

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.

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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 *Homonoselaps* 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 ana-

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

(continued on next page)

Table 1(continued)

Family	Subfamily	Genus and species	Museum	Voucher number	GenBank cyt b/c-mos
		<i>Amphiesma stolata</i>	CAS	206560	AF471030/AF471097
		<i>Natriciteres olivacea</i>	CAS	220640	AF471058/AF471146
		<i>Natrix natrix</i>	CAS	175878	AF471059/AF471121
		<i>Psammodynastes pulverulentus</i>	CAS	213503	AF471031/AF471157
		<i>Regina rigida</i>	CAS	165994	AF471052/AF471120
		<i>Rhabdophis tigrinus</i>	LSUMZ	37418	AF471051/AF471119
		<i>Storeria dekayi</i>	CAS	106039	AF471050/AF471154
		<i>Thamnophis godmani</i>	MZFC	10202	AF420135/AF471165
		<i>Xenochrophis punctulatus</i>	CAS	201594	AF471079/AF471106
	Pareatinae	<i>Pareas macularius</i>	CAS	206620	AF471082/AF471150
	Psammophiinae	<i>Malpolon monspessulanus</i>	MVZ	186256	AY058965/AY058936
		<i>Mimophis mahfalensis</i>	HLMD	J68	AY188032/AY187993
		<i>Psammophis condanarus</i>	CAS	205003	AF471075/AF471104
	Pseudoxenodontinae	<i>Pseudoxenodon karlschmidti</i>	ROM	30627	AF471080/AF471102
	Pseudoxyrhopiinae	<i>Ditytophis vivax</i>	HLMD	RA-2972	AY188013/AY188052
		<i>Leioheterodon modestus</i>	LSUMZ	H1991	AY058967/AY058933
		<i>Alluaudina bellyi</i>	MRSN FAZC	10622	AY188005/AY187966
		<i>Ithyocyphus miniatus</i>	MRSN FAZC	10680	AY188019/AY187980
	Xenodermatinae	<i>Oxyrhabdium leporinum</i>	SURC	No number	AF471029/DQ112081
	Xenodontinae	<i>Alsophis portoricensis</i>	CAS	200813	AF471085/AF471126
		<i>Arrhyton exiguum</i>	CAS	200732	AF471071/AF471117
		<i>Farancia abacura</i>	CAS	184359	U49307/AF471141
		<i>Helicops angulatus</i>	LSUMZ	H3346	AF471037/AF471160
		<i>Heterodon simus</i>	CAS	195959	AF217840/AF471142
		<i>Hydrops triangularis</i>	LSUMZ	H3105	AF471039/AF471158
		<i>Hypsigena torquata</i>	CAS	205337	AF471038/AF471159
Elapidae		<i>Bungarus fasciatus</i>	CAS	207988	AY217830/AY058924
		<i>Sinomicrourus japonicus</i>	CAS	204980	AY217831/AY058926
		<i>Dendroaspis polylepis</i>	CAS	220644	AY217832/AY058928
		<i>Laticauda colubrina</i>	CAS	220643	AF217834/AY058932
		<i>Micrurus fulvius</i>	CAS	178659	AY217839/AY058935
		<i>Naja kaouthia</i>	CAS	206602	AF217835/AY058938
		<i>Notechis ater</i>	SAM	R31604	AF217836/AF058937
		<i>Ophiophagus hannah</i>	CAS	206601	AF217842/AF058940
Viperidae		<i>Agkistrodon piscivorus</i>	CAS	214406	AF471074/AF471096
		<i>Atheris nitschei</i>	CAS	201708	AF471070/AF471125
		<i>Bitis nasicornis</i>	CAS	207874	AF471069/AF471130
		<i>Cerastes cerastes</i>	No voucher		AF471028/AF471131
		<i>Crotalus viridis</i>	CAS	200713	AF471066/AF471135
		<i>Daboia russelli</i>	CAS	205255	AF471076/AF471156
Acrochordidae		<i>Acrochordus granulatus</i>	No voucher		AF217841/AF471124
Outgroups		<i>Boa constrictor</i>	No voucher		AF471036/AF471115
		<i>Casarea dussumieri</i>	No voucher		U69755/AF471114
		<i>Cylindrophis ruffus</i>	CAS	206622	AF471032/AF471113
		<i>Uropeltis phillipsi</i>	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 Mg²⁺ 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 Mg²⁺ 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 Mg²⁺ 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 Phylml 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 (Kolaczowski 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 \leftrightarrow C = 0.1180, A \leftrightarrow G = 3.6011, A \leftrightarrow T = 0.2997, C \leftrightarrow G = 0.28963, C \leftrightarrow T = 3.6011, and G \leftrightarrow 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 \leftrightarrow C = 1.1636, A \leftrightarrow G = 6.0069, A \leftrightarrow T = 0.8762, C \leftrightarrow G = 1.5445, C \leftrightarrow T = 6.0069, and G \leftrightarrow 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 \leftrightarrow C = 0.1213, A \leftrightarrow G = 3.5348, A \leftrightarrow T = 0.2995, C \leftrightarrow G = 0.4042, C \leftrightarrow T = 3.5348, and G \leftrightarrow 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

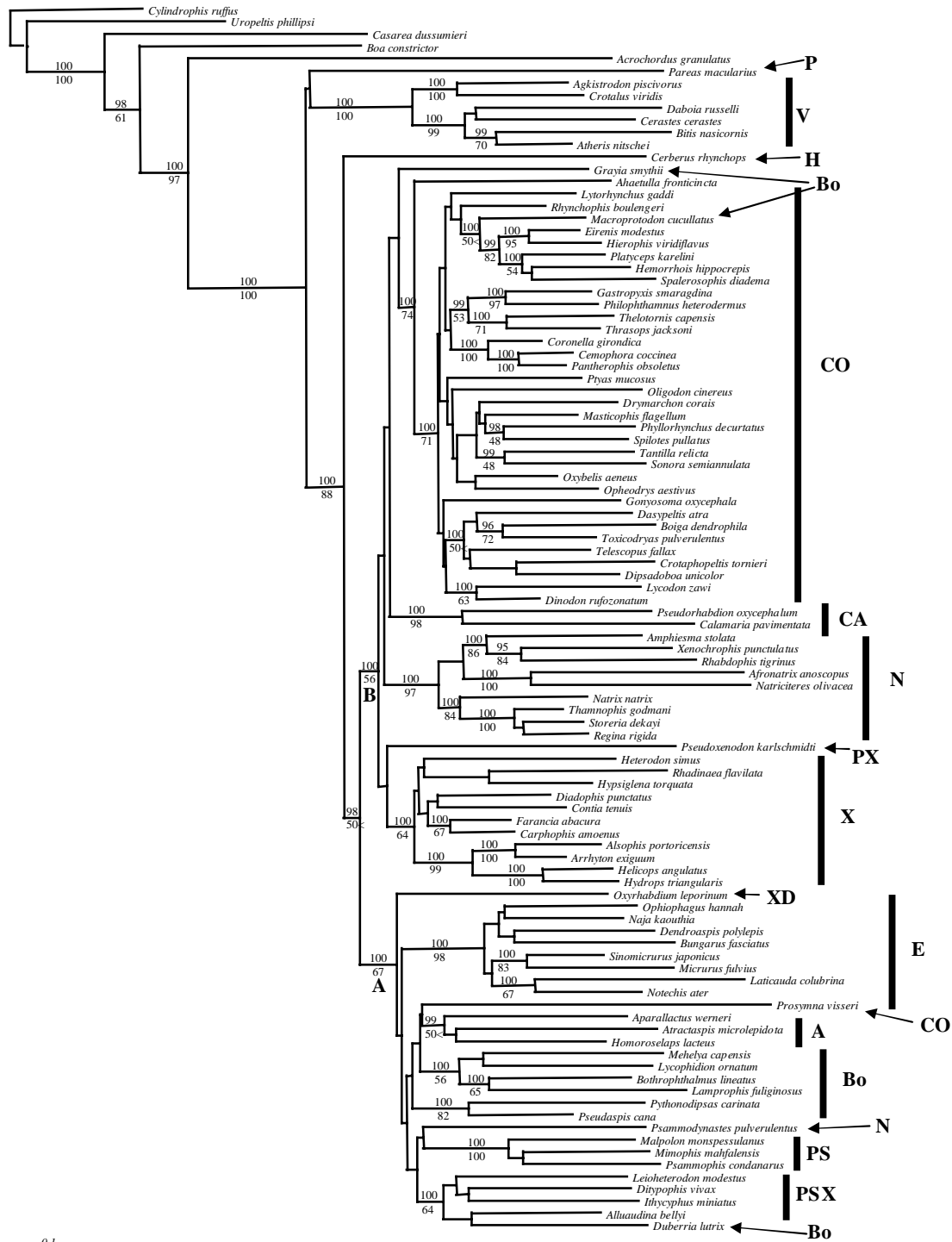


Fig. 1. Maximum likelihood inferred phylogeny of the combined data. Values above branches indicate the percent posterior probability support and values below branches indicate percent nonparametric bootstrap support. The labels (A) and (B) on two internal nodes identify the two main clades discussed 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(GTR + Γ + I)

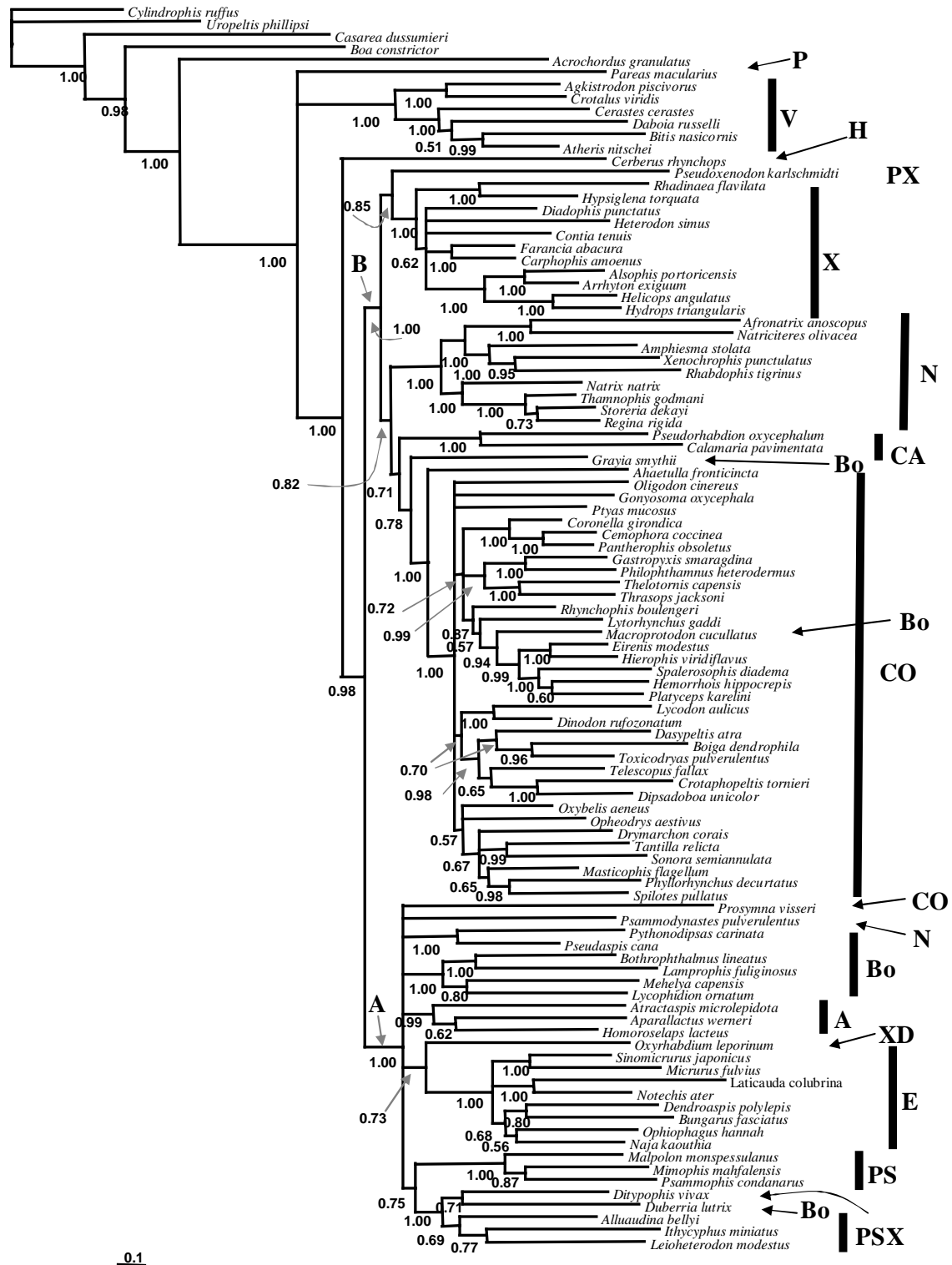


Fig. 2. Bayesian inference of phylogeny of the combined data. The values assigned to the internodes indicate posterior probability support. The labels (A) and (B) on two internal nodes identify the two main clades discussed in the text. The letter labels refer to the current taxonomic assignment of the 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

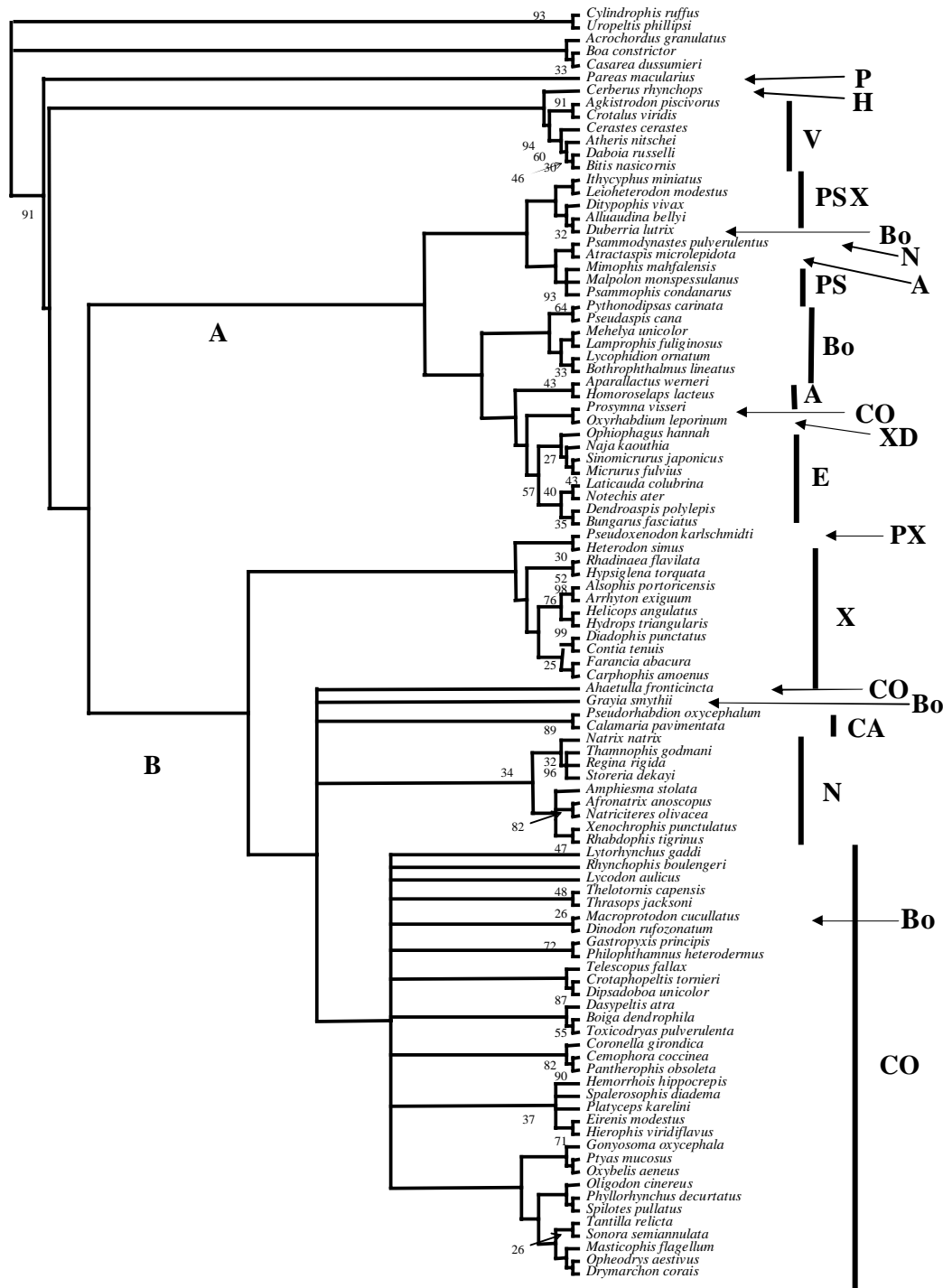


Fig. 3. Maximum parsimony inferred phylogeny of the combined data. This is a strict consensus tree derived from 11 most parsimonious trees. Numbers above branches indicate support from 1000 nonparametric bootstraps. See Tables 3 and 4 for clade character support. The labels (A) and (B) on two internal 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, Pseudoxyrhopiinae.

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 <i>b</i>	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
Pseudoxyrhopiinae (with <i>Duberria</i>)	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 <i>Macroprotodon</i>)	0.96	0.99	1.00
Colubrinae (with <i>Macroprotodon</i> and <i>Grayia</i>)	0.98	0.86	0.78
Natricinae (without <i>Psammodynastes</i>)	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 <i>b</i>	
	<i>n</i>	%	<i>n</i>	%
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 4

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

	c-mos	cyt <i>b</i>
Colubroids + <i>Pareas</i>	0	17
Colubroids	17	39
Clade A + Clade B	1	41
Clade A	4	42
Pseudoxyrhopiinae + Psammophiinae	6	25
Pseudoxyrhopiinae	0	22
Psammophiinae	0	70
Elapidae + Atractaspididae + Boodontinae	6	43
Elapidae + Atractaspididae	0	28
Elapidae + <i>Oxyrhabdium</i> + <i>Prosymna</i>	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

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.

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, pseudoxyrhopiines, psammophiines, atractaspidids, elapids, and xenodermatines, labeled Clade A in Figs. 1–3, and a group composed of natricines, calamariines, 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 Pseudoxyrhopiinae 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 inter-nested 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 pseudoxyrhopiines (Figs. 1–3).

Within the Colubroidea, the Viperidae appear monophyletic and may be related to the Preatinae, 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 Preatinae 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 Preatinae and Viperidae (Fig. 1). The BI is unresolved with regard to viperids, preatines, and colubroids. The monophyly of the Preatinae 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 preatines, *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 *Sinomicrourus* 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 flouces, *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 *Macrotodon* 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*, *Hemorrhais*, *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 Colubrinae. 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 Colubrinae). 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*, *Philophthammus*, *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 uncaped (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 Calamariinae, 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	<i>Calamaria</i>
		<i>Calamorhabdium</i>
		<i>Collorhabdium</i>
		<i>Etheridgeum</i>
		<i>Macrocalamus</i>
		<i>Pseudorabdion</i>
		<i>Rabdion</i>
		Colubrinae
	<i>Ahaetulla</i>	
	<i>Argyrogena</i>	
	<i>Arizona</i>	
	<i>Bogertophis</i>	
	<i>Boiga</i>	
	<i>Cemophora</i>	
	<i>Chilomeniscus</i>	
	<i>Chionactis</i>	
	<i>Chironius</i>	
	<i>Chrysopelea</i>	
	<i>Coelognathus</i>	
	<i>Coluber</i>	
	<i>Conopsis</i>	
	<i>Coronella</i>	
	<i>Crotaphopeltis</i>	
	<i>Cryptophidion</i>	
	<i>Cyclophiops</i>	
	<i>Dasypeltis</i>	
	<i>Dendrelaphis</i>	
	<i>Dendrophidion</i>	
	<i>Dinodon</i>	
	<i>Dipsadoboa</i>	
	<i>Dispholidus</i>	
	<i>Dryadophis</i>	
	<i>Drymarchon</i>	
	<i>Drymobius</i>	
	<i>Drymoluber</i>	
	<i>Dryocalamus</i>	
	<i>Dryophiops</i>	
	<i>Eirenis</i>	
	<i>Elachistodon</i>	
	<i>Elaphe</i>	
	<i>Euprepiophis</i>	
	<i>Ficimia</i>	
	<i>Gastropyxis</i>	
	<i>Geagras</i>	
	<i>Gonyophis</i>	
	<i>Gonyosoma</i>	
<i>Grayia</i>		
<i>Gyalopion</i>		
<i>Hapsidophrys</i>		
<i>Hemerothis</i>		
<i>Hemorrhoidis</i>		
<i>Hierophis</i>		
<i>Lampropeltis</i>		
<i>Leptodrymus</i>		
<i>Leptophis</i>		
<i>Lepturophis</i>		
<i>Liopeltis</i>		
<i>Lycodon</i>		
<i>Lycognathophis</i>		

Table 5 (continued)

Family	Subfamily	Genus
		<i>Lytorhynchus</i>
		<i>Macroprotodon</i>
		<i>Masticophis</i>
		<i>Mastigodryas</i>
		<i>Meizodon</i>
		<i>Oligodon</i>
		<i>Oocatachus</i>
		<i>Opheodrys</i>
		<i>Orthiophis</i>
		<i>Oreophis</i>
		<i>Oxybelis</i>
		<i>Pantherophis</i>
		<i>Philothamnus</i>
		<i>Phyllorhynchus</i>
		<i>Pituophis</i>
		<i>Platycephalus</i>
		<i>Pseudelaphe</i>
		<i>Pseudoficimia</i>
		<i>Pseustes</i>
		<i>Ptyas</i>
		<i>Rhamnophis</i>
		<i>Rhinechis</i>
		<i>Rhinobothryum</i>
		<i>Rhinocheilus</i>
		<i>Rhynchocalamus</i>
		<i>Rhynchophis</i>
		<i>Salvadora</i>
		<i>Scaphiodontophis</i>
		<i>Scolecophis</i>
		<i>Senticolis</i>
		<i>Sibynophis</i>
		<i>Simophis</i>
		<i>Sonora</i>
		<i>Spalerosophis</i>
		<i>Spilotes</i>
		<i>Stegonotus</i>
		<i>Stenorrhina</i>
		<i>Stilosoma</i>
		<i>Symphimus</i>
		<i>Sympholis</i>
		<i>Tantilla</i>
		<i>Tantillita</i>
		<i>Telescopus</i>
		<i>Thelotornis</i>
		<i>Thrasops</i>
		<i>Trimorphodon</i>
		<i>Xenelaphis</i>
		<i>Zamenis</i>
		<i>Zaocys</i>
	Natricinae	<i>Adelophis</i>
		<i>Afronatrix</i>
		<i>Amphiesma</i>
		<i>Amphiesmoides</i>
		<i>Anoplohydrus</i>
		<i>Aspidura</i>
		<i>Atretium</i>
		<i>Balanophis</i>
		<i>Clonophis</i>
		<i>Hologerrhum</i>
		<i>Hydrablades</i>
		<i>Hydraethiops</i>
		<i>Iguanognathus</i>

(continued on next page)

Table 5 (continued)

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

Table 5 (continued)

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

Table 5 (continued)

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

Table 5 (continued)

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

(continued on next page)

Table 5 (continued)

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

Table 5 (continued)

Family	Subfamily	Genus
		<i>Monatatheris</i>
		<i>Montivipera</i>
		<i>Proatheris</i>
		<i>Pseudoceraste</i>
		<i>Vipera</i>

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).

dentinae, 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 Pseudoxyrhophiinae. 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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