

Support for the Hypothesis of Anguimorph Ancestry for the Suborder Serpentes from Phylogenetic Analysis of Mitochondrial DNA Sequences

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Snakes represent one of the most ubiquitous and successful groups of terrestrial vertebrates; however, many aspects of their evolutionary relationships remain uncertain. Previous research, which utilized morphological and immunological data, has not resolved the origin of snakes or clearly delineated the relationships between snakes and other lizards. A DNA sequence data set from the mitochondrial ND4 gene and the histidine, serine, and leucine tRNAs has been generated for use in the examination of these relationships. Parsimony analyses employing multiple outgroups resolve snakes within the lizard clade. *Varanus* is the sister group to the snakes in 81% of bootstrap replications using *Bos* as the outgroup specified, 85% using *Trachemys*, 57% using *Alligator*, and 80% using all three outgroups. The primitive, fossorial snake genera *Leptotyphlops* and *Typhlops* are basal to the other snakes and provide tentative molecular evidence in support of a fossorial or subfossorial origin of limblessness for the suborder Serpentes. © 1995 Academic Press, Inc.

The evolutionary origin of snakes is obscure and has remained a central issue in herpetology since the beginning of the 19th century. This uncertainty has delayed understanding of the evolutionary relationships among snakes, as well as between snakes and other reptiles. Snakes, lizards, and amphisbaenids are the extant members of the order Squamata, and their ancestors first appeared approximately 150 million years ago in the early Cretaceous (Zug, 1993). Subsequent to Cope's (1869) conclusion that the sister group of snakes was the extinct mosasaurs, two main theories have emerged to explain the origin of snakes. One theory suggests that snakes and lizards are sister groups and proposes a basal divergence of lizards and snakes from

an unknown ancestor (Hoffstetter, 1968; Rieppel, 1983; Underwood, 1970). A second theory suggests that snakes arose from within the lizard clade, although the particular alliance within the Lacertilia is unclear (Brock, 1941; McDowell and Bogert, 1954; Senn and Northcutt, 1973; Rieppel, 1988).

Unfortunately, definitive evidence for these hypotheses has not been provided by morphological examination or by fossil evidence. Most of the investigations that have proposed an origin of snakes have been plagued by conflicting evidence from different sets of morphological characters. McDowell and Bogert (1954) concluded that snakes arose from varanoid lizards, which are represented today by monitor lizards such as *Varanus* and *Lanthanotus*, based on morphological characters such as the retractile foretongue, the pattern of tooth replacement, and similarities of the intramandibular joint in varanids and snakes. Skull and braincase features led other researchers to the conclusion that snakes arose from scincomorphs (Brock, 1941; Senn and Northcutt, 1973); however, convergent miniaturization of the skull in burrowing lizards and snakes may account for these similarities (Rieppel, 1988). The amphisbaenids have been proposed as the sister group to the snakes based on cloacal ribs and several soft anatomical characters (Rieppel, 1988), although other studies have concluded that the amphisbaenids are too radically different in skull morphology to have given rise to the snakes (Bellairs and Underwood, 1951). Estes and Pregill (1988) provide the most complete recent analysis of the squamata. Their morphological analyses placed snakes as the sister group to the varanoids (inclusive of the helodermatids); however, they chose to reject this result based on the dibamids and amphisbaenids being "drawn along" with the snakes as a result of character bias due to limblessness in each of these groups (Estes and Pregill 1988). Estes and Pregill (1988) instead place the three limbless squamate clades within a new group, the Scleroglossa. This new level of squamate taxonomy is basally divergent from the division Autarchglossa, which includes the Anguimorpha.

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There is little consensus regarding snake phylogeny. A conservative presentation of the phylogeny of snakes depicts an early divergence of the blind snakes (Typhlopidae and Leptotyphlopidae) from the other large diverse familial assemblages (Fig. 1). This level of classification is expressed by placement of the blind snakes in the infraorder Scolecophidia separate from other snakes. Indeed, the blind snakes so closely resemble fossorial lizards that Bellairs and Underwood (1951) concluded that they are more properly lizards and not snakes at all. The remaining snakes represent many divergent families and comprise the infraorders Henophidia and Caenophidia, and both of these groups are currently in a period of revisal. Members of the family Boidae, including the type species *Boa constrictor* used in this investigation, form the major component of the Henophidia. Unfortunately, grouping of the boas and pythons into the Boidae is based more on shared primitive characters than on shared derived characters, and thus the group is potentially paraphyletic (Goin *et al.*, 1978; Cadle, 1987). The third major radiation of snakes, or Caenophidia, includes the families Colubridae, Elapidae, and Viperidae. The colubrids are a very successful group and include such familiar snakes as the North American garter snakes, *Thamnophis*, and the European water snake, *Natrix*. The elapids and viperids are venomous snakes and include the cobras and rattlesnakes, respectively.

The limited and fragmentary fossil evidence, in combination with character convergence between limbless lizards and snakes, has acted to prevent an accurate determination of the ancestral relationships of snakes and lizards. In addition, recent immunological comparisons have failed to clarify the situation (Dessauer *et*

al., 1987). One technique which provides a potential resolution to the problem of snake origins is the analysis of mitochondrial DNA (mtDNA) sequence data. This molecular perspective on the evolution of Squamates circumvents the problems caused by morphological convergence between lizards and snakes. Mitochondrial DNA in reptiles is similar to that of other vertebrates (Brown, 1983, 1985) in that it possesses highly conservative gene order and rapid substitution rates relative to nuclear sequences. Different regions of the mtDNA genome have been shown to evolve at different rates (Brown, 1983; Miyamoto and Boyle, 1989), allowing a choice of temporal scale by selection of an appropriate region of the molecule (Hedges *et al.*, 1991; Disotell *et al.*, 1992). A highly conserved region can be used to answer systematic questions at the ordinal level (Adkins and Honeycutt, 1991), while a variable region is useful for species or even population level evaluations (Lamb and Avise, 1992). Because few molecular phylogenies exist for Squamates, ND4 was chosen for sequencing based on an appropriate rate of divergence observed in lizards (Arévalo *et al.*, 1994) and because the region is considered to be evolving at a moderate rate in comparison with other mitochondrial protein genes (Cracraft and Helm-Bychowski, 1991).

MATERIALS AND METHODS

The segment of the snake mtDNA sequenced in this study includes 264 codons of the ND4 gene as well as the histidine, serine, and leucine tRNAs (Fig. 2). This region corresponds to the positions 11150 to 12146 on the bovine mitochondrial map (Anderson *et al.*, 1982). The 12 taxa chosen for this study included five lizards and seven snakes. The lizard genera *Cyclura clarki* (Iguanidae), *Sceloporus grammicus* (Phrynosomatidae), *Varanus niloticus* (Varanidae), *Hemidactylus turcicus* (Gekkonidae), and *Scincella lateralis* (Scincidae) and the snake genera *Leptotyphlops dulcis* (Leptotyphlopidae), *Typhlops punctatus* (Typhlopidae), *Acrantophis madagascariensis* and *Boa constrictor* (Boidae), *Lampropeltis mexicana* and *Heterodon nasicus* (Colubridae), and *Aghistrodon contortrix* (Viperidae) were selected as representative of the two Squamate suborders. Several outgroups were utilized (Farris, 1982; Maddison *et al.*, 1984), including testudine (*Trachemys scripta*), archosaur (*Alligator mississippiensis*), and mammal (*Bos taurus*).

DNA was extracted from whole blood, tissues, and alcohol-preserved specimens (Maniatis *et al.*, 1982). This template DNA was amplified in 100 μ l reactions using oligonucleotide primers created in our laboratory for use with Sceloporine lizards (Arévalo *et al.*, 1994). The locations of both the region amplified and the internal sequencing primers are depicted in Fig. 2. *Thermophilus aquaticus* (Taq) polymerase (Perkin Elmer-Cetus) was used in a Perkin Elmer-Cetus ther-

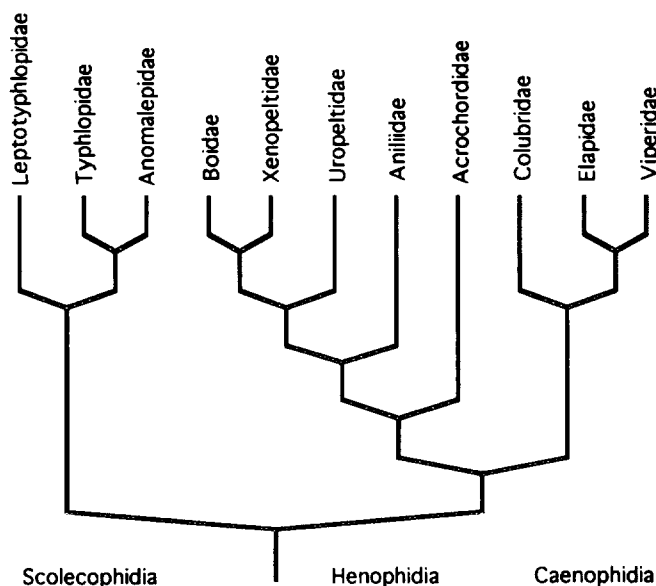


FIG. 1. Conservative assessment of the relationships among snake families (Zug, 1993).

<i>Bos</i>	TGACTACCTA	AAGCTCACGT	AGAAGCCCCC	ATCGCAGGCT	CCATAGTCTC	TGCAGCAGTT
<i>Acrantophis</i>C..A.C..T.AG.	.T....A..	A.....TA.C
<i>Boa</i>A.T.T..AC..G.	.A....A..	G.....A.C
<i>Leptotyphlops</i>A.T.AG.A..	A.....CA.C
<i>Typhlops</i>A.T.A..A	G.T.....	.A....A..	A.....TA.C
<i>Lampropeltis</i>A.T.T..A	.T....A..A..	A..C...A.C
<i>Heterodon</i>A.T.AT..G..AT.	A..C...A.C
<i>Agkistrodon</i>A.T.TT..T.	.A....AT.	A.....A..
<i>Varanus</i>A.T.T....A..T...A.C
<i>Sceloporus</i>C..A.T..A	.T....T..	.A.....	A..C...TA..
<i>Cyclura</i>A.T.A	.T..C....A..	A..C...CA.C
<i>Hemidactylus</i>A.T.A	.T.....	.A..A....	A.....C..A
<i>Scincella</i>A.T.TG..AT.	A.....TA..
<i>Trachemys</i>A.T.AA..	.A..A....	A.....A..
<i>Alligator</i>C..C.C....T..	.T.....	.T.....??	?..T....G
<i>Bos</i>	CTACTAAAAC	TAGGGGGGTA	CGGTATGCTA	CGAATCACAC	TAATTCTAAA	CCCTATGACC
<i>Acrantophis</i>	T..T.G....	.C..C..A..	...C..TA.T	.C..A.T..	A.G.CT.GCC	AA.A.CA.AA
<i>Boa</i>	.C..T....	.C....T..	T....AA.CA.T..	A.....GCC	.A.A.CA.AA
<i>Leptotyphlops</i>	.C.....	...C..A..	T..A..AA.CGG.C.	A...C..TCC	A..A.CAGAA
<i>Typhlops</i>	.C..T...A	.G.....AA.C	.C..TT.C.	A.GC...GCC	A..C...AA
<i>Lampropeltis</i>C.....	T..G..TA.CA.T..	A...A..CC	AATA..AA
<i>Heterodon</i>G.	.T..A..T.	T..C..TA.TA.T..	A..CA..GCC	AATA..AA
<i>Agkistrodon</i>C..C....TA.C	.C..A.T..	AG..C...CC	.A.A.CA.AA
<i>Varanus</i>	.C..C...A	...T..A..	...C..A.T	.C..TT..	CT.CCT..C	.T.CC.C...
<i>Sceloporus</i>	.C.....	.T..T..T.CA.TT..A	.TCA..T.T	A..A..A...
<i>Cyclura</i>G..A..C..	...A..CA.CC.	...CAT..T	G..A..A..A
<i>Hemidactylus</i>	.C.....	.T..T..A..	...G..CA..A...	C..C..A...C	.A.A.CA...
<i>Scincella</i>T..T..T..	...A..TA.TT..C	CT.....	...AC.TT..
<i>Trachemys</i>C...TA..	T..A..CA.TT..T.	C..CA....	...CC.AT.A
<i>Alligator</i>	..TA.T....	...C..T..	T..CC.A...	...G.AGT.A	.TT.A..C.C	TGAGCAA.T.
<i>Bos</i>	GACTTTATAG	CATACCCATT	CATTATACTC	TCCTATGAG	GCATAATTAT	AACCAGCTCA
<i>Acrantophis</i>	ACAGA...T	TCATA....	TC..A..T..AG.	..GC...T.	.G.T.A.CT.
<i>Boa</i>	ACAGA..T..T	TTATA..C..	...A..T..G	.AT.....	..GC..CC.	TG...A.CT.
<i>Leptotyphlops</i>	ACAAACTC.A	TCACA...A.	.C.A.CC..A	...A.C....A..	.G.T.A.CT.
<i>Typhlops</i>	AC.GACT.CT	TTAC.ATC..	AC.AG..A..	..TA.....C.	...A....
<i>Lampropeltis</i>	ACAGACT..T	TTCTA....	T..G.C..T	G.T..T...	.AGC..CAC.	.G.T.A..T.
<i>Heterodon</i>	ACAGAC...T	TC.TA..T..	T..CG.T...T...	.GGC..CAC.	.G.T.ATCTT
<i>Agkistrodon</i>	AC.GAC...T	TCCT.....	.G.CG..T.A	G...G..G.	.A.GC..CC.	.G...A..T.
<i>Varanus</i>	CCA.CAC.CT	T..T..G..	AC.A.CCA.A	G..ACC....	...T..C.CT.
<i>Sceloporus</i>	CCAAAAC.CT	AC.....C..	...A..TT.A	G...C....	.A..CG.C..	...A..T...
<i>Cyclura</i>	C.AAAAC..T	AC...T.C..	...G..CT.A	G.....	.A..CG.A..	G.....
<i>Hemidactylus</i>	C.AACCGCCT	AC.....C..	...CG..T.AA..G.G..	...A...CT.
<i>Scincella</i>	ATAAA.C.CT	AC..T....	T...TT.A	G.AAC....	.T..T....	...T...T
<i>Trachemys</i>	A.AACAC.CT	.C.....	T..AG...A	G.AT.....	.AG.....	...TG.T...
<i>Alligator</i>	TT.ACA..CT	ACCC...A.	TT.AGGC..A	G.G..C..G.	.GGC.C....	G..A...CTC
<i>Bos</i>	ATCTGCCTCC	GTCAAACGGA	CCTAAAATCA	CTCATCGCAT	ACTCCTCTGT	AAGCCACATA
<i>Acrantophis</i>	.CA....A.	AA....A..	...C....C	.A....T.	...A..AA.	C.....
<i>Boa</i>	.CA..TT.A.	AA....A..C	.A.....	...A..AA.	C.....G
<i>Leptotyphlops</i>	.C...T..T.	AA....A..G..C	.A....C.	.T..A..A.	.G.....G
<i>Typhlops</i>	.C...TT.A.	AA....A..	T..T...GC	.A.....	...A..AA.	CG.A..T...
<i>Lampropeltis</i>	.C.....A.	AA....T..CA..A.
<i>Heterodon</i>	.C.....A.	AA....A..	...T....C	.A..T....	...A..CA.	C..T..T..G
<i>Agkistrodon</i>	.CA....A.	AA..G..A..G..T	.A.....?	...CA..	C..T..T...
<i>Varanus</i>	.C....T.A.	.C....T..T	.A....T.	...A..A..G
<i>Sceloporus</i>	.T..A.A.	.A....A..T..C.	.T..A....	T.....
<i>Cyclura</i>A.A.	.A....GC.A.	C.....
<i>Hemidactylus</i>A.	.A....A..	A..G...C.	.T..A..A.T...
<i>Scincella</i>	.T.....A.	.A...G.A..G	...T..T.A.
<i>Trachemys</i>A.	.A....A..	TT.....	.A..T..T.	...A..A..
<i>Alligator</i>T.G.	.A.....C	.A.C...T.	.T..A..G.	T...T..G

FIG. 3. Mitochondrial DNA sequences from ND4 through the leucine tRNA (positions 11150 to 12146 on the bovine map) for the 15 taxa utilized in this analysis. The taxa are *Acrantophis madagascariensis*, *Boa constrictor*, *Leptotyphlops dulcis*, *Typhlops punctatus*, *Lampropeltis mexicana*, *Heterodon nasicus*, *Agkistrodon contortrix*, *Varanus niloticus*, *Sceloporus grammicus*, *Cyclura clarki*, *Hemidactylus turcicus*, *Scincella lateralis*, *Trachemys scripta*, *Bos taurus*, and *Alligator mississippiensis*.

<i>Bos</i>	GCACTCGTTA	TCGTAGCCAT	CCTTATCCAG	ACACCTTGAA	GCTACATAGG	AGCAACCGCC
<i>Acrantophis</i>	.G...A.A.	...C...A.	TA.A.T.A	.C....GG	CTCTAGCC..TAA..
<i>Boa</i>	.G...A.A.	.T.C...A.	TA.A...A	.T.AC...G	CTCTGGC...TA..A
<i>Leptotyphlops</i>	.GCT.G.A.	...G...A.	...A.C...A	T...AAC..	..ACAGC...	G..C.TAA.A
<i>Typhlops</i>	AGT..T.A.	.TTC...A.	T..A.T.AA..G.	...TA.C...TACTA
<i>Lampropeltis</i>	.GC..A.A.	.T.CC...A.	TA...T.AAA...	...TATC...C.TA...
<i>Heterodon</i>	.GTT.A...	.T.CC....	A.....A.CAA..G.	..CTATG...	G..C.TA...
<i>Agkistrodon</i>	.GC..A..AG	.A.CC...A.	.A.C.T.AA..GG	..ACT.TC...	G..C.TA...
<i>Varanus</i>	.GC..A.A.	.TC...A.	...A.CA..A	..TAACCT.G	.A.TA.C...	C..T.TACTT
<i>Sceloporus</i>	.G.T.A..AG	.T.C...ATG	...A....AA....	...TT.C...	G.T.TAAT.
<i>Cyclura</i>	.G...A..C.	...CT...TGA....AA....	...ATT.C...T.TAA..
<i>Hemidactylus</i>	.G...T....	.TAC.....	...A....AA....	...AT..CC..	G...TGCTA
<i>Scincella</i>	.GC..G..A.	.TAC...ATC	TA.A.T.AAA...	...TA.CC...	G...GTT.TG
<i>Trachemys</i>	.GT..T....	.T.CT..A.C	A..A.CA...	..C.AA...G	CA...C...	T..T.TTA.A
<i>Alligator</i>	.GC..A..A.	CA.CT..A.	T..C.C...A	..CAAC.A.T	C.C?G....	GT...TAAT.
<i>Bos</i>	CTTATGATTG	CCCACGGCCT	CACATCCTCC	ATACTTTTCT	GTCTAGCAAA	CTCAAACCTAC
<i>Acrantophis</i>	..A..A..C.AT.	...T..A.TA.T.	.C.....C..	TATC.C...
<i>Boa</i>	..A..A..C.	.A.....AT.	T.....A.AA....	.C.....	ATC.CG..T
<i>Leptotyphlops</i>	..A..A..C.AT.	.T....G...	GCC..A...	.C.....	ATC.....
<i>Typhlops</i>	T.A..A....T.	T.....T.	C....A.T.	...TA....	AT.TC...T
<i>Lampropeltis</i>	T.A..A....	...T.GT.	T..T....T	GC...A.T.	...T..G..	TA.T.C...T
<i>Heterodon</i>	T.A..A..C.	...T..T.	...T....A	GCC..C...	.C.....T..	.A.C.C...T
<i>Agkistrodon</i>	..A..A..C.	.T.....T.	T..C....A	GC.....	.C.....C..	.A..C...T
<i>Varanus</i>	..C..A..C.	.A.....AT.	A..C..G...	C.T.G..T.	.C.....	A.T.....
<i>Sceloporus</i>	..A..A....	...T....	...T..A.A	..T.A..T.	.C.....	A.....
<i>Cyclura</i>	..A..A....	...T..TT.	A.....T...	...A.A....	.C.....C..	.A.C.....
<i>Hemidactylus</i>	T.A..A..C.A..	A..T....AA....	.C..T..T..	TA.C.....
<i>Scincella</i>	T.A..A....	...T..A.	T..T..A.AA..T.A.....T
<i>Trachemys</i>	...A..C.	...T..AT.	A.....A.AC....	.C.....C..	A.....
<i>Alligator</i>	..A.....	...T..A..	A.....T..T	...T.A....	..T.....	..TC.G...T
<i>Bos</i>	GAACGAATCC	ACAGCCGAAC	CATAATTCTA	GCTCGAGGCC	TACAAACGCT	CCTTCCACTA
<i>Acrantophis</i>T.CA.	...CA..T.T	TC.T..A...	A.C....AT	.C..T.ACA.	TA.A...A..
<i>Boa</i>CG.	...CA...T	.C.T..A...	A.A....GT	.C..C.ACA.	T..C...A..
<i>Leptotyphlops</i>C.C.	...C.....	.C..GC..C	A.G.A...ATGCA.	...G.....
<i>Typhlops</i>C..	...C...CCT	TC....C.C	A.AAA...	.T..C.T.AC	A..A..C..C
<i>Lampropeltis</i>CTA	.T.C.....C..T	T.A....AT	.C..C.ATA.	T..A...A..
<i>Heterodon</i>CAA	.A.C....T	T....C..T	T...C..AT	.C..C.ACA.	...A..A..
<i>Agkistrodon</i>T.CA.	.C....T	TC.....T	A.A....T.	.T..C.ATA.	...G..CA..
<i>Varanus</i>	...A..C..	.A.CA..G..	.C.CC.AAC.	A.C..C...C.TCG.	...C..C...
<i>Sceloporus</i>C.	...C.....	A..G.A..T	.C..C...T	.C...TTA.	TT.....
<i>Cyclura</i>C.	...C.....	...C..	A.A.....	.C...TCA.C...
<i>Hemidactylus</i>C.A	.T.CA....	AC.T..AA.	A.....A.TTAC	...A..C...
<i>Scincella</i>CA.	...T....	...T.AT.	.C....A.TAA.	T...T..T.
<i>Trachemys</i>C.	.T.....	AC..C.AT.	.C...AA.ACTA.	ATAC...T..
<i>Alligator</i>CGC.	...C..C..	AC.TC.CGC.	ATA.A...AACTTAC	.ACA..TACT
<i>Bos</i>	ATAGCCACCT	GATGACTACT	AGCAAGTCTA	ACCAACTTAG	CTCTACCCCC	AACAATCAAC
<i>Acrantophis</i>	...A.AG.T.T.GGC	C..T.A...	.TA...A.C.	..A.T..A..	..GC..T...
<i>Boa</i>	...A..G.A.GG.	...C.AC...	.TA...A.T.	.A.C.....	C.GC..A...
<i>Leptotyphlops</i>	.C.A.A..A.AC	.A...C...	.TA...A.C.	.A.....
<i>Typhlops</i>	...A.T..A.T....	T..T....C	.T...C.T.	A....A..	C...A..T
<i>Lampropeltis</i>	C.CA.A....T....	.ATT.AC...	.TA..TA.T.	.AAAC..A..	...T..A...
<i>Heterodon</i>	G.CA.A..A.	.G.....C..	.A.C..C...	.TA...A.C.	.AAAC..A..	..GC..A...
<i>Agkistrodon</i>	GCTA.A....T.	.A.C.AC..T	.TA..TA.C.	.CG.T.....	C..T..A..
<i>Varanus</i>	...A..CT..CTA	C.....CT..	G.A...A..	.C.....	.T.C.....
<i>Sceloporus</i>	...T.A....TT.A.T.A...	.A..G....	GT...T..T
<i>Cyclura</i>	...A..T.A.G....	...C.TC..C	.A...A..	.C..G....	G..T..T...
<i>Hemidactylus</i>	...A...A.TA.	...C.AC...	.TA...A..	.A...G...	...G.A...
<i>Scincella</i>	...A.AG.T.	.G...T....	G..T.AC...	.TA..TA..	.A..T..A..	...T..T...
<i>Trachemys</i>	...AG.CTA.	C..T..CT.	G...A...	.AA.T..A..	...T..T...
<i>Alligator</i>	T..A.AT.A.	.G...T.T..	...T..CA..	.TA..TA..	.C..G....	...T...

FIG. 3—Continued

<i>Bos</i>	TTAATTGGAG	AMCTATTTGT	AGTAATGTCA	ACCTTTTCAT	GATCTAACAT	TACAATTATT
<i>Acrantophis</i>	.C.CCG....TC.AA.	TA..TCAA.CAAC.	...G.CCA.C	A..C..C..C
<i>Boa</i>	..T.CA..T.C.AA.	CA..TCA...	CTA..CAAT.	...G.CCA.C	A.....C..C
<i>Leptotyphlops</i>	..C.CA....TC.AA.	CA..CTCA..C	CTA..CAAC.	...CGC.CC	A.....T..C
<i>Typhlops</i>	..T.CA....	..A.CA.AA.	TA..TCAG.CAAC.CC	A..T.....
<i>Lampropeltis</i>	..T.CA....AA.	T.C.TCC...	CTA..CAAC.	...GCCCA.C	A.....C...
<i>Heterodon</i>CA....	..T..C.AA.	T.C.TCC..C	TTG..CAAC.	...G.CCG.C	A.....C...
<i>Agkistrodon</i>	..T.CA..C.AA.	TA.GTCCG.C	TTA..CAAC.	...GCCCA.C	A.....C..C
<i>Varanus</i>	C.C..G....	..T.....A.	TA.C.CC...	CTA.....	..G..CCC.C	C.....
<i>Sceloporus</i>	C...A..G.TC.CA.	CA.TG.T...	TTA..CAAC.CC	A.....T..A
<i>Cyclura</i>	A...A....	..A.TA.GA.	CA..CTCCG.C	CTA..CAAT.	...CTCACC	A.....C
<i>Hemidactylus</i>	C..C.A..G.A.AA.	CA..C..CT..C	..AC.AAAC.	..AAC.CA.C	A.....C..C
<i>Scincella</i>	...C.A....C..AA.	TA..T.CA...	..T...AAT.	..G...T.TT	A..T.....C
<i>Trachemys</i>	C...A....	..T..ACCA.	CA..C..CC...	CTA..CAAC.	..G..A.....C..A
<i>Alligator</i>	..GTGA..T.GC.C.	CA..T..CC...	CTT...AAC.AG.G.C	G...C.AC.A
<i>Bos</i>	CTAATAGGAG	TAAATATAGT	AATCACCGCC	CTATATTCTC	TATACATGCT	AATTATAACC
<i>Acrantophis</i>	T..C.C...C	..TCC...C.	...T..A..A	ACC.....A.	..CC.T..AT.	CC.ATC...A
<i>Boa</i>	..GT.G..G.	..GTC....C.A..A	AC...C..A.	..C...AT.	CC.ATC...A
<i>Leptotyphlops</i>	..C.C.....C	..T.GC.C...	T.....A...	GCT..CA.C.	..CC.T..A..	CC.CTC...A
<i>Typhlops</i>	..ATC.C...C	..GGA.C.C.A..A	ACC..CA.C.	..CC...AT.	T.CCTC...A
<i>Lampropeltis</i>	A..T.C...C	..TCA...C.	T..T..A..A	TC.....	..TC.T..ATA	TC.ATC...A
<i>Heterodon</i>	A..CT.T...T	..TCA...C.	...T..A...	T...C..C.	..C...ATA	TC..TC...A
<i>Agkistrodon</i>	A..GC...C	..TCA...C.	...T.....	TCC..C...	..C.T...T.	CT.ATC...A
<i>Varanus</i>	..GCC...A	C.GGA.CCC.	CT.A..A..A	TCT..CA.C.	..C..T..AT.	CC.ATC...A
<i>Sceloporus</i>	T...C...C	..GGA.CCC.	...T..A..A	GC...C..A.	..TC.T..AT.	CC.C.CT...
<i>Cyclura</i>	T...C...CC	..GGA.CCC.A...	G.G..C..AT	..C...AT.	CC.A.C...A
<i>Hemidactylus</i>	..T.C...A	..T.CA.C.C.	..C.G.....T	AC...C..A.	...T..CT.	C..A.CC...A
<i>Scincella</i>	A...C...CC	..GGA.CCT.	..T.A..A..T	ACT..C...AT.	CT.A.C...A
<i>Trachemys</i>	A...C...TT	C.GGA.CCA.	..C..T...T	AC...A.C.	...T..AT.	..TCC.C...A
<i>Alligator</i>	T...C...GC	..C..CTCTAC	C..T..T..A	A.C..CA.G.	..C...AT.	CTCGTC....
<i>Bos</i>	CAACGAGGAA	AATATACCTA	CCACATTAAT	AATATCTCGC	CTTCCTTTAC	ACGGGAAAAT
<i>Acrantophis</i>	...GC.....	..CCCT.ACC	AA..C.A..C	..C..C.---	..CA..CA...	T..A...C..
<i>Boa</i>	...AC.....	..ACAT.A.C	AA..TC.A..C	..C..A.---	..AG.ACAC..	C..A...C..C
<i>Leptotyphlops</i>	...AC...T.	..CCAC.AA.	..A..C.A..A	CTC.A.AA..	..G.TCAC..	...A..GC.C
<i>Typhlops</i>	??????????	??????????	??????????	TT..AACAA.	..AA..CACT.	...A...C..C
<i>Lampropeltis</i>	...AT...T.	CGCC.CTA.T	AA...C..TA	..C.CAA---	..AA.ACACT.	...A...C..
<i>Heterodon</i>	...AT...T.	C..CCC.ACT	AA..T.C.C.A	..TC.CA---	..AA.ACAC..	G..A...C..C
<i>Agkistrodon</i>	...AT...C	C.ACCCTGCT	AA...ACC.A	..CAGAA---	..AA.ACACT.	...A...C..C
<i>Varanus</i>	..GAA.TCT.	..CTGC..CC	..A.....CC	TTATC.---	..CAATCAC..	C..A..GC.C
<i>Sceloporus</i>AAC.	..CTCC.AAC	AA.....C	CT.TCTGAT.	..AA..CA...	...A...C..
<i>Cyclura</i>AAC.	TCATGC.ACT	T...C..TC	..CC.CAGAC.	..AA.ACAC..	C..A...C..
<i>Hemidactylus</i>	...AC...-	..CCACAAC.	A..T.CCCCA	TTAC.AC...	..C..ACAC..	C..A...C..
<i>Scincella</i>	...A...T.	..TA..AA.CA.CA	C.A..AATA.	..CA.ACA...	C..T...C..C
<i>Trachemys</i>	...T...G	GGACAC..C	AT.T..C..A	..CA..AC.A.	..AA..CA...	...A...C..C
<i>Alligator</i>	...A.....	C..CTAC.AATCCCTC	..TCC...A.	..CA..CAA...	C...C...C..C
<i>Bos</i>	GCACCTCATAT	CATTACACAT	CCTACCCTA	CTACTCCTAA	CCCTAAACCC	AAAAATTATT
<i>Acrantophis</i>	CT.T.A...A	TTA...TC.	AA...A...	..C..AA.CT	..AA...A..	..G..C..AG.A
<i>Boa</i>	CT...A...A	..CA...T...	..GCC..A..T	G..T.AA.CT	..TT...A..	..G..T..A..C
<i>Leptotyphlops</i>	CT...A...A	..C.T...T...	..T.G..C	..A.AA.CT	..AGC...A..	..GCC.A..C
<i>Typhlops</i>	CT...AGC.A	..C...T...	TA..C..A..T	A..T..TT..T	..AT...A..	..G..C..A..A
<i>Lampropeltis</i>	CT...T...G	T.C.....	..A.T..A...	A...AA.CTA..	..G..C..G...
<i>Heterodon</i>	CTCT.AT..A	..C.T...T.	TA..C...T..	A..T...A.TT	..AT...A..	..G..C..AG.A
<i>Agkistrodon</i>	CT...A...A	..CC...C.	..A.C..AT..	A..A.AA.CT	..A...A..	..G..C..AG.C
<i>Varanus</i>	CTC..A...A	..CC.C...C.	AA.C...C	A..T...C.	..TCT..A..	..G..T..A..C
<i>Sceloporus</i>	CT.T.A...G	T.C.T...C.	GGCC..A...	GCT...A.T	..TTAC...A..	..GCC.A...
<i>Cyclura</i>	CTCA...G	..CC...C	T...C...C	G.T..A...	..T...T..A..	CG.TC.A..C
<i>Hemidactylus</i>	CT...A..TG	..CA...C	AA...A...	GC...A...	..TAAC.....	...C..CGCA
<i>Scincella</i>	TT.T.A...A	..C.....	...T..AA..	A..T..TT..T	..TA...A..	..C.GT.A...
<i>Trachemys</i>	CTT...A	TCC..C..T.	...C...A..	AC...A..G	..TAA...A..	..G..C..A..C
<i>Alligator</i>	CTCT.A...C	T.....C	A.....ATC.	A..TA...C.	..T.AC.....	..CG.C.....

FIG. 3—Continued

<i>Bos</i>	CTAGGACCTC	TATAC-----	----TGTA	TATAGTTAA	CAAAA-ACAT	T-AGATTGTG
<i>Acrantophis</i>	T.-----	-----	.AGA--GTGT	GTGTAA..T.	A...A.T.C	C...GCC...
<i>Boa</i>	-----	-----	.AGG--GTGC	.TGTAA..T.	G...A.T..	...AGC...
<i>Leptotyphlops</i>	A.G-----	-----	.AG--GT..	CTGTAG..T.	AC.T.A...	C...CC...
<i>Typhlops</i>	...TA.AAC-	-----	.GG--GT.T	ACGTAG..T.	AC...A.T..	...GACC...
<i>Lampropeltis</i>	A.C-----	-----	.AG..-GTGT	ATGTAA..T.	A...AGT..	C...AGC...
<i>Heterodon</i>	A.T-----	-----	.AGA--GTGT	GGGTAA..T.	A...A.T..	C...AGC...
<i>Agkistrodon</i>	A.C-----	-----	.AGA--GTGT	GTGTAA..T.	A...A.T..	C...AG...
<i>Varanus</i>	AC.TTCT.C-	-----	.TAACCGTG.	GCATAG..T.	ATC..A...	C...C...
<i>Sceloporus</i>	TC...TTAA	.CAT.....	.T.--GTT.	GTATAG..T.	AC...A...	...GCA...
<i>Cyclura</i>	TC.AACTACT	.CGC.....	.T.--GTT.	GCATAG..T.	AT...A...	...GA...
<i>Hemidactylus</i>	...TT..AA-	-----	.AG--GC.G	GTATAG..T.	ACC..A..C.	...AGC...
<i>Scincella</i>	A...C...T	.T.C.....-ATT.	ACATAG..T.	ACT..A...C	C...G...
<i>Trachemys</i>	TG...A..T	.TGCT.....-GTT.	ATATAG..T.	A..C.A...	...C...
<i>Alligator</i>	AACC..GAGA	G..C.GCGCA	GAAC..CT..	.T---CC..G	.CCCC..G.C	...-A.CC...

<i>Bos</i>	AATCTAACAA	TAGAACTCA	TTACCTTCTT	ATTTACC---	GAAAAAGTAT	GC-----A
<i>Acrantophis</i>	.CC..G.A..C	.A---C...C	.CAC.....	.G..G..C	T-----
<i>Boa</i>	.CCT.G.A..CAC..	A-----C	.AGC.....	.G..T.A.A	T-----
<i>Leptotyphlops</i>	.G.CG.A..	C..C..A--	-----GCT.C	A.GGG..A.A	A.A.....C
<i>Typhlops</i>	.G.C..AG.	C.....A-	-----AATC.	GCG.....	A..GGCTGTA	A.C.....C
<i>Lampropeltis</i>	.CC..G...T	...G.CAAA-	-----CC.C	.AC.....	.GGGGT.ATG	T-----
<i>Heterodon</i>	.CCT.G.TC.	.G..T.T.	.C---C..GC	.CACC.....	A.G.GG.AGC	CAA.....
<i>Agkistrodon</i>	.CC..G.A..	...C..C.-	-----C..GC	--AC.....	.G.GGA.TA	-----
<i>Varanus</i>	.C..G.A..	C...G.C..	CCC.....	GCCC.....	A.G..GACCC	AAGC...C
<i>Sceloporus</i>	GCC...A..	C...GT.TC	AAC.....	.CAA.....	A.GGGGTGT.	TTGAACACC.
<i>Cyclura</i>	GCC...A..	A...GTG-C	AAC.....	GCGA.....	.G.GGTGT.	TAGAACACT.
<i>Hemidactylus</i>	G.CT...A..	.A.G...A.	.C.-CCT..	.CC.....	...GC-GC.	CAA.....C
<i>Scincella</i>	G.C..G.A..	...GGGT.-	AAG..CC...	G..A.T...	.G.GGTAC.	ATACA....
<i>Trachemys</i>	GC...A..	...G.GT.-	AA.....C	..AA.....	.G.GGTGT.	T.ACAAT...
<i>Alligator</i>	GGCTCGGT..	GTAT.GT.T.	-----	---A...-	A...C.T..G	A-----

<i>Bos</i>	AGAACTGCTA	ATTCTATGCT	CCCATATCTA	ATAGTATGGC	TTTTTCGA--	-ACTTTTAAA
<i>Acrantophis</i>	...C.....	.C...T.AA.	.TGGC..TA-	.ATAACCA.T	C..C.TC..	.T..AC..G.
<i>Boa</i>	...C.....	.C...T.TA.	.TGTCC.TA-	.C...CA..	...C..T..	.T..ACC...
<i>Leptotyphlops</i>A..TAAAA	.AGAGCTAT	.ACCCC...	CCCC.T--
<i>Typhlops</i>	T..G.....	.C...TACTC	.TGGA.ATA-	.CCAACCA..	GCC-----
<i>Lampropeltis</i>C...T.AA.	.TGGA.CTA-	.C.CACCA.-	-C.CC.--	.T..ACC...
<i>Heterodon</i>	...T.....	.C...T.AAC	.TGGA..TA-	.C-TACCA.-	-CCCC.--	.T..ACC...
<i>Agkistrodon</i>	...C.....	.C...TCAA.	.TGG--TA-	.A.TACCA..	CC.C..T..	.T..A.C...
<i>Varanus</i>	T.....	...AGGC.C	.TAGG.CTA-	.ACCCC.A.A	C..C.T--	.G.....
<i>Sceloporus</i>TACTA	.TG.AG.TA	.ATCCTCA.A	CC.C.T--
<i>Cyclura</i>TAT.C	.TG.AG.TA	..TCCTCA.A	CCCC--
<i>Hemidactylus</i>	CAG.....	.CCTGG.A..	.A.A.CTA-	...C.T...	CC...C..	...C...
<i>Scincella</i>	...G.....	.C..CT..TA	.TGGGT.TA.	..TTCC...G	CC.....	...C.....
<i>Trachemys</i>CTATAC	.TG.G.ATA.	T.CCCTCA..	.C.C...-	.G.....
<i>Alligator</i>	T.TGAAC...	.AAA..G.AG	A.T.CT..CC	T..C.CACCT	...CC.--	...C.T.

<i>Bos</i>	GGATAGT-AG	TTTATCCGTT	GGTCTTAGGA	ACCAAAAAA-	TTGGTGCAAC	TCCAAATAAA
<i>Acrantophis</i>	.AT..ACAGC	CA-TC.AC.G	.TCT.AG.A	C.A-C.GCCCA	...G...
<i>Boa</i>	...A.A.AGA	CA-TC.AC.G	.TCT.AG.A	C.A-...CCCA	...G...
<i>Leptotyphlops</i>	..GA.TAAGA	.A-CC.AT.G	.T.T.AG.C	C.A-.C...CA	...G...
<i>Typhlops</i>	..GG.A.AGA	CA-CC.AT.G	.T.T.AG.C	C.A-.C...CA	...G...
<i>Lampropeltis</i>A.AGT	-A.TC.AC.G	.TCT.AG.C	C.A-T..TCCA	...G...
<i>Heterodon</i>A.AGT	-A.TC.AC.G	.TCT.AG.C	C.A-.C.TCCA	...G...
<i>Agkistrodon</i>	...G.A.AGT	.ACTC..T.G	.TCT.AG.CG	C.A-...CTCA	...G...
<i>Varanus</i>ACAGC	CA-TC.AC.G	-C..AG.AG	C.A-CCT.TCA	...G...
<i>Sceloporus</i>AAAGT	AA-TC.AC.G	.TCT.AG.AG	C.A-...CCA	...G...
<i>Cyclura</i>AAAGC	AA-TC.AC.G	.T.T.AG.C	C.A-...TCA	...G...
<i>Hemidactylus</i>	...CCCCAGC	CG-CC.AT.A	AT.T.AG.A	TTA.C.C..CA	...G...
<i>Scincella</i>GACAGC	.ATC.AT.A	.TCT.AG.A	CTT-...TCCA	...G...
<i>Trachemys</i>AAGT	A-ATC.AT.G	.T.T.AGA..	C.ATCC.CCCA	...G...
<i>Alligator</i>ACT..	GAC...CC.	.C.....	G...T..TCG...

FIG. 3—Continued

<i>Bos</i>	AGTAATA
<i>Acrantophis</i>G
<i>Boa</i>G
<i>Leptotyphlops</i>G
<i>Typhlops</i>G
<i>Lampropeltis</i>G
<i>Heterodon</i>G
<i>Agkistrodon</i>G
<i>Varanus</i>G
<i>Sceloporus</i>G
<i>Cyclura</i>G
<i>Hemidactylus</i>G
<i>Scincella</i>G
<i>Trachemys</i>G
<i>Alligator</i>G

FIG. 3—Continued

cause large divergence between outgroup and ingroup taxa due to mutational saturation is undesirable for phylogenetic analyses, alternative rootings for the tree were obtained using other vertebrate orders singly and as a multiple outgroup. Unfortunately, the obvious choice for an outgroup, *Sphenodon*, the only surviving Rynchocephalian, was not available for this initial survey.

A single most parsimonious tree resulted from the analysis of all informative characters in a heuristic search from 100 random starting points. This tree indicated the origin of snakes from within the lizards (Fig. 4). Using *Alligator* as the outgroup, the tree is 2216 steps in length, with a consistency index (CI) of 0.497 (excluding uninformative characters) and a retention index (RI) of 0.384. The solution using the turtle, *Trachemys*, has tree length 2062, a CI of 0.496, and RI of 0.411. The cow, *Bos*, as the outgroup resolves a tree of 2132 steps, CI of 0.495, and RI of 0.394. Finally, using the combined outgroups, the tree is 2590 steps, has a CI of 0.463, and RI of 0.385. The distribution of 10,000 random trees was left skewed ($g_1 = -0.5349$) $P < 0.01$ (Hillis and Huelsenbeck, 1992). An additional analysis which weighted transversions over transitions (5:1) and deleted all third positions produced one most parsimonious tree which has the same topology as the analysis with all data (Fig. 4). The sister relationship between *Varanus* and the snake clade (Fig. 4) remains intact in all rootings and, in fact, tree topology remains unchanged with all outgroups. A final analysis of amino acid replacement also preserved the sister relationship between snakes and *Varanus* and the topology depicted in Fig. 4. Bootstrap values provide further support of the monophyly of the snakes and their relationship to *Varanus* (Fig. 4). Only three branches on the tree show a lack of resolution in the bootstrap, and these are branches which resolve relationships between ancient lizard groups or within the Scolecophidia (Fig. 4). The placement of *Scincella* outside the Iguania is strongly supported by the outgroup *Trachemys*, but poorly supported by other outgroups. Therefore, this placement is best left as ambig-

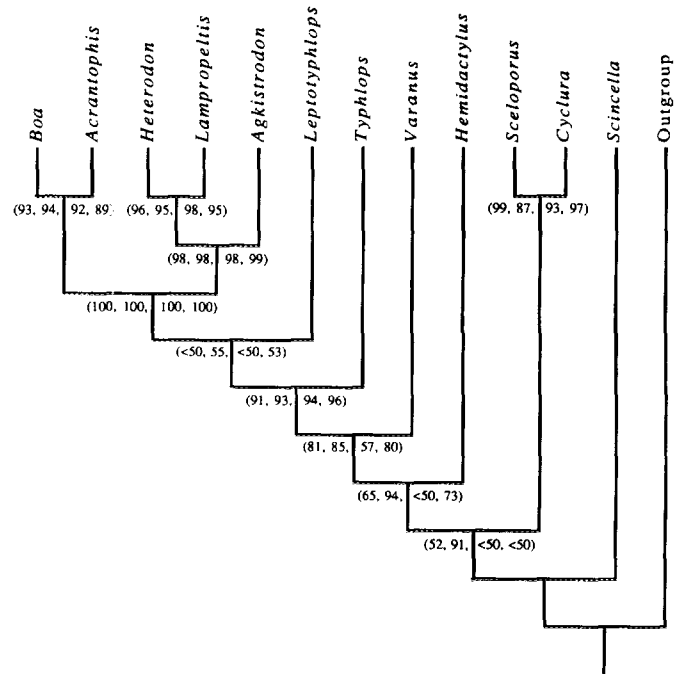


FIG. 4. Most parsimonious topology for all characters using multiple outgroups (*Trachemys scripta*, *Bos taurus*, and *Alligator mississippiensis*) for the mitochondrial DNA sequences for *Acrantophis madagascariensis*, *Boa constrictor*, *Leptotyphlops dulcis*, *Typhlops punctatus*, *Lampropeltis mexicana*, *Heterodon nasicus*, *Agkistrodon contortrix*, *Varanus niloticus*, *Sceloporus grammicus*, *Cyclura clarki*, *Hemidactylus turcicus*, and *Scincella lateralis*. The tree using all three outgroups is 2590 steps in length and has a CI of 0.463 (exclusive of uninformative characters) and an RI of 0.385. Bootstrap support values are presented at each node based upon 200 replicates and indicate analyses utilizing *Bos*, *Trachemys*, *Alligator*, and all three outgroups, respectively. Analyses using transversions only, positional weighting, and amino acid replacements preserve the sister group relationship of *Varanus* and the snake clade.

uous. Finally, the bootstrap values do not indicate strong support of the paraphyly in Scolecophidia.

In his textbook on herpetology, Zug (1993) states "Snakes are lizards, . . . only our uncertainties of the specific alliances has kept them separate from the other lizards. . . ." The molecular data support this conclusion, providing evidence that the snakes arose from within the lizard clade and are allied with Anguimorph lizards, represented here by *Varanus*. This is in agreement with several suites of morphological characters, including the presence of hemipenes, an elongate neck and consequent carotid vessels (Camp, 1923; Bellairs and Underwood, 1951), presence of zygosphenes, the long retractile tongue, and the lack of a urinary bladder (Bellairs and Underwood, 1951). The last three characters are found in other lizards, but are found in combination only in snakes and in *Varanus* (Bellairs and Underwood, 1951). Snakes share 7 of the 22 anguimorph synapomorphies and 6 of the 37 varanoid synapomorphies used by Estes and Pregill (1988) in their analyses.

The evidence from the sequences analyzed here supports the general structure of snake classification within the suborder Serpentes (Fig. 4). The Leptotyphlopidae and Typhlopidae represent extant ancestral lineages; however, the tree does not support the widely held sister group relationship between these two groups. The Scolecophidia is basal and sister to the other two infraordinal groups, the Henophidia and Caenophidia. The advanced snake clade is supported in all bootstrap replicates, whereas *Varanus* is the sister group to the snakes is 81% of bootstrap replications using *Bos* as the outgroup specified, 85% using *Trachemys*, 57% using *Alligator*, and 80% using all three outgroups (Fig. 4). Using the molecular phylogeny as a historical backdrop for the radiation of the snakes, we can address several questions about the earliest snakes. Hypotheses of the ecological adaptations of the ancestral snakes have varied from aquatic (Nopsca, 1923) to fossorial (McDowell and Bogert, 1954). Camp (1923) argued against a truly fossorial ancestry for the snakes because the number of posterior vertebrae in many advanced arboreal colubrid snakes would seem to require addition of postsacral vertebrae subsequent to an initial fossorial reduction in number. However, the distinction between truly fossorial and sheltering lifestyles is not clear. A sheltering, or subfossorial, lifestyle, which is common in modern snakes in all families, may be sufficient to lead to limb loss. Camp (1923) provides just such an intermediate scenario in which grass-living terrestrial lizards, such as the modern genus *Ophisaurus*, underwent limb degeneration to facilitate better locomotion among the stems. The basal position of the two groups of burrowing snakes, although poorly supported in the bootstrap analyses, lends support to an origin of the serpents from a fossorial lizard (Fig. 4). The fossorial specialists that represent the two most primitive snake groups may have acquired those traits independently subsequent to the split from the main snake lineages. However, many of the other primitive snake families share morphological characteristics associated with fossorial or subfossorial lifestyles. For example, the genera *Loxocemus* and *Calabaria* (two enigmatic primitive snakes) and many other members of the Henophidia have morphological traits congruent with a derivation from a fossorial ancestor. The molecular evidence favors a fossorial or near fossorial lifestyle for the ancestral lizard stock which led to the limbless condition found in snakes.

Snakes and lizards are acknowledged as the most successful of the surviving reptile lineages both in terms of described taxa and global distribution (Zug, 1993). The loss of limbs was a very significant evolutionary advance which allowed subsequent exploitation of many ecological niches. Examination of the historical radiation of snakes is an evaluation of an evolutionary innovation which allowed an explosive divergence of taxa. Their diversification and special-

izations have contributed to the difficulties in assessments of the relationships using traditional techniques. Molecular support of the sister relationship of snakes and the anguimorphs will allow a reexamination of the morphological characters in the hope of obtaining a better understanding of their structural evolution. Although previous authors (Walls, 1940; Bellairs and Underwood, 1951) have proposed the sister relationship of Anguimorpha and Serpentes, the evidence has been inconclusive and internally conflicting (Rieppel, 1988). The evidence presented here helps to clarify the position of the suborder Serpentes by providing a morphologically independent character set from which to gain phylogenetic insight. Our results are in agreement with Estes and Pregill (1988) regarding the position of snakes within the lizards and more specifically as sister to the Anguimorphs. Although this preliminary survey does not include all of the taxa necessary to clarify specific alliances (e.g., dibamids and amphisbaenids), it does unambiguously resolve Serpentes within the lizard clade. The addition of taxa to the molecular data set will allow us to determine whether snakes are actually sister to varanids, varanoids, anguimorphs, dibamids, amphisbaenids, or some higher grouping of these taxa.

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