Kelly et al., 2009).

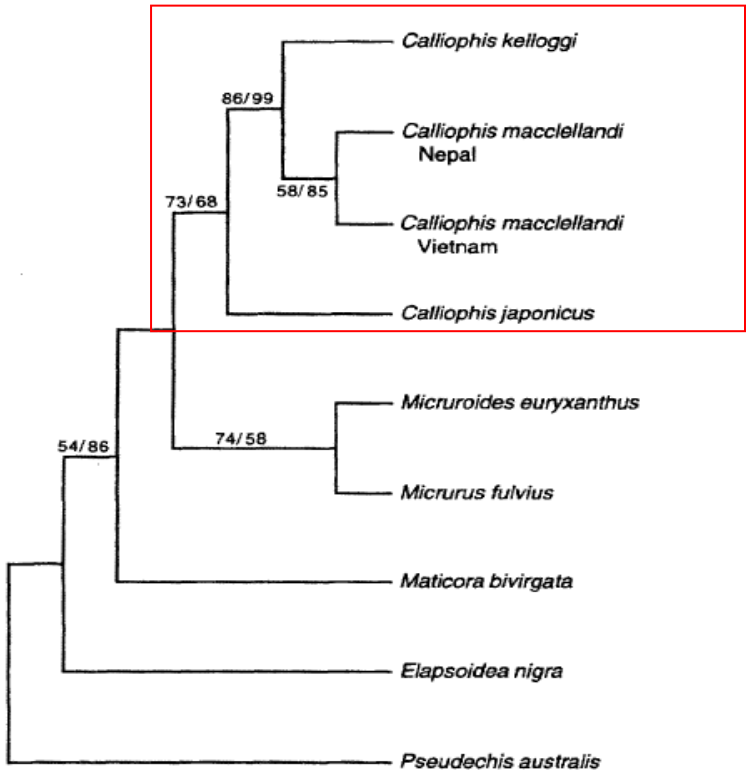


Figure 4.1. The optimal parsimony and maximum likelihood tree from the cytochrome b data. Bootstrap values >50% are shown along the interior branches (parsimony/maximum likelihood). The red square highlights the clade that was assigned the genus *Sinomicrurus* (modified from Slowinski et al al. 2001).

Currently the genus *Sinomicrurus* comprises of five species distributed across subtropical India, China, Indochina, with the majority of the diversity centered on the Taiwan - Ryukyu archipelago, south of Japan. Among the five species, *Sinomicrurus macclellandi* exhibits the largest range spanning across northeastern India, Nepal, Myanmar, Thailand, Vietnam, China and the Taiwan-Ryukyu archipelago. Four subspecies are presently recognized viz. *Sinomicrurus macclellandi macclellandi*, *Sinomicrurus macclellandi swinhoei* from Taiwan, *Sinomicrurus macclellandi univirgatus* from India and Nepal *and Sinomicrurus macclellandi iwasakii* from the Ishigaki island group in the Ryukyus.

With three subspecies of its own, *Sinomicrurus japonicus* is endemic to the Amami and Okinawa groups of the central Ryukyus in Japan (Ota et al., 1999). *Sinomicrurus japonicus japonicus* is restricted to the Amami group, while *Sinomicrurus japonicus boettgeri* and *Sinomicrurus japonicus takarai* are found on both Amami and Okinawa islands. *Sinomicrurus hatori* and *Sinomicrurus satori* are both endemic to Taiwan. And lastly *Sinomicrurs kelloggi* has been recorded from northern Laos, Vietnam and eastern China.

Two broad groups can be inferred based on color pattern in these snakes - the striped *Sinomicrurus* restricted to the Taiwan-Ryukyu island arc (*S. sauteri, S. hatori, S. japonicus, S. boetgeri* and *S. takarai*) and the unstriped *Sinomicrurus* found on the Asian continent (with the exception of *S. iwasakii*, which is an unstriped species found in the southern Ryukyus). Given the disjunct distributions, color pattern polymorphism and absence of molecular data, a thorough systematic classification of these East Asian coralsnakes has been problematic (Ota et al., 1999).

Biogeographic patterns in the Taiwan-Ryukyu archipelago

Since its centre of diversity appears to be the continental islands of Taiwan and Ryukyu, an investigation into the spatial and temporal evolution of this genus has the potential to provide an insight into the mechanisms that drove the faunal diversification of the Taiwan-Ryukyu archipelago. As one of the most threatened biodiversity hotpsots in the world, the Taiwan-Ryukyu island arc is known to harbor a lot of endemic taxa (Ota, 1998; Honda et al., 2014; Kaito and Toda, 2016; Xu et al., 2016). Several competing paleogeographical hypotheses have been put forward explaining the diversification of the extant taxa of these islands (Ota, 1998; Kimura, 2000; Kaito and Toda, 2016; Su et al., 2016a; Xu et al., 2016). The one thing that all studies agree on is the division of the Ryukyu archipelago into three biogeographic groups: 1) the northern Ryukyus, comprising of the Osumi and Tokara islands with strong faunistic affinities to Japan; 2) the central Ryukyus, consisting of the southern Tokara island and the Amami and Okinawa island group, home to highly endemic taxa; 3) the southern Ryukyu group of islands that typically show strong faunal kinship to Taiwan and/or mainland Asia. The biogeographic break between northern Ryukyus and the central Ryukyus corresponds to the Tokara gap – a deep submarine trench that runs perpendicular to the island arc. Similarly the Kerama submarine trench divides the central group from southern Ryukyus and Taiwan. Shallow seas separate the southern Ryukyus from Taiwan and Taiwan from mainland China (Osozawa et al., 2012) (fig. 4.2). Despite their current isolation, these islands are all known to have been connected to the mainland multiple times during the past 2 Mya.

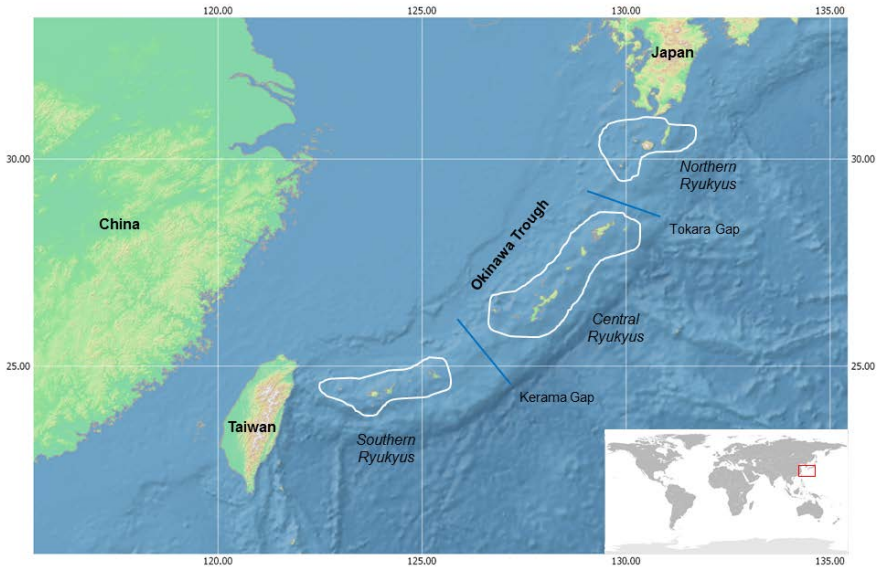


Figure 4.2. Map of the Taiwan-Ryukyu Archipelago highlighting biogeographic breaks and corresponding island groups.

There is considerable disparity within literature regarding the geological developments of the Cenozoic that drove the speciation dynamics within the Taiwan-Ryukyu archipelago and between the archipelago and mainland Asia. Available literature can be broadly divided into two themes – the Pleistocene and the pre-Pleistocene diversification hypotheses (Su et al., 2016). The Pleistocene diversification postulates that the rifting of the Okinawa Trough in the early Pleistocene led to the separation of the Taiwan-Ryukyu arc from the mainland between 1.7 – 1.4 Mya (Osozawa et al., 2012). This was followed by the formation of two land bridges; the first land bridge connecting the eastern margin of China to the Central Ryukyus, formed between 1.3-1.0 Mya while the second extended from the mainland all the way up to mainland Japan around 0.2 – 0.04 Mya. Hence the biotic diversification of the Taiwan-Ryukyu archipelago, according to this hypothesis, was largely the result of fluctuating sea-levels and formation of land-bridges with the Eurasian continent during the Quaternary glacial cycles. Studies that

have evoked Pleistocene vicariance, have thus far only focused on comparatively young taxa, and avoided the use of model-based biogeographic inference relying instead on ad-hoc explanations for the observed distributional patterns.

According to the pre-Pleistocene proposition, the Ryukyu archipelago, once a continental arc in the eastern margin of Chinese continent, rifted away with the opening of the Okinawa Trough in the late Miocene (11.2-5.3 Mya) (Ota, 1998; Kimura, 2000). Thereafter, the Ryukyu island arc arrived at its modern configuration in the early Pliocene (5.3-3.6 Mya) with Taiwan emerging above sea level almost synchronously (Huang et al. 2006; Wang et al. 2014). This geological scenario, it is claimed, provided sufficient opportunities for previously isolated biota to migrate southward into Taiwan during the formation of the Quaternary land-bridges. It also provided ample time for allopatric speciation on the isolated islands and the development of endemic taxa. Recent papers investigating the biogeographic dynamics of spiders (Su et al., 2016b; Xu et al., 2016), geckos (Honda et al., 2014) and natricine snakes (Kaito and Toda, 2016) have shown diversification in the Taiwan-Ryukyu archipelago to have been driven by pre-Pleistocene historical processes .

The relative influence of the two temporally distinct sets of historical processes can be evaluated by testing their predictions using the snakes of the genus *Sinomicrurus*. These subtropical coralsnakes make an ideal study system to test the impacts of paleo-geography of the area on biotic diversification because a) they comprise of species that are endemic to multiple islands of the Taiwan-Ryukyu archipelago and b) they are fossorial and have limited dispersal capacities over marine environments. If most cladogenetic events in the evolutionary history of this genus were a result of the formation of land-bridges in the Quaternary then they should all have occurred within the last ~2.0 Myr with ancestral lineages derived from China and/or Taiwan. On the contrary,

if the diversification of these snakes was primarily pre-Pleistocene, then the divergence times between lineages found on the islands should be much earlier than the Pleistocene, with the lineages endemic to the Central Ryukyu being ancestral to the rest. Given that the two aforementioned scenarios are not mutually exclusive, it is also likely that species accumulation and endemism in these islands was driven by a combination of pre-Pleistocene and Pleistocene processes, in which case roughly one half of the cladogenetic events would coincide with the Pleistocene and the other half with an earlier time.

Having accumulated multiple samples for every species and subspecies of *Sinomicrurus* snakes from across their range, in this chapter, we investigated their evolutionary relationships and history using a multilocus data set. We employed both supermatrix and coalescent approaches to elucidate the systematic relationships and clarified species boundaries using a phylogenetic species delimitation method. Additionally, we also used model-based approaches investigating geographical range evolution and evaluated alternative biogeographic hypotheses regarding the ancestral range and evolutionary diversification of these subtropical coralsnake lineages.

Methods

Taxon sampling

With a dataset consisting of a total of 23 taxa comprising every known subspecies and multiple individuals (where possible) of every known species of *Sinomicrurus* , our study incorporates the most comprehensive sampling of *Sinomicrurus* species to date. This included 10 individuals of *Sinomicrurus macclellandi* from across their range; two individuals of *Sinomicrurus sauteri*, three individuals of *Sinomicrurus hatori*; four individuals of *Sinomicrurus japonicus*; and one individual of *Sinomicrurus*

kelloggi. *Micrurus fulvius*, used as an immediate outgroup, was also included to facilitate secondary calibration using biogeographic data for divergence time analysis. *Hemibungarus calligaster* and a more divergent *Calliophis nigrescens* were used as distant outgroup taxa to help root the phylogenetic tree (see appendix.

Molecular data

Tissues were collected from blood, liver, muscle, or shed skin for each individual. Genomic DNA was isolated from tissues using a Qiagen DNeasy kit (Qiagen, Valencia, California, USA) and GoTaq® Green Master Mix, 2X (Promega Corporation, Madison, Wisconsin, USA) amplification reactions was used for all samples. Thermal cycling was performed on a GeneAmp® PCR System 9700 machine (Applied BioSciences, Foster City, California, USA). The ND4 + tRNA fragments were amplified using an initial 5 min denaturation cycle at 95°C, followed by 30s denaturing at 94°C, 45s annealing at 52°C and 1 min extension at 72°C f or 38 cycles, and a final 5 min extension at 72°C. The cyt-b fragments were amplified using an initial 2 min denaturation cycle at 95°C, followed by 30s denaturing at 94°C, 30s annealing a t 53°C and 1 min 15s extension at 72°C for 2 cycles, followed by 30s denaturing at 94°C, 30s annealing at 52°C and 1 min 15s extension at 72°C for 3 cycles, followed by 30s denaturing at 94 °C, 30s annealing at 51°C and 1 min 15s extension at 72°C for 5 cycles, followed by 30s denaturing at 94°C, 30s annealing at 50°C and 1 min 15s extension at 72°C for 30 cycles, followed by a 7 min extension at 72°C. The NT3 fragment was amplified using an initial 1 min 30s denaturation cycle at 94°C, followed by 30s denaturing at 94°C, 30s annealing at 51°C and 1 min 30s extension at 72°C for 5 cycles, followed by 30s denaturing at 94°C, 30s annealing a t 50°C and 1 min 30s extension at 72°C for 5 cycles, followed by 30s denaturing at 94°C, 30s a annealing at 49°C and 1 min 30s extension at 72°C for 10

cycles, followed by 30s denaturing at 9 4°C, 30s annealing at 48°C and 1 min 30s extension at 72°C for 30 cycles, followed by a 7 mi n extension at 72°C. After quantification of PCR product was visualized on 1% agarose gel, successfully amplified PCR products were prepared for sequencing by using AMPure XP beads following the Agencourt protocol (Beckman Coulter). A BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) was used for sequencing following the manufacturer's protocol and using PCR primers. The samples were sequenced on a ABI PRISM 3100xl Genetic Analyzer in the Genomics Core Facility at the University of Texas at Arlington, USA. Alignments were constructed using the program Sequencher 4.8 (Gene Codes, Ann Arbor, Michigan, USA), and edited by eye using the program MEGA.

Supermatrix and species tree inference

Sequences were translated to amino acid sequences to verify the absence of stop codons and proper alignment, and edited by eye for accuracy. No internal stop codons were detected and the new sequences were deposited in GenBank. The concatenated data set was partitioned by gene and codon position. Optimum partitioning schemes and substitution models for the phylogenetic analysis were identified based on Bayesian Information Criterion (BIC) using the 'greedy' search algorithm in Partition-Finder v1.1.0 (Lanfear et al., 2012). Five unlinked partitions were estimated for the data by the best partitioning scheme: 1) K80+I for Cytb Codon1 and Cytb Codon 3; 2) GTR+I+G for Cytb Codon 2, ND4 Codon 3 and NT3 Codon 2; 3) HKY+G for ND4 codon 1 and NT3 Codon 3; 4) HKY+I+G for ND4 Codon 2; 5) TrN for NT3 Codon 1. We used a consensus of maximum likelihood (ML) and bayesian inference (BI) methods. Gaps in alignments were treated as missing data for all phylogenetic analyses.

ML analysis employing the rapid bootstrapping algorithm was conducted using the program RAxML 8.00 (Stamatakis, 2014) on the CIPRES Science Gateway server v3.2 (Miller et al., 2010); the model GTR+G was used instead of GTR+I+G because the 25 discrete rate categories appear to better estimate invariant sites (Stamatakis, 2006). Nodal support for ML was provided by bootstrapping (1000 pseudoreplicates), with bootstrap support (BS) values ≥0.70 considered strong support (Hillis and Bull, 1993).

Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analyses were conducted on a partitioned alignment using MrBayes v. 3.3(Ronquist and Huelsenbeck, 2003). Two simultaneous runs of four MCMC analyses, consisting of one cold and three incrementally heated chains, were initiated with random trees for a total of 1×10^8 generations (sampling every 500 generations). Burn-in was set to the default values of 25%, hence discarding the initial 50K generations. Stationarity was examined using trace plots and ESS values (>200) on TRACER v. 1.7 (Rambaut & Drummond, 2009). A 50% majority rule consensus tree with estimates of Bayesian support was constructed using the remaining sampled trees and posterior probabilities (PP) provided nodal support for Bayesian analyses, with PP values ≥0.95 considered strong support (Alfaro et al., 2003; Huelsenbeck and Rannala, 2004; Mulcahy et al., 2011). The graphical viewer Figtree V 1.3.1 (Rambaut, 2007) was used to view and manipulate the trees from RAxML and Mr Bayes analyses.

We inferred a species tree using *BEAST part of the BEAST V2.1.0 software packet (Bouckaert et al., 2014) from the nuclear locus plus a concatenated mitochondrial dataset (Cytb and ND4) which was treated as a second locus. The Yule model was used for the tree prior while all other priors were set to default values. Analyses were run in duplicate, each for 1×10^9 generations, sampling every 20,000 generations, for a total of 5×10^4sampled trees. TRACER v. 1.7 (Rambaut & Drummond, 2009) was used to assess

convergence after which we used Tree Annotator v1.7.4 to discard the first10% of the samples as burn-in and to construct the Maximum-clade credibility tree from the posterior distribution of species trees. We used DensiTree v2.1.10 (Bouckaert, 2010) to inspect variation in the topologies obtained from the coalescent analysis. The graphical viewer Figtree V 1.3.1 (Rambaut, 2007) was used for tree visualization and manipulation.

Species delimitation

We used the Bayesian version of PTP (bPTP; Zhang et al., 2013), which is a single-locus species delimitation method using just nucleotide substitution information; it implements a model assuming gene tree branch lengths produced by two independent Poisson process categories (within- and among-species substitution events).

The bPTP species delimitation analysis was executed on the bPTP web server (Zhang et al., 2013). For the input file we used the maximum likelihood phylogeny obtained from a RAxML analysis on a concatenated Cytb and ND4 dataset. We ran the PTP analysis for 200000 MCMC generations, with a thinning value of 100, a burn-in of 25%. To improve species delimitation, we removed the most distant outgroup taxa. Convergence of the MCMC chain was visually confirmed after analysis as recommended by the authors (Zhang et al., 2013).

Divergence Time analysis

We used three different schemes to evaluate the timeframe of diversification of major lineages of *Sinomicrurus* snakes : 1) the root of the tree (the split between the outgroup *Calliophis nigrescens* and all other OTUs) was set to 28.55 Ma (95% HPD = 24.0-35.0), a date derived from our analysis on the diversification of Elapidae (Smart et al. in prep); the invasion of the New World by coralsnakes is said to have happened

during the Early Miocene (c. 23.7 Ma) when the Beringian land bridge was at its optimum (Kelly et al., 2009a; Slowinski et al., 2001). To reflect this information, the split between *Micrurus fulvius* and the *Sinomicrurus* species was set to 23 Ma (95% HPD = 18.76-28.20) (Smart et al. in prep); 2) the vertebrate mitochondrial clock which is said to evolve at a general rate of 0.001 to 1.0 substitutions per million year (mean = 0.5) (Drummond and Bouckaert, 2015); 3) a combination of the aforementioned secondary calibration points and rate of molecular divergence. The feasibility of each of these temporal frameworks was evaluated by comparing the fit of their individual results to the geo-historical record.

Divergence times were estimated using a concatenated data matrix with BEAST 2 (Drummond and Rambaut, 2007). We treated the two mitochondrial genes as a single linked unit and the nuclear gene fragment was treated as a second unlinked locus. The strict molecular clock was used in conjunction with a Yule tree prior and all other parameters were set to default values. A Normal distribution was used for calibration of each of the two time-calibrated nodes. For every temporal framework, we ran two independent runs with a chain length of 1×10^{9} and a sampling frequency of $1\times 10^{4,}$ resulting in ESS >200. Convergence for both runs was diagnosed using TRACER v. 1.7 (Rambaut & Drummond, 2009). A final maximum clade credibility tree was constructed using TreeAnnotator v. 1.7.4. FigTree was used to visualize tree topology.

Geographic range evolution

We used the R package BioGeoBears (Matzke, 2013a) to infer geographic range evolution and estimate putative modes of speciation. BioGeoBears implements several popular models in a likelihood framework and allows the user to compare the fit of different biogeography models to their data using standard statistical techniques (Matzke,

2013b). To this end we implemented three likelihood-based models: Dispersal-Extinction-Cladogenesis (DEC; Ree and Smith, 2008), DIVALIKE or the likelihood version of dispersal-vicariance (DIVA; Ronquist, 1997) and BAYAREALIKE or the likelihood version of BayArea model (Landis et al., 2013). To simulate the process of founder events, an additional *j* parameter was added in each model for a total of six models of range evolution. We took the ultrametric tree generated from our BEAST analyses and programmed each terminal with a distribution range. These ranges were demarcated based on known biogeographic zones, endemic areas and current ranges: R (Central Ryukyus), T (Taiwan+Southern Ryukyus), C (China), I (Indochina i.e. Northeast India to Laos), S (Indian Subcontinent), P (Philippines) and A (Americas).

To factor-in the historical components that could have influenced biogeographic processes, we constrained our analyses by stratifying them according to time and assigning relative probabilities of dispersal between ranges based on our knowledge of the geological history of the area. An age of 5Mya was adopted to reflect the subaerial emergence of Taiwan due to the collision between the Luzon arc and the Eurasian continental margin (Huang et al., 2006; Osozawa et al., 2012). A date of 1.70 Mya was assigned to the simultaneous separation of the Ryukyu archipelago and Taiwan from the Eurasian mainland (Osozawa et al., 2012). The formation of the first land-bridge connecting China to the to the Central Ryukyus was assigned to a time frame of 1.3 Mya followed by total inundation of the Ryukyu ark at 1.0 Mya implying reduced dispersal of organisms between the mainland and the archipelago (Osozawa et al., 2012). The second and final land-bridge was said to have occurred at 0.2 Mya and we incorporated this date into the analysis to represent the re-initiation of gene flow between Eurasia and the island arc with a final cessation of dispersal between the two areas at 0.04 Mya when the archipelago is supposed to have attained its current configuration (Kimura, 2000;

Osozawa et al., 2012). Furthermore, to preserve the chronological sequence of the emergence of landmasses, we deleted areas going back in time and recalculated likelihoods based on the dispersal only between areas allowed.

Results

Supermatrix and species tree analyses

Our ML analyses produced a single tree (lnL = -9364.98) which resulted in an identical topology as our Bayesian consensus phylogram (fig. 4.3). The striped and the unstriped coralsnakes are recovered as reciprocally monophyletic with high support (PP = 1; BS = 100). Within the striped *Sinomicrurus*, two main lineages were recovered i.e. a clade containing the Taiwanese species *C. hatori* and *C. sauteri* forming a strongly supported (PP = 1; BS = 100) sister group to individuals of *S. japonicus*. The latter are further subdivided into two distinct clades with individuals of *S. japonicus japonicus* sister to a group (PP = 1; BS = 100) containing *S. japonicus takarai* and *S. japonicus boettgeri*. The unstripped *Sinomicrurus* clade comprising of species *S. kelloggi* and *S. macclellandi* can be broadly divided into three well supported (PP=1; BS = 100 for all three nodes) monophyletic groups i.e. *S. kelloggi*, island *S. macclellandi* and mainland *S. macclellandi*. Within the island subspecies *S. macclellandi iwasakii* of Southern Ryukyus, *S. macclellandi swinhoei* from Taiwan and an individual from Libo County, China each form distinct lineages (PP=1; BS = 100 for all three nodes). These island lineages together form a strong sister clade (PP=1; BS = 100) to mainland *S. macclellandi*, which are additionally sectioned into two reciprocally monophyletic groups (PP=1; BS = 100); a group comprising of *S. macclellandi* west of the Himalayan foothills (from Bhutan, Northeast India and Myanmar) that descend into southeast Asia and another group comprising of individuals from south-eastern China and north-eastern Vietnam. Our

coalescent species tree analysis recovered an identical and highly supported topology as our analyses on the concatenated data set.

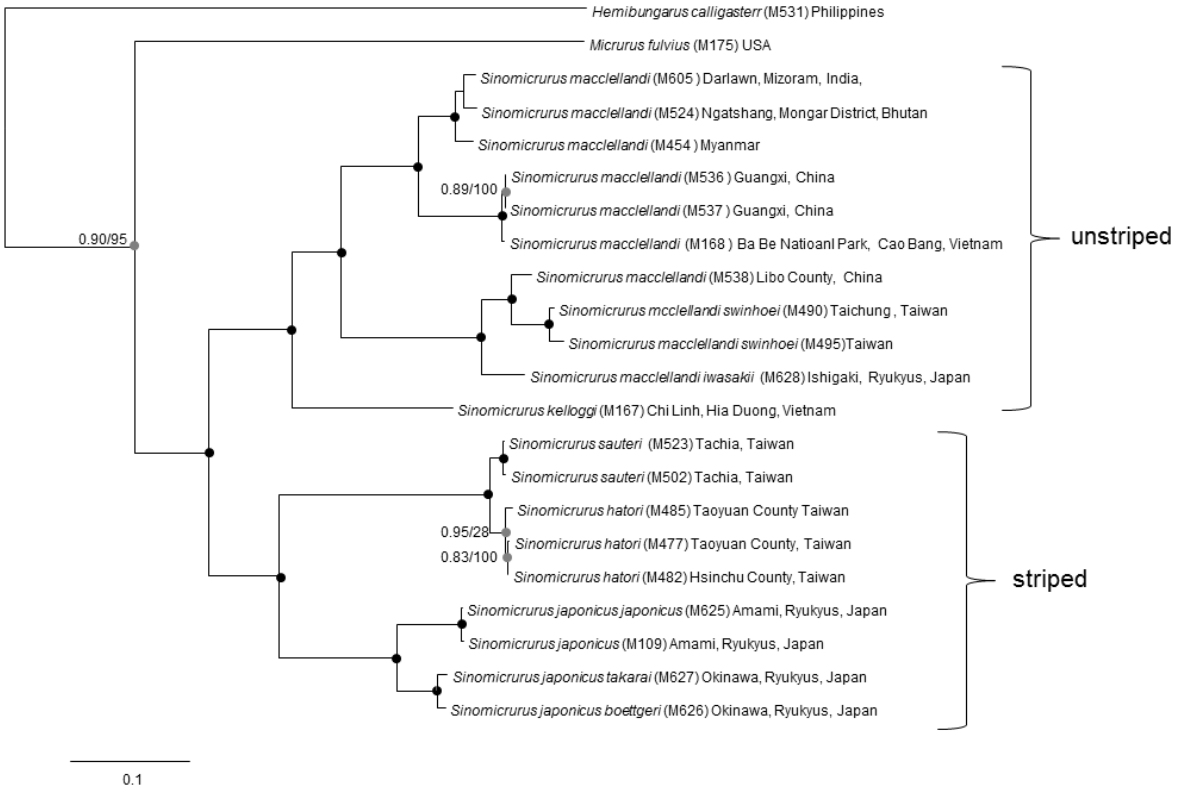


Figure 4.3. Phylogeny of *Sinomicrurus* species based on a concatenated data set of nuclear and mitochondrial loci depicted as the 50% majority rule consensus Bayesian tree. Black circles denote strong nodal support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate strong support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value.

Species delimitation

The bPTP species delimitation results were in general highly congruent with morphologically described species and recovered nine evolutionary significant units (fig. 4.4). The subspecies *S. japonicus boettgeri* and *S. japonicus takarai* were recovered as a single evolutionary lineage, as were the species *S. hatori* and *S. sauteri*. On the contrary the subspecies that comprise of the Okinawa *S. japonicus takarai-boetgeri* bPTP group formed a distinct group from the Amami subspecies *S. japonicus japonicus* with high support. Similarly the Northeast Indian-Indochinese *S. macclellandi* clade together formed a discrete evolutionary lineage as did their sister group from southern China and northwest Vietnam. The island *S. macclelandi* comprised of three bPTP groups which broadly validated the morphological species delimitation with *S. macclelandi swinhoei* and *S. macclelandi iwasakii*recovered as distinct species. The third putative species in the clade was represented by the individual from Libo County, China.

Divergence time analysis

All three schemes resulted in near identical divergence times for major nodes (Table 4.1), hence herein produce the tree calibrated using scheme 3 (fig. 4.5). The age of the oldest node representing the split between the Ryukyu-Taiwan endemics (striped) and the primarily mainland species (unstriped) as expressed by the 95% HPD intervals greatly pre-dated the Pleistocene (Node 1: 18.63-28.52 Mya). Contrary to biogeographical propositions, the split between the Okinawa and Amami lineages also significantly pre-dated the Pleistocene (Node 3: 10.76-17.23 Mya) during which time the strait between the two island groups of the Central Ryukyus is said to have formed (Kizaki and Oshiro, 1977; Ota, 1998). Similarly the divergence times

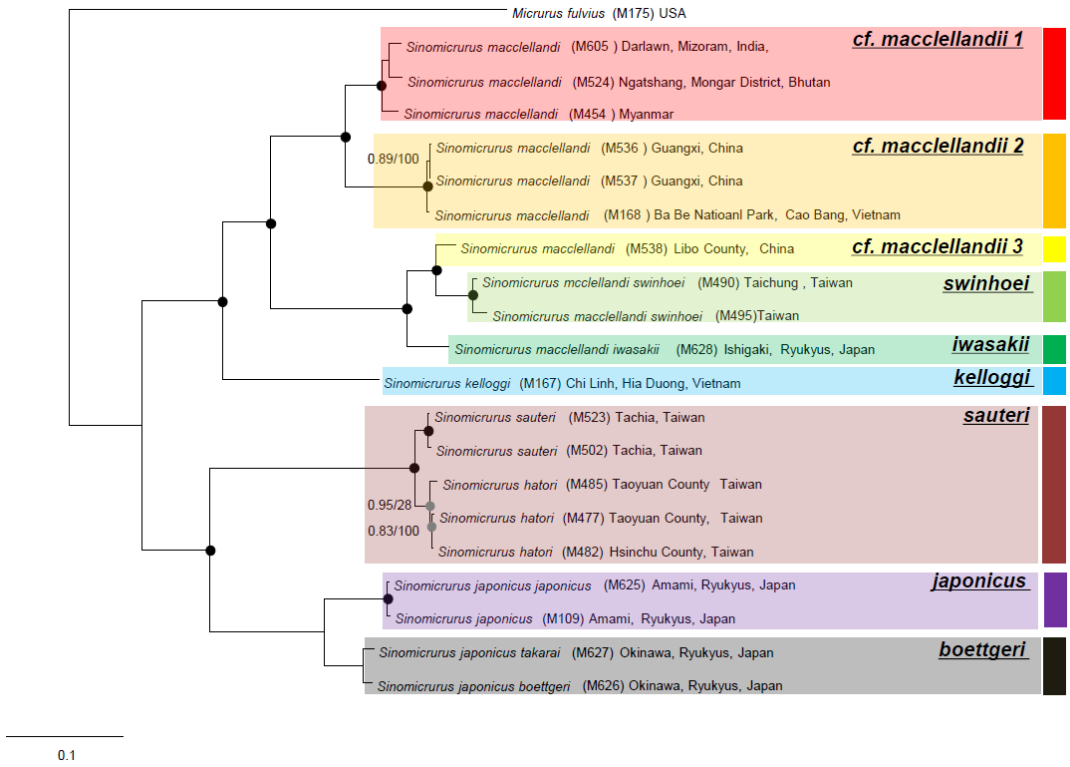


Figure 4.4. bPTP species delimitation results superimposed on 50% majority rule consensus Bayesian tree of *Sinomicrurus* species. Black circles denote strong nodal support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate strong support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value. Each candidate species is coded in a separate color with its specific epithet. While most subspecies have been raised to species status, *S. hatori* is sunk to *S. sauteri* and *S. japonicus takarai* is sunk into *S. japonicus boetgeri*.

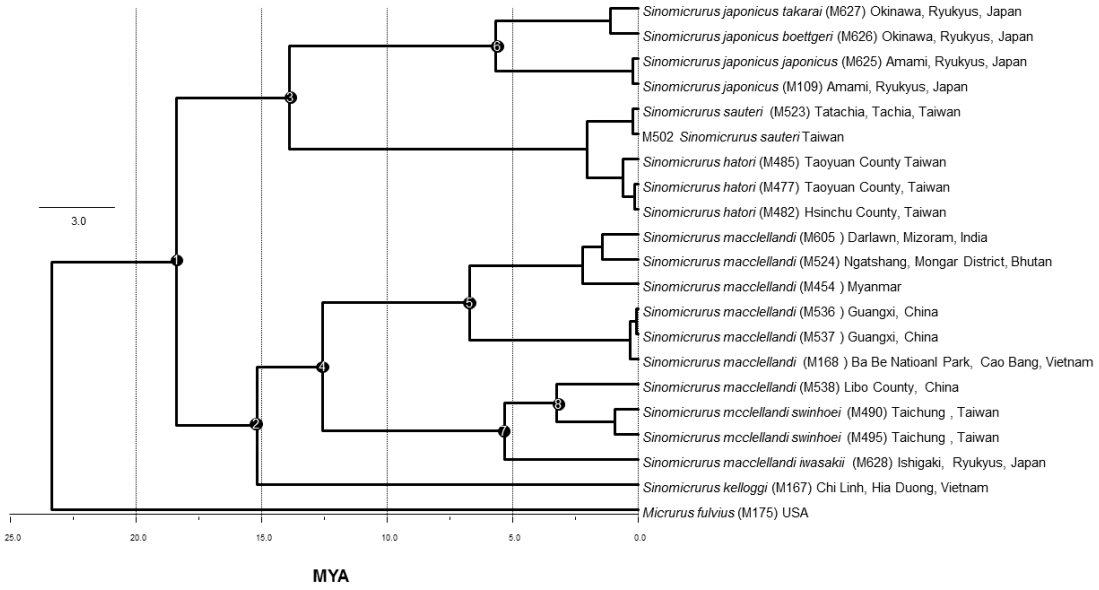


Figure 4.5. BEAST chronogram calibrated using a combination of the vertebrate mitochondrial clock and secondary calibrations. Major cladogenetic events are numbered 1 through 8; corresponding divergence estimates and posterior probability for each node can be found in table 4.1

Table 4.1. Posterior probabilities and divergence times with confidence intervals at each major node for the three different calibration schemes

Node	Posterior probability	Divergence time 1 (95%HPD)	Divergence time 2	Divergence time 3 (95%HPD)
1	1.00	18.82 (19.55-28.52)	18.59	18.41 (14.34-22.30)
2	1.00	15.53 (12.41-18.53)	15.34	15.18 (11.85-18.59)
3	1.00	14.20 (11.36-17.09)	13.98	13.88 (10.76-17.23)
4	1.00	12.85 (10.46-15.59)	12.70	12.57 (9.82-15.58)
5	1.00	6.87 (5.24-8.47)	6.78	6.71 (5.02-8.45)
6	1.00	5.87 (4.59-7.08)	5.73	5.68 (4.18-7.29)
7	1.00	5.44 (4.13-6.82)	5.39	5.32 (3.98-6.78)
8	1.00	3.32 (2.37-4.27)	3.28	3.25 (2.33-4.27)

between the endemics of Taiwan and the Southern Ryukyus pre-dated the formation and collapse of the Pleistocene land bridges by over 4 Mya (Node 7: 3.98-6.82 Mya). In summary, all nodes representing major cladogenetic events in the genus rejected the Pleistocene divergence hypothesis as the principal driver of diversification.

Geographic range evolution

The best fitting biogeographical model identified by the BioGeoBEARS analyses was DIVALIKE+J, Matzke's (2013) likelihood implementation of the DIVA model (table 4.2) which incorporates dispersal to a geographic area not observed in the parental lineage. This model of range evolution hints at an ancestral population occupying an area that comprised of the Ryukyu-Taiwan archipelago and continental Eurasian landmasses combined. A basal vicariance event gave rise to the two broadest clades of *Sinomicrurus* species (striped island and unstriped mainland lineages) in the early Miocene (fig. 4.4). The striped *Sinomicrurus*, thereafter restricted to the island arch, experienced an additional vicariant event when the Taiwanese population splits from the Central Ryukyu endemics approximately 14 Mya. Approximately at the same time a lineage of unstriped *Sinomicrurus* from the mainland dispersed into the adjacent biogeographic zone comprising of Taiwan (*S. swinhoei*) and the southern Ryukyus (*S. iwasakii*) with a back dispersal into mainland China (*S. cf. macclellandi*) during the late Pliocene. The lineages

of Northeast India were founded by dispersal from an ancestral population situated in the continental margin of China in the late Miocene.

Table 4.2. Results of biogeographic model selection procedure for *Sinomicrurus* in BioGeoBEARS via Akaike Information Criterion assessment of model fit

Model	Log likelihood	Number of parameters	*d*	*e*	*j*	AIC	AIC weight
DIVALIKE+J	-20.97440	3	0.02572741	1.000000e-12	0.8596484	47.94880	8.888585e-01
DEC+J	-23.70692	3	0.02127798	2.977935e-03	1.3430922	53.41383	5.782494e-02
DIVALIKE	-24.82532	2	0.06188233	1.000000e-12	0.0000000	53.65064	5.136796e-02
BAYAREALIKE+J	-28.03420	3	0.02202454	3.779556e-02	0.6509281	62.06840	7.634838e-04
DEC	-28.59619	2	0.04577633	1.000000e-12	0.0000000	61.19238	1.183112e-03
BAYAREALIKE	-34.98769	2	0.07518495	7.468100e-02	0.0000000	73.97538	1.982582e-06

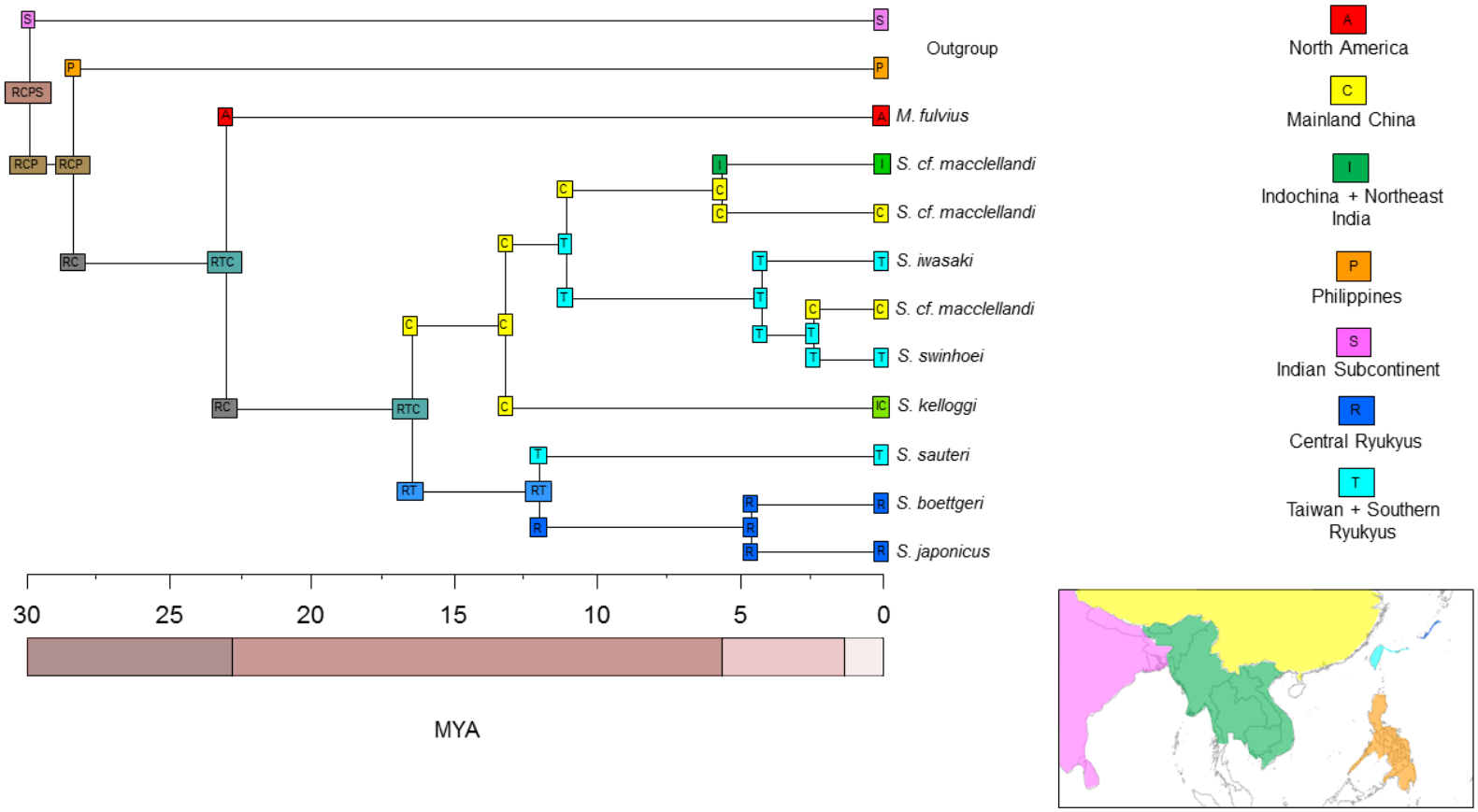


Figure 4.6. Chronogram depicting ancestral range evolution of *Sinomicrurus* species according to the best-fitting biogeographical model (DIVALIKE+J) proposed by BioGeoBEARS. Colored squares with letters at nodes depict most likely states (ancestral ranges). Map on lower right corner depicts color coded biogeographic areas used for the analysis (excluding North America).

Discussion

Systematics and species boundaries

Our gene tree is completely congruent with our coalescent based species tree phylogeny and all nodes are well resolved in both analyses. These phylogenetic analyses provide molecular validation to the grouping of two major clades based on the presence/absence of stripes and reveal morphologically cryptic lineages in both groups. The wide-spread *S. macclellandi* has traditionally been assigned four subspecies based on morphology and color pattern; our analyses show that the subspecies *S. macclellandi swinhoei* and *S. macclellandi iwasakii* are substantially divergent genetically and should be raised to species level based on our quantitative evaluation of species boundaries within the genus (i.e. *S. swinhoei* and *S. iwasakii*). Our species delimitation analyses further expose the presence of three additional distinct lineages within the *S. macclellandi* clade. Thus we propose to raise the Northeast Indian-Indochinese animals as well as their sister group found west of the trailing Himalayan ranges to two separate species. A third species, closely related to, yet sufficiently distinct from *S. swinhoei*, is identified from Libo County in southeastern China. In the absence of available names, these three species will be provided new names based on the amended International Code of Zoological Nomenclature.

The striped *Sinomicrurus* of the Taiwan-Ryukyu archipelago have conventionally been comprised of three species, namely the Taiwanese *S. hatori* and *S. sauteri* and *S. japonicus* which is restricted to the Central Ryukyus. According to our molecular analyses *S. hatori* and *S. sauteri* fail to garner support to be considered distinct species and we herein sink *S. hatori* into the senior species *S. sauteri*. The *S. japonicus* clade, on the

other hand, displays the presence of two genetically differentiated species. Thus, to reflect species boundaries in this clade, we propose to sink the junior subspecies *S. japonicus takarai* into *S. japonicus boettgeri* while simultaneously raising *S. japonicus boettgeri* to species status i.e. *S. boettgeri*, distinct from *S. japonicus*.

Biogeographic implications

Early biogeographic works focusing on the Taiwan-Ryukyu archipelago have often evoked dispersal via Pleistocene land-bridges and subsequent allopatric speciation as the primary drivers of diversification and endemism (e.g. Kizaki and Oshiro, 1977; Ota, 1998; Ota et al., 1999). Empirical evidence evaluated in the light of biogeographic hypothesis testing in recent years however, is increasingly hinting at diversification in varying organisms of the area, to have occurred before the Pleistocene (Honda et al., 2014; Kaito and Toda, 2016; Su et al., 2016b; Xu et al., 2016). Our research lends further support to the pre-Pleistocene diversification hypothesis as it clearly establishes that all major speciation events in the genus *Sinomicrurus* occurred between the Early Miocene and late Pliocene. As predicted by the pre-Pleistocene hypothesis, the divergence times between lineages found on the islands in our analyses are significantly earlier than the Pleistocene, and the *Sinomicrurus* lineages endemic to the Central Ryukyu are the first ones to diverge.

The timing of the split between the Taiwan-Ryukyu and the mainland clade however, pre-dates every available geologic estimate of the rifting between the island arc and mainland China. The earliest estimates of the separation of the arc from the continent are put at 8 MYA (Kizaki and Oshiro, 1977), which is 10 MYA later than the divergence between the mainland and archipelagic *Sinomicrurus* (19-16 MYA). The split does however coincide with the first phase expansion of the Okinawa trough that

occurred in the Mid-Late Miocene (Sibuet et al., 1995). However, this discordance between biological and geological data is not unprecedented; Honda et al. (2013) in their study of *Goniurosaurus* geckos from the Central Ryukyus reported an early Eocene divergence between the island and Eurasian mainland, while Xu et al. (2016) identify the early Miocene (95%HPD: 26.6-18.4) as the time of divergence between island and continental clades of East Asian Liphistiide spiders.

It is to be noted that even though Taiwan only obtained its current configuration around 5 MYA (Huang et al., 2006), Taiwan island represents the exposed accretionary prism, developed via arc-continent collision around 16 MYA ago with the intra-oceanic subduction of the South China sea crust beneath the Philippine Sea plate (Huang et al., 2006, 2012). During the Paleogene and through the early Neogene, the Taiwan-Ryukyu island-arc was part of the eastern Eurasian continental margin and our biogeographic appraisal shows that the ancestor of *Sinomicrurus* snakes were broadly distributed around the same area in the early Miocene (fig. 4.4). It is thus conceivable that the extensive volcanism, which is said to have accompanied the subduction event (Huang et al., 2006, 2012), isolated the continental population from the snakes that would go on to evolve into the island-arc population. This vicariant event would have presumably been followed by an episode of prolonged isolation as the Okinawa Trough began to open up in the late Miocene, forcing the island-arch to rift farther away from the Eurasian mainland. This scenario could explain the early divergence of the *S. sauteri-S. boettgeri-S. japonicus* clade from the rest of the *Sinomicrurus* and their endemism to the island-arch. Correspondingly, if the split between the Taiwan and Central Ryukyu species (95% HPD: 10.76-17.23) signifies the formation of the Kerama Gap, then our data once again predate the geological estimates of 10-1.5 MYA (Hikida and Ota, 1997; Ota, 1998). Similarly the divergence between the *Sinomicrurus* of the Central Ryukyus (*S. japonicus*

vs *S. boettgeri*) significantly predates the formation of the strait that split the Okinawa island group from the Amami group. Once again, this pattern resembles the one seen in spiders (Xu et al., 2016) and amphibians (Honda et al., 2012; Matsui et al., 2005) of the region. Collectively, these results suggest one of two possibilities: 1) vicariant histories in these islands may have formed as a result of barriers that are more ancient than those highlighted by geology and that hitherto remain unknown; 2) divergences in the island lineages are non-vicariant.

In the unstriped clade (primarily restricted to the mainland) the first invasion into Taiwan from mainland China occurred during the latter half of the Miocene (i.e. *S. swinhoei*). The 95% HPD interval (9.82-15.58) of this split includes the 10-5 MYA window that's associated with the emergence of Taiwan above sea level (Huang et al., 2006). According to our biogeographic model, the invasion probably occurred via oversea dispersal and involved founder event speciation. From Taiwan, the invading snakes could only disperse as far as Ishigaki Island in the Southern Ryukyus (i.e. *S. iwasaki*), since, according to our model, the biogeographic break between the Central Ryukyus and the Southern Ryukyus and Taiwan had already been established along the Kerama Gap, between 11.85 and 18.59 MYA. Overall, our results indicate that the Quaternary drop in sea level and the corresponding formation of land-bridges did not contribute to the diversification of *Sinomicrurus* lineages. The opportunity of dispersal to and from the mainland, provided by these ephemeral Pleistocene connections, as well as the subsequent prospect of founding were probably curbed by the lack of empty niches, as they would have likely been occupied by congeners since the early Miocene.

The biogeographic drivers of cladogenetic events, further south and east in the continent, are more difficult to elucidate given the absence of distinct biogeographic breaks relative to the archipelago. The most likely barrier segregating genetic variation

between the two undescribed species of *S. macclellandi* in China and Indochina appears to be the Hengduan Mountains that descend southwards from the Tibetan Plateau and into the Yunnan province of southern China. However, this postulate requires a more thorough sampling of *S. macclellandi* especially from Thailand.

In summary, ours is the first study to tackle the molecular systematics and biogeography of understudied subtropical *Sinomicrurus* coralsnakes. Our phylogenetic and species delimitation results broadly corroborate clades assigned on the basis of color patterns and morphology while revealing several instances of cryptic speciation in wide-spread species. Our biogeographic analyses reveal that divergence in the genus *Sinomicrurus* significantly pre-dates the outbreak of Quaternary diversification and were probably triggered by more ancient events. Thus adding to a small but growing number of studies, which hint that the Miocene epoch might have been responsible for high levels or endemism that we see in the Taiwan-Ryukyu archipelago fauna today.

Chapter 5

Higher-level relationships and historical biogeography of the family Elapidae

Introduction

A diversity of charismatic yet highly venomous snakes make up the family Elapidae, including cobras, mambas, coralsnakes, kraits, Australopapuan endemics and their marine relatives. Collectively called elapids, these snakes have also been of particular interest to biologists because of their wide geographic distribution, morphological diversity and the clinical importance of their venom and envenomation of humans (Keogh, 1998; Campbell et al., 2004; Kelly et al., 2009). Today, with approximately 360 species in over 60 genera (Uetz, P. & Jirí Hošek (eds.), The Reptile Database, http://www.reptile-database.org, accessed August 13, 2015), the family Elapidae ranges across global tropics and subtropics and contains over 80% of the world's snake species (Keogh, 1998a).

Almost one third of the species diversity within the Elapidae is comprised by coralsnakes, which are be broadly divided into two groups based on geographic distribution, namely New World and Old World coralsnakes. Usually small, shy and fossorial, Old World coralsnakes are amongst the rarest venomous Asian snakes and their sampling in molecular studies has been sparse (Castoe et al.2007). In this study, finally equipped with a comprehensive representation of Asian coralsnakes we provide mitochondrial and nuclear data for all the major lineages of *Sinomicrurus*, Sundaland and Indian *Calliophis*. Leveraging this dataset, we build a fossil calibrated phylogeny to revisit the higher level relationships and historical biogeography of elapids. Specifically, we examine molecular phylogenetic evidence, for 1) the monophyly of *Calliophis*, 2) the

phylogenetic placement of Indian coralsnakes within the Elapidae and 3) elucidate the divergence times and ancestral ranges of basal elapid lineages.

A review of higher level elapid relationships

For systematists, the monophyly of Elapidae has never been controversial, but rampant incongruity and poor resolution have plagued most studies that attempted to confidently determine basal branching patterns within the family (Slowinski et al., 1997; Keogh, 1998a; Slowinski and Keogh, 2000a; Zaher et al., 2009; Pyron et al., 2011). The only broad consistency between various authors has been recognition of the two subfamilies, namely Hydrophiinae (i.e. marine and terrestrial Australo-Malenesian species) and the terrestrial Elapinae (i.e. the remaining African, Asian and American species) (McDowell, 1986b; Slowinski et al., 1997; Slowinski and Keogh, 2000a; Scanlon and Lee, 2004a; Castoe et al., 2007a). Unlike the flurry of studies investigating the evolutionary relationships amongst the hydrophiines (Mengden, 1985; Schwaner et al., 1985; Keogh, 1998a; Slowinski and Keogh, 2000a; Scanlon and Lee, 2004a; Lukoschek and Keogh, 2006) , the phylogeny of nominal groupings within the Elapinae has received relatively little attention (Slowinski and Keogh, 2000a; Slowinski et al., 2001; Castoe et al., 2007). In a thorough phylogenetic treatment of Elapinae, Castoe et al. (2007) used molecular phylogenetic and descriptive morphological evidence to investigate the phylogenetic affinity of *Hemibungarus*, which at the time was considered to be a coralsnake. In doing so they also provided the most recent update of relationships amongst the enigmatic elapine genera conventionally known as “coralsnakes”. Their data divided the Elapinae into two clades: 1) Hemibungarini for the Afro-Asian cobras and their relatives including *Hemibungarus* and Calliophini for the coralsnakes (*Calliophis, Sinomicrurus, Micrurus, Micruroides and Leptomicrurus*). The implications of their results

were largely consistent with previous work based on morphology and molecular data regarding the close affinity between Asian coralsnakes and American coralsnakes (McDowell, 1969, 1970, 1986b; McDowell and Cogger, 1967), specifically the monophyly of *Sinomicrurus* and the American clade containing *Micrurus*, *Micruroides* and *Leptomicrurus* (Keogh, 1998; Slowinski and Keogh, 2000).

However their study only included one species of *Calliophis* (*C*. *bivirgata*), which is a species of coralsnake from the Sunda Shelf. It was explicitly assumed, based primarily on morphological synapomorphies presented by Slowinski et al. (2001), that all other *Calliophis* species not included in the study (including the Indian taxa), formed a clade with *C. bivirgata*. The authors concluded by acknowledging the need to test their assumption of monophyly in *Calliophis* when a more comprehensive sampling of these rare Asian coralsnake species became available.

With the recent availability of nucleotide sequences for more *Calliophis* species, some studies on higher level systematics of Caenophida have recovered this genus to be paraphyletic, with the Sundaland species *C. bivirgata* sister to the remaining coralsnakes (genera *Micrurus* and *Sinomicrurus*) while the Indian radiation is recovered as a sister lineage to all other elapids (Pyron et al., 2013, 2013). But these relationships are equivocal at their best, given their inadequate support values, thus discouraging authors from discussing the phylogenetic and taxonomic implications of the basal position of *Calliophis* coralsnakes.

Given that suboptimal taxon sampling is known to negatively affect the overall accuracy of reconstructed phylogenies (Rannala et al., 1998; Pollock et al., 2002; Zwickl and Hillis, 2002), the traditional limiting factor in studies on higher level elapid phylogenetics may indeed be the availability of Old World coralsnake samples. With the

exclusion of all major coralsnake lineages from South and Southeast Asia, we anticipate a better resolution of basal nodes in this family.

A review of the biogeographic origin and history of elapids

The lack of strong resolution of higher level relationships among elapid clades has hitherto confounded a clear understanding of the biogeogeographic history of these diverse and widely distributed snakes. Thus few authors have been able to speculate on which modern day elapids most closely bear a resemblance to the ancestral stock or where their ancestors might have lived. Most recent authors tend to lean towards Asia (as opposed to Africa) as the centre of origin for the Elapidae with subsequent dispersal to Africa and the Americas (Keogh, 1998; Slowinski and Keogh, 2000; Kelly et al., 2009). However, they all together caution against discounting a Gondwanan origin for the elapids in the face of unresolved basal relationships (especially in the American-Asian coralsnake clades) and the absence of a proper temporal scale. Kelly et al. (2009), provisionally refuting a Gondwanan origin based on their results, went so far as to contemplate on the putative drivers of early elapid diversification. In this context, their temporal and biogeographic analyses lead them to speculate about an Indian origin for Elapidae, with early cladogenesis in the family triggered not by the development of the proteroglyphous condition but by the collision of the Indian and Eurasian plates. While several studies have further explored the biogeography of major elapid radiations such as Afro-Asian cobras (Wüster et al., 2007; Kelly et al., 2009) and Australasian elapids (Keogh, 1998; Scanlon et al., 2003; Scanlon and Lee, 2004), the spatio-temporal evolution of the wide-spread genera *Calliophis* and *Sinomicrurus* has remained elusive due to lack of comprehensive sampling. Kelly et al. (2005) briefly touch on the group and provide an age of 31 (35.6-26.9) Mya for the divergence of the coralsnake clade

postulating that it originated in the Oriental region early in elapid history. Like most authors before them, Kelly et al. (2005) also support a dispersal of coralsnakes from the Old World into the Americas via the Bering Land Bridge during the Oligocene-Miocene boundary (approximately 23 Mya).

The primary shortfall of the aforementioned studies is the limited use of fossil data to constrain divergences within the Elapidae, despite of the availability of several elapid fossils. Herein we employ for the first time, three ingroup fossils in addition to two fossil age constraints outside of the Elapidae.

Material and Methods

Taxon sampling

Nucleotide sequences of four gene fragments for a total of 41 terminals (39 species) were included in this study (see Appendix, Supplementary Tables 6 and 7). This comprised sequences from 27 members of the Elapidae; for a thorough representation of Asian coralsnakes, our sampling included for the first time: sequences of both known species of Sundaland coralsnakes as well as representatives from each of the three coralsnakes radiations from India (i.e. as demonstrated in Chapter 2); and sequences for three major lineages of *Sinomicrurus* (striped, unstriped and *S. kelloggi*, as established in Chapter 4). Members of each of the three American coralsnake genera *Micrurus*, *Leptomicrurus* and *Micruroides* were also represented. Eight species across six genera represented all major groups of the tribe Hemibungarini and the subfamily Hydrophinae was represented by three genera. Lamprophiidae, the sister group to Elapidae, was represented by four species across three genera. This included both species of the genus *Oxyrhabdium*, the exclusively Asian lineage within the family, which several previous studies have placed as the sister lineage to elapids but always with low support.

Our outgroup sampling consisted of nine outgroup taxa from the remainder of Xenophidae (e.g. Colubrids and Vipers).

DNA sequencing and alignment

Genomic DNA for all individuals was isolated from tissue samples of either the liver, skin (preserved in ethanol), or sheds using a Qiagen DNeasy kit (Qiagen, Valencia, California, USA) or "serapure" magnetic beads (Rohland and Reich, 2012). Two mitochondrial and two nuclear genes were sequenced via PCR for each sample. The mtDNA cytochrome-b (cyt-b) gene fragment was amplified using the primers Gludg and AtrCB3 (Parkinson et al., 2002), The fourth subunit of the NADH dehydrogenase mitochondrial gene (ND4) and adjacent tRNAHis, tRNASer and tRNALeu were amplified using the primers ND4 and LEU (Arèvalo et al., 1994). The nuclear oocyte maturation factor (c-mos) gene fragment was amplified using the primers S77 and S78 following Lawson et al. (2005). We amplified another nuclear locus, the Recombination-activating gene 1 (*RAG1*) in two steps given the target length of about 2600 bp; we amplified it via two different overlapping fragments that we call RAG1F and RAG1R. Both CMOS and RAG1 are "stock" genes that are used regularly in many phylogenetic studies of snakes (Lawson et al., 2005; Slowinski and Lawson, 2002; Vidal et al., 2007a, 2007b).

GoTaq® Green Master Mix, 2X (Promega Corporation, Madison, Wisconsin, USA) was used for all amplification reactions on a GeneAmp® PCR System 9700 (Applied BioSciences, Foster City, California, USA). All PCR products were checked visually for successful amplification on agarose gels, and successful PCR products were purified using AMPure XP beads following the Agencourt protocol (Beckman Coulter). The Genomic Core Facility at the University of Texas at Arlington completed the sequencing reactions with an ABI PRISM 3100xl Genetic Analyzer.

The raw gene fragment sequences were assembled and cleaned using Sequencher 4.8 (Gene Codes, Ann Arbor, Michigan, USA). The alignment of sequences was obtained using Clustal W (Larkin et al., 2007). Sequences were translated to amino acid sequences to verify the absence of stop codons and proper alignment, and edited by eye for accuracy. The three tRNAs (histidine, serine and leucine) were aligned by eye guided by the model provided by (Kumazawa and Nishida, 1993) and using pre-aligned tRNAs for members of the genus Micrurus as a template.

Supermatrix and coalescent phylogenetic analyses

The concatenated data set was partitioned by gene and codon position. Optimum partitioning schemes and substitution models for the phylogenetic analysis were identified based on Bayesian Information Criterion (BIC) using the 'greedy' search algorithm in Partition-Finder v1.1.0 (Lanfear et al., 2012). The best partitioning scheme divided the data into five unlinked partitions: 1) GTR+I+G for codon position 1 of genes Cytb and ND4; 2) GTR+I+G for codon position 2 of genes Cytb and ND4; 3) GTR+I+G for codon position 3 of genes Cytb and ND4; 4) GTR+I+G for codon positions 1 and 2 for genes CMOS and RAG1; 5) GTR+G for codon position 3 for genes CMOS and RAG1.

We used a consensus of maximum likelihood (ML) and bayesian inference (BI) methods. Gaps in alignment were treated as missing data for all phylogenetic analyses. ML analysis employing the rapid bootstrapping algorithm was conducted using the program RAxML 8.00 (Stamatakis, 2014) on the CIPRES Science Gateway server v3.2 (Miller et al., 2010); the model GTR+G was used instead of GTR+I+G because the 25 discrete rate categories appear to better estimate invariant sites (Stamatakis, 2006). Nodal support for ML was provided by bootstrapping (1000 pseudoreplicates), with bootstrap (BS) values ≥0.70 considered strong support (Hillis and Bull, 1993).

Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analyses were conducted on a partitioned alignment using MrBayes v. 3.3(Ronquist and Huelsenbeck, 2003). Two simultaneous runs of four MCMC analyses, consisting of one cold and three incrementally heated chains, were initiated with random trees for a total of 1×10^8 generations (sampling every 500 generations). Burn-in was set to the default values of 25%, hence discarding the initial 50K generations. Stationarity was examined using trace plots and ESS values (>200) on TRACER v. 1.7 (Rambaut & Drummond, 2009). A 50% majority rule consensus tree with estimates of Bayesian support was constructed using the remaining sampled trees and posterior probabilities (PP) provided nodal support for Bayesian analyses, with PP values ≥0.95 considered strong support (Alfaro et al., 2003; Huelsenbeck and Rannala, 2004; Mulcahy et al., 2011). The graphical viewer Figtree V 1.3.1 (Rambaut, 2007) was used to view and manipulate the trees from RAxML and Mr Bayes analyses.

Anticipating conflicts amongst different gene trees we also carried out coalescent based species tree analyses to more accurately assess species relationships. We inferred a species tree using *BEAST part of the BEAST V2.1.0 software packet (Bouckaert et al., 2014). We used the Yule Model for the tree prior while all other priors were set to default values. Analyses were run in duplicate, each for 1×10^9 generations, sampling every 20,000 generations, for a total of 5×10^4sampled trees. We then used Tree Annotator v1.7.4 to discard the first 10% of the samples as burn-in and to construct the Maximum-clade credibility tree from the posterior distribution of species trees. We used DensiTree v2.1.10 (Bouckaert, 2010) to inspect variation in the topologies obtained from the coalescent analysis. The graphical viewer Figtree V 1.3.1 (Rambaut, 2007) was used for tree visualization and manipulation.

Divergence dating and fossil calibrations

We estimated divergence times across the elapid phylogeny using BEAST 2 (Drummond and Rambaut, 2007). To accommodate possible rate variation among lineages we applied an uncorrelated log–normal relaxed clock model. For the tree prior we used the Fossilized Birth Death (FBD) model (Heath et al., 2014); the FBD method provides an alternative to the problematic approach of specifying calibration constraints and associated probability distributions assigned to interior nodes; it does this by directly incorporating all fossil data in a Bayesian framework (Heath et al., 2014). Since the runs failed to achieve stationarity under the models suggested by PartitionFinder, we decided to use the simpler HKY model of sequence evolution for all partitions.

Dates used to constrain nodes were obtained from estimates based on the fossil record and literature. We used 5 fossil calibrations for our divergence estimates (table 5.1): 1) the youngest unequivocal colubroid fossil dates back approximately 40 Mya to the Eocene of Asia (Head et al., 2005). To reflect we applied a constraint of 40 Mya to the value for the origin (i.e. MRCA of Colubroidea and Viperidae). Assuming the origin time to be drawn from a log-normal distribution, we set the mean to 17.0, the standard deviation to 0.25 and fixed the offset of the log-normal distribution to 23.5 (i.e. the approximate age of the oldest fossil used for calibration, following Heath et al., 2014) for a final median age of 40 Mya; 2) we constrained the root of the elapid clade with a date of 23 Mya based on the age of the oldest known elapid fossil (Scanlon et al., 2003; Lukoschek et al., 2012); 3) fossils of European '*Naja*' species from the Middle Miocene have typically been used to date the spilt between Asian and African *Naja*, however based on the suggestion by Szyndlar and Rage (1990) and Lukoschek et al. (2012) we used them to constrain the stem *Naja* clade (e.g. the split between *Bungarus* and *Naja*) with a date of 19 Mya; 4) we used a date of 13.60 to constrain the stem Croatalinae

based on a Barstovian crotalin fossil from Nebraska (Rage and Holman, 1984); and 5) stem Micrurus were constrained with a date of 12.50 using a Micrurus fossil also from the Barstovian of Nebraska (Rage and Holman, 1984).

Two independent analyses were run for 1×10^8 generations, sampling every 1000 generations. We used the program Tracer v. 1.5 (Drummond and Rambaut, 2007) to confirm stationarity of the Markov chain Monte Carlo (MCMC) analysis, with acceptable effective sample sizes of the posterior above >200 for each estimated prior. For each independent analysis, we also executed a run that samples only from priors while ignoring sequence data, which allowed us to visually compare marginal prior distributions on each parameter and assess the informativeness of the priors. After discarding burn-in samples, the trees and parameter estimates from the independent runs were combined using LogCombiner v. 1.7.4 (Bouckaert et al., 2014). We used the program TreeAnnotator v. 1.7.4 to summarize parameter values of the samples from the posterior on a maximum clade credibility tree.

Biogeographic analyses

Geographic range evolution was inferred using a consensus of several different models. This included models based on both parametric and parsimony methods as implemented in the R package BioGeoBEARS (Matzke, 2013) and RASP 3.2 (Yu et al., 2015). Within the program RASP we used the parametric Bayesian Binary MCMC (BBM) analysis which infers geographic distributions at ancestral nodes employing a full hierarchical Bayesian approach (Ronquist, 2004). Ancestral range evolution was modeled on the ultrametric tree obtained from the *BEAST analysis for both programs. However in order to improve likelihood values we pruned of the Viperid and Colubrid outgroups. We also removed two species of sea snakes (*Laticauda colubrina* and

hydrophis platurus) since being oceanic they did not adhere to any distinct biogeographic zone. To investigate whether different models of State Frequency (i.e. F81 and JC) and Among-Site rate variation (i.e. Gamma and Equal) resulted in drastically different estimates we ran four independent analyses exhausting all possible combinations of the said models. All analyses employed ten simultaneous chains and were run for 50000 cycles sampling every 100 and discarding 100. BioGeoBEARS infers range evolution employing several standard models but does so in a likelihood framework (Matzke, 2013c, 2014); in this context we used likelihood versions of the DIVA (or DIVALIKE), LAGRANGE (DEC) and BAYAREA (or BAYAREALIKE) models. Moreover BioGeoBEARS also allows users to add "founder-event speciation" termed "*J"* as a cladogenetic process, in addition to the two default processes (dispersal "*d"* and extinction "*e*"). Thus our geographic range evolution analyses included a total of six models i.e. DIVALIKE, DIVALIKE+*J*, DEC, DEC+*J*, BAYAREALIKE and BAYAREALIKE +*J*. We then used the Akaike Information Criterion (AIC) to directly compare the fit of different models to the data. For all analyses the terminals were coded with known zoogeographic zones based on their modern day distributions. These included A) Oriental Region, B) Indian Subcontinent, C) Africa, D) Australasia and E) Americas. Conventionally, the Indian Subcontinent is considered part of the Oriental Region, however given that previous hypotheses of basal elapid cladogenesis involved the collision of the Indian Subcontinent with Laurasia as trigger (Kelly et al., 2009), we considered the subcontinent as a separate biogeographic unit. For all analyses, ancestral ranges were constrained to two or fewer regions based on the fact that all taxa used in the study are confined to two or fewer regions.

Results

Phylogenetic analyses

Our ML analyses produced a single tree (lnL = -29284.00) which resulted in an identical topology as our Bayesian consensus phylogram (fig. 5.1). Unlike recent studies (Pyron et al., 2013a) Lamprophiidae is recovered as paraphyletic with the two species of Oxyrahbdium being sister to all elapids while the remaining lamprophiids were sister to a clade containing both Elapidae+*Oxyrahbdium*. The total Elapoidea group is recovered as monophyletic with high support (PP=1.00; BS=100), but the node joining Oxyrahbdium species to a sister elapid clade does not have adequate support values (PP<95; BS<100). The monophyly of Elapidae is strongly supported in both BI and ML analyses (PP=1.00; BS=100). The overall *Calliophis* clade is retrieved as monophyletic with mediocre support (PP <95; BS <70) but the sister Sundaland and Indian *Calliophis* clades are individually both well supported (PP =1.00; BS=100). The *Calliophis* in turn are sister to a strongly supported group (PP=1.00; BS=83) consisting of all other elapids including the American coralsnake genera and *Sinomicrurus* thus essentially rendering the functional group known as coralsnakes paraphyletic and the tribe Calliophini untenable. Within the Indian *Calliophis*, *C. bibroni* is sister to a clade containing C. *melanurus* (blue-tailed coralsnake) and C. *nigrescens* (striped coralsnake). Consistent with previous research (Keogh, 1998; Slowinski & Keogh, 2000), the snakes of the Old World genus *Sinomicrurus* and the New World coralsnakes (genera *Micrurus*, *Leptomicrurus* and *Micruroides*) form a strongly supported clade (PP =1.00; BS=100). Consistent with previous studies based on morphology and molecules (Pyron et al., 2013a; Slowinski, 1995) *Micruroides* is strongly retrieved as basal (PP =1.00; BS=100) to a sister clade containing *Leptomicrurus* and *Micrurus*. Relationship within the *Micrurus* broadly agreed with previous research with the primarily North American monadal

coralsnakes sister (PP=1.0; BS=100) to South American triadal members. Sister to this exclusive coralsnake clade is the monophyletic group (PP=1.00; BS=100) that consists of what Castoe et. al. (2007) referred to as the elapine tribe Hemibungarini + the subfamily Hydrophinae (the Australasian elapid radiation). It is clear given the polyphyly that arises considering the basal position of *Calliophis*, that the subfamily Elapinae is untenable. There is high support for the monophyly of the tribe Hemibungarini (PP=99; BS=87) and the traditional genera contained therein are retrieved as monophyletic. The Asian elapid genus Bungarus is recovered as basal to all other Hemibungarini and the African and Asian *Naja* species are consistently recovered as a monophyletic group having a sister relationship with the African shield-nose cobras of the genus *Aspidelaps* (PP=1.00; BS=100). The relationships that remain unresolved in this clade include the placement of the Philippine endemic *Hemibungarus calligaster* which appears as a polytomy in the BI and has exceptionally low support in the ML; the inadequately supported sister relationships between the Asian genus *Ophiophagus* and the African *Dendroaspis*. Overall, despite of the presence of several unresolved crown nodes, the support for major relationships is high.

Even though nodal support was much lower in general, the *BEAST species tree analyses of the combined dataset recovered an almost identical topology as our ML and BI analyses, with one major difference: the genus *Oxyrhabdium* is nested within the Lamprophiidae but with insufficient support (PP=0.71).

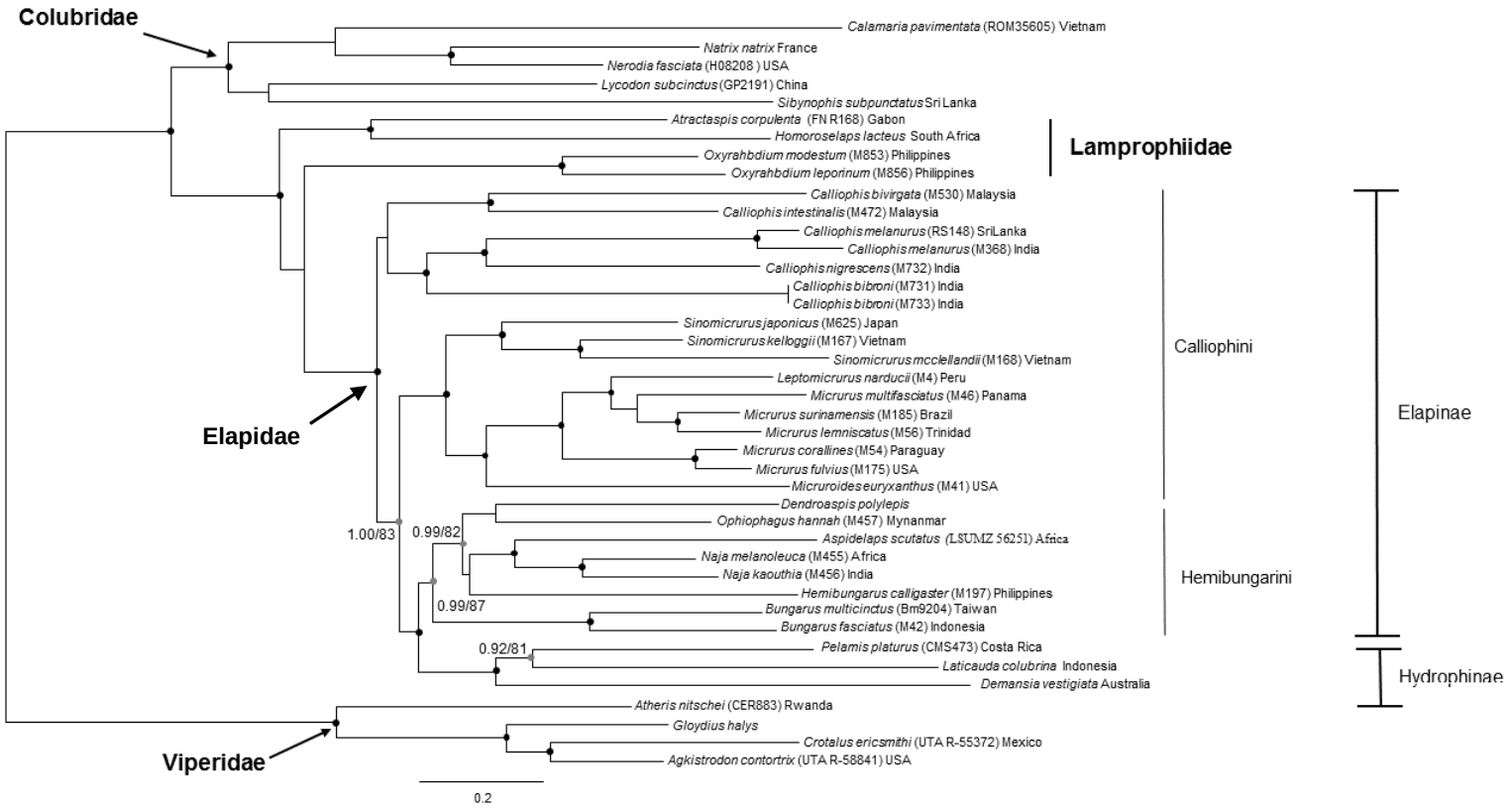


Figure 5.1. Phylogeny of Elapidae depicted as a maximum-likelihood consensus tree with Viperidae and Colubridae as distant and Lamprophiidae as proximate outgroups. Black circles denote strong nodal support (≥0.95 PP and ≥0.70 ML bootstrap). Gray circles indicate full support by at least one method (PP/ML). Lack of circles indicates support below the cutoff value.

Divergence dating

The divergence estimates from the BEAST analysis (fig. 5.2) places the basal split between Lamprophiides and elapids (i.e. the root of the superfamily Elapoidea) at the boundary between the Eocene and the Oligocene around 33.00 (95% HPD =27.42-41.97) million years. The first cladogenetic event in the Elapidae, namely the splitting of the genus *Calliophis* from the rest of the elapids, does not occur until the end of the Late Oligocene around 28.38 million (95% HPD =23.50-35.46) years ago. All the major branching events that follow, also all occur in the Late Oligocene in rapid succession within a time span of around five million years. The first major divergence is between the true coralsnakes (genera *Sinomicrurus* and *Micrurus*) and all other elapids (remaining members of the subfamily Elapinae and all of the Hydrophinae), which occurs at 26.57 (95% HPD = 21.73-32.47) million years before present. The subsequent split between the Elapines and the Hydrophines is placed at 25.21 (95% HPD = 20.86-31.09) million years while the age estimate of the stem of true coralsnakes (i.e. the split between the *Sinomicrurus* and *Micrurus*) is right at the boundary between the Oligocene and the Miocene around 23.78 (95% HPD = 18.73-28.06) million years ago. It is important to note that the tree topology of our BEAST run differed from the our MrBayes analysis in that just as in the *BEAST analysis, the genus *Oxyrhabdium* was found to be sister to the Lamprophiids, only this time with good posterior support (PP=0.98).

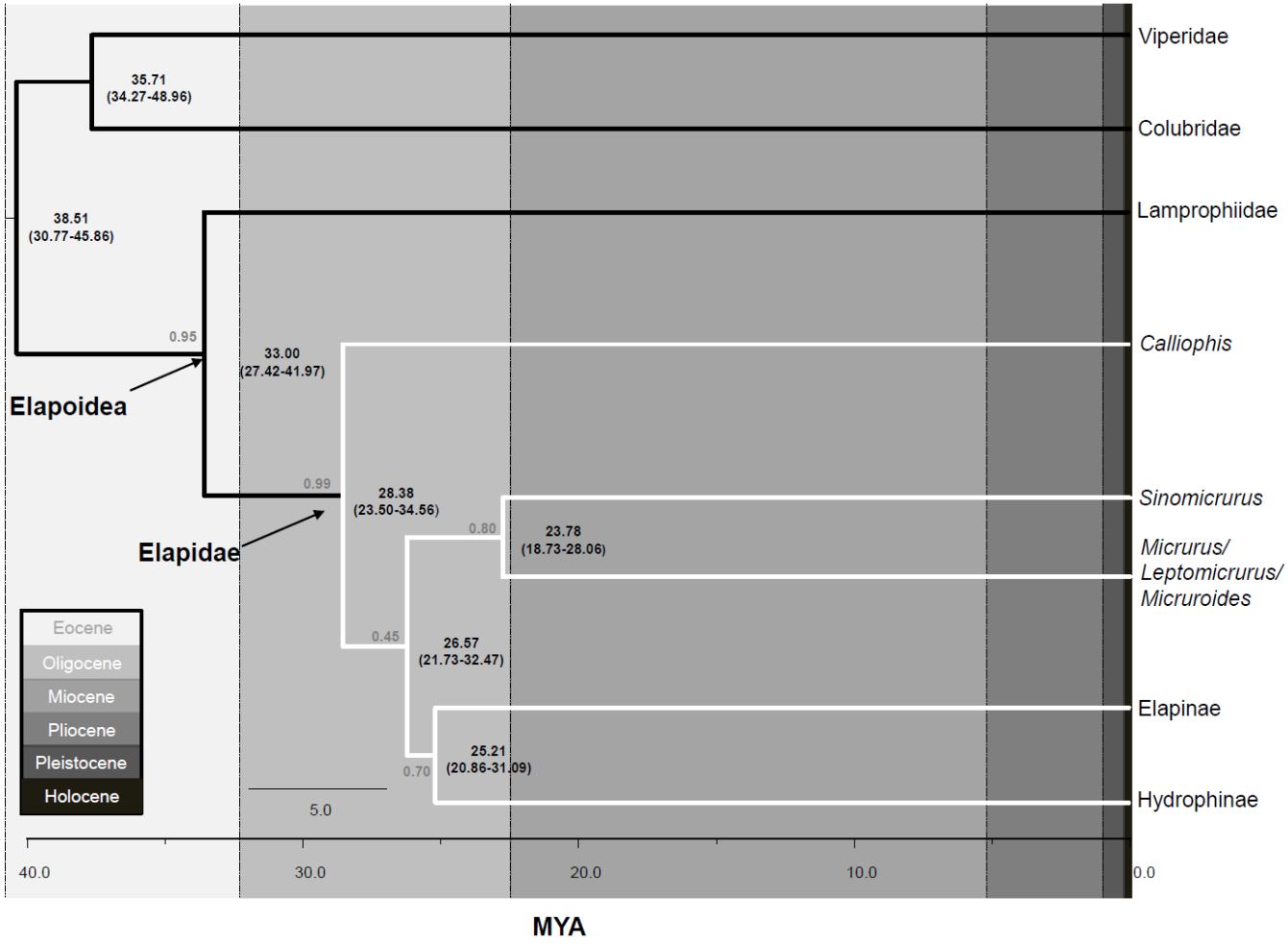

Viperidae
Colubridae
Lamprophiidae
Calliophis
Sinomicrurus
Micrurus/
Leptomicrurus/
Micruroides
Elapinae
Hydrophinae
35.71
(34.27-48.96)
38.51
(30.77-45.86)
0.95
33.00
(27.42-41.97)
Elapoidea
0.99
28.38
(23.50-34.56)
Elapidae
0.80
23.78
(18.73-28.06)
0.45
26.57
(21.73-32.47)
0.70
25.21
(20.86-31.09)
Eocene
Oligocene
Miocene
Pliocene
Pleistocene
Holocene
5.0
40.0
30.0
20.0
10.0
0.0
MYA

Figure 5.2 (previous page). Fossil calibrated chronogram of constructed using the Fossilized Birth Death model in BEAST. Black text at nodes represent divergence time estimates for major splits in the superfamily Elapoidea (with 95% HPD values in brackets below). Posterior support for major nodes is depicted in grey text. The different shades of grayscale represent distinct epochs of the Cenozoic era that are displayed in the legend.

Table 5.1. The five calibration points used in the dated phylogenetic analysis, with age and 95% HPD values in brackets (when available), their placement in the tree and the corresponding reference.

Fossil	**Age (95% HPD)**	**Placement**	**Reference**
Unidentified colubroid	40 (37-60) MYA	Origin	Lukoschek et al. 2011
Oldest elapid vertebra	23 (21-30) MYA	Crown elapids	Sanders and Lee 2008; Lukoschek et al. 2011
Naja from Europe	19 (17-30) MYA	Stem *Naja*	Szyndlar and Rage 1990; Lukoschek et al. 2011
Crotalinae from Nebraska	14.00 – 13.60 MYA	Stem Crotalinae	Rage and Holman 1984
Micrurus from Nebraska	13.60 – 12.50 MYA	Stem *Micrurus*	Rage and Holman 1984

Biogeographic analyses

Both programs produced identical estimates of range evolution. All runs of the BBM analysis converged on the same results. The Divalike +J was identified as the best-fit model of range evolution by the BioGeoBEARS analyses (table 5.2; fig. 5.3). The estimated ancestral range for the Elapoidea MRCA was indicated to be the Oriental Region. According to our model, extant elapids achieved their current cosmopolitan distribution via a four step dispersal scheme, wherein all but one instances of range evolution involved dispersals out of the Oriental Region and into other biogeographic realms. The common ancestor of the Lamprophiid genera *Oxyrhabdium* and *Atractaspis*+*Homoroselaps* occupied the Oriental Region until around 30 million years ago during which time it invaded Africa giving rise to the radiation of African lamprophiids. Another basal elapid dispersal, this time out of the Oriental region and into the Indian Subcontinent, occurred approximately 27 million years ago, right around the time of India's hard collision with Laurasia. This dispersal gave rise to the Indian *Calliophis* while the lineage that remained in the Oriental region went on to become the Sundaland *Calliophis*. At 25 million years before present came the next dispersal out of Oriental Region and into Australasia, which corresponds to the putative origin of the Hydrophinae radiation. The cladogenetic event at this node temporally corresponds to the collision of the Australian shelf with Sundaland. The elapid invasion of the New World occurred at the boundary of the Oligocene and the Miocene with a dispersal, most likely across the Beringial landbridge, out of the Orient and into the Americas. A second dispersal into Africa happened 22 million years ago, this time involving a derived elapid lineage i.e. the MRCA of the cobras; and the final migration occurred around 17 million years ago when the MRCA of *Dendroaspis* and *Ophiophagus* also spread into the African continent. Non

Calliophis elapids such as *Bungarus fasciatus, Naja kaouthia, Ophiophagus hannah* and the subtropical coralsnakes *Sinomicrurus macclelandii*, all dispersed out of the Oriental Region and into India only in the last five million years or so according to our analyses.

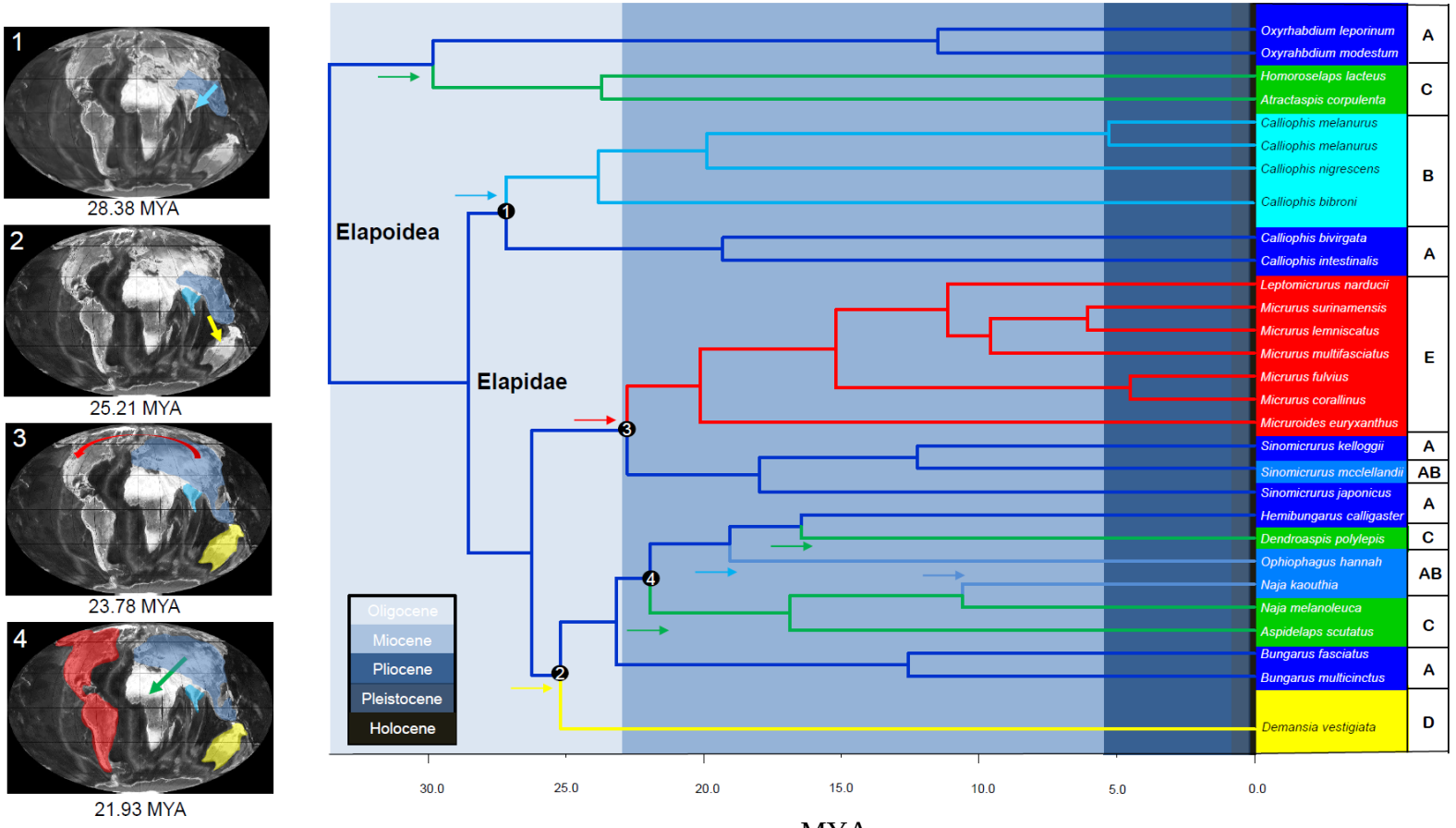
Discussion

A well supported phylogenetic framework of higher level elapid relationships

Despite of prolonged interest by several cohorts of authors, the basal relationships of Elapidae had hitherto remained obscure. In their attempt at resolving deeper nodes of the Elapoidea tree of life, Kelly et al. (2009) had associated this lack of resolution to an “explosive diversification” early in the evolutionary history of these snakes, implying that short internal branches typically associated with radiations may be responsible for the notorious difficulty in attaining good nodal support. Kelly and colleagues tried to overcome this impasse by increasing the breath of their molecular and taxonomic sampling using a larger number of molecular characters (4354 base-pairs) and also a larger number of terminals (96 OTUs for a total of 94 species) compared to studies before them (e.g. Slowinski and Keogh, 2000; Slowinski et al., 2001; Castoe et al., 2007); they also paid careful attention to their phylogenetic methods and models. Despite of this meticulous effort, their phylogenetic reconstruction though generally informative, failed, as other studies before them, to28 confidently resolve branching patterns in basal elapids.

Our approach to resolving the deeper nodes of the elapid tree was three-pronged. Our first and foremost emphasis was on acquiring our own molecular dataset rather than re-cycling material from GenBank, like most studies before us (Castoe et al., 2007; Kelly et al., 2009; Pyron et al., 2013). This gave us confidence in the quality and identity of our sequences, since the GenBank database is known to be problematic with

mis-labeled or chimeric sequences which could make for a noisy or downright erroneous phylogeny (see Reyes-Velasco et al., 2013). Another difference between our study and the work of others was the inclusion of a large number of nuclear characters, which being slower than mitochondrial loci can prove to be more apposite at resolving deeper phylogenetic patterns (Nabhan and Sarkar, 2012). Our nuclear dataset included close to 3000 base pairs (compared to around 572 in both Castoe et. al. 2007 and Kelly et al. 2009) the majority of which belonged to the RAG1 gene not previously utilized in phylogenetic works focusing specifically on elapids. We chose to include this gene based on a more widespread presence of segregating sites relative to CMOS; trees constructed using the latter usually showed insufficient phylogenetic structure in this group of snakes during our preliminary sequencing efforts. Lastly, instead of simply attempting to increase the number of terminals across all of the Elapidae, we focused on acquiring at least two representatives from every single major evolutionary lineage in the family. In our case, this included for the first time, taxa from each of the three very divergent groups of Indian *Calliophis* (i.e. *C. bibroni*, Blue-tailed coralsnakes and Striped coralsnakes) alongside both the Southeast Asian *Calliophis* species that are traditionally used in phylogenetic studies to represent Asian coralsnakes. Thus, although our taxonomic sampling was not as dense, it was theoretically more comprehensive than all of the earlier studies. Given that *Calliophis* are in fact basal elapids, it is quite probable that their inclusion significantly bolstered support values at the root of the tree. We believe that the combination of the said approaches helped us achieve the resolution of deeper nodes in the elapid tree of relationships.



A – Oriental Region; B – Indian Subcontinent; C – Africa; D – Australsia; E – Americas

Figure 5.3 (previous page). Chronogram depicting ancestral range evolution of basal elapid lineages according to the best-fitting biogeographical model (DIVALIKE+J) proposed by BioGeoBEARS. Each biogeographical realm is assigned a letter and a color and each lineage (branch) is colored according to the geographic range it occupies. Colored arrows represent dispersals out of the Oriental Region. The four major dispersal events are numbered and depicted on a corresponding maps to the left.

Table 5.2. Results of biogeographic model selection procedure for the superfamily Elapoidea in BioGeoBEARS via Akaike Information Criterion assessment of model fit

Model	LnL	numparams	d	e	j	AIC	AIC_wt
DEC	-50.09967	2	0.007683654	4.131641e-03	0.00000000	104.19935	5.338330e-03
DEC+J	-46.79114	3	0.000000100	9.974785e-03	0.04279229	99.58229	5.370157e-02
DIVALIKE	-49.76657	2	0.008592703	1.571256e-03	0.00000000	103.53315	7.448508e-03
DIVALIKE+J	-43.99488	3	0.003755548	1.000000e-12	0.03994967	93.98976	8.798100e-01
BAYAREALIKE	-62.14148	2	0.007451636	2.316035e-02	0.00000000	128.28296	3.145683e-08
BAYAREALIKE+J	-46.79114	3	0.000000100	9.974785e-03	0.04279229	99.58229	5.370157e-02

Problematic taxa

There were however, a number of taxa that still failed to associate clearly with any of the major elapid groups in our study. These included the usual suspects, such as the genus *Oxyrhabdium*, the only strictly Oriental representative of the African Lamprophiid radiation; and the clade containing the Sundaland *Calliophis*. The phylogenetic affinity of these groups have traditionally been difficult to resolve. *Oxyrhabdium*, has always represented in elapoide phylogenies by a single *O. leporinum* sample making the rounds of publications ever since it was generated by Lawson et al. (2005). According to the results of Kelly et al. (2009), *Oxyrhabdium* was placed as *incertae sedis* since it was positioned as basal to the Elapidae but outside of the African Lamprophiid radiation with weak support. Two subsequent studies (Pyron et al., 2011, 2013a) recovered it nested within the Lamprophiidae but with poor nodal support, and the authors had to maintain the *status quo* regarding it phylogenetic position. In fact, even the most recent and largest estimate of snake phylogenetic relationships (Figueroa et al., 2016) was unsuccessful at resolving the systematic affinity of *Oxyrhabdium*.

Ours is the first study to include both species of this Asian Lamprophiid genus, sequences for which were generated *de-novo* by us. Interestingly, despite of being weakly placed as sister to the elapids but outside of the Lamprophiids in RAxML and Mr. Bayes analyses, our *Beast and BEAST analyses positioned it sister to all other Lamprophiids, with inadequate posterior support in the former but high support in the latter (PP=0.98). In this context it might be worth noting that the BEAST analyses were run using a simple HKY model for all partitions due to the lack of convergence that resulted from using models recommended by PartitionFinder. It's likely that the discrepancy with the position of *Oxyrhabdium* is an artefact of the difference between models used in the BEAST and Mr. Bayes analyses. The other conceivable reason for this topological discrepancy could be the inherent differences in the manner with which both programs model molecular evolution. BEAST, for example, explicitly models the rate of evolution on each branch of the tree while also incorporating rate heterogeneity across lineages (e.g. by using the relaxed molecular clock) (Drummond and Rambaut, 2007), something that Mr. Bayes is not programed to do. Irrespective of the reasons behind the topological inconsistency of *Oxyrhabdium*, given its deep divergence and distributional distinctiveness, we believe that this genus should be placed in its own family.

The other group whose placement was hard to resolve were the Sundaland *Calliophis* species, *C. intestinalis* and *C. bivirgata*. Even though consistently cladding with the Indian *Calliophis*, their nodal support left much to be desired. It was hard to assess the monophyly of the *Calliophis* until sequences for Indian *Calliophis* species until Pyron et al. (2012) made it available. All studies thereafter consistently recovered the genus as paraphyletic with the *C. melanurus* and other Indian coralsnakes being sister to the total elapid clade, while the Sundaland *Calliophis* were nested within it (Pyron et al., 2011, 2013; Lee et al., 2016). In our analyses *Calliophis* is recovered as monophyletic, being sister to all other elapids but with inadequate nodal support. We suspect the addition of more *Calliophis* representatives from the *C. intestinalis* species complex (Smart et. al. in prep) and the inclusion of *C. gracilis* (the type for the genus that has hitherto not been included in any molecular study) may help shed light once and for all on the monophyly of *Calliophis*.

Based on data of internal and external morphology, we have strong reasons to believe that *C. gracilis*, whose phylogenetic affinity remains to be investigated with molecular data, might be highly divergent evolutionary lineage from the other *Calliophis* species of Sundaland (Smith pers com.). *Calliophis gracilis* is distinctive from all other coralsnakes in having single hemipenes (as opposed to being bi-lobed), substantially higher number of ventrals and not possessing the characteristics long venom glands found in other Sundaland coralsnakes.

The remaining inadequately resolved nodes include the placement of the Philippine taxon *Hemibungarus calligaster* which is recovered as sister to the cobras and the sister relationship between the King Cobra *Ophiophagus hannah* and the African boomslang *Dendroaspis polylepis*. This however was not unexpected since these have proven to be problematic taxa in previous studies and further work is required to investigate their taxonomic status.

Spatio-temporal evolution of basal elapids

The only study before ours to integrate information from both spatial and temporal dimensions, while inferring the evolutionary history of the Elapidae was the work by Kelly et al. (2009). Yet, as mentioned earlier, their proposals were provisionary given the lack of resolution at the base of the tree. Another study to explicitly investigate divergence time in the elapids was the recent work by Lee et al. (2016) which used four elapid fossils to constrain divergence dates within the ingroup. However, since the primary aim of their study was to infer rates of speciation and phenotypic

evolution, the authors did not deem it necessary to delve into the spatio-temporal implications of their divergence time analyses. In general, the divergence estimates of these two studies are closer to each other than either one is to our study. The temporal estimates in our study are consistently about three to four million years younger than the estimates provided by Kelly et al. (2009) and Lee et al. (2016). This is not surprising since calibrating molecular phylogenies using published literature is known to be problematic; the absence of discreet apomorphies and un-quantified variation in morphology makes taxonomic assignments questionable thus leading to highly incongruent divergence estimates (Head et al., 2016). For example, Lee et al. (2016) use the putative coralsnake fossil *Micrurus gallicus* (Rage and Holman, 1984) from the Miocene, to constrain the split between *Micrurus* and *Sinomicrurus*, possibly assuming it was an ancestral form of the Micrurus+*Sinomicrurus* clade, since it comes from Eurasia. However, given that it was described in absence of comparative material from other Asian coralsnakes (i.e. *Calliophis*) its true taxonomic affinity might deviate substantially from expectation.

Overall, the temporal sequence of dispersal out of an ancestral Laurasian distribution and into other continents/biogeographic realms is consistent with global geological and climatic history as well as some of the previous estimates (Slowinski et al., 2001; Kelly et al., 2009b). The basal most cladogenetic event in the elapids, corresponding to the invasion of the Indian subcontinent in the Oligocene at ~27 million years before present, falls well within the recent estimates of India's "hard" collision with Laurasia between 35-20 million years ago (Aitchison et al., 2007; Ali and Aitchison, 2008; Hinsbergen et al., 2012). Provided that ancestral elapids, like the majority of extant *Calliophis*, inhabited forest ecosystems, the most likely geographic source of dispersal into India would have been Sundaland. This scenario would have facilitated the dispersal of these snakes, since both India and Sundaland are purported to have had large swaths of megathermal monsoonal forests according to Oligocene paleoclimate reconstructions (Bush et al., 2011). The eventual uplifit of the Himalayas which led to the onset of a monsoonal climate and corresponding aridification, most likely contracted the distribution of a once-widespread Indian *Calliophis*, to their current range in and around the Western Ghats Mountains where ancient forests still persist to this day.

The second important dispersal involved the incursion of the Australasian region at approximately 25 million years ago. Once again, this date corresponds well with the geological record (Hall, 2012) and previous estimates of Hydrophiine origin (Kelly et al., 2009). According to tectonic

reconstructions by Hall (2012), at 25 million years before the present, the northern most extension of the Australian plate was poised to make its first contact with the Southeast Asia, at the area that currently is the island of Sulawesi. This tectonic accretion most likely let to the rapid invasion and subsequent diversification of elapids that we see in the region today.

The dispersal of elapids in to the New World, was unique in the sense that it does not seem to have involved tectonic events, but rather a change in the paleo-environment. According to paleo-reconstructions, by about 23 million years ago, right at the boundary between the Miocene and Oligocene, tropical rain forests covering Southeast Asia extended much further north as far as Southern Japan (Tsuda et al. 1984). Similarly, warm temperate forests covered northern Eurasia and North America during this time as evidenced by fossil palms and crocodylid species from northern Europe (Sill, 1968; Scotese, Paleomap Project http://www.scotese.com/climate.htm). Considering this data, it's clear that coralsnake-like elapids extended all the way into Germany and France as demonstrated by the fossil record (Rage and Holman, 1984). Based on our analyses, we conjecture that during this time, while the distribution of the basal elapids was at its widest along the east-west axis, these snakes migrated into the new world via the Bering landbridge. This persuasive proposition of invasion of the New World via the Bering landbridge at the end of the Oligocene, has in fact been invoked by several authors before us, not just for coralsnakes (Slowinski et al., 2001) but also for vipers (Alencar et al., 2016) and ratsnakes (Burbrink and Lawson, 2007; Pyron and Burbrink, 2009). One can assume that when early elapids invaded the New World, they would have been provided with tremendous diversification opportunities given the depauperate snake fauna of the region at the time (Holman, 2000); an assumption well supported by the prodigious diversity observed in extant New World elapids.

Shortly after the Asian elapids invaded the New World, a group of them also dispersed to the African continent for the first time. According to tectonic and paleontological data there were two major instances of faunal exchange between Eurasia and Africa. The initial one was during the early Miocene (between 23 and 18 Ma), when the counter clockwise rotation of Africa-Arabia lead to a collision with the Anatolian plate of Eurasia (Bernor et al., 1987; Rögl, 1999). This event was the precursor to the renowned Gomphotherium landbridge which facilitated the dispersal of large mammals between Asia and Africa (Tassy, 1990). It is during this window of time that stem cobras appear to have migrated into the African continent according to our ancestral area reconstructions.

After a temporary period of disconnection, the bridge was re-established and seems to have been continuously present since about 15 million years ago (Rögl, 1997, 1999). It is only after this (~10 million years ago) that elapids back-dispersed into the Asian continent as derived cobras and went on to attain their current diversity and distribution in the region. Also during this time (~16 million years ago) there occurred a second migration into Africa of elapids from Asia when the ancestor of *Dendroaspis* or African mambas split from a clade containing the exclusively Asian lineages of the King Cobra *Ophiopahgus hannah* and *Hemibungarus calligaster*. This setup however should be taken as provisional since the aforementioned clade suffers from poor nodal resolution and is topologically inconsistent between different tree-inference methods.

The split between Elapidae and Lamprophiidae occurred around 33 million years ago, with an apparent Asian MRCA dispersal to Africa. Despite of some historical support for this hypothesis (Cox, 2000), previous estimates (Kelly et al., 2009) and the concentration of high, extant diversity of Lamprophiides in Africa, raise some doubt about the validity of such a scenario. Moreover, given that *Oxyrhabdium* is prone to chronic topological inconsistency, the ancestral distribution for the MRCA of Elapoidea should be considered uncertain for the time being. After a hiatus of about five million years, the first cladogenetic event within the elapids began with the splitting of the Indian *Calliophis* from its congeners, triggered by the collision of the Indian and Laurasian tectonic plates. The remaining major speciation events within the family rapidly follow in the heel of this event and in a span of roughly seven million years the elapids diversified and achieved their current pan-global distribution. A modest journey that thus began in ancient Asia with possibly small and non-descript fossorial snakes, spurred on by powerful historical events, explosively culminated into a worldwide conquest of diverse habits and habitats by the charismatic elapid species we see today.

Revised higher-level elapid relationships

Though most studies since Castoe and colleagues (2007) have recovered a paraphyletic coralsnake group, no author has explicitly commented on the major taxonomic implications thereof, probably because of lack of proper resolution of basal nodes. Given that our phylogenetic tree is completely resolved at that level, we propose a classification system below that resolves taxonomic conflicts (i.e. paraphyly of the subfamily Elapinae) while accurately reflecting the current understanding of elapid evolution in space and time.

Elapoidea Boie, 1827

Elapidae Boie, 1827

subfam. nov. (Basal Indian ‘coralsnakes’) this study

gen. nov. (Bibron’s ‘coralsnakes’) this study

Calliophis bibroni (Jan 1858)

gen. nov. (Blue-tailed ‘coralsnakes’) this study

Calliophis haematoetron Smith, Manamendra-Arachchi & Somaweera, 2008

Calliophis maculicpes (Günther, 1858)

Calliophis melanurus (Shaw, 1802)

gen. nov. (Striped ‘coralsnakes’) this study

Calliophis castoe E.N. Smith, Ogale, Deepak & Giri, 2012

Calliophis nigrescens (Günther, 1862)

subfam. nov. (Basal Sundaland ‘coralsnakes’) this study

Calliophis Gray, 1834

Calliophis gracilis Gray, 1834

Maticora Laurenti, 1768

Maticora bivirgata (Stejneger, 1922)

Maticora intestinalis (Loveridge, 1944)

subfam. nov. (True coralsnakes) this study

Leptomicrurus

Micruroides K.P. Schmidt, 1928

Micrurus Wagler, 1824

Sinomicrurus Slowinski, Boundy & Lawson, 2001

Sinomicrurus boetgeri comb. nov. (Fritze, 1894)

Sinomicrurus iwasakii comb. nov. (Maki, 1935)

Sinomicrurus japonicus (Günther, 1868)

Sinomicrurus kelloggi (Pope, 1928)

Sinomicrurus cf. macclelandii 1 this study

Sinomicrurus cf. macclelandii 2 this study

Sinomicrurus cf. macclelandii this study

Sinomicrurus sauteri (Reinhardt, 1844)

Sinomicrurus swinhoei comb. nov. (Van Denburgh, 1912)

Elapinae Boie, 1827 (with modifications from Pyron and Wiens 2013)

Aspidelaps Fitzinger, 1843

Boulengerina Dollo, 1886

Bungarus Daudin, 1803

Dendroaspis Schlegel, 1848

Elapsoidea Bocage, 1866

Hemachatus Fleming, 1822

Hemibungarus Peters, 1862

Naja Laurenti, 1768

Ophiophagus Günther, 1864

Paranaja Loveridge, 1944

Pseudohaje Günther, 1858

Walterinnesia Lataste, 1887

Hydrophinae Fitzinger, 1843 (*sensu* Strickland et al., 2016)

Appendix

Supplementary Tables and Figures

Supplementary Table 1. Names and sequences of the primers used for the amplification and sequencing of gene fragments for Chapters 2-5.

Locus	Primer	Reference	Primer sequence (5' -3')
Cytb	ATRCB3	Parkinson et al. 2002	TGAGAAGTTTTCYGGGTCRTT
	GLUDG	Palumbi, 1996	TGACTTGAARAACCAYCGTTG
ND4	ND4	Arévalo et al. 1994	CACCTATGACTACCAAAAGCTCATGTAGAAGC
	Leu	Arévalo et al. 1994	CATTACTTTTACTTGGATTTGCACCA
C-mos	S77	Cox et al. 2012	CATGGACTGGGATCAGTTATG
	S78	Cox et al. 2012	CCTTGGGTGTGATTTTCTCACCT
NT3	NT3-F3	Noonan & Chippindale, 2006	ATATTTCTGGCTTTTCTCTGTGGC
	NT3-R4	Noonan & Chippindale, 2006	GCGTTTCATAAAAATATTGTTTGACCGG
RAG-1	RAG 1F - 290F	This study	TGA ATA AAA ATA GCT TGG CAR GAG AG
	RAG 1F - 2000R	This study	TTA CAA CAC AAC TCT GAA TTG GG
	RAG 1R - 1430 F	This study	TCA TCC AGC TGT TTG TTT GGC
	RAG 1R - 2700R	This study	AAA GGT CCA TTA ATT CTC TGA GGG

Supplementary Table 2. Specimen voucher and locality information for Indian *Calliophis* specimens used in Chapter 2.

Taxon Name	**Voucher/Museum #**	**Locality**
Calliophis bibroni	M816	Waynad, Kerala, India
Calliophis bibroni	M817	Trivandrum/Thiruvananthapuram, Kerala, India
Calliophis bibroni	M819	Kannur, Kerala, India
Calliophis melanurus	M367	Mumbai, Maharashara, India
Calliophis melanurus	M368	Pune, Maharashtra, India
Calliophis melanurus	RS148	Matale Dist., Dambulla, Sri Lanka
Calliophis maculiceps	M814	Langkawai Islands, Kedah, Malaysia
Calliophis maculiceps	M815	Langkawai Islands, Kedah, Malaysia
Calliophis castoe	M706	Ambe Ghat, South Goa District, Goa, India
Calliophis nigrescens khandalensis	BNHS 3475	Amboli, Maharashtra, India
Calliophis nigrescens khandalensis	M648	Goa, India
Calliophis nigrescens pentalineatus	VDEG19	Megamalia, Tamil Nadu, India
Calliophis nigrescens	M820	Waynad, Kerala, India

Supplementary Table 3. Specimen voucher and locality information for Indian *Calliophis bivirgata* specimens used in Chapter 3.

Taxon Name	**Voucher /Museum #**	**Locality**
Calliophis bivirgata flaviceps	M274	Parapat, Sumatera Utara, Sumatra, Indonesia
Calliophis bivirgata flaviceps	M530	West Malaysia
Calliophis bivirgata bivirgata	M769	Jawa Tengah, Java, Indonesia
Calliophis bivirgata tetrataenia	M832	Kubah National Park, Matang, Sarawak, Borneo, Malaysia

Supplementary Table 4. Specimen voucher and locality information for Indian *Calliophis intestinalis* specimens used in Chapter 3.

Taxon Name	**Voucher/Museum #**	**Locality**
Calliophis intestinalis	M351	Terengganu, Malaysia
Calliophis intestinalis	M352	Cameron Highlands, Malaysia
Calliophis intestinalis	M472	Malaysia
Calliophis intestinalis	M473	Malaysia
Calliophis intestinalis	M757	Aceh, Sumatra, Indonesia
Calliophis intestinalis	M761	Bogor, Java Barat, Java Indonesia
Calliophis intestinalis	M762	Bogor, Java Barat, Java Indonesia
Calliophis intestinalis	M763	Gunung Penrissen, Sarawak, Borneo, Malaysia
Calliophis intestinalis	M768	Sarawak, Borneo, Malaysia
Calliophis intestinalis	M771	Singapore
Calliophis intestinalis	M831	Kuching Peat Swamp Area, Sarawak, Borneo, Malaysia
Calliophis intestinalis	M850	Anna Rais, Kuching, Sarawak, Borneo, Malaysia
Calliophis intestinalis	M851	UNIMAS, Kuching, Sarawak, Borneo, Malaysia
Calliophis intestinalis	M861	Kecamatan Bangko, Jambi, Sumatra, Indonesia
Calliophis intestinalis	M876	Pulau Pinang, Penang, Malaysia

Supplementary Table 5. Specimen voucher and locality information for *Sinomicrurus* specimens used in Chapter 4.

Taxon Name	**Voucher/Museum #**	**Locality**
Sinomicrurus japonicus	M109	Amami, Ryukyus, Japan
Sinomicrurus japonicus japonicus	M625	Amami, Ryukyus, Japan
Sinomicrurus japonicus boettgeri	M626	Okinawa, Ryukyus, Japan
Sinomicrurus japonicus takarai	M627	Okinawa, Ryukyus, Japan
Sinomicrurus hatori	M477	Taoyuan County, Taiwan, China
Sinomicrurus hatori	M485	Taoyuan County Taiwan, China
Sinomicrurus hatori	M482	Hsinchu County, Taiwan, China
Sinomicrurus sauteri	M523	Tachia, Taiwan, China
Sinomicrurus sauteri	M502	Tachia, Taiwan, China
Sinomicrurus mcclellandii	M168	Ba Be Natioanl Park, Cao Bang, Vietnam
Sinomicrurus mcclellandii	M524	Ngatshang, Mongar District, Bhutan
Sinomicrurus mcclellandii	M454	Myanmar
Sinomicrurus mcclellandii	M605	Darlawn, Mizoram, India
Sinomicrurus mcclellandii	M536	Guangxi, China
Sinomicrurus mcclellandii	M537	Guangxi, China
Sinomicrurus mcclellandii	M538	Libo County, China
Sinomicrurus mcclellandii swinhoei	M490	Taichung, Taiwan
Sinomicrurus mcclellandii swinhoei	M495	Taiwan
Sinomicrurus mcclellandii iwaskkii	M628	Ishigaki, Ryukyus, Japan
Sinomicrurus kelloggi	M167	Chi Linh, Hia Duong, Vietnam

Supplementary Table 6. Specimen voucher and locality information for elapid species used in Chapter 5.

Taxon Name	Voucher/Museum #	Locality
Agkistrodon contortrix	UTA R-58841	U.S.A
Aspidelaps scutatus	LSUMZ 56251	Africa
Atheris nitschei	CER883	Rwanda
Atractaspis corpulenta	FN R168	-
Bungarus fasciatus	M42	-
Bungarus multicinctus	Bm9204	-
Calamaria pavimentata	ROM35605	Vietnam
Calliophis bibroni	M731	India
Calliophis bibroni	M733	India
Calliophis bivirgata	M530	West Malaysia
Calliophis intestinalis	M472	Malaysia
Calliophis melanurus	RS148	Sri Lanka
Calliophis melanurus	M368	Pune, Maharashtra, India
Calliophis nigrescens	M732	India
Crotalus ericsmithi	UTA R-55372	-
Demansia vestigiata	ABTC:11765	Australia
Dendroaspis polylepis	Unnumbered	Africa
Gloydius halys	Unnumbered	-
Hemibungarus calligaster	M197	Munic. Malinao, Luzon, Philippines
Homoroselaps lacteus	IPMB J157	-
Laticauda colubrina	CAS:HERP:220643	Indonesia
Leptomicrurus narducii	WED-54125	Peru
Lycodon subcinctus	GP2191	China
Micruroides euryxanthus	M41	Hillsborough County, Tampa, Florida, U.S.A
Micrurus corallinus	M54	Parque Nacional San Rafael, Itapua, Paraguay
Micrurus fulvius	M175	Florida, U.S.A
Micrurus lemniscatus	M56	St. Patrick, Trinidad
Micrurus multifasciatus	M46	La Mina, Distrito de Penonomé, Cocle, Panama
Micrurus surinamensis	M185	Parque Estadual Guajara-Mirim, Rondonia, Brazil
Naja kaouthia	CAS 206602	Ayeyarwady Div., Myanmar
Naja melanoleuca	CAS 207871	Equatorial Guinea
Natrix natrix	MTD:D 35933	-
Nerodia fasciata	H08208	-
Ophiophagus hannah	CAS 206601	Ayeyarwady Div., Myanmar
Oxyrahbdium leporinum	M856	Philippines
Oxyrahbdium modestum	M853	Philippines
Pelamis platurus	CMS473	Costa Rica
Sinomicrurus japonicus	KUZ R33074	Amami, Ryukyus, Japan
Sinomicrurus kelloggi	ROM37080	Chi Linh, Hia Duong, Vietnam
Sinomicrurus mcclellandii	M168	Ba Be Natioanl Park, Cao Bang, Vietnam
Sibynophis subpunctatus	RAP0491	-

Supplementary Table 7. Specimen voucher and accession numbers for elapid species used in Chapter 5 that have been previously deposited in GenBank. Loci without an accession number either don't have data or were sequenced in the current study but have not been deposited into GeneBank yet.

Taxon Name	Voucher/Museum #	Cytb	ND4	CMOS	RAG1
Aspidelaps scutatus	LSUMZ 56251		AY058969	AY058923	
Bungarus multicinctus	Bm9204	AJ565002	AJ830249		
Calamaria pavimentata	ROM35605	AF471081		AF471103	
Calliophis bivirgata	M530		KX130756		
Calliophis melanurus	RS148	KC347458	KC347502	KC347391	KC347429
Crotalus ericsmithi	UTA R-55372		KF410289		
Demansia vestigiata	ABTC:11765		EU547003	EU546911	EU546872
Homoroselaps lacteus	IPMB J157	AY611992		AY611901	
Laticauda colubrina	CAS:HERP:220643	AF217834	AY058977		
Leptomicrurus narducii	WED-54125	EF137412	EF137404	EF137420	
Lycodon subcinctus	GP2191	KC733203	KC733236	KC733220	KC733188
Micruroides euryxanthus	M41	EF137416	EF137408	EF137423	
Naja kaouthia	CAS 206602		AY058982	AY058938	
Naja melanoleuca	CAS 207871		KX130765		
Natrix natrix	MTD:D 35933	HF679955	HF679649		
Nerodia fasciata	NFASC1	KF258648	KF258631		
Ophiophagus hannah	CAS 206601		AY058984	AY058940	
Sibynophis subpunctatus	RAP0491	KC347471	KC347516	KC347449	KC347411
Sinomicrurus kelloggi	ROM37080	EF137417	EF137409	EF137424	

Supplementary Table 8. Raw genetic distances of Cytb sequences used in phylogenetic analyses, number of base substitutions per site (below diagonal) among Indian coralsnakes of the genus *Calliophis*. All codon positions were included except when containing gaps and missing data. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

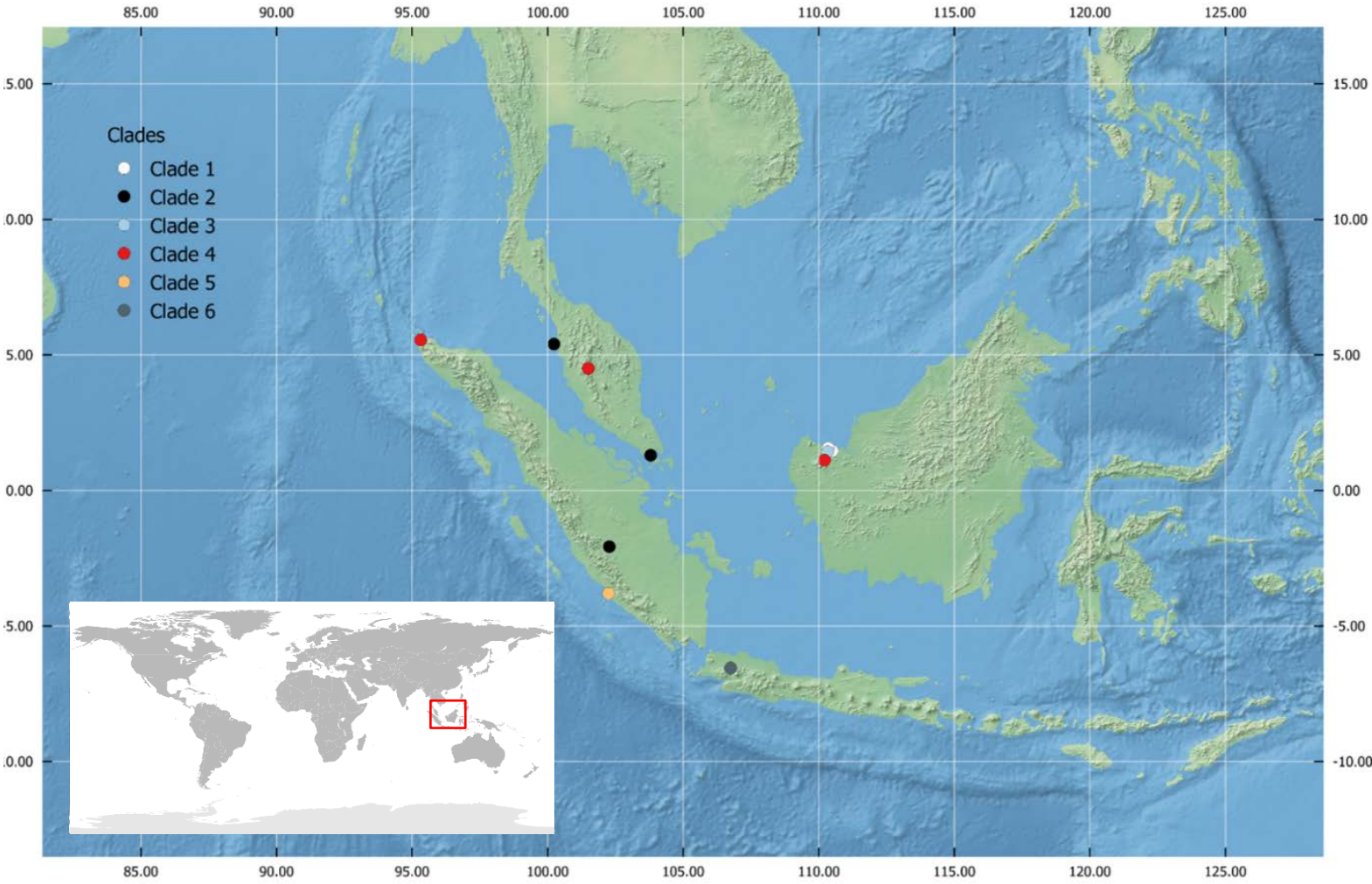
	M816 *Calliophis bibroni* Waynad	M817 *Calliophis bibroni* Trivandrum	M819 *Calliophis bibroni* Kannur	VDEG19 *Calliophis nigrescens* Megamalai	M820 *Calliophis nigrescens* Waynad	BNHS 3475 *Calliophis nigrescens khandalensis* Goa	18.M648 *Calliophis nigrescens khandalensis*	M706 *Calliophis castoe* Goa	M367 *Calliophis melanurus* Mumbai	M368 *Calliophis melanurus* Pune	RS148 *Calliophis melanurus* SriLanka	M814 *Calliophis maculiceps* Langkawi Malaysia	M815 *Calliophis maculiceps* Langkawi Malaysia
M816 *Calliophis bibroni* Waynad													
M817 *Calliophis bibroni* Trivandrum	0.006												
M819 *Calliophis bibroni* Kannur	0.002	0.005											
VDEG19 *Calliophis nigrescens* Megamalai	0.202	0.201	0.201										
M820 *Calliophis nigrescens* Waynad	0.201	0.199	0.199	0.014									
BNHS 3475 *Calliophis nigrescens khandalensis* Goa	0.210	0.206	0.209	0.044	0.045								
18.M648 *Calliophis nigrescens khandalensis*	0.202	0.201	0.201	0.020	0.006	0.051							
M706 *Calliophis castoe* Goa	0.210	0.215	0.212	0.121	0.125	0.117	0.131						
M367 *Calliophis melanurus* Mumbai	0.234	0.235	0.235	0.196	0.193	0.184	0.192	0.190					
M368 *Calliophis melanurus* Pune	0.234	0.235	0.235	0.196	0.193	0.184	0.192	0.190	0.000				
RS148 *Calliophis melanurus* SriLanka	0.217	0.217	0.215	0.182	0.181	0.179	0.181	0.188	0.087	0.087			
M814 *Calliophis maculiceps* Langkawi Malaysia	0.195	0.192	0.193	0.176	0.174	0.165	0.173	0.182	0.193	0.193	0.187		
M815 *Calliophis maculiceps* Langkawi Malaysia	0.192	0.188	0.190	0.173	0.174	0.162	0.173	0.179	0.192	0.192	0.185	0.003	

Supplementary Table 9. Raw genetic distances of Cytb sequences used in phylogenetic analyses, number of base substitutions per site (below diagonal) among Sundaland coralsnakes of the genus *Calliophis*. All codon positions were included except when containing gaps and missing data. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

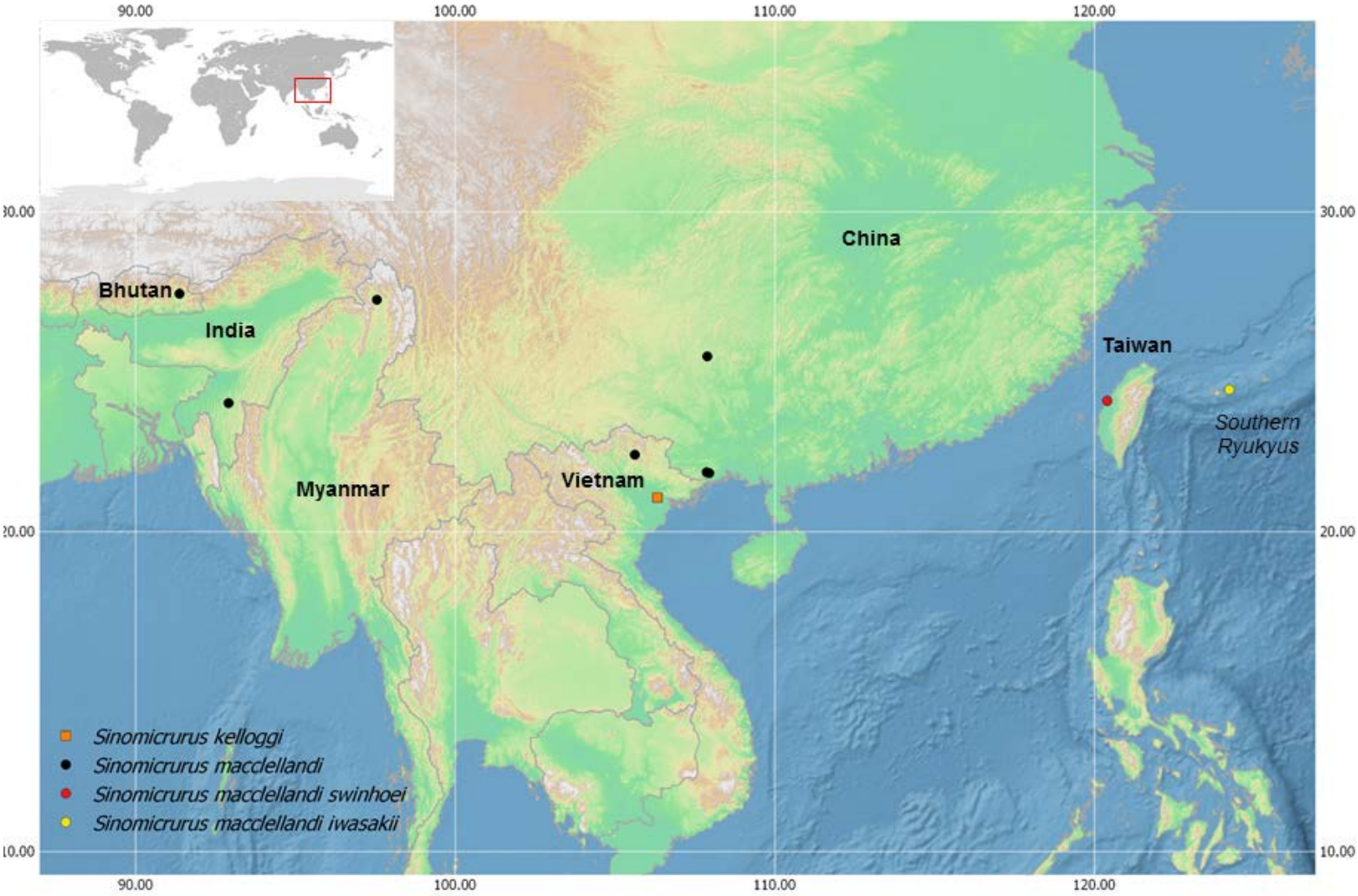
	M876_*Calliophis_intestinalis*_Penang	M472_Lowland_Malaysia	M861_Lowland_Jambi	M771_Singapore	M768_Sarawak_Malaysia	M851C_Kucing_Malay	M850C_Kucing_Malay	M757__*lineata*_Aceh_Indo	M352_Highland_Cameron_Malaysia	M473_Highland_Cameron_Malaysia	M763_Malaysia	M761_Jawa_Barat_Indo	M762_Jawa_Barat_Indo	18529_Lowland_Benkulu_Indo	M831_Sarawak
M876_*Calliophis_intestinalis*_Penang															
M472_Lowland_Malaysia	0.038														
M861_Lowland_Jambi	0.041	0.034													
M771_Singapore	0.036	0.013	0.043												
M768_Sarawak_Malaysia	0.047	0.047	0.051	0.049											
M851C_Kucing_Malay	0.047	0.047	0.051	0.049	0.000										
M850C_Kucing_Malay	0.111	0.111	0.117	0.105	0.119	0.119									
M757__*lineata*_Aceh_Indo	0.141	0.151	0.151	0.149	0.154	0.154	0.158								
M352_Highland_Cameron_Malaysia	0.147	0.156	0.154	0.154	0.156	0.156	0.162	0.024							
M473_Highland_Cameron_Malaysia	0.147	0.156	0.154	0.154	0.156	0.156	0.162	0.024	0.000						
M763_Malaysia	0.139	0.149	0.149	0.147	0.149	0.149	0.153	0.030	0.028	0.028					
M761_Jawa_Barat_Indo	0.153	0.158	0.158	0.158	0.168	0.168	0.169	0.151	0.158	0.158	0.147				
M762_Jawa_Barat_Indo	0.154	0.160	0.160	0.160	0.169	0.169	0.171	0.149	0.156	0.156	0.145	0.002			
18529_Lowland_Benkulu_Indo	0.156	0.156	0.158	0.154	0.154	0.154	0.160	0.137	0.137	0.137	0.130	0.090	0.089		
M831_Sarawak	0.047	0.047	0.051	0.049	0.000	0.000	0.119	0.154	0.156	0.156	0.149	0.168	0.169	0.154	
M862_*Calliophis_bivirgata*_West_Malaysia	0.175	0.179	0.190	0.177	0.181	0.181	0.181	0.179	0.175	0.175	0.181	0.207	0.205	0.192	0.181

Supplementary Table 10. Raw genetic distances of Cytb sequences used in phylogenetic analyses, number of base substitutions per site (below diagonal) among Sundaland coralsnakes of the genus *Calliophis*. All codon positions were included except when containing gaps and missing data. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

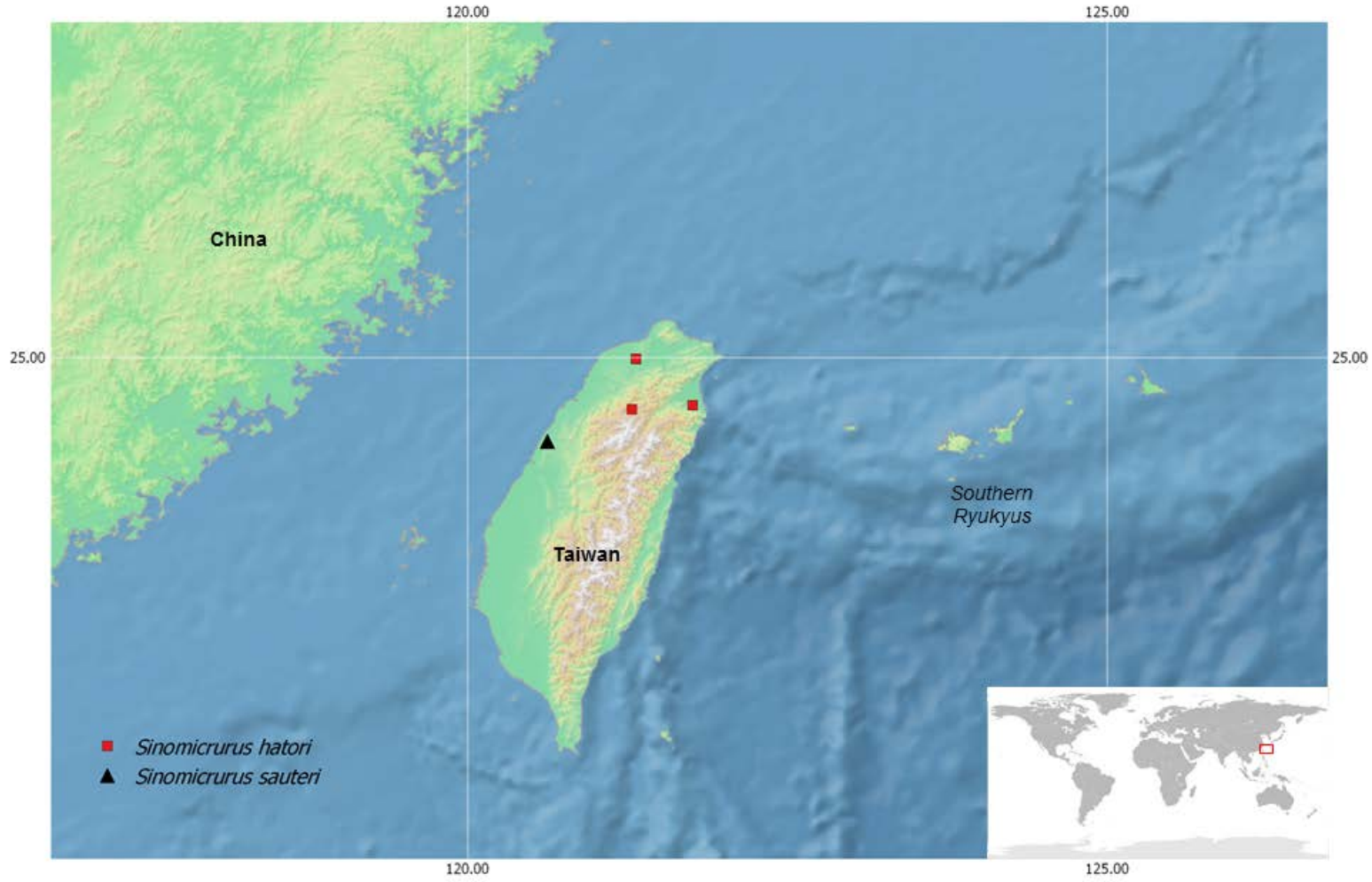
	M167 *S. kelloggi*	M605 *S. cf. macclellandi 3*	M524 *S. cf. macclellandi 3*	M454 *S. cf. macclellandi* 3	M536 *S. cf. macclellandi* 2	M537 *S. cf. macclellandi* 2	M168 *S. cf. macclellandi* 2	M538 *S. cf. macclellandi* 1	M490 *S. swinhoei*	M495 *S. swinhoei*	M628 *S. iwasakii*	M502 *S. sauteri*	M523 *S. sauteri*	M485 *S. sauteri*	M477 *S. sauteri*	M482 *S. sauteri*	M625 *S. japonicus*	M109 *S. japonicus*	M627 *S. boettgeri*	M626 *S. boettgeri*
M167 *S. kelloggi*																				
M605 *S. cf. macclellandi 3*	0.130																			
M524 *S. cf. macclellandi 3*	0.132	0.018																		
M454 *S. cf. macclellandi* 3	0.137	0.025	0.030																	
M536 *S. cf. macclellandi* 2	0.147	0.083	0.087	0.083																
M537 *S. cf. macclellandi* 2	0.147	0.083	0.087	0.083	0.000															
M168 *S. cf. macclellandi* 2	0.149	0.084	0.089	0.084	0.002	0.002														
M538 *S. cf. macclellandi* 1	0.147	0.149	0.153	0.149	0.165	0.165	0.167													
M490 *S. swinhoei*	0.147	0.135	0.134	0.137	0.149	0.149	0.150	0.046												
M495 *S. swinhoei*	0.144	0.135	0.134	0.137	0.149	0.149	0.150	0.043	0.003											
M628 *S. iwasakii*	0.144	0.134	0.139	0.137	0.147	0.147	0.149	0.054	0.069	0.069										
M502 *S. sauteri*	0.149	0.157	0.158	0.158	0.168	0.168	0.170	0.188	0.180	0.177	0.186									
M523 *S. sauteri*	0.149	0.157	0.158	0.158	0.168	0.168	0.170	0.188	0.180	0.177	0.186	0.000								
M485 *S. sauteri*	0.152	0.158	0.157	0.157	0.163	0.163	0.165	0.178	0.173	0.170	0.175	0.020	0.020							
M477 *S. sauteri*	0.145	0.158	0.157	0.157	0.163	0.163	0.165	0.185	0.177	0.173	0.182	0.018	0.018	0.008						
M482 *S. sauteri*	0.145	0.158	0.157	0.157	0.163	0.163	0.165	0.185	0.177	0.173	0.182	0.018	0.018	0.008	0.000					
M625 *S. japonicus*	0.155	0.165	0.165	0.167	0.168	0.168	0.170	0.172	0.160	0.160	0.160	0.147	0.147	0.152	0.149	0.149				
M109 *S. japonicus*	0.157	0.167	0.167	0.168	0.170	0.170	0.172	0.173	0.162	0.162	0.162	0.149	0.149	0.153	0.150	0.150	0.002			
M627 *S. boettgeri*	0.162	0.168	0.165	0.168	0.175	0.175	0.177	0.173	0.172	0.172	0.162	0.139	0.139	0.134	0.137	0.137	0.073	0.074		
M626 *S. boettgeri*	0.160	0.170	0.167	0.168	0.175	0.175	0.177	0.172	0.167	0.167	0.163	0.140	0.140	0.132	0.135	0.135	0.073	0.074	0.018	
M15 *M. fulvius*	0.198	0.195	0.196	0.205	0.196	0.196	0.198	0.221	0.215	0.211	0.224	0.205	0.205	0.203	0.205	0.205	0.205	0.206	0.210	0.200



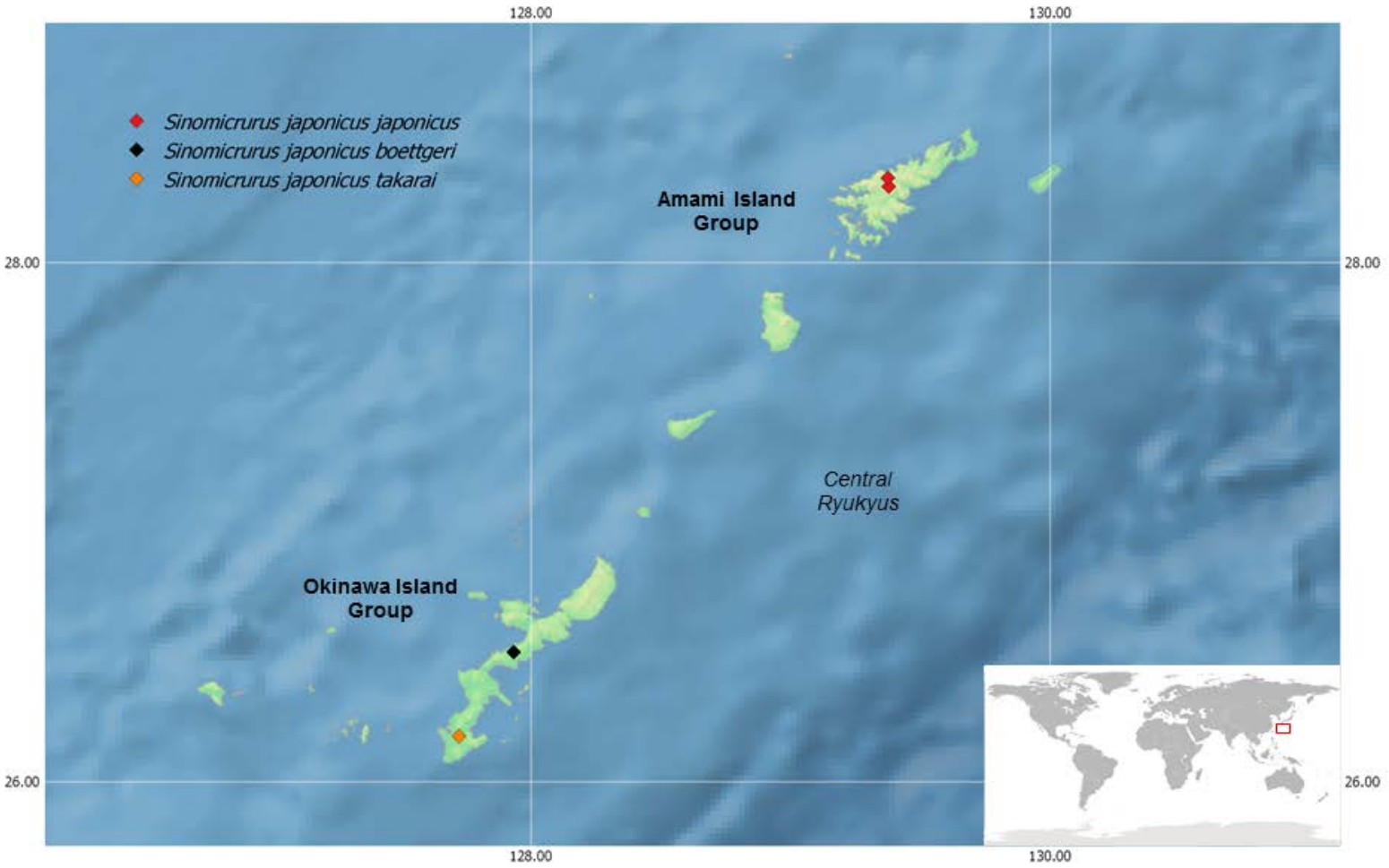
Supplementary Figure 1. Geographic distribution of *Calliophis intestinalis* samples from across Sundalanad used in Chapter 3. The samples are categorized into six broad clades based on molecular phylogenetic analyses.



Supplementary Figure 2. Geographic distribution of sampled individuals of *Sinomicrurus kelloggi, S. macclellandi swinhoei* and *S. macclellandi iwasakii* from mainland Asia and the Taiwan-Ryukyu Archipelago used in Chapter 4.



Supplementary Figure 3. Geographic distribution of sampled individuals of *Sinomicrurus hatori* and *S. sauteri* from the Taiwan-Ryukyu Archipelago used in Chapter 4.



Supplementary Figure 4. Geographic distribution of *Sinomicrurus japonicus* samples from the central Ryukyus used in Chapter 5.

Supplementary Table 11. Matrices of time-stratified dispersal multipliers for BioGeoBears analysis executed in Chapter 4. R=Central Rykyus; T=Taiwan + Southern Ryukyus; I=Indochina + Northeast India; C=Mainland China; P=Philippines; A=North America; S=Indian Subcontinent.

R	T	I	C	P	A	S
1	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
0.0000001	1	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001.5	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	1	0.0000001	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001.5	0.0000001	1	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	1

R	T	I	C	P	A	S
1	1	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
1	1	0.0000001	1	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001	0.0000001
0.0000001	1	1	1	0.0000001	0.0000001.5	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	1	0.0000001	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001.5	0.0000001	1	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	1

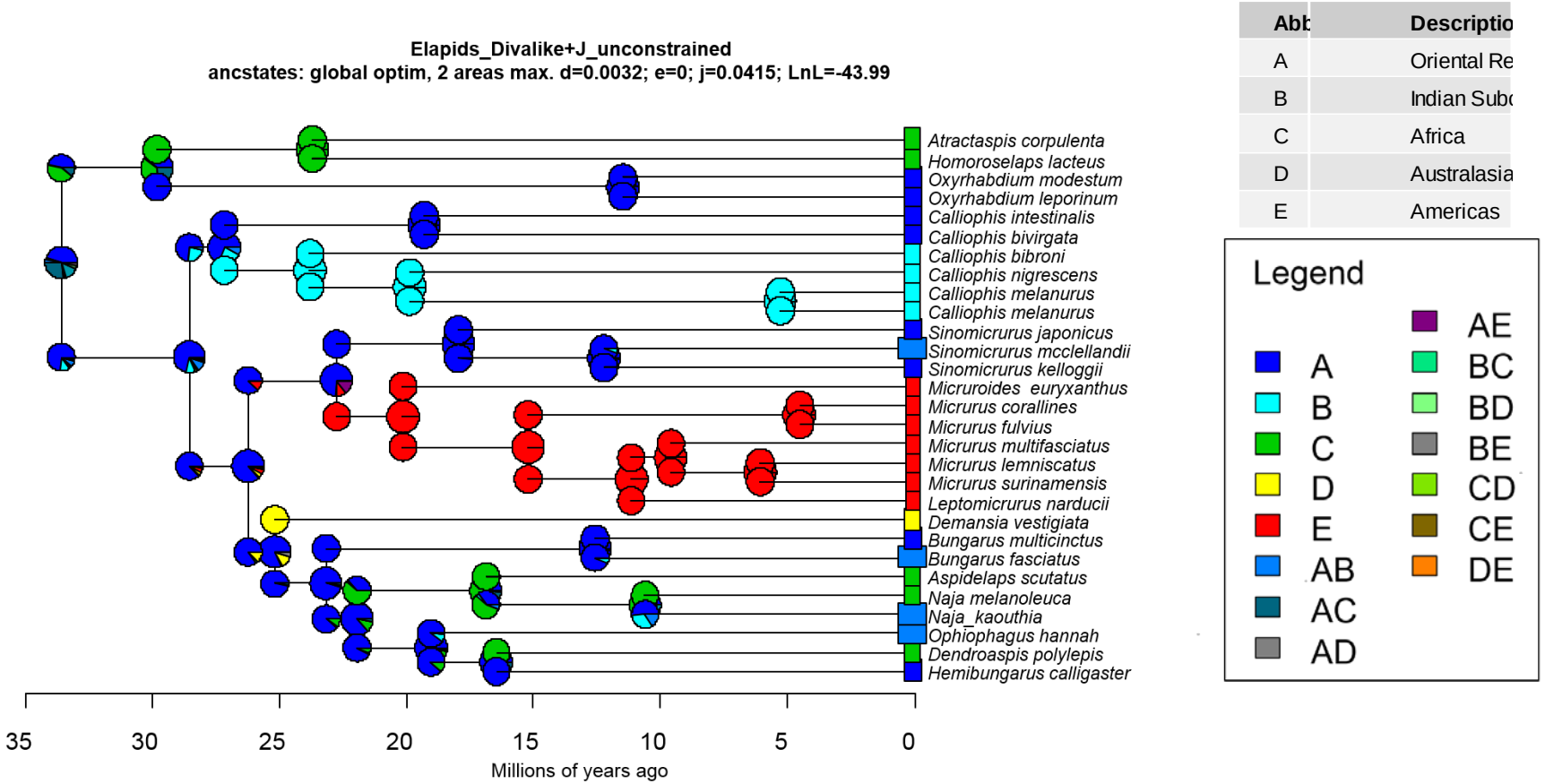
R	T	I	C	P	A	S
0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
0.0000001	1	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001.5	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	1	0.0000001	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001.5	0.0000001	1	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	1

R	T	I	C	P	A	S
1	1	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
1	1	0.0000001	1	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001.5	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	1	0.0000001	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001.5	0.0000001	1	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	1

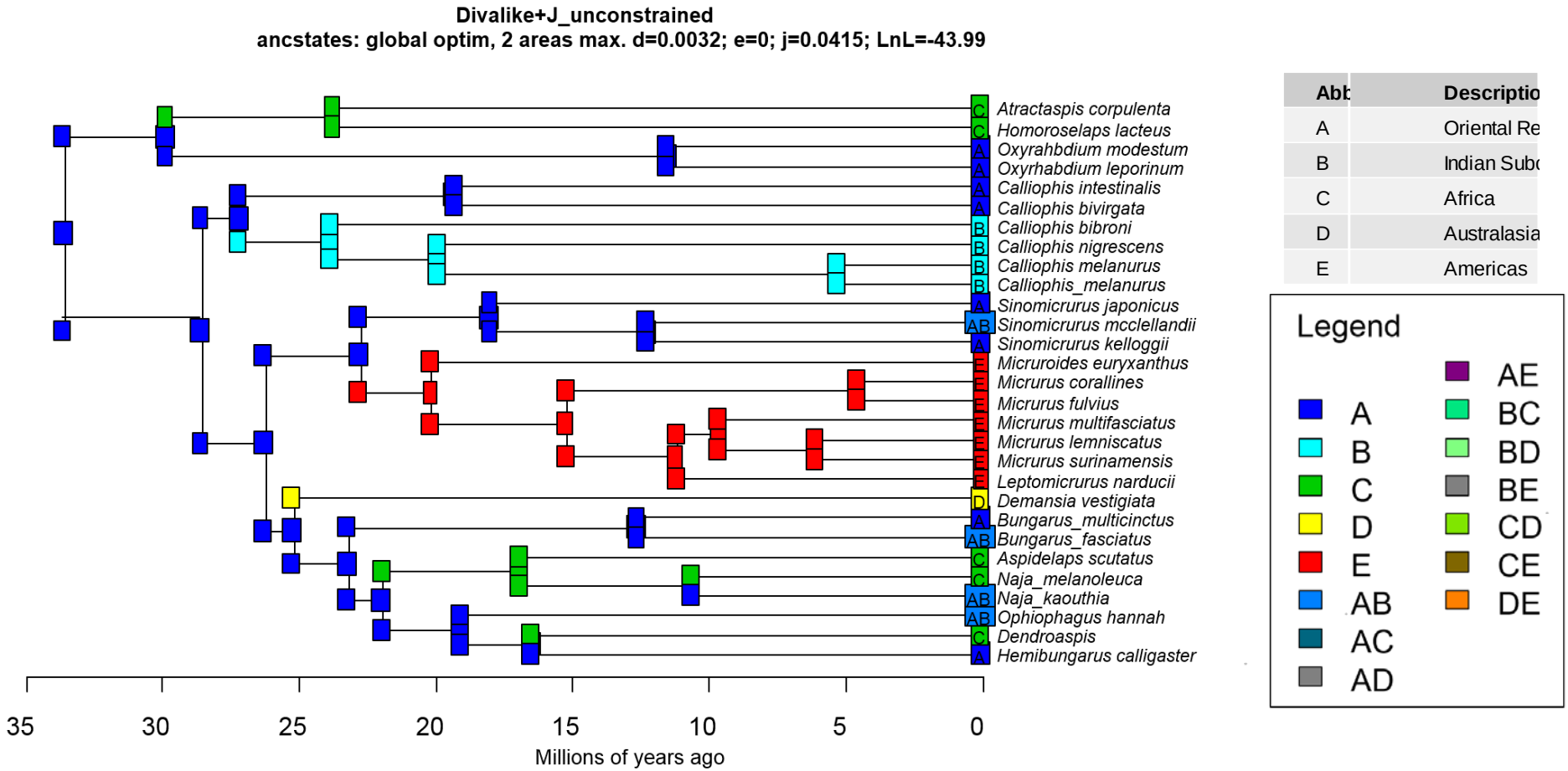
R	T	I	C	P	A	S
1	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
0.0000001	1	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001.5	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	1	0.0000001	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001.5	0.0000001	1	0.0000001
0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	1

R	T	I	C	P	A	S
0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
0.0000001	1	0.0000001	0.0000001.5	0.0000001	0.0000001	0.0000001
0.0000001	0.0000001	1	1	0.0000001	0.0000001	0.0000001

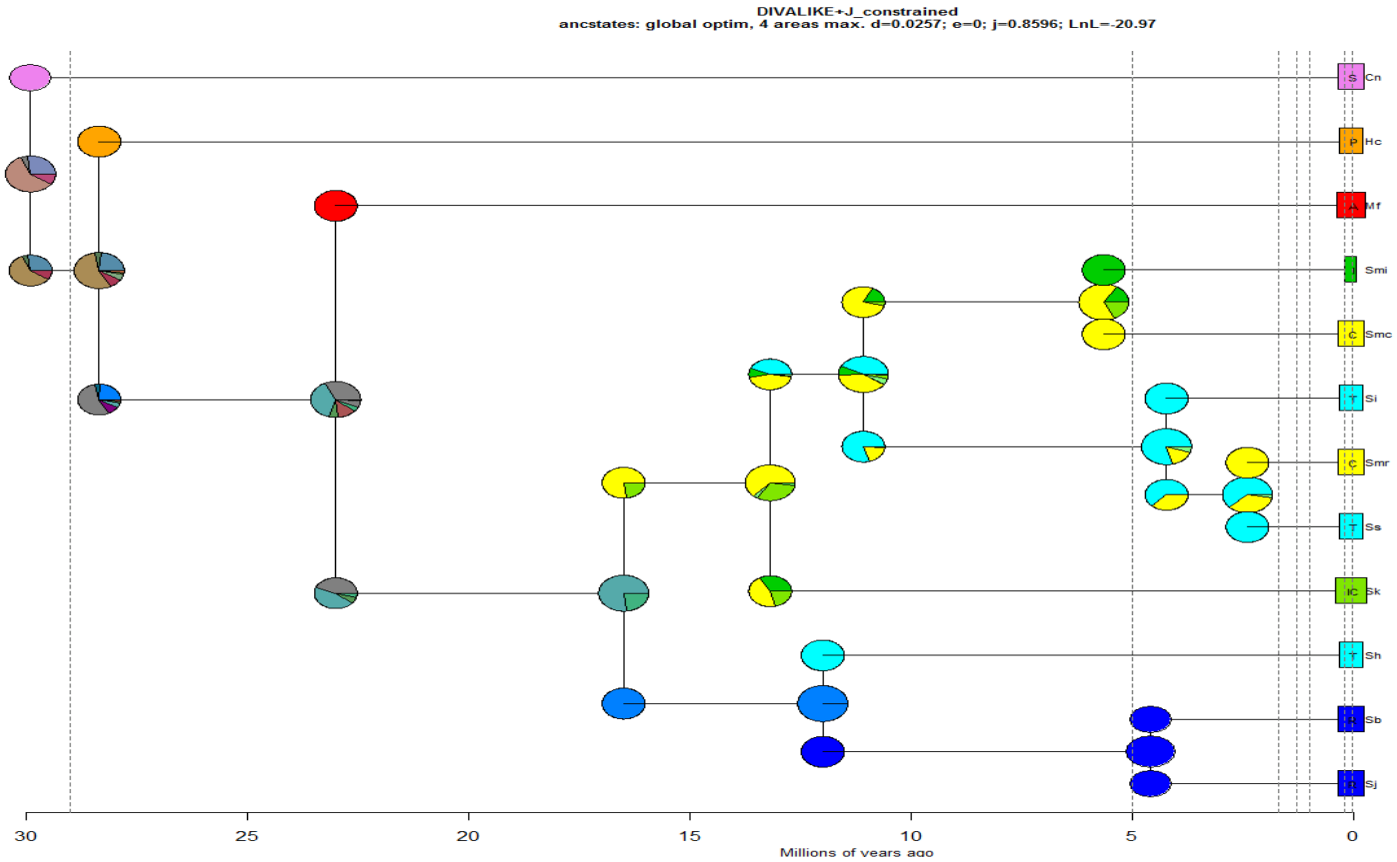
```
0.0000001    0.0000001.5    1    1    0.0000001    0.0000001.5    0.0000001
0.0000001    0.0000001    0.0000001    0.0000001    1    0.0000001    0.0000001
0.0000001    0.0000001    0.0000001    0.0000001.5    0.0000001    1    0.0000001
0.0000001    0.0000001    0.0000001    0.0000001    0.0000001    0.0000001    1

R    T    I    C    P    A    S
0.0000001    0.0000001    0.0000001    0.0000001    0.0000001    0.0000001
    0.0000001
0.0000001    1    0.0000001    0.0000001.5    0.0000001    0.0000001    0.0000001
0.0000001    0.0000001    1    1    0.0000001    0.0000001    0.0000001
0.0000001    0.0000001.5    1    1    0.0000001    0.0000001.5    0.0000001
0.0000001    0.0000001    0.0000001    0.0000001    1    0.0000001    0.0000001
0.0000001    0.0000001    0.0000001    0.0000001.5    0.0000001    1    0.0000001
0.0000001    0.0000001    0.0000001    0.0000001    0.0000001    0.0000001    1

END
```



Supplementary Figure 5. Reconstruction of ancestral ranges as provided by the best-fit biogeographical model (Divalike+J) in the BioGeoBEARS analyses in Chapter 5. Pie charts depict the relative probability of ancestral ranges. d=dispersal rate/unit time; e=extinction rate/unit time; j=jump dispersal rates/unit time; LnL=log likelihood value of model.



Supplementary Figure 6. Reconstruction of ancestral ranges as provided by the best-fit biogeographical model (Divalike+J) in the BioGeoBEARS analyses. Colored squares at nodes depict most likely ancestral range. d=dispersal rate/unit time; e=extinction rate/unit time; j=jump dispersal rates/unit time; LnL=log likelihood value



Supplementary Figure 7. Reconstruction of ancestral ranges as provided by the best-fit biogeographical model (Divalike+J) in the BioGeoBEARS analyses in Chapter 4. Pie charts depict the relative probability of ancestral ranges. d=dispersal rate/unit time; e=extinction rate/unit time; j=jump dispersal rates/unit time; LnL=log likelihood value of model; R=Central Rykyus; T=Taiwan + Southern Ryukyus; I=Indochina + Northeast India; C=Mainland China; P=Philippines; A=North America; S=Indian Subcontinent.

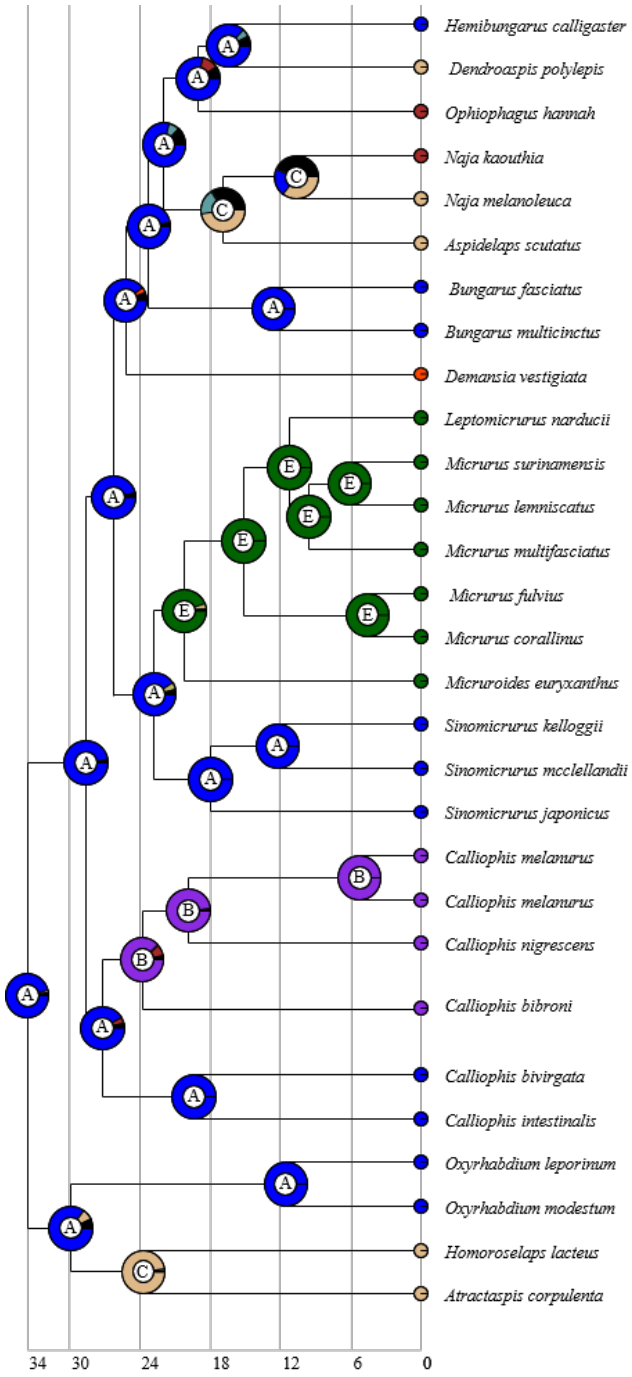
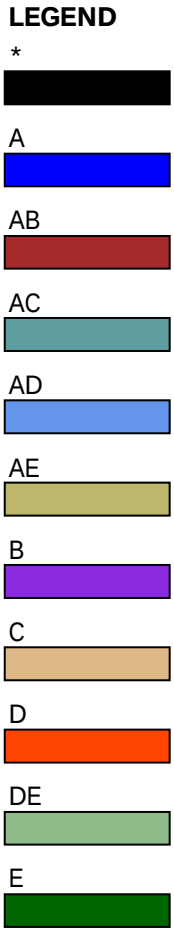


Abb	Descriptio
A	Oriental Re
B	Indian Subc
C	Africa
D	Australasia
E	Americas



Supplementary Figure 8. Reconstruction of ancestral ranges as provided by BBM for the biogeographical analyses in Chapter 5. Pie charts depict the relative probability of ancestral ranges.

References

Aitchison, J.C., Ali, J.R., and Davis, A.M. (2007). When and where did India and Asia collide? J. Geophys. Res. Solid Earth *112*, B05423.

Alencar, L.R.V., Quental, T.B., Grazziotin, F.G., Alfaro, M.L., Martins, M., Venzon, M., and Zaher, H. (2016). Diversification in vipers: Phylogenetic relationships, time of divergence and shifts in speciation rates. Mol. Phylogenet. Evol. *105*, 50–62.

Alfaro, M.E., Zoller, S., and Lutzoni, F. (2003). Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol. Biol. Evol. *20*, 255–266.

Ali, J.R., and Aitchison, J.C. (2008). Gondwana to Asia: Plate tectonics, paleogeography and the biological connectivity of the Indian sub-continent from the Middle Jurassic through latest Eocene (166–35 Ma). Earth-Sci. Rev. *88*, 145–166.

Arèvalo, E., Davis, S.K., and Sites, J.W. (1994). Mitochondrial DNA Sequence Divergence and Phylogenetic Relationships among Eight Chromosome Races of the Sceloporus Grammicus Complex (Phrynosomatidae) in Central Mexico. Syst. Biol. *43*, 387–418.

Bernor, R.L., Brunet, M., Ginsburg, L., Mein, P., Pickford, M., Rögl, F., Sen, S., Steininger, F., and Thomas, H. (1987). A consideration of some major topics concerning Old WorldMiocene Mammalian chronology, migrations and paleogeography. Geobios *20*, 431–439.

Bouckaert, R.R. (2010). DensiTree: making sense of sets of phylogenetic trees. Bioinformatics *26*, 1372–1373.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., and Drummond, A.J. (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Comput Biol *10*, e1003537.

Burbrink, F.T., and Lawson, R. (2007). How and when did Old World ratsnakes disperse into the New World? Mol. Phylogenet. Evol. *43*, 173–189.

Bush, M., Flenley, J., and Gosling, W. (2011). Tropical rainforest responses to climatic change (Springer Science & Business Media).

Castoe, T.A., Smith, E.N., Brown, R.M., and Parkinson, C.L. (2007). Higher-level phylogeny of Asian and American coralsnakes, their placement within the Elapidae (Squamata), and the systematic affinities of the enigmatic Asian coralsnake Hemibungarus calligaster (Wiegmann, 1834). Zool. J. Linn. Soc. *151*, 809–831.

Cox, C.B. (2000). Plate tectonics, seaways and climate in the historical biogeography of mammals. Mem. Inst. Oswaldo Cruz *95*, 509–516.

Das, I. (2010). The Field Guide to the Reptiles of Thailand & South-East Asia (Asia Books).

Deepak, V., Harikrishnan, S., Vasudevan, K., and Smith, E.N. (2010). Redescription of Bibron's coralsnake, *Calliophis bibroni* Jan 1858 with notes and new records from south of the Palghat and Shencottah gaps of the Western Ghats, India. Hamadryad *35*, 1–10.

Drummond, A.J., and Bouckaert, R.R. (2015). Bayesian evolutionary analysis with BEAST (Cambridge University Press).

Drummond, A.J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. *7*, 214.

Figueroa, A., McKelvy, A.D., Grismer, L.L., Bell, C.D., and Lailvaux, S.P. (2016). A Species-Level Phylogeny of Extant Snakes with Description of a New Colubrid Subfamily and Genus. PLOS ONE *11*, e0161070.

Ganesh, S.R., and Ramanujam, E. (2014). Rediscovery of Beddome's coralsnake *Calliophis* beddomei Smith, 1943 from the type locality. J. Threat. Taxa *6*, 5580–5582.

Hall, R. (1996). Reconstructing Cenozoic SE Asia. Geol. Soc. Lond. Spec. Publ. *106*, 153–184.

Hall, R. (2012). Late Jurassic–Cenozoic reconstructions of the Indonesian region and the Indian Ocean. Tectonophysics *570–571*, 1–41.

Head, J.J., Mahlow, K., and Müller, J. (2016). Fossil calibration dates for molecular phylogenetic analysis of snakes 2: Caenophidia, Colubroidea, Elapoidea, Colubridae. Palaeontol. Electron. *19*, 1–21.

Heath, T.A., Huelsenbeck, J.P., and Stadler, T. (2014). The fossilized birth–death process for coherent calibration of divergence-time estimates. Proc. Natl. Acad. Sci. *111*, E2957–E2966.

Hillis, D.M., and Bull, J.J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. *42*, 182–192.

Hinsbergen, D.J.J. van, Lippert, P.C., Dupont-Nivet, G., McQuarrie, N., Doubrovine, P.V., Spakman, W., and Torsvik, T.H. (2012). Greater India Basin hypothesis and a two-stage Cenozoic collision between India and Asia. Proc. Natl. Acad. Sci. *109*, 7659–7664.

Holman, J.A. (2000). Fossil snakes of North America: origin, evolution, distribution, paleoecology (Indiana University Press).

Honda, M., Matsui, M., Tominaga, A., Ota, H., and Tanaka, S. (2012). Phylogeny and biogeography of the Anderson's crocodile newt, Echinotriton andersoni (Amphibia: Caudata), as revealed by mitochondrial DNA sequences. Mol. Phylogenet. Evol. *65*, 642–653.

Honda, M., Kurita, T., Toda, M., and Ota, H. (2014). Phylogenetic Relationships, Genetic Divergence, Historical Biogeography and Conservation of an Endangered Gecko, Goniurosaurus kuroiwae (Squamata: Eublepharidae), from the Central Ryukyus, Japan. Zoolog. Sci. *31*, 309–320.

Huang, C.-Y., Yuan, P.B., and Tsao, S.-J. (2006). Temporal and spatial records of active arc-continent collision in Taiwan: A synthesis. Geol. Soc. Am. Bull. *118*, 274–288.

Huang, C.-Y., Yen, Y., Zhao, Q., and Lin, C.-T. (2012). Cenozoic stratigraphy of Taiwan: Window into rifting, stratigraphy and paleoceanography of South China Sea. Chin. Sci. Bull. *57*, 3130–3149.

Huelsenbeck, J.P., and Rannala, B. (2004). Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Syst. Biol. *53*, 904–913.

Jones, G.R. (2014). STACEY: species delimitation and phylogeny estimation under the multispecies coalescent. bioRxiv 010199.

Jones, G., Aydin, Z., and Oxelman, B. (2015). DISSECT: an assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. Bioinformatics *31*, 991–998.

Kaito, T., and Toda, M. (2016). The biogeographical history of Asian keelback snakes of the genus Hebius (Squamata: Colubridae: Natricinae) in the Ryukyu Archipelago, Japan. Biol. J. Linn. Soc. n/a-n/a.

Kelly, C.M., Barker, N.P., Villet, M.H., and Broadley, D.G. (2009). Phylogeny, biogeography and classification of the snake superfamily Elapoidea: a rapid radiation in the late Eocene. Cladistics *25*, 38–63.

Keogh, J.S. (1998). Molecular phylogeny of elapid snakes and a consideration of their biogeographic history. Biol. J. Linn. Soc. *63*, 177–203.

Kimura, M. (2000). Paleogeography of the Ryukyu Islands. Tropics *10*, 5–24.

Kizaki, K., and Oshiro, I. (1977). Paleogeography of the Ryukyu Islands. Mar. Sci. Mon. *9*, 542–549.

Kumazawa, Y., and Nishida, M. (1993). Sequence evolution of mitochondrial tRNA genes and deep-branch animal phylogenetics. J. Mol. Evol. *37*, 380–398.

Landis, M.J., Matzke, N.J., Moore, B.R., and Huelsenbeck, J.P. (2013). Bayesian Analysis of Biogeography when the Number of Areas is Large. Syst. Biol. syt040.

Lanfear, R., Calcott, B., Ho, S.Y., and Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. *29*, 1695–1701.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., and Lopez, R. (2007). Clustal W and Clustal X version 2.0. Bioinformatics *23*, 2947–2948.

Lawson, R., Slowinski, J.B., Crother, B.I., and Burbrink, F.T. (2005). Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes. Mol. Phylogenet. Evol. *37*, 581–601.

Lee, M.S.Y., Sanders, K.L., King, B., and Palci, A. (2016). Diversification rates and phenotypic evolution in venomous snakes (Elapidae). R. Soc. Open Sci. *3*, 150277.

Lukoschek, V., and Keogh, J.S. (2006). Molecular phylogeny of sea snakes reveals a rapidly diverged adaptive radiation. Biol. J. Linn. Soc. *89*, 523–539.

Lukoschek, V., Keogh, J.S., and Avise, J.C. (2012). Evaluating Fossil Calibrations for Dating Phylogenies in Light of Rates of Molecular Evolution: A Comparison of Three Approaches. Syst. Biol. *61*, 22–43.

Manthey, U., and Grossmann, W. (1997). Amphibien & Reptilien Südostasiens (Natur-und-Tier-Verlag).

Matsui, M., Shimada, T., Ota, H., and Tanaka-Ueno, T. (2005). Multiple invasions of the Ryukyu Archipelago by Oriental frogs of the subgenus Odorrana with phylogenetic reassessment of the related subgenera of the genus Rana. Mol. Phylogenet. Evol. *37*, 733–742.

Matzke, N.J. (2013a). BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R Scripts. R Package Version 02 *1*.

Matzke, N.J. (2013b). Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing (University of California, Berkeley).

Matzke, N.J. (2013c). BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R Scripts. R Package Version 02 *1*.

Matzke, N.J. (2014). Model Selection in Historical Biogeography Reveals that Founder-event Speciation is a Crucial Process in Island Clades. Syst. Biol. syu056.

McDowell, S.B. (1969). Toxicocalamus, a New Guinea genus of snakes of the family Elapidae. J. Zool. *159*, 443–511.

McDowell, S.B. (1970). On the status and relationships of the Solomon Island elapid snakes. J. Zool. *161*, 145–190.

McDowell, S.B. (1986). The Architecture of the Corner of the Mouth of Colubroid Snakes. J. Herpetol. *20*, 353–407.

McDowell, S.B., and Cogger, H.G. (1967). Aspidomorphus, a genus of New Guinea snakes of the family Elapidae, with notes on related genera. J. Zool. *151*, 497–543.

Mengden, G.A. (1985). Australian elapid phylogeny: A summary of the chromosomal and electrophoretic data. Biol. Australas. Frogs Reptil. 185–192.

Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Gateway Computing Environments Workshop (GCE), 2010, pp. 1–8.

Moyle, R.G. (2005). Phylogeny and biogeographical history of Trogoniformes, a pantropical bird order. Biol. J. Linn. Soc. *84*, 725–738.

Moyle, R.G., Taylor, S.S., Oliveros, C.H., Lim, H.C., Haines, C.L., Rahman, M.A., and Sheldon, F.H. (2011). Diversification of an Endemic Southeast Asian Genus: Phylogenetic Relationships of the Spiderhunters (Nectariniidae: Arachnothera). The Auk *128*, 777–788.

Mulcahy, D.G., Beckstead, T.H., and Sites Jr, J.W. (2011). Molecular systematics of the Leptodeirini (Colubroidea: Dipsadidae) revisited: species-tree analyses and multi-locus data. Copeia *2011*, 407–417.

Nabhan, A.R., and Sarkar, I.N. (2012). The impact of taxon sampling on phylogenetic inference: a review of two decades of controversy. Brief. Bioinform. *13*, 122–134.

Osozawa, S., Shinjo, R., Armid, A., Watanabe, Y., Horiguchi, T., and Wakabayashi, J. (2012). Palaeogeographic reconstruction of the 1.55 Ma synchronous isolation of the Ryukyu Islands, Japan, and Taiwan and inflow of the Kuroshio warm current. Int. Geol. Rev. *54*, 1369–1388.

Ota, H. (1998). Geographic patterns of endemism and speciation in amphibians and reptiles of the Ryukyu Archipelago, Japan, with special reference to their paleogeographical implications. Res. Popul. Ecol. *40*, 189–204.

Ota, H., Ito, A., and Lin, J.-T. (1999). Systematic Review of the Elapid Snakes Allied to Hemibungarus *japonicus* (Günther, 1868) in the East Asian Islands, with Description of a New Subspecies from the Central Ryukyus. J. Herpetol. *33*, 675–687.

Parkinson, C.L., Campbell, J.A., Chippindale, P.T., and Schuett, G.W. (2002). Multigene phylogenetic analysis of pitvipers, with comments on their biogeography. Biol. Vipers *9*, 3–110.

Peters, W. (1871). Über neue Reptilien aus Ostafrica und Sarawak (Borneo), vorzüglich aus der Sammlung des Hrn. Marquis J. Doria zu Genua. Monatsbericht K. Akad. Wiss. Zu Berl. *1871*, 566–581.

Pollock, D.D., Zwickl, D.J., McGuire, J.A., and Hillis, D.M. (2002). Increased Taxon Sampling Is Advantageous for Phylogenetic Inference. Syst. Biol. *51*, 664–671.

Pyron, R.A., and Burbrink, F.T. (2009). Neogene diversification and taxonomic stability in the snake tribe Lampropeltini (Serpentes: Colubridae). Mol. Phylogenet. Evol. *52*, 524–529.

Pyron, R.A., Burbrink, F.T., Colli, G.R., de Oca, A.N.M., Vitt, L.J., Kuczynski, C.A., and Wiens, J.J. (2011). The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. Mol. Phylogenet. Evol. *58*, 329–342.

Pyron, R.A., Burbrink, F.T., and Wiens, J.J. (2013a). A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evol. Biol. *13*, 1–53.

Pyron, R.A., Kandambi, H.K.D., Hendry, C.R., Pushpamal, V., Burbrink, F.T., and Somaweera, R. (2013b). Genus-level phylogeny of snakes reveals the origins of species richness in Sri Lanka. Mol. Phylogenet. Evol. *66*, 969–978.

Rage, J.-C., and Holman, J.A. (1984). Des serpents (Reptilia, Squamata) de type nord-américain dans le Miocène Français. Évolution parallèle ou dispersion? Geobios *17*, 89–104.

Rambaut, A. (2007). FigTree, a graphical viewer of phylogenetic trees. See Httptree Bio Ed Ac Uksoftwarefigtree.

Rannala, B., and Yang, Z. (2013). Improved reversible jump algorithms for Bayesian species delimitation. Genetics *194*, 245–253.

Rannala, B., Huelsenbeck, J.P., Yang, Z., and Nielsen, R. (1998). Taxon Sampling and the Accuracy of Large Phylogenies. Syst. Biol. *47*, 702–710.

Ree, R.H., and Smith, S.A. (2008). Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. Syst. Biol. *57*, 4–14.

Reyes-Velasco, J., Meik, J.M., Smith, E.N., and *Castoe*, T.A. (2013). Phylogenetic relationships of the enigmatic longtailed rattlesnakes (Crotalus ericsmithi, C. lannomi, and C. stejnegeri). Mol. Phylogenet. Evol. *69*, 524–534.

Rögl, F. (1997). Palaeogeographic considerations for Mediterranean and Paratethys seaways (Oligocene to Miocene). Ann. Naturhistorischen Mus. Wien Ser. Für Mineral. Petrogr. Geol. Paläontol. Anthropol. Prähistorie 279–310.

Rögl, F. (1999). Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). Geol. Carpathica *50*, 339–349.

Rohland, N., and Reich, D. (2012). Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. Genome Res. *22*, 939–946.

Ronquist, F. (1997). Dispersal-Vicariance Analysis: A New Approach to the Quantification of Historical Biogeography. Syst. Biol. *46*, 195–203.

Ronquist, F., and Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics *19*, 1572–1574.

Scanlon, J.D., and Lee, M.S.Y. (2004a). Phylogeny of Australasian venomous snakes (Colubroidea, Elapidae, Hydrophiinae) based on phenotypic and molecular evidence. Zool. Scr. *33*, 335–366.

Scanlon, J.D., Lee, M.S.Y., and Archer, M. (2003). Mid-Tertiary elapid snakes (Squamata, Colubroidea) from Riversleigh, northern Australia: early steps in a continent-wide adaptive radiation. Geobios *36*, 573–601.

Schwaner, T.D., Baverstock, P.R., Dessauer, H.C., and Mengden, G.A. (1985). Immunological evidence for the phylogenetic relationships of Australian elapid snakes. Biol. Australas. Frogs Reptil. 177–184.

Sheldon, F.H., Moyle, R.G., and Kennard, J. (2001). Ornithology of Sabah: history, gazetteer, annotated checklist, and bibliography (American Ornithologists' Union).

Sheldon, F.H., Lim, H.C., Nais, J., Lakim, M., Tuuga, A., Malim, P., Majuakim, J., Lo, A., Schilthuizen, M., and Hosner, P.A. (2009). Observations on the ecology, distribution, and biogeography of forest birds in Sabah, Malaysia. Raffles Bull. Zool. *57*, 577–586.

Sibuet, J.-C., Hsu, S.-K., Shyu, C.-T., and Liu, C.-S. (1995). Structural and Kinematic Evolutions of the Okinawa Trough Backarc Basin. In Backarc Basins, B. Taylor, ed. (Springer US), pp. 343–379.

Sill, W.D. (1968). The Zoogeography of the Crocodilia. Copeia *1968*, 76–88.

Slowinski, J.B. (1995). A Phylogenetic Analysis of the New World Coral Snakes (Elapidae: Leptomicrurus, Micruroides, and Micrurus) Based on Allozymic and Morphological Characters. J. Herpetol. *29*, 325–338.

Slowinski, J.B., and Keogh, J.S. (2000). Phylogenetic Relationships of Elapid Snakes Based on Cytochrome b mtDNA Sequences. Mol. Phylogenet. Evol. *15*, 157–164.

Slowinski, J.B., and Lawson, R. (2002). Snake phylogeny: evidence from nuclear and mitochondrial genes. Mol. Phylogenet. Evol. *24*, 194–202.

Slowinski, J.B., Knight, A., and Rooney, A.P. (1997). Inferring Species Trees from Gene Trees: A Phylogenetic Analysis of the Elapidae (Serpentes) Based on the Amino Acid Sequences of Venom Proteins. Mol. Phylogenet. Evol. *8*, 349–362.

Slowinski, J.B., Boundy, J., and Lawson, R. (2001). The Phylogenetic Relationships of Asian Coral Snakes (Elapidae: *Calliophis* and Maticora) Based on Morphological and Molecular Characters. Herpetologica *57*, 233–245.

Smith, M.A. (1943). The Fauna of British India. Reptilia and Amphibia. Vol. 3. Serpentes. Taylor Fran-Cis Lond. 402.

Smith, E.N., Manamendra-Arachchi, K., and Somaweera, R. (2008). A new species of coralsnake of the genus *Calliophis* (Squamata: Elapidae) from the Central Province of Sri Lanka. Zootaxa 19–33.

Smith, E.N., Ogale, H., Deepak, V., and Giri, V.B. (2012). A new species of coralsnake of the genus *Calliophis* (Squamata: Elapidae) from the west coast of peninsular India. Zootaxa *3437*, 51–68.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics *22*, 2688–2690.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics *30*, 1312–1313.

Strickland, J.L., Carter, S., Kraus, F., and Parkinson, C.L. (2016). Snake evolution in Melanesia: origin of the Hydrophiinae (Serpentes, Elapidae), and the evolutionary history of the enigmatic New Guinean elapid Toxicocalamus. Zool. J. Linn. Soc.

Su, Y.-C., Brown, R.M., Chang, Y.-H., Lin, C.-P., and Tso, I.-M. (2016). Did a Miocene–Pliocene island isolation sequence structure diversification of funnel web spiders in the Taiwan-Ryukyu Archipelago? J. Biogeogr. n/a-n/a.

Szyndlar, Z., and Rage, J.-C. (1990). West Palearctic cobras of the genus Naja (Serpentes: Elapidae): interrelationships among extinct and extant species. Amphib.-Reptil. *11*, 385–400.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. *28*, 2731–2739.

Tassy, P. (1990). The "Proboscidean Datum Event:" How Many Proboscideans and How Many Events? In European Neogene Mammal Chronology, (Springer), pp. 237–252.

Vidal, N., Delmas, A.-S., and Hedges, S.B. (2007a). The higher-level relationships of alethinophidian snakes inferred from seven nuclear and mitochondrial genes. Biol. Boas Pythons 27–33.

Vidal, N., Delmas, A.-S., David, P., Cruaud, C., Couloux, A., and Hedges, S.B. (2007b). The phylogeny and classification of caenophidian snakes inferred from seven nuclear protein-coding genes. C. R. Biol. *330*, 182–187.

Vogel, G. (2006). Venomous snakes of Asia (Edition Chimaira).

Whitaker, R., Captain, A., and Ahmed, F. (2004). Snakes of India (Draco Books).

Wüster, W., Crookes, S., Ineich, I., Mané, Y., Pook, C.E., Trape, J.-F., and Broadley, D.G. (2007). The phylogeny of cobras inferred from mitochondrial DNA sequences: Evolution of venom spitting and the phylogeography of the African spitting cobras (Serpentes: Elapidae: Naja nigricollis complex). Mol. Phylogenet. Evol. *45*, 437–453.

Xu, X., Liu, F., Chen, J., Ono, H., Agnarsson, I., Li, D., and Kuntner, M. (2016). Pre-Pleistocene geological events shaping diversification and distribution of primitively segmented spiders on East Asian margins. J. Biogeogr. n/a-n/a.

Yang, Z. (2015). The BPP program for species tree estimation and species delimitation. Curr. Zool. *61*, 854–865.

Yang, Z., and Rannala, B. (2014). Unguided species delimitation using DNA sequence data from multiple loci. Mol. Biol. Evol. msu279.

Yu, Y., Harris, A.J., Blair, C., and He, X. (2015). RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. Mol. Phylogenet. Evol. *87*, 46–49.

Zaher, H., Grazziotin, F.G., Cadle, J.E., Murphy, R.W., Moura-Leite, J.C. de, and Bonatto, S.L. (2009). Molecular phylogeny of advanced snakes (Serpentes, Caenophidia) with an emphasis on South American Xenodontines: a revised classification and descriptions of new taxa. Papéis Avulsos Zool. São Paulo *49*, 115–153.

Zhang, C., Zhang, D.-X., Zhu, T., and Yang, Z. (2011). Evaluation of a Bayesian coalescent method of species delimitation. Syst. Biol. syr071.

Zhang, J., Kapli, P., Pavlidis, P., and Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. Bioinformatics *29*, 2869–2876.

Zwickl, D.J., and Hillis, D.M. (2002). Increased Taxon Sampling Greatly Reduces Phylogenetic Error. Syst. Biol. *51*, 588–598.

Biographical Information

Utpal Smart was born in the port city of Surat, the economical capital of the state of Gujarat, western India in May 31 1984. At age four he and his parents moved to Pondicherry, a quaint little town in the southern Indian state of Tamil Nadu where he enrolled into The Sri Aurobindo International Centre of Education, an institute known for its unique, non-traditional approach to education. He spent the subsequent 17 years in the same institute (primary school through college) graduating with a B.A (majoring in English literature) at the age of 21 in 2005. Spurred on by a long-standing fascination and curiosity for the natural world, Utpal then decided (almost on a whim) to abandon his dreams of moving to Oxford and pursuing the life of a novelist, and instead turned his attention to Biology. He enrolled into a M.S in ecology at Pondicherry University (not exactly Oxford, but close enough), and for the next two years he immersed himself in various local projects, working with diverse fauna including sea turtles, birds, butterflies and snakes. His journey as a herpetologist began during his last semester when he spent six years studying elusive Cane-turtles in the dense, evergreen forests of the Western Ghats biodiversity hotspot. During this time he developed a more holistic appreciation for the fascinating breadth and diversity of herpetofauna. In 2016 Utpal received his PhD in Quantitative Biology from the University of Texas at Arlington. Utpal has authored several peer-reviewed and popular articles based on his research and hopes to continue working on the systematics, integrative taxonomy and biogeography of global herepetofauna with his postdoctoral research.