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Phylogenetic relationships of colubroid snakes based on mitochondrial DNA sequences

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We used a 694 bp length of the mitochondrial ND4 gene from 40 genera to infer phylogenetic relationships among colubroid snakes. The goals of this study were to identify conserved subsets of ND4 sequence data that could be used to address (1) which nominal higher-level colubroid taxa are monophyletic, and (2) the relationships among the monophyletic lineages identified. Use of transversions only proved the most reliable and efficient means of retrieving colubroid relationships. Transversion parsimony and neighbour-joining analyses identify similar monophyletic higher-level taxa, but relationships among these lineages differ considerably between the two analyses. These differences were affected by the inclusion/exclusion of (1) transitions, (2) autapomorphies, and (3) the boid outgroups. Saturation effects among the transitions, uninformativeness of autapomorphies for clustering taxa, and long-branch and base-compositional problems among the boids lead us to regard the tree resulting from transversion parsimony analysis rooted with Acrochordus as the best current estimate of colubroid phylogenetic relationships. However, several aspects of this proposed phylogeny need further testing (e.g. the apparent diphyly of Natricinae is especially controversial). Relationships retrieved using all colubroid taxa are not obtained when sparsely or unevenly sampled experimental subsets of taxa are used instead, suggesting that long-branch problems can severely compromise elucidation of colubroid relationships if limited taxonomic sampling strategies are followed. We discuss the importance of this finding for previous molecular attempts to assess colubroid relationships. Our analyses confirm the historical validity of several nominal colubroid families and subfamilies, establish polyphyly of a few, but generally fail to resolve relationships among the monophyletic taxa we identify. More conservative character information will be required to confidently resolve the last issue.

ADDITIONAL KEY WORDS:—systematics – venomous snakes – Colubridae – Elapidae – Viperidae.

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INTRODUCTION

The 380 genera and almost 2000 species of Colubroidea, or advanced snakes, comprise approximately 80% of recent snake species diversity (Dowling & Duellman, 1978). In its most conservative, traditional arrangement, this taxon is divided into three families: the Elapidae, or snakes bearing erect, immobile anterior fangs; the Viperidae, or snakes bearing erectable anterior fangs; and the Colubridae, including all higher snakes bearings fangs or grooved teeth on the posterior of the maxilla, or lacking fangs or grooved teeth entirely (Dowling, 1959). The Elapidae includes five subfamilies with some 200 terrestrial and marine species, and, with the possible exception of *Homoroselaps*, appears to be monophyletic on the basis of morphological evidence (McDowell, 1968; McCarthy, 1985). The Viperidae consists of three subfamilies and approximately 250 species, and appears to be monophyletic (Liem, Marx & Rabb, 1971) if the African genus *Atractaspis* is excluded. The Colubridae is a heterogeneous assemblage of approximately 1500 species divided into a number of subfamilies and tribes (see e.g. Underwood, 1967; Dowling & Duellman, 1978; McDowell, 1987). Some of these appear monophyletic on the basis of morphological evidence, but the historical reality of several of the largest proposed subfamilies is tenuous and there is no evidence for the monophyly of the family as a whole. Most commonly in recent years, names have been applied to ten colubrid subfamilies, although there is considerable overlap among some of these (e.g. Boodontinae *sensu* McDowell [1987], and Lycodontinae *sensu* Dowling & Duellman [1978]). Including the three venomous families mentioned above, approximately 13 putative higher-level taxa are subsumed within the Colubroidea (Table 1).

TABLE 1. Taxa used in analysis of Mitochondrial ND4 gene

Family/Subfamily	Species
Atractaspididae	*Atractaspis bibroni*
Colubridiae	
Aparallactinae	*Aparallactus werneri*
Boodontinae	*Leioheterodon madagascariensis, Madagascarophis colubrina*
Calamariinae	*Oreocalamus hanitschi*
Colubrinae	*Boiga dendrophila, Chilomeniscus cinctus, Coluber constrictor, Dendrelaphis pictus, Dispholidus typus, Elaphe flavolineata, Lampropeltis mexicana*[1]
Homalopsinae	*Cerberus rhynchops, Enhydris plumbea*
Lycodontinae	*Lycodon capucinus, Oligodon octolineata*
Natricinae	*Macropisthodon rudis, Nerodia taxispilota, Rhabdophis subminiata, Sinonatrix trianguligera, Storeria occipitomaculata, Thamnophis butleri*
Pareatinae	*Aplopeltura boa, Pareas nuchalis*
Xenoderminae	*Achalinus rufescens, Xenodermus javanicus*
Xenodontinae	*Alsophis portoricensis, Farancia abacura, Helicops pictiventris, Heterodon nasicus*[1], *Hypsiglena torquata*
Elapidae	*Bungarus fasciata, Micrurus fulvius, Pelamis platurus*
Viperidae	*Azemiops feae, Calloselasma rhodostoma, Causus rhombeatus*
Outgroups	*Acrochordus granulatus, Boa constrictor*[1], *Trachyboa boulengeri*

[1] Sequences taken from Forstner *et al.* (1995).

Historically, two separate problems have been encountered in attempts to determine evolutionary relationships among the advanced snakes. The first is the identification and diagnosis of monophyletic lineages within the Colubroidea. The second is the determination of phylogenetic relationships among any lineages so identified. Boulenger (1893) and Cope (1893, 1894a, 1894b, 1895) used dental, lung, and hemipenis characters for their respective classifications. Unfortunately, the phylogenies derived from these different character systems were not congruent, resulting in conflicting classifications. This early discordance has characterized, to a large extent, the state of colubroid classification up to the present, although some success has been had in characterizing a few of the smaller monophyletic groups within the Colubridae (Inger & Marx, 1965; Underwood, 1967; Gyi, 1970; McDowell, 1987).

Most previous attempts at identifying monophyletic groups within and resolving relationships among colubroids have involved either extensive comparisons of morphological characters or immunological comparisons among representatives of only a few of the proposed higher-level colubroid taxa (e.g. Dowling *et al.*, 1983; Cadle, 1988). The morphological approach has been limited by two factors. The first is the difficulty of comprehensively surveying many morphological characters across the 380 genera and 2000 species of the Colubroidea. The second is that many of those characters investigated are highly homoplastic within different colubroid lineages (see e.g. Underwood, 1967) and would seem to be unreliable for resolving relationships across these presumptive lineages. The immunological approach has been limited by incomplete sampling of higher-level taxa and by the employment of assumptions of clocklike evolution for the interpretation of the data. DNA data have been partly brought to bear on this problem too, but the sole study to date (Heise *et al.*, 1995) is flawed by poor alignment, large amounts of sequence saturation, incomplete taxonomic sampling, poor data analysis, and misreading of dendrograms. These flaws are the subject of a separate review by the senior author and will not be considered further here. To avoid the limitations of previous approaches we employed DNA character data from all proposed higher-level colubroid taxa to resolve relationships among colubroids. In particular, we wished to address two questions: (1) for which of the putative higher-level colubroid taxa proposed by such recent authors as Underwood (1967), Dowling & Duellman (1978), and McDowell (1987) do we find preliminary evidence of monophyly; and (2) what are the phylogenetic relationships among the groups so identified?

It must be recognized that critical attempts at character analysis are necessary if DNA sequence data are to be effectively used in phylogenetic inference (e.g. Penny *et al.*, 1990), because the direct evaluations of within-taxon character variation that typify the use of morphological data are generally not available. Under conditions of rapid evolution, DNA sequences saturate quickly (Brown *et al.*, 1982; Aquadro & Greenberg, 1983), thereby reducing the original phylogenetic signal, and highly saturated sequences may behave largely as random strings of nucleotides in phylogenetic inference algorithms, clustering with other taxa on the basis of branch length (Felsenstein, 1978; Penny, 1988; Hendy & Penny, 1989; Miyamoto & Boyle, 1989; Huelsenbeck & Hillis, 1993) or similarity in base composition (Penny *et al.*, 1990; Lockhart *et al.*, 1992a, b, 1994).

One obvious way to avoid this problem is to employ only genes that exhibit a properly low degree of intertaxon variation for the study group in question (Friedlander, Regier & Mitter, 1992, 1994; Graybeal, 1994). The appropriateness of a gene is roughly indicated by its sequence divergence among the taxa in question;

however, because this is an ensemble measure across an entire sequence, it is insensitive to differences in the freedom of different sites within that sequence to vary (Fitch, 1986; Palumbi, 1989; Collins, Wimberger & Naylor, 1994). A limitation of this approach is that divergences can be measured only *a posteriori*, providing no knowledge to guide the *a priori* selection of genes.

Alternatively, because positional and substitutional classes of DNA sequence data have different evolutionary dynamics (Brown *et al.*, 1982; Holmquist, Pearl & Jukes, 1982; Jukes & Bhushan, 1986), with careful character analysis, single genes can provide information over a range of hierarchical levels. Consequently, one may potentially obtain reliable phylogenetic signal from more rapidly evolving genes by focusing on conservative subsets of the data. This is the intent, for example, behind use of only transversion substitutions (Hasegawa, Kishino & Yano, 1985; Miyamoto & Boyle, 1989; Huelsenbeck & Hillis, 1993). For protein-encoding genes, one may expect phylogenetic signal at the three different codon positions to be hierarchically arranged, because of both redundancy in the genetic code and differences in rates of replacement (Fitch, 1980; Hasegawa *et al.*, 1985; Irwin, Kocher & Wilson, 1991). Hence, a significant amount of saturation in subsets of the primary DNA sequence does not necessarily preclude the retrieval of phylogenetic signal from it. With careful analysis of conserved subsets of sequence data, one may theoretically retrieve a considerable amount of phylogenetic resolution from even rapidly evolving genes. Such subsets may be identified *a priori* using a variety of biological criteria to identify putative 'conservativeness'. We have investigated the potential of this approach for our present attempt at understanding evolutionary relationships among colubroid snakes.

MATERIAL AND METHODS

We sequenced 694 base pairs of the mitochondrial ND4 gene from 37 species representing the Atractaspididae, Viperidae, Elapidae, 10 proposed subfamilies of Colubridae, and two outgroups (Table 1, Appendix). Sequence data for the same region in *Lampropeltis mexicana*, *Heterodon nasicus*, and *Boa constrictor* were taken from Forstner, Davis & Arévalo (1995). We included representatives of all proposed colubrid subfamilies except McDowell's (1987) Pseudoxenodontinae, erected to contain two small southeast Asian genera of 'natricines'. Considerable morphological evidence supports the placement of the three species of the Acrochordidae as the sister taxon to the Colubroidea (Underwood, 1967; Groombridge, 1979a–c, 1984; Rieppel, 1988; Kluge, 1991; Cundall, Wallach & Rossman, 1993); hence, we used *Acrochordus* as the proximal outgroup to the Colubroidea. The 'boid' taxa *Trachyboa* and *Boa* were chosen as representatives of successively more distantly related outgroup lineages, based on the topology depicted by Kluge (1991). It should be noted that no character matrix for Kluge's (1991) topology has been published, but those data are available from that author.

The dideoxy-termination method of Sanger *et al.* (1977) was used to sequence single-stranded, PCR-amplified DNA. We used the amplification primers labeled ND4 and Leu by Arévalo, Davis & Sites (1994). Sequencing primers were designed to conserved regions within the ND4 gene. Sequence variation in the regions to which the primers were designed required the construction of several different,

taxon-specific primers in order to sequence all taxa. Ninety to 95% of the 694 bp was sequenced on both strands in all taxa. The remaining 5–10% constituted the region 3′ to the ND4 amplification primer, which could not be sequenced along the light strand. However, because this is the most conserved region of our ND4 sequences, we believe that the lack of verification from the second strand does not compromise the accuracy of the character determination.

Sequences were aligned by eye to conserved amino acid positions. No insertions/deletions were introduced into the data set. Sequences are deposited in GenBank (U41865–66, U41878, U49295–328). Base compositional frequencies were calculated by codon position, using MEGA, version 1.01 (Kumar, Tamura & Nei, 1993). Percent divergences were calculated and phylogenetic analyses performed using PAUP, version 3.1.1 (Swofford, 1993), MacClade, version 3.0 (Maddison & Maddison, 1992), and MEGA, version 1.01. Parsimony searches were conducted heuristically using 10 different random addition sequence replicates, except for reduced data sets having 11 or fewer taxa, in which case the branch-and-bound algorithm was used. Successive approximation searches, when used, based revised weightings on character retention indices (RI). In our analyses we did not constrain the ingroup to be monophyletic. Estimates of tree distribution skewness (g_1 of Huelsenbeck, 1991; Hillis & Huelsenbeck, 1992) were drawn in each case from samples of one million random trees. Significant values of g_1 at $P<0.05$ depend on numbers of taxa, characters, and character states, but are approximately -0.2 for all data sets, as determined from figure 7 of Hillis & Huelsenbeck (1992). Bremer support indices (Bremer, 1988) were calculated using the 'converse constraints' method in PAUP. In one comparison involving only three ingroup taxa, support for the most-parsimonious tree relative to its nearest competitor was assessed with a binomial test (Prager & Wilson, 1988) instead of sign test. Le Quesne character compatibilities (Wilkinson, 1992; Meacham, 1994a) were calculated using COMPROB (Meacham, 1994b). Neighbour-joining (NJ) searches were done to assess the extent to which recovered snake relationships were congruent across estimation methods making different operating assumptions. Neighbour-joining employed MEGA, version 1.01, using a variety of distance-estimation methods (Jukes & Cantor, 1969; Kimura, 1980; Tamura, 1992; Tamura & Nei, 1993).

Parsimony analyses were done with varying numbers of ingroup taxa in order to investigate the effect of sampling density on the phylogenetic conclusions. For this purpose, analyses with 4, 6, 11, 13, 21, 27, 38, and all 40 taxa were performed. The analyses with fewer taxa (4, 6, 11, 13) were intended to simulate the taxonomic sampling regimes of earlier studies (Knight & Mindell, 1994; Dowling *et al.*, 1983; Cadle, 1988, respectively), and employed only representatives of the same family/subfamily-level taxa sampled in those studies. The analyses using 11 and 21 taxa were to investigate the effects of sampling one *versus* two representatives of each of the colubroid families/subfamilies. Investigations employing 38 and 40 taxa were to compare effects of two of the three outgroups on tree topology. All attempts to identify conservative subsets of the sequence data, other than those employing simple transversion parsimony, used 38 taxa (i.e. only one outgroup), for reasons explained below.

RESULTS

Pairwise divergences ranged from 10.5% to 29.8% for all data and from 1.7% to 17.0% for transversions only. The number of parsimony-informative characters

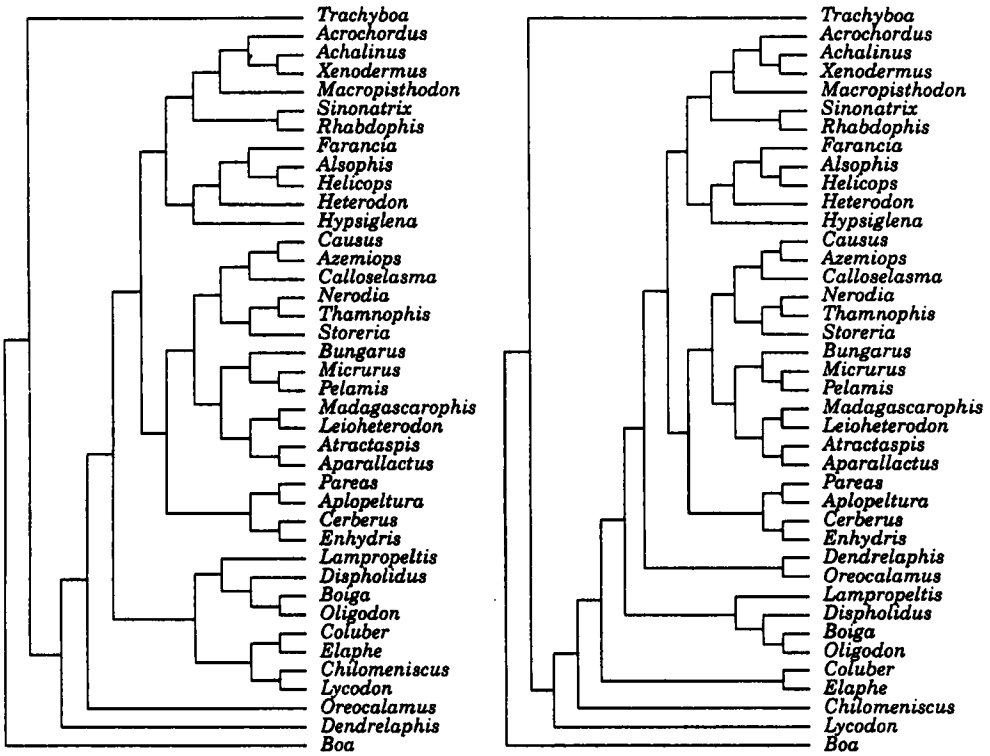

Figure 1. Two equally parsimonious trees obtained using transversion parsimony and employing all outgroups. L = 1470; R.I. = 0.388.

is 391 if all character states are included, and 291 if only transversions are analysed. By codon position, divergences varied from 4.3% to 25.9% at first positions, 1.3% to 14.3% at second positions, and 23.8% to 59.7% at third positions for all substitutions, and from 0.9% to 14.7% at first positions, 0% to 5.2% at second positions, and 3.5% to 37.2% at third positions for transversions only. Transition/transversion ratios (TS/TV) varied from 0.55 to 5.09 for the sequence as a whole, with all but seven pairwise comparisons being less than 2.0 and most centering around 1.0. By position, TS/TV varied from 0.50 to 6.50 at first positions, 0.57 to 20.00 at second positions, and 0.35 to 5.87 at third positions. The poor fossil record of colubroids (Rage, 1984, 1987) doesn't allow us to observe saturation directly from a plot for these taxa, so we are constrained to assess its importance otherwise. From the percent divergences and TS/TV ratios we infer that transitions are saturated (Brown *et al.*, 1982; Aquadro & Greenberg, 1983); hence, we used more conserved subsets of the data for inferring phylogenetic history. We chose to use potentially conserved subsets of the total sequence data for these analyses so as to avoid the limitations inherent in using *a posteriori* character-state weighting methods (Mishler *et al.*, 1988; Collins, Kraus & Estabrook, 1994) such as differentially weighting TSs versus TVs.

Analysis of all transversions produced two most-parsimonious trees with identical ingroup relationships and the outgroups attaching at two different parts of the tree (Fig. 1). The boids root at the colubrines *Dendrelaphis* and *Lycodon*, while *Acrochordus* attaches to the xenodermines. The splitting of the three outgroups onto separate parts of the tree, the variable placement of the boids, the distant relationship of the

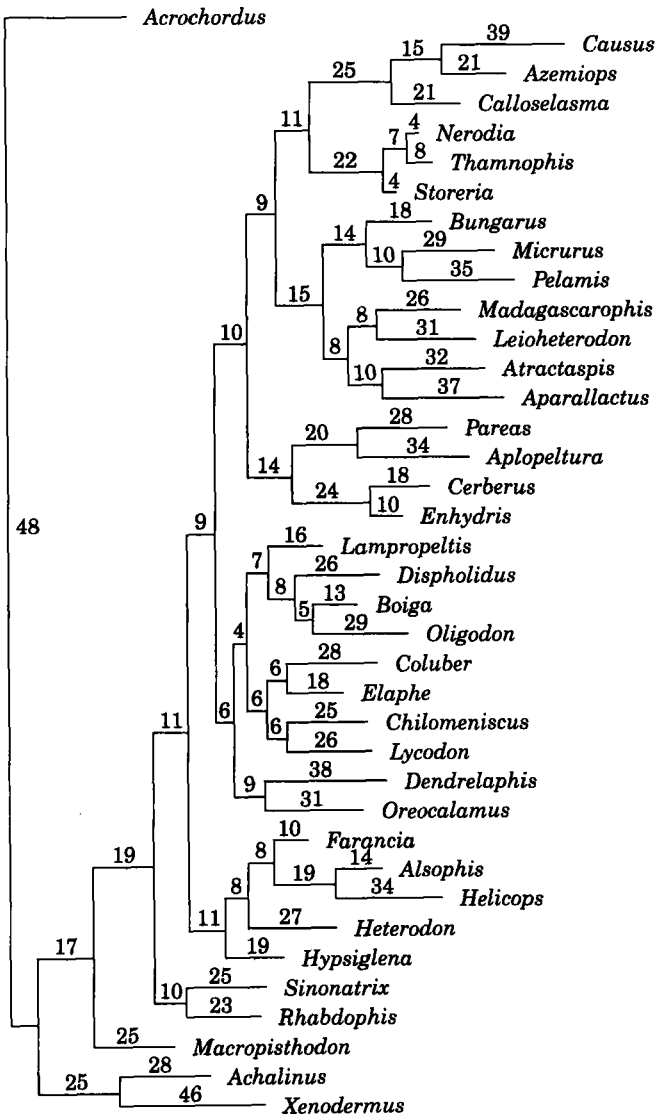

Figure 2. Phylogram of most-parsimonious tree obtained using transversion parsimony and employing only *Acrochordus* as an outgroup. Numbers along branches indicate inferred branch lengths as measured by average numbers of changes. L = 1360; R.I. = 0.396.

boids to the ingroup (Rieppel, 1988; Kluge, 1991), and the fact that they join (in the case of *Dendrelaphis*) to one of the longest branches in the tree (Fig. 2) raise the possibility that the attachment of the boids to the colubrines is an artifact of homoplasy. If true, one might expect their placement to be a reflection of overall base-compositional similarity to the colubrine taxa. Hence, clustering the taxa on the basis of overall base-compositional similarity (constructing a so-called 'GC tree'; see Lockhart *et al.*, 1994) should match to some extent the clustering of the presumed random sequences seen in the most-parsimonious tree. Constructing a Euclidean distance matrix of the base compositional data and clustering those distances using neighbour-joining (Saitou & Nei, 1987), as recommended by Lockhart *et al.* (1994),

indeed places the two boid taxa with *Dendrelaphis* or *Elaphe*, depending on whether the distance matrix is calculated using all four nucleotide states as the starting compositional information or whether the two transversional states 'R' and 'Y' are used. This suggests the boid rooting of the tree at the colubrines in general, and at *Dendrelaphis* in particular, is largely an artifact of base-compositional similarity and probably influenced by long branch length (Fig. 2) as well.

Neither the ingroup relationships in the most-parsimonious tree nor the placement of *Acrochordus* with the xenodermines is evident in the GC tree, which suggests that these topological relationships result from phylogenetic signal instead of artifactitious association due to long-branch attraction. If the latter were a general problem in the data set, one might expect to see the longest branches attaching to each other in descending order of length within the ingroup (Miyamoto & Boyle, 1989). As Figure 2 shows, this is not the case; the longest branches in the tree all connect terminal taxa to relatively short internal nodes. Although *Acrochordus* connects to the longest internal branch of the tree, this appears to reflect phylogenetic signal rather than shared base-compositional similarity.

Use of *Acrochordus* as the only outgroup produces one most-parsimonious tree (Fig. 3) with the same ingroup topology as that produced by the inclusion of the boid taxa, but with the root at the base of the xenodermines. The same result is obtained if all outgroups are retained and the ingroup is constrained to be monophyletic. Successive weighting using RI scores from Figure 3 results in no change in tree topology. Bremer support indices vary considerably across this tree, with most terminal, higher-level taxa being rather well-supported, but most internal nodes joining higher-level taxa having little support (Fig. 3).

The data set has a highly significant g_1 of -0.365. This may be interpreted as indicating that a large amount of phylogenetic signal resides in the data (Hillis & Huelsenbeck, 1992), but says nothing about the location of that signal. A reasonable surmise is that most or all of the signal is contained by the nodes diagnosing the well-supported monophyletic families and subfamilies (Fig. 3). To assess this hypothesis we took one representative of each of the smaller, generally better-supported clades (Viperidae, Elapidae, Boodontinae [*sensu stricto*], Atractaspididae, Pareatinae, Xenoderminae, Homalopsinae, and Thamnophiini), added to them all the representatives of the more questionably monophyletic taxa (e.g. Colubrinae, Xenodontinae), and produced a new data set having 27 taxa, with a significant g_1 of -0.227, analysis of which resulted in 12 most-parsimonious trees (Fig. 4A). This skewness measure is still highly significant and the strict consensus of these most-parsimonious trees suggests that much of this signal may lie in the nodes specifying relationships within the assorted colubrines, joining the South American xenodontines together, and separating *Xenodermus* and *Macropisthodon* from the remaining colubroids (Fig. 4A). To assess the importance of the first two, only one representative of each of the four colubrine clades evident in Figure 4A and only one representative of the South American xenodontines (*sensu* Cadle, 1984a) were used in a subsequent analysis, conducted as above. The rationale for tentatively accepting the reality of these smaller clades is their consistent appearance in each of the phylogenetic analyses of 40, 38, and 27 taxa. Repeating this procedure produces a data set of 21 taxa, which again has a significant g_1 of -0.215, and results in a single most-parsimonious tree (Fig. 4B). Presumably the signal in this case lies mainly in the nodes connecting the xenodontines together, that joining *Sinonatrix* and *Rhabdophis*, and in those joining the elapids, boodontines, and atractaspidids. The remaining

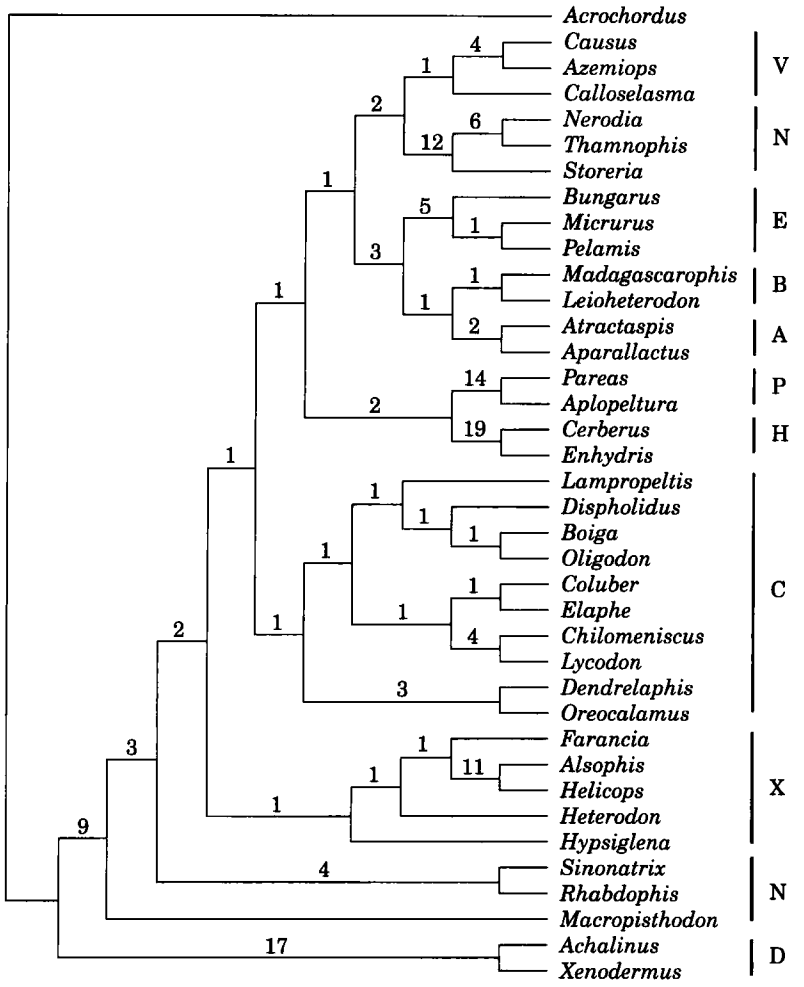

Figure 3. Most-parsimonious tree obtained using transversion parsimony and employing only *Acrochordus* as an outgroup. Taxonomic nomenclature of lineages is shown on right. L = 1360; R.I. = 0.396; $g_1 = -0.365$. Taxon abbreviations are: A = Atractaspididae, B = Boodontinae, C = Colubrinae, D = Xenoderminae, E = Elapidae, H = Homalopsinae, N = Natricinae, P = Pareatinae, V = Viperidae, and X = Xenodontinae.

relationships are sufficiently unlike those obtained from the total analysis (Fig. 3) that they probably represent an artifact of reduced taxonomic sampling instead of real phylogenetic affinity.

Further attempts to refine a more conservative subset of characters out of the primary sequence data result in consensus trees with little resolution or with phylogenetic results that contradict morphologically well-established monophyletic groups. Several approaches were taken (Table 2). Tree skewness statistics for most of these data sets were significant, though some were marginal (Table 2, analyses D, G, J, K, L) and one (Table 2, analysis B) was not significantly different from random. Bremer support analyses could not be done for many of the data sets because of prohibitively long run times (e.g. analyses G, H) or because the heuristic

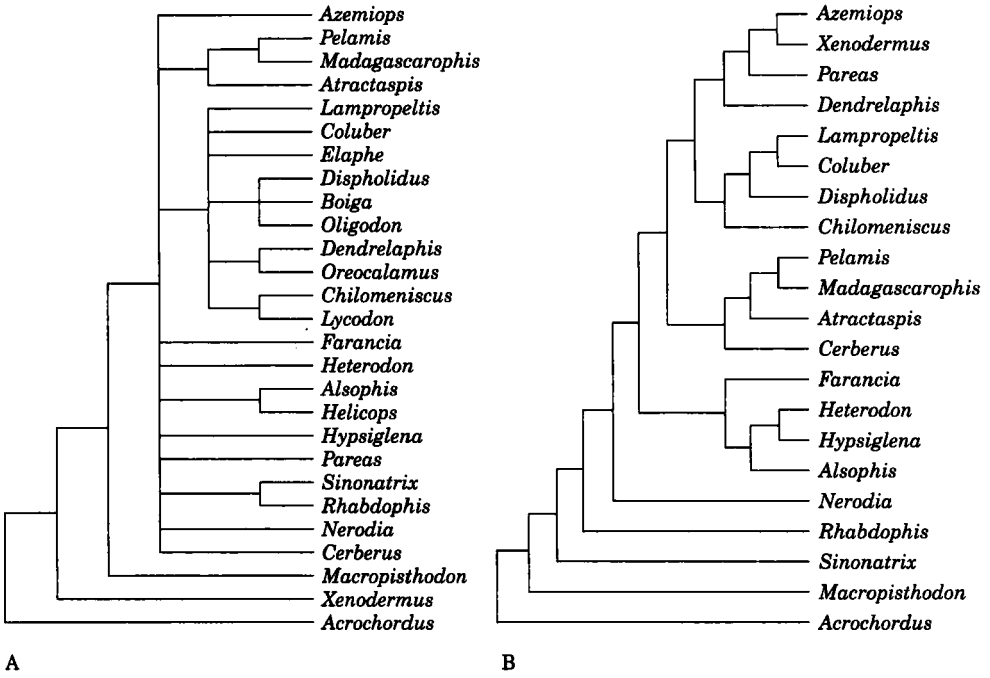

Figure 4. A, strict consensus of 12 equally parsimonious trees obtained using transversion parsimony and including only one representative each of Viperidae, Elapidae, Atractaspididae, Boodontinae, Pareatinae, Xenoderminae, and Thamnophiini. L=1039; R.I.=0.322; $g_1 = -0.227$. B, most-parsimonious tree obtained using same analysis as in (A) but including only one representative of South American Xenodontinae and select Colubrinae. L=867; R.I.=0.307; $g_1 = -0.215$.

searches became trapped in local, though not global, optima (e.g. analyses I, J, K). However, for those data sets for which such support measures could be calculated reliably (analyses A, B, C, E), the only nodes with Bremer support indices greater than 2 were the same taxa well-diagnosed in the transversion parsimony analysis (Fig. 3), e.g. Viperidae, Xenoderminae, Pareatinae, etc. In sum, the best these analyses did was to confirm several of the well-established relationships clear from the transversion parsimony analysis and previous morphological studies. At worst, they resolved relationships clearly non-sensical in light of previous morphological data.

Use of one representative species each of the Colubrinae, Elapidae, and Viperidae, which simulates the taxonomic sampling of Knight & Mindell (1994), produced one most-parsimonious tree of length 192 when rooted with a boid outgroup, and grouped *Coluber* ('Colubridae') with *Calloselasma* (Viperidae) to the exclusion of Bungarus (Elapidae). The two alternative trees have lengths of 193 and 194; hence, there is not significantly greater evidence in support of the most-parsimonious tree over its alternatives (binomial test, $P=0.125$).

Use of six taxa (three colubrines, one xenodontine, one New World natricine, and one 'boodontine'), which simulates the colubroid representation of the study of Dowling *et al.* (1983), produced a single most-parsimonious tree: (*Madagascarophis*(*Thamnophis*(*Heterodon*(*Lampropeltis*(*Coluber*, *Elaphe*))))). The g_1 for these data is

TABLE 2. Results of phylogenetic analyses using only putatively conservative subsets of data

Data set	#MP trees	Statistics[1]	Strictly resolved nodes
(A) 1st & 2nd codon positions only	88	929/0.346/-0.426/8	((*Achalinus, Xenodermus*) (*Oreocalamus*(all other taxa))) ((*Causus, Azemiops*) *Calloselasma*) ((*Bungarus, Atractaspis*)(*Pelamis, Micrurus*) *Aparallactus, Madagascarophis, Leioheterodon*) (*Cerberus, Enhydris*) (*Pareas, Aplopeltura*) (*Rhabdophis* (*Nerodia, Storeria, Thamnophis*)) (*Macropisthodon, Sinonatrix*)
(B) Ditypic characters only[2]	198	565/0.346/-0.100/4	(*Macropisthodon, Calloselasma*(*Causus, Azemiops*)) (*Bungarus, Pelamis*) ((*Rhabdophis*(*Pareas, Aplopeltura*)(*Heterodon, Coluber*)) (*Elaphe*(*Nerodia*(*Thamnophis, Storeria*)))) (*Dispholidus, Madagascarophis,* (*Aparallactus, Leioheterodon*), ((*Achalinus,* *Xenodermus*)(*Chilomeniscus*(*Oreocalamus, Hypsiglena*)))) ((*Alsophis, Oligodon*)(*Lycodon, Helicops*))
(C) Hydrophobic transversions only[3]	6	939/0.404/-0.322/15	((*Achalinus, Xenodermus*)(*Macropisthodon*(all other taxa))) (*Causus, Azemiops, Calloselasma*) (*Madagascarophis, Leioheterodon*) (*Aparallactus, Atractaspis*) ((*Pareas, Aplopeltura*)(*Cerberus, Enhydris*)) (*Lampropeltis*(*Coluber, Boiga*)) ((*Rhabdophis, Sinonatrix*)(*Storeria*(*Thamnophis, Nerodia*))) (*Dendrelaphis, Oreocalamus*) (*Chilomeniscus, Lycodon*) (*Alsophis, Helicops*)
(D) Hydrophilic transversions only[4]	23	392/0.432/-0.229/9	(*Achalinus*(*Macropisthodon*(all other taxa))) (*Cerberus, Enhydris*) (*Aplopeltura, Xenodermus*) *Sinonatrix*) (*Pareas, Aparallactus*) *Dendrelaphis*) (((*Alsophis, Helicops*) *Heterodon*)(*Farancia, Hypsiglena* (*Dispholidus* ((*Chilomeniscus, Lycodon*)(*Coluber* ((*Elaphe, Oreocalamus*)(*Boiga, Oligodon*))))))) (((*Nerodia, Thamnophis*) *Storeria*)(((*Causus, Azemiops*) *Calloselasma*)(((*Bungarus, Pelamis*) *Micrurus*) ((*Madagascarophis, Atractaspis*) *Leioheterodon*))))

(*continued*)

TABLE 2. (*continued*)

Data set	#MP trees	Statistics[1]	Strictly resolved nodes
(E) All nucleotide states ($P<0.05$[5])	51	283/0.483/-0.423/6	(*Rhabdophis* (*Storeria*(*Thamnophis*, *Nerodia*))) (*Lycodon*(*Chilomeniscus*(*Cerberus*, *Enhydris*))) (*Pelamis*, *Madagascarophis*) (*Aparallactus*(*Micrurus*, *Macropisthodon*)) (*Bungarus*(*Atractaspis*(*Coluber*, *Elaphe*))) (*Xenodermus*, *Achalinus*(*Calloselasma*(*Azemiops*, *Causus*)), (all other taxa))
(F) All nucleotide states ($P<0.10$)	96	452/0.456/-0.453/7	(*Cerberus*, *Enhydris*) (*Aparallactus*, *Lycodon*) (*Coluber*, *Elaphe*) ((*Bungarus*, *Leioheterodon*) (*Micrurus*(*Pareas*(*Calloselasma*(*Azemiops*, *Causus*))))) ((*Achalinus*, *Xenodermus*)(all other taxa)) ((((((*Nerodia*, *Storeria*) *Thamnophis*) *Sinonatrix*) *Rhabdophis*) *Pelamis*) *Madagascarophis*)
(G) Transversions only ($P<0.05$)	194	124/0/632/-0.247/6	((*Azemiops*, *Calloselasma*)*Causus*) (*Lampropeltis*, *Heterodon*) (*Oreocalamus*, *Aparallactus*) (*Alsophis*, *Helicops*) (*Hypsiglena*(*Cerberus*, *Enhydris*)) (*Sinonatrix*, *Rhabdophis*) (*Xenodermus*, *Achalinus*) ((((*Thamnophis*, *Storeria*) *Nerodia*) *Madagascarophis*) *Leioheterodon*)
(H) Transversions only ($P<0.10$)	58	181/0.599/-0.277/11	(*Thamnophis*, *Nerodia*, *Storeria*) (*Sinonatrix*, *Rhabdophis*) (*Pareas*, *Aplopeltura*) (*Hypsiglena*(*Cerberus*, *Enhydris*)) (*Alsophis*, *Helicops*) (*Lycodon*(*Boiga*(*Oligodon*, *Dispholidus*))) (*Coluber*, *Elaphe*) (*Causus*(*Calloselasma*, *Azemiops*)) (*Atractaspis*((*Madagascarophis*, *Leioheterodon*) (*Bungarus*(*Micrurus*, *Pelamis*))))

(*continued*)

TABLE 2. (*continued*)

Data set	#MP trees	Statistics[1]	Strictly resolved nodes
(I) Amino acids (20 states)[6]	378	696/0.401/-0.448/10	((*Xenodermus*, *Achalinus*)((*Pareas*, *Aplopeltura*) (all other taxa))) (*Calloselasma*(*Azemiops*, *Causus*)) (*Nerodia*, *Storeria*, *Thamnophis*) ((*Atractaspis*, *Aparallactus*)(*Bungarus*, *Micrurus*, *Pelamis*) (*Leioheterodon*, *Madagascarophis*)) (*Cerberus*, *Enhydris*) (*Dispholidus*, *Lycodon*) (*Alsophis*, *Heterodon*) (*Macropisthodon*(*Sinonatrix*, *Rhabdophis*))
(J) Amino acids (2 states)[7]	167	199/0.475/-0.220/4	(*Azemiops*, *Helicops*) (*Boiga*, *Rhabdophis*) (*Atractaspis*, *Aparallactus*) (*Achalinus*, *Xenodermus*) (*Nerodia*, *Thamnophis*, *Storeria*) (*Cerberus*, *Enhydris*)
(K) Amino acids (4 states)[8]	3335	245/0.467/-0.241/6	(*Achalinus*, *Xenodermus*) ((*Cerberus*, *Enhydris*)(*Storeria*(*Nerodia*, *Thamnophis*))) ((((*Micrurus*, *Pelamis*) *Bungarus*, *Madagascarophis*) (*Atractaspis*, *Aparallactus*)))
(L) Amino acids (5 states)[9]	1110	415/0.428/-0.255/8	(*Azemiops*, *Causus*, *Calloselasma*) ((*Atractaspis*, *Aparallactus*)(*Micrurus*(*Pelamis*, *Bungarus*)) *Madagascarophis*) ((*Elaphe*, *Chilomeniscus*) *Hypsiglena*) (*Oligodon*, *Lycodon*) (*Sinonatrix*, *Rhabdophis*) (*Pareas*, *Aplopeltura*) ((*Cerberus*, *Enhydris*)(*Storeria*(*Nerodia*, *Thamnophis*)))

[1] L/RI/g_1/number of nodes (out of 35 possible) shared in common with the MP tree (Fig. 3). Significance values ($P<0.05$) for g_1 are approximately -0.2 (from fig. 7 of Hillis & Huelsenbeck [1992]).
[2] minus C-T transitions at second-codon positions (cf. Naylor *et al.*, 1995).
[3] includes only TVs from the hydrophobic, membrane-spanning regions of the ND4 molecule (cf. Fearnely & Walker, 1992).
[4] includes only TVs from the hydrophilic, emergent regions of the ND4 molecule (cf. Fearnely & Walker, 1992).
[5] P values are LeQuesne values from compatibility analyses (Meacham, 1984, 1994a; Wilkinson, 1982).
[6] all amino acids are treated as separate states with equal probabilities of change amongst themselves.
[7] categories from Kyte & Doolittle (1982); otherwise treated as for analysis I.
[8] categories from Lewin (1994); otherwise treated as for analysis I.
[9] categories from Lehninger *et al.* (1993); otherwise treated as for analysis I.

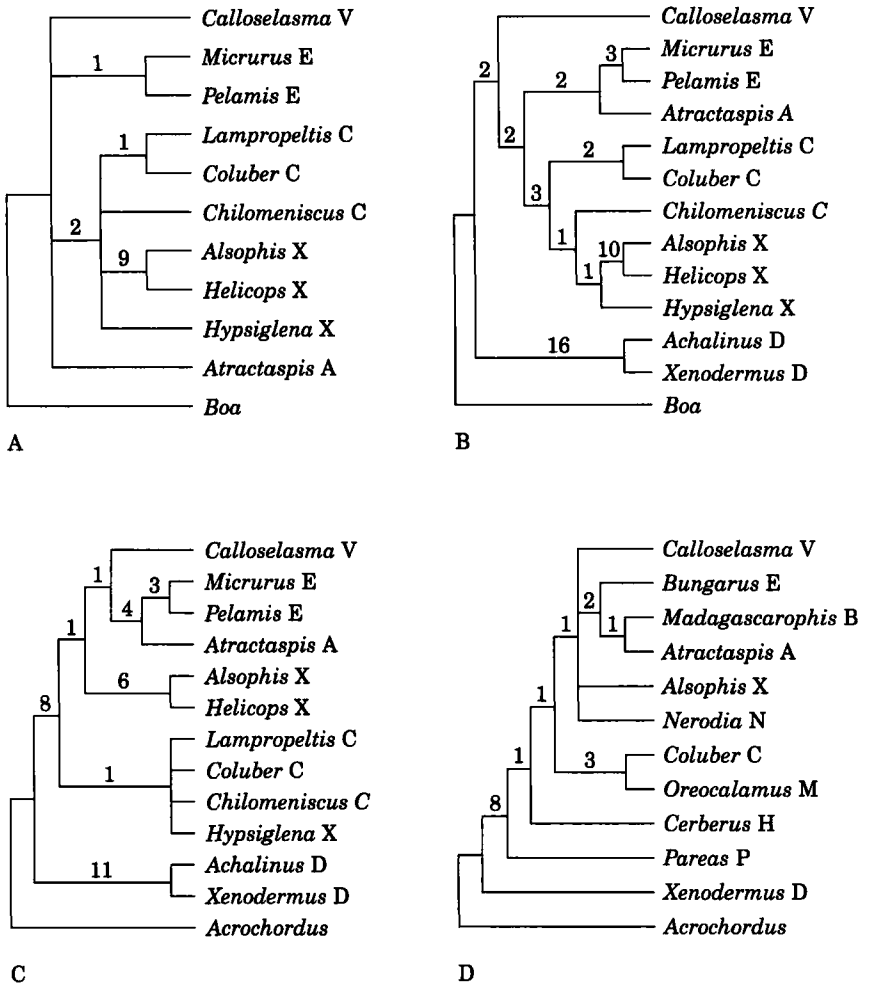

Figure 5. Trees obtained from parsimony analyses of restricted sets of taxa. A, strict consensus of three most-parsimonious trees obtained using transversion parsimony and employing only 11 representatives of the five higher-level colubroid taxa employed by Cadle (1988) and rooted with his outgroup taxon; L=452; R.I.=0.361; $g_1 = -0.540$. B, single most-parsimonious tree obtained using transversion parsimony and employing the same taxa as in (A), but to which have been added the two xenodermine taxa; L=567; R.I.=0.376; $g_1 = -0.691$. C, strict consensus of three most-parsimonious trees obtained using transversion parsimony and employing the same taxa as in (B), but to which *Acrochordus* has been substituted as an outgroup; L=566; R.I.=0.374; $g_1 = -0.765$. D, strict consensus of two most-parsimonious trees obtained using transversion parsimony and employing only one representative of each higher-level colubroid lineage identified from the overall transversion parsimony analysis (Fig. 3); L=572; R.I.=0.290; $g_1 = -0.257$. Taxon abbreviations as in Fig. 3, except also M=Calamariinae. Numbers along branches are Bremer support indices.

-0.046, which is not significant, suggesting that little phylogenetic structure is present.

Use of 11 taxa, which simulates the colubroid and outgroup representation in Cadle's (1988) study (sampling the Crotalinae, Elapidae, Atractaspididae, South American lineage of Xenodontinae, Central American lineage of Xenodontinae, and Colubrinae; see fig. 7 of Cadle [1988]) produced three equally parsimonious trees (Fig. 5A), with a significant $g_1 = -0.540$. When the two xenodermine taxa are

added to these analyses, one most-parsimonious tree (Fig. 5B) is produced, with $g_1 = -0.691$, but the relatively basal position of the viperid representative is not changed. However, if *Acrochordus* is used in place of *Boa* as an outgroup and the xenodermines are included, three equally parsimonious trees with substantially different topologies are produced (Fig. 5C), with $g_1 = -0.765$. The consensus of these three trees has a topology more like that seen in the transversion parsimony analysis of all taxa, with viperids placed far away from the base of the tree as sister taxon to (Elapidae + *Atractaspis*). The significant g_1 statistics for all these data sets suggest considerable structure within each, although the structure may well reside only in clustering the multiple representatives of Colubrinae and Xenodontinae together.

Use of one representative from each of the 11 colubroid families/subfamilies produced two equally parsimonious trees (Fig. 5D) that reproduce several of the nodes (6 of 9 and 4 of 9) obtained by including all ingroup taxa (Fig. 3). For these data, $g_1 = -0.257$, which is marginally significant. Use of two representatives of each of these higher taxa (except for the lone available representative of the Calamariinae) produced one most-parsimonious tree (not shown) that replicates the results of the analysis with all taxa (Fig. 3) except for the placement of atractaspidids basal to (Elapidae + Boodontinae) and the placement of *Alsophis* away from *Hypsiglena*. The g_1 of these data is -0.534.

Clustering of taxa by the neighbour-joining method (Saitou & Nei, 1987) applied to distance data corrected with Kimura's two-parameter model (Kimura, 1980) produced a tree (Fig. 6) that recovers most of the same monophyletic higher-level taxa seen from the transversion parsimony analysis (Fig. 3). Exceptions are that *Dendrelaphis* and *Oreocalamus* are removed from the body of colubrines, the natricines are monophyletic, and problems arise at the base of the colubroids: either *Acrochordus* is joined with the xenodermines if the two boid genera are used for rooting purposes (Fig. 6), or Xenoderminae becomes paraphyletic if *Acrochordus* alone is used for rooting. However, relationships among the monophyletic, higher-level taxa differ considerably from those obtained using transversion parsimony. Of particular interest is that the viperids are moved to a more basal position with colubroids (Fig. 6). These same groupings are obtained whether applied to distances measured from the entire sequences, from parsimony-informative sites only, from first- and second-codon positions only, using all character-state data, or using transversions only (Kumar *et al.*, 1993). The results are largely consistent across other distance estimation methods (Jukes & Cantor, 1969; Tamura, 1992; Tamura & Nei, 1993) as well. However, when the two boid outgroups are removed from the analyses, the natricines frequently move to a more basal position, as in the parsimony trees discussed above. This is the case when transversions alone are used for calculating the distances, whether using only parsimony-informative sites, all first- and second-position transversions, or only first- and second-position parsimony-informative sites. These results, again, are generalizable across several distance estimation methods. However, in every analysis involving transition information, even with the removal of the boid taxa, the viperids are again placed relatively basally in the tree.

The results of the neighbour-joining distance analyses, with regard to the placement of viperids, may be partly replicated by differential weighting of transversions and transitions in a parsimony analysis. If transversions are weighted six times as much as transitions, the same tree as a transversion-only analysis (Fig. 3) is obtained, with the exception that the (Pareatinae + Homalopsinae) clade is placed as sister taxon to the Colubrinae instead of the clade (Viperidae + New World Natricinae +

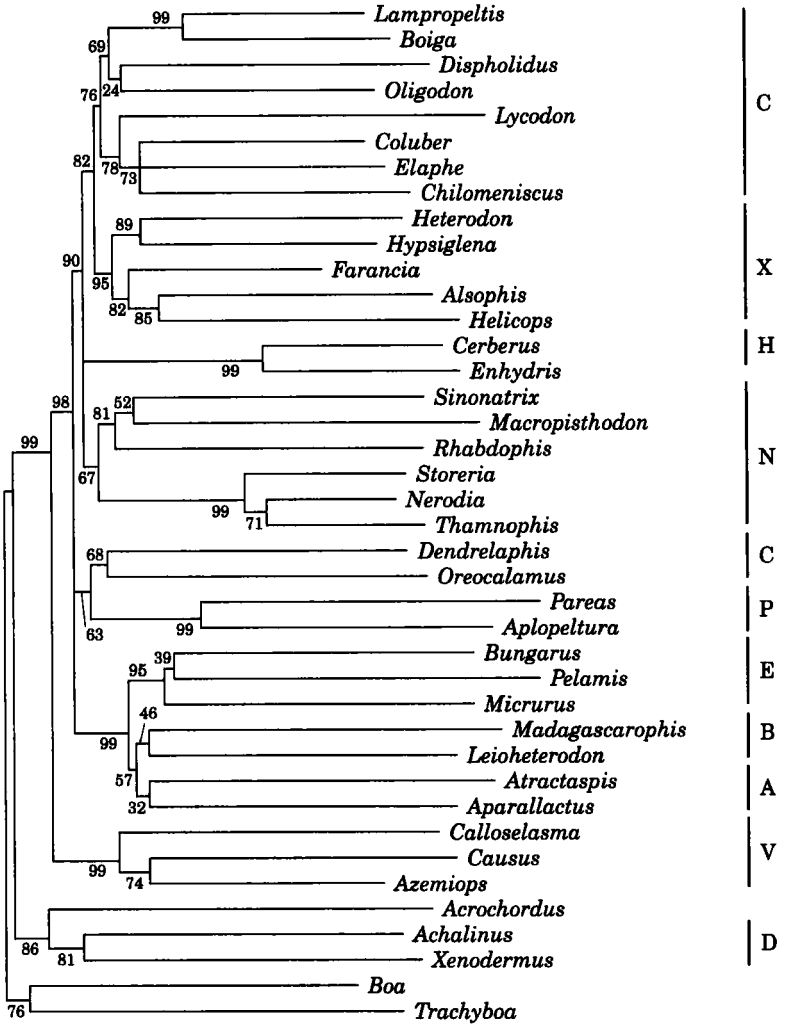

Fig. 6. Neighbour-joining tree obtained using Kimura's (1980) two-parameter model for estimating distances. Distances are based on all character-state data and all variable sites. All outgroups are included. Numbers along branches are branch lengths. Taxon abbreviations as in Fig. 3.

Elapidae + Boodontinae + Atractaspididae). As the relative weight given to transitions increases (TV:TS of 4:1 and 2:1), the viperids are relocated to a more basal position in the tree, branching off after the Old World natricines. These parsimony analyses more closely approximate the rooting results generally obtained from the neighbour-joining analyses employing transitions only, although the viperids are never placed as basally as in those analyses.

DISCUSSION

The search for conservative characters

For our data set, we could not identify conserved subsets of characters more reliable for phylogenetic inference than standard transversion parsimony analysis.

This failure was manifested in two ways. First, many of the data sets (A, B, D-G, J of Table 2) cluster genera that clearly are not sister taxa based on morphological evidence and our own transversion parsimony analysis (Fig. 3). This strongly suggests that the examined methods are inappropriate for our data. Second, all methods provide considerably less resolution of relationships than does the transversion parsimony analysis, though this may partially reflect that these analyses have reduced numbers of characters that are insufficient for complete phylogenetic resolution of 38 taxa. And in the case of analysis I, the large number of potentially available character states (21) results in a great increase in the number of autapomorphic states and a concomitant loss of information for phylogenetic resolution. Only three analyses (D, H, I) provide even moderate resolution of relationships relative to the total transversion parsimony analysis, and these also do not contradict well-established relationships based on morphology (Table 2). Hence, these approaches may warrant further examination by others. For all 'conserved-character' analyses, resolved nodes with Bremer support indices greater than 3 were all of families and subfamilies that have relatively few constituent species and are well-diagnosed by morphological evidence and our transversion parsimony analysis (e.g. Xenoderminae, Pareatinae, Homalopsinae). Thus, subsets of presumptive conservative characters served at best to recover the more divergent lineages, but were of little use in retrieving evolutionary signal relevant to the more problematic relationships.

Interpretation of the merit of such approaches to phylogenetic analysis largely depends on whether relationships obtained are congruent with other evidence. Character subsets producing phylogenetic nonsense may reasonably be concluded to be lacking in adequate phylogenetic signal. Character subsets providing congruence with other phylogenetic data (e.g. analyses D, H, I) may indicate some potential for successful phylogenetic inference, although a greater number of characters may be needed to provide complete resolution of relationships. Pursuit of this approach may require the collection of large amounts of sequence data in order to amplify weak phylogenetic signal from genes with moderate levels of saturation (e.g. Olmstead & Sweere, 1994). A caveat, however, is that simply adding more of the same kind of data is not guaranteed to lead to enhanced resolution, because additional characters having the same evolutionary dynamics as those already demonstrated to be of limited phylogenetic utility may present the same ambiguity of signal, thus leading to no increased resolution or to convergence on a wrong tree (Felsenstein, 1978; Lanyon, 1988; Huelsenbeck & Hillis, 1993). Such characters may be expected, on average, to add to the evidence in support of nodes already diagnosed. Polytomies may remain unresolved, because the same levels of noise leading to their original lack of resolution can be expected in the additional sequences. In any event, for the present data, standard transversion parsimony analysis proved more efficient and informative than alternative approaches to identify conservative phylogenetic characters.

Number of taxa and long branches

Given the transversion parsimony tree (Fig. 3) is our best current estimate of colubroid relationships, the question remains as to how reliable it is, or to what degree it avoids systemic errors due to long-branch attraction or spurious base-compositional similarity among taxa.

The former problem may sometimes be avoided or minimized by judicious selection of taxa so as to break up long branches (Hendy & Penny, 1989; Penny *et al.*, 1990), but this strategy can work only for groups in which annectant forms exist (e.g. see Lanyon, 1988). In the case of colubroid snakes, the diversity of species available in most of the named families and subfamilies (with the possible exceptions of the Xenoderminae and Pareatinae) allows such a dense sampling approach to be taken. One goal of our study was to achieve a dense enough sampling of the major lineages of colubroid diversity to offset most long-branch problems, although it is possible we are only just approaching the sampling density needed to achieve this. The fact that most taxa (with the possible exception of the xenodermines) do not seem to cluster on the basis of branch lengh similarities (Fig. 2) suggests that this goal may have been largely met.

In the present study, light-strand base compositions for all taxa diverged significantly from an even 25% ratio, though deviations from stationarity (Saccone, Pesole & Preparata, 1989) were not great. Base compositional deviations of purines and pyrimidines from 50% were less pronounced because an over-representation of As was largely complemented by an under-representation of Gs. Thus, base-compositional differences within and across taxa seem to be of little importance in influencing the phylogenetic conclusions derived from the transversion analyses. This conclusion holds for trees obtained from both parsimony and neighbour-joining analyses, and is most compellingly supported by the failure of the GC trees to show any concordance with the results obtained from phylogenetic analysis, aside from the previously discussed problem with the boid outgroups.

Thus, we believe we have reduced the likelihood of systemic error resulting from long-branch attraction and spurious base-compositional similarity in the present study. Consequently, we interpret the significant skewness statistics and larger Bremer support indices (three or greater) of the transversion parsimony analyses as indicating that a considerable amount of phylogenetic structure resides in those data. Note, however, that we do not consider all resolved nodes to be equally reliable. Our data best serve to diagnose monophyletic higher-level taxa, but are generally less reliable in establishing relationships among those taxa. We discuss this in greater detail below.

In contrast, all earlier molecular studies of colubroid snakes have failed to pursue a dense and even taxonomic sampling strategy so as to reduce long-branch-attraction problems. Instead, these studies have included three (Knight & Mindell, 1994), four (Dowling *et al.*, 1983), five (Cadle, 1988), and nine (Heise *et al.*, 1995) of the 13 putative higher-level colubroid taxa, and sampling within those higher-level taxa represented has been insufficient. For example, while Heise *et al.*'s (1995) sample of higher-level taxa is greater than the other studies, three of these taxa (Natricinae, Homalopsinae, and 'Lycodontinae') were represented by single genera. We believe such sampling schemes to be inadequate for elucidating higher-level colubroid relationships and likely to lead to erroneous conclusions. In particular, the xenodermines appear critical for breaking up the long-branch attaching outgroups to the remaining colubroids and they have a major effect on root placement if outgroups not too distantly related to colubroids are used (e.g. in Fig. 5 compare A and B with C). But xenodermines were not included in earlier molecular analyses of colubroid relationships, and this may account in part for the frequent conclusion that viperids represent the most basal colubroid taxon (e.g. Cadle, 1988; Heise *et al.*, 1995).

The importance of taxonomic sampling strategy is further supported by our experiments in tree-building using abbreviated samples of taxa, which resulted in trees with topologies that are widely variant from that obtained when all taxa are used (Fig. 5A–C vs. Fig. 3). As well, the experiments using the smallest samples of taxa (three and six representatives) yielded topologies that could not be distinguished from those expected from chance alone, as inferred from binomial and g_1 statistics. Phylogenetic estimates more closely approximated that obtained from the total transversion parsimony analysis (Fig. 3) when sampling was conducted more evenly across all higher-level lineages (Fig. 5D), especially when the sample size was doubled. This illustrates most clearly the need for dense and even sampling of taxa in attempts to resolve highly diverse and divergent clades such as the Colubroidea. In the present case, results very similar to the analysis involving all taxa were had when only two representatives of each higher-level taxon were sampled. This may suggest a rough rule of thumb in sampling strategies for future studies, although denser sampling should, of course, provide even more reliable results in general.

Differences between phylogeny-estimation methods

The transversion parsimony and neighbour-joining analyses give very different estimates of phylogenetic relationships among higher-level colubroid lineages, although they identify virtually the same set of higher-level taxa (compare Figs 3 and 6). The degrees to which information about transitions, the boid outgroups, and autapomorphic sites are incorporated into the analyses seem to account for the differences. None of these factors in isolation is sufficient to explain the observed differences in tree topologies, though inclusion/exclusion of transition information seems to be the most important. In the extreme, for neighbour-joining analyses, when all variable sites, character states and taxa are used in estimating distances, a topology identical with or very similar to that shown in Figure 6 is obtained. This holds no matter what distance estimate is used. At the other extreme, if distance estimates are based only on parsimony-informative transversions and the boids omitted (as in the parsimony analysis), trees more similar to the parsimony tree (Fig. 3) are obtained. As these restrictive parameters are expanded, either by including the boids, transition information, or invariant and unique characters, trees more like Figure 6 are obtained, i.e. having *Acrochordus* clustered with the xenodermines and the viperids as the next-most-basal lineage within the colubroids. Similar results can be had by means of parsimony analysis that includes information on transitions. When transitions are weighted equal to transversions, the results are similar to the distance trees (viperids occupy a basal position in the tree, branching off one node after the xenodermines). As weighting of transversions is doubled or quadrupled relative to transitions, trees with viperids placed in the middle of the tree are obtained. Weighting transversions six times as heavily as transitions produces a tree virtually identical to that obtained using only transversions (Fig. 3). Hence, including information on transitions, whether using parsimony or distance methods, is a major determinant of which of the two alternative tree topologies is obtained.

Clearly, which of the two general topologies one finds most convincing will depend on the relative value one places on information derived from transitions, unique positions, and the boid outgroups. The evidence that transitions as a class are saturated with mutations and that the boids show indications of acting as random

outgroups leads us to question the quality of the information these provide. Under conditions of saturation, and with the high TS:TV bias characteristic of mtDNA (Brown *et al.*, 1982; Aquadro & Greenberg, 1983), transversion parsimony is expected to be least susceptible to estimating the wrong tree (Huelsenbeck & Hillis, 1993; Hillis *et al.*, 1994a). Also, it is more efficient than uniform-parsimony and neighbour-joining methods (Hillis *et al.*, 1994a, b). Although we recognize that we may be discarding some useful phylogenetic information in ignoring transitions, we believe that the likelihood of being systematically misled by including them is higher. Hence, we consider the transversion-parsimony tree (Fig. 3) to be the best current estimate of colubroid relationships available from the ND4 data, even though it may not be a correct reflection of phylogenetic history in every instance. An important caveat, though, is that this cladogram is rooted using only a single outgroup and, hence, the validity of the root is only as good as the assumption derived from morphological evidence that *Acrochordus* indeed represents the sister-group to all Colubroidea. Should it in fact be a highly derived member of the Colubroidea, the root estimate would prove incorrect, although we note that the neighbour-joining analysis, which includes all outgroup taxa, does not support a claim that *Acrochordus* is a highly derived colubroid convergent in morphology with more ancestral snake taxa. Nonetheless, alternative interpretations of colubroid relationships such as this and those shown in Figure 6 must be given serious consideration in future evaluations of the history of this group, and these alternatives may well serve to inform the search for further diagnostic synapomorphies.

Taxonomy

Two factors have contributed to our historically poor understanding of colubroid evolutionary relationships: the great diversity of species involved and the relatively limited range of morphological characters investigated. The confluence of these two factors has resulted in various systems of higher-level colubroid classification. Most attention has been devoted to defining which genera belong to which higher-level lineage, although this undertaking has met with only limited success because of the frequent absence of synapomorphies for diagnosing putative lineages (e.g. see Dowling & Duellman, 1978; McDowell, 1987). Less attention has been given to determining relationships among the putative lineages so identified.

The present study was designed to include as broad a taxonomic range within each proposed lineage as possible, to provide the most severe test of the monophyly of each presumptive higher-level lineage. Because our sample includes divergent representatives of all higher-level colubroid taxa, it is our belief that, with a few possible exceptions discussed below, our sampling is sufficiently broad in taxonomic scope to provide useful tests of monophyly for the proposed higher-level taxa. The confidence we place in our taxonomic conclusions depends on the consistency with which monophyletic groups were retrieved across different analytical methods and the degree of Bremer support each garners in our preferred tree (Fig. 3).

Monophyletic taxa

Our best current phylogenetic estimate of colubroid relationships (Fig. 3), as well as the alternate topology obtained using transitions (Fig. 6), provides evidence

for the monophyly of the Viperidae, Elapidae, Xenoderminae, Homalopsinae, Pareatinae, Thamnophiini (New World natricines), Xenodontinae, Colubrinae (redefined), and Boodontinae (redefined). Monophyly of the first six of these is well-supported by morphological and/or karyological data (Gyi, 1970; Rossman & Eberle, 1977; McCarthy, 1985; McDowell, 1987), and our molecular data serve to strengthen it. Note, however, that our evidence (as well as the morphological studies of Groombridge, 1979a–c, 1984; Rieppel, 1988) falsifies the inclusion of Natricinae, Homalopsinae, and Acrochordidae together as one group (contra Dowling, 1975; Dowling & Duellman, 1978). Also, our evidence concerning the monophyly of the Elapidae is inconsistent with Savitzky's (1979) suggestion that the coral snakes (*Micrurus*) represent an independent derivation from xenodontines (see McCarthy, 1985, for discussion). Our results regarding the apparent monophyly of the Xenodontinae, Colubrinae, and Boodontinae are more interesting, because they are at variance with recent conclusions made by other researchers; however, it should be recalled that the monophyly of these taxa is rather weakly supported, as measured by the small number of additional steps needed to render each paraphyletic.

The Xenodontinae was originally proposed by Cope (1893) to include a diversity of genera (approximately 95; see Dowling & Duellman, 1978) of mainly South and Central American snakes. A handful of genera occur in North America north of Mexico. While xenodontines generally have morphologically distinct hemipenes (Dowling, 1975; Jenner & Dowling, 1985; McDowell, 1987), these differences are not clearly synapomorphic (Cadle, 1984c) and the monophyly of the proposed subfamily has not been clearly established. In a study of microcomplement fixation (MC'F) of albumins, Cadle (1988) concluded that the xenodontines represent two ancient and diverse lineages, referred to as the South American and Central American lineages, plus six North American genera that are as divergent from each other and from the two primary lineages as those two lineages are from each other (Cadle, 1984a, c). He concluded that the six North American genera (*Carphophis*, *Conophis*, *Contia*, *Diadophis*, *Farancia*, and *Heterodon*) represent ancient lineages of uncertain placement and, hence, that the Xenodontinae was a potentially paraphyletic cluster of extremely old New World colubroids. In contrast, our data indicate that the Xenodontinae is a monophyletic cluster of moderately, but not extremely, ancient divergence. Our sample includes two representatives of the South American and one of the Central American lineages, and two representatives of the *incertae sedis* genera of Cadle (1984a–c).

We believe this discrepancy may result from variation in evolutionary rates and from substitutional saturation. Phenetic measures such as MC'F will infer historical branching patterns only if evolution of the molecules studied proceeds in a more-or-less clocklike fashion. However, available evidence suggests that proteins do not evolve in a clocklike fashion (Avise & Aquadro, 1982; Scherer, 1990), and demonstrations of putative clocklike behaviour based on relative rate tests are biased toward finding rate regularity (Fitch, 1976; Scherer, 1990; Cunningham & Collins, 1994). Adoption of an erroneous assumption of rate regularity will lead one to interpret lineages as ancient that are, instead, divergent because of accumulation of autapomorphies. This may be sufficient to account for the discrepancies between our conclusions and Cadle's regarding xenodontine evolution.

Additionally, proteins are subject to the effects of replacement saturation (Dayhoff & Eck, 1968; Fitch, 1976; Kimura, 1987) as a result of functional constraints upon structure. But the importance of saturation cannot be ascertained in immunological

distance studies because homoplastic substitutions cannot be directly evaluated. Saturation effects may explain why taxa that in our analysis appear to be rather closely related often show the same approximate MC'F distances as seen in representatives from different families and subfamilies (in the approximate range of 75–100 units [Cadle, 1983, 1984a–c, 1988, 1994]). It is possible that the varying albumin domains in snakes have largely saturated by the times such divergences are obtained, resulting in specious clustering of taxa on the basis of homoplasy and symplesiomorphy. If so, then albumin MC'F data may not be useful for determining relationships among the higher-level colubroid groups. This suggestion is supported by the observation that the distances separating major colubroid lineages examined with MC'F are smaller than the non-reciprocity errors associated with the pairwise distance measurements (Cadle, 1984a, 1984c, 1988). The interaction of these rate-variation and sequence-saturation problems in immunological studies may largely account for the nonmetricity of immunological distances and the consequent fact that they cannot be interpreted as evolutionary path lengths (Farris, Kluge & Mickevich, 1979; Farris, 1981). Given that immunological distances cannot be interpreted as amounts of evolution, it remains uncertain exactly how they should be interpreted and what they may indicate about phylogeny (Farris *et al.*, 1979; Farris, 1981).

The Colubrinae is another large and diverse lineage for which we find evidence of monophyly. The subfamily had hitherto been diagnosed as having an apparently synapomorphic, asymmetric hemipenis, though not all taxa belonging to this lineage have this feature (McDowell, 1987). Of particular interest in our analysis is that *Lycodon* and *Oligodon,* identified by Dowling (1975) and by Dowling & Duellman (1978) as representing two tribes of the 'Lycodontinae', clearly belong with the colubrines, suggesting that other members of Dowling and Duellman's tribes Lycodontini and Oligodontini probably do as well. McDowell (1987) reached the same conclusion regarding the placement of these two genera within Colubrinae on the basis of careful comparisons of hemipenial morphology, and Cadle (1994) reached an identical conclusion for *Lycodon* based on MC'F albumin comparisons.

We also find evidence suggesting that the Calamariinae is a subgroup of the Colubrinae, although we note that we had only one representative of this group available for analysis. This conclusion is congruent with the classification of Dowling & Duellman (1978) which recognizes this apparent clade (Inger & Marx, 1965) as a tribe of the Colubrinae. However, other classifications (e.g. McDowell, 1987) recognizing these snakes as a separate subfamily apparently obscure these relationships and would make the Colubrinae paraphyletic. Hence, until contrary evidence is presented, we suggest relegating the 'calamarines' to the Colubrinae, recognized as a separate tribe if so desired. It is not our intent in this study to place much emphasis on the apparent relationships within the major colubroid lineages, because our sampling within those lineages was not comprehensive enough to justify such inference. However, we note the consistent placement of *Oreocalamus* as the sister-taxon to *Dendrelaphis*, our sole representative of Dowling & Duellman's (1978) colubrine tribe Philothamni. Inasmuch as both Calamarini and Philothamni are, respectively, entirely or largely restricted to Southeast Asia, their suggested close relationship may be a fruitful area for future study.

Polyphyletic taxa

Despite finding support for the monophyly of most proposed higher-level colubroid taxa, there are two large groups (Lycodontinae, as used by Dowling [1975] and by

Dowling & Duellman [1978]; and Boodontinae, as used by McDowell [1987]) that are polyphyletic based on our data. This interpretation holds whether the transversion parsimony (Fig. 3) or neighbour-joining (Fig. 6) tree serves as the basis for phylogenetic inference. The Lycodontinae was originally proposed by Bonaparte (1845), and has been most recently detailed by Dowling & Duellman (1978) as including 11 tribes of morphologically diverse snakes from Asia and Africa. As noted above, two of these presumed tribes are clearly members of the Colubrinae. Following McDowell (1987), three others have been treated in this study as separate subfamilies (Aparallactinae, Pareatinae, Xenoderminae), and they clearly show no close relationship to each other. The remaining tribes (of which we have two representatives [*Leioheterodon* of the Pseudoxyrhophini, and *Madagascarophis* of the Geodipsadini]) are of African snakes, and will be discussed under the treatment of the Boodontinae which follows. It is clear from the topology of our phylogenetic estimates that the assorted taxa referred by Dowling & Duellman (1978) to the Lycodontinae form a polyphyletic assemblage of distantly related snakes. Hence, this name does not refer to a taxon of any historical validity. Further, the type genus of this taxon is a colubrine (see above). Consequently, we concur with Cadle (1994) that the name 'Lycodontinae' be relegated to the synonymy of Colubrinae.

McDowell (1987) did not follow Dowling & Duellman's (1978) use of Lycodontinae as a lineage within Colubroidea; instead, he recognized Pareatinae and Xenoderminae as separate subfamilies, subsumed Oligodontini and Lycodontini within the Colubrinae as discussed above, referred most of the aparallactine genera to the Atractaspididae, and placed the remaining (entirely African) taxa within the subfamily Boodontinae. He also included within Boodontinae several Asian genera historically assigned to the Natricinae (Dowling & Duellman, 1978). Our findings refute the recognition of the Boodontinae, as defined by McDowell (1987), as a clade. Of greatest importance in this regard is that *Sinonatrix* groups with other Old World natricines instead of with the African representatives of the Boodontinae included in this study (*Leioheterodon*, *Madagascarophis*). This suggests that the other Old World genera typically assigned to the Natricinae, but included by McDowell within his Boodontinae (e.g. *Opisthotrophis*, *Rhabdops*), are also likely to cluster with the natricines when those relationships are investigated in more detail. Our findings, however, tentatively suggest that with the removal of several of these problematic genera, the Boodontinae may consist of a monophyletic radiation of strictly African genera. It is also possible that the Boodontinae may prove to be a paraphyletic assemblage of African snakes that subtend the Atractaspididae or Elapidae (e.g. see Cadle, 1994).

The status of Natricinae is left undecided by our data. Like most 'colubrid' subfamilies, this has been recognized largely on the basis of a combination of phenetic similarity and hemipenial and vertebral character states of uncertain polarity (Dunn, 1928; Bogert, 1943; Malnate, 1960; Dowling *et al.*, 1983). In our study, the Natricinae are variously found to be polyphyletic or monophyletic, depending on whether transitions are included in the analyses. The transversion evidence alone suggests a monophyletic and highly derived Thamnophiini (New World natricines) related to the vipers, and a paraphyletic assemblage of Old World genera near the base of the tree (Fig. 3). Inclusion of transitions places a monophyletic Natricinae in the middle of the tree. The proposed natricine polyphyly evident in the parsimony tree is concordant with the biogeography of the group; however, if true, it represents a remarkable case of phenotypic convergence. Indeed, one of the New World taxa (*Nerodia*) and one of the Old World taxa (*Sinonatrix*) were, until recently (Rossman &

Eberle, 1977), placed in the same genus (*Natrix*). Thus, it may be that polyphyly of natricines is an artifact of homoplasy in the ND4 transversion data. Whether true or not, the proposed diphyly provides an explicit hypothesis that can be tested with future data.

While the transversion parsimony tree may be viewed as providing questionable resolution of natricine relationships that resolve along traditional lines if transitions are included, the converse problem is encountered in considering relationships among xenodermines and acrochordids. Morphological evidence suggests that colubroids are monophyletic and that *Acrochordus* is the proximate outgroup to this clade (Underwood, 1967; Groombridge, 1979a–c, 1984; Rieppel, 1988; Kluge, 1991). In contradistinction to this, the neighbour-joining analyses that both include transitions and use boids as outgroups consistently place Acrochordus as sister taxon to the xenodermines, and this entire clade as basal to the remaining colubroids (Fig. 6). While this result is contrary to our best estimate of colubroid phylogeny (Fig. 3) and to current morphological evidence, our finding that xenodermines are consistently placed as the most basal clade within colubroids makes the potential sister-taxon relationships of acrochordids and xenodermines a reasonable hypothesis requiring future testing. This hypothesis could not be tested by transversion parsimony analysis of the ND4 data, because the random behavior of our more distantly related outgroups necessitated use of only a single outgroup.

Relationships among monophyletic taxa

In assessing the reality of proposed higher-level colubroid taxa, neither transversion parsimony nor neighbour-joining analysis provides complete and unambiguous (when viewed in relation to morphological evidence) identification of which groups are monophyletic, although they agree on the monophyly or polyphyly of most proposed taxa. But they provide quite different notions of the relationships among the monophyletic groups identified. Because of this, we will discuss only those among-family/subfamily relationships that are identically resolved between the two approaches.

First among these is the close apparent association between *Atractaspis* and the 'aparallactines', as herein represented by *Aparallactus*. This relationship was first proposed by Bourgeois (1968), who argued (along with Kochva, Shayer-Wollberg & Sabel, 1967) that the historical interpretation of *Atractaspis* as a viperid was incorrect. More recently, the association between atractaspidids and 'aparallactines' has been further promoted on the basis of morphological evidence (McDowell, 1986; Underwood & Kochva, 1993). McDowell (1986) argued that atractaspidids should be taken to include the 'aparallactine' snakes, though he removed the genera *Aparallactus* and *Macrelaps* to the Boodontinae. The evidence from our study suggests that *Aparallactus* does indeed have a close relationship with *Atractaspis*; hence, McDowell's (1986) removal of that genus and *Macrelaps* from association with the other 'aparallactines' may be in error. Underwood & Kochva (1993) reached an identical conclusion on the basis of morphological evidence. Whether the aparallactines form a monophyletic or paraphyletic group relative to *Atractaspis* is left unresolved by our data. However, Underwood & Kochva's (1993) morphological data suggest that recognition of these taxa as separate families or subfamilies (e.g. Heymans, 1975) would leave the Aparallactinae polyphyletic. More data are needed, but there is currently no basis for recognizing a separate Aparallactinae. Hence, we follow

McDowell (1986, 1987) and Underwood & Kochva (1993) in treating that name as a synonym of Atractaspididae, and we concur with Underwood & Kochva (1993) in a more expanded definition of the family that includes *Aparallactus* and *Macrelaps*.

The relationship among families/subfamilies that is most consistently and strongly obtained in all our analyses is the placement of the Elapidae as sister taxon to the African snakes of the Boodontinae and Atractaspididae. Typically, the latter two lineages appear as sister taxa to each other and the elapids as sister taxon to this more-inclusive group. However, we consider our taxonomic sampling too limited to establish the sister-taxon relationship of Boodontinae and Atractaspididae with certainty, inasmuch as more extensive sampling may show boodontines to be a paraphyletic assemblage relative to the atractaspidids (Cadle, 1994). It is clear from our data, however, that the elapids are closely related to these African lineages, irrespective of how the latter may be related among themselves. As far as we can determine, this close relationship of elapids and (Boodontinae + Atractaspididae) has not previously been explicitly proposed in the literature, although it certainly provides a clarifying perspective on the controversy regarding whether the South African *Homoroselaps* represents a primitive elapid or an 'aparallactine' (McDowell, 1968, 1986; McCarthy, 1985; Underwood & Kochva, 1993). The controversy apparently hinges on whether higher-level taxa are defined on the basis of synapomorphies or symplesiomorphies. McCarthy (1985) focused on the former, and McDowell (1968, 1986) based his conclusions on overall similarity. The close apparent relationship between elapids and atractaspidids makes identification of diagnostic synapomorphies for each group crucial for resolution of the status of *Homoroselaps*.

In our analyses, the boodontines, atractaspidids, and elapids form a single monophyletic group that is not among the most primitive colubroids. In all analyses based on transversions alone these snakes form a highly derived clade (e.g. Fig. 3), although inclusion of transitions places this clade in a somewhat more basal position in the tree (e.g. Fig. 6). In either event we consider the strong and consistent resolution of these relationships by what is a relatively rapidly evolving gene as suggestive that these taxa are among the more recently diverged higher-level colubroid taxa. In contrast, each of these three taxa has been considered by one author or another to represent ancient, basal lineages of colubroids. McDowell (1987) considered the proteroglyphs (elapids and atractaspidids) primitive grades of colubroids that retained the supposedly ancestral characteristics of that clade, but he did not presume either group to be monophyletic. Underwood & Kochva (1993) made the same claim for the Atractaspididae. Cadle (1983, 1994) considered the boodontines and the atractaspidids to represent a series of ancient, not necessarily monophyletic, lineages within the colubroids, basing this on the large immunological distances separating these species from each other and from some other colubroid lineages. The conclusions of these earlier authors may be affected by two problems. First, the morphological data have been interpreted by character argumentation schemes lacking explicit attempts to determine character polarities, and simultaneous analyses of all relevant data have not been performed. Consequently, phylogenetic conclusions seem to have been largely based on consideration of symplesiomorphic attributes (McDowell, 1987; Underwood & Kochva, 1993), which we view as an unreliable means of ascertaining historical relationships. Second, as discussed earlier, it seems likely that the albumin evidence upon which Cadle (1983, 1988, 1994) based his conclusions may suffer from problems associated with amino acid saturation, use of symplesiomorphies, and/or long-branch-attraction problems. If true, relatively

recent divergences may appear ancient if albumin MC'F's upper limit of resolving power has been reached or if particular clades have experienced accelerated evolutionary rates that provide large immunological distances. This conflict between the DNA and immunological data sets can be resolved conclusively only by application of more character data, and it may serve as a useful stimulus to further research.

An equally consistent result of our analyses is the well-supported basal position of the Xenoderminae within the Colubroidea, although there is some question as to whether Acrochordus belongs in a clade with the xenodermines or is the proximate outgroup to Colubroidea, as presented by Kluge (1991). Current knowledge of xenodermines is limited because of their rarity, but further research on this obscure group is needed to clarify this issue.

It will be immediately apparent from the above discussion that the current classification of Colubroidea is in need of considerable revision. We have proposed certain changes above relating to synonymization or redefinition of clearly polyphyletic taxa. We are also of the opinion that most of the taxa referred to in this paper as subfamilies will eventually need to have their rank raised to familial status in order to provide a taxonomy based solely on monophyletic groups. We do not formally propose doing so, however, until the status of the questionable taxa discussed above is resolved and until the relationships among these higher-level taxa are better established. We emphasize, though, the importance of recognizing that 'Colubridae', as traditionally understood, is not a natural taxon. While the traditional use of this name will, no doubt, temporarily persist as a nomenclatural convenience, it should be understood that it does not refer to a single historical lineage. Its continued use should be obviated upon obtaining more data to justify the taxonomic revisions referred to above.

Most prior attention given to higher-level colubroid relationships has been focused on the origin(s) of the venomous snakes and has been framed in terms of the origins of the Elapidae and Viperidae relative to the 'Colubridae'. The situation as regards the placement of the venomous Atractaspididae has been discussed above and will not be reiterated here. As concerns the three traditional colubroid families 'Colubridae', Elapidae, and Viperidae, four general hypotheses (i.e. all possible) have been proposed to explain their interrelationships. These are (1) elapids and viperids are the result of a single origin from an aglyphous or proteroglyphous ancestral 'colubrid' (Cope, 1900; Mosauer, 1935; Bogert, 1943; Johnson, 1955; Marx & Rabb, 1965); (2) elapids are the (possibly paraphyletic) sister group to the remaining colubroids, and viperids are of uncertain placement within the latter (McDowell, 1986, 1987); (3) viperids are the sister group to the remaining colubroids, and elapids are of uncertain placement within the latter (Haas, 1938, 1952; Kochva & Gans, 1970; Rage, 1984; Cadle, 1987, 1988); and (4) elapids and viperids are independently derived from different 'colubrid' lineages (Anthony, 1955; Kardong, 1980, 1982). The first two hypotheses are contradicted by our results. Elapids and viperids do not form a monophyletic group, although they appear to be somewhat closely related (Fig. 3); and elapids do not form the most primitive lineage within Colubroidea, as discussed above. The last two hypotheses cannot be conclusively evaluated by our data. Use of only transversions supports Hypothesis 4, but inclusion of transitions produces a tree (Fig. 6) more in line with Hypothesis 3 (if one overlooks the basal position of xenodermines). As stated earlier, we consider that transversions

alone probably provide a more reliable estimate of colubroid evolutionary relationships, so we view the evidence from ND4 to be more in line with Hypothesis 4 than Hypothesis 3. Other recent molecular evidence is interpreted as favoring Hypothesis 3 (Cadle, 1988; Heise *et al.*, 1995), though xenodermines were not included in those analyses. The data of Heise *et al.* (1995) are being reanalysed, as noted earlier, and will be reported on separately, but the reanalysed data do not support Hypothesis 3. Despite our misgivings about MC'F, the fact that the MC'F data are congruent with our analyses when all substitutions are used highlights the need for this alternative hypothesis to be given due consideration when further data are brought to bear on colubroid relationships. Beyond noting the apparently independent origins of venom-delivery systems in colubroid snakes, we do not currently have enough confidence in the phylogenetic relationships of the elapids and viperids to each other and to most of the remaining colubroids to discuss or refute particular evolutionary scenarios relating to the development of these morphological novelties.

One last issue regarding the evolutionary history of colubroid snakes may be briefly touched upon—the geographical origin of that clade. We believe it likely that colubroids arose and underwent much of their early diversification in Southeast Asia. This conclusion is supported by several lines of evidence: (1) the most primitive colubroids based on our study, the xenodermines, are restricted to Southeast Asia; (2) the presumed proximate outgroup of Colubroidea, Acrochordidae, is restricted to this region; (3) Southeast Asia has the largest diversity of higher-level colubroid taxa in the world (7 of 10 presumptive lineages); and (4) four of the ten currently identified higher-level colubroid lineages are restricted to this region (other regions have at most one or two endemic taxa). The earliest colubroid fossils are from the Eocene of Europe (Rage, 1987), but there is a general paucity of early colubroid fossils and, more particularly, a lack of such fossils from regions other than Europe and North America. Thus, the current usefulness of fossils for testing this hypothesis appears limited.

We believe we have only just reached the threshold of information—in terms of both representative taxa and amount of sequence per taxon—needed to begin resolving the evolutionary history of colubroids using DNA sequences. Nonetheless, resolution of several outstanding problems has been assisted by analysis of the DNA sequence data presented here. These include the identification of most proposed higher-level colubroid families and subfamilies as monophyletic (in a few cases, within redefined limits), identification of xenodermines as the most primitive living colubroids, and partial resolution of the origins of the venomous snakes (uncertainty primarily remains with the position of the Viperidae). In many cases, the phylogenetic framework presented here is sufficiently strong to allow for testing of specific phylogenetic hypotheses in an explicit and focused fashion. In other cases, enough confidence may be had in the monophyly and nearest relationships of particular lineages to allow for resolution of lower-level relationships within these groups and the informed choice of close outgroups for rooting purposes. Clearly, several problems remain in further clarifying colubroid phylogenetic relationships. These include determination of (1) the exact branching order among most of the monophyletic higher-level lineages, (2) the root of the colubroids that remain after the split with the Xenoderminae, (3) whether the Natricinae are monophyletic, and (4) whether Acrochordus truly represents an outgroup to the colubroids or is a member of the Xenoderminae. Further evidence in support of the monophyly of Colubrinae

and Xenodontinae would also be desirable, as would more precise resolution of relationships within the clade (Elapidae + Atractaspididae + Boodontinae). These questions should be resolvable by recourse to additional DNA data from more conservative genes and by more careful, and explicitly cladistic, analyses of morphological features.

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APPENDIX

Specimens examined

Achalinus rufescens—Hong Kong: Lantau Is.: Ngong Ping (SL 13929).

Acrochordus granulatus—Philippines: Luzon: Batangas Prov: Talisay (USNMFS 056632).

Alsophis portoricensis—British Virgin Is.: Guana Is. (FK 2440).

Aparallactus werneri—Tanzania: Tanga Region: Korongwe Dist.: 11 km NW of Korongwe (FMNH 250440).

Aplopeltura boa—Brunei: Belait Dist.: junc. Sg. Ingai and Sg. Belait (UMMZ 201905).

Atractaspis bibroni—Zimbabwe (UMMZ 209986).

Azemiops feae—China Guangxi Prov. (UTA R-32069).

Boiga dendrophila—Brunei: Brunei-Maura Dist.: Pulau Berambang, Brunei River (FK 2964).

Bungarus fasciata—Brunei: Temburong Dist.: 5 km W of Labu (UMMZ 201916).

Calloselasma rhodostoma—No data (UMMZ 184314).

Causus rhombeatus—Zimbabwe.

Cerberus rhynchops—Malaysia: Sabah: Telipok Dist.: Kg. Giling Laut (FMNH 251594).

Chilomeniscus cinctus—USA: Arizona: Maricopa Co.: Sossaman and Guadalupe Roads. (UMMZ 200750).

Coluber constrictor—USA: Michigan: Washtenaw Co.: Univ. Michigan Botanical Gardens (UMFS 4634).

Dendrelaphis pictus—Philippines: Panay Prov.: Iloilo (UMMZ 200268).

Dispholidus typus—Tanzania: Tanga Region: Muheza Dist.: East Usambara Mountains, 8 km NNW of Amani (FMNH 250444).

Elaphe flavolineata—Brunei: Temburong Dist.: 1.4 km N of Bukok (UMMZ 201910).

Enhydris plumbea—Malaysia: Sabah: Telipok Dist.: Kg. Kayumadang (FMNH 251883).
Farancia abacura—USA: Florida: Washington Co.: on U.S. 90, 6.4 km E of Choctawhatchee River (UMMZ 205023).
Helicops pictiventris—Brazil: Rio Grande do Sul: Torres: Rio Cornelios at Estrada Praia do Barco/Terra de Areia (UMMZ 205992).
Hypsiglena torquata—USA: Arizona: Maricopa Co.: Usery Pass (UMMZ 200753).
Leioheterodon madagascariensis—Madagascar: Toliara: Sakaraha, Zombitsy Forest (RAN 42543).
Lycodon capucinus—Philippines: Panay Prov.: Iloilo (USNM 340053).
Macropisthodon rudis—Taiwan: Taipei: Yanmingshan Natl. Park (UMMZ 190534).
Madagascarophis colubrina—Madagascar: Antsiranana: Ambanja: Ambalafary, Manangarivo Reserve (UMMZ 209591).
Micrurus fulvius—USA: Florida: Putnam Co.: Interlachen (UF 72716).
Nerodia taxispilota—USA: Florida: Marion Co.: Oklawaha River 0.8 km S of State Route 316 (UMMZ 190958).
Oligodon octolineatus—Brunei: Tutong Dist.: Kampong Keriam, 3 km E of Tutong (UMMZ 201913).
Oreocalamus hanitschi—Malaysia: Sabah: Sipitang Dist.: Mendolong (FMNH 243938).
Pareas nuchalis—Brunei: Belait Dist.: junc. Sg. Ingai and Sg. Belait (FK 2626).
Pelamis platurus—Panama: Chiriquí Prov.: Golfo de Chiriquí (UMMZ 209799).
Rhabdophis subminiata—Hong Kong: Lantau Is.: Keung Shan (SL 13908).
Sinonatrix trianguligera—Brunei: Temburong Dist.: 3.2 km S of Bukok (FK 2807).
Storeria occipitomaculata—USA: Michigan: Mackinac Co.: off Worth Road, 1.6 km E of Brevoort River (UMMZ 205803).
Thamnophis butleri—USA: Michigan: Jackson Co.: Jackson (UMMZ 205026).
Trachyboa boulengeri—No data (live specimen at Cincinnati Zoo).
Xenodermis javanicus—Malaysia: Sabah: Lahad Datu Dist.: Danum Valley (FMNH 230073).