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Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes

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Abstract

The Colubroidea contains over 85% of all the extant species of snakes and is recognized as monophyletic based on morphological and molecular data. Using DNA sequences (cyt *b*, c-mos) from 100 species we inferred the phylogeny of colubroids with special reference to the largest family, the Colubridae. Tree inference was obtained using Bayesian, likelihood, and parsimony methods. All analyses produced five major groups, the Pareatidae, Viperidae, Homalopsidae, the Elapidae, and the Colubridae. The specific content of the latter two groups has been altered to accommodate evolutionary history and to yield a more stable taxonomy. We propose an updated classification based on the reallocation of species as indicated by our inferred phylogeny.

Keywords: Colubroidea; cyt *b*; c-mos; Bayesian; Likelihood; Parsimony; Classification

1. Introduction

The Colubroidea represents nearly 2500 species of extant snakes (Pough et al., 2004) and is understood to be monophyletic based on both morphological (Lee and Scanlon, 2002; Rieppel, 1988; Zaher, 1999) and molecular data (Cadle, 1988; Gravlund, 2001; Heise et al., 1995; Kraus and Brown, 1998; Slowinski and Lawson, 2002, 2005; Wilcox et al., 2002). A trend among herpetological lexicographers is to subdivide Colubroidea into the families Viperidae, Elapidae, Atractaspididae, and Colubridae (Pough et al., 2004), although Dowling and Jenner (1988) restricted the superfamily to just the Colubridae and Natricidae and in the process erected four other superfamilies that contain traditional colubroid groups. The proposed classification by Dowling and Jenner (1988) was not accompanied by supporting data and is not considered further. Morphological characters related to their respective venom-delivery systems (Cadle, 1992; Jackson and Fritts, 1995; Kochva, 1978; Underwood and Kochva, 1993; Zaher, 1999) and several gene sequences (Heise et al., 1995; Kelly et al., 2003; Scanlon and Lee, 2004; Slowinski and Lawson, 2002, 2005; Vidal and Hedges, 2002) identify Elapidae and Viperidae as monophyletic groups. Within the Colubroidea, the Viperidae may be the sister group to all other colubroids (Cadle, 1988; Kelly et al., 2003; Kraus and Brown, 1998). However, other molecular studies by Dowling et al. (1996), Kraus and Brown (1998), and Gravlund (2001) are ambiguous with regard to the deepest divisions within the Colubroidea. Additionally, the recognition of Elapidae may render the largest colubroid

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family, Colubridae, paraphyletic (Heise et al., 1995; Kelly et al., 2003; Kraus and Brown, 1998). The monophyly of the other family, the Atractaspididae, is supported by some studies (Bourgeois, 1968; Heymans, 1975; McDowell, 1968, 1987; Underwood and Kochva, 1993; Zaher, 1999) but rejected by others (Cadle, 1988, 1994; Kelly et al., 2003). The generic composition of Atractaspididae with respect to the inclusion of *Homoroselaps* in this family or Elapidae has been debated for over three decades (Cadle, 1994; Kelly et al., 2003; McCarthy, 1985; McDowell, 1968; Slowinski and Keogh, 2000; Underwood and Kochva, 1993; Zaher, 1999). As with Elapidae, the Atractaspididae may also render Colubridae paraphyletic.

The family Colubridae is the most diverse, widespread, and species-rich family within all of Serpentes, occupying all continents except Antarctica and consisting of greater than 1800 species (Pough et al., 2004). The composition of this group, the putative paraphyly of the family, and the hierarchical structuring into subfamilies remain contentious issues (Dowling and Duellman, 1978; Heise et al., 1995; Kelly et al., 2003; Kraus and Brown, 1998; McDowell, 1987; Meirte, 1992; Nagy et al., 2003a; Vidal and Hedges, 2002; Williams and Wallach, 1989). Zaher (1999) and Zug et al. (2001) each published recent taxonomic allocations of all colubrid genera into subfamilies, based in part on lists and research by Dowling and Duellman (1978), McDowell (1987), Williams and Wallach (1989), and Meirte (1992). The 12 subfamilies comprising the Colubridae referred to in Zaher (1999) are Xenodermatinae, Pareatinae, Calamariinae, Homalopsinae, Boodontinae, Pseudoxyrhophiinae, Colubrinae, Psammophiinae, Pseudoxenodontinae, Natricinae, Dipsadinae, and Xenodontinae. The monophyly of the subfamilies Colubrinae, Natricinae, Psammophiinae, and Xenodontinae appears to be common to several molecular studies (Cadle, 1988; Dowling et al., 1996; Gravlund, 2001; Kelly et al., 2003; Kraus and Brown, 1998, (in part)).

Assessment of the monophyly and relationships among the families and subfamilies of Colubroidea has been hampered in the past by lack of thorough sampling and collection of data from independent sources. Therefore, our goals in this paper are to determine whether the four colubroid families each represent monophyletic groups and, if so, to examine the relationships among them using a diverse sampling of taxa. We also investigate relationships among the members of the family Colubridae and assess whether they conform to the subfamilies of Zaher (1999). To meet these goals, we infer a phylogeny for the group from a taxonomically wide range of species within the Colubroidea using the nucleotide sequences of two unlinked and independently evolving genes: the single-copy nuclear c-mos gene (Graybeal, 1994; Harris et al., 1999; Saint et al., 1998) and the mitochondrial cytochrome *b* gene. We have analyzed the genes separately and together using Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP). Separate analyses of unlinked genes allows the identification of areas of congruence, which, because of the low probability of shared clades by chance alone, can be considered supported with a high degree of confidence (de Queiroz et al., 1995; Hendy et al., 1988; Miyamoto and Fitch, 1995). Combined datasets were analyzed using BI, ML, and MP. Current computational implementations of Bayesian methods allow phylogenetic inference of combined datasets where separate model parameters may be applied to individual genes. Recommendations for taxonomic changes that support monophyletic arrangements within Colubroidea are constrained by phylogenies produced here and previous evidence from independent studies of morphology and molecular data.

2. Methods and materials

Snakes collected for this study were humanely euthanized following protocols approved by the California Academy of Sciences Animal Welfare Committee and the three North American herpetological societies (American Society of Ichthyologists and Herpetologists 1987).

2.1. DNA extraction, amplification, and sequencing

We extracted DNA from liver tissue, tail tip biopsies, or shed skin from 89 species representing all families of Colubroidea and all subfamilies of Colubridae (Table 1). All tissues were treated by the standard method of proteinase K digestion in lysis buffer followed by phenol/chloroform extraction (see Burbrink et al., 2000; for details). Template DNA for the polymerase chain reaction (PCR) was also prepared as in Burbrink et al. (2000). For amplification of the entire mitochondrial cytochrome *b* gene we used primers L14910 (de Queiroz et al., 2002) and H16064 (Burbrink et al., 2000). Our cycle sequencing protocol for the cytochrome *b* gene was identical to that given in Burbrink et al. (2000). For sequencing we used primers L15584 (de Queiroz et al., 2002) H16064 and L14919 (Burbrink et al., 2000), H15149 (Kocher et al., 1989), and H15716 (Slowinski and Lawson, 2002). Taxon-specific sequencing primers (available from the senior author) were required for *Alsophis portoricensis*, *Bitis nasicornis*, *Pareas macularius*, and *Coronella girondica*. This combination of primers allowed us to sequence both strands of the approximately 1116 nucleotides making up the cytochrome *b* gene of snakes.

For the c-mos gene, we developed primers that allow the amplification and sequencing in snakes of a 570–576 bp segment exclusive of the primers. In the develop-

Table 1
List of taxa and associated museum and voucher number used in the study

Family	Subfamily	Genus and species	Museum	Voucher number	GenBank cyt *b*/c-mos
Atractaspididae					
		Aparallactus werneri	CAS	168914	AF471035/AF471116
		Atractaspis microlepidota	MVZ	228653	AF471045/AF471127
		Homoroselaps lacteus	LSUMZ	55386	AF217833/AY058931
Colubridae					
	Boodontinae	*Bothrophthalmus lineatus*	CAS	201746	AF471090/AF471129
		Duberria lutrix	CAS	201763	AF471061/AF471138
		Grayia smythii	LSUMZ	H9143	DQ112077/DQ112080
		Lamprophis fuliginosus	CAS	168909	AF471060/AF471143
		Lycophidion ornatum	CAS	201648	AF471086/AF471144
		Macroprotodon cucullatus	MVZ	186073	AF471087/AF471145
		Mehelya capensis	No voucher		AF471077/AF471099
		Pseudaspis cana	LSUMZ	37426	AY458068/AY058942
		Pythonodipsas carinata	PEM	R8234	AY188036/AY187297
	Calamarriinae	*Calamaria pavimentata*	ROM	35605	AF471081/AF471103
		Pseudorhabdion oxycephalum	CMNH	5802	AF471073/DQ112083
	Colubrinae	*Ahaetulla fronticincta*	CAS	204916	AF471082/AF471161
		Boiga dendrophila	No voucher		AF471089/AF471128
		Cemophora coccinea	CAS	203080	AF471091/AF471132
		Coronella girondica	MVZ	178073	AF471088/AF471113
		Crotaphopeltis tornieri	CAS	168957	AF471093/AF471112
		Dasypeltis atra	CAS	201641	AF471065/AF471136
		Dinodon rufozonatum	LSUMZ	44977	AF471063/AF471163
		Dipsadoboa unicolor	CAS	201660	AF471062/AF471139
		Drymarchon corais	CAS	198327	AF471064/AF471137
		Eirenis modestus	HLMD	J159	AY486933/AY486957
		Gastropyxis smaragdina	CAS	219171	DQ112075/DQ112078
		Gonyosoma oxycephala	No voucher		AF471084/AF471105
		Hemorrhois hippocrepis	MNCN	11988	AY486916/AY486940
		Hierophis viridiflavus	MVZ	178418	AY486925/AY486949
		Lycodon zawi	CAS	210323	AF471040/AF471111
		Lytorhynchus gaddi	MVZ	234500	DQ112076/DQ112079
		Masticophis flagellum	CAS	219734	AY486928/AY486952
		Oligodon cinereus	CAS	205028	AF471033/AF471101
		Opheodrys aestivus	CAS	172661	AF471057/AF471147
		Oxybelis aeneus	CAS	175557	AF471056/AF471148
		Pantherophis obsoletus	CAS	208631	AF283577/AF471140
		Philophthamnus heterodermus	CAS	201619	AF471055/AF471149
		Phyllorhynchus decurtatus	No voucher		AF471083/AF471098
		Platyceps karelini	CAS	184636	AY486918/AY486942
		Prosymna visseri	CAS	214753	AY188033/AY187994
		Ptyas mucosus	CAS	208434	AF471055/AF471151
		Rhynchophis boulengeri	No voucher		AF471053/AF471153
		Salvadora mexicana	No voucher		AY486934/AY486958
		Sonora semiannulata	CAS	206503	AF471048/AF471164
		Spalerosophis diadema	CAS	220641	AF471049/AF471155
		Spilotes pullatus	No voucher		AF471041/AF471110
		Tantilla relicta	CAS	200845	AF471045/AF471107
		Telescopus fallax	LSUMZ	37967	AF471043/AF471108
		Thelotornis capensis	LSUMZ	22073	AF471042/AF471109
		Thrasops jacksoni	LSUMZ	37488	AF471044/DQ112083
		Toxicodryas(*Boiga*) *pulverulenta*	CAS	220642	AF471047/AF471118
	Dipsadinae	*Carphophis amoenus*	CAS	160710	AF471067/DQ112082
		Contia tenuis	CAS	202582	AF471095/AF471134
		Diadophis punctatus	CAS	184351	AF471094/AF471122
		Rhadinaea flavilata	CAS	199634	AF471078/AF471152
	Homalopsinae	*Cerberus rhynchops*	CAS	206574	AF471092/AF471162
	Natricinae	*Afronatrix anoscopus*	ROM	19842	AF420073/AF471123

(*continued on next page*)

Table 1(*continued*)

Family	Subfamily	Genus and species	Museum	Voucher number	GenBank cyt *b*/c-mos
		Amphiesma stolata	CAS	206560	AF471030/AF471097
		Natriciteres olivacea	CAS	220640	AF471058/AF471146
		Natrix natrix	CAS	175878	AF471059/AF471121
		Psammodynastes pulverulentus	CAS	213503	AF471031/AF471157
		Regina rigida	CAS	165994	AF471052/AF471120
		Rhabdophis tigrinus	LSUMZ	37418	AF471051/AF471119
		Storeria dekayi	CAS	106039	AF471050/AF471154
		Thamnophis godmani	MZFC	10202	AF420135/AF471165
		Xenochrophis punctulatus	CAS	201594	AF471079/AF471106
	Pareatinae	*Pareas macularius*	CAS	206620	AF471082/AF471150
	Psammophiinae	*Malpolon monspessulanus*	MVZ	186256	AY058965/AY058936
		Mimophis mahfalensis	HLMD	J68	AY188032/AY187993
		Psammophis condanarus	CAS	205003	AF471075/AF471104
	Pseudoxenodontinae	*Pseudoxenodon karlschmidti*	ROM	30627	AF471080/AF471102
	Pseudoxyrhophiinae	*Ditypophis vivax*	HLMD	RA-2972	AY188013/AY188052
		Leioheterodon modestus	LSUMZ	H1991	AY058967/AY058933
		Alluaudina bellyi	MRSN FAZC	10622	AY188005/AY187966
		Ithycyphus miniatus	MRSN FAZC	10680	AY188019/AY187980
	Xenodermatinae	*Oxyrhabdium leporinum*	SURC	No number	AF471029/DQ112081
	Xenodontinae	*Alsophis portoricensis*	CAS	200813	AF471085/AF471126
		Arrhyton exiguum	CAS	200732	AF471071/AF471117
		Farancia abacura	CAS	184359	U49307/AF471141
		Helicops angulatus	LSUMZ	H3346	AF471037/AF471160
		Heterodon simus	CAS	195959	AF217840/AF471142
		Hydrops triangularis	LSUMZ	H3105	AF471039/AF471158
		Hypsiglena torquata	CAS	205337	AF471038/AF471159
Elapidae		*Bungarus fasciatus*	CAS	207988	AY217830/AY058924
		Sinomicrurus japonicus	CAS	204980	AY217831/AY058926
		Dendroaspis polylepis	CAS	220644	AY217832/AY058928
		Laticauda colubrina	CAS	220643	AF217834/AY058932
		Micrurus fulvius	CAS	178659	AY217839/AY058935
		Naja kaouthia	CAS	206602	AF217835/AY058938
		Notechis ater	SAM	R31604	AF217836/AF058937
		Ophiophagus hannah	CAS	206601	AF217842/AF058940
Viperidae		*Agkistrodon piscivorus*	CAS	214406	AF471074/AF471096
		Atheris nitschei	CAS	201708	AF471070/AF471125
		Bitis nasicornis	CAS	207874	AF471069/AF471130
		Cerastes cerastes	No voucher		AF471028/AF471131
		Crotalus viridis	CAS	200713	AF471066/AF471135
		Daboia russelli	CAS	205255	AF471076/AF471156
Acrochordidae		*Acrochordus granulatus*	No voucher		AF217841/AF471124
Outgroups		*Boa constrictor*	No voucher		AF471036/AF471115
		Casarea dussumieri	No voucher		U69755/AF471114
		Cylindrophis ruffus	CAS	206622	AF471032/AF471113
		Uropeltis phillipsi	LSUMZ	H5788	AF471034/AF471100

The following abbreviations for museums are used: CAS, California Academy of Sciences; HLMD, Hessisches Landesmuseum Darmstadt, Germany; MZFC, Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México, México; MNCN, Museo Nacional de Ciencias Naturales, Madrid , Spain; MRSN, Museo Regionale di Scienze Naturali, Turin, Italy; MVZ, Museum of Vertebrate Zoology at the University of California-Berkeley; LSUMZ, The Louisiana State University Museum of Natural Science; ROM, Royal Ontario Museum; SAM, South Australian Museum; PEM, Port Elizabeth Museum, Port Elizabeth, South Africa; CMNH, Carnegie Museum of Natural History; SURC, Silliman University Reference Collection, Philippines.

ment of these primers, the following rationale and procedure was used. With genomic DNA from 10 taxonomically diverse snakes, we first performed PCRs using primers G73 and G78 (Saint et al., 1998) in reaction mixtures containing 4 mM Mg^{2+} and at a 55 °C annealing temperature. We reamplified the resulting products using primers G73 and G74 (Saint et al., 1998) with either 4 or 1.5 mM Mg^{2+} also with a 55 °C annealing temperature. The products of these reactions were then sequenced using the G73 and G74 primers, enabling us to design the new internal primers G73S and G74S. Two sets of amplifications were carried out on our original templates, one under our standard condition (Burbrink et al., 2000) using G74S and G77 as primers and the

other with G73S and G78 as primers with 4 mM Mg^{2+} in the reaction mixture. The products of these reactions were then sequenced using primers G73S and G74S enabling us to read sequences just internal to the original G77 and G78 primers. These new primers we have designated S77 (5′-CAT GGA CTG GGA TCA GTT ATG-3′) and S78 (5′-CCT TGG GTG TGA TTT TCT CAC CT-3′). PCR amplifications with these primers were done under standard conditions with negative controls.

Cytochrome *b* and c-mos PCR products were purified using Promega Wizardprep^{R} PCR Preps DNA Purification System according to manufacturer's instructions. Cycle sequencing was performed on purified PCR products using the Perkin-Elmer Big Dye^{R} reaction premix for 50 cycles of 96 °C for 10 s; 45 °C for 5 s; and 60 °C for 4 min. Nucleotide sequences were determined using either an ABI model 310 or 3100 Genetic Analyzer. Because the position of indels in the snake cytochrome *b* gene is known (Burbrink et al., 2000; Campbell, 1997; Slowinski and Keogh, 2000) and there are few in the c-mos sequence, alignment can be performed by eye using the computer software Xesee version 3.2 (Cabot and Beckenbach, 1989) and Sequencher 4.0 (Gene Codes Corporation, Inc., Ann Arbor, MI, 1999). However, to check these alignments, sequences were converted to amino acids and aligned using Clustal X (Thompson et al., 1997) in DAMBE (Xia, 2000; see Lawson et al., 2004; for technique). The original DNA sequences were stored and their positions were maintained according to their amino acid alignments. Additionally, the sequences were examined by eye using Sequencher 4.1.2 (Genecodes, 2000), and an open reading frame for these protein coding genes was determined. We verified that our cytochrome *b* sequences were not pseudogenes by confirming that there were no internal stop codons. All sequences newly generated for this study have been deposited in GenBank Accession Nos. AF471028–AF471165 and DQ112075–DQ112083.

2.2. Phylogenetic analysis

Analyses were conducted on each gene separately and combined using ML, BI, and for the combined data only, MP. Additionally, BI was used to examine combined data using a mixed-model analysis.

2.3. Separate gene analyses

For ML analyses, the appropriate model for each gene was selected using Akaike Information Criterion (AIC) in the program Modeltest v3.06 (Posada and Crandall, 1998), with a starting tree obtained using the neighbor joining algorithm. After producing an initial ML estimate using PAUP* 4.10b (Swofford, 2003), parameters were re-estimated using this tree, and another ML tree (the best ML tree) was estimated using these updated parameters. For the ML analyses, tree space was searched heuristically using the TBR algorithm in PAUP* 4.10b (Swofford, 2003). Support for ML trees is derived from 1000 nonparametric bootstraps using the GTR + Γ + I model in the program Phyml v.2.4.4 (Guindon and Gascuel, 2003) and from posterior probability values obtained from BI analyses (see below).

Bayesian inference (BI) using Mr. Bayes 3.0b4 (Huelsenbeck and Ronquist, 2001) was also used to yield trees and to assess support on both genes separately and combined. Two statistical evolutionary models were evaluated using Bayes factors prior to tree inference. A codon-position-specific model was chosen for each of these genes. Each codon position was separated into three partitions corresponding to the first, second, and third codon position using the GTR + Γ + I model. For this codon-position-model, abbreviated 3(GTR + Γ + I), a single tree and branch lengths were estimated for all three codon partitions simultaneously, but all other model parameters were unlinked among partitions. The GTR + Γ + I model was also used in a separate analysis where the codon positions were not specified. Four independent Metropolis-coupled Markov chain Monte Carlo (MCMCMC) searches were performed for each of the models to ensure convergence. All runs consisted of one cold and three heated Markov chains estimated for 10 million generations, with every 1000th sample being retained. No specified priors were applied to any parameter. Parameter stationarity is assumed to have occurred when $-\ln L$ values for chains converged on similar harmonic mean values in all four replicates for each individual model. Trees prior to stationarity were discarded. To use Bayes factors to determine the appropriate model given the data, the harmonic mean for the model likelihood $f(X|M_i)$ following the methodology of Newton and Raftery (1994) and Nylander et al. (2004) was estimated from the values in the stationarity phase of the MCMC run. When comparing models, Bayes factors followed the form $2\log_e B_{10}$, where B_{10} is the ratio of model likelihoods. Given the interpretation of Bayes factors from Kass and Raftery (1995), a value greater than 10 is considered strong evidence for the more parameter-rich model. Tree inference(s) was derived from the single appropriate model. Also, trees and posterior probability support was then compared between both genes, and similarities in relationships in these trees was considered highly credible evidence for the evolution of the Colubroidea. These results were also compared to the ML results discussed above.

2.4. Combined gene analyses

Both genes were combined and a ML analysis was conducted in the same manner described above for the single genes using AIC to choose the appropriate model.

To infer trees that use evolutionary information specific to both genes without compromising parameter information specific to each dataset, a mixed-model analysis was performed using Bayesian inference with MrBayes 3.0 (Huelsenbeck and Ronquist, 2001; Nylander et al., 2004). Two models were used to partition the data. The less parameter-rich model applies a separate GTR + Γ + I model for each gene partition, 2(GTR + Γ + I) , and the more parameter rich model applies a separate GTR + Γ + I model across each codon position of each gene, 6(GTR + Γ + I). Additionally, a simple analysis using GTR + Γ + I model across both partitions was performed. Method-specific MCMCMC searches were identical to those described for single gene analyses described above. Bayes factors as described in the previous section were used to choose among the three models for the combined genes (Nylander et al., 2004).

For reasons noted by Kluge and others (e.g., Kluge, 1989, 1998) and because of recent challenges to the consistency of parametric methods of phylogenetic inference (Kolaczkowski and Thornton, 2004) an unweighted parsimony analysis of the combined data were also performed. The most parsimonious tree was inferred using a heuristic search with global branch swapping (TBR) and 10,000 random additions on equally weighted characters using PAUP* (Swofford, 2003). Support for the MP tree was derived from 100,000 nonparametric bootstraps using the fast-heuristic algorithm.

3. Results

3.1. Cytochrome b and c-mos sequence characteristics

Alignment by eye of the cytochrome *b* sequences resulted in 1125 sites. The nucleotide sequence of the cytochrome *b* gene of all snakes examined in this study commences with the ATG methionine codon, as has been previously reported for boid, elapid, and colubrid snakes (Burbrink et al., 2000; Campbell, 1997; Slowinski and Lawson, 2002; Slowinski and Keogh, 2000) and is apparently universal in squamates. The cytochrome *b* gene of the snakes varies between 1101 and 1131 nucleotides, this variation being largely due to length variation in codon numbers at the 3′ terminus of the gene. Because of this variation, the alignment at the end of the cytochrome *b* sequences was ambiguous, and therefore, we only used the first 1100 bp for the phylogenetic analyses. In the snakes examined herein, the signal for termination of translation varies among taxa and is either a post-transcriptionally polyadenylated thymine or one of the mitochondrial stop codons TAA or TAG. This is in keeping with Campbell (1997) and Slowinski and Lawson (2002). The 10 internal codon deletions within the snake cytochrome *b* gene relative to that of other vertebrates are identical in number and position to those reported by Campbell (1997), Slowinski and Keogh (2000), and Burbrink et al. (2000). Exceptions to this, relative to all other snakes examined here, are an additional codon deletion at codon position three in *Homoroselaps lacteus*, one at codon position four in *Bitis nasicornis*, and one at codon position 207 in *Daboia russelli*.

Alignment by eye of the c-mos sequences resulted in 570 sites. Several indels were located in a one-to-two codon-wide area of the c-mos gene at nucleotide positions 299–304 in the aligned sequences. An absence of two amino acids in this region was noticed for *Aparallactus*. An absence of a single amino acid at position 302–304 was noticed for Calamarinae, Colubrinae, Pseudoxenodontinae, Natricinae, Xenodontinae, *Grayia*, *Macroprotodon*, *Bitis*, and *Acrochordus*. All taxa displaying this single amino acid absence, excluding *Bitis* and *Acrochordus*, form a monophyletic group. All other snakes in this study have a 3-bp gap at positions 302–304. There was no significant base compositional variation among species for the c-mos gene ($\chi^2 = 27.18$, df = 255, $P = 1.0$), but there was compositional variation among codon sites.

3.2. Maximum likelihood results

Results from Modeltest using AIC suggested that the following parameters were most appropriate for the cytochrome *b* gene under the TvM + I + Γ model: base frequency (A = 0.3967, C = 0.3610, G = 0.0449, and T = 0.224); rate matrix A ⇔ C = 0.1180, A ⇔ G = 3.6011, A ⇔ T = 0.2997, C ⇔ G = 0.28963, C ⇔ T = 3.6011, and G ⇔ T = 1.0; I = 0.2934 and Γ = 0.4342. For the c-mos gene, Modeltest using AIC chose the TvM + I + Γ model but with different parameters: base frequency (A = 0.3238, C = 0.1840, G = 0.1813, and T = 0.3109); rate matrix A ⇔ C = 1.1636, A ⇔ G = 6.0069, A ⇔ T = 0.8762, C ⇔ G = 1.5445, C ⇔ T = 6.0069, and G ⇔ T = 1.0; I = 0.3310 and Γ = 1.7071. For both cytochrome *b* and c-mos combined and unpartitioned, Modeltest using AIC also chose the TvM + I + Γ with the following parameters: base frequency (A = 0.4113, C = 0.3460, G = 0.0491, and T = 0.1936); rate matrix A ⇔ C = 0.1213, A ⇔ G = 3.5348, A ⇔ T = 0.2995, C ⇔ G = 0.4042, C ⇔ T = 3.5348, and G ⇔ T = 1.0; I = 0.3516 and Γ = 0.4435.

The basic structure of all ML trees for combined and separate analyses using the models and parameters chosen using AIC were similar to BI and MP trees (Figs. 1–3). These similarities along with support for various groupings are discussed below.

3.3. Bayesian inference results

Burnin occurred prior to one million generations for the simplest models (those that do not consider different codon positions) regardless if genes were analyzed separately or combined. Harmonic means were therefore

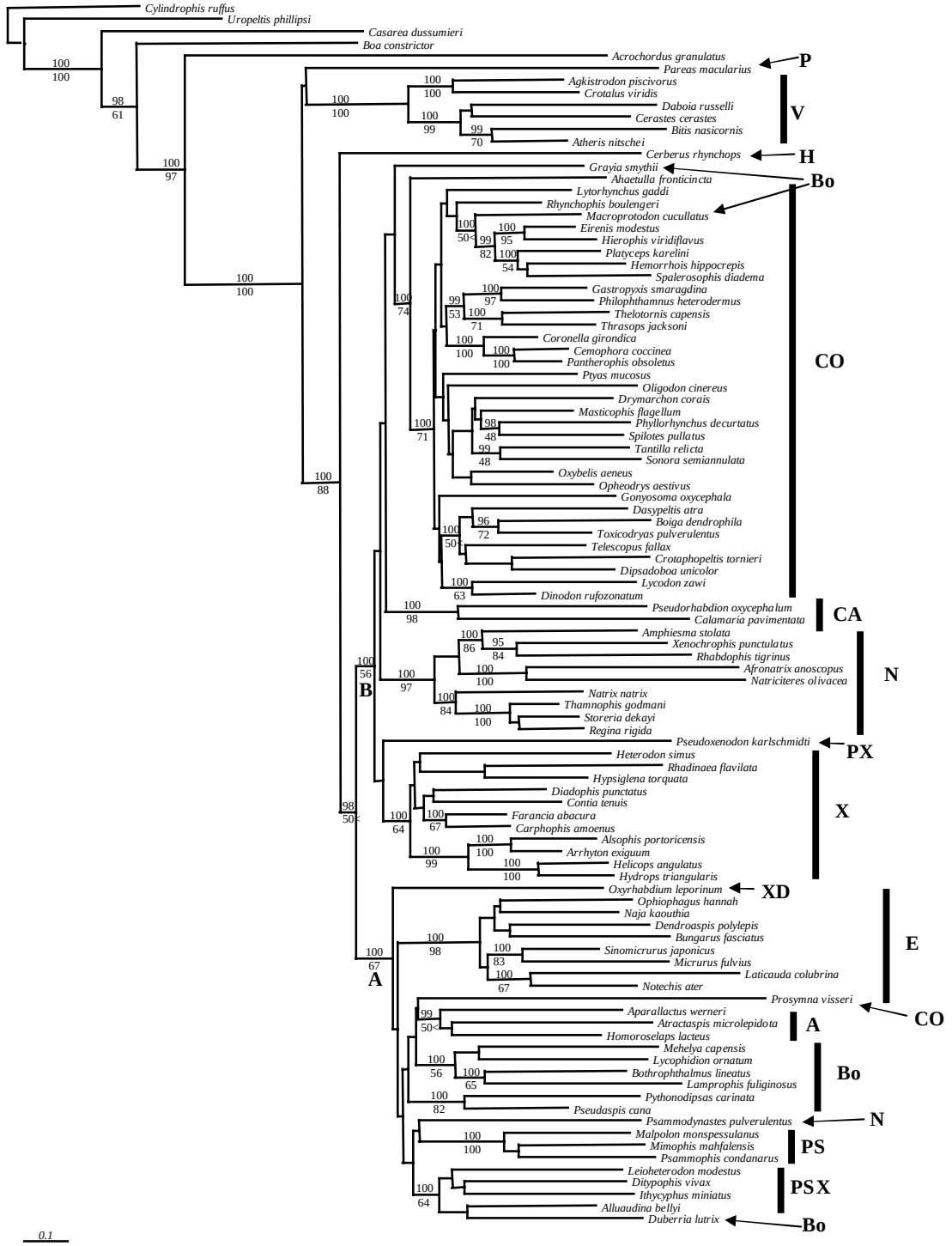

Fig. 1. Maximum likelihood inferred phylogeny of the combined data. Values above branches indicate the percent posterior probability support and values below branches indicate percent nonparametric bootstrap support. The labels (A) and (B) on two internal nodes identify the two main clades discussed in the text. The letter labels refer to the current taxonomic assignment of the designated taxon/taxa. P, Pareatinae; V, Viperidae; H, Homalopsinae; Bo, Boodontinae; CO, Colubrinae; CA, Calamariinae; N, Natricinae; PX, Pseudoxenodontinae; X, Xenodontinae; XD, Xenodermatinae; E, Elapidae; A, Atractaspididae; PS, Psammophiinae; PSX, Pseudoxyrhophiinae.

estimated from the remaining 9 million generations. Burnin for the site specific models occurred within two to six million generations. The two-partition model of the combined gene analysis burned-in within the first 2 million generations. Bayes factors always chose the most complex models at a value greater than 100 for each gene separately and combined. Therefore, the posterior probabilities were derived from the $3(\mathrm{GTR}+\Gamma+I)$

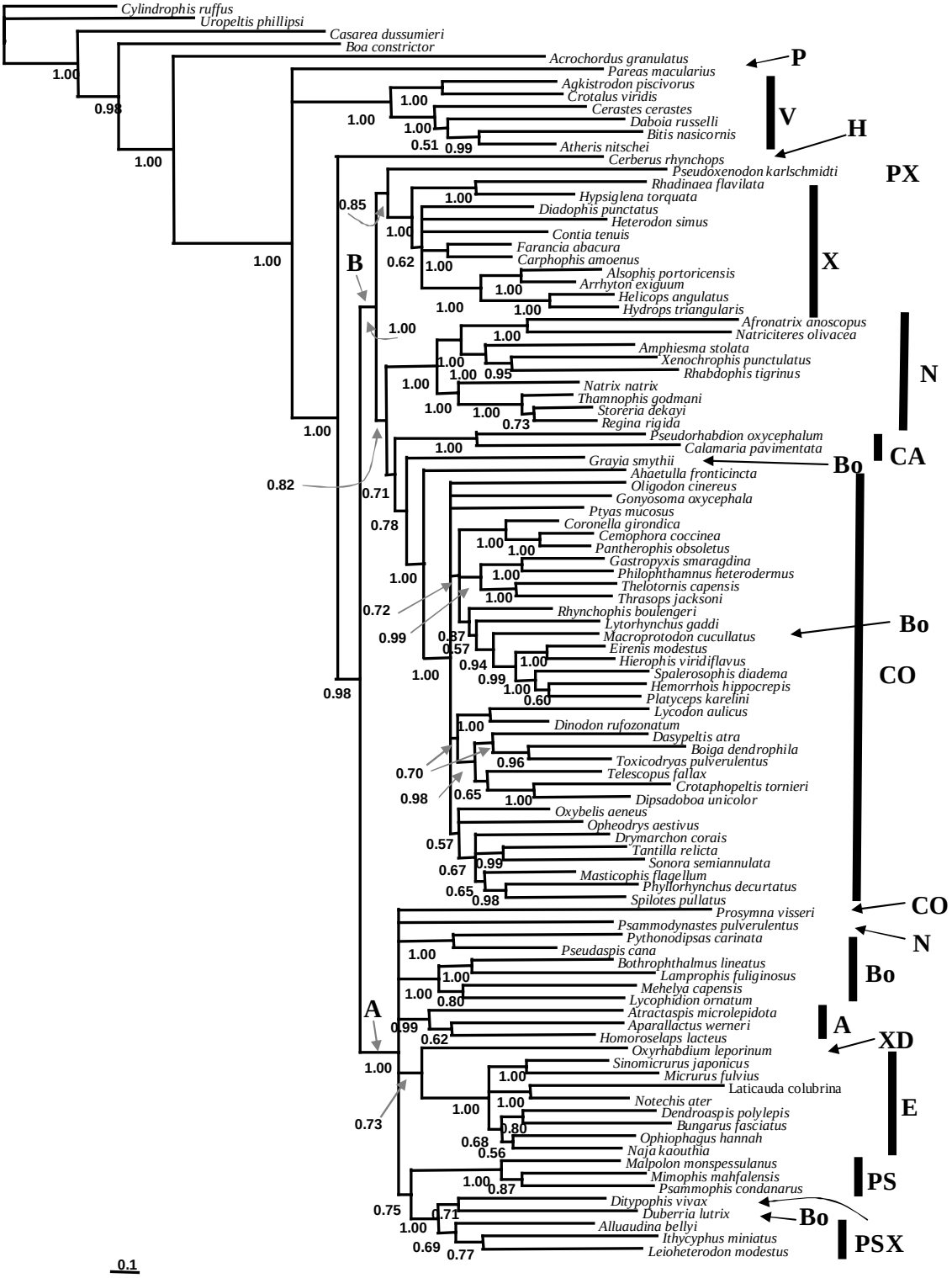

Fig. 2. Bayesian inference of phylogeny of the combined data. The values assigned to the internodes indicate posterior probability support. The labels (A) and (B) on two internal nodes identify the two main clades discussed in the text. The letter labels refer to the current taxonomic assignment of the designated taxon/taxa. P, Pareatinae; V, Viperidae; H, Homalopsinae; Bo, Boodontinae; CO, Colubrinae; CA, Calamariinae; N, Natricinae; PX, Pseudoxenodontinae; X, Xenodontinae; XD, Xenodermatinae; E, Elapidae; A, Atractaspididae; PS, Psammophiinae; PSX, Pseudoxyrhophiinae.

model for each gene analyzed separately and the 6(GTR + Γ + I) model for the genes combined. The combined, mixed model trees are similar in structure to the ML and MP trees (Figs. 1–3). Posterior probability values equal to or greater than 95% are considered credible support for a bipartition. Additionally, support for

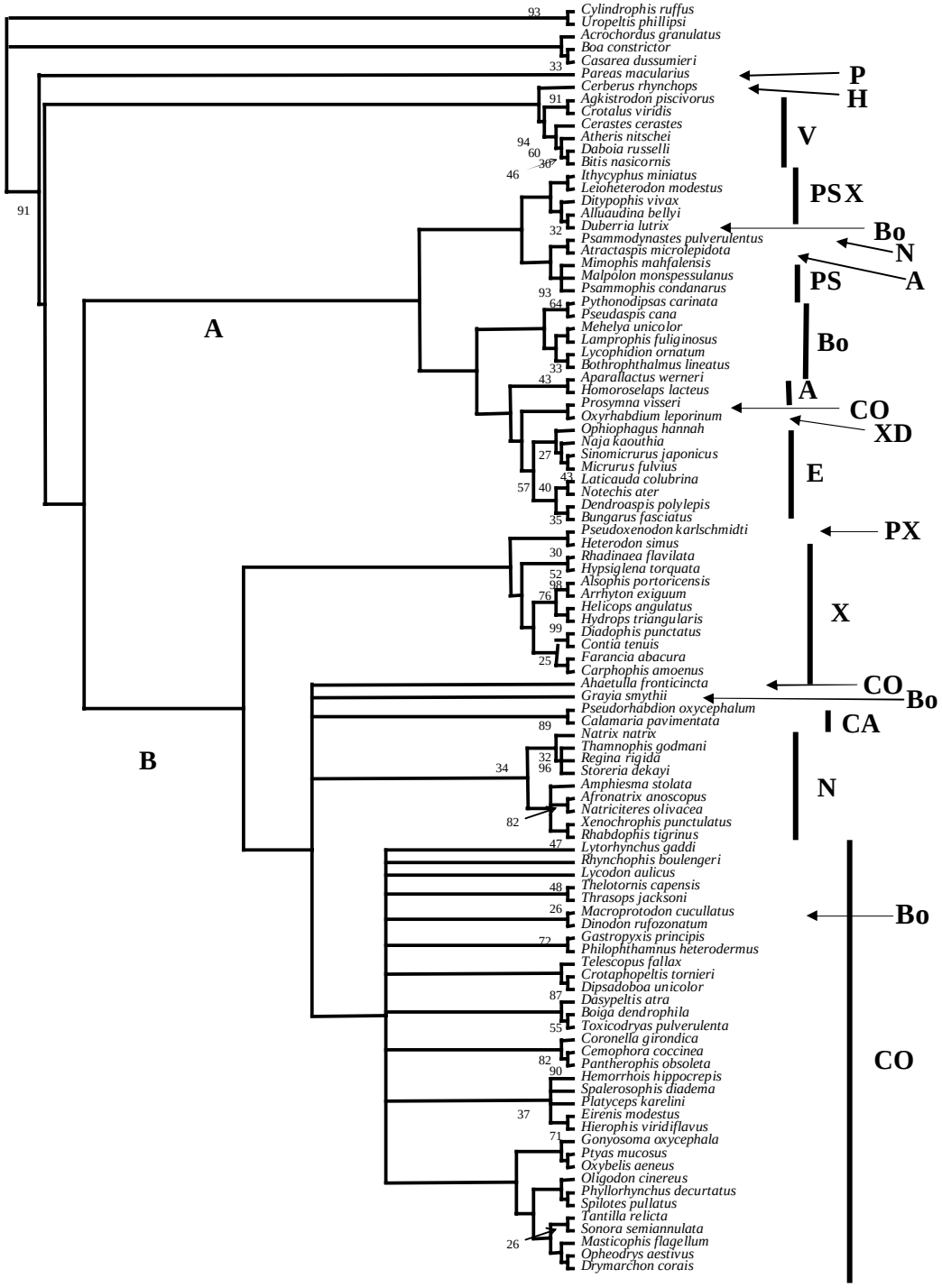

Fig. 3. Maximum parsimony inferred phylogeny of the combined data. This is a strict consensus tree derived from 11 most parsimonious trees. Numbers above branches indicate support from 1000 nonparametric bootstraps. See Tables 3 and 4 for clade character support. The labels (A) and (B) on two internal nodes identify the two main clades discussed in the text. The letter labels refer to the current taxonomic assignment of the designated taxon/taxa. P, Pareatinae; V, Viperidae; H, Homalopsinae; Bo, Boodontinae; CO, Colubrinae; CA, Calamariinae; N, Natricinae; PX, Pseudoxenodontinae; X, Xenodontinae; XD, Xenodermatinae; E, Elapidae; A, Atractaspididae; PS, Psammophiinae; PSX, Pseudoxyrhophiinae.

major groups among combined and separate gene analyses was similar (Table 2). Support values between the genes for major clades in Table 2 were significantly correlated ($P < 0.04$) as indicated by Spearman's rank correlation test (Statistica 6, 2003).

3.4. Maximum parsimony results

The most parsimonious tree was inferred using an heuristic search with global branch swapping (TBR) and 10,000 random additions. Ten islands were recovered

Table 2
A comparison of BI posterior probability (as estimated from the appropriate statistical models for each gene) among individual and combined genes for particular clades

Group	c-mos	cyt *b*	Combined
Acrochordus + Colubroidea	1.00	1.00	1.00
Viperidae	1.00	1.00	1.00
Viperidae + Pareatinae	0.57	0.64	0.50<
Homalopsinae + (Clade A and Clade B)	1.00	0.99	1.00
Clade A + Clade B	0.96	0.84	0.98
Clade A	1.00	1.00	1.00
Atractaspididae	1.00	0.50<	0.99
Elapidae	1.00	1.00	1.00
Psammophiinae	1.00	1.00	1.00
Pseudoxyrhophiinae (with *Duberria*)	1.00	0.83	1.00
Boodontinae	0.50<	0.50<	0.50<
Clade B	1.00	0.50<	1.00
Calamariinae	1.00	1.00	1.00
Colubrinae (with *Macroprotodon*)	0.96	0.99	1.00
Colubrinae (with *Macroprotodon* and *Grayia*)	0.98	0.86	0.78
Natricinae (without *Psammodynastes*)	1.00	1.00	1.00
Xenodontinae	1.00	0.99	1.00
Xenodontinae + Pseudoxenodontinae	0.76	0.99	0.85

Table 3
The following illustrates the apparent asymmetry in character support between the two genes

	c-mos		cyt *b*	
	n	%	*n*	%
PI	177	31	668	59
PU	105	18	86	7.6
C	288	50	371	33

Of the 570 bases of c-mos, 68% are uninformative whereas only 40.6% of the 1125 cyt *b* bases are uninformative. The following values are displayed: Parsimony informative characters (PI), variable but parsimony uninformative (PU) characters, and constant characters (C).

(one with two trees and nine with one tree) with a total of 11 most parsimonious trees (mpts) of 14,261 steps. These mpts exhibited the following descriptive statistics: CI = 0.37, RI = 0.32, RC = 0.04. Out of the combined data, there were 850 parsimony informative characters. A strict consensus of these trees produced the same basic structure as those found with the ML and BI analyses (Figs. 1–3). Additionally, when comparing the number of characters from each gene supporting the MP tree, it was revealed that the longer cytochrome *b* gene had more parsimony informative characters, less parsimony uninformative characters, and fewer constant characters than c-mos (Table 3). Clades are also supported by a higher number of characters in the cytochrome *b* gene than in the c-mos gene (Table 4).

3.5. Topological results

Acrochordus is the sister genus to a monophyletic Colubroidea according to both genes analyzed separately and together using ML and BI (Figs. 1 and 2; Table 2). All analyses reveal that the Viperidae form a monophyletic group. Although poorly supported, cytochrome *b* and c-mos genes analyzed separately using ML and BI show a sister group relationship between the Viperidae and the colubrid subfamily Pareatinae (Table 2). Maximum likelihood analysis of combined data suggests that the Viperidae and the Pareatinae are sister groups, but this grouping is poorly supported. The combined analyses using BI and MP place the Pareatinae in an unresolved position outside of the remaining Colubridae, Elapidae, and Atractaspididae (Figs. 2 and 3). This result, along with the following evidence, suggests that Colubridae as currently defined is not monophyletic.

Table 4
The number of characters by gene that support a clade under the MP criterion

	c-mos	cyt *b*
Colubroids + *Pareas*	0	17
Colubroids	17	39
Clade A + Clade B	1	41
Clade A	4	42
Pseudoxyrhophiinae + Psammophinae	6	25
Pseudoxyrhophiinae	0	22
Psammophiinea	0	70
Elapidae + Atractaspididae + Boodontinae	6	43
Elapidae + Atractaspididae	0	28
Elapidae + *Oxyrhabdium* + *Prosymna*	1	43
Elapidae	4	36
Atractaspididae	5	34
Boodontinae	2	36
Clade B	11	70
Colubrinae + Natricinae + Calamariinae	0	21
Colubrinae + Calamariinae	2	26
Colubrinae	4	26
Natricinae	1	50
Xenodontinae	3	30
Viperidae	1	92

Both ML and BI combined data analyses suggest that the genus *Cerberus*, from the colubrid subfamily Homalopsinae, is the sister group to a clade composed of the Colubridae, the Elapidae, and the Atractaspididae (Figs. 1 and 2). Cytochrome *b* using BI includes the colubrid *Prosymna* as the sister genus to *Cerberus*, and MP analysis of combined data places *Cerberus* as the sister group to the Viperidae (Fig. 3). Within this assemblage of Colubridae, Elapidae, and Atractaspididae, both the combined and c-mos results and all methodologies suggest a basal split between a group composed mostly of boodontines, pseudoxyrhophiines, psammophiines, atractaspidids, elapids, and xenodermatines, labeled Clade A in Figs. 1–3, and a group composed of natricines, calamarines, colubrines, xenodontines, and pseudoxenodontines, labeled Clade B in Figs. 1–3. These divisions into Clade A and B are found in all analyses, but poorly supported using nonparametric bootstraps.

Within Clade A, only the Psammophiinae, Atractaspididae, and Elapidae each form monophyletic groups in

all analyses but MP (Figs. 1–3). The Atractaspididae does not form a monophyletic group according to cytochrome *b* results analyzed using BI (Table 2) and the combined analyses using MP (Fig. 3). According to the cytochrome *b* analyses using BI, the genus *Atractaspis* may be most closely related to *Psammodynastes*, and a PP value of 0.87 places them as the sister group to the psammophiines. The other two taxa from the Atractaspididae, *Homoroselaps* and *Aparallactus*, are members of the same group in all analyses, but not always well supported (Figs. 1–3). Both genes analyzed together or separately demonstrate that the Pseudoxyrhophiinae from Madagascar and Socotra are not a monophyletic group with respect to the African boodontine *Duberria*. This result, along with the placement of *Grayia* as the sister genus to the Colubrinae and with *Macroprotodon* located within the Colubrinae, suggests that African boodontines are not monophyletic either. Although the boodontine genera *Bothrophthalmus*, *Lamprophis*, *Mehelya*, and *Lycophidion* form a clade in all analyses, this monophyletic group excludes other boodontines: *Pseudaspis*, *Pythonodipsas*, *Macroprotodon*, *Grayia*, and *Duberria*. The position of the Xenodermatinae is not clear in any analysis, but always falls within Clade A.

The other large assemblage, Clade B, composed of natricines, calamarines, colubrines, xenodontines, and pseudoxenodontines is also well-supported among all phylogenetic analyses of combined genes (Figs. 1–3). However, relationships among these subfamilies are uncertain. Excluding *Psammodynastes*, the Natricinae are a monophyletic group according to both combined gene and cytochrome *b* analyses using BI. However, support for the Natricinae as a monophyletic group using c-mos is low. A monophyletic group composed mostly of Colubrinae, with the sister taxon being *Grayia*, a boodontine (Zaher, 1999), is found in all analyses. *Prosymna*, classified as a colubrine (Zaher, 1999), does not appear part of this monophyletic group in any analysis. The position of *Ahaetulla*, a colubrine, is not certain according to c-mos, and the boodontine *Macroprotodon* (Zaher, 1999) is always placed within the Colubrinae. The two members of Calamariinae always form a clade. All genera classified as xenodontines form a monophyletic group sometimes including the Pseudoxenodontinae as a member or as the sister group (Figs. 1–3).

4. Discussion

Basic inferences are similar among combined and separate gene analyses as shown by ML, BI, and MP methods (Figs. 1–3; Table 2). Where there is disparity between separate and combined analyses, the hypotheses based on all the evidence is preferred. All results suggest that the Colubroidea are a monophyletic group. Based on the phylogeny inferred from the combined and separate data the Colubridae is polyphyletic. Both the Elapidae and Viperidae are monophyletic. The Atractaspididae, while monophyletic in the ML and BI results, is internested within Clade A in all three inferences. Our following discussion and taxonomic conclusions are confined to the results from the combined data analyses.

4.1. Basic topology of the Colubroidea

The outgroup to the Colubroidea appears to be Acrochordidae. This result corroborates morphological analyses (Cundall et al., 1993; Greene, 1997; Kluge, 1991; Lee and Scanlon, 2002; Pough et al., 2004; Rieppel, 1988; Tchernov et al., 2000) and DNA sequence data (Kelly et al., 2003; Slowinski and Lawson, 2002; Vidal and Hedges, 2002). Kelly et al. (2003) suggested that both Xenodermatinae and Acrochordidae form the root to the rest of the Colubroidea. Our data place the Xenodermatinae within a group (Clade A) composed of elapids, atractaspidids, psammophiines, boodontines, and pseudoxyrophiines (Figs. 1–3).

Within the Colubroidea, the Viperidae appear monophyletic and may be related to the Pareatinae, to the exclusion of the remaining Colubroidea. The monophyly of Viperidae is supported by the character of the single rotating fang on the premaxillary (Cadle, 1992; Kochva, 1978; Underwood and Kochva, 1993; Zaher, 1999). Various molecular studies using immunological and DNA sequence data have also found the Viperidae to be monophyletic (Cadle, 1988; Kelly et al., 2003; Lenk et al., 2001; Parkinson, 1999). The position of the Viperidae as the sister group to the remaining Colubroidea is supported by Kelly et al. (2003) and the immunological studies of Cadle (1988). The Viperinae and Crotalinae each form monophyletic groups within the Viperidae in all analyses presented here.

The basal division of the Pareatinae and the remaining Colubroidea has been presented in Slowinski and Lawson (2002), Kelly et al. (2003), and Vidal and Hedges (2002) and is inferred in the MP tree. Our ML results, although not well supported, suggest a sister group relationship between Pareatinae and Viperidae (Fig. 1). The BI is unresolved with regard to viperids, pareatines, and colubroids. The monophyly of the Pareatinae was not evaluated in our study, but has been considered morphologically well-defined by McDowell (1987). Additionally, Vidal and Hedges (2002) and Kelly et al. (2003) found that the pareatines, *Pareas* and *Aplopeltura*, formed a clade.

The Homalopsinae is the sister taxon to the remaining Colubroidea. Earlier studies using molecular data (Heise et al., 1995) and some of the analyses of Kelly et al. (2003) place them in this position. Other studies have suggested that the Homalopsinae are related to the Natricinae and Acrochordidae (Dowling and Duellman, 1978), *Dasypeltis* (Rasmussen, 1985; Underwood, 1967), the Boigini (Underwood, 1967), or the Viperidae

(Gravlund, 2001; Rasmussen, 1985). Although recognized by Underwood (1967) and McDowell (1987), no distinct morphological synapomorphies have been identified for the Homalopsinae, and its monophyly has yet to be evaluated. However, a study by Voris et al. (2002) using DNA sequence data showed eight genera of the Homalopsinae to be closely related. Thus, the preponderance of molecular evidence supports a relatively early separation of the Homalopsinae from other colubroids and this result is in keeping with McDowell's (1987) proposal based on morphological data.

The phylogenetic position of the African genus *Prosymna*, a colubrine by Zaher (1999), is unresolved and placed within Clade A by Bayesian inference, whereas ML places it as the sister genus to the Atractaspididae and MP as the sister group to the xenodermatine *Oxyrhabdium* (Figs 1–3). All inferences share the hypothesis that *Prosymna* may be misclassified as a colubrine and is instead a member of Clade A (see below).

The remaining Colubroidea are separated into two divisions, Clades A and B (Figs. 1–3). Clade A is composed of the Elapidae, Xenodermatinae, Atractaspididae, Psammophiinae, Boodontinae, and Pseudoxyrhophiinae. Clade B is composed of the Xenodontinae, Pseudoxenodontinae, Natricinae, Calamariinae, and the Colubrinae. These basic divisions were also found in Vidal and Hedges (2002) and Kelly et al. (2003).

4.2. Clade A

Within Clade A, Elapidae, Psammophiinae, and Atractaspididae each form monophyletic groups in the ML and BI trees. The MP analysis inferred the placement of *Atractaspis* with the psammophines but kept *Aparallactus* and *Homoroselaps* as sister taxa. Monophyly of Elapidae has been supported by various morphological characters associated with the venom delivery system and hemipenial morphology (Jackson and Fritts, 1995; Zaher, 1999). Within the Elapidae, the sister taxon pairs of *Sinomicrurus* and *Micrurus*, *Notechis* and *Laticauda*, and *Dendroaspis* and *Bungarus* are found in all analyses and consistent with previous molecular studies (Slowinski et al., 2001; Slowinski and Keogh, 2000; Slowinski and Lawson, 2005).

The recognition of the Psammophiinae as a monophyletic group has traditionally relied on the unique maxillary dentition and characteristically reduced ornamentation on the hemipenes (Bogert, 1940; Bourgeois, 1968; Dowling and Duellman, 1978; Underwood, 1967). The recognition of this subfamily as a monophyletic group is supported by our study and by the mtDNA study of Gravlund (2001). Although, the monophyly of the Psammophiinae seems unassailable, what remains elusive is their position relative to other lineages in Clade A and the taxonomic level at which they should be recognized. Although their position within Clade A is poorly supported in all analyses, MP places them as the sister taxon to a clade composed of *Atractaspis* and *Psammodynastes*, ML as the sister group to *Psammodynastes* and BI as the sister subfamily to the Pseudoxyrhophiinae and *Duberria* (a boodontine).

A monophyletic Atractaspididae has historically been based on skull morphology or jaw musculature (Bourgeois, 1968; Heymans, 1975; McDowell, 1968, 1987; Underwood and Kochva, 1993). Underwood (1967) and Dowling and Duellman (1978) placed *Atractaspis* in the Viperidae, with *Aparallactus* and *Homoroselaps* in the lycodontine tribe Aparallactini. Interestingly, Dowling and Duellman (1978) argued for the transfer of *Atractaspis* from the Viperids to the Aparallactini. The cytochrome *b* and ND4 analyses of Kelly et al. (2003) support a monophyletic Atractaspididae (with the exclusion of *Homoroselaps*), whereas Cadle (1988, 1994) using immunological distances found no close relationship between the two atractaspidid genera *Atractaspis* and *Aparallactus*. Although results in our study indicate that *Homoroselaps* is related to *Atractaspis* or *Aparallactus*, this taxon has not always been considered a member of the Atractaspididae. McDowell (1968) and Bourgeois (1968) placed *Homoroselaps* within the Atractaspididae, whereas Underwood and Kochva (1993) and McCarthy (1985) believed it to be an elapid. Slowinski and Keogh (2000) and Slowinski and Lawson (2005) positioned it outside Elapidae.

Zaher (1999) included all of the non-Colubrine, non-Elapidae, non-Atractaspididae, non-Malagasy colubrids (pseudoxyrhophiines), and sub-Saharan colubrids into a group referred to as the Boodontinae by Dowling et al. (1983). This group is equivalent to the combined Boaedontini and Lycophidini of Dowling and Duellman (1978). Additionally, all of the Malagasy and Socotran non-colubrine colubrids were placed into the Pseudoxyrhophiinae (Zaher, 1999). Our results agree with Nagy et al. (2003a) that neither of these groups are monophyletic. *Duberria*, a boodontine (*insertae sedis* according to Zaher, 1999), falls within the Pseudoxyrhophiinae. Vidal and Hedges (2002) report a sister taxon relationship between *Duberria* and *Leioheterodon* (a member of the Pseudoxyrhophiinae). This result supports the hypothesis proposed by Nagy et al. (2003a) that the Malagasy colubrid snake fauna is derived from multiple lineages, and it is fully in keeping with McDowell's (1987) observation that in such features as a lack of hemipenial flounces, *Duberria* is more similar to Malagasy boodontines than it is to other African boodontine snakes. The remaining boodontines are made polyphyletic by the placement of *Macroprotodon* and *Grayia* within Clade B. Within the Boodontinae, the clade composed of the sister taxon pairs of *Bothrophthalmus* and *Lamprophis* and *Mehelya* and *Lycophidion* is inferred by all three methods of analysis, thus strongly supporting Bogert's (1940) association of these taxa based on shared character

states of the posterior hypophyses, a forked sulcus and no grooved teeth. Dowling and Duellman (1978) placed all four taxa in their tribe Boaedontini. This clade is associated with the sister taxon pair of *Pseudaspis* and *Pythonodipsas*, and the Atractaspididae, but the exact relationships vary according to the different methods of data analysis.

The Asian genus *Psammodynastes* has had a confusing and contentious taxonomic history. McDowell (1987) referred it to the Natricinae based on characteristics of the sulcus spermaticus. After studying a variety of morphological characters, Rassmussen (1990) concluded that the systematic position of *Psammodynastes* remained unresolved. Zaher (1999) placed *Psammodynastes* as *insertae sedis* within the Natricinae. Our phylogenies, each differing in details of branching sequence, all place *Psammodynastes* within Clade A and associated with mostly non-Asian groups, the Atractaspididae, the Psammophiinae, the Boodontinae, and the Pseudoxyrhophiinae.

Only one sample of the last colubrid subfamily within Clade A was examined during this study, the Xenodermatinae, thus preventing the evaluation of the monophyly of this group. McDowell (1987) suggested that members of the group all share a suite of characters associated with facial morphology. Zaher (1999) claimed that these were not unique to this group. Studies by Kraus and Brown (1998) and Kelly et al. (2003) using sequences from the ND4 gene place them with Acrochordidae. All analyses of our combined data place this subfamily as a member of Clade A, but ML places it as the sister group to all other members of Clade A, BI as the sister taxon to the Elapidae and MP as the sister subfamily to *Prosymna*. Thus, given the poor support within each method and the topological differences among each method with regard to *Prosymna*, their taxonomic position remains ambiguous.

4.3. Clade B

The largest subfamily within Colubridae, the Colubrinae, forms a monophyletic group. A majority of members within this monophyletic assemblage have been diagnosed as Colubrinae prior to this study and the monophyly of the subfamily is in agreement with earlier studies of DNA sequence data (Gravlund, 2001; Heise et al., 1995; Kelly et al., 2003; Kraus and Brown, 1998; Vidal et al., 2000) and is supported by the morphological synapomorphy of asymmetric hemipenes with a simple sulcus (Dowling and Duellman, 1978). Nevertheless, a number of taxonomic groupings are supported by at least two out of three methods of analysis of our data. These include a clade formed of the Old World (OW) racers with the genera *Eirenis*, *Macroprotodon*, and *Spalerosophis*, this being congruent with the studies of Nagy et al. (2003a,b, 2004) and Schätti and Utiger (2001). Although poorly supported, our ML and BI analyses show that *Lytorhynchus* and *Rhynchophis* form the sister group to a clade composed of OW racers (*Platyceps*, *Hemorrhois*, *Spalerosophis*, *Hierophis*, and *Eirenis*). The close relationship between the OW genus *Coronella* with the New World genus *Pantherophis* and the other Lampropeltini has also been suggested by Dowling and Duellman (1978), who placed both *Pantherophis* (then *Elaphe*) and *Coronella* within the colubrid tribe Colubrini. As discussed above, the genus *Prosymna* is not found within the Colubrinae, but rather is nested within Clade A. The position of *Prosymna* outside the Colubrinae agrees with Nagy et al. (2003a). The Colubrinae are monophyletic in the results of Kelly et al. (2003), and according to Zaher (1999) they are diagnosed as having a loss of a branch of the sulcus spermaticus. This morphological synapomorphy is not homologous to the condition found in the hemipenes of the Natricinae (McDowell, 1961, 1987; Rossman and Eberle, 1977).

Among colubrine snakes, unique features of hemipenial morphology of the Nearctic *Phyllorhynchus* and the Oriental *Oligodon* have been used to provide evidence of a close phylogenetic relationship between these two geographically well separated genera (Dowling and Duellman, 1978). Based on shared derived allelomorphs and morphological characters, Dowling et al. (1996) placed *Phyllorhynchus* and *Oligodon* into the tribe Oligodontini, thus recognizing them as distinct from other colubrines (tribe Colubrini). In contrast, our analyses of combined data produced a poorly supported clade composed of New World (NW) colubrines including the genera *Phyllorhynchus*, *Spilotes*, *Tantilla*, *Sonora*, *Masticophis*, *Opheodrys*, and *Drymarchon* (MP), plus *Oxybelis* (ML and BI). Although not well supported in our ML analyses, *Oligodon* appears as the sister taxon to this group of NW colubrines. While our data offer no support for the Oligodontini as a phylogenetic entity (Dowling and Duellman, 1978; Dowling et al., 1996), there may be a close phylogenetic relationship between *Oligodon* and certain NW colubrines.

Other groups within the Colubrinae found using ML and BI (but only well supported using BI) are an African clade composed of *Gastropyxis*, *Philophthamnus*, *Thelotornis*, and *Thrasops*; and a clade composed of genera which are among those considered to constitute the boigines *sensu* Bourgeois (1968) (Fig. 2). This clade includes *Dasypeltis*, *Boiga*, *Toxicodryas*, *Telescopus*, *Crotaphopeltis*, *Dipsadoboa*, *Lycodon*, and *Dinodon*.

The sister taxon to all other Colubrinae is the genus *Grayia* as indicated by our BI and ML analyses, although support is low for this hypothesis. This taxon was classified as a boodontine by Zaher (1999) and seems only distantly related to the majority of colubrine snakes. This genus was found in a similar position as the sister group to Colubrinae in some of analyses presented in Kelly et al. (2003) and in Vidal and Hedges (2002).

The sister taxon to the remaining Colubrinae is the genus *Ahaetulla* as indicated by BI and ML analyses (Figs. 1 and 2).

The Natricinae is also monophyletic according to all results presented here, with the OW genus *Natrix* as sister taxon to North American taxa, and the remaining OW taxa forming a separate monophyletic group. Within this OW clade, the African genera *Afronatrix* and *Natriciteres* are sister taxa by all analyses. The natricines are characterized as having a distinctive hemipenis with a highly centripetal sulcus spermaticus with broadly divergent branches (Zaher, 1999). Several morphological studies (McDowell, 1987; Rossman and Eberle, 1977; Zaher, 1999), molecular studies using allozyme data (Dowling et al., 1996) and DNA sequence data (Kelly et al., 2003; Kraus and Brown, 1998) have demonstrated that Natricinae is a monophyletic group. However, Kraus and Brown's (1998) preferred tree depicted them as diphyletic. Two authors have suggested that the Natricinae is closely related to the Colubrinae (Cadle, 1994; Dowling et al., 1996). Those two subfamilies are grouped in the same large clade (Clade B) in our results, but a sister group relationship is not inferred. The inclusion of the genus *Psammodynastes* in the Natricinae appears spurious as discussed above under Clade A. The genera *Xenochrophis* and *Natriciteres* placed by Zaher (1999) as *insertae sedis* are by our data firmly nested within the Natricinae.

All members classified into the New World subfamily Xenodontinae also form a monophyletic group. Both BI and ML trees (Figs. 1 and 2) place the Asian Pseudoxenodontinae as the sister genus to Xenodontinae, although this arrangement is poorly supported. Considered a monophyletic group by McDowell (1987), the Pseudoxenodontinae was not evaluated in our study. The Xenodontinae are generally characterized by a forked sulcus spermaticus, or if single, the hemipenis is unicaped (Jenner, 1981). Some authors (Cadle, 1984, 1985; Dowling et al., 1983, 1996) suggested that the Xenodontinae represent an ancient lineage within the Colubridae. Our data, along with Kraus and Brown (1998), Vidal et al. (2000), and Kelly et al. (2003) disagree with this assumption, and it appears that xenodontines are in fact a derived colubroid lineage. However, relative to other colubrids in Clade B, the xenodontines and pseudoxenodontines form the sister group as inferred from the ML and BI trees. Cadle (1984, 1985) found the Xenodontinae monophyletic and recognized two geographically defined clades. He restricted the name Xenodontinae for the South American group and used Dipsadinae for the Central America group. Our sampling is not extensive enough to evaluate these groupings. Crother (1999) employed allozymes and was in broad agreement with the geographic split in Cadle (1984, 1985). Zaher (1999) also recognized these two clades and placed various North American taxa into each group. Vidal et al. (2000) using 12S rRNA and 16S rRNA sequence data found the Xenodontinae monophyletic and suggested a distinct South American, Central American, and North American origin for various taxa. Zaher (1999) was uncertain where to group the North American genera *Contia*, *Carphophis*, and *Diadophis* and placed them as *insertae sedis* within the Dipsadinae. From our analyses, it is certain that these genera are part of the larger group of the xenodontines. These genera along with other North American xenodontine genera (*Farancia*, *Hypsiglena*, and *Heterodon*) may form a monophyletic group.

Finally, the Calamariinae are defined as having the border of the orbital foramen formed by the frontal and parasphenoid bones to the exclusion of the parietal bone (McDowell, 1987). Zaher (1999) found similarities in the hemipenes of two of the genera and Kelly et al. (2003) associated them with members of the Colubrinae. Our ML and BI analyses always place both genera of Calamariinae as sister taxa within Clade B and together as the sister group to the Colubrinae plus *Grayia*.

4.4. Taxonomic recommendations

This study, along with evidence from other research using morphological, immunological, and DNA sequence data, demonstrates that recognition of Colubridae as a monophyletic group is not warranted. This is particularly evident where the recognition of Atractaspididae and Elapidae renders Colubridae paraphyletic. In an attempt to provide taxonomic stability derived from our analyses of data using two independent genes with the consideration of other datasets, we offer the following and provide a table of all currently recognized extant genera of Colubroidea putatively placed in our classifications system (Table 5). The recognition of the traditional Linnean endings are maintained here for convenience, although we recognize that this particular hierarchy is irrelevant when comparing groups with similar Latin endings.

Viperidae should remain unaltered and include the three subfamilies Azemiopinae, Crotalinae, and Viperinae (Liem et al., 1971). Because the currently recognized family Colubridae is a polyphyletic group (evolved once as clade B and evolved multiple other times within clade A; the position of *Pareas* also renders the Colubridae (*sensu lato*) polyphyletic) by the recognition of Atractaspididae and Elapidae, we restrict the family (Colubridae) to all members of Clade B. Therefore, Colubridae (Clade B) would now include the subfamilies Calimariinae, Colubrinae, Natricinae, Pseudoxenodontinae, and Xenodontinae. This leaves Clade A unnamed. We propose the oldest family name for Clade A, Elapidae (Boie, 1827). This family would include the subfamilies Boo

Table 5
A list of all extant genera of Colubroidea classified into families and subfamilies

Family	Subfamily	Genus
Colubridae		
	Calamariinae	*Calamaria*
		Calamorhabdium
		Collorhabdium
		Etheridgeum
		Macrocalamus
		Pseudorabdion
		Rabdion
	Colubrinae	*Aeluroglena*
		Ahaetulla
		Argyrogena
		Arizona
		Bogertophis
		Boiga
		Cemophora
		Chilomeniscus
		Chionactis
		Chironius
		Chrysopelea
		Coelognathus
		Coluber
		Conopsis
		Coronella
		Crotaphopeltis
		Cryptophidion
		Cyclophiops
		Dasypeltis
		Dendrelaphis
		Dendrophidion
		Dinodon
		Dipsadoboa
		Dispholidus
		Dryadophis
		Drymarchon
		Drymobius
		Drymoluber
		Dryocalamus
		Dryophiops
		Eirenis
		Elachistodon
		Elaphe
		Euprepiophis
		Ficimia
		Gastropyxis
		Geagras
		Gonyophis
		Gonyosoma
		Grayia
		Gyalopion
		Hapsidophrys
		Hemerophis
		Hemorrhois
		Hierophis
		Lampropeltis
		Leptodrymus
		Leptophis
		Lepturophis
		Liopeltis
		Lycodon
		Lycognathophis
		Lytorhynchus
		Macroprotodon
		Masticophis
		Mastigodryas
		Meizodon
		Oligodon
		Oocatachus
		Opheodrys
		Orthiophis
		Oreophis
		Oxybelis
		Pantherophis
		Philothamnus
		Phyllorhynchus
		Pituophis
		Platyceps
		Pseudelaphe
		Pseudoficimia
		Pseustes
		Ptyas
		Rhamnophis
		Rhinechis
		Rhinobothryum
		Rhinocheilus
		Rhynchocalamus
		Rhynchophis
		Salvadora
		Scaphiodontophis
		Scolecophis
		Senticolis
		Sibynophis
		Simophis
		Sonora
		Spalerosophis
		Spilotes
		Stegonotus
		Stenorrhina
		Stilosoma
		Symphimus
		Sympholis
		Tantilla
		Tantillita
		Telescopus
		Thelotornis
		Thrasops
		Trimorphodon
		Xenelaphis
		Zamenis
		Zaocys
	Natricinae	*Adelophis*
		Afronatrix
		Amphiesma
		Amphiesmoides
		Anoplohydrus
		Aspidura
		Atretium
		Balanophis
		Clonophis
		Hologerrhum
		Hydrablabes
		Hydraethiops
		Iguanognathus

(*continued on next page*)

Table 5 (*continued*)

Family	Subfamily	Genus
		Macropisthodon
		Natriciteres
		Natrix
		Nerodia
		Opisthotropis
		Parahelicops
		Pararhabdophis
		Regina
		Rhabdophis
		Seminatrix
		Sinonatrix
		Storeria
		Thamnophis
		Tropidoclonion
		Tropidonophis
		Virginia
		Xenochrophis
	Natricinae *insertae sedis*	*Amplorhinus*
		Limnophis
	Pseudoxenodontinae	*Plagiopholis*
		Pseudoxenodon
	Xenodontinae (Dipsadinae)	*Adelphicos*
		Amastridium
		Atractus
		Carphophis
		Contia
		Chersodromus
		Coniophanes
		Cryophis
		Diadophis
		Dipsas
		Eridiphas
		Geophis
		Hypsiglena
		Imantodes
		Leptodeira
		Ninia
		Pseudoleptodeira
		Rhadinaea
		Sibon
		Sibynomorphus
		Tretanorhinus
		Trimetopon
		Tropidodipsas
		Urotheca
	Xenodontinae (Dipsadinae) *insertae sedis*	*Calamodontophis*
		Crisantophis
		Diaphorolepis
		Echinanthera
		Emmochliophis
		Enuliophis
		Enulius
		Gomesophis
		Hydromorphus
		Nothopsis
		Pseudotomodon

Table 5 (*continued*)

Family	Subfamily	Genus
		Ptychophis
		Rhadinophanes
		Synophis
		Tachymenis
		Taeniophallus
		Tantalophis
		Thamnodynastes
		Tomodon
		Xenopholis
	Xenodontinae (Xenodontinae)	*Alsophis*
		Antillophis
		Apostolepis
		Arrhyton
		Boiruna
		Clelia
		Conophis
		Darlingtonia
		Ditaxodon
		Drepanoides
		Elapomorphus
		Erythrolamprus
		Farancia
		Helicops
		Heterodon
		Hydrodynastes
		Hydrops
		Hypsirhynchus
		Ialtris
		Liophis
		Lystrophis
		Manolepis
		Oxyrhopus
		Phalotris
		Philodryas
		Phimophis
		Pseudablabes
		Pseudoboa
		Pseudoeryx
		Psomophis
		Rhachidelus
		Saphenophis
		Siphlophis
		Tropidodryas
		Umbrivaga
		Uromacer
		Uromacerina
		Waglerophis
		Xenodon
		Xenoxybelis
	Xenodontinae *insertae sedis*	*Cercophis*
		Lioheterophis
		Sordellina
Colubridae *insertae sedis*		*Blythia*
		Cercaspis
		Cyclocorus
		Elapoidis
		Gongylosoma
		Haplocercus
		Helophis

Table 5 (*continued*)

Family	Subfamily	Genus
		Myersophis
		Oreocalamus
		Poecilopholis
		Rhabdops
		Tetralepis
		Thermophis
		Trachischium
Elapidae	Atractaspidinae	*Amblyodipsas*
		Aparallactus
		Atractaspis
		Brachyophis
		Chilorhinophis
		Elapotinus
		Hypoptophis
		Macrelaps
		Micrelaps
		Polemon
		Xenocalamus
	Boodontinae	*Boaedon*
		Bothrolycus
		Bothrophthalmus
		Chamaelycus
		Dendrolycus
		Dipsina
		Dromophis
		Gonionotophis
		Hormonotus
		Lamprophis
		Lycodonomorphus
		Lycophidion
		Macroprotodon
		Mehelya
		Pseudaspis
		Pseudoboodon
		Pythonodipsas
		Scaphiophis
	Boodontinae *insertae sedis*	*Buhoma*
		Montaspis
	Elapinae	*Aspidelaps*
		Boulengerina
		Bungarus
		Calliophis
		Dendroaspis
		Elapsoidea
		Hemachatus
		Hemibungarus
		Homoroselaps
		Maticora
		Micruroides
		Micrurus
		Naja
		Ophiophagus
		Paranaja
		Pseudohaje
		Sinomicrurus
		Walterinnesia
	Hydrophiinae	*Acalyptophis*
		Acanthophis
		Aipysurus
		Aspidomorphus

Table 5 (*continued*)

Family	Subfamily	Genus
		Astrotia
		Austrelaps
		Cacophis
		Demansia
		Denisonia
		Drysdalia
		Echiopsis
		Elapognathus
		Emydocephalus
		Enhydrina
		Ephalophis
		Furina
		Hemiaspis
		Hoplocephalus
		Hydrelaps
		Hydrophis
		Kerilia
		Kolphophis
		Lapemis
		Laticauda
		Loveridgelaps
		Micropechis
		Notechis
		Ogmodon
		Oxyuranus
		Parahydrophis
		Parapistocalamus
		Pelamis
		Praescutata
		Pseudechis
		Pseduonaja
		Rhinoplocephalus
		Salomonelaps
		Simoselaps
		Suta
		Thalassophis
		Toxicocalamus
		Tropidechis
		Vermicella
	Psammophiinae	*Hemirhagerrhis*
		Malpolon
		Mimophis
		Psammophis
		Psammophylax
		Rhamphiophis
	Pseudoxyrhophiinae	
		Alluaudina
		Brygophis
		Compsophis
		Ditypophis
		Dromicodryas
		Duberria
		Exallodontophis
		Geodipsas
		Heteroliodon
		Ithycyphus
		Langaha
		Leioheterodon
		Liophidium
		Liopholidophis
		Lycodryas
		Madagascarophis

(*continued on next page*)

Table 5 (*continued*)

Family	Subfamily	Genus
		Micropisthodon
		Pararhadinaea
		Pseudoxyrhopus
		Stenophis
	Xenodermatinae	*Achalinus*
		Fimbrios
		Oxyrhabdium
		Stoliczkia
		Xenodermus
		Xylophis
Elapidae *insertae sedis*		*Prosymna*
		Psammodynastes
Homalopsidae		*Bitia*
		Cantoria
		Cerberus
		Enhydris
		Erpeton
		Fordonia
		Gerarda
		Heurnia
		Homalopsis
		Myron
Homalopsidae *insertae sedis*		*Brachyorrhos*
Pareatidae		*Aplopeltura*
		Internatus
		Pareas
Viperidae	Azemiopinae	*Azemiops*
	Crotalinae	*Agkistrodon*
		Atropoides
		Bothriechis
		Bothrops
		Calloselasma
		Cerrophidion
		Crotalus
		Cryptelytrops
		Deinagkistrodon
		Garthius
		Gloydius
		Himalayophis
		Hypnale
		Lachesis
		Ophryacus
		Ovophis
		Parias
		Peltopelor
		Popeia
		Porthidium
		Sistrurus
		Trimeresurus
		Tropidolaemus
		Viridovipera
	Viperinae	*Adenorhinos*
		Atheris
		Bitis
		Causus
		Cerastes
		Daboia
		Echis
		Eristocophis
		Macrovipera
		Monatatheris
		Montivipera
		Proatheris
		Pseudoceraste
		Vipera

Along with original research provided in this study, this list is compiled from Zaher (1999), Zug et al. (2001), Utiger et al. (2002), Malhotra and Thorpe (2004), Schätti and Utiger (2001), Nagy et al. (2004), Voris et al. (2002), Garrigues et al. (2005), and Scanlon and Lee (2004).

dontinae, Elapinae, Hydrophiinae, Pseudoxyrhophiinae, Atractaspidinae, and Xenodermatinae. We recognize that the Boodontinae is a polyphyletic assemblage and have made the following taxonomic rearrangements to rectify this currently undesirable grouping: *Grayia* and *Macroprotodon* have been moved to Colubrinae and *Duberria* has been moved to Pseudoxyrophiinae. Due to poor sampling, it is not clear if the remaining boodontines form a natural group. *Prosymna* (a former colubrine) and *Psammodynastes* (a former natricine) have been placed as *insertae sedis* within the family Elapidae.

Unfortunately, sampling is not adequate to evaluate the monophyly of the Homalopsinae or the Pareatinae. However, given our analyses, they do not appear to group with either the elapids (Clade A) or the colubrids (clade B). At this time we elevate them to Homalopsidae and Pareatidae, fully recognizing their uncertain position within the Colubroidea.

4.5. Final note

A stable phylogeny and consequent classification of colubroids remains elusive. Our data and analyses corroborated a number of hypotheses from previous work but also produced some novel hypotheses that reject old familiar notions of relationship and evolutionary history. We expect these controversial hypotheses will continue to be tested with the ultimate goal of yielding a stable picture of colubroid evolutionary history.

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References

Bogert, C.M., 1940. Herpetological results of the Vernay Angola Expedition, with notes on African reptiles in other collections. Part I.—Snakes, including an arrangement of African Colubridae. Bull. Am. Mus. Nat. Hist. 77, 1–107.

Boie, F., 1827. Bemerkungen über Merrem's Versuch eines Systems der Amphibien. I-te Lieferung: Ophidier. Isis von Oken, Jena 20, 508–566.

Bourgeois, M., 1968. Contribution à la morphologie comparée du crâne des ophidiens de l'Afrique Centrale. Publ. Univ. Off. Congo, Lubumbashi, 18, 1–293.

Burbrink, F.T., Lawson, R., Slowinksi, J.B., 2000. MtDNA phylogeography of the North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54, 2107–2118.

Cabot, E.L., Beckenbach, A.T., 1989. Simultaneous editing of nucleic acid and protein sequences with EESE. Comput. Appl. Biosci. 5, 233–234.

Cadle, J.E., 1984. Molecular systematics of neotropical xenodontine snakes: III. Overview of xenodontine phylogeny and the history of New World snakes. Copeia 1984, 641–652.

Cadle, J.E., 1985. The neotropical colubrid snake fauna (Serpentes: Colubridae): lineage components and biogeography. Syst. Zool. 34, 1–20.

Cadle, J.E., 1988. Phylogenetic relationships among advanced snakes: a molecular perspective. University of California Publication in Zoology 119, 1–70.

Cadle, J.E., 1992. Phylogenetic relationships among vipers: immunological evidence. In: Campbell, J.A., Brodie, Jr., E.D. (Eds.), Biology of the Pitvipers. Selva Natural History Book Publishers, Tyler, TX, pp. 41–48.

Cadle, J.E., 1994. The colubrid radiation in Africa (Serpentes: Colubridae): phylogenetic relationships and evolutionary patterns based on immunological data. Zool. J. Linn. Soc. 110, 103–140.

Campbell, B.N., 1997. Hic sunt serpentes: molecular phylogenetics and the Boidae (Serpentes: Booidea). Unpublished Ph.D. dissertation, Queens University, Ontario, Canada.

Crother, B.I., 1999. Phylogenetic relationships among West Indian xenodontine snakes (Serpentes: Colubridae) with comments on the phylogeny of some mainland xenodontines. Contemporary Herpetology 1999 <http://dataserver.calacademy.org/herpetology/herpdocs/index.html>.

Cundall, D., Wallach, V., Rossman, D.A., 1993. The systematic relationships of the snake genus *Anomochilus*. Zool. J. Linn. Soc. 109, 275–299.

de Queiroz, A., Donoghue, M.J., Kim, J., 1995. Separate versus combined analysis of phylogenetic evidence. Ann. Rev. Ecol. Syst. 26, 657–681.

de Queiroz, A., Lawson, R., Lemos-Espinal, J.A., 2002. Phylogenetic relationships of North American garter snakes (*Thamnophis*) based on four mitochondrial genes: how much DNA is enough? Mol. Phylogenet. Evol. 22, 315–329.

Dowling, H.G., Duellman, W.E., 1978. Systematic Herpetology: A Synopsis of Families and Higher Categories. HISS Publications, New York.

Dowling, H.G., Highton, R., Maha, G.C., Maxson, L.R., 1983. Biochemical evaluation of colubrid snake phylogeny. J. Zool., Lond. 201, 309–329.

Dowling, H.G., Jenner, J.V., 1988. Snakes of Burma. Checklist of reported species and bibliography. Smithsonian Herpetological Information Service 76.

Dowling, H.G., Hass, C.A., Hedges, S.B., Highton, R., 1996. Snake relationships revealed by slow-evolving proteins: a preliminary survey. J. Zool., Lond. 240, 1–28.

Garrigues, T., Dauga, C., Ferquel, E., Choumet, V., Failloux, A.B., 2005. Molecular phylogeny of *Vipera* Laurenti, 1768 and the related genera *Macrovipera* (Reuss, 1927) and *Daboia* (Gray, 1842), with comments about neurotoxic *Vipera aspis aspis* populations. Mol. Phylogenet. Evol. 35, 35–47.

Gravlund, P., 2001. Radiation within the advanced snakes (Caenophidia) with special emphasis on African opistoglyph colubrids, based on mitochondrial sequence data. Biol. J. Linn. Soc. 72, 99–114.

Graybeal, A., 1994. Evaluating the phylogenetic utility of genes: a search for genes informative about deep divergences among vertebrates. Syst. Biol. 43, 174–193.

Greene, H.W., 1997. Snakes: The Evolution of Mystery in Nature. University of California Press, Berkeley, CA.

Guindon, Gascuel, 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Harris, D.J., Arnold, E.N., Thomas, R.H., 1999. A phylogeny of the European lizard genus *Algyroides* (Reptilia: Lacertidae) based on DNA sequences, with comments on the evolution of the group. J. Zool., Lond. 249, 49–60.

Heise, P.J., Maxson, L.R., Dowling, H.G., Hedges, S.B., 1995. Higher-level snake phylogeny inferred from mitochondrial DNA sequences of 12S rRNA and 16S rRNA genes. Mol. Biol. Evol. 12, 259–265.

Hendy, M.D., Steel, M.A., Penny, D., Henderson, I.M., 1988. Families of trees and consensus. In: Bock, H.H. (Ed.), Classification and Related Methods of Analysis. Elsevier Science Publishers B.V., North Holland, pp. 355–362.

Heymans, J.C., 1975. La musculature mandibulaire et le groupe parotidien des *Aparallactinae* et *Atractaspinae* (Serpents *Coubridae* á majorité fouisseurs). Rev. Zool. Afr. 89, 889–905.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes 2.01 (Bayesian Analysis of Phylogeny).

Jackson, K., Fritts, T.H., 1995. Evidence from tooth surface morphology for a posterior maxillary origin of the proteroglyph fang. Amphibia-Reptilia 16, 273–288.

Jenner J.V., 1981. A zoogeographic study and the taxonomy of the xenodontine colubrid snakes. Unpublished D.Phil. Thesis, New York University.

Kass, R.E., Raftery, A.E., 1995. Bayes factors and model uncertainty. J. Am. Stat. Assoc. 90, 773–795.

Kelly, C.M.R., Barker, N.P., Villet, M.H., 2003. Phylogenetics of advanced snakes (Caenophidia) based on four mitochondrial genes. Syst. Biol. 52, 439–459.

Kluge, A.G., 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). Syst. Zool. 38, 7–25.

Kluge, A.G., 1991. Boine snake phylogeny and research cycles. Misc. Pub. Mus. Zool, Univ. Michigan 178, 1–58.

Kluge, A.G., 1998. Total evidence or taxonomic congruence: cladistics or consensus classification. Cladistics 14, 151–158.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Kochva, E., 1978. Oral glands of reptiles. In: Gans, C., Gans, K.A. (Eds.), Biology of the Reptilia, vol. 9. Academic Press, London, UK, pp. 43–161.

Kolaczkowski, B., Thornton, J.W., 2004. Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. Nature 431, 980–984.

Kraus, F., Brown, W.M., 1998. Phylogenetic relationships of colubroid snakes based on mitochondrial DNA sequences. Zool. J. Linn. Soc. 122, 455–487.

Lawson, R., Slowinski, J.B., Burbrink, F.T., 2004. A molecular approach to discerning the phylogenetic placement of the enigmatic snake *Xenophidion schaeferi* among the Alethinophidia. J. Zool. Lond. 263, 1–10.

Lee, M.S.Y., Scanlon, J.D., 2002. Snake phylogeny based on osteology, soft anatomy and behaviour. Biol. Rev. 77, 333–401.

Lenk, P., Kalyabina, S., Wink, M., Joger, U., 2001. Evolutionary relationships among the true vipers (Reptilia: Viperidae) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 19, 94–104.

Liem, K.F., Marx, H., Rabb, G.B., 1971. The viperid snake *Azemiops* and its comparative anatomy and phylogenetic position in relation to Viperinae and Crotalinae. Fieldiana Zool. 59, 67–126.

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.

McCarthy, C.J., 1985. Monophyly of elapid snakes (Serpentes: Elapidae). An assessment of the evidence. Zool. J. Linn. Soc. 83, 79–93.

McDowell, S.B., 1961. Systematic division and evolution of the colubrid snake genus *Natrix*, with comments on the subfamily Natricinae, by E.V. Malnate. Copeia 1961, 502–506.

McDowell, S.B., 1968. Snakes usually called *Elaps lacteus* and *E. dorsalis*. J. Linn. Soc. (Zoology) 47, 561–578.

McDowell, S.B., 1987. Systematics. In: Seigel, R.A., Collins, J.T., Novak, S.S. (Eds.), Snakes: Ecology and Evolutionary Biology. Macmillan Publishing, New York, NY, pp. 3–50.

Meirte, D., 1992. Clés de détermination des serpentes d'Afrique. Mus. R. Afr. Cent. Tervuren Belg. Ann. Ser. Octavo Sci. Zool. 267, 1–152.

Miyamoto, M.M., Fitch, W.M., 1995. Testing species phylogenies and phylogenetic methods with congruence. Syst. Biol. 44, 64–76.

Nagy, Z.T., Joger, U., Wink, M., Glaw, F., Vences, M., 2003a. Multiple colonization of Madagascar and Socotra by colubrid snakes: evidence from nuclear and mitochondrial gene phylogenies. Proc. R. Soc. Lond. B. 270, 2613–2621.

Nagy, Z.T., Schmidtler, J.F., Joger, U., Wink, M., 2003b. Systematik der Zwergnattern (Reptilia: Colubridae: *Eirenis*) und verwandter Gruppen anhand von DNA-Sequenzen und morphologischen Daten. Salamandra 39, 149–168.

Nagy, Z.T., Lawson, R., Joger, U., Wink, M., 2004. Molecular systematics of racers, whipsnakes and relatives (Reptilia: Colubridae) using mitochondrial and nuclear markers. J. Zool. Syst. Evol. Res. 42, 223–233.

Newton, M.A., Raftery, A.E., 1994. Approximate Bayesian inference with the weighted likelihood bootstrap. J. Roy. Stat. Soc. Ser. B 56, 3–48.

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

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

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Pough, H.F., Andrews, R.M., Cadle, J.E., Crump, M.L., Savitsky, A.H., Wells, K.D., 2004. Herpetology. Pearson Prentice Hall, Upper Saddle River, NJ.

Rasmussen, J.B., 1985. A new species of *Crotaphopeltis* from East Africa, with remarks on the identity of *Dipsas hippocrepis* Reinhardt, 1843 (Serpentes: Boiginae). Steenstrupia 11, 113–129.

Rassmussen, J.B., 1990. The retina of *Psammodynastes pulverulenta* (Boie, 1827) and *Telescopus fallax* (Fleischmann, 1831) with a discussion of the phylogenetic significance (Colubroidea, Serpentes). Z. Zool. Syst. Evol.—forsch. 28, 269–276.

Rieppel, O., 1988. A review of the origin of snakes. Evol. Biol. 22, 37–130.

Rossman, D.A., Eberle, W.G., 1977. Partition of the genus *Natrix*, with preliminary observations on evolutionary trends in natricine snakes. Herpetologica 33, 34–43.

Saint, K.M., Austin, C.C., Donnellan, S.C., Hutchinson, M.N., 1998. C-mos, a nuclear marker useful for squamate phylogenetic analysis. Mol. Phylogenet. Evol. 10, 259–263.

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

Schätti, B., Utiger, U., 2001. *Hemerophis*, a new genus for *Zamenis socotrae* Günther, and a contribution to the phylogeny of Old World racers, whip snakes, and related genera (Reptilia: Squamata: Colubrinae). Rev. Suisse Zool. 108, 919–948.

Sequencher., 1999. Gene Codes Corporation, Inc., Ann Arbor, MI.

Slowinski, J.B., Boundy, J., Lawson, R., 2001. The phylogenetic relationships of Asian coral snakes (Elapidae: *Calliophis* and *Maticora*) based on morphological and molecular characters. Herpetologica 57, 233–243.

Slowinski, J.B., Keogh, J.S., 2000. Phylogenetic relationships of elapid snakes based on cytochrome *b* mtDNA sequences. Mol. Phylogenet. Evol. 16, 157–164.

Slowinski, J.B., Lawson, R., 2005. Elapid relationships. In: Donnelly, M.A., Crother, B.I., Guyer, C., Wake, M.H., White, M.E. (Eds.), Ecology and Evolution in the Tropics: Essays in tribute to J.M. Savage. University of Chicago Press, Chicago, pp. 174–189.

Slowinski, J.B., Lawson, R., 2002. Snake phylogeny: evidence from nuclear and mitochondrial genes. Mol. Phylogenet. Evol. 23, 194–202.

Statistica 6., 2003. Statsoft, Inc. Tulsa, OK.

Swofford, D.L., 2003. PAUP*bv10: Phylogenetic analysis using parsimony (* and other methods) v. 4. Sinauer Assoc., Sunderland, MA.

Tchernov, E., Rieppel, O., Zaher, H., Polcyn, M.J., Jacobs, L.L., 2000. A fossil snake with limbs. Science 287, 2010–2012.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Underwood, G., 1967. A Contribution to the Classification of Snakes. British Museum (Natural History), London, UK.

Underwood, G., Kochva, E., 1993. On the affinities of the burrowing asps *Atractaspis* (Serpentes: Atractaspididae). Zool. J. Linn. Soc. 107, 3–64.

Utiger, U., Helfenberger, N., Schätti, B., Schmidt, C., Ruf, M., Ziswiler, V., 2002. Molecular systematics and phylogeny of Old and New World ratsnakes, *Elaphe* Auct., and related genera (Reptilia, Squamata, Colubridae). Russian J. Herp. 9, 105–124.

Vidal, N., Hedges, S.B., 2002. Higher-level relationships of caenophidian snakes inferred from four nuclear and mitochondrial genes. Comptes Rendus de l'Academie des Sciences, Paris Biologies 325, 987–995.

Vidal, N., Kindl, S.G., Wong, A., Hedges, S.B., 2000. Phylogenetic relationships of xenodontine snakes inferred from 12s and 16s ribosomal RNA sequences. Mol. Phylogenet. Evol. 15, 389–402.

Voris, H.K., Alfaro, M.E., Karns, D.R., Starnes, G.L., Thompson, E., Murphy, J.C., 2002. Phylogenetic relationships of the Oriental-Australian rear-fanged water snakes (Colubridae: Homalopsinae) based on mitochondrial DNA sequences. Copeia 2002, 906–915.

Wilcox, T.P., Zwickl, T.A., 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. 23, 361–371.

Williams, K., Wallach, V., 1989. Snakes of the World, vol. 1. Synopsis of snake generic names. Krieger, Malabar, FL.

Xia, X., 2000. Data Analysis in Molecular Biology and Evolution. Kluwer Academic, Berlin.

Zaher, H., 1999. Hemipenial morphology of the South American xenodontine snakes, with a proposal for a monophyletic Xenodontinae and a reappraisal of colubroid hemipenes. Bull. Am. Mus. Nat. Hist. 240, 1–168.

Zug, G.R., Vitt, L.J., Caldwell, J.P., 2001. Herpetology: An Introductory Biology of Amphibians and Reptiles. Academic Press, New York, NY.