

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)

TODD A. CASTOE¹*, ERIC N. SMITH², RAFE M. BROWN³ and CHRISTOPHER L. PARKINSON¹

¹Department of Biology, University of Central Florida, 4000 Central Florida Blvd, Orlando, Florida 32816-2368, USA

²Department of Biology, University of Texas at Arlington, Arlington, Texas, USA

³Department of Evolutionary Biology, University of Kansas, Lawrence, Kansas, USA

Received July 2006; accepted for publication April 2007

The snake family Elapidae contains over 60 genera (about 300 species) of highly venomous snakes. About one-third of the alpha-taxonomic diversity of the Elapidae comprises coralsnakes: a major radiation of colourful venomous snakes including six genera distributed in Asia and the Americas. In this study, we examine molecular phylogenetic and descriptive morphological evidence for the placement of the monotypic coralsnake genus *Hemibungarus* (*H. calligaster* (Wiegmann)) among the elapids, and clarify the relationships among genera traditionally referred to as 'coralsnakes'. We use two mitochondrial gene fragments (ND4 and cyt-*b*) and a nuclear gene fragment (*c-mos*) to estimate relationships among elapids and other colubroid snakes, based on parsimony and likelihood methods, as well as Bayesian phylogenetic methods incorporating complex partitioned models of nucleotide evolution. As different phylogenetic methods provided alternative results, we include an extensive examination of molecular phylogenetic analyses to facilitate a transparent and thorough exploration of the data. Additionally, we highlight external morphological and hemipenial characters that appear to further support molecular hypotheses for the placement of *Hemibungarus*, and relationships among the Elapinae. Owing to conflicting descriptions of morphological characters in the literature, and the unavailability of comparative morphological data for certain key species, we include detailed descriptions of the hemipenes of *Bungarus caeruleus* (Schneider), *B. fasciatus* (Schneider), *Calliophis nigrescens* Günther, *Dendroaspis polylepis* (Günther), *H. calligaster* (Wiegmann), *Naja naja* (Linnaeus) and *Ophiophagus hannah* (Cantor). We present evidence that Asian and American coralsnakes (*Calliophis*, *Sinomicrurus*, *Micruroides*, *Micrurus* and *Leptomicrurus*) form an exclusive clade, distantly related to *Hemibungarus*. Thus, despite long-held beliefs of systematic affinities based on morphology and colour pattern, our results suggest that *Hemibungarus* is not (phylogenetically) a coralsnake, but instead shares an exclusive common ancestor with Afro-Asian elapine genera (*Elapsoidea*, *Dendroaspis* and *Ophiophagus*). Results of our molecular phylogenetic analyses also support the recognition of two primary clades of elapids corresponding to the subfamilies Elapinae and Hydrophiinae. Additionally, we provide evidence that the Elapinae consists of two main clades: (1) coralsnakes *s.s.* (*Calliophis*, *Sinomicrurus*, *Micruroides*, *Micrurus*, *Leptomicrurus*), and (2) the remaining genera of Afro-Asian species, including cobras, kraits, mambas and *Hemibungarus*. We suggest a new classification for these two elapine clades: Calliophini for the coralsnakes (*Calliophis*, *Sinomicrurus*, *Micrurus*, *Micruroides* and *Leptomicrurus*), and Hemibungarini for the remaining Afro-Asian elapine species, including *Hemibungarus*. © 2007 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2007, 151, 809–831.

ADDITIONAL KEYWORDS: Calliophini – *Calliophis* – Elapinae – Hemibungarini – hemipenial morphology – *Micrurus* – molecular phylogeny.

*Corresponding author. Current address: University of Colorado Health Science Center, Department of Biochemistry & Molecular Genetics, PO Box 6511, 12801 E. 17th Ave., Aurora, CO 80045, USA. E-mail: todd.castoe@uchsc.edu

INTRODUCTION

The family Elapidae comprises a large and diverse group of terrestrial and marine species of highly venomous snakes, with over 60 genera and about 300 species (Golay *et al.*, 1993). Although various authors have treated the higher level taxonomy of the group differently, there is broad agreement that two main groups exist, the first containing marine and Australo-Melanesian species, and a second containing the remaining African, Asian and American terrestrial species (e.g. McDowell, 1986, 1987; Slowinski, Knight & Rooney, 1997; Slowinski & Keogh, 2000; Scanlon & Lee, 2004). These two main groups of elapids have been considered subfamilies by most recent authors, Hydrophiinae for the marine and Australo-Melanesian species, and Elapinae for the latter (e.g. Slowinski & Keogh, 2000). Whereas relationships among members of the Hydrophiinae have received considerable attention (e.g. Mengden, 1985; Schwaner *et al.*, 1985; Keogh, 1998; reviewed in Slowinski & Keogh, 2000; Scanlon & Lee, 2004), relationships among the Elapinae have received considerably less attention (e.g. Slowinski & Keogh, 2000; Slowinski, Boundy & Lawson, 2001).

The subfamily Elapinae contains African and Asian species including cobras, kraits and mambas (e.g. *Dendroaspis*, *Bungarus*, *Naja*, *Ophiophagus*, *Elapsoidea*), as well as the Asian and American coralsnakes (e.g. *Calliophis*, *Hemibungarus*, *Sinomicrurus*, *Micrurus*). Identification of higher-level relationships and the monophyly of these nominal groupings within the Elapinae have yet to be determined conclusively, and several recent studies have provided mostly contradictory results that collectively have suffered from poor resolution and low support (e.g. Slowinski *et al.*, 1997; Keogh, 1998; Slowinski & Keogh, 2000). Based on morphological evidence, McDowell (1967, 1969, 1986, 1987) suggested a close relationship between Asian coralsnakes (previously recognized as *Calliophis* and *Maticora*) and the New World coralsnakes (*Micrurus*, *Micruroides* and *Leptomicrurus*). Subsequently, molecular data also supported a monophyletic Asian–New World coral-snake clade (Keogh, 1998; Slowinski & Keogh, 2000), although the sampling of coralsnakes in these molecular studies was sparse.

Based on a detailed study of the anatomy of the corner of the mouth, McDowell (1987) divided Asian coralsnakes into four parts: (1) *Calliophis bibroni* (Jan), *C. gracilis* Gray and *C. melanurus* (Shaw); (2) *Maticora bivirgata* (Boie), *M. intestinalis* (Laurenti), *M. maculiceps* (Günther) and *M. nigrescens* (Günther); (3) *C. hatori* Takahashi, *C. japonicus* Günther, *C. kelloggi* (Pope), *C. maclellandi* (Reinhardt) and *C. sauteri* (Steindachner); and (4) *H.*

(*Calliophis*) *calligaster* (Wiegmann). Based on this evidence, McDowell (1987) resurrected the genus *Hemibungarus* Peters, 1862 for *H. calligaster*. *Calliophis beddomei* Smith was not allocated by McDowell (1987), but it is very similar to *M. nigrescens*.

Slowinski *et al.* (2001) were the first to conduct a detailed phylogenetic analysis of the Asian coral-snakes, and they found strong evidence for the distinctiveness of *H. calligaster* from other species of *Calliophis* and *Maticora*. Congruent with the results of McDowell (1987), they also identified a clade of *Calliophis* closely related to the New World coral-snakes (*Micrurus*, *Micruroides* and *Leptomicrurus*) and erected the genus *Sinomicrurus* for members of this clade (*S. hatori*, *S. japonicus*, *S. kelloggi*, *S. maclellandi*, and *S. sauteri*). Additionally, Slowinski *et al.* (2001) recommended the synonymy of *Maticora* (*M. bivirgata*, *M. intestinalis*, *M. maculiceps* and *M. nigrescens*) with the remaining *Calliophis* (*C. beddomei*, *C. bibroni*, *C. gracilis* and *C. melanurus*) because recognition of *Maticora* apparently rendered *Calliophis* paraphyletic. They also provided evidence for the uniqueness of *H. calligaster* in relation to other lineages of coralsnakes. Throughout the analyses of Slowinski *et al.* (2001), the explicit assumption was made that Asian and New World coralsnakes are monophyletic, and all phylogenetic analyses were constrained to produce a monophyletic group of coral-snakes. This assumption appeared reasonable at the time, but part of the present effort involves an explicit test of the assumption that coralsnakes, including *H. calligaster*, form a monophyletic group.

The conservative external morphology of snakes makes classification based on morphology difficult. Contrastingly, the hemipenes of snakes are highly elaborate and diverse across species; they thus serve an important role in snake classification and systematics (e.g. Cope, 1895; Dowling & Savage, 1960; Myers & Cadle, 2003). Several studies have addressed hemipenial morphology of Asian coralsnakes (McDowell, 1986; Slowinski *et al.*, 2001), American coralsnakes (Campbell & Lamar, 1989, 2004; Slowinski, 1995; Roze, 1996), other Asian Elapids (e.g. McDowell, 1986; Slowinski, 1994) and African Elapids (e.g. Bogert, 1940). The hemipenis of *H. calligaster* was described by Leviton (1964) and McDowell (1986). As pointed out by Slowinski *et al.* (2001), McDowell (1986) described the hemipenes of this species as single, although Leviton (1964) had remarked that it was slightly bifurcated. To investigate the role of hemipenial characters in defining the elapine clades, and to clarify conflicting descriptions of characters, we examined the hemipenial morphology of *Bungarus caeruleus*, *B. fasciatus*, *C. nigrescens*, *Dendroaspis polylepis*, *H. calligaster*, *Naja naja* and *Ophiophagus hannah*. We use these data, together with hemipenial

characters for additional taxa taken from the literature and from direct examination of museum specimens, to identify potential synapomorphies in order to compare hemipenial morphology with molecular phylogenetic results.

In this study we examine molecular phylogenetic and descriptive/comparative morphological evidence for the placement of *H. calligaster* among elapids, evaluating evidence that this species forms an exclusive monophyletic group with other Asian and New World coralsnakes. We use two mitochondrial gene fragments and a nuclear gene fragment to estimate relationships among elapids and other colubroid snakes, based on parsimony, likelihood and Bayesian phylogenetic methods incorporating complex partitioned models of evolution. We include extensive analytical treatment of the molecular data to explore thoroughly the phylogenetic signal in this data set in a methodologically transparent and justified fashion. With the explicit caveat that we have not thoroughly sampled all major lineages of elapids (particularly hydrophiine elapids), we take advantage of the data set also to explore evidence for higher-level relationships among elapids, including: (1) the validity of the subfamilies Hydrophiinae and Elapinae, (2) relationships among the Elapinae and the placement of coralsnakes, and (3) the relationships among Asian and New World coralsnakes.

METHODS

MOLECULAR TAXON SAMPLING

Nucleotide sequences of three gene fragments from a total of 37 snake species were included in this study. This included sequences from 26 members of the Elapidae, three members of the Atractaspididae and ten members of the Colubridae (Table 1, Appendix 4). Our sampling of elapids included at least a single representative of each genus in the subfamily Elapinae. Two colubrid species (*Farancia abacura* Holbrook and *Ptyas korros* Schlegel) were used as outgroup taxa based on recent estimates of the phylogeny of colubroids (Lawson *et al.*, 2005), and specified as such for rooting phylogenetic trees.

DNA SEQUENCING AND ALIGNMENT

A majority of nucleotide sequences used in this study have been published previously (Slowinski & Keogh, 2000; Slowinski & Lawson, 2002; Lawson *et al.*, 2005), and were obtained from GenBank. Laboratory methods for novel sequences generated for this study are provided below. Genomic DNA was isolated from tissue samples (liver or skin preserved in ethanol) using the Qiagen DNeasy extraction kit and protocol. One nuclear gene and two mitochondrial (mtDNA) gene fragments were independently amplified via

PCR and sequenced, per sample. The mtDNA cytochrome-*b* (*cyt-b*) gene fragment was amplified using the primers Gludg and AtrCB3 (described in Parkinson, Chippindale & Campbell, 2002), and the mtDNA NADH dehydrogenase subunit 4 (ND4) gene fragment was amplified via PCR using the primers ND4 and LEU as described in Arévalo, Davis & Sites (1994). The nuclear oocyte maturation factor (*c-mos*; also known as the serine/threonine protein kinase *mos*) gene fragment was amplified using the primers S77 and S78 following Lawson *et al.* (2005). Amplified PCR products were excised from agarose electrophoretic gels and purified using the GeneCleanIII kit (BIO101). Purified PCR products were sequenced in both directions with the amplification primers. Sequencing was accomplished using the CEQ Dye Terminator Cycle Sequencing Quick Start Kit (Beckman-Coulter) and run on a Beckman CEQ8000 automated sequencer. Raw sequence chromatographs were edited using Sequencher 4.2 (Gene Codes Corp.). Sequences of each fragment were aligned manually in GeneDoc (Nicholas & Nicholas, 1997). Alignment of these three protein-coding gene fragments was straightforward and included six indels in *c-mos* that represented deletions or insertions of complete codons (and no indels in mtDNA fragments). No internal stop codons were found in any of the three fragments. Novel sequences were deposited in GenBank (all accession numbers are provided in Table 1).

PHYLOGENETIC INFERENCE

Gaps in alignment were treated as missing data for all phylogenetic analyses. Maximum parsimony (MP), maximum likelihood (ML) and Bayesian Metropolis-Hastings coupled Markov chain Monte Carlo (MCMC) phylogenetic methods were used to reconstruct phylogenies. Both MP and MCMC methods were initially used to compare phylogenetic reconstructions estimated for each gene fragment independently. In general, we expect that the two mtDNA gene fragments (*cyt-b* and ND4) should contain phylogenetic signal supporting a common phylogeny because mtDNA haplotypes are inherited maternally as a single linkage unit. We verified this assumption, prior to combining mtDNA gene data, by reconstructing phylogenies of each gene independently and searching for strongly supported incongruent relationships across gene trees (e.g. Wiens, 1998). We paid particular attention to the congruence between the mtDNA and *c-mos*-based estimates of phylogeny to identify areas of apparent conflicting signal indicated by strongly supported alternative placement of taxa, as these two sets of characters are independently inherited. We considered estimates of relationships to be strongly supported if they received > 90% posterior

Table 1. Species used in phylogenetic analyses along with GenBank accession numbers

Species	Voucher	GenBank accession		
		cyt- <i>b</i>	ND4	c-mos
<i>Aparallactus werneri</i>		AF471035	AWU49315	AF471116
<i>Aspidelaps scutatus</i>		AY188007	AY058969	AY187968
<i>Atractaspis bibroni</i>		AY188008	ABU49314	AY187969
<i>Boulengerina annulata</i>		AY188010	AY058970	AY187971
<i>Bungarus fasciatus</i>		AF217830	U49297	AY058924
<i>Bungarus multicinctus</i>		AJ565002	AJ830249	AF435021
<i>Calliophis bivirgata</i>		AF217812	AY058979	AY058934
<i>Demansia atra</i>		AY058966	AY058973	AY058927
<i>Dendroaspis polylepis</i>		AF217832	AY058974	AY058928
<i>Elapognathus coronatus</i>		AF217819	AY058972	AY058929
<i>Elapsoidea nigra</i>		AF217820	AY05897	AY058930
<i>Farancia abacura</i>		FAU69832	FAU49307	AF471141
<i>Hemibungarus calligaster</i>	TNHC-62483	EF137411*	EF137403*	EF137419*
<i>Homoroselaps lacteus</i>		AF217833	AY058976	AY058931
<i>Lamprophis fuliginosus</i>		AF471060	AF544664	AF471143
<i>Laticauda colubrina</i>		AF217834	AY058977	AY058932
<i>Leioheterodon madagascariensis</i>		AY188022	LMU49318	AY187983
<i>Leioheterodon modestus</i>		AY058967	AY058978	AY058933
<i>Leptomicrurus narducii</i>	KU 202955	EF137412*	EF137404*	EF137420*
<i>Malpolon monspessulanus</i>		AY058965	AY058989	AY058936
<i>Micruroides euryxanthus</i>	AMNH R-128233	EF137416*	EF137408*	EF137423*
<i>Micrurus fulvius</i>	CAS-214347	EF137413*	EF137405*	EF137421*
<i>Micrurus mipartitus</i>	CH-5377	EF137414*	EF137406*	
<i>Micrurus surinamensis</i>	OMNH 37596	EF137415*	EF137407*	EF137422*
<i>Mimophis mahfalensis</i>		AY188032	AF544662	AY187992
<i>Naja naja</i>		AY713376	AY713378	AF435020
<i>Naja kaouthia</i>		AF217835	AY058982	AY058938
<i>Naja nivea</i>		AF217827	AY058983	AY058939
<i>Notechis ater</i>		AF217836	AY058981	AY058937
<i>Ophiophagus Hannah</i>		AF217842	AY058984	AY058940
<i>Paranaja multifasciata</i>		AF217837	AY058985	AY058941
<i>Psammophis condanarus</i>		AF471075	AY058987	AF471104
<i>Pseudaspis cana</i>		AY058968	AY058986	AY058942
<i>Ptyas korros</i>		AY486929	AY487062	AY486953
<i>Sinomicrurus japonicus</i>		AF217831	AY058971	AY058926
<i>Sinomicrurus kelloggii</i>	ROM-37080	EF137417*	EF137409*	EF137424*
<i>Sinomicrurus mccllellandii</i>	ROM-35245	EF137418*	EF137410*	EF137425*
<i>Walterinnesia aegyptia</i>		AF217838	AY058988	AY058943

Novel sequences generated in this study are indicated with a '*'. Museum acronyms for specimen vouchers for these novel sequences follow Leviton *et al.* (1985) except for the following: CH = Círculo Herpetológico de Panamá.

probability support (PP) in MCMC analyses, or > 70% bootstrap support (BSS) in MP or ML analyses (Hillis & Bull, 1993).

All MP phylogenetic analyses were conducted using PAUP* version 4.0b10 (Swofford, 2002). All characters were treated as equally weighted in MP searches. We used a heuristic search with tree bisection reconnection (TBR) branch-swapping and 1000 random-taxon-addition sequences to search for optimal MP trees.

Bootstrap support for nodes in MP was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 1000 full heuristic pseudo-replicates (ten random-taxon-addition sequence replicates per bootstrap pseudo-replicate).

MrModeltest v2.2 (Nylander, 2004) was used to select an appropriate model of evolution for ML and MCMC analyses because this program only considers nucleotide substitution models that are currently

Table 2. Description of complex partitioned models used in the analysis of the combined data set

Data set	Model	Partitions	Free model parameters	Description of partitions	Harmonic mean of marginal likelihood	Akaike weight (A_w)	Relative Bayes Factor (RBF)
mtDNA	mt1x	1	10	single model for all mtDNA	-22 722.85	0.000	–
mtDNA	mt2xA	2	22	ND4; cyt- <i>b</i>	-22 715.38	0.000	1.25
mtDNA	mt2xB	3	22	codon pos. 1 + 2; pos. 3	-22 108.39	0.000	102.41
mtDNA	mt3x	4	33	codon pos. 1; pos. 2; pos. 3	-21 984.70	0.999	64.19
mtDNA	mt4x	4	43	ND4 pos. 1 + 2; ND4 pos. 3 cyt- <i>b</i> pos. 1 + 2; cyt- <i>b</i> pos. 3	-22 093.91	0.000	38.12
mtDNA	mt6x	5	59	each codon pos. of each gene with independent model	-21 966.11	0.001	30.89*
c-mos	c-mos1x	1	5	single model for c-mos	-2289.89	0.000	–
c-mos	c-mos2x	2	15	c-mos pos. 1 + 2; c-mos pos. 3	-2251.13	0.011	7.75
c-mos	c-mos3x	3	20	c-mos pos. 1 + 2; c-mos pos. 3	-2241.60	0.989	6.44*
All Data	All-1x	1	10	single model for all data (mtDNA and c-mos)	-25 555.36		–
All Data	All-9x	9	80	all codon positions of each gene allocated independent model (P_1 – P_9 in Table 3)	-24 297.03		17.97

Each partition identified below was allocated the model selected by AICs estimated in MrModeltest. Models favoured by Bayes factors are indicated with an asterisk (*); see Appendix 2 for Bayes factor comparisons between models. The number of free model parameters includes the rate scalars for partitioned models.

available in MrBayes v3.1 (Ronquist & Huelsenbeck, 2003). PAUP* was used to calculate model likelihoods for use in MrModeltest. Based on arguments presented by Posada & Buckley (2004), we used Akaike's information criterion (AIC; Akaike, 1973, 1974; Sakamoto, Ishiguro & Kitagawa, 1986) to select best-fit models in MrModeltest. In addition to the combined data set, putative a priori partitions of the data set (Table 2) were independently analysed using MrModeltest to estimate best-fit models of nucleotide evolution.

We used ML to estimate the phylogeny based on the combined (mtDNA + c-mos) data set. Estimates based on ML were conducted in PAUP* using a heuristic search initiated with a starting tree estimated by neighbour joining, with TBR branch swapping. Model selection based on AICs suggested the general time-reversible model (Tavaré, 1996) with gamma-distributed among-site rate variation (Yang, 1996) incorporating an estimate of the proportion of invariant sites (GTR + Γ) as best fitting the data (Table 2). This model (and starting parameters estimated by MrModeltest/PAUP*) was used to initiate ML searches. Nodal support for ML was estimated by conducting 150 full-heuristic bootstrap pseudoreplicates, each consisting of a heuristic search initiated with a tree estimate based on neighbour joining.

All MCMC phylogenetic analyses were conducted in MrBayes 3.0b4 (Ronquist & Huelsenbeck, 2003) with vague priors and three incrementally heated chains in addition to the cold chain (as per the program's defaults). Each MCMC analysis was conducted with the default settings including two parallel MCMC runs (each run was conducted in duplicate), and each conducted for a total of 6.0×10^6 generations (sampling trees and parameters every 100 generations). Conservatively, the first 3.0×10^6 generations from each run were discarded as burn-in. Summary statistics and consensus phylograms with PP support were estimated from the combination of the two parallel MCMC runs per analysis (see justification below).

The best-fit models for each partition (based on AICs; Table 3) were implemented as partition-specific models within partitioned mixed-model analyses of the mtDNA, c-mos and combined data set (e.g. Brandley, Schmitz & Reeder, 2005; Castoe, Sasa & Parkinson, 2005). These partitioned MCMC analyses were designed to allow independent models of nucleotide evolution to be applied to partitions of the combined data set. This was accomplished by dividing the data set into a priori partitions (Table 3) and specifying that an independent (partition-specific) model be used for each putative partition (using the 'unlink' and 'ratepr = variable' commands in MrBayes). These

Table 3. Results of AIC model selection conducted in MrModeltest for partitions of the data set

Partition	AIC model
All data	GTR + Γ + I
mtDNA (ND4 + cyt- <i>b</i>)	GTR + Γ + I
mtDNA – 1st pos.	GTR + Γ + I
mtDNA – 2nd pos.	GTR + Γ + I
mtDNA – 1st + 2nd pos.	GTR + Γ + I
mtDNA – 3rd pos.	GTR + Γ + I
ND4	GTR + Γ + I
ND4–1st pos. [<i>P</i> ₁]	GTR + Γ
ND4–2nd pos. [<i>P</i> ₂]	GTR + Γ + I
ND4–1st + 2nd pos.	GTR + Γ + I
ND4–3rd pos. [<i>P</i> ₃]	GTR + Γ
cyt- <i>b</i>	GTR + Γ + I
cyt- <i>b</i> –1st pos. [<i>P</i> ₄]	GTR + Γ + I
cyt- <i>b</i> –2nd pos. [<i>P</i> ₅]	HKY + Γ + I
cyt- <i>b</i> –1st + 2nd pos.	GTR + Γ + I
cyt- <i>b</i> –3rd pos. [<i>P</i> ₆]	GTR + Γ + I
c-mos	HKY + Γ
c-mos – 1st pos. [<i>P</i> ₇]	HKY + I
c-mos – 2nd pos. [<i>P</i> ₈]	HKY
c-mos – 1st + 2nd pos.	HKY + I
c-mos – 3rd pos. [<i>P</i> ₉]	GTR

The partitions of the complex partitioned Bayesian MCMC model used to analyse the combined data set are labelled *P1–P9* in parentheses.

mixed models partitioned the combined data set based on gene fragment origin (nuclear vs. mtDNA), gene and codon position (Table 2). To identify which model partitioning scheme best fit the data, we first analysed partitioned models for the mtDNA and c-mos data sets independently (Table 2). Based on these results, we used the best partitioning scheme for mtDNA and c-mos in the analysis of the combined dataset.

We used three statistics to evaluate model partitioning schemes: (1) Bayes factors (BFs), (2) relative Bayes factors (RBFs) and (3) Akaike weights (A_w ; as in Castoe *et al.*, 2005). Each of these criteria allows objective evaluation of non-nested partitioned models, which is important here because several alternative partitioned models are non-nested. Bayes factors were calculated using the harmonic mean approximation of the marginal model likelihood following Nylander *et al.* (2004; see also Kass & Raftery, 1995), and we report the results in the form of $2\ln B_{10}$. Evidence for model M_1 over M_0 was considered very strong (and considered sufficient) if $2\ln B_{10} > 10$ (Kass & Raftery, 1995; see also Nylander *et al.*, 2004).

RBFs (Castoe *et al.*, 2005) were used to quantify the average impact that each free model parameter had on increasing the fit of the model to the data. These values

were also used to estimate the ratio of parameters to posterior evidence of increasingly complex partitioned models, and RBF values may be essentially interpreted as the average component of the BF difference between models contributed by each added free model parameter. This may provide a simple means for comparing the parameter richness of candidate models tested in relation to how complex a model may be justified by the size and heterogeneity of a data set (Castoe *et al.*, 2005; Castoe & Parkinson, 2006).

Akaike weights (A_w) were employed as a means of confirming model choice, together with $2\ln B_{10}$ estimates. To estimate A_w , we used the harmonic mean of the model likelihood from the MCMC analyses (harmonic mean of the two parallel runs combined) to incorporate an estimate of the marginalized likelihood of models (Castoe *et al.*, 2005); the higher the A_w for a model, the higher the relative support for that model.

Once a tentative best-fit model was chosen for the combined data, this model was checked for evidence of parameter identifiability, failed convergence and unreliability (which would suggest the model may be parametrically over-fit; e.g. Huelsenbeck *et al.*, 2002; Rannala, 2002; Castoe, Doan & Parkinson, 2004). We investigated the performance of models (using Tracer; Rambaut & Drummond, 2003) by examining cold chain likelihood and parameter estimate burn-in, as well as the shapes and overlap of posterior distributions of parameters. We looked for evidence that model likelihood and parameter estimates ascended directly and rapidly to a stable plateau, and that independent runs converged on similar likelihood and parameter posterior distributions (considered evidence that a model was not over-fit). We also used the potential scale reduction factor (PSRF; Gelman & Rubin, 1992) to identify that independent runs under the same model converged with regard to estimates of phylogeny and parameters. We considered runs to have converged when the PSRF of parameters dropped below 1.02 (1 indicating 100% convergence of estimates between runs). Based on this criterion, we chose the conservative burn-in period of 3×10^6 because independent runs of all analyses had converged to PSRF < 1.02 by this period (most parameters had PSRFs < 1.002 by 3×10^6 generations).

METHODS FOR COLLECTION OF HEMIPENIAL DATA

Hemipenes of select species were dissected and everted from preserved specimens deposited in the herpetological collections of the Bombay Natural History Society (BNHS), California Academy of Sciences (CAS) and the Amphibian and Reptile Diversity Research Center of The University of Texas at Arling-

ton (UTA). Locality and museum information for specimens illustrated or for which hemipenes were examined are presented in Appendix 1. The hemipenes of *H. calligaster*, *C. nigrescens*, *B. caeruleus*, *B. fasciatus*, *N. naja*, *O. hannah* and *D. polylepis* were everted and described in detail. Terminology for hemipenes follows Savage (2002). Preparation of hemipenes follows Myers & Cadle (2003) and Zaher & Prudente (2003), except as explained below. Previous experience everting bilobed hemipenes with long and thin lobes, as well as very thin-walled organs (e.g. *Calliophis* and most *Micrurus* spp.) have forced us to reduce the initial time these hemipenes are immersed in KOH to 5 min, and also reduce the concentration of the KOH solution to 1%. If the organs were left for more time they tended to disintegrate, not become 'rubbery' as stated by Zaher & Prudente (2003). After immersion in KOH some small or difficult organs were immersed in hot water (70–75 °C) to attain the proper pliability and ('rubbery') firm consistency. Alternatively, some were exchanged between hot water and KOH or 70% ethanol through the process to maintain ideal manageability. During this process, the KOH softens the fixed tissue to make it more easily inverted and the water acts to hydrate and prevent the disintegration of the tissue. We used KOH mixed with Alizarin red in order to colour the ossified structures in the hemipenes, and glycerin stained with blue candle dye to expand the organs. Coloration of the spines with Alizarin red varied considerably, and appears to be less noticeable with some older specimens. In some older specimens, the whole tissue tends to become reddish and the distinction between calcified tissue and regular tissue (via Alizarin red) is not achieved. Due to this bias, which appears to be related to the age of the specimen, we do not describe the Alizarin staining of calcified tissues.

RESULTS

MOLECULAR DATA SET CHARACTERISTICS

The concatenated alignment of all three gene fragments contained 1946 characters, 792 of which were parsimony-informative characters (PIC) and 960 were constant. The combined mtDNA data contained a total of 1374 characters (PIC = 707), with 663 from ND4 (PIC = 356) and 711 from *cyt-b* (PIC = 351). The nuclear c-mos data set contained 572 characters (PIC = 85).

BAYESIAN MCMC MODEL SELECTION AND EVALUATION

We used a combination of A_w and BF to compare the support for alternative partitioning strategies of the

data in MCMC analyses. These two different model selection criteria suggested different partitioning schemes for the combined mtDNA data set (Table 2; Appendix 2). While A_w placed the highest support (greatest weight) under the mt3x model, BF instead strongly supported the most complex model, mt6x, with a BF value nearly 30-fold higher than our threshold value supporting the mt6x over the mt3x model (BF = 290; Appendix 2; see also Table 2). Also, RBF values provided evidence of a very strong average contribution of free parameters in the mt6x model (RBF = 30.89; Table 2). In general, A_w penalizes competing models more for added parameters, and it is not surprising that it selected a slightly simpler model for the mtDNA data. Preliminary analyses alternatively using these two models for the mtDNA data showed that both provided extremely similar estimates of nodal support (PP), and both models converged equally well with very small PSR values (implying identifiability of added parameters was not a problem). Given these results and suggestions that even overly complex models retain their accuracy (Huelsenbeck & Rannala, 2004; Lemmon & Moriarty, 2004), whereas underparameterized models tend to suffer from inaccuracy in MCMC analyses (e.g. Erixon, Britton & Oxelman, 2003; Huelsenbeck & Rannala, 2004; Lemmon & Moriarty, 2004; Castoe & Parkinson, 2006), we used the mt6x model as the preferred MCMC partitioning scheme for the mtDNA data.

Selection among partitioning schemes for the c-mos data set was straightforward, and both A_w and BF provided strong support for the most complex partitioned model, c-mos3x (all BF > 19; A_w = 98.9%; Table 2; Appendix 2). The average contribution of added model parameters in the c-mos partitioned models was not as high as observed with the mtDNA data (c-mos3x RBF = 6.44; Table 2); these results are consistent, however, with the overall low amount of variation in this slowly evolving nuclear gene, in comparison with the highly variable mtDNA data. This partitioned model (c-mos3x) was used to analyse the c-mos data set for subsequent MCMC analyses.

The combined mtDNA + c-mos data set was analysed under the partitioning scheme determined for each independent data set (Table 2). Essentially, we combined the mt6x and the c-mos3x partitioned models into a combined 'All-9x' partitioned model (with nine total partitions) for the combined data MCMC analyses in which each codon position of each gene fragment was allocated an independent model, each selected by AICs (Table 2). These partition-specific models included the GTR and HKY (Hasegawa, Kishino & Yano, 1985) substitution models with or without Γ and I model components (Table 2).

INDEPENDENT DATA SET PHYLOGENIES

Phylogenetic estimates based on ND4 and *cyt-b* were very similar with no strongly supported alternative placements of taxa, although both MP and MCMC estimates based on these single gene fragments were poorly resolved and generally associated with low nodal support (topologies not shown). These results supported our expectation that these two gene fragments were appropriate to combine given their similar phylogenetic signal, as expected with mtDNA genes.

The MP analysis of the combined mtDNA data found eight equally parsimonious trees (5729 steps). The homoplasy index (HI = 0.751) and the rescaled consistency index (RCI = 0.075) based on this analysis suggest substantial homoplasy present in the mtDNA data. Essentially all deeper relationships in the mtDNA data set received less than 50% MP-BSS, and the high homoplasy observed in this data set probably greatly obscures resolution of relationships based on mtDNA alone at these deeper levels of phylogeny (particularly under MP). The strict consensus of the eight MP trees resulted in a polytomy among 18 branches encompassing nearly all deep relationships including the placement of *Hemibungarus*. Similarly, the MCMC analyses of the mtDNA data (under the mt6x model; Table 2) resulted in a majority of deep nodes being associated with weak PP support values. *Demansia atra* was inferred as the sister lineage to the remaining elapidae with PP = 100. Coralsnakes, excluding *Hemibungarus*, formed a weak clade (PP = 55), and *Hemibungarus* formed a clade with other African and Asian elapine genera (PP = 63). Within this non-coralsnake elapine clade, *Hemibungarus* was grouped with *Elapsoidea*, *Dendroaspis* and *Ophiophagus* (PP = 52).

The MP analysis of the c-mos data set resulted in 3020 equally parsimonious trees (227 steps), and the overall phylogenetic signal in this data set does not appear to suffer from substantial homoplasy (HI = 0.185, RCI = 0.657). The strict consensus of c-mos MP trees estimated a monophyletic Elapidae (MP-BSS = 84), except for *Demansia* and *Elapognathus*, which formed a clade at the base of the tree. The lengths of the branches leading to these two species in the c-mos tree were quite long (nearly two-fold longer than other taxa), implying long branch attraction phenomena may significantly bias the placement of these taxa. Based on the MP estimate, the Elapidae formed two clades, one containing the Asian and New World coralsnakes excluding *Hemibungarus* (*Calliophis*, *Sinomicrurus*, *Micruroides*, *Micrurus* and *Leptomicrurus*; MP-BSS = 61), and a second clade containing the remaining elapids including *Hemibungarus* (MP-BSS = 76). The rela-

tionships among lineages within this second elapid clade were not resolved based on the strict consensus of the MP trees, resulting in a polytomy of nearly all taxa in this clade (including *Hemibungarus*). Like the MP estimate, the c-mos MCMC estimate showed a deep divergence of a clade including *Demansia* and *Elapognathus* (with colubrids), and an otherwise monophyletic Elapidae (PP = 100). A deep bipartition among the Elapidae was inferred, with one clade including coralsnakes (exclusive of *Hemibungarus*; PP = 85) and the other including the remaining elapids (including *Hemibungarus*; PP = 100). The position of *Hemibungarus* among this second non-coralsnake elapid clade was unresolved.

Comparing phylogeny estimates based on the mtDNA and the c-mos data sets, only one strongly supported relationship was in conflict. The phylogenetic placement of *Elapognathus coronatus* Schlegel and *Demansia atra* Macleay differed substantially between estimates, with c-mos indicating these two species did not form an exclusive clade with other elapids, and mtDNA suggesting *Elapognathus* groups with other Australian elapids and sea snakes (hydrophiines), while *Demansia* formed the sister lineage to the remaining elapids. Otherwise, all other estimates of relationships suggested that the c-mos and mtDNA did not display obviously incongruent phylogenetic signal and should be combined. It is possible that insufficient phylogenetic signal (potentially compounded by problems placing long branches), rather than actual incongruent evolutionary histories of different loci, are responsible for the alternative placement of these taxa in different data sets. To examine this further, we decided to proceed tentatively with analysing the combined data set, including *Demansia* and *Elapognathus*, pending the results of the combined estimate. Generally, we expected these two taxa to group with the other hydrophiine Australian elapid and sea snake included (*Notechis* and *Laticauda*, respectively; e.g. Keogh, 1998; Keogh, Scott & Scanlon, 2000; Slowinski & Keogh, 2000), and evidence for *Demansia* and *Elapognathus* grouping with these taxa in the combined analyses would suggest independent data set estimates may have been a spurious result based on insufficient or misleading phylogenetic signal.

COMBINED DATA MP PHYLOGENETIC ANALYSIS

The MP heuristic search of the combined data set found a single shortest tree with 5999 steps (Fig. 1). BSS values for nodes, based on the combined data set (as well as the mtDNA and c-mos data sets), are provided. A substantial degree of character-state homoplasy was inferred within the combined data set, based on the HI (0.732) and RCI (0.83); a majority of

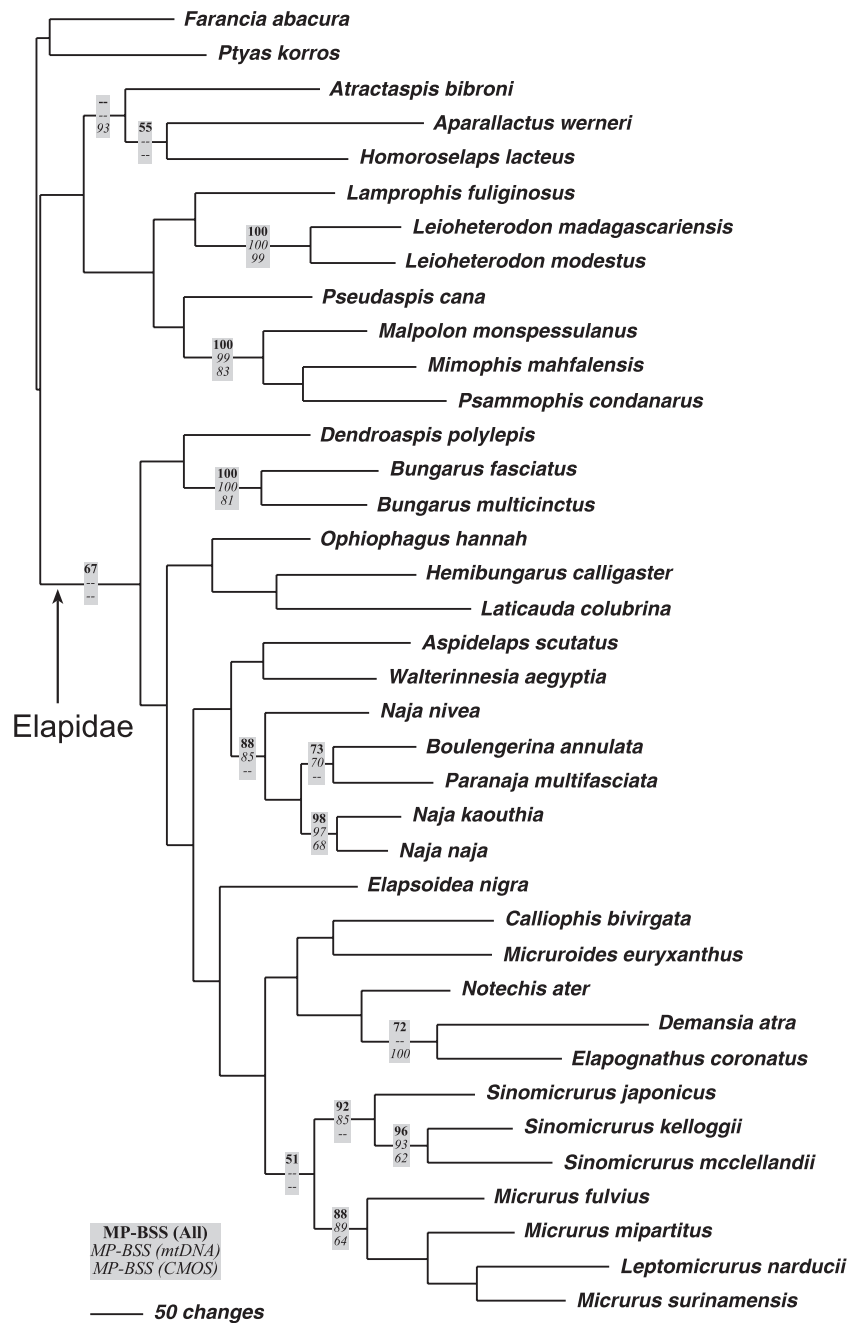
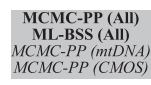


Figure 1. Phylogram of the single shortest tree resulting from a heuristic maximum parsimony search of the combined (mtDNA + c-mos) data. Bootstrap support (BSS) for nodes (> 50%) are provided in grey rectangles adjacent to nodes based on parsimony analyses of the combined data set (top of rectangle, bold), the mtDNA data set (middle of rectangle, italics), and c-mos data set (bottom of rectangle, italics). Support values (BSS) for nodes < 50% are either not indicated or are indicated by a dashed line.

this homoplasy is contributed by the mtDNA data, as pointed out above.

Overall, MP phylogenetic estimates provide a well-resolved but very poorly supported phylogeny of the Elapidae, and only a few deeper branches within the

phylogeny received BSS above 50% (Fig. 1). The Elapidae did form a weakly supported (BSS = 67) monophyletic group. *Hemibungarus calligaster* formed a poorly supported clade with *Ophiophagus hannah* and *Laticauda colubrina* Schneider. This estimate of the



position of *Hemibungarus* based on MP is suspect, and may be biased by long branch attraction, as *L. colubrina*, and a number of other elapids, appear to have particularly long branches and substantial branch length variation (especially apparent in the MCMC and ML phylograms; e.g. Fig. 2). In general, we are not

© 2007 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2007, **151**, 809–831

of phylogeny are more likely to provide accurate estimates and we confine a detailed discussion of phylogeny estimates to these results (additional justification outlined below).

COMBINED DATA ML PHYLOGENETIC ANALYSIS

The single best-fit model of evolution selected (by AICs) was a GTR + Γ I model with the starting parameters estimated in PAUP* during the model selection process. This model and starting parameters were used to analyse the combined (mtDNA + c-mos) data set. This ML estimate (Fig. 2) provides a substantially different topology, generally with higher BSS values across nodes, compared with the MP analyses (Fig. 1). The ML topology suggests a monophyletic Elapidae (ML-BSS = 91), and also implies a primary division among elapid snakes that correspond to the Elapinae and Hydrophiinae (although ML-BSS values were below 50% for each clade). Also, within the Hydrophiinae, *Demansia atra* and *Elapognathus coronatus* form a clade (ML-BSS = 99) and group with other hydrophiine genera (Fig. 2). These results imply that alternative placement of these taxa based on individual gene fragment data sets (i.e. not forming a clade with other Hydrophiine genera) may have been due to insufficient (or misleading) phylogenetic signal of these smaller data sets rather than incongruent evolutionary histories of c-mos and mtDNA data. The ML estimate placed *Hemibungarus* as the sister lineage to a clade containing *Elapsoidea*, *Dendroaspis* and *Ophiophagus*, although support for this was low (Fig. 2). Also, Asian coralsnakes (*Calliophis* and *Sinomicrurus*) and New World coralsnakes (*Micrurus*, *Micruroides* and *Leptomicrurus*) formed a clade (ML-BSS = 68) exclusive of *Hemibungarus*.

COMBINED DATA MCMC PHYLOGENETIC ANALYSIS

The chosen partitioning scheme for allocating independent (partitioned) models of evolution to portions of the combined data set (the All-9x model; Table 2) was a mixed nucleotide substitution model with a total of nine independent partitions (labelled P1–P9 in Table 3). This 'All-9x' model allocates independent partitions to each codon position of each gene used (Table 2), and was the most complex model tested.

The complex All-9x model showed no evidence of parametric over-fitting based on analysis of convergence and mixing. Cold chain likelihoods and parameter estimates showed a rapid convergence of estimates between independent runs, based on visualization (in Tracer) and on PSRF values. Similarly, by approximately 10^6 generations, the average standard deviation of split frequencies compared between runs decreased to below 0.001 (as reported by MrBayes), indicating convergence of independent

runs on essentially identical posterior distributions of trees. Despite apparent convergence occurring around 10^6 generations, we conservatively discarded the first 3×10^6 generations (as burn-in) and used the posterior distributions of the second 3×10^6 generations (of each run) to generate estimates of phylogeny and parameters.

To investigate the impact of our choice to partition models according to BF rather than A_w (as there was a difference between criteria), we also conducted analyses of the combined data set under the partitioning scheme suggested by A_w (mt3x + c-mos3x; a six-partition combined data model) to investigate the difference between estimates based on this and our chosen All-9x model. Estimates of phylogeny were nearly identical between this six-partition and the All-9x partitioned model; between models, the 50% majority rule topology was identical, and PP support for nodes was consistently either identical or differed only by a few percentage points (with no weakly supported nodes becoming strongly supported, and vice versa). Given the insignificance of the differences between alternative model partitioning schemes, and arguments for erring on the side of higher model complexity (e.g. Huelsenbeck & Rannala, 2004; Lemmon & Moriarty, 2004), we limit our discussion of MCMC results to those from the All-9x model. In further support of partitioning, posterior distributions of model parameter estimates of the All-9x model showed relatively little overlap among partitions, and also were quite distinct from the model parameter estimates of the unpartitioned (All-1x) model (Appendix 3). These findings seem to suggest that our partitioning strategy effectively captures/characterizes a large component of the natural patterns of sequence evolution. The main reason for using partitioned models is to extract phylogenetic signal more accurately from heterogeneous data sets by taking into account variance in nucleotide evolutionary patterns across such data (e.g. Castoe *et al.*, 2004; Nylander *et al.*, 2004; Brandley *et al.*, 2005; Castoe & Parkinson, 2006). Based on this idea, we expect support values based on the partitioned (All-9x) MCMC analysis to provide the most accurate assessment of phylogenetic support for nodes, and we use these support values as the primary means of assessing support for the phylogeny.

The topology based on the partitioned analysis of the combined data was nearly identical to the estimate from ML (Fig. 2), and the 50% majority rule consensus topology of the MCMC estimate was completely consistent with the ML topology with regard to relationships among elapids (there were some minor topological differences within the non-elapid colubroids included). As the topology based on MCMC and ML estimates were so similar, we focus here on

the differences in relative support for nodes based on the MCMC estimate (see Fig. 2).

The All-9x MCMC estimate shows relatively stronger support for many deep nodes, compared with the corresponding ML-BSS estimates (Fig. 2). Support for the monophyly of the Elapidae was similar and high for both ML and MCMC (ML-BSS = 91; PP = 100). Based on our limited sampling of elapid diversity, both ML and MCMC estimates suggest a primary split among elapids that corresponds to the Hydrophiinae and Elapinae, as well as a deep split among elapines that corresponds to the coralsnakes and the non-coralsnake genera (except for the placement of *Hemibungarus* with non-coralsnakes; Fig. 2). Support for the monophyly of the Hydrophiinae increased dramatically (from ML-BSS < 50 to PP = 98) in the MCMC estimate, as did support for the *Laticauda/Notechis* clade (ML-BSS = 76, PP = 100). Support for the placement of *Hemibungarus* with *Elapsoidea*, *Dendroaspis* and *Ophiophagus* increased substantially in the MCMC estimate (ML-BSS < 50, PP = 93). Most importantly, support for this group forming a clade with other elapines (*Bungarus*, *Aspidelaps*, *Walterinesia*, *Naja*, *Boulengerina* and *Paranaja*) that excluded the Asian and New World coralsnakes was very strong in the MCMC estimate (ML-BSS < 50, PP = 99). In other words, the All-9x MCMC estimate provided strong support for the exclusion of *Hemibungarus* from an otherwise monophyletic group of Asian and American coralsnakes (Fig. 2). Relationships among Asian and American coralsnakes, excluding *Hemibungarus*, also received notably higher support in the MCMC estimate, including the monophyly of this group (ML-BSS = 66, PP = 96), the sister group relationship between *Sinomicros* and American coralsnakes (ML-BSS = 88, PP = 99), and the monophyly of American coralsnakes (ML-BSS = 58, PP = 86). Also, similar to ML, the MCMC estimate provided strong support for the paraphyly of *Micrurus* due to the nested placement of *Leptomicrurus* (Fig. 2).

DESCRIPTIONS OF HEMIPENES OF SELECT ELAPINE SPECIES

Because of the importance of hemipenial characters in snake systematics, and the absence of detailed descriptions of these important structures in existing literature, we provide descriptions of hemipenes prepared and examined for this study. When hemipenes were not partially or fully everted, they are described *in situ*.

Hemibungarus calligaster calligaster (CAS SU 7243)
A medium-sized individual of 538 mm in total length. The tail is very short, 34 mm (6.3% of total length)

and only 21 subcaudals (sc) long (not counting tip). The left hemipenis was already dissected *in situ* and a portion of the description below is based on this, prior to being everted. Both left and right *musculus retractor penis magnus* inserts first to a vertebra at the level of subcaudal 16 (sc 16). The spines start at the level of sc 2, they increase abruptly in size at the end of sc 2, and continue moderately enlarging to the level of sc 4. The largest spines at the level of sc 4 are approximately two-thirds the length of sc 4, 1.0 and 1.5 mm, respectively. After this level the spines abruptly reduce in size (to about one-eighth of sc 4) and the hemipenis becomes calyculate. The *sulcus spermaticus* stays ventro-sinistral from the organ's base to the level of sc 4, where it divides dorsally and ventrally, and proceeds towards each lobe for about half of one sc length (about 0.7 mm). The hemipenis ends at the level of sc 6 and appears just slightly bilobed.

Above the hemipenis is the cloacal scent gland (or 'anal gland' of earlier usage). This gland is elongated and reaches the level of the middle of sc 4. The right hemipenis when removed and fully everted and expanded (Fig. 3A) is very short, about 10 mm in length and 6 mm in width. The organ includes a short and naked pedicel for about 2 mm, and spines then appear and gradually increase in size for the next 4 mm. At 7 mm the spines are relatively well separated from each other; there are 17 spines around the organ at this level. The spines then become very small and restricted to the rim of calyces all the way to the tip of the organ. The *sulcus spermaticus* bifurcates at the level of calyculature of the hemipenis and it is centripetal.

The right hemipenes of four other specimens were examined uneverted *in situ* (BMNH RR, BMNH 1964.664, BMNH 72.10.11.13, and BNHS 72.10.11.18). These additional specimens showed little variation in relation to the CAS specimen described above. In BMNH 1964.664, the right hemipenis showed 15 large spines at the last row around the organ and the hemipenis of BMNH 72.10.11.13 was slightly longer, extending to the end of sc 7.

Calliophis nigrescens (BNHS 3348)

Individual of moderate size, 503 mm in total length. The tail is moderately long, 64 mm (12.7% of total length) and 39 subcaudals long (not counting tip). The left hemipenis was dissected and examined *in situ* prior to being everted. The left hemipenis bifurcates at the beginning of sc 5 and terminates at the end of the same scale. The left hemipenis, removed and fully everted and expanded (Fig. 3B), is bilobed, about 6 mm in length and 1.7 mm in width, at apices. The organ includes a pedicel with tiny spines for the first 1.5 mm. Between 1.5 mm and the tips of the lobes

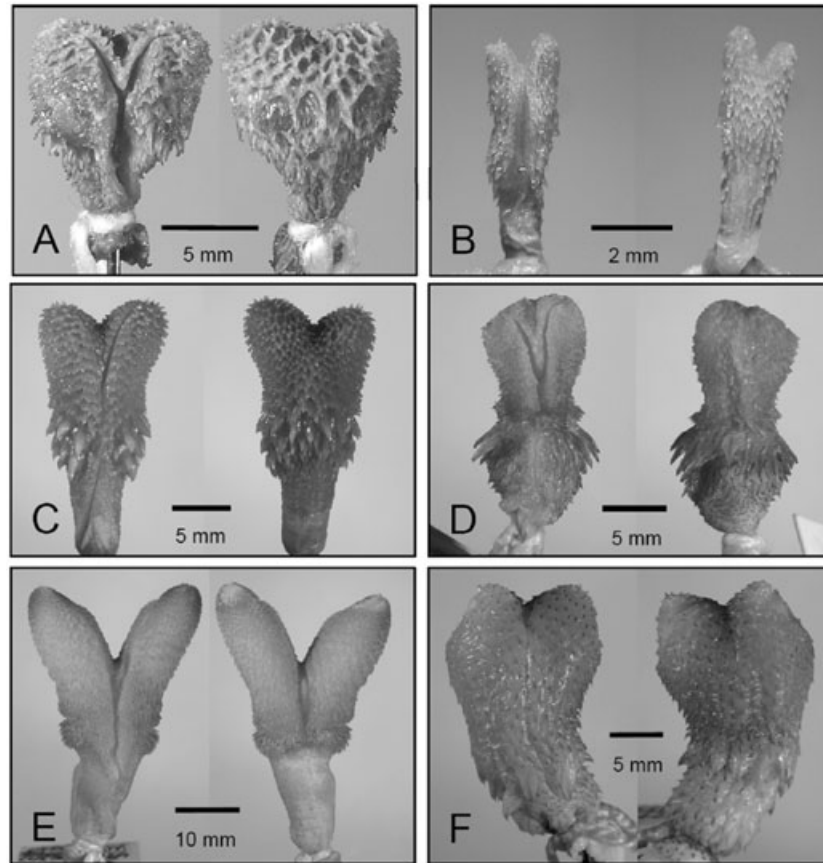


Figure 3. Selected hemipenes of Asian and African Elapidae. A, *Hemibungarus calligaster*, UTA R-7243, right hemipenis; B, *Calliophis nigrescens*, BNHS 3348, left hemipenis; C, *Bungarus caeruleus*, UTA R-7168, right hemipenis; D, *Bungarus fasciatus*, UTA R-24697, right hemipenis; E, *Naja naja*, UTA R-24702, right hemipenis; F, *Dendroaspis polylepis*, UTA R-25373, right hemipenis. Sulcate side shown on the left, asulcate on the right.

there are spines all around the hemipenis. At mid-hemipenis there are 17 spines around the organ. Spines diminish in size distally, from about 0.3 mm at about 2 mm from the base to about 0.1 mm near the tips. The hemipenis bifurcates 0.8 mm before the terminus and the *sulcus spermaticus* bifurcates approximately 0.3 mm before the hemipenial furcation. The *sulcus spermaticus* is bordered at the base (dextrally) by a flap-like fold, is centripetal and terminates distally on each lobe. There are no grooves, flounces, papillae or calyces.

Bungarus caeruleus (UTA R-7168)

A large individual, 1410 mm in total length. The tail is moderately long, 182 mm (12.9% of total length) and 49 sc long (not counting tip). The left hemipenis was dissected and the description is based on inspection *in situ* and everted. The *m. retractor penis magnus* inserts first to a vertebra at the level of sc 17. The hemipenis ends at level of sc 6. The right hemipenis was examined uneverted *in situ*. It complies

with the above description of the left organ. The spines of the right hemipenis are extremely small at the pedicel, like tiny embedded spicules. They abruptly become larger at the level of sc 3, and attain maximal size at level of sc 4. From this point on, the hemipenis becomes calyculate and the spines become small and restricted to the rim of the calyces. At the level sc 4, spines reach 4.5 mm in length. The *sulcus spermaticus* remains ventro-dextral from the base to the level of the end of sc 4, where it divides dorsally and ventrally, and proceeds onto each lobe, extending to the end of the organ. The hemipenis terminates at the level of the caudal edge of sc 6 and appears moderately bilobed.

The cloacal scent gland is elongated and reaches the level of the middle of sc 5 on the left side, and the end of sc 4 on the right. The right hemipenis, when removed and fully everted and expanded (Fig. 3C), is about 25 mm in length and 10 mm in width. The organ begins with a short and naked pedicel for about 5 mm. Spines appear very tiny for the next 5 mm and

then abruptly increase in size. For the next 5 mm, the spines are large and seem slightly larger and hook-like close to the *sulcus spermaticus*. Spines then become small and restricted to calyces. Calyces appear papillated towards the tip of the lobes. The organ is bilobed. At 7 mm the spines are relatively well separated from each other. There are 13 large spines below the calyculate distal portion. The *sulcus spermaticus* bifurcates within the calyculate portion, at one-fifth the distance to the tip, and it is centripetal. There is no groove demarcating a capitulum (Slowinski, 1994).

Bungarus fasciatus (UTA R-24697)

A specimen of 1240 mm in total length with a relatively short tail, 112 mm (9.0% of total length) and 34 subcaudals long (not counting tip). The left hemipenis is described *in situ* and everted. The *m. retractor penis magnus* of both left and right hemipenes insert first to vertebrae at the level of sc 20. The cloacal scent gland is elongated and reaches the end of sc 5. The right hemipenis when removed and fully everted and expanded (Fig. 3D) is about 22 mm in length and 14 mm in width. The organ consists of a short and naked pedicel for about 3 mm. Spines appear very small for the next 4 mm and then begin increasing in size. The spines are largest and hook-like at 12 mm, with larger spines occurring on the lateral and asulcate surfaces of the organ. They are particularly small close to the *sulcus spermaticus*. There are 21 hooks on the last row before the distal calyculate area; the largest of these hooks is 4.6 mm in length. There is a groove demarcating the beginning of the calyculate area. Spines are very small distal to the groove and become even smaller and restricted to the calyces distally. The lobes contain calyces with no spines or papillae at the tips. The organ is slightly bilobed. The *sulcus spermaticus* bifurcates about one-third before the end of the hemipenis, within the calyculate portion. The hemipenis is centripetal.

Naja naja (UTA R-24702)

A medium-sized individual, 1536 mm in total length. The tail is very long, 237 mm (15.4% of total length) and 52 subcaudals long (not counting tip). The right hemipenis was dissected and is described *in situ*, as well as removed and everted. The *m. retractor penis magnus* inserts first to a vertebra at the level of sc 29. The cloacal scent gland is relatively short, thin and ovoid, reaching sc 3. The right hemipenis, when removed and fully everted and expanded (Fig. 3E), is about 40 mm in length and 25 mm in width at the apices. The organ consists of a pedicel with small spines for the first 15 mm, except for a sunken, flat and oval-shaped surface 10 mm long and 2 mm wide on the inner side (sinistral). This oval surface is

bordered on each side by a thick fold. Between 15 and 20 mm from the base, there is a bulging area around the hemipenis with abruptly enlarged spines (1.0–1.5 mm) in 3–6 rows. This area is delimited above by a groove and is thinner on the mid-asulcate area, and wider on sides. There are about 54 hooks below the groove and in between the borders of the *sulcus spermaticus*. Above the groove spines gradually diminish in size towards the tip of the organ, from 1.0 to 0.1 mm, and are gradually restricted to the rim of calyces. The *sulcus spermaticus* bifurcates about 7 mm before the bifurcation of the organ, is centripetal and terminates towards the asulcate side. There are no flounces.

Dendroaspis polylepis (UTA R-25373)

This is a large individual, measuring 2440 mm in total length. The tail is long, extending 500 mm (20.5% of total length) and 117 subcaudals (not counting tip). The right hemipenis was dissected, described *in situ* and based on the everted organ. The *m. retractor penis magnus* inserts first to a vertebra at the level of sc 25. The cloacal scent gland is thin and very long, reaching the level of the middle of sc 7 on the left and right. The right hemipenis, when removed and fully everted and expanded (Fig. 3F), is about 30 mm in length and 14 mm in width. The organ begins as a pedicel with small spines that gradually increase in size (approximately 0.1–1.0 mm in length) for about 10 mm. At this level a row of 13 hooks appear encircling the hemipenis, with the largest hooks nearest the *sulcus spermaticus* (about 4.3 mm long). Spines gradually decrease in size towards the tips of the organ, to about 0.5 mm. The hemipenis has lateral apical discs, with a smooth central area and a calyculate rim. Each disc is about 7 mm in diameter with no spines or papillae. The *sulcus spermaticus* bifurcates within the spinuous portion, at 8 mm before the terminus of the organ, and it is centripetal. The *sulcus spermaticus* terminates in small fleshy papillae that are directed distally. There is no groove demarcating a capitulum and no flounces, but there is some constriction below and above the row of hooks.

Ophiophagus hannah (UTA R-6813)

A large individual, 4065 mm in total length, with a very long tail measuring 877 mm (21.6% of total length) and 112 subcaudals (not counting tip). The right hemipenis was examined superficially, uneverted and *in situ*. The *m. retractor penis magnus* of the right hemipenis inserts first to a vertebra at the level of sc 69. The hemipenis bifurcates at the level of sc 4. Both lobes of the hemipenis end at different subcaudal scale levels, the dorsal lobe at sc 23 and the ventral at sc 22. The *m. retractor penis magnus*

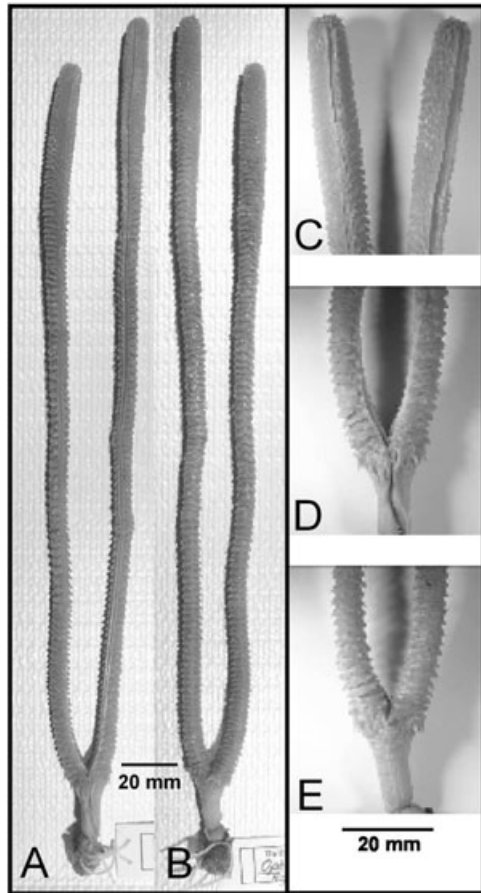


Figure 4. Selected views of right hemipenis of *Ophiophagus hannah*, UTA R-6813. A, sulcate view of complete organ; B, asulcate view of complete organ; C, sulcate inner view of lobes; D, sulcate view of base and furcation; E, asulcate view of base and furcation.

remains divided as lobular branches until the level of sc 39. This characteristic is correlated to the long lobes and early furcation of the organ. The spines of the right hemipenis are extremely small at the pedicel, like tiny embedded spicules. Above the hemipenis is the cloacal scent gland. This gland is oval in shape and reaches the end of sc 3 on the right side, and the middle of sc 4 on the left.

The right hemipenis, once removed and fully everted and expanded (Fig. 4), is approximately 310 mm in length and 16 mm in width at the level of bifurcation. The organ consists of a naked pedicel for the first 30 mm. Spines appear very tiny and sparse for the next 5 mm and then some abruptly increase in size, forming hooks for the next 5 mm. The number of hooks is low; the only row has 13 around the organ. The largest hooks are next to the *sulcus spermaticus* (4.5 mm) and opposite to this on the asulcate face (4.0 mm). The organ and its *sulcus spermaticus* bifur-

cate at the same distance from the base, 40 mm. The lobes are encircled completely by flounces to a distance of 27 mm on the left lobe and 25 mm on the right lobe. The flounces are nearly perpendicular to the main axis of the organ, although they tend to be slightly slanted towards the *sulcus spermaticus*. The flounces continue on the outside of the lobes until about 15 mm before the terminus of the lobes. Flounces are replaced by calyces and the tips consist of small and papillated calyces. There is no groove demarcating a capitulum at the end of the flounced area. The *sulcus spermaticus* ends on the inner surface of the lobe, not on the tips, and is centripetal.

DISCUSSION

ALTERNATIVE ESTIMATES OF PHYLOGENY AMONG METHODS

We used MP, ML and MCMC methods to analyse the molecular phylogenetic data in this study. We identified evidence for a substantial degree of homoplasy (in the mtDNA), potential instances of long branch attraction (Felsenstein, 1978; Huelsenbeck, 1997), and clear heterogeneity of the patterns and rates of nucleotide evolution across the molecular data (e.g. Appendix 3, especially values for the rate scalar [m] and gamma shape [Γ] parameters). These characteristics of the data suggest that the MP (and the unpartitioned ML, but to a lesser extent) may not provide an accurate phylogenetic estimate. For these reasons, and the observation that ML and particularly the partitioned MCMC estimates provide a topology that is much more consistent with previous DNA-based studies and with morphological evidence (elaborated below), we treat ML- and MCMC-based estimates based on the combined data set (which are very similar) as our preferred phylogenetic hypotheses, and use these as a primary basis for discussion of estimates of phylogeny. Furthermore, based on evidence for complex (including partitioned) models providing increased accuracy in phylogenetic analyses (e.g. Huelsenbeck & Rannala, 2004; Lemmon & Moriarty, 2004; Castoe & Parkinson, 2006), we suggest that PP support values based on the All-9x MCMC analyses probably provide the most accurate assessment of nodal support (e.g. Alfaro, Zoller & Lutzoni, 2003; Erixon *et al.*, 2003).

PHYLOGENETICALLY, *HEMIBUNGARUS CALLIGASTER* IS NOT A CORALSNAKE

The taxonomy of Asian elapids, particularly the recognition of genera, has remained in flux for many decades, and has yet to be definitively inferred with strong well-supported phylogenetic evidence. Slowinski *et al.* (2001) were the first to use explicit phylo-

genetic methodology to estimate relationships among Asian and American coralsnakes including *H. calligaster*, although DNA sequence data for this species were unavailable and its relationships were assessed solely based on morphological data. Furthermore, their study included only a few non-coralsnake elapids (two for molecular and three for morphological phylogenetic analyses), and they constrained the monophyly of 'coralsnakes' (including *Hemibungarus*), which was essentially necessary due to the limited inclusion of elapid outgroups in their study. However, this constraint had the effect of limiting possible alternative, non-traditional topological hypotheses to emerge from their analyses, including the phylogenetic clustering of *H. calligaster* with non-coralsnake elapines, rather than coralsnake genera, as we have found in this study.

In this study, although we have not included a majority of the (hydrophiine) genera of elapids, we have included a broad representation of essentially all known major elapid lineages (including all genera of elapines), as well as a diversity of colubroid outgroup taxa to avoid having to constrain elapid relationships (either explicitly or indirectly through limited outgroup inclusion). We found evidence that the coralsnakes exclusive of *Hemibungarus* (*Calliophis*, *Sinomicrurus*, *Micruroides*, *Micrurus* and *Leptomicrurus*) form a clade (ML-BSS = 68, PP = 96). Instead of being grouped with other Asian and American coralsnakes, ML and MCMC estimates place *Hemibungarus* with other elapine genera (ML-BSS < 50, PP = 99). *Hemibungarus* forms a clade with *Elapsoidea*, *Dendroaspis* and *Ophiophagus* (ML-BSS < 50, PP = 93), although support for relationships among members of this clade is weak (Fig. 2). A close relationship between *Elapsoidea*, *Dendroaspis* and *Ophiophagus*, and a distant relationship between these and other non-coralsnake elapines is generally similar to previous estimates (Slowinski & Keogh, 2000). Our molecular phylogenetic results support the recognition of the monotypic *Hemibungarus* as a genus clearly distinct from *Sinomicrurus* or *Calliophis* (e.g. McDowell, 1987; Slowinski *et al.*, 2001). Furthermore, our phylogenetic estimates suggests that *H. calligaster* is not phylogenetically a coralsnake, but rather shares an exclusive common ancestor with the Afro-Asian genera *Ophiophagus*, *Dendroaspis* and *Elapsoidea*.

MORPHOLOGICAL DISTINCTIVENESS OF *HEMIBUNGARUS* AND THE REINTERPRETATION OF HEMIPENIAL, COLORATION AND SCUTELLATION CHARACTERS IN ELAPINE SYSTEMATICS

Molecular phylogenetic evidence presented here provides strong support for: (1) the phylogenetic distinc-

tiveness of *Hemibungarus calligaster*, (2) a distant relationship between other coralsnake genera and *Hemibungarus*, and (3) evidence that the Elapinae comprises two main clades, one including exclusively coralsnake genera, and a second including non-coralsnake genera and *Hemibungarus*. In addition to molecular phylogenetic evidence, further evidence for these three conclusions is apparent when we reconsider the interpretation of hemipenial morphology, colour pattern and head scalation.

Examination of the hemipenes of exemplar Asian and African elapid species reveals substantial variation. The everted hemipenes of *H. calligaster*, *Bungarus caeruleus*, *B. fasciatus*, *Dendroaspis polylepis*, *Naja naja* and *Ophiophagus hannah* (Figs 3, 4) illustrate calyculature not present on the hemipenes of 'true' Asian coralsnakes (see *Calliophis nigrescens*, Fig. 3B) and also absent in American coralsnakes (Slowinski, 1994; Campbell & Lamar, 2004; E. N. Smith, unpubl. data). This calyculature may be restricted to apical discs (*D. polylepis*, Fig. 3F) or include only the tips of the organs (*N. naja*, Fig. 3D), but appears to be always present in non-coralsnake elapine species. Contrastingly, the hemipenes of Asian coralsnakes of the genus *Calliophis* lack calyculature, and their only ornamentation are spines (see Fig. 3B; Slowinski *et al.*, 2001). Slowinski *et al.* (2001), citing Leviton (1964), pointed out that McDowell (1986, 1987) was mistaken in reporting that *H. calligaster* possessed single (non-bifurcated) hemipenes, and here we confirm and illustrate the bilobed condition in *H. calligaster*. We have also found that the hemipenes of *C. nigrescens* are in fact bilobed, unlike *C. gracilis*, *contra* McDowell (1986, e.g. BMNH 98.4.2.27; fig. 5 in Slowinski *et al.*, 2001 [AMNH 2870]). Additionally, we have observed that the hemipenes of both *Calliophis* species have no calyces (contrary to McDowell, 1986), and it appears that the hemipenes of all Asian coralsnakes (*Sinomicrurus* and *Calliophis* spp.) lack calyces. Collectively, the hemipenial character data suggest a close phylogenetic affinity of *H. calligaster* with non-coralsnake elapines, calyculate species.

Slowinski *et al.* (2001) diagnosed *H. calligaster*, in part, as possessing a 1/1 temporal formula and a raised 6th supralabial; however, our observations lead us to an alternative interpretation. We interpret the raised sixth labial as a lower temporal of the first row, rendering the number of labials one less (five) and the temporal formula as 2/2 or 2/3 (Fig. 5). Leviton (1964) also regarded *H. calligaster* as having two primary temporals and six (rarely seven) supralabials. These character conditions are similar to those found in some other species of the Elapinae, particularly non-coralsnake elapine species that we estimate to be most closely related to *H. calligaster* using molecular data (Fig. 2). Many cobras and mambas possess an

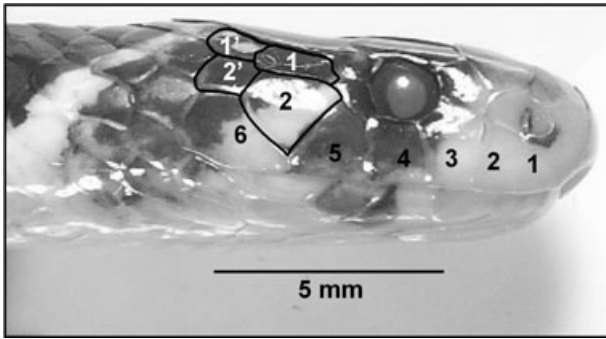


Figure 5. Right lateral view of the head of *Hemibungarus calligaster*, TNHC 62483, showing delineated primary and secondary temporal plates as interpreted in this paper and by Leviton (1964). Lower primary temporal (larger) was interpreted as a sixth raised labial by Slowinski *et al.* (2001). Supralabials and temporals are indicated by numerals.

enlarged lower primary temporal, multiple secondary temporals and as low as six supralabials. Slowinski *et al.* (2001) also pointed out the uniqueness of the band arrangement in *H. calligaster*, consisting of black bands in dyads, as being a diagnostic character. The only other elapid that may possess this arrangement in the tail is *Bungarus flaviceps baluensis* from Borneo (see illustration in Vogel, 2006: 23), implying another potential link between *Hemibungarus* and noncoralsnake elapines.

TOWARD AN UNDERSTANDING OF RELATIONSHIPS AMONG CORALSNAKES

As successive studies have added more resolution regarding relationships among Asian and New World coralsnakes, there are still a number of important (major) lineages that have yet to be confidently placed to frame inclusively the evolutionary history of coralsnakes. This study is, however, the first phylogenetic investigation to include one or more examples of all recognized genera of the Elapinae. Our ML and MCMC results provide strong support (ML-BSS = 88, PP = 99) for a clade consisting of temperate/tropical Asian (*Sinomicrurus*) and New World (*Micruroides*, *Micrurus* and *Leptomicrurus*) coralsnakes, and these estimates (unlike the MP estimates) are in strong agreement with morphological data. Specific morphological synapomorphic evidence for this grouping (*Sinomicrurus* + American coralsnakes) has been identified: the loss of the postorbital bone (McDowell, 1986), the presence of the basal pocket of the hemipenis (Toriba, 1993), a uniformly spinose hemipenis (Slowinski *et al.*, 2001) and the bipartite origin of the *m. adductor mandibulae externus superficialis* muscle

(McDowell, 1986; Slowinski *et al.*, 2001). Additionally, the lack of calyculation on the hemipenes might be considered a synapomorphic character, given that most non-coralsnake Elapinae and Hydrophiinae are calyculate. We also found moderate support (based on ML and MCMC) for the monophyly of American coralsnakes (Fig. 2; ML-BSS = 58, PP = 86), as have other studies based on molecular data (Slowinski & Keogh, 2000; Slowinski *et al.*, 2001).

Aside from the multiple forms of evidence linking *Sinomicrurus* with American coralsnakes, a clear understanding of the relationships of the other Asian coralsnakes (*Calliophis*, *sensu* Slowinski *et al.*, 2001) is lacking. Based on the musculature of the corner of the mouth, McDowell (1987) placed these remaining species into two groups: (1) *Calliophis bibroni*, *C. gracilis* and *C. melanurus*; and (2) *Maticora bivirgata*, *M. intestinalis*, *M. maculiceps* and *M. nigrescens*. Slowinski *et al.* (2001) placed *Maticora* in the synonymy of *Calliophis* based on results of their morphological phylogeny estimate that arrived at a polytomy among members of these two genera. Our study, as well as the molecular data set of Slowinski *et al.* (2001), only included one species of *Calliophis* (*C. [Maticora] bivirgata*) that we estimated to be the sister lineage of the remaining Asian and New World coralsnakes (ML-BSS = 66, PP = 96; Fig. 2; as in Slowinski *et al.*, 2001).

The morphological data set of Slowinski *et al.* (2001) does provide phylogenetic evidence that *Calliophis* (including *Maticora*) is monophyletic, and the monophyly of *Calliophis* is supported by morphological synapomorphies: possession of a single elongate temporal scale, *m. adductor mandibulae externus superficialis* originating at the Harderian gland, and the posterodorsal extension of the Harderian gland (Slowinski *et al.*, 2001). These data suggest that all *Calliophis* species not included in this study form a clade with *C. bivirgata*, collectively implying that a monophyletic *Calliophis* forms the sister group to all other American and Asian coralsnakes. The hypothesis of a monophyletic coralsnake clade (excluding *Hemibungarus*), and the validity of *Calliophis* (*sensu* Slowinski *et al.*, 2001), however, remain to be tested further with molecular data including expanded sampling of Asian coralsnake species.

Our sampling of New World coralsnakes included only a few representatives of this diverse group, although we have purposefully included a representative of each putative 'major' clade identified by Slowinski (1995). Our ML and MCMC estimates are congruent with nearly all previous studies based on molecular and morphological data in placing *Micruroides* as the sister lineage to all other New World coralsnakes (ML-BSS = 58, PP = 86; e.g. Roze & Bernal-Carlo, 1987; Slowinski, 1995; Slowinski *et al.*,

2001; see also Gutberlet & Harvey, 2004). Our results also agree with Slowinski (1995; contrary to Roze & Bernal-Carlo, 1987) by providing well-supported evidence that *Leptomicrurus* renders *Micrurus* paraphyletic. Although Slowinski (1995) suggested the synonymy of *Leptomicrurus* with *Micrurus*, we have retained the use of the name *Leptomicrurus* (see also Campbell & Lamar, 2004) because this group of coral-snakes is distinctive (morphologically and genetically) and probably monophyletic (Roze & Bernal-Carlo, 1987; Slowinski, 1995; Campbell & Lamar, 2004), and a thorough understanding of coral-snake relationships may eventually facilitate the dissection of the large genus *Micrurus* (~ 70 spp.) into multiple distinct genera (and the continued recognition of *Leptomicrurus*). Recently, some of us (Smith, Parkinson, J. A. Campbell and Castoe) have initiated a large-scale investigation of relationships among Asian and New World coral-snakes. Our research continues to investigate the relationships among Asian *Calliophis* and *Sinomicrurus*, as well as the relationships among the New World coral-snakes using morphological and molecular phylogenetic data, and should eventually resolve many of the outstanding questions surrounding coral-snake evolution and systematics.

HIGHER-LEVEL RELATIONSHIPS AMONG ELAPIDS

A majority of authorities divide the Elapidae into two groups, the Hydrophiinae and Elapinae (Hydrophiidae and Elapidae), based largely on characters associated with the kinetic morphology of the skull (McDowell, 1970), differentiating between the 'palatine draggers' and the 'palatine erectors'. McDowell (1970) showed that the Afro-Asian cobras, Asian kraits, Asian and American coral-snakes, *Laticauda* and *Parapristocalamus* were 'palatine erectors' (Elapinae), and the remaining Australo-Paupan terrestrial elapids and sea snakes were 'palatine draggers' (Hydrophiinae). Subsequently, other studies have provided evidence for *Laticauda* being an early diverging lineage within the Hydrophiinae (palatine draggers, e.g. Cadle & Gorman, 1981; Schwaner *et al.*, 1985; Slowinski *et al.*, 1997; Keogh, Shine & Donnellan, 1998; Scanlon & Lee, 2004), implying that the palatine characteristics in *Laticauda* and probably *Parapristocalamus* (Scanlon & Lee, 2004) may be either convergent or symplesiomorphic.

Based on analyses of our molecular data, some differences in phylogenetic signal for higher-level relationships among elapids were evident between the mtDNA and c-mos data set. Like the combined data analysis, the mtDNA data supported (Hydrophiinae (coral-snake elapines, Afro-Asian non-coral-snake elapines)). The c-mos data suggested an alternative arrangement with the Hydrophiinae nested within

the Elapinae, and a more basal divergence of coral-snakes: (coral-snake elapines (Hydrophiinae, Afro-Asian elapines)). It is important to note, however, that we observed drastic variation in branch lengths based on c-mos across elapids, particularly among the hydrophiine genera, which may lead to inaccurate phylogenetic estimates when this locus is used as the sole data set for phylogenetic reconstruction.

The results of our combined data ML and MCMC analyses (and particularly the MCMC) support the existence of the Hydrophiinae and Elapinae as the two primary lineages of elapid snakes (Fig. 2). Support values for the monophyly of the Elapinae (ML-BSS < 50, PP = 85) and the monophyly of the Hydrophiinae (ML-BSS < 50, PP = 98) were low based on ML, but moderately to substantially higher based on the partitioned MCMC analyses of the combined data set (Fig. 2). We also found support for the Elapinae being composed of two main clades: the coral-snakes (excluding *Hemibungarus*; ML-BSS = 66, PP = 96), and a second clade containing the Afro-Asian cobras, Asian kraits, and mambas (ML-BSS < 50, PP = 99; Fig. 2). This deep division among elapine snakes was also suggested by some of the results of Slowinski & Keogh (2000), and is compatible with the partially resolved phylogenetic topologies estimated from sequences of venom proteins (Slowinski *et al.*, 1997). This division of elapine taxa is also indicated by hemipenial characters (particularly the presence or absence of calyces as discussed above; see also Keogh, 1999).

Given the concordance of several lines of evidence (our phylogenetic estimates, previous phylogenetic estimates and hemipenial data), we suggest that the two main clades of elapines identified in ML and MCMC analyses be assigned taxonomic status. We suggest the name **Hemibungarini** for the clade encompassing the common ancestor of *Hemibungarus* (Peters, 1862; designated as the type genus) and the Afro-Asian kraits, mambas and cobras (Fig. 2). These snakes are palatine draggers possessing calyculate hemipenes. We suggest the name **Calliophini** (*Calliophis*, Gray, 1835; designated as the type genus) for the second of these elapine clades that contains the Asian and American coral-snakes. This second group consists of palatine draggers that possess no calyulation of the hemipenes as a synapomorphy. As such, both clades are assigned tribal status under the Elapinae. This new tribal-level taxonomy for members of the Elapinae should provide added convenience for future reference.

ACKNOWLEDGEMENTS

We thank Jill Castoe and Juan Daza for constructive comments on and suggested corrections to the manu-

script. Funding for this project was provided by a UCF startup package to C.L.P., a grant from Bioclon to E.N.S., and an NSF Collaborative Research grant to C.L.P. and E.N.S. (DEB-0416000, 0416160). Funding for R.M.B.'s fieldwork was provided by grants from the American Society of Ichthyologists and Herpetologists, the Society for the Study of Amphibians and Reptiles, the Society of Systematic Biologists, and the National Science Foundation. Collin McCarthy at BMNH kindly provided space and material to E.N.S. for nearly a month, and Varad Giri at BNHS kindly provided space and material to E.N.S. and T.A.C. during several weeks of examining coral snakes. Travis LaDuc (TNHC) and Jens Vindum (CAS) kindly provided loans of specimens under their care. Several researchers provided tissues under their care and obtained during sponsored research, including Laurie Vitt (University of Oklahoma, obtained through NSF grants DEB-9200779 and DEB-9505518), Jonathan Campbell (University of Texas at Arlington, obtained through NSF grants DEB-9705277 and DEB-0102383), Cesar Jaramillo (Círculo Herpetológico de Panamá), William Duellman (KU) and Robert Murphy (ROM).

REFERENCES

- Akaike H. 1973.** Information theory and an extension of the maximum likelihood principle. In: *Second International Symposium on Information Theory*. Budapest: Akademiai Kiado, 673–681.
- Akaike H. 1974.** A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**: 716–723.
- Alfaro ME, Zoller S, 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. *Molecular Biology and Evolution* **20**: 255–266.
- Arévalo ES, Davis SK, Sites JW Jr. 1994.** Mitochondrial DNA sequence divergence and phylogenetic relationships of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology* **43**: 387–418.
- Bogert CM. 1940.** Herpetological results of the Vernay Angola Expedition: with notes on African reptiles in other collections. Part 1, Snakes, including an arrangement of African Colubridae. *Bulletin of the American Museum of Natural History* **77**: 1–107.
- Brandley MC, Schmitz A, Reeder TW. 2005.** Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. *Systematic Biology* **54**: 373–390.
- Cadle JE, Gorman GC. 1981.** Albumin immunological evidence and relationships of sea snakes. *Journal of Herpetology* **15**: 329–334.
- Campbell JA, Lamar WW. 1989.** *The venomous reptiles of Latin America*. Ithaca: Cornell University Press.
- Campbell JA, Lamar WW. 2004.** *The venomous reptiles of the Western Hemisphere*. Ithaca: Cornell University Press.
- Castoe TA, Doan TM, Parkinson CL. 2004.** Data partitions and complex models in Bayesian analysis: the phylogeny of gymnophthalmid lizards. *Systematic Biology* **53**: 448–469.
- Castoe TA, Parkinson CL. 2006.** Bayesian mixed models and the phylogeny of pitvipers (Serpentes: Viperidae). *Molecular Phylogenetics and Evolution* **39**: 91–110.
- Castoe TA, Sasa M, Parkinson CL. 2005.** Modeling nucleotide evolution at the mesoscale: the phylogeny of the Neotropical pitvipers of the *Porthidium* group (Viperidae: Crotalinae). *Molecular Phylogenetics and Evolution* **37**: 881–898.
- Cope ED. 1895.** The classification of the ophidia. *Transactions of the American Philosophical Society* **18**: 186–219.
- Dowling HG, Savage JM. 1960.** A guide to the snake hemipenis: a survey of basic structure and systematic characteristics. *Zoologica* **45**: 17–30.
- Erixon SP, Britton B, Oxelman B. 2003.** Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology* **52**: 665–673.
- Felsenstein J. 1978.** Cases in which parsimony or compatibility methods will be positively misleading. *Systematic Zoology* **27**: 401–410.
- Felsenstein J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.
- Gelman A, Rubin DB. 1992.** Inference from iterative simulation using multiple sequences (with discussion). *Statistical Science* **7**: 457–511.
- Golay P, Smith HM, Broadley DG, Dixon JR, McCarthy C, Rage J-C, Schatti B, Toriba M. 1993.** *Endoglyphs and other major venomous snakes of the world: a checklist*. Geneva: Azemiops.
- Gray JE. 1832–1835.** *Illustrations of Indian Zoology; Chiefly Selected from the Collection of Major-General Hardwicke*, Vol. II. (parts XI–XX). London: Adolphus Richter and Co. & Parbury, Allen, and Co.
- Gutberlet RL Jr, Harvey MB. 2004.** The evolution of New World venomous snakes. In: Campbell JA, Lamar WW, eds. *The venomous reptiles of the Western Hemisphere*. Ithaca: Cornell University Press, 634–682.
- Hasegawa M, Kishino H, Yano T. 1985.** Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **21**: 160–174.
- Hillis DM, Bull JJ. 1993.** An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**: 182–192.
- Huelsenbeck JP. 1997.** Is the Felsenstein zone a fly trap? *Systematic Biology* **46**: 69–74.
- Huelsenbeck JP, Larget B, Miller R, Ronquist F. 2002.** Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology* **51**: 673–688.
- Huelsenbeck JP, Rannala B. 2004.** Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology* **53**: 904–913.
- Kass RE, Raftery AE. 1995.** Bayes factors. *Journal of the American Statistical Association* **90**: 773–795.

- Keogh JS. 1998.** Molecular phylogeny of elapid snakes and a consideration of their biogeographic history. *Biological Journal of the Linnean Society* **63**: 177–203.
- Keogh JS. 1999.** Evolutionary implications of hemipenial morphology in the terrestrial Australian elapid snakes. *Zoological Journal of the Linnean Society* **125**: 239–278.
- Keogh JS, Scott IAW, Scanlon JD. 2000.** Molecular phylogeny of viviparous Australian elapid snakes: affinities of *Echiopsis atriceps* (Storr, 1980) and *Drysdalia coronatus* (Schlegel, 1837), with description of a new genus. *Journal of Zoology, London* **252**: 315–326.
- Keogh JS, Shine R, Donnellan SC. 1998.** Phylogenetic relationships of terrestrial Australo-papuan elapid snakes (subfamily Hydrophiinae) based on Cytochrome b and 16s rRNA sequences. *Molecular Phylogenetics and Evolution* **10**: 67–81.
- Lawson R, Slowinski JB, Crother BI, Burbrink FT. 2005.** Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. *Molecular Phylogenetics and Evolution* **37**: 581–601.
- Lemmon AR, Moriarty EC. 2004.** The importance of proper model assumption in Bayesian phylogenetics. *Systematic Biology* **53**: 265–277.
- Leviton AE. 1964.** Contributions to a review of Philippine snakes III. The genera *Maticora* and *Calliophis*. *Philippine Journal of Science* **92**: 523–550.
- Leviton AE, Gibbs RH Jr, Heal E, Dawson CE. 1985.** Standards in herpetology and ichthyology: part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. *Copeia* **1985**: 802–832.
- McDowell SB. 1967.** *Aspidomorphus*, a genus of New Guinea snakes in the family Elapidae, with notes on related genera. *Journal of Zoology, London* **151**: 497–543.
- McDowell SB. 1969.** *Toxicocalamus*, a New Guinea genus of snakes of the family elapidae. *Journal of Zoology, London* **159**: 443–511.
- McDowell SB. 1970.** On the status and relationships of the Solomon Island elapid snakes. *Journal of Zoology, London* **161**: 145–190.
- McDowell SB. 1986.** The architecture of the corner of the mouth of colubroid snakes. *Journal of Herpetology* **20**: 353–407.
- McDowell SB. 1987.** Systematics. In: Seigel RA, Collins JT, Novak SS, eds. *Snakes: ecology and evolutionary biology*. New York: MacMillan, 3–50.
- Mengden GA. 1985.** Australian elapid phylogeny: a summary of the chromosomal and electrophoretic data. In: Grigg G, Shine R, Ehmann H, eds. *Biology of Australian frogs and reptiles*. Sydney: Surrey Beatty and Sons, 185–192.
- Myers CW, Cadle JE. 2003.** On the snake hemipenis, with notes on Psomophis and techniques of eversion: a response to Dowling. *Herpetological Review* **34**: 295–302.
- Nicholas KB, Nicholas HB Jr. 1997.** *GeneDoc: a tool for editing and annotating multiple sequence alignments*. Available at <http://www.cris.com/~Ketchup/genedoc.shtml>
- Nylander JAA. 2004.** *MrModeltest v2*. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.
- Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL. 2004.** Bayesian phylogenetic analysis of combined data. *Systematic Biology* **53**: 47–67.
- Parkinson CL, Campbell JA, Chippindale PT. 2002.** Multigene phylogenetic analyses of pitvipers; with comments on the biogeographical history of the group. In: Schuett GW, Höggren M, Douglas ME, Greene HW, eds. *Biology of the vipers*. Salt Lake City: Eagle Mountain Publishing, 93–110.
- Peters WCH. 1862.** Präparate vor zur craniologischen Unterscheidung der Schlangengattung *Elaps* und machte eine Mittheilung über eine neue Art der Gattung *Simotes*, *S. semicinctus*. *Monatsberichte der Deutschen Akademie der Wissenschaft zu Berlin* **1862**: 635–638.
- Posada D, Buckley TR. 2004.** Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**: 793–808.
- Rambaut A, Drummond AJ. 2003.** *Tracer*, Version 1.0.1. Available at <http://evolve.zoo.ox.ac.uk/>
- Rannala B. 2002.** Identifiability of parameters in MCMC Bayesian inference of phylogeny. *Systematic Biology* **51**: 754–760.
- Ronquist F, Huelsenbeck JP. 2003.** MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Roze JA. 1996.** *Coral snakes of the Americas: biology, identification, and venoms*. Malabar, FL: Kreiger.
- Roze JA, Bernal-Carlo A. 1987.** Las serpientes corales venenosas del género *Leptomicrurus* (Serpentes, Elapidae) de Suramérica con descripción de una nueva subespecie. *Bolletino del Museo Regionale di Scienze di Torino* **5**: 573–608.
- Sakamoto Y, Ishiguro M, Kitagawa G. 1986.** *Akaike information criterion statistics*. New York: Springer.
- Savage JM. 2002.** *The amphibians and reptiles of Costa Rica*. Chicago: The University of Chicago Press.
- Scanlon JD, Lee MSY. 2004.** Phylogeny of Australasian venomous snakes (Colubroidea, Elapidae, Hydrophiinae) based on phenotypic and molecular evidence. *Zoologica Scripta* **33**: 335–366.
- Schwaner TD, Baverstock PR, Dessauer HC, Mengden GA. 1985.** Immunological evidence for the phylogenetic relationships of Australian elapid snakes. In: Grigg G, Shine R, Ehmann H, eds. *Biology of Australian frogs and reptiles*. Sydney: Surrey Beatty and Sons, 177–184.
- Slowinski JB. 1994.** A phylogenetic analysis of *Bungarus* (Elapidae) based on morphological characters. *Journal of Herpetology* **28**: 440–446.
- Slowinski JB. 1995.** A phylogenetic analysis of the New World coral snakes (Elapidae: *Leptomicrurus*, *Micruroides*, and *Micrurus*) based on allozymatic and morphological characters. *Journal of Herpetology* **29**: 325–338.
- Slowinski JB, Boundy J, Lawson R. 2001.** The phylogenetic relationships of Asian coral snakes (Elapidae: *Calliophis* and *Maticora*) based on morphological and molecular evidence. *Herpetologica* **57**: 233–245.

- Slowinski JB, Keogh JS. 2000.** Phylogenetic relationships of elapid snakes based on cytochrome *b* mtDNA sequences. *Molecular Phylogenetics and Evolution* **15**: 157–164.
- Slowinski JB, Knight A, Rooney AP. 1997.** Inferring species trees from gene trees: a phylogenetic analysis of the Elapidae (Serpentes) based on the amino acid sequences of venom proteins. *Molecular Phylogenetics and Evolution* **8**: 349–362.
- Slowinski JB, Lawson R. 2002.** Snake phylogeny: evidence from nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution* **24**: 194–202.
- Swofford DL. 2002.** *PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.0b10*. Sunderland, MA: Sinauer Associates.
- Tavaré S. 1996.** Some probabilistic and statistical problems on the analysis of DNA sequences. In: Miura RM, ed. *Some mathematical questions in biology—DNA sequence analysis*. Providence, RI: American Math Society, 57–86.
- Toriba M. 1993.** A karyotypic and morphological comparison of *Calliophis japonicus* with coral snakes. *Japan Journal of Herpetology* **15**: 91–92.
- Vogel G. 2006.** *Venomous snakes of Asia, Giftschlangen Asiens*. Frankfurt am Main: Edition Chimaira.
- Wiegmann AFA Jr. 1834.** Beiträge zur Zoologie, gesammelt auf einer Reise um die Erd von F. J. F. Meyen, M. D., A. D. N. Siebente Abhandlung. *Amphibien Nova Acta Leopoldina* **17**: 185–268.
- Wiens JJ. 1998.** Combining data sets with different phylogenetic histories. *Systematic Biology* **47**: 568–581.
- Yang Z. 1996.** Maximum likelihood models for combined analyses of multiple sequence data. *Journal of Molecular Evolution* **42**: 587–596.
- Zaher H, Prudente ALC. 2003.** Hemipenes of *Siphlophis* (Serpentes, Xenodontinae) and techniques of hemipenial preparation in snakes: a response to Dowling. *Herpetological Review* **34**: 302–307.

APPENDIX 1

Specimens examined for comparative morphological characters. Museum acronyms follow Leviton *et al.* (1985).

Bungarus caeruleus: UNKNOWN: (UTA R-7168).
Bungarus fasciatus: THAILAND: (UTA R-24697).
Calliophis gracilis: SINGAPORE: (BMNH 98.4.2.27).
Calliophis nigrescens: INDIA: *Maharashtra*: Amboli (BNHS 3348).
Dendroaspis polylepis: BURUNDI: (UTA R-25373).
Hemibungarus calligaster: PHILIPPINES: *Negros Oriental*: Dumaguete (CAS SU 7243); NE slope Cuernos de Negros (BMNH 1964.664); *Unknown*: (BMNH 72.10.11.13, 72.10.11.18, RR); *Luzon*: Albay Province: Municipality Malinao: Barangay Tagoytoy, Sitio Kumangingking, Mt. Malinao, 700 m (TNHC 62483).
Naja naja: THAILAND: (UTA R-24702).
Ophiophagus hannah: UNKNOWN: (UTA R-6813).

APPENDIX 2

Bayes factors ($2\ln B_{10}$) across alternative models for the mtDNA and c-mos data sets. Values above the diagonal show the Bayes factor support for model M_1 over model M_0 (values considered 'strong evidence' for M_1 over M_0 appear in bold). Values below the diagonal show Bayes factor ($2\ln B_{10}$) support for M_0 over M_1 (bold indicates 'strong evidence' for M_0 over M_1). See text for justification of critical values for interpreting Bayes factors and descriptions of models.

M_0	M_1 mtDNA-1x	mtDNA-2xA	mtDNA-2xB	mtDNA-3x	mtDNA-4x	mtDNA-6x
mtDNA-1x	–	14.94	1228.92	1476.3	1257.88	1513.48
mtDNA-2xA	–14.94	–	1213.98	1461.36	1242.94	1498.54
mtDNA-2xB	–1228.92	–1213.98	–	247.38	28.96	284.56
mtDNA-3x	–1476.3	–1461.36	–247.38	–	–218.42	290.62
mtDNA-4x	–1257.88	–1242.94	–28.96	218.42	–	255.6
mtDNA-6x	–1513.48	–1498.54	–284.56	–37.18	–255.6	–
M_0	c-mos-1x		c-mos-2x		c-mos-3x	
c-mos-1x	–		77.52		96.58	
c-mos-2x	–77.52		–		19.06	
c-mos-3x	–96.58		–19.06		–	

APPENDIX 3

Median and 95% credibility interval (in parentheses) of model parameters from Bayesian MCMC analyses of the combined data set conducted under the All-1x (unpartitioned; shaded) and All-9x (partitioned) models. Parameter estimates for each model are based on a total of 6×10^6 post-burnin generations combined from two independent MCMC runs. Partitions of the All-9x model ($P_1 - P_9$) are defined in Table 3. The PSRF of all model parameters were < 1.01 (most were < 1.001) except rate scalar parameters that were < 1.02 .

Model – partition	Ti/Tv	r(C–T)	r(C–G)	r(A–T)	r(A–G)	r(A–C)	r(G–T)
All-1x	–	0.60 (0.56–0.65)	0.02 (0.01–0.02)	0.06 (0.05–0.07)	0.24 (0.20–0.28)	0.04 (0.03–0.04)	0.04 (0.03–0.06)
All-9x- P_1	–	0.42 (0.51–0.34)	0.00 (0.00–0.01)	0.09 (0.05–0.11)	0.34 (0.26–0.42)	0.04 (0.03–0.06)	0.11 (0.07–0.17)
All-9x- P_2	–	0.36 (0.24–0.49)	0.08 (0.05–0.15)	0.04 (0.01–0.08)	0.41 (0.26–0.57)	0.08 (0.04–0.14)	0.01 (0.00–0.03)
All-9x- P_3	–	0.46 (0.35–0.65)	0.07 (0.02–0.11)	0.04 (0.02–0.06)	0.37 (0.22–0.48)	0.00 (0.00–0.01)	0.04 (0.00–0.11)
All-9x- P_4	–	0.47 (0.37–0.57)	0.01 (0.00–0.03)	0.09 (0.06–0.12)	0.29 (0.20–0.40)	0.10 (0.06–0.14)	0.04 (0.02–0.08)
All-9x- P_5	22.32 (13.67–38.67)	–	–	–	–	–	–
All-9x- P_6	–	0.50 (0.34–0.67)	0.02 (0.00–0.05)	0.03 (0.01–0.04)	0.38 (0.24–0.53)	0.00 (0.00–0.01)	0.06 (0.01–0.12)
All-9x- P_7	4.45 (2.39–8.69)	–	–	–	–	–	–
All-9x- P_8	4.41 (2.48–8.22)	–	–	–	–	–	–
All-9x- P_9	–	0.31 (0.23–0.40)	0.14 (0.07–0.22)	0.03 (0.01–0.05)	0.35 (0.26–0.45)	0.09 (0.05–0.16)	0.04 (0.03–0.06)
Model – partition	pi(A)	pi(C)	pi(G)	pi(T)	Γ	pInvar. (I)	m (rate scalar)
All-1x	0.38 (0.36–0.40)	0.31 (0.30–0.33)	0.09 (0.09–0.11)	0.21 (0.20–0.22)	0.57 (0.48–0.65)	0.42 (0.38–0.45)	–
All-9x- P_1	0.42 (0.37–0.47)	0.26 (0.21–0.31)	0.12 (0.09–0.15)	0.20 (0.16–0.24)	0.34 (0.27–0.43)	–	0.32 (0.26–0.42)
All-9x- P_2	0.17 (0.13–0.22)	0.31 (0.25–0.37)	0.14 (0.10–0.18)	0.39 (0.33–0.45)	1.13 (0.31–3.73)	0.58 (0.27–0.70)	0.08 (0.06–0.12)
All-9x- P_3	0.48 (0.44–0.52)	0.31 (0.28–0.34)	0.04 (0.04–0.05)	0.17 (0.15–0.19)	1.64 (1.32–2.05)	–	3.07 (2.61–3.61)
All-9x- P_4	0.35 (0.30–0.40)	0.23 (0.19–0.28)	0.17 (0.12–0.22)	0.25 (0.20–0.30)	0.72 (0.40–1.24)	0.39 (0.22–0.50)	0.26 (0.21–0.37)
All-9x- P_5	0.19 (0.14–0.23)	0.31 (0.26–0.36)	0.11 (0.08–0.14)	0.40 (0.34–0.45)	0.60 (0.30–1.40)	0.62 (0.46–0.73)	0.12 (0.08–0.16)
All-9x- P_6	0.41 (0.37–0.46)	0.35 (0.31–0.38)	0.04 (0.03–0.05)	0.20 (0.18–0.22)	1.34 (1.09–1.68)	0.02 (0.01–0.05)	4.49 (3.96–4.95)
All-9x- P_7	0.28 (0.23–0.34)	0.22 (0.17–0.28)	0.30 (0.24–0.36)	0.20 (0.15–0.25)	–	0.63 (0.36–0.77)	0.02 (0.01–0.03)
All-9x- P_8	0.32 (0.27–0.39)	0.17 (0.13–0.22)	0.22 (0.17–0.27)	0.29 (0.23–0.35)	–	–	0.02 (0.02–0.03)
All-9x- P_9	0.27 (0.22–0.32)	0.17 (0.14–0.22)	0.15 (0.12–0.20)	0.40 (0.35–0.46)	–	–	0.06 (0.05–0.08)

APPENDIX 4

Locality data for specimens sequenced. Museum acronyms follow Leviton *et al.* (1985) except for CH = Círculo Herpetológico de Panamá.

Hemibungarus calligaster: PHILIPPINES: Luzon: Albay Province: Municipality Malinao: Barangay Tagoytoy, Sitio Kumangingking, Mt Malinao, 700 m (TNHC 62483).

Leptomicrurus narducii: ECUADOR: Napo: 0.7 km S Arosemena Tola. (KU 202955).

Micruroides euryxanthus euryxanthus: USA: Arizona: Cochise: Portal, 4800 ft elev. (AMNH R-128233;

LSU tissue H-14737, DNA isolated by Robin Lawson, CAS).

Micrurus fulvius: USA: Florida: Liberty Co., Forest road 105 at Camel Lake. 30°16'39.6"N, 84°59'28.5"E. (CAS-214347).

Micrurus mipartitus: PANAMA: Coclé: Distrito de Penonomé: La Mina (Noroeste del Canal de Panamá, Vertiente Atlántica). (CH-5377)

Micrurus surinamensis: BRAZIL: Rondonia: Rio Formoso, Parque Estadual Guajará Mirim, approx. 90 km N Nova Mamoré. 10°19'17.2"S, 64°33'47.9"W (OMNH 37596; LJV-7110).