Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular Phylogenetics and Evolution 39 (2006) 91–110
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Bayesian mixed models and the phylogeny of pitvipers (Viperidae: Serpentes)

Todd A. Castoe, Christopher L. Parkinson *

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

Received 6 June 2005; revised 2 December 2005; accepted 26 December 2005

Abstract

The subfamily Crotalinae (pitvipers) contains over 190 species of venomous snakes distributed in both the Old and New World. We incorporated an extensive sampling of taxa (including 28 of 29 genera), and sequences of four mitochondrial gene fragments (2.3 kb) per individual, to estimate the phylogeny of pitvipers based on maximum parsimony and Bayesian phylogenetic methods. Our Bayesian analyses incorporated complex mixed models of nucleotide evolution that allocated independent models to various partitions of the dataset within combined analyses. We compared results of unpartitioned versus partitioned Bayesian analyses to investigate how much unpartitioned (versus partitioned) models were forced to compromise estimates of model parameters, and whether complex models substantially alter phylogenetic conclusions to the extent that they appear to extract more phylogenetic signal than simple models. Our results indicate that complex models do extract more phylogenetic signal from the data. We also address how differences in phylogenetic results (e.g., bipartition posterior probabilities) obtained from simple versus complex models may be interpreted in terms of relative credibility. Our estimates of pitviper phylogeny suggest that nearly all recently proposed generic reallocations appear valid, although certain Old and New World genera (*Ovophis*, *Trimeresurus*, and *Bothrops*) remain poly- or paraphyletic and require further taxonomic revision. While a majority of nodes were resolved, we could not confidently estimate the basal relationships among New World genera and which lineage of Old World species is most closely related to this New World group.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Bayesian phylogeny; Crotalinae; Data partitions; MCMC; Mixed models; Pitvipers; Posterior probability credibility; Viperidael

1. Introduction

1.1. Pitvipers and their contemporary systematics

The venomous snake family Viperidae (asps, moccasins, rattlesnakes, and true vipers) includes about 260 species in four subfamilies: Azemiopinae, Causinae, Crotalinae, and Viperinae (McDiarmid et al., 1999). The Crotalinae (pitvipers) is the most species rich of the four subfamilies, containing over 190 species (≈75% of viperid species) allocated to 29 genera (Gutberlet and Campbell, 2001; Malhotra and Thorpe, 2004; McDiarmid et al., 1999; Zhang, 1998; Ziegler et al., 2000). Among viperid groups, pitvipers are also the most widely distributed subfamily, with major radiations of species in the Old World and the New World (Campbell and Lamar, 2004; Gloyd and Conant, 1990; McDiarmid et al., 1999).

Pitviper species produce a wide diversity of proteinaceous venom toxins, and many species are capable of inflicting fatal bites to humans (e.g., Russell, 1980). Accordingly, a valid taxonomy and a robust understanding of relationships among these venomous species are important for systematics, in addition to the fields of medicine, pharmacology, and toxicology (e.g., >3000 citations on PubMed [National Center for Biotechnical Information] for "pit viper venom"). The phylogeny and taxonomy of this group has received substantial research attention that has lead to many revisions to make taxonomy consistent with estimates of phylogeny (see reviews in Campbell and Lamar, 2004; Gutberlet and Harvey, 2004; Malhotra and Thorpe, 2004; Parkinson et al., 2002). Of the 29 generic names in

* Corresponding author. Fax: +1 407 823 5769.
E-mail address: cparkins@mail.ucf.edu (C.L. Parkinson).

1055-7903/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2005.12.014

use, 19 have been recognized in the last three decades (Burger, 1971; Campbell and Lamar, 1989, 1992; Gutberlet and Campbell, 2001; Hoge and Romano-Hoge, 1981, 1983; Malhotra and Thorpe, 2004; Werman, 1992; Zhang, 1998; Ziegler et al., 2000).

The deepest phylogenetic divergences among pitvipers have yet to be resolved with strong support. Current evidence indicates either: (1) a clade containing *Hypnale*, *Calloselasma*, *Deinagkistrodon*, and *Tropidolaemus* as the sister group to the remaining pitvipers (Malhotra and Thorpe, 2004; Parkinson et al., 2002) or, (2) a clade comprised of *Deinagkistrodon* and *Tropidolaemus* as the sister group to the remaining pitvipers (Knight et al., 1992; Parkinson, 1999; Parkinson et al., 2002; Vidal and Lecointre, 1998.

The Old World genus *Trimeresurus* (*sensu lato*; e.g., Burger, 1971) was found to be polyphyletic by a number of studies (e.g., Malhotra and Thorpe, 2000; Parkinson, 1999), and was subsequently dissected into a total of 11 genera, including: *Protobothrops* (Hoge and Romano-Hoge, 1983), *Ovophis* (Burger, 1971; Hoge and Romano-Hoge, 1981), *Zhaoermia* (described as *Ermia* by Zhang, 1993, changed to *Zhaoermia* by Gumprecht and Tillack, 2004), *Triceratolepidophis* (Ziegler et al., 2000), and *Cryptelytrops*, *Garthius*, *Himalayophis*, *Parias*, *Peltopelor*, *Popeia*, and *Viridovipera* (Malhotra and Thorpe, 2004). Despite these changes, recent pitviper phylogenetic estimates suggest that *Ovophis* and *Trimeresurus* (*sensu stricto*) remain polyphyletic (e.g., Malhotra and Thorpe, 2000, 2004; Parkinson et al., 2002).

Kraus et al. (1996) hypothesized that New World pitvipers are monophyletic, and recent molecular studies have shown increasing support for this clade (e.g., Malhotra and Thorpe, 2004; Parkinson, 1999; Parkinson et al., 2002). This contradicts all morphology-based phylogenetic hypotheses (not constraining New World pitviper monophyly) which find a polyphyletic origin of New World pitvipers (Brattstrom, 1964; Burger, 1971; Gloyd and Conant, 1990). Currently, there are twelve genera of New World pitvipers recognized (Campbell and Lamar, 2004) and the relationships among these remain poorly understood and inconsistent across studies. Certain molecular studies (Parkinson, 1999; Parkinson et al., 2002), and the morphological data set of Gutberlet and Harvey (2002), support the earliest New World divergence as being between a temperate North American clade and a Neotropical clade. Within this temperate clade, rattlesnakes (*Crotalus* and *Sistrurus*) have been consistently inferred to be monophyletic, and to be the sister group to a clade containing the cantils/copperheads/moccasins (*Agkistrodon*; Knight et al., 1992; Murphy et al., 2002; Parkinson, 1999; Parkinson et al., 2002; Vidal et al., 1999).

Few relationships among the tropical New World genera are supported by multiple studies, although several notable relationships have been repeatedly identified. A primarily South American bothropoid clade, with *Bothrocophias* inferred as the sister group to *Bothrops* plus *Bothriopsis*, has been found by both morphological and molecular-based studies (Castoe et al., 2005; Gutberlet and Campbell, 2001; Parkinson et al., 2002). Results of several studies have agreed on the paraphyly of *Bothrops* (*sensu stricto*) with respect to *Bothriopsis* (Gutberlet and Campbell, 2001; Knight et al., 1992; Parkinson, 1999; Parkinson et al., 2002; Salomão et al., 1997, 1999; Vidal et al., 1997, 1999; Wüster et al., 2002). Although studies incorporating morphological data disagree (Gutberlet and Harvey, 2002; Werman, 1992), several molecular studies have inferred a clade comprising the primarily Middle American genera *Porthidium*, *Atropoides*, and *Cerrophidion* (Castoe et al., 2003, 2005; Parkinson, 1999; Parkinson et al., 2002).

1.2. Challenges and strategies for resolving pitviper phylogeny

Despite the efforts of numerous authors, phylogenetic relationships within the subfamily Crotalinae remain controversial, particularly at the intergeneric level (e.g., Gutberlet and Harvey, 2004; Malhotra and Thorpe, 2004; Parkinson et al., 2002). Three issues have likely played major roles in the generation of inconsistent conclusions or poor resolution across studies: (1) Only four (Kraus et al., 1996; Malhotra and Thorpe, 2004; Parkinson, 1999; Parkinson et al., 2002) of nearly twenty inter-generic molecular-based studies have included most of the proposed crotaline genera. No study has included a large representation of both Old World and New World genera and species. Limited taxonomic sampling can be problematic in phylogenetic analyses (Hillis, 1998; Poe, 1998; Poe and Swofford, 1999; Salisbury and Kim, 2001), and when only a few representatives of a diverse group are sampled, the resulting phylogenies may represent sampling artifacts (e.g., due to long-branch attraction) rather than accurate and objective phylogenetic reconstructions (Graybeal, 1998; Hillis, 1996, 1998). (2) Many studies (particularly earlier studies) employed only a small gene region to infer inter-generic relationships providing few informative characters. (3) Most DNA-based studies to date have analyzed relationships based on mitochondrial gene sequences. Mitochondrial-based phylogenetics has proven very successful largely because of the rapid rate of sequence evolution characteristic of this genome (Brown et al., 1979; Caccone et al., 1997; Vidal et al., 1999), yielding large proportions of potentially informative (variable) sites. This strength becomes problematic, however, because the probability of continued sequence turnover at sites increases with phylogeny depth. Confident estimation of deeper relationships becomes increasingly difficult as the phylogenetic signal-to-noise ratio becomes unfavorable. This problematic feature of molecular evolution, combined with limited taxon sampling and limited character sampling has synergistically weighed against previous attempts to reconstruct crotaline phylogeny.

Here, we use DNA sequences from four mitochondrial gene regions sampled from a large array of pitviper taxa (including 28 of 29 genera) to estimate pitviper phylogeny.

Our extensive taxonomic sampling design targets difficulties that limited taxon sampling may impose on recovering accurate phylogenetic estimates. Our sampling of gene regions (mitochondrial genes), however, remains potentially susceptible to problems associated with the high rate of sequence evolution characteristic of mitochondrial genes, leading to excessive homoplasy and obscured phylogenetic signal at deeper nodes. We target this latter problem analytically through complex-partitioned modeling of nucleotide evolution during phylogenetic analyses.

Model-based phylogenetic methods (including Bayesian phylogenetic techniques) are particularly useful for reconstructing phylogenies from divergent sequences because they incorporate probabilistic models of DNA substitution that should be less likely to be misled by complexities of DNA evolution (Huelsenbeck, 1995; Huelsenbeck and Crandall, 1997). Multigene datasets, as in this study, may contain partitions (e.g., multiple genes, rRNA versus protein coding genes, codon positions, and types of RNA secondary structures) that evolve under different models (or patterns) of evolution. In these cases, using a single likelihood model for the entire dataset forces a compromise in parameter estimates that must (under a single model) be averaged over the entire dataset. This compromise may lead to systematic error and mislead phylogenetic conclusions (Brandley et al., 2005; Huelsenbeck and Rannala, 2004; Lemmon and Moriarty, 2004; Reeder, 2003; Wilgenbusch and de Queiroz, 2000). Important for our phylogenetic problem, a single compromise model may not capture the range of complexities in nucleotide substitution across the entire mixed dataset. In turn, this compromise may result in increased error identifying substitutions with high likelihoods of change (and homoplasy), versus substitutions with low likelihoods of change (with higher probabilities of containing phylogenetic signal). This type of modeling compromise may also increase the error in reconstructing ancestral states. This problematic compromise may be avoided by allocating independent models of nucleotide evolution to partitions of a heterogeneous dataset (e.g., Nylander et al., 2004; Pagel and Meade, 2004; Yang, 1996).

Model choice may affect both phylogenetic topology (e.g., Huelsenbeck, 1995, 1997; Sullivan and Swofford, 2001) and posterior probability estimation (e.g., Buckley, 2002; Castoe et al., 2004; Erixon et al., 2003; Suzuki et al., 2002). Complex partitioned models may have important effects in the resolution of deeper nodes, a majority of which receive increased support under complex models (Brandley et al., 2005; Castoe et al., 2004, 2005). Complex models appear to be more effective at estimating patterns of molecular evolution when sequences are highly divergent and phylogenetic signal is otherwise obscured by multiple substitutions (Brandley et al., 2005; Castoe et al., 2005; see also Huelsenbeck and Rannala, 2004; Lemmon and Moriarty, 2004).

In this study, we combine taxon sampling and analytical strategies to estimate a robust hypothesis for the phylogeny of pitvipers. Along with maximum parsimony analyses, we implement complex partitioned models of nucleotide evolution (in a Bayesian MCMC framework) to help counter problems likely to have biased previous analyses of pitviper phylogeny. We compare phylogeny and parameter estimates between simple and complex models to identify the impacts that complex models have on phylogenetic inference and on modeling patterns of nucleotide evolution. Based on our estimates of pitviper phylogeny we evaluate the current genus-level taxonomy and discuss the relevance of our estimates to previous phylogenetic and taxonomic hypotheses.

2. Materials and methods

2.1. Taxon sampling

A total of 167 terminals were included in this study. We base our taxonomic assignment of species and genera on Malhotra and Thorpe (2004); McDiarmid et al. (1999) and Campbell and Lamar (2004), unless specifically noted (see, Appendix A). The ingroup, members of the subfamily Crotalinae (pitvipers), were represented by 157 terminals comprising 116 currently recognized species, including 45 Old World, and 71 New World species (Appendix A). Collectively, our sampling included representatives of 28 of 29 genera, excluding only the monotypic Old World genus *Peltopelor*. Outgroup taxa including representatives of the three other subfamilies of viperids (Causinae, Viperinae, and Azemiopinae) were also included so that the monophyly of the Crotalinae could be assessed. We rooted phylogenies with members of the genus *Causus* based on previous suggestions that the Causinae is the sister group to all other viperids (McDiarmid et al., 1999).

2.2. DNA sequencing and sequence alignment

A majority of sequences used in this study have been published previously (Castoe et al., 2003, 2005; Kraus et al., 1996; Malhotra and Thorpe, 2004; Murphy et al., 2002; Parkinson, 1999; Parkinson et al., 1997, 2000, 2002). 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. Four mitochondrial gene fragments were independently PCR amplified and sequenced per sample. The 12s gene was amplified using the primers L1091 and H1557, and the 16s gene was amplified using the primers L2510 and H3059 (described in Parkinson et al., 1997; Parkinson, 1999). The cyt-*b* fragment was PCR amplified using the primers Gludg and AtrCB3 (described in Parkinson et al., 2002) and the ND4 fragment was amplified via PCR using the primers ND4 and LEU or ND4 and HIS as described in Arévalo et al. (1994). Positive 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 (and for ND4, an additional internal primer

HIS; Arévalo et al., 1994). In cases where PCR products were too weak to sequence directly, they were cloned using the Topo TA cloning kit (Invitrogen). Plasmids were isolated from multiple clones per individual using the Qiaquick spin miniprep kit (Qiagen) and sequenced using M13 primers. All 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). Sequences of each fragment were aligned manually in GeneDoc (Nicholas and Nicholas, 1997). Alignment of protein-coding genes was straightforward and included several indels that represented deletions or insertions of complete codons. No internal stop codons were found in either protein coding fragment. Alignment of rRNA genes was based on models of secondary structure for snake mitochondrial rRNAs (Parkinson, 1999). A total of 24 sites were excluded because positional homology was not obvious (all occurred in loop structural regions of rRNA genes), including 10 sites from 12s and 14 sites from 16s. Novel sequences were deposited in GenBank (Accession Nos. DQ305409–DQ305489; Table 1) and the final nucleotide alignment is available online at http://biology.ucf.edu/~clp/.

2.3. Phylogenetic reconstruction

Gaps in alignment were treated as missing data for all phylogenetic reconstructions. Maximum parsimony (MP) and Bayesian Metropolis-Hastings coupled Markov chain Monte Carlo (MCMC) phylogenetic methods were used to reconstruct phylogenies. Both methods were initially used to compare phylogenetic reconstructions based on each gene fragment independently. In general, we expect that mitochondrial loci should all contain phylogenetic signal supporting a common phylogeny because mitochondrial haplotypes are inherited maternally as a single linkage unit. We verified this assumption, prior to combining data, by reconstructing phylogenies of each gene independently and searching for strongly supported incongruent relationships across gene trees (e.g., Wiens, 1998).

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 the heuristic search option with tree bisection reconnection (TBR) branch-swapping option, and 1000 random-taxon-addition sequences to search for optimal trees. Support for nodes in MP reconstructions was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 1000 full heuristic pseudo-replicates (10 random-taxon-addition sequence replicates per bootstrap pseudo-replicate).

MrModeltest v.2.2 (Nylander, 2004) was used to select an appropriate model of evolution for MCMC analyses because this program only considers nucleotide substitution models that are currently available in MrBayes v3.04b (Ronquist and Huelsenbeck, 2003). PAUP* was used to calculate model lilkelihoods for use in MrModeltest. Based on arguments presented by Posada and Buckley (2004), we used AIC (Akaike, 1973, 1974; Sakamoto et al., 1986) to select best-fit models in MrModeltest. In addition to the combined dataset, putative a priori partitions of the dataset

Table 1
Description of complex partitioned models used in the analysis of the combined dataset

Model	Partitions	Free model parameters	Description of partitions	Harmonic mean of marginal likelihood	Akaike weight (A_w)	Relative Bayes Factor (RBF)
1×	1	11	Single model for the entire dataset	−66557.76	0.0000	—
2×	2	22	Protein coding genes; rRNA genes	−66405.69	0.0000	27.65
3×	3	33	Codon positions 1 + 2; codon position 3; rRNA genes	−66337.62	0.0000	20.01
4×A	4	44	12s; 16s; codon positions 1 + 2; codon position 3	−66300.39	0.0000	15.60
4×B	4	44	12s; 16s; ND4; cyt-*b*	−66342.22	0.0000	13.06
5×A	5	51	rRNA stems, rRNA loops, codon position 1; codon position 2; codon position 3	−66195.33	0.0000	18.12
5×B	5	55	12s; 16s; codon position 1; codon position 2; codon position 3	−66255.71	0.0000	13.73
5×C	5	55	rRNA genes; ND4 position 1 + 2; ND4 position 3; cyt-*b* position 1 + 2; cyt-*b* codon position 3	−66043.64	0.0000	23.37
8×	8	84	12s; 16s; ND4 position 1; ND4 position 2; ND4 position 3; cyt-*b* position 1; cyt-*b* position 2; cyt-*b* position 3	−65842.18	0.0000	19.60
10×	10	94	All codon positions or stem and loop regions of each gene allocated independent model (labeled *P*1–10 in Table 2)	−65737.02	1.0000	19.78

Each partition identified above was allocated the model selected by AIC criteria estimated in MrModeltest.

were independently analyzed using MrModeltest to estimate best-fit models of nucleotide evolution. These best-fit models for each partition were implemented as partition-specific models within partitioned-model analyses of the combined dataset, similar to the suggestions of Brandley et al. (2005).

All MCMC phylogenetic analyses were conducted in MrBayes 3.0b4 (Ronquist and 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 in triplicate, with three independent runs initiated with random trees, and run for a total of 4.0×10^6 generations (sampling trees every 100 generations). Conservatively, the first 1.0×10^6 generations from each run were discarded as burn-in. Summary statistics and consensus phylograms with nodal posterior probability support were estimated from the combination of the triplicate set of runs per analysis.

An initial set of MCMC runs (for the individual and combined datasets) was conducted using the model estimated by AIC in MrModeltest for each dataset. In addition to the unpartitioned model selected by AIC for the entire dataset, the combined dataset was subjected to additional MCMC analyses under nine alternative evolutionary models. These additional MCMC analyses were designed to allow independent models of nucleotide evolution to be applied to partitions of the combined dataset. This was accomplished by dividing the dataset into a priori assumed biologically relevant partitions and specifying that an independent (partition-specific) model be used for each partition (using the "unlink" command in MrBayes). For these complex-partitioned models, only branch lengths and topology remained linked between partitions. These mixed models partitioned the combined dataset based on gene fragment type (protein coding or rRNA), gene, codon position (for protein encoding genes), and stem and loop secondary structure (for rRNA genes). The names and details of all models used to analyze the combined dataset are summarized in Table 2. MrBayes blocks containing the settings for various MCMC analyses are available from the authors upon request.

We used three statistics to choose the best-fit partitioned model for analysis of the combined data: (1) Bayes factors (B_{10}), (2) relative Bayes factors (RBF), and (3) Akaike weights (A_w) (as in Castoe et al., 2005). Each of these criteria allow objective evaluation of non-nested partitioned models, which is important here because several alternative 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 and Raferty, 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 for our purposes) if $2\ln B_{10} > 10$ (Kass and Raferty, 1995, see also Nylander et al., 2004).

Relative Bayes factors (RBF; 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 prior modification by the data) of increasingly complex partitioned models. This may provide a simple means of determining the parameter richness of candidate models tested in relation to how complex a model may be justified by the size and heterogeneity of a dataset (Castoe et al., 2005). We calculated the RBF of each complex model by calculating $2\ln B_{10}$ between the base model and each complex (partitioned) model and dividing this by the difference in the number of free model parameters between the base and complex model (Castoe et al., 2005).

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 estimator of the model likelihood from MCMC analyses to incorporate an estimate of the marginalized likelihood of models (following 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., Castoe et al., 2004; Huelsenbeck et al., 2002; Rannala, 2002). We investigated the performance of models (using Tracer; Rambout and Drummond, 2003) by examining features of model 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

Table 2
Results of AIC model selection conducted in MrModeltest for partitions of the dataset

Partition	AIC model
All data	GTR + ΓI
All rRNA	GTR + ΓI
All rRNA, stems	SYM + ΓI
All rRNA, loops	GTR + ΓI
12s	GTR + ΓI
12s, stems (= *P*1)	SYM + ΓI
12s, loops (= *P*2)	HKY + ΓI
16s	GTR + ΓI
16s, loops (= *P*3)	GTR + ΓI
16s, stems (= *P*4)	SYM + ΓI
All protein coding	GTR + ΓI
Positions 1 + 2	GTR + ΓI
Position 1	GTR + ΓI
Position 2	GTR + ΓI
Position 3	GTR + ΓI
cyt-*b*	GTR + ΓI
cyt-*b*, positions 1 + 2	GTR + ΓI
cyt-*b*, position 1 (= *P*5)	GTR + ΓI
cyt-*b*, position 2 (= *P*6)	HKY + ΓI
cyt-*b*, position 3 (= *P*7)	GTR + ΓI
ND4	GTR + ΓI
ND4, positions 1 + 2	GTR + ΓI
ND4, position 1 (= *P*8)	GTR + ΓI
ND4, position 2 (= *P*9)	GTR + ΓI
ND4, position 3 (= *P*10)	GTR + ΓI

posterior distributions (considered evidence that a model was not over-fit). We also examined the model parameter estimates to confirm that the shape of their posterior distributions reflected a substantial modification of the priors (indicating their identifiability based on the data). As a secondary validation that the partitioning of the dataset was justified, we graphically compared posterior distributions of parameter estimates across partitions to confirm that, in fact, different partitions demonstrated unique posterior distributions of parameter estimates.

3. Results

3.1. Properties of the dataset

The final alignment of all four gene fragments concatenated consisted of a total of 2306 aligned positions: 417 from 12s, 503 from 16s, 717 from cyt-*b*, and 669 from ND4. This alignment contained 1105 parsimony-informative characters and 906 invariant characters.

The greatest pairwise sequence divergence (uncorrected percent divergence) across all taxa was 20.8% (*Causus resimus* and *Bothrops atrox*), and 17.7% among crotaline taxa (*Calloselasma rhodostoma* and *Sistrurus miliarius*). The maximum divergence among Old World pitvipers was 16.4% (*C. rhodostoma* and *Cryptelytrops venustus*), and 16.2% among New World pitvipers (*Porthidium porrasi* and *Crotalus transverses*). The mean divergence between Old and New World pitvipers was 12.9%.

Individual gene phylogenies generally suffered from poor resolution and low support under MP and MCMC analyses. No instances of strongly supported differences across individual gene trees were observed, providing evidence for the assumption that individual genes supported a common phylogeny and are appropriate for combined data analysis. Previous studies that have analyzed many of the sequences used in this study have come to the same general conclusion supporting the combinability of these four gene fragments (e.g., Castoe et al., 2005; Malhotra and Thorpe, 2004; Murphy et al., 2002; Parkinson, 1999; Parkinson et al., 2002). Hereafter, we focus exclusively on analyses of the combined dataset of four gene fragments.

3.2. Maximum Parsimony phylogenetic analyses

The MP heuristic search found 12 equally-parsimonious trees, each with 14,816 steps. These trees had a consistency index of 0.162, a retention index of 0.568, and a homoplasy index of 0.838. The strict consensus of these 12 trees, along with nodal bootstrap support (BS hereafter) values, is provided (Fig. 1).

Maximum parsimony phylogenetic estimates (Fig. 1) show strong support for a clade containing the monotypic Azeimopinae (*Azemiops feae*) and the Crotalinae (BS = 100), as well as the sister-group relationship of these two subfamilies (BS = 89). Three ancient clades of pitvipers are inferred by MP analyses: two exclusively Old World clades, and a third containing both Old and New World species, although support for these clades is low. The deepest phylogenetic split among pitvipers is estimated as being between a clade including *Hypnale* and *Calloselasma* and the remaining Crotalinae. Following this divergence, a clade including *Deinagkistrodon*, *Garthius*, and *Tropidolaemus* is estimated to be the sister group to the third ancient pitviper clade comprising the remaining Asiatic and New World species (Fig. 1).

A large clade containing nearly all members of *Trimeresurus sensu lato* was strongly supported (BS = 89), as were a majority of intra and intergeneric relationships within this clade (Fig. 1). *Trimeresurus sensu stricto* is inferred to be polyphyletic, with *Trimeresurus gracilis* distantly related to the remaining members. Monophyly of *Popeia*, *Viridovipera*, and *Parias* received moderate to strong (BS > 74) support, although *Cryptelytrops* was found to be polyphyletic, with a clade containing *C. venustus* and *Cryptelytrops macrops* distantly related to the remaining *Cryptelytrops* species (Fig. 1). *Ovophis* was found to be polyphyletic, with *Ovophis monticola* estimated to be the sister lineage to a clade containing *Triceratolepidophis*, *Zhaoermia*, and *Protobothrops* (Fig. 1). The other representative of this genus included in this study, *Ovophis okinavensis*, was strongly supported as the sister taxon to *T. gracilis*, both forming the sister clade to *Gloydius*. This clade was weakly supported as the sister taxon to a moderately supported (BS = 76) clade including all New World genera (Fig. 1).

The deepest phylogenetic relationships among New World genera were poorly resolved by MP analyses (Fig. 1). The temperate New World genera (*Agkistrodon*, *Sistrurus*, and *Crotalus*) did not form a clade (Fig. 1). *Ophryacus* and *Lachesis* formed a weakly supported clade, inferred as the sister group to *Agkistrodon*. Monophyly of *Ophryacus*, *Lachesis*, and *Agkistrodon* were all strongly supported (BS > 96), and monophyly of *Bothriechis* received weak support (BS = 58). The primarily Middle American genera *Atropoides*, *Cerrophidion*, and *Porthidium* formed a strongly supported (BS = 95) clade inferred to be the sister group to a clade (BS = 100) containing the primarily South American genera *Bothrocophias*, *Bothrops*, and *Bothriopsis*. Within the Middle American group, monophyly of *Porthidium* was well supported (BS = 100). *Atropoides* was inferred to be paraphyletic (BS = 72) with respect to *Cerrophidion* and *Porthidium*, with *Atropoides picadoi* distantly related to other *Atropoides* species. Within the South American group, a *Bothrocophias* clade (BS = 100) was inferred to be the sister taxon to a clade containing a *Bothriopsis* clade (BS = 100) and paraphyletic clustering of *Bothrops* species. Monophyly of the rattlesnakes, *Sistrurus* and *Crotalus*, was strongly supported (BS = 100), with a monophyletic (BS = 89) *Sistrurus* forming the sister taxon to a weakly supported (BS = 57) monophyletic *Crotalus*. Deep phylogenetic relationships among *Crotalus* species generally received weak support (Fig. 1).

Fig. 1. Strict consensus cladogram of 12 equally-parsimonious trees obtained from maximum parsimony analysis of 2306 bp of mitochondrial DNA sequences (14,816 steps, consistency index = 0.162, retention index = 0.568, homoplasy index = 0.838). Bootstrap support for nodes above 50% is given adjacent to nodes; nodes receiving bootstrap support of 100% are indicated by gray-filled circles.

3.3. Selection, evaluation, and comparison of Bayesian MCMC models

The single (unpartitioned) best-fit model for the combined dataset identified by AIC criteria was the GTR + ΓI model (Tavaré, 1996; Table 2; "1×" model in Table 1). In addition to this unpartitioned model, nine other models that allocated an independent model of nucleotide evolution to various partitions of the dataset within a combined data analysis were examined (Table 1). Partition-specific best-fit models selected using AIC criteria in MrModeltest are shown in Table 2, and included one of three different models selected for various partitions: the GTR + ΓI (11 free model parameters), the HKY + ΓI (Hasegawa et al., 1985; 7 free parameters), and SYM + ΓI (a GTR model with fixed equal base frequencies; 7 free parameters).

Across all models for the combined dataset, Akaike weights ($A_w = 1.0000$; Table 1) and Bayes factors ($2\ln B_{10} > 210$; Table 3) provided extremely strong support for the most complex partitioned model examined, 10×, as the best-fit to the combined data. Relative Bayes factors demonstrate that, despite the large number of free model parameters in the 10× model, the average contribution of each parameter to increasing the overall likelihood remains high (RBF = 19.78), compared across other partitioned models (Table 1). Only one model, the 2× model in which protein-coding and rRNA genes were allocated separate models, had a RBF (27.65; Table 1) substantially higher than the 10× model.

The best-fit 10× model showed no indications of being parametrically overfitted, or of poor mixing or convergence. The three independent runs of the 10× model produced identical tree topologies, extremely similar posterior probability estimates (all values within three percentage points, most less than three), and model likelihoods and parameter estimates that were nearly identical. Plots of the model likelihoods through generations from independent runs all show a rapid and direct ascent to a stationary plateau by no later than 200,000 generations (suggesting that burn-in occurred by this period), implying that our exclusion of the first 10^6 generations (as "burn-in") was conservative. Similar to plots of model likelihoods through time, plots of parameter estimates all demonstrated a direct approach to a stationary range, occurring at approximately the same number of generations as likelihood values appeared to reach stationarity (as visualized using Tracer). Based on our model-selection criteria, combined with our inability to identify any problems indicating that the 10× model is excessively parameter rich, we treat phylogenetic estimates based on the 10× model as our favored phylogenetic hypothesis hereafter.

Substantial differences in parameter estimates were observed between the 1× model and the parameters of the 10× partitions, as well as among different partitions of the 10× model (based on parameter means and 95% credibility intervals, CI hereafter; Appendix B). A subset of parameter estimates is shown in Fig. 2. For each of the five parameters plotted across models and partitions, at least two partition-specific parameter estimates (based on CIs) from the 10× model do not overlap with the CI of the analogous parameter from the 1× model (Fig. 2). Among parameter CIs that do overlap between the 1× and 10× partitions, many partitions have parameter estimates in which a majority of posterior density is concentrated outside the 95% CI of the 1× model estimates (Fig. 2). Among model parameters, estimates of the gamma shape parameter (and I parameter, pInvar.) show the least overlap between 10× partitions and the 1× model, followed in magnitude by nucleotide frequencies, and then by parameters of the GTR substitution matrix (Fig. 2; Appendix B).

3.4. Bayesian phylogenetic hypotheses based on 10× partitioned model

Bayesian phylogenetic estimates under the 10× partitioned model inferred a strongly supported clade ($Pp = 100$) comprising the Azemiopinae (*Azemiops*) and the Crotalinae, with the Crotalinae forming its own monophyletic group ($Pp = 100$; Fig. 3). This MCMC phylogeny implied the same three early phylogenetic splits among pitvipers as did MP, although the relationships between the three were unresolved (Fig. 3). The first of these clades ($Pp = 100$) includes *Hypnale* and *Calloselasma*. The second of these clades ($Pp = 92$) includes *Deinagkistrodon*, *Garthius*, and *Tropidolaemus*. The third basal pitviper clade ($Pp = 100$) includes all remaining Old World and New World genera (Fig. 3).

A large clade containing almost all members of *Trimeresurus* (*sensu lato*) is strongly supported ($Pp = 100$). *Trimeresurus sensu stricto* was inferred to be polyphyletic (with strong support across several intervening nodes), with *T. gracilis* distantly related to a strongly supported clade ($Pp = 100$) containing the remaining members of *Trimeresurus* (Fig. 3). Monophyly of *Popeia* ($Pp = 100$), *Viridovipera* ($Pp = 98$), and *Parias* ($Pp = 100$) received strong support. *Cryptelytrops* was found to be monophyletic,

Table 3
Bayes factors ($2\ln B_{10}$) across alternative models for the combined dataset

M_1										
M_0	1×	2×	3×	4×A	4×B	5×A	5×B	5×C	8×	10×
1×	—	**304.14**	**440.28**	**514.74**	**431.08**	**724.86**	**604.10**	**1028.24**	**1431.16**	**1641.48**
2×	−304.14	—	**136.14**	**210.60**	**126.94**	**420.72**	**299.96**	**724.10**	**1127.02**	**1337.34**
3×	−440.28	−136.14	—	**74.46**	−9.20	**284.58**	**163.82**	**587.96**	**990.88**	**1201.20**
4×A	−514.74	−210.60	−74.46	—	−83.66	**210.12**	**89.36**	**513.50**	**916.42**	**1126.74**
4×B	−431.08	−126.94	9.20	83.66	—	**293.78**	**173.02**	**597.16**	**1000.08**	**1210.40**
5×A	−724.86	−420.72	−284.58	−210.12	−293.78	—	−120.76	**303.38**	**706.30**	**916.62**
5×B	−604.10	−299.96	−163.82	−89.36	−173.02	120.76	—	**424.14**	**827.06**	**1037.38**
5×C	−1028.24	−724.10	−587.96	−513.50	−597.16	−303.38	−424.14	—	**402.92**	**613.24**
8×	−1431.16	−1127.02	−990.88	−916.42	−1000.08	−706.30	−827.06	−402.92	—	**210.32**
10×	−1641.48	−1337.34	−1201.20	−1126.74	−1210.40	−916.62	−1037.38	−613.24	−210.32	—

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.

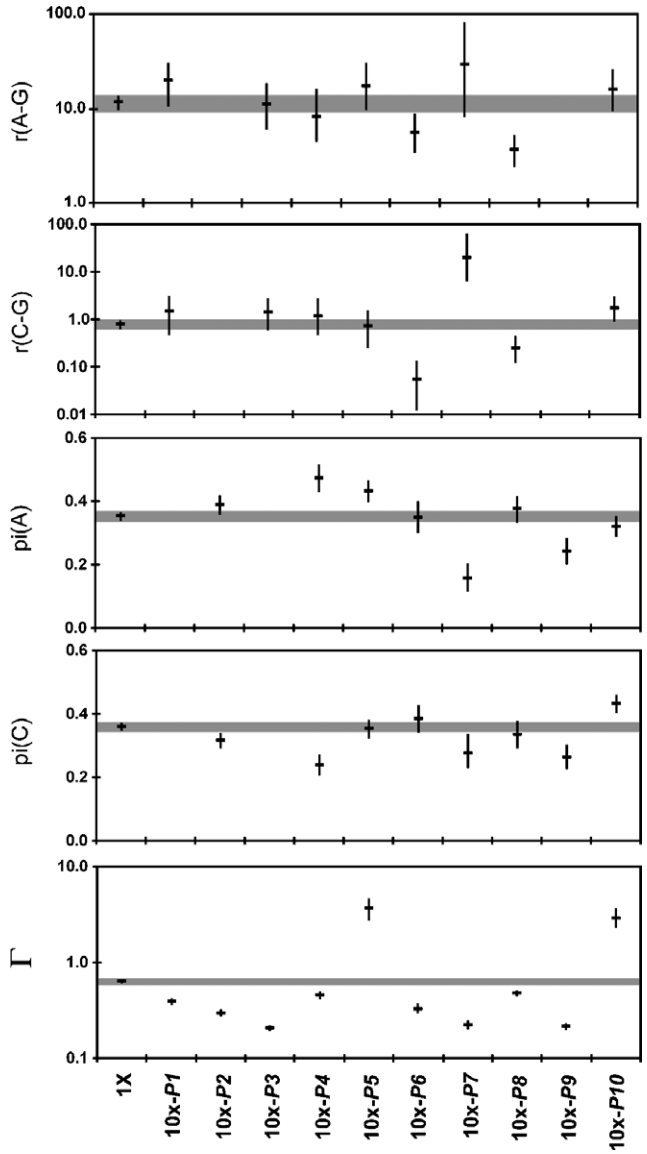

Fig. 2. Comparisons of means and 95% credibility intervals (CI) of selected nucleotide model parameters estimated from Bayesian MCMC analyses conducted under the 1× (unpartitioned) and the 10× (partitioned) models. Partitions of the 10× model are designated *P*1–*P*10 and correspond with Table 2. Gray-shaded bands indicate the 95% CI of parameters estimated under the 1× model. (Note: some models used for various partitions of the 10× dataset do not employ the particular selected parameters shown in plots, and for this reason are blank for such parameters.)

unlike in the MP tree, but with low support (*Pp* = 63). *Ovophis* was estimated to be polyphyletic, with *O. monticola* placed as the sister lineage (*Pp* = 97) to a clade containing *Triceratolepidophis*, *Zhaoermia*, and *Protobothrops*. Within this clade, *Zhaoermia* was inferred as the sister lineage (*Pp* = 70) to a monophyletic (*Pp* = 100) *Protobothrops* clade. *O. okinavensis* was strongly supported (*Pp* = 100) as the sister lineage to *Trimeresurus gracilis* (both taxa placed far from congeneric species); collectively, this clade formed the sister group to a monophyletic (*Pp* = 100) *Gloydius* (Fig. 3). The sister group to all New World genera was not resolved, with a polytomy uniting three clades (*Pp* = 100) including: a *Gloydius*, *O. okinavensis*, *T. gracilis* clade; an *O. monticola*, *Triceratolepidophis*, *Zhaoermia*, and *Protobothrops* clade; and a third clade (*Pp* = 100) including all New World genera (Fig. 3).

The earliest phylogenetic divisions among New World pitvipers were generally inferred with weak support and poor resolution. The earliest divergence within New World genera was estimated between a clade (*Pp* = 100) including Middle and South American bothropoid genera (*Atropoides*, *Cerrophidion*, *Porthidium*, *Bothrocophias*, *Bothrops*, and *Bothriopsis*) and a weakly supported clade (*Pp* = 64) containing the remaining temperate and tropical New World genera (Fig. 3). The Middle American genera *Atropoides*, *Cerrophidion*, and *Porthidium* formed a clade inferred to be the sister group to a clade comprising the South American genera *Bothrocophias*, *Bothrops*, and *Bothriopsis* (*Pp* = 100). Within the Middle American clade, the monophyly of *Porthidium* received strong support (*Pp* = 100). *Atropoides* was estimated to be paraphyletic (*Pp* = 78) with respect to *Cerrophidion* and *Porthidium*, due to *A. picadoi* not being grouped with other *Atropoides* species (Fig. 3). Among South American bothropoids, a monophyletic (*Pp* = 100) *Bothrocophias* formed the sister group to a clade containing a monophyletic (*Pp* = 100) *Bothriopsis* and a paraphyletic *Bothrops* group.

Relationships among members of the second basal clade of New World genera (including tropical and temperate genera) were unresolved, with a polytomy between three clades: a clade (*Pp* = 51) containing a monophyletic *Ophryacus* (*Pp* = 100) and a monophyletic *Lachesis* (*Pp* = 100), a clade (*Pp* = 100) including all *Bothriechis* species, and a clade (*Pp* = 52) containing the temperate New World genera (*Agkistrodon*, *Sistrurus*, and *Crotalus*). Monophyly of *Agkistrodon* and *Sistrurus* received strong support (both *Pp* = 100) and *Crotalus* monophyly received weak support (*Pp* = 75). *Agkistrodon* was weakly inferred to be the sister taxon (*Pp* = 52) to a clade including *Crotalus* and *Sistrurus* (*Pp* = 100). Deep phylogenetic relationships among *Crotalus* species received poor support (Fig. 3).

3.5. Differences in MCMC phylogenetic estimates between 1× and 10× partitioned analyses

Consensus topology and nodal posterior probabilities from the 1× model analyses that differed notably (*Pp* difference >5 for weakly supported clades, >3 for *Pp* values above 90) from that of the 10× model are indicated in Fig. 3. A majority of the differences between the MCMC phylogeny based on the unpartitioned 1× model, compared to the partitioned 10× model, represented changes in the posterior probability for moderately or weakly supported nodes. No nodes receiving 100% *Pp* under one model received less than 97% *Pp* support under the other model. Posterior probabilities that differed notably between the 1× and 10× estimates tended to show higher *Pp* estimates in the 10× model, although examples to the contrary were observed. This trend of increased *Pp* support under the 10× model was more pronounced at deeper nodes (Fig. 3).

Fig. 3. Bayesian MCMC 50% majority-rule consensus phylogram compiled from analyses of 2306 bp of mitochondrial DNA sequences analyzed under the best-fit "10×" partitioned model (see text for model definition and selection). Consensus phylogram and posterior probabilities (shown adjacent to nodes) were estimated from a total of 9×10^6 post-burn-in generations (from three independent MCMC runs). Nodes receiving posterior probability support of 100% are indicated by gray-filled circles; otherwise, posterior probability support for nodes based on the 10× model is shown in black print. Posterior probability estimates based on the unpartitioned 1× model that differed notably from those from the 10× model are shown in black rectangles with white print (black boxes with dashes indicate clades that were not present in the consensus topology of the 1× tree).

There were no major changes in the tree topology between the 1× and 10× analyses (considering moderate to well supported clades). The 50% majority rule consensus topology, however, did show several differences in resolution of poorly supported clades between estimates. The only important difference in the majority-rule consensus topology among Old World pitvipers was the collapse of the internode supporting *C. venustus* plus *C. macrops* as sister to the remaining members of the genus, hence the failure of the 1× model to infer/resolve the monophyly of *Crypteletrops* ($1\times - Pp < 50$, $10\times - Pp = 63$). Deep phylogenetic relationships among New World pitvipers, based on the 50% majority-rule consensus of the 1× analyses, suggest a different (yet poorly supported) topology with a primary phylogenetic division occurring between a clade containing *Sistrurus* and *Crotalus* (the rattlesnakes; $Pp = 100$), and the remaining New World genera ($Pp = 51$), similar to that seen in the MP tree. Within this second large New World clade, there was a polytomy of three lineages in the 1× tree including the following clades: (1) an *Agkistrodon* clade, (2) a *Lachesis* and *Ophryacus* clade, and (3) a clade containing *Bothriechis* as the sister group ($Pp = 56$) to Middle and South American bothropoid genera. Relationships among several *Crotalus* species also show alternative consensus topology between models, largely resulting from the placement of *Crotalus enyo* shifting from the sister taxon to *Crotalus willardi* in the 1× tree ($Pp = 59$), to the sister lineage ($Pp = 78$) of a clade containing *Crotalus molossus*, *Crotalus basiliscus*, *Crotalus unicolor*, *Crotalus durissus*, and *Crotalus "vegrandis"* in the 10× tree.

4. Discussion

4.1. Strengths and limitations of complex partitioned models

Model specification in Bayesian MCMC analyses is inherently critical to the accuracy of phylogeny estimates since Bayesian *Pp*s represent estimates of bipartition support that are dependent on the model (and priors) and the data (Huelsenbeck et al., 2002; Larget and Simon, 1999; also see Huelsenbeck and Rannala, 2004). In general, *Pp*s have been shown to be less conservative than bootstrap values (Douady et al., 2003; Erixon et al., 2003; Leaché and Reeder, 2002; see also Cummings et al., 2003). Nonetheless, broad claims that bipartition *Pp*s represent over-inflated estimates of phylogenetic confidence (e.g., Simmons et al., 2004; Suzuki et al., 2002) are not necessarily justifiable. Available evidence suggests, instead, that *Pp* values provide a more powerful estimate of phylogenetic structure present in aligned sequences than do BS values (Alfaro et al., 2003; Wilcox et al., 2002), provided major assumptions of the method are not violated (e.g., Suzuki et al., 2002). Many studies agree that Bayesian analyses conducted using overly simplistic models suffer from decreased *Pp* accuracy (e.g., Erixon et al., 2003; Huelsenbeck and Rannala, 2004; Suzuki et al., 2002; Wilcox et al., 2002). In contrast, simulation studies have shown that when Bayesian analyses are conducted using models more complex than that used to generate simulated data, *Pp* accuracy remains high (Huelsenbeck and Rannala, 2004; Lemmon and Moriarty, 2004). Collectively, these conclusions suggest that using a "compromise" model, in which multiple unique patterns of evolution are modeled using a single set of parameters, appears to be a major concern for phylogenetic estimation. Partitioning models of evolution across portions of a dataset provides a straightforward means of reducing the biases inherent with oversimplified modeling in Bayesian phylogenetic analyses. Generally, favoring the use of more complex models offers the best chance of recovering an accurate Bayesian phylogenetic estimate, as long as parameters can be accurately identified from the data (see also Huelsenbeck and Rannala, 2004). The upper limit of model complexity imposed by the need for parameters to be estimatable (or identifiable; see Castoe et al., 2004; Huelsenbeck et al., 2002; Rannala, 2002) is the primary justification for employing methods of model selection (e.g., Bayes factors, Akaike weights) and post hoc MCMC run evaluation in Bayesian phylogenetic analyses.

To what extent is an unpartitioned model forced to compromise estimates of model parameters in the analysis of a combined multi-gene dataset (as in our case), versus a model like the 10× that contains several partitions? Our results suggest that this compromise is extreme in some cases, and is evident across different classes of model parameters. Comparisons of the 95% CI of parameter estimates derived from the 1×, versus partitions of the 10× model (Fig. 2, Appendix B), show many instances where 95% CIs of partitions do not overlap those based on the 1× model. Furthermore, many CIs that do overlap do not coincide for a majority of their posterior densities. These findings point directly at the elevated potential for an unpartitioned model to fall into the trap identified in simulation studies where an oversimplified model suffers from decreased posterior probability accuracy. Collectively, available evidence supports not only the use of complex models (including partitioned models), but implies that these may be crucial for accurate phylogenetic estimates (see also Huelsenbeck and Rannala, 2004).

Across the models we tested for the combined data, all model-selection criteria supported the most complex partitioned model by a large margin (the 10× model). A majority of Bayes factors provided extremely strong support for increasingly complex models (Table 3). Relative Bayes factors (RBF) for increasingly complex models remained high, suggesting high returns on parameter addition even with increasing model complexity (Castoe et al., 2005). Collectively, these results seem to suggest that even more complex models than those tested here are likely to have been favored by model-selection criteria. Our most complex candidate model exhausted our a priori conceptions of biologically meaningful partitions of the data, placing an upper limit on the models examined. Future studies that investigate additional partitioning schemes (e.g., identify heterogeneous patterns within genes not examined here) may

provide additional suggestions for partitioning heterogeneous datasets (Faith and Pollock, 2003; Huelsenbeck et al., 2004).

How should the differences in phylogenetic hypotheses between simple and complex models be interpreted? We found complex models to result in changes in *Pp*s of clades that, in some instances, altered the Bayesian consensus topology. These changes tended to provide higher *Pp*s in the complex (10×) model, with a majority of changes concentrated at deeper nodes (e.g., Brandley et al., 2005; Castoe et al., 2004, 2005; see also Alfaro et al., 2003). This observation raises two possibilities, either complex models result in over-inflated *Pp* support, or they provide (at least on average) more accurate estimates of nodal support. Three points of evidence suggest that complex models do generally provide more accurate, rather than over-inflated, posterior probability estimates: (1) the results of simulation studies discussed above, (2) empirical studies, including this one, demonstrating that even though a majority of nodes may increase, some decrease under complex model analyses (see also Brandley et al., 2005; Castoe et al., 2004, 2005; Nylander et al., 2004), and (3) results that show a coincidence between clades that show increased *Pp* support under complex-model analyses and are also supported by other independent data (noted below; see also examples in Castoe et al., 2005).

4.2. Phylogeny and systematics of pitvipers

In agreement with previous studies (e.g., Kraus et al., 1996; Malhotra and Thorpe, 2004; Parkinson et al., 2002), our results provide strong support for the monophyly of the Crotalinae (BS = 100, *Pp* = 100) and the Azemiopinae as its sister lineage (BS = 89, *Pp* = 100). We found evidence of three early-diverging lineages of pitvipers, two exclusively Old World clades, and a third containing both Old and New World species, although the branching pattern and order among these three clades was poorly resolved (Figs. 1 and 3). Strong support for two exclusively Old World clades, *Hypnale* plus *Calloselasma*, and *Deinagkistrodon*, *Garthius*, and *Tropidolaemus*, was found by MP and MCMC analyses, although it remains unclear whether these two clades are sister groups (Figs. 1 and 3). The third early-diverging pitviper group included all other Old and New World genera (Fig. 3), including a clade containing all members of *Trimeresurus sensu lato* (except *T. gracilis*) inferred to be the sister lineage to the remaining Old and New World genera.

The recent generic subdivision of *Trimeresurus* (Malhotra and Thorpe, 2004) is supported by our results. Monophyly of *Popeia*, *Viridovipera*, and *Parias* received strong support under MCMC (*Pp* > 97) and MP (BS > 74) analyses. Although *Cryptelytrops* was paraphyletic under MP (Fig. 1) and unresolved in the 1× MCMC tree, the 10× MCMC tree weakly supported the monophyly of this new genus (*Pp* = 63; Fig. 3). Monophyly of *Cryptelytrops* is additionally supported by the presence of long, slender, deeply-bifurcated papillose hemipenes (and other external morphological characters) in members of this genus (Malhotra and Thorpe, 2004). Interestingly, the monophyly of *Viridovipera*, united by the possession of spinose "type 2" hemipenes (Malhotra and Thorpe, 2004), also received increased support under the 10× (*Pp* = 98) versus the 1× model (*Pp* = 84; Fig. 3). We found strong support for the validity of two newly described monotypic genera, *Triceratolepidophis* (Ziegler et al., 2000) and *Zhaoermia* (Zhang, 1993; Gumprecht and Tillack, 2004), which formed a clade with *Protobothrops* (BS < 50, *Pp* = 100). *Zhaoermia* was inferred with weak to moderate support (BS = 73, 1× − *Pp* = 88, 10× − *Pp* = 70) as the sister lineage to a clade (BS = 97, *Pp* = 100) comprising *Protobothrops* species.

All analyses provided strong evidence that *Trimeresurus sensu stricto* is rendered polyphyletic by *T. gracilis* being placed distantly from remaining members of *Trimeresurus*. Similarly, the placement of *O. okinavensis* (distant from the type species *O. monticola*) renders the genus *Ovophis* polyphyletic. These two enigmatic species, *O. okinavensis* and *T. gracilis*, formed a strongly supported clade in all analyses (BS = 100, *Pp* = 100). Our results supporting the close relationship of *T. gracilis* and *O. okinavensis*, and the distant relationship of these taxa to congeneric species, is in agreement with previous studies based on mitochondrial gene sequences (Malhotra and Thorpe, 2000, 2004) as well as sequences of a nuclear intron (Giannasi et al., 2001). The close relationship of these two species is particularly surprising because *T. gracilis* (like a majority of pitvipers) gives live birth to offspring, whereas *O. okinavensis* is among the few egg-laying species. Malhotra and Thorpe (2004) discussed possible actions to rectify the current generic allocation of *O. okinavensis* and *T. gracilis* (i.e., recognition of these species as a new genus versus allocating them to the genus *Gloydius*). These authors deferred taxonomic action until they could amass additional hemipenal and other morphological characters (work in progress by Malhotra and Thorpe), and we follow their decision.

Which lineage is the sister group to the New World pitvipers is an important question, with numerous ramifications relative to biogeography and trait evolution, yet no two studies have yielded identical results. Among molecular-based hypotheses, four Old World genera (*Protobothrops*, *Ovophis*, *Trimeresurus*, and *Gloydius*) have been variously estimated as the sister group to the New World clade (Knight et al., 1992; Malhotra and Thorpe, 2004; Parkinson, 1999; Parkinson et al., 2002). Although support was weak, our MP tree inferred a clade containing *Gloydius*, *O. okinavensis*, and *T. gracilis* as the sister group to all New World genera (Fig. 1). Bayesian estimates did not resolve this relationship (based on the 50% majority-rule consensus), and yielded a polytomy between three clades: (1) a clade including all New World genera, (2) a *Gloydius*, *O. okinavensis*, and *T. gracilis* clade, and (3) a clade containing *Protobothrops*, *Zhaoermia*, *Triceratolepidophis*, and *O. monticola*.

Early pitviper systematic studies suggested a close relationship between terrestrial pitvipers with large head

shields (rather than many small head scales) in the Old World and New World, recognizing a trans-continental genus *Agkistrodon* (e.g., Gloyd and Conant, 1990). Several studies, including our results, indicate that New World and Old World *Agkistrodon* (*sensu lato*) do not form a clade exclusive of other New World pitvipers (e.g., Knight et al., 1992; Kraus et al., 1996; Parkinson et al., 1997, 2002), supporting the recognition of *Gloydius* (Hoge and Romano-Hoge, 1981) for the Asiatic members of *Agkistrodon sensu lato*. Despite the polyphyly of *Agkistrodon sensu lato*, *Gloydius* is relatively close phylogenetically to New World pitvipers (Figs. 1 and 3).

All non-crotaline members of the Viperidae are distributed exclusively in the Old World. Here, as in other studies (Kraus et al., 1996; Malhotra and Thorpe, 2004; Parkinson, 1999; Parkinson et al., 2002), we find strong evidence for multiple early-diverging lineages of Old World pitvipers, and the relatively recent origin of a monophyletic clade of New World pitvipers. Kraus et al. (1996) were the first to provide molecular evidence for the monophyly of all New World pitvipers and suggest a historical biogeographic scenario for pitvipers including a single dispersal event from the Old World into the New World, and subsequent studies have supported this hypothesis (Kraus et al., 1996; Parkinson, 1999; Parkinson et al., 2002; see also Gutberlet and Harvey, 2002, 2004).

Phylogenetic estimates based on both MP and MCMC did not resolve the deep phylogenetic relationships among New World genera with any decisive levels of support (Figs. 1 and 3). We did not find evidence for a temperate (*Agkistrodon*, *Sistrurus*, and *Crotalus*) clade as the sister group to the remaining New World (Neotropical) genera, as has been suggested by several studies (e.g., Gutberlet and Harvey, 2002; Parkinson et al., 2002). The Bayesian 10× tree placed the earliest New World phylogenetic split between a clade ($Pp = 100$) including the Middle and South American bothropoid genera (*Atropoides*, *Cerrophidion*, *Porthidium*, *Bothrocophias*, *Bothrops*, and *Bothriopsis*) and a weakly supported clade ($Pp = 64$) containing the remaining temperate and tropical New World genera (Fig. 3).

Morphological and molecular studies have found strong support for the monophyly of the primarily temperate genera (*Agkistrodon*, *Sistrurus*, and *Crotalus*; e.g., Gutberlet and Harvey, 2002; Parkinson et al., 2002). Although MP and Bayesian analyses under the 1× model did not resolve this temperate clade, this clade was weakly supported ($Pp = 52$) under the 10× MCMC model. Monophyly of *Agkistrodon* and the rattlesnakes (*Sistrurus* and *Crotalus*) was strongly supported by both MP and MCMC analyses. The monophyly of the rattlesnake genera was supported by both MP and MCMC, although *Crotalus* monophly received weak support (BS = 57, $1\times - Pp = 81$, $10\times - Pp = 75$). Our estimates of *Crotalus* phylogeny differ notably from estimates of Murphy et al. (2002, based only on MP including many of the same sequences as this study), although many deep phylogenetic relationships among *Crotalus* species received weak support under MP and MCMC analyses (Figs. 1 and 3). Both MP and MCMC inferred *C. polystictus* to be the sister taxon to the remaining *Crotalus* species, instead of *C. ravus* as suggested by Murphy et al. (2002). Other novel relationships in our trees include the early divergence of *C. cerastes*, and the placement of *C. enyo* as the sister taxon to a clade containing *C. molossus*, *C. basiliscus*, *C. unicolor*, *C. durissus*, and *C. "vegrandis"* (Fig. 3; rather than nested within it). Despite the inclusion of nearly all *Crotalus* species by Murphy et al. (2002), and in this study, our understanding of relationships among rattlesnakes remains incomplete.

Several molecular studies have supported a clade comprising the primarily Middle American genera *Porthidium*, *Atropoides*, and *Cerrophidion* (Castoe et al., 2003, 2005; Parkinson, 1999; Parkinson et al., 2002), although studies incorporating morphological data disagree (Gutberlet and Harvey, 2002; Werman, 1992; see also Gutberlet and Harvey, 2004). These Middle American genera formed a strongly supported clade (BS = 96, $Pp = 100$) inferred as the sister group to a clade comprising the South American genera *Bothrocophias*, *Bothrops*, and *Bothriopsis* (as in Castoe et al., 2005; Parkinson et al., 2002). Within the Middle American group, *Atropoides* appeared paraphyletic (BS = 72, $1\times - Pp = 73$, $Pp = 78$) with respect to *Cerrophidion* and *Porthidium*, with *A. picadoi* distantly related to other *Atropoides* species (Fig. 3). Based on results of several studies, the phylogenetic status of *Atropoides* appears to be a difficult problem to solve with molecular data (Castoe et al., 2003, 2005; Kraus et al., 1996; Parkinson, 1999; Parkinson et al., 2002). A recent study using two mitochondrial gene sequences (ND4 and cyt-*b*) for a large sample of Middle American pitvipers did resolve *Atropoides* monophyly with moderate support (Castoe et al., 2005), as had been found by studies based on morphology (Gutberlet and Harvey, 2002) and morphology plus allozymes (Werman, 1992). This example demonstrates the potential impact of taxon sampling and inclusion of morphological characters on estimating pitviper phylogeny.

As the sister group to Middle American pitvipers in all analyses, the South American bothropoid genera formed a strongly supported clade (BS = 100, $Pp = 100$) with *Bothrocophias* estimated to be the sister taxon to a clade containing a monophyletic (BS = 100, $Pp = 100$) *Bothriopsis* and a paraphyletic *Bothrops* grouping. The problem of the recognition of *Bothriopsis*, rendering *Bothrops* paraphyletic, has been noted by many studies (e.g., Gutberlet and Campbell, 2001; Gutberlet and Harvey, 2002; Parkinson, 1999; Salomão et al., 1997; Wüster et al., 2002), with some suggesting that *Bothriopsis* should not be recognized (e.g., Salomão et al., 1997; Wüster et al., 2002). Currently, *Bothrops* contains a large and diverse assemblage (around 40 species; Campbell and Lamar, 2004) of primarily South American pitvipers, and some have argued that the genus *Bothriopsis* should be retained and *Bothrops* be subdivided to rectify the current paraphyly of the genus. The subdivision of *Bothrops* is most consistent with recent trends in pitviper systematics characterized by the recognition of genera that include restricted numbers of eco-

logically and morphologically similar species, rather than recognition of genera including a broad diversity and large number of species (e.g., Campbell and Lamar, 1992; Gutberlet and Campbell, 2001; Malhotra and Thorpe, 2004). Neither this study, nor previous studies, have sufficiently sampled *Bothrops* species to the extent that new generic allocations from within *Bothrops* are obvious. Our results do suggest, however, that subdivision of *Bothrops* may be accomplished by recognition of at least the three major groups receiving strong support throughout our analyses, including: (1) *B. ammodytoides*, *B. cotiara*, and *B. alternatus*, (2) *B. jararacussu*, *B. atrox*, and *B. asper*, and (3) *B. insularis*, *B. erythromelas*, and *B. diporus* clades (see also Parkinson, 1999; Parkinson et al., 2002; Salomão et al., 1997, 1999; Werman, 1992; Wüster et al., 2002). The challenge of placing unsampled species within these groups, and confirming that these three groups are monophyletic, needs to be confronted before a valid taxonomy can be proposed (see also Gutberlet and Harvey, 2004).

Studies incorporating morphological data have inferred *Ophryacus* to be the sister taxon to *Bothriechis* (Gutblerlet, 1998; Gutberlet and Harvey, 2002; Werman, 1992), although no DNA-sequence-based evidence has supported this relationship (Kraus et al., 1996; Parkinson, 1999; Parkinson et al., 2002; see discussion in Gutberlet and Harvey, 2004). Our phylogenies place *Ophryacus* in a clade with *Lachesis* with weak support (BS < 50, 10× model $Pp = 51$). It is interesting to note that the 10× MCMC analyses showed decreased *Pp* support for this relationship compared to the 1× model ($Pp = 78$), vaguely suggesting convergence of the 10× model on trees that are more in agreement with morphological studies (that reject the existence of this clade). Neither MP nor MCMC results resolved the sister lineage to *Bothriechis*, but both supported monophyly of the genus (BS = 66, $Pp = 100$).

4.3. Future directions for pitviper systematics

Over thirty years of intense research on pitviper systematics, including works by numerous authors, have produced a phylogeny that is nearing resolution and a current taxonomy that is approaching stability. Sampling of molecular phylogenetic characters has, to date, been largely restricted to mitochondrial gene data, except for studies restricted to particular groups (Creer et al., 2003; Giannasi et al., 2001). Although mitochondrial gene sequences provide a large number of variable characters, homoplasy due to the high divergence of mitochondrial sequences probably substantially hinders estimates of deep relationships among pitvipers. Sequences of nuclear genes may hold valuable synapomorphies required to solidify estimates of relationships at deeper nodes that are not confidently resolved in this study. Additionally, no studies have combined morphological and molecular data to estimate pitviper relationships. These future directions have the potential for establishing robust synapomorphic evidence for relationships, particularly at the inter-generic level, that comprise a majority of the currently outstanding questions in pitviper phylogeny and systematics.

Acknowledgment

We sincerely thank California Academy of Sciences, Jonathan Campbell, David Cannatella, Robert Espinoza, Harry Greene, Hans-Werner Herrmann, David Hillis, Ulrich Kuch, and Bill Lamar, Louisiana State University Museum of Zoology, Scott Moody, Mahmood Sasa, and Eric Smith for providing us with tissue samples. A large number of samples were collected in the field under NSF support to Jonathan Campbell (DEB–9705277, DEB–0102383). We thank Jonathan Campbell, Ron Gutberlet, and Marcio Martins for sharing their constructive insight into pitviper phylogeny. We thank the following for helpful comments on the manuscript: Jack Degner, Jill Stainkamp, Josh Reece, and Robert Ruggiero. Funding for this project was provided by a UCF startup package, and by an NSF Grant to CLP (DEB–0416000).

Appendix A

Taxon sampling with voucher information, locality data, and Genbank accession numbers for gene fragments

Taxon and sample identifier	Voucher	Locality	Genbank numbers (12s, 16s, cyt-*b*, ND4)
Causus rhombeatus		Africa	DQ305409*, DQ305432*, DQ305455*, DQ305473*
Causus resimus	Moody 515	Africa	AY223649, AY223662, AY223555, AY223616
Causus defilippi	CLP154	Tanzania	AF057186, AF057233, AY223556, AY223617
Atheris ceratophora			DQ305410*, DQ305433*, DQ305456*, DQ305474*
Atheris nitchei	CAS201653	Tanzania	AY223650, AY223663, AY223557, AY223618
Bitis nasicornis	CAS207874		DQ305411*, DQ305434*, DQ305457*, DQ305475*
Bitis peringueyi	CAS193863		DQ305412*, DQ305435*, DQ305458*, DQ305476*
Bitis arietans		Togo	AF057185, AF57232, AY223558, AY223619
Daboia russelii	CAS205253		DQ305413*, DQ305436*, DQ305459*, DQ305477*
Azemiops feae	CLP-157	China	AF057187, AF057234, AY223559, AFU41865
Calloselasma rhodostoma	UTA-R22247		AF057190, AF057237, AY223562, U41878
Cryptelytrops albolabris (A165)	AM A165	Thailand, Loei Prov.	AF517169, AF517182, AF517185, AF517214
Cryptelytrops albolabris (A229)	AM A229	Thailand, Pha Yao Prov.	AY059544, AY059560, AY059566, AY059583
Cryptelytrops albolabris (B22)	AM B22	Thailand, Nonthaburi	AF517165, AF517178, AF517189, AF517221
Cryptelytrops albolabris (B47)	AM B47	Thailand, Phetburi Prov.	AF517160, AF517173, AF517187, AF517216

Appendix A (*continued*)

Taxon and sample identifier	Voucher	Locality	Genbank numbers (12s, 16s, cyt-*b*, ND4)
Cryptelytrops albolabris (B6)	AM B6	Indonesia, Java, Cilacap	AF517158, AF517171, AF517186, AF517213
Cryptelytrops albolabris (MCZR)	MCZR-177966	Hong Kong, Port Shelter Is., Yim Tin Tsi	AF057195, AF057242, AY223567, U41890
Cryptelytrops andersonii	AM A77	India, Andaman Is.	AY352801, AY352740, AF171922, AY352835
Cryptelytrops cantori (A85)	AM A85	India, Nicobar Is.	AY352802, AY352741, AF171889, AY352836
Cryptelytrops cantori (CLP)		India, Nicobar Is., Kamurta	AF057196, AF057243, -AY223568, U41891
Cryptelytrops erythrurus (A209)	AM A209	Myanmar, Rangoon	AF517161, AF517174, AF171900, AF517217
Cryptelytrops erythrurus (B220)	AM B220	Bangladesh, Chittagong	AY352800, AY352739, AY352768, AY352834
Cryptelytrops insularis (A109)	AM A109	Indonesia, Java	AY352799, AY352738, AY352767, AY352833
Cryptelytrops insularis (B7)	AM B7	Indonesia, Timor	AY059534, AY059550, AY059568, AY059586
Cryptelytrops macrops	AM B27	Thailand, Bangkok	AF517163, AF517176, AF517184, AF517219
Cryptelytrops purpureomaculatus (A83)	AM A83	Thailand, Satun Prov.	AF517162, AF517175, AF517188, AF517218
Cryptelytrops purpureomaculatus (B418)	CAS212246	Myanmar, Ayeyarwade	AY352807, AY352746, AY352772, AY352746
Cryptelytrops septentrionalis (A100)	AM A100	Nepal, Mahattari Dist.	AY059543, AY059559, AF171909, AY059592
Cryptelytrops septentrionalis (B487)	AM B487	Nepal, Kathmandu Dist.	AY352784, AY352724, AY352755, AY352818
Cryptelytrops venustus	AM A241	Thailand, Thammarat Prov.	AY293931, AY352723, AF171914, AY293930
Deinagkistrodon acutus	CLP-28	China	AF057188, AF057235, AY223560, U41883
Garthius chaseni	AM B306	Malaysia, Sabah	AY352791, AY352729, AY352760, AY352825
Gloydius halys		Kazakhstan	AF057191, AF057238, AY223564, AY223621
Gloydius shedaoensis	ROM-20468	China, Liaoning	AF057194, AF057241, AY223566, AY223623
Gloydius strauchi	ROM-20473	China, Jilin, Waqie Sichuan	AF057192, AF057239, AY223563, AY223620
Gloydius ussuriensis	ROM-20452	China, Jilin, Kouqian	AF057193, AF057240, AY223565, AY223622
Himalayophis tibetanus	ZMB-65641	Nepal, Helambu Prov.	AY352776, AY352715, AY352749, AY352810
Hypnale hypnale	CLP-164	Sri Lanka, Columbo	AF057189, AF057236, AY223561, U41884
Ovophis monticola (A87)	AM A87	Taiwan	AY059545, AY059561, AF171907, AY059582
Ovophis monticola (JBS)	CAS215050	China, Yunnan Prov., Nu Jiang Prefecture	DQ305416*, DQ305439*, DQ305462*, DQ305480*
Ovophis monticola (MAK)	NTNU B200800		DQ305417*, DQ305440*, DQ305463*, DQ305481*
Ovophis monticola (ROM)	ROM-7798	Vietnam	AY223652, AY223665, AY223572, AY223626
Ovophis okinavensis (162)	CLP-162	Japan, Okinawa	AF057199, AF057246, AY223573, U41895
Ovophis okinavensis (FK)	FK		DQ305418*, DQ305441*, DQ305464*, U41895
Parias flavomaculatus (B289)	AM B289	Philippines, Batan Is.	AY371756, AY371795, AY371831, AY371858
Parias flavomaculatus (B3)	AM B3	Philippines, Luzon	AY059535, AY059551, AF171916, AY059584
Parias flavomaculatus (B4)	AM B4	Philippines, Mindanao	AY352796, AY352734, AY352764, AY352830
Parias hageni (B33)	AM B33	Thailand, Songhkla Prov.	AY059536, AY059552, AY059567, AY059585
Parias hageni (B364)	AM B364	Indonesia, Sumatra, Bengkulu Prov.	AY371763, AY371790, AY371825, AY371863
Parias malcomi	AM B349	Malaysia, Sabah	AY371757, AY371786, AY371832, AY371861
Parias schultzei	AM B210	Philippines, Palawan	AY352785, AY352725, AY352756, AY352819
Parias sumatranus (B347)	AM B347	Malaysia, Sabah	AY371759, AY371788, AY371823, AY371859
Parias sumatranus (B367)	AM B367	Indonesia, Sumatra, Bengkulu Prov.	AY371765, AY371791, AY371824, AY371864
Popeia popeiorum (A203)	AM A203	Thailand, Thammarat Prov.	AY059537, AY059553, AY371796, AY059588
Popeia popeiorum (B196)	FMNH-258950	Laos, Phongsaly Prov.	AY059538, AY059554, AY059571, AY059590
Popeia popeiorum (B246)	AM B246	Malaysia, Selangor	AY059540, AY059556, AY059570, AY059589
Popeia popeiorum (B34)	AM B34	Thailand, Phetburi Prov.	AY059542, AY059558, AY059572, AY059591
Protobothrops cornutus	ZFMK75067	Vietnam, Phong Nha-Ke NP	AY294272, AY294262, AY294276, AY294267
Protobothrops elegans	UMMZ-199970	Japan, Ryuku Is., Ishigaki	AF057201, AF057248, AY223575, U41893
Protobothrops falvoviridis	UMMZ-199973	Japan, Ryuku Is., Tokunoshima	AF057200, AF057247, AY223574, U41894
Protobothrops jerdonii	CAS215051	China, Nu Jiang, Yunnan	AY294278, AY294269, AY294274, AY294264
Protobothrops mucrosquamatus (2717)	ROM-2717	Vietnam	AY223653, AY223666, AY223577, AY223629
Protobothrops mucrosquamatus (B106)	AM B106	Vietnam, Vin Phuc Prov.	AY294280, AY294271, AY294275, AY294266
Protobothrops tokarensis	FK-1997	Japan, Ryuku Is., Takarajima	AF057202, AF057249, AY223576, AY223628

(*continued on next page*)

Appendix A (*continued*)

Taxon and sample identifier	Voucher	Locality	Genbank numbers (12s, 16s, cyt-*b*, ND4)
Triceratolepidophis sieversorum (B162)	AM B162	Vietnam	AY352782, AY352721, AY352753, AY352816
Triceratolepidophis sieversorum (CLP)	ZFMK 75066	Vietnam, Phong Nha-Quang Ping Province	DQ305414*, DQ305437*, DQ305460*, DQ305478*
Trimeresurus borneensis	AM B301	Malaysia, Sabah	AY352783, AY352722, AY352754, AY352817
Trimeresurus gracilis (A86)	AM A86	Taiwan	AY352789, AY352728, AF171913, AY352823
Trimeresurus gracilis (NTUB)	NTNUB 200515		DQ305415*, DQ305438*, DQ305460*, DQ305478*
Trimeresurus gramineus (A220)	AM A220	India, Tamil Nadu	AY352793, AY352731, AY352761, AY352827
Trimeresurus gramineus (B261)	AM B261	India, Maharashtra	AY352794, AY352732, AY352762, AY352828
Trimeresurus malabaricus (A218)	AM A218	India, Tamil Nadu	AY059548, AY059564, AY059569, AY059587
Trimeresurus malabaricus (B260)	AM B260	India, Maharashtra	AY352795, AY352733, AY352763, AY352829
Trimeresurus puniceus	AM B213	Indonesia	AF517164, AF517177, AF517192, AF517220
Trimeresurus trigonocephalus	AM A58	Sri Lanka, Balangoda	AY059549, AY059565, AF171890, AY059597
Tropidolaemus wagleri (B132)	AM B132	Malaysia, Perak	AF517167, AF517180, AF517191, AF517223
Tropidolaemus wagleri (B311)	AM B311	Malaysia, Sabah	AY352788, AY352727, AY352759, AY352822
Tropidolaemus wagleri (141)	CLP-141	Indonesia, West Kalimantan	AF057198, AF057245, AY223571, AY223625
Viridovipera gumprechti (A164)	AM A164	Thailand, Loei Prov.	AF517168, AF517181, AY352766, AF157224
Viridovipera gumprechti (B15)	NMNS-3113	China, Yunnan Prov.	AY352798, AY352736, AY3521487, AY352736
Viridovipera gumprechti (B174)	FMNH-255579	Vietnam, Nghe An Prov.	AY059547, AY059563, AY059573, AY059595
Viridovipera medoensis	CAS 221528	Myanmar, Kachin	AY352797, AY352735, AY352765, AY352831
Viridovipera stejnegeri (A160)	AM A160	Taiwan, Taipei	AY059539, AY059555, AF171896, AY059593
Viridovipera stejnegeri (A222)	NMNS-3651	China, Fujian Prov.	AY059541, AY059557, AF277677, AY059594
Viridovipera stejnegeri (UMMZ)	UMMZ-190532	Taiwan, Taipei	AF057197, AF057244, AY223570, U41892
Viridovipera vogeli (B97)	AM B97	Thailand, Ratchasima Prov.	AY059546, AY059562, AY059574, AY059596
Viridovipera vogeli	ROM-7234		AY223651, AY223664,AY223569, AY223624
Zhaoermia mangshanensis	AM B300	China, Hunan Prov.	AY352787, AY352726, AY352758, AY352821
Agkistrodon bilineatus	WWL	Costa Rica, Guanacaste	AF156593, AF156572, AY223613, AF156585
Agkistrodon contortrix	Moody 338	USA, Ohio, Athens Co.	AF057229, AF057276, AY223612, AF156576
Agkistrodon piscivorus	CLP-30	USA, South Carolina	AF057231, AF057278, AY223615, AF156578
Agkistrondon taylori	CLP-140	Mexico, Tamaulipas	AF057230, AF057230, AY223614, AF156580
Atropoides mexicanus	CLP-168	Costa Rica	AF057207, AF057254, AY223584, U41871
Atropoides nummifer	ENS-10515	Mexico. Puebla, San Andres Tziaulan	DQ305422*, DQ305445*, DQ061195, DQ061220
Atropoides occiduus	UTA-R29680	Guatemala, Escuintla	DQ305423*, DQ305446*, AY220315, AY220338
Atropoides olmec	JAC-16021	Mexico, Veracruz	AY223656, AY223669, AY220321, AY220344
Atropoides picadoi	CLP-45	Costa Rica, Alajuella	AF057208, AF057255, AY223593, U41872
Bothriechis aurifer	UTA-R35031	Guatemala	DQ305425*, DQ305448*, DQ305466*, DQ305483*
Bothriechis bicolor	UTA-R34156		DQ305426*, DQ305449*, DQ305467*, DQ305484*
Bothriechis lateralis	MZUCR-11155	Costa Rica, Acosta	AF057211, AF057258, AY223588, U41873
Bothriechis marchi	UTA-R52959	Guatemala: Zacapa: Cerro del Mono	DQ305428*, DQ305451*, DQ305469*, DQ305486*
Bothriechis nigroviridis	MZUCR-11151	Costa Rica, San Gerondo de Dota	AF057212, AF057259, AY223589, AY223635
Bothriechis rowleyi	JAC 13295	Mexico: Cerro Baúl	DQ305427*, DQ305450*, DQ305468*, DQ305485*
Bothriechis schlegelii	MZUCR-11149	Costa Rica, Cariblanco de Sarapiquí	AF057213, AF057260, AY223590, AY223636
Bothriechis supraciliaris		San Vito, Costa Rica	DQ305429*, DQ305452*, DQ305470*, DQ305487*
Bothriechis thalassinus	UTA-R52958	Guatemala: Zacapa	DQ305424*, DQ305447*, DQ305465*, DQ305482*
Bothriopsis bilineata		Colombia, Letícia	AF057214, AF057261, AY223591, U41875
Bothriopsis chloromelas	LSUMZ 41037	Peru, Pasco Dept.	DQ305430*, DQ305453*, DQ305471*, DQ305488*
Bothriopsis taeniata		Suriname	AF057215, AF057262, AY223592, AY223637
Bothrocophias hyoprora		Colombia, Letícia	AF057206, AF057253, AY223593, U41886
Bothrocophias microphthalmus	LSUMZ H-9372	Peru, Pasco Dept.	AY223657, AY223670, AY223594, AY223638
Bothrops alternatus	DLP-2879		AY223660, AY223673, AY223601, AY223642
Bothrops ammodytoides	MVZ-223514	Argentina, Neuguen	AY223658, AY223671, AY223595, AY223639
Bothrops asper	MZUCR-11152	Costa Rica	AF057218, AF057265, AY223599, U41876
Bothrops atrox	WWW-743		AY223659, AY223672, AY223598 AY223641
Bothrops cotiara	WWW	Brazil	AF057217, AF057264, AY223597, AY223640
Bothrops diporus	PT3404	Depto. Castro Barros, Prov. La Rioja, Argentina	DQ305431*, DQ305454*, DQ305472*, DQ305489*
Bothrops erythromelas	RG-829	Brazil, Algóas, Piranhas	AF057219, AF057266, -AY223600, U41877

Appendix A (*continued*)

Taxon and sample identifier	Voucher	Locality	Genbank numbers (12s, 16s, cyt-*b*, ND4)
Bothrops atrox	WWW-743		AY223659, AY223672, AY223598 AY223641
Bothrops insularis	WWW	Brazil, São Palo, Iiha Queimada Grande	AF057216, AF057263, AY223596, AF188705
Bothrops jararacussu	DPL-104		AY223661, AY223674, AY223602, AY223643
Cerrophidion godman (CR)	MZUCR-11153	Costa Rica, San Jose	AF057203, AF057250, AY223578, U41879
Cerrophidion godmani (GM)	UTAR-40008	Guatemala: Baja Verapaz	DQ305419*, DQ305442*, AY220348, AY220325
Cerrophidion petlalcalensis	ENS-10528	Mexico, Veracruz, Orizaba	DQ305420*, DQ305443*, DQ061202, DQ061227
Crotalus adamanteus	CLP-4	USA, Florida, St. Johns Co.	AF057222, AF057269, AY223605, U41880
Crotalus aquilus	ROM-18117	Mexico, San Luis Potosi	AF259232, AF259125, AF259162, —
Crotalus atrox	CLP-64	USA, Texas, Jeff Davis Co.	AF0572225, AF057272, AY223608, AY223646
Crotalus basiliscus	ROM-18188	Mexico, Nyarit	AF259244, AF259136, AF259174, —
Crotalus catalinensis	ROM-18250, BYU-34641-42	Mexico, Baja California Sur, Isla Santa Catalina	AF259259, AF259151, AF259189, —
Crotalus cerastes	ROM-FC-20099, ROM-19745	USA, California, Riverside Co.	AF259235, AF259128, AF259165, —
Crotalus durissus	ROM-18138	Venezuala	AF259248, AF259140, AF259178, —
Crotalus enyo	ROM-FC411, ROM13648	Mexico, Baja California Sur	AF259245, AF259137, AF259175, —
Crotalus "exsul"[a]	BYU-34753-54	Mexico, Baja California, Isla de Cedros	AF259260, AF259152, AF259190, —
Crotalus horridus (AR)	UTA-R14697	USA, Arkansas	AF259252, AF259144, AF259182, —
Crotalus horridus (NY)	ROM-18132-33	USA, New York	AF259251, AF259143, AF259181, —
Crotalus intermedius	ROM-FC223, ROM-18164	Mexico, Veracruz	AF259238, AF259131, AF2589205, —
Crotalus lepidus	ROM-18128	Mexico, Chihuahua	AF259230, AF259123, AF259160, —
Crotalus mitchelli	ROM-18178	USA, California, Imperial Co.	AF259250, AF259142, AF259180, —
Crotalus molossus	CLP-66	USA, Texas, El Paso Co.	AF057224, AF057271, AY223607, AY223645
Crotalus oreganus	ROM-19656	USA, California, Los Angeles Co.	AF259253, AF259145, AF259183, —
Crotalus polystictus	ROM-FC263, ROM-18139	Mexico, Districto Federal	AF259236, AF259129, AF259166, —
Crotalus pricei	ROM-FC2144, ROM-18158	Mexico, Nuevo Leon	AF259237, AF259130, AF259167, —
Crotalus pusillus	ROM-FC271	Mexico, Michoacan	AF259229, AF259122, AF259159, —
Crotalus ravus	UTA-live	Mexico, Puebla, Zapotitlán	AF057226, AF057273, AY223609, AY223647
Crotalus ruber	ROM-18197-98, ROM18207	USA, California, Riverside CO.	AF259261, AF259153, AF259191
Crotalus scutulatus	ROM-18210, ROM-18218	USA, Arizona, Mojave Co.	AF259254, AF259146, AF259184, —
Crotalus tigris	CLP169	USA, Arizona, Pima Co.	AF057223, AF057270, AY223606, AF156574
Crotalus tortugensis	ROM-18192, ROM-18195	Mexico, Baja California Sur, Isla Tortuga	AF259257, AF259149, AF259187, —
Crotalus transversus	KZ-shed skin	Mexico	AF259239, AF259206, AF259169, —
Crotalus triseriatus (LG)	ROM-18114	Mexico, Districto Federal, Llano Grande	AF259231, AF259124, AF259161, —
Crotalus triseriatus (TO)	ROM-18121	Mexico, Districto Federal, Toluca	AF259233, AF259126, AF259163, —
Crotalus triseriatus (XO)	ROM-18120	Mexico, Districto Federal, Xochomiko	AF259234, AF259127, AF259164, —
Crotalus unicolor	ROM-18150	Aruba Island	AF259246, AF259138, AF259176, —
Crotalus "vegrandis"[b]	ROM-18261	Venezuela	AF259247, AF259139, AF259177, —
Crotalus willardi (2575)	HWG-2575	USA, Arizona, Coshise Co.	AF259242, AF259134, AF259172, —
Crotalus willardi (413)	ROM-FC363, KZ-413	USA, Arizona, Santa Cruz Co.	AF259241, AF259133, AF259171, —
Crotalus willardi (ROM)	ROM-18183, ROM-18185	Mexico, Sonora	AF259240, AF259132, AF259170, —

(*continued on next page*)

Appendix A (*continued*)

Taxon and sample identifier	Voucher	Locality	Genbank numbers (12s, 16s, cyt-*b*, ND4)
Lachesis muta	Cadle 135	Peru	AF057221, AF057268, AY223604, AY223644
Lachesis stenophrys		Costa Rica, Limón	AF057220, AF057267, AY223603, U41885
Ophryacus melanurus	UTA-R34605	Mexico	AF057210, AF057257, AY223587, AY223634
Ophryacus undulatus	CLP-73	Mexico	AF057209, AF057256, AY223586, AY223633
Porthidium arcose	WWW-750	Ecuador	AY223655, AY223668, AY223582, AY223631
Porthidium dunni	ENS-9705	Mexico, Oaxaca	AY223654, AY223667, AY223581, AY223630
Porthidium nasutum	MZUCR-11150	Costa Rica	AF057204, AF057251, AY223579, U41887
Porthidium ophryomegas	UMMZ-210276	Costa Rica, Guanacaste Prov.	AF057205, AF057252, AY223580, U41888
Porthidium porrasi	MSM	Costa Rica, Puntarenas	DQ305421*, DQ305444*, DQ061214, DQ061239
Sistrurus catenatus	Moody-502	USA, Texas, Haskel Co.	AF057227, AF057274, AY223610, AY223648
Sistrurus miliarius	UTA-live	USA, Florida, Lee Co.	AF057228, AF057275, AY223611, U41889

An asterisk is used to indicate novel sequences generated in this study.

Voucher acronyms are as follows: AM, A. Malhotra; BYU, Brigham Young University; Cadle, J. Cadle; CAS, California Academy of Sciences; CLP, C.L. Parkinson; ENS, E.N. Smith; FK, F. Kraus; FMNH, Field Museum of Natural History; HWG, H.W. Greene; JAC, J.A. Campbell; KZ, K. Zamudio; LSUMZ, Louisiana State University Museum of Zoology; MCZ, Berkeley Museum of Comparative Zoology; Moody, S.M. Moody; MZUCR, Universidad de Costa Rica Museo de Zoología; NMNS, Taiwan National Museum of Natural Science; NTNU, National Taiwan Normal University, ROM, Royal Ontario Museum; UMMZ, University of Michigan Museum of Zoology; UTA, University of Texas at Arlington; WWL, W.L. Lamar; WWW, W.W. Wüster; ZFMK, Zoologisches Forschungsinstitut und Museum Alexander Koenig (Bonn); ZMB, Museum für Naturkunde, Humboldt-Universität (Berlin).

[a] *Crotalus "exsul"* was considered a junior synonym of *C. ruber* by Campbell and Lamar (2004).

[b] *Crotalus "vegrandis"* was considered a junior synonym of *C. durissus* by Campbell and Lamar (2004).

Appendix B

Mean and 95% credibility interval (in parentheses) of model parameters from Bayesian phylogenetic analyses of the combined data set conducted under the 1× and 10× models

Model-partition	Ti:Tv	r(C–T)	r(C–G)	r(A–T)	r(A–G)	r(A–C)
1×	—	7.21 (6.12–8.61)	0.77 (0.60–0.96)	0.83 (0.68–1.01)	11.63 (9.63–13.70)	0.57 (0.47–0.70)
10×-*P*1	—	70.32 (34.36–98.54)	1.50 (0.47–3.24)	4.70 (2.25–7.94)	19.61 (10.35–30.26)	6.33 (2.99–10.82)
10×-*P*2	11.44 (9.89–13.18)	—	—	—	—	—
10×-*P*3	—	10.16 (5.97–16.51)	1.37 (0.59–2.69)	3.26 (1.74–5.58)	10.93 (6.02–18.78)	1.68 (0.75–3.17)
10×-*P*4	—	12.90 (6.78–26.99)	1.18 (0.47–2.67)	1.40 (0.71–2.95)	8.14 (4.41–16.01)	0.99 (0.48–2.07)
10×-*P*5	—	11.46 (6.01–21.67)	0.70 (0.25–1.52)	0.95 (0.45–1.82)	16.93 (9.51–30.88)	0.54 (0.29–1.03)
10×-*P*6	—	4.19 (2.59–6.86)	0.05 (0.01–0.14)	0.66 (0.35–1.18)	5.47 (3.33–8.88)	0.30 (0.17–0.52)
10×-*P*7	—	17.08 (4.12–60.87)	20.04 (6.09–65.38)	1.71 (0.34–6.17)	28.82 (8.15–82.77)	3.85 (0.65–14.12)
10×-*P*8	—	3.40 (2.27–5.09)	0.25 (0.12–0.44)	0.50 (0.31–0.78)	3.63 (2.44–5.29)	0.21 (0.12–0.35)
10×-*P*9	7.27 (5.50–9.48)	—	—	—	—	—
10×-*P*10	—	6.05 (3.78–9.73)	1.70 (0.90–2.99)	0.63 (0.35–1.08)	15.74 (9.38–26.20)	0.36 (0.21–0.59)

Model-partition	pi(A)	pi(C)	pi(G)	pi(T)	Γ	pInvar.
1×	0.35 (0.34–0.37)	0.36 (0.35–0.37)	0.07 (0.06–0.07)	0.22 (0.21–0.23)	0.63 (0.60–0.66)	0.31 (0.28–0.33)
10×-*P*1	—	—	—	—	0.39 (0.36–0.42)	0.29 (0.22–0.38)
10×-*P*2	0.39 (0.36–0.42)	0.31 (0.29–0.34)	0.08 (0.07–0.09)	0.22 (0.20–0.24)	0.30 (0.27–0.32)	0.08 (0.03–0.14)
10×-*P*3	—	—	—	—	0.21 (0.19–0.22)	0.17 (0.08–0.25)
10×-*P*4	0.47 (0.43–0.52)	0.24 (0.21–0.27)	0.07 (0.05–0.09)	0.22 (0.19–0.25)	0.46 (0.41–0.5)	0.32 (0.26–0.37)
10×-*P*5	0.43 (0.40–0.47)	0.35 (0.32–0.38)	0.06 (0.05–0.06)	0.16 (0.15–0.18)	3.63 (2.76–4.62)	0.03 (0.00–0.07)
10×-*P*6	0.35 (0.30–0.40)	0.38 (0.34–0.43)	0.10 (0.08–0.13)	0.17 (0.14–0.20)	0.33 (0.29–0.37)	0.21 (0.15–0.28)
10×-*P*7	0.16 (0.12–0.20)	0.28 (0.23–0.34)	0.11 (0.06–0.15)	0.46 (0.40–0.52)	0.22 (0.20–0.25)	0.41 (0.33–0.49)
10×-*P*8	0.37 (0.33–0.42)	0.33 (0.29–0.38)	0.09 (0.07–0.11)	0.20 (0.18–0.23)	0.48 (0.44–0.51)	0.34 (0.28–0.39)
10×-*P*9	0.24 (0.20–0.29)	0.26 (0.22–0.30)	0.11 (0.09–0.14)	0.38 (0.34–0.43)	0.21 (0.20–0.23)	0.31 (0.23–0.38)
10×-*P*10	0.32 (0.29–0.35)	0.43 (0.40–0.46)	0.04 (0.03–0.04)	0.21 (0.20–0.23)	2.89 (2.31–3.62)	0.03 (0.00–0.07)

Parameter estimates for each model are based on a total of 9×10^6 generations combined from three independent MCMC runs. Partitions of the 10× model (*P*1–*P*10) are defined in Table 2.

References

Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. In: Second International Symposium on Information Theory. Akademiai Kiado, Budapest, pp. 673–681.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Automat. Control 19, 716–723.

Alfaro, M.E., 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. Mol. Biol. Evol. 20, 255–266.

Arévalo, E.S., Davis, S.K., Sites Jr., J.W., 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Syst. Biol. 43, 387–418.

Brandley, M.C., Schmitz, A., Reeder, T.W., 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. 54, 373–390.

Brattstrom, B.H., 1964. Evolution of pit vipers. Trans. San Diego Soc. Nat. Hist. 13, 185–268.

Brown, W.M., George Jr., M., Wilson, A.C., 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76, 1967–1971.

Buckley, T.R., 2002. Model misspecification and probabilistic tests of topology: evidence from empirical data sets. Syst. Biol. 51, 509–523.

Burger, W.L., 1971. Genera of pitvipers. Ph.D. Dissertation, University of Kansas, Lawrence, KS.

Caccone, A., Milinkovitch, M.C., Sbordoni, V., Powell, J.R., 1997. Mitochondrial DNA rates and biogeography in European newts (genus *Euproctus*). Syst. Biol. 46, 126–144.

Campbell, J.A, Lamar, W.W., 1989. The Venomous Reptiles of Latin America. Cornell University Press, Ithaca, NY.

Campbell, J.A., Lamar, W.W., 1992. Taxonomic status of miscellaneous neotropical viperids with the description of a new genus. Occ. Pap. Texas Tech. Univ. 153, 1–31.

Campbell, J.A, Lamar, W.W., 2004. The Venomous Reptiles of the Western Hemisphere. Cornell University Press, Ithaca, NY.

Castoe, T.A., Chippindale, P.T., Campbell, J.A., Ammerman, L.A., Parkinson, C.L., 2003. The evolution and phylogeography of the Middle American jumping pitvipers, genus *Atropoides*, based on mtDNA sequences. Herpetologica 59, 421–432.

Castoe, T.C., Doan, T.M., Parkinson, C.L., 2004. Data partitions and complex models in Bayesian analysis: the phylogeny of gymnophthalmid lizards. Syst. Biol. 53, 448–469.

Castoe, T.C., Sasa, M., Parkinson, C.L., 2005. Modeling nucleotide evolution at the mesoscale: The phylogeny of the Neotropical pitvipers of the *Porthidium* group (Viperidae: Crotalinae). Mol. Phylogent. Evol. 37, 881–898.

Creer, S., Malhotra, A., Thorpe, R., 2003. Assessing the phylogenetic utility of four mitochondrial genes and a nuclear intron in the Asian pit viper genus, *Trimeresurus*: separate, simultaneous, and conditional data combination analyses. Mol. Biol. Evol. 20, 1240–1251.

Cummings, M.P., Handley, S.A., Myers, D.S., Reed, D.L., Rokas, A., Winka, K., 2003. Comparing bootstrap and posterior probability values in the four-taxon case. Syst. Biol. 52, 477–487.

Douady, C.J., Delsuc, F., Boucher, Y., Doolittle, W.F., Douzery, E.J.P., 2003. Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. Mol. Biol. Evol. 20, 248–254.

Erixon, S.P., Britton, B., Oxelman, B., 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. Syst. Biol. 52, 665–673.

Faith, J.J., Pollock, D.D., 2003. Likelihood analysis of asymmetrical mutation bias gradients in vertebrate mitochondrial genomes. Genetics 165, 735–745.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Giannasi, N., Malhotra, A., Thorpe, R., 2001. Nuclear and mtDNA phylogenies of the *Trimeresurus* complex: implications for the gene versus species tree debate. Mol. Phylogenet. Evol. 19, 57–66.

Gloyd, H.K., Conant, R., 1990. Snakes of the *Agkistrodon* complex: a monographic review. SSAR Contributions to Herpetology 6, Ithaca, NY.

Graybeal, A., 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Syst. Biol. 47, 9–17.

Gumprecht, A., Tillack, F., 2004. Proposal for a replacement name of the snake genus *Ermia* Zhang, 1993. Russ. J. Herpetol. 11, 73–76.

Gutblerlet Jr., R.L., 1998. The phylogenetic position of the Mexican blacktailed pitviper (Squamata: Viperidae: Crotalinae). Herpetologica 54, 184–206.

Gutberlet Jr., R.L., Campbell, J.A., 2001. Generic recognition for a neglected lineage of South American pitvipers (Squamata: Viperidae: Crotalinae), with the description of a new species from the Colombian Chocó. Am. Mus. Novit. 3316, 1–15.

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

Gutberlet Jr., R.L., Harvey, M.B., 2004. The evolution of New World venomous snakes. In: Campbell, J.A., Lamar, W.W. (Eds.), The Venomous Reptiles of the Western Hemisphere. Cornell Univiversity Press, Ithaca, NY, pp. 634–682.

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 21, 160–174.

Hillis, D.M., 1996. Inferring complex phylogenies. Nature 383, 130–131.

Hillis, D.M., 1998. Taxonomic sampling, phylogenetic accuracy, and investigator bias. Syst. Biol. 47, 3–8.

Hoge, A.R., Romano-Hoge, S.A.R.W., 1981. Poisonous snakes of the World. Part I. Check list of the pitvipers: Viperoidea, Viperidae, Crotalinae. Mem. Inst. Butantan 42/43, 179–310 [dated 1979].

Hoge, A.R., Romano-Hoge, S.A.R.W., 1983. Notes on micro and ultrastructure of "oberhauschen" in Viperoidea. Mem. Inst. Butantan 44/45, 81–118 [dated 1981].

Huelsenbeck, J.P., 1995. The performance of phylogenetic methods in simulation. Syst. Biol. 44, 17–48.

Huelsenbeck, J.P., 1997. Is the Felsenstein zone a fly trap? Syst. Biol. 46, 69–74.

Huelsenbeck, J.P., Crandall, K.A., 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annu. Rev. Ecol. Syst. 28, 437–466.

Huelsenbeck, J.P., Larget, B., Miller, R., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 51, 673–688.

Huelsenbeck, J.P., Larget, B., Alfaro, M.E., 2004. Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. Mol. Biol. Evol. 21, 1123–1133.

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

Kass, R.E., Raferty, A.E., 1995. Bayes factors. J. Am. Stat. Assoc. 90, 773–795.

Knight, A., Densmore, L.D., Rael, E.D., 1992. Molecular systematics of the *Agkistrodon* complex. In: Campbell, J.A., Brodie, Jr, E.D. (Eds.), Biology of the Pitvipers. Selva, Tyler, TX, pp. 49–70.

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

Larget, B., Simon, D.L., 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16, 750–759.

Lemmon, A.R., Moriarty, E.C., 2004. The importance of proper model assumption in Bayesian phylogenetics. Syst. Biol. 53, 265–277.

Leaché, A.D., Reeder, T.W., 2002. Molecular systematics of the eastern fence lizard (*Sceloporus undulates*): a comparison of parsimony, likelihood, and Bayesian approaches. Syst. Biol. 51, 44–68.

Malhotra, A., Thorpe, R.S., 2000. A phylogeny of the *Trimeresurus* group of pit vipers: new evidence from a mitochondrial gene tree. Mol. Phylogenet. Evol. 16, 199–211.

Malhotra, A., Thorpe, R.S., 2004. A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers (*Trimeresurus* and *Ovophis*). Mol. Phylogenet. Evol. 32, 83–100.

McDiarmid, R.W., Campbell, J.A., Touré, T.A., 1999. Snake Species of the World: A Taxonomic and Geographical Reference, Vol. 1. The Herpetologists' League, Washington, D.C.

Murphy, R.W., Fu, J., Lathrop, A., Feltham, J.V., Kovac, V., 2002. Phylogeny of the rattlesnakes (*Crotalus* and *Sistrurus*) inferred from sequences of five mitochondrial genes. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salt Lake City, UT, pp. 69–92.

Nicholas, K.B., Nicholas Jr., H.B., 1997. GeneDoc: a tool for editing and annotating multiple sequence alignments. Available from <http://www.cris.com/~Ketchup/genedoc.shtml/>.

Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.

Pagel, M., Meade, A., 2004. A phylogenetic mixture model for detecting pattern-heterogeneity in gene sequence or character data. Syst. Biol. 53, 571–581.

Parkinson, C.L., 1999. Molecular systematics and biogeographical history of pitvipers as determined by mitochondrial ribosomal DNA sequences. Copeia, 1999, 576–586.

Parkinson, C.L., Campbell, J.A., Chippindale, P.T., 2002. Multigene phylogenetic analyses of pitvipers; with comments on the biogeographical history of the group. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salt Lake City, UT, pp. 93–110.

Parkinson, C.L., Moody, S.M., Ahlquist, J.E., 1997. Phylogenetic relationships of the "*Agkistrodon* Complex" based on mitochondrial DNA sequence data. In: Thorpe, R.S., Wüster, W., Malhotra, A. (Eds.), Venomous Snakes: Ecology, Evolution, and Snakebite. The Zoological Society of London, Clarendon Press, Oxford, UK, pp. 63–78.

Poe, S., 1998. Sensitivity of phylogeny estimation to taxonomic sampling. Syst. Biol. 47, 18–31.

Poe, S., Swofford, D.L., 1999. Taxon sampling revisited. Nature 398, 299–300.

Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.

Rambout, A., Drummond, A.J., 2003. Tracer. Version 1.0.1. Available from http://evolve.zoo.ox.ac.uk/.

Rannala, B., 2002. Identifiability of parameters in MCMC Bayesian inference of phylogeny. Syst. Biol. 51, 754–760.

Reeder, T.W., 2003. A phylogeny of the Australian *Sphenomorphus* group (Scincidae: Squamata) and the phylogenetic placement of the crocodile skinks (*Tribolonotus*): Bayesian approaches to assessing congruence and obtaining confidence in maximum likelihood inferred relationships. Mol. Phylogenet. Evol. 4, 203–222.

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

Russell, F.E., 1980. Snake Venom Poisoning. Lippincott, Philadelphia, PA.

Sakamoto, Y., Ishiguro, M., Kitagawa, G., 1986. Akaike Information Criterion Statistics. Springer, NY.

Salisbury, B.A., Kim, J., 2001. Ancestral state estimation and taxon sampling density. Syst. Biol. 50, 557–564.

Salomão, M.G., Wüster, W., Thorpe, R.S., Butantan-British *Bothrops* Systematics Project, 1999. MtDNA phylogeny of Neotropical pitvipers of the genus *Bothrops* (Squamata: Serpentes: Viperidae). Kaupia 8, 127–134.

Salomão, M.G., Wüster, W., Thorpe, R.S., Touzet, J.M., Butantan-British *Bothrops* Systematics Project, 1997. DNA evolution of South American pitvipers of the genus *Bothrops*. In: Thorpe, R.S., Wüster, W., Malhotra, A. (Eds.), Venomous Snakes: Ecology, Evolution, and Snakebite. The Zoological Society of London, Clarendon Press, Oxford, UK, pp. 89–98.

Simmons, M.P., Pickett, K.M., Miya, M., 2004. How meaningful are Bayesian support values? Mol. Biol. Evol. 21, 188–199.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods). Version 4.0b10. Sinauer, Sunderland, MA.

Sullivan, J., Swofford, D.L., 2001. Should we use model-based methods for phylogenetic inference when we know assumptions about among-site rate variation and nucleotide substitution pattern are violated? Syst. Biol. 50, 723–729.

Suzuki, Y., Glazko, G.V., Nei, M., 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. Proc. Nat. Acad. Sci. USA 99, 16138–16143.

Tavaré, S., 1996. Some probabilistic and statistical problems on the analysis of DNA sequences. In: Miura, R.M. (Ed.), Some Mathematical Questions in Biology—DNA Sequence Analysis. American Math Society, Providence, RI, pp. 57–86.

Vidal, N., Lecointre, G., 1998. Weighting and congruence: a case study based on three mitochondrial genes in pitvipers. Mol. Phylogenet. Evol. 9, 366–374.

Vidal, N., Lecointre, G., Vié, J.C., Gasc, J.P., 1997. Molecular systematics of pitvipers: paraphyly of the *Bothrops* complex. C. R. Acad. Sci. Paris, Science de la vie 320, 95–101.

Vidal, N., Lecointre, G., Vié, J.C., Gasc, J.P., 1999. What can mitochondrial gene sequences tell us about intergeneric relationships of pitvipers? Kaupia 8, 113–126.

Werman, S., 1992. Phylogenetic relationships of Central and South American pitvipers of the genus *Bothrops* (*sensu lato*): cladistic analyses of biochemical and anatomical characters. In: Campbell, J.A., Brodie, Jr., E.D. (Eds.), Biology of the Pitvipers. Selva, Tyler, TX, pp. 21–40.

Wiens, J.J., 1998. Combining data sets with different phylogenetic histories. Syst. Biol. 47, 568–581.

Wilcox, T.P., Zwickl, D.J., Heath, T.A., Hillis, D.M., 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol. Phylogenet. Evol. 25, 271–361.

Wilgenbusch, J., de Queiroz, K., 2000. Phylogenetic relationships among the phrynosomatid sand lizards inferred from mitochondrial DNA sequences generated by heterogeneous evolutionary processes. Syst. Biol. 49, 592–612.

Wüster, W., da Graca Salomão, M., Quijada-Mascareñas, J.A., Thorpe, R.S., Butantan-British *Bothrops* Systematics Project, 2002. Origin and evolution of the South American pitviper fauna: evidence from mitochondrial DNA sequence data. In: Schuett, G.W., Höggren, M., Douglas, M.E., Greene, H.W. (Eds.), Biology of the Vipers. Eagle Mountain Publishing, Salt Lake City, UT, pp. 111–128.

Yang, Z., 1996. Maximum likelihood models for combined analyses of multiple sequence data. J. Mol. Evol. 42, 587–596.

Zhang, F.J., 1998. Description of the distinct pitviper of genus *Ermia* (Serpentes: Viperidae) of China. Russ. J. Herpetol. 5, 83–84.

Ziegler, T., Herrmann, H.-W., David, P., Orlov, N.L., Pauwels, S.G., 2000. *Triceratolepidophis sieversorum*, a new genus and species of pitviper (Reptilia: Serpentes: Viperidae: Crotalinae) from Vietnam. Russ. J. Herpetol. 7, 199–214.