# Snake relationships revealed by slow-evolving proteins: a preliminary survey

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(With 6 figures in the text)

We present an initial evaluation of relationships among a diverse sample of 215 species of snakes (8% of the world snake fauna) representing nine of the 16 commonly-recognized families. Allelic variation at four slow-evolving, protein-coding loci, detected by starch-gel electrophoresis, was found to be informative for estimating relationships among these species at several levels. The numerous alleles detected at these loci [*Acp*-2 (42 alleles), *Ldh*-2 (43), *Mdh*-1 (29), *Pgm* (25)] provided unexpected clarity in partitioning these taxa. Most congeneric species and several closely-related genera have the same allele at all four loci or differ at only a single locus. At the other extreme are those species with three or four unique alleles: these taxa cannot be placed in this analysis. Species sharing two or three distinctive alleles are those most clearly separated into clades. Typhlopids, pythonids, viperids, and elapids were resolved into individual clades, whereas boids were separated into boines and erycines, and colubrids appeared as several distinct clades (colubrines, natricines, psammophines, homalopsines, and xenodontines). Viperids were recognized as a major division containing three separate clades: Asian and American crotalines, Palaearctic and Oriental viperines, and Ethiopian causines. The typhlopids were found to be the basal clade, with the North American erycine boid *Charina* and the West Indian woodsnakes *Tropidophis* near the base. A number of species and some small clades were not allocated because of uninformative (common, unique, or conflicting) alleles. Of the 215 species examined, five to eight appear to have been misplaced in the analysis of these electrophoretic data.

## Introduction

Snakes are placed in their own suborder of the reptilian order Squamata and number approximately 2700 species in 16 families (McDowell, 1987; Zug, 1993). Although their morphology has been investigated scientifically for more than 200 years, there are still major gaps in our knowledge of the relationships of these animals. With their loss of external ears, eyelids, legs, and other structural elements, evolution has had a relatively narrow range of morphological features in which to work. This has led to a very large amount of parallelism and convergence (homoplasy) in morphological characters, with most anatomical features, especially those involved with feeding and locomotion, demonstrating ecological adaptations rather than providing evidence for phylogenetic relationships. In many cases it appears that a few taxa have entered a continental (or zoogeographic) region having many unfilled niches, and then radiated

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to fill those niches, thus leading to similar morphological adaptations within vastly different evolutionary lineages such as the snail-eaters *Dipsas* (Neotropical dipsadine) and *Pareas* (Oriental lamprophiine) and frog/toad-eaters *Heterodon/Xenodon/Lioheterodon* (Nearctic relict, Neotropical xenodontine, and Madagascan endemic, respectively). While some morphological characters continue to provide useful information for snake relationships, biochemical data have proven especially valuable in this morphologically conservative group (e.g. Dowling *et al.*, 1983; Dessauer, Cadle & Lawson, 1987; Cadle, 1988).

The technique of protein electrophoresis has contributed greatly to resolving systematic problems in many groups of organisms (Avise, 1994). However, the necessity of side-by-side mobility comparisons and the large number of alleles often encountered at variable loci has limited the number of species that can be examined in a typical study to about 20–30. This limitation can be overcome if only the most slowly-evolving loci—those with the fewest electromorphs (alleles)—are used. Such an approach was applied to frogs of the genus *Eleutherodactylus* (84 species, six loci; Hedges, 1989), and lizards of the genera *Anolis* (50 species, 12 loci; Burnell & Hedges, 1990) and *Sphaerodactylus* (56 species, 15 loci; Hass, 1991). Here we apply this same approach on a larger scale and compare 215 species of snakes (8% of the 2700 extant species), representing nine of the 16 families, at four protein loci.

## Materials and methods

Snake tissues were collected over a period of more than 10 years. Animals were anaesthetized with ether; both blood (plasma and red blood cells) and other tissue samples (heart, liver, kidney, skeletal muscle) were taken. Tissue samples currently are in the frozen ($-70$ °C) tissue collections of R. Highton and S. B. Hedges. Voucher specimens (**Appendix**), preserved intact or as a skin and skeleton, have been deposited in the United States National Museum of Natural History, Washington, D.C. (USNM), but they have not been incorporated into their collections as yet. Hemipenes currently are retained in the H. G. Dowling HISS Collection, but will be deposited in the USNM.

Samples to be examined electrophoretically were prepared by homogenizing a portion of each of the 4 tissues separately in $dH_2O$. These homogenates were then centrifuged to remove cellular debris, equal portions of each tissue supernatant were mixed, and the supernatants of both this mixture and the separate tissues were stored frozen. Four loci, determined to be the most slowly evolving (i.e. having the fewest electromorphs) after an initial survey of more than 20 loci, were used. These are acid phosphatase [*Acp-2*; E.C. 3.1.2.2 (IUBMBNC, 1992)], L-lactate dehydrogenase (*Ldh-2*; 1.1.1.27), malate dehydrogenase (*Mdh-1*; 1.1.1.37), and phosphoglucomutase (*Pgm*; 5.4.2.2). Horizontal starch-gel electrophoresis was performed as described by Harris & Hopkinson (1976), using the buffers shown in Hass (1991), under the following conditions: *Acp-2*—Tris-versene-borate, pH 8.0, 250 v, 6 h; *Ldh-2*—Tris-citrate-EDTA, pH 7.0, 250 v, 6 h; *Mdh-1*—Tris-citrate, pH 8.0, 130 v, 6 h; *Pgm*—Poulik, 1.0 ml NADP added to gel, 350 v, until borate line migrates 12 cm anodally from the origin.

Comparisons of electromorph mobility were made across all taxa and were performed by repeatedly alternating the different taxa with electromorphs whose mobility appeared identical in side-by-side comparisons on the same gel. This ensured detection of even slight differences in mobility that might have gone undetected if the samples were not run on the same gel. Electromorphs were numbered sequentially from cathode to anode. Attempts to use additional electrophoretic loci were unsuccessful because the large number of electromorphs precluded accurate comparisons.

Because of the large number of taxa in this study, we were limited in our choice of data analyses. A UPGMA phenogram was constructed using Nei's (1972) coefficients of genetic identity (I) values. Nei's genetic distance values (D) were not used because some I values are zero, giving a D value of infinity. A phenogram of Cavalli-Sforza & Edwards's (1967) chord distances was virtually identical to that shown here (see **Results**).

## Results

The electrophoretic data demonstrated great uniformity of electromorphs (alleles) within genera, usually congeneric species had identical alleles at all four loci or, rarely, there were one or two allelic differences within a genus. There were also many genera within some clades that shared identical alleles. Members of clades generally recognized as belonging to distinct families or subfamilies, however, usually showed marked differences at more than one locus, often having alleles unique to a higher taxon (genus, subfamily, family). Because slow-evolving loci were used, only 16 heterozygotes were found among the 864 individual genotypes observed.

The phenogram constructed from genetic similarities of the 215 species has been separated into five segments (Figs 1–5). Taxa connected by vertical lines have identical alleles at all four loci (Nei I value equal to 1). The similarities decrease to the left on this tree; some taxa having only a single allele, or no alleles in common with other taxa. This provides nested groups from species level to that of families (consult Table VI for a summary of clades). For ease of comparison, the genotypes of each taxon at each locus are grouped into five tables (Tables I–V), which correspond to Figs 1–5.

Species currently recognized as colubrine colubrids form a single group (Fig. 1; Table I). This group (I) is comprised of three clades. A small, poorly-resolved clade (A) consists of two Neotropical colubrines, *Pseustes poecilonotus* and *Leptophis ahaetulla*, each of which possesses one unique and one rare allele. A Neotropical dipsadine (*Tretanorhinus nigroluteus*) with a unique allele and an unusual combination of other alleles is also placed here, apparently in error (see **Discussion**). The two main clades are (B) Neotropical and Old World racers and their relatives (*Chironius carinatus* through *Spalerosophis cliffordi*) and (C) Holarctic ratsnakes and Nearctic racers and their relatives (*Coluber constrictor* through *Senticolis triaspis*). Many of these species have identical alleles at all four loci, and most genera vary by no more than a single allele.

The watersnakes and their relatives, the natricine colubrids, form the second major clade (Group II) as shown in Fig. 2. A small group (A) of two Asian genera (*Rhabdophis, Xenochrophis*) is distinct from the major group (B) of (mainly) North American species. Except for two species of *Regina* that share a unique allele, the American gartersnakes and watersnakes, including *R. alleni*, have identical alleles at all four loci. There are also two Asian species (*Amphiesma stolata* and *Sinonatrix annularis*) that differ by single unique alleles from the group of North American species (Table II).

Figure 3 shows four clusters of species (Groups III–VI). The first (Group III) consists of two unusual Oriental tree-racers, *Chrysopelea* and *Dendrelaphis*, that share a unique *Ldh* allele but have a mixture of other alleles that give no clear indication of outside relationships (see Table III and **Discussion**).

The second (Group IV) is a miscellaneous group of relict genera consisting of: (A) an Oriental fossorial snake (*Calamaria*) with unique alleles at two loci; (B) two sandsnakes of Ethiopian derivation (*Psammophis* and *Rhamphiophis*) with four identical alleles, two of them unique (Table III); and (C) a cluster of four Nearctic species belonging to three genera (*Carphophis, Diadophis*, and *Farancia*).

The next clade (Group V) consists of a cluster of Oriental rear-fanged treesnakes and tree-racers (boigin and philothamnin colubrines), and also includes a Neotropical tree-racer (*Oxybelis*) and two Ethiopian snakes, *Telescopus* and *Philothamnus*.

A distinctive clade of Oriental watersnakes is defined next (Group VI), with an indication of a rather distant relationship of the aglyphodont Oriental Wartsnake *Acrochordus* (A) to the

## TABLE I

*Alleles seen in Group I, ratsnakes, and racers (Colubridae: Colubrinae: Colubrini)*

| | Acp | Ldh | Mdh | Pgm |
|---|---|---|---|---|
| **I (A)** | | | | |
| *Pseustes poecilonotus* | **28**[1] | 31 | 07 | 08 |
| *Tretanorhinus nigroluteus* | **09** | 27 | 07 | 08 |
| *Leptophis ahaetulla* | **35** | 11[2] | 07 | 08 |
| **I (B)** | | | | |
| *Chironius carinatus* | 38 | 18 | 07 | 08 |
| *Chironius* sp. | 38 | 18 | 07 | 08 |
| *Chironius cinnamomeus* | 38 | 29 | 07 | 08 |
| *Chironius multiventris* | 38 | 29 | 07 | 08 |
| *Mastigodryas bifossatus* | 38 | 29 | 07 | 08 |
| *Pseustes* sp. | 38 | 29 | 07 | 08 |
| *Pseustes sulphurus* | 38 | 29 | 07 | 08 |
| *Ptyas korros* | 38 | 29 | 07 | 08 |
| *Salvadora grahami* | 38 | 29 | 07 | 08 |
| *Zaocys carinatus* | 38 | 29 | 07 | 08 |
| *Dasypeltis scaber* | 38 | 29 | 07 | 08 |
| *Drymarchon corais* | 38 | 33 | 07 | 08 |
| *Drymoluber dichrous* | 38 | 30 | 07 | 08 |
| *Entechinus semicarinatus* | 38 | **08** | 07 | 08 |
| *Leptophis mexicanus* | 38 | **12** | 07 | 08 |
| *Coluber ventromaculatus* | 38 | 29 | **06** | 08 |
| *Gonyosoma oxycephalum* | 38 | 29 | **12** | 08 |
| *Spalerosophis cliffordi* | 38 | 29 | **03** | 08 |
| **I (C)** | | | | |
| *Coluber constrictor* | 39 | 29 | 07 | 08 |
| *Masticophis lateralis* | 40 | 29 | 07 | 08 |
| *Liochlorophis vernalis* | 40 | 29 | 07 | 08 |
| *Elaphe dione* | 40 | 29 | 07 | 08 |
| *Elaphe flavolineata* | 40 | 29 | 07 | 08 |
| *Elaphe longissima* | 40 | 29 | 07 | 08 |
| *Elaphe rufodorsata* | 40 | 29 | 07 | 08 |
| *Ptyas mucosus* | **17** | 29 | 07 | 08 |
| *Elaphe scalaris* | 40 | 29 | 07 | **04** |
| *Opheodrys aestivus* | 40 | **19** | 07 | 08 |
| *Tantilla coronata* | 40 | **14** | 07 | 08 |
| *Arizona elegans* | 40 | 29 | **01** | 08 |
| *Cemophora coccinea* | 40 | 29 | 01 | 08 |
| *Elaphe* (13 species)[3] | 40 | 29 | 01 | 08 |
| *Bogertophis subocularis* | 40 | 29 | 01 | 08 |
| *Lampropeltis getulus* | 40 | 29 | 01 | 08 |
| *Lampropeltis mexicanus* | 40 | 29 | 01 | 08 |
| *Lampropeltis calligaster* | 40 | 29 | 01 | **18** |
| *Lampropeltis triangulum* | 40 | 29 | 01 | 18 |
| *Rhinocheilus lecontei* | 40 | 29 | 01 | 18 |
| *Pituophis melanoleucus* | 40 | 29 | 01 | **08/18** |
| *Senticolis triaspis* | **33** | 29 | 01 | 08 |

[1] Boldface numbers indicate alleles that are unique to this species

[2] Underlined numbers indicate alleles that are unique to the taxonomic group (here, Colubrinae) or a part of it. Some members of this subfamily (e.g. tree-racers, boigins, philothamnins, oligodontins) are found in Tables III and IV

[3] *Elaphe bimaculata, climacophora, flavirufa, guttata, moellendorffi, obsoleta, quadrivirgata, quatorlineata, radiata, schrencki, situla, taeniura, vulpina*

TABLE II

*Alleles in Group II, watersnakes and allies (Colubridae, Natricinae)*

|  | Acp | Ldh | Mdh | Pgm |
|---|---|---|---|---|
| **II (A)** | | | | |
| *Rhabdophis chrysargus* | 05 | **13** | 13 | 08 |
| *Rhabdophis* sp. | 05 | 30 | 13 | 08 |
| *Xenochrophis flavipunctatus* | 04 | 30 | 13 | 08 |
| *Xenochrophis piscator* | 04 | 30 | 13 | 08 |
| *Xenochrophis punctulatus* | 04 | 30 | 13 | 08 |
| **II (B)** | | | | |
| *Amphiesma stolata* | **06** | 29 | 13 | 08 |
| *Clonophis kirtlandi* | 21 | 29 | 13 | 08 |
| *Nerodia* (8 sp.)[1] | 21 | 29 | 13 | 08 |
| *Regina alleni* | 21 | 29 | 13 | 08 |
| *Seminatrix pygaea* | 21 | 29 | 13 | 08 |
| *Storeria occipitomaculata* | 21 | 29 | 13 | 08 |
| *Thamnophis* (16 sp.)[2] | 21 | 29 | 13 | 08 |
| *Virginia striatula* | 21 | 29 | 13 | 08 |
| *Virginia valeriae* | 21 | 29 | 13 | 08 |
| *Sinonatrix annularis* | 21 | 29 | **19** | 08 |
| *Regina rigida* | 21 | 29 | 14 | 04 |
| *Regina septemvittata* | 21 | 29 | 14 | 08 |

[1] *N. compressicauda, cyclopion, erythrogaster, fasciata, harteri, rhombifera, sipedon, taxispilota*

[2] *T. brachistoma, butleri, chrysocephalus, couchi, elegans, eques, marcianus, melanogaster, mendax, ordinoides, proximus, radix, rufipunctatus, sauritus, sirtalis, sumicrasti*

rear-fanged homalopsine colubrids (B). Also included in this clade is an obviously misplaced Ethiopian viper, *Bitis arietans*.

The next clade (Group VII) containing the Neotropical xenodontine snakes (A) is seen in Fig. 4 (also Table IV). Included with them is an Ethiopian lamprophiine, *Lamprophis fuliginosus*, and two Neotropical dipsidines, *Dipsas* and *Rhadinaea*. A xenodontine with three unique alleles, *Darlingtonia*, appears as a separate subclade (A'). A surprising feature of the clade is the clustering of the elapids (B) with the xenodontines.

Basal to this cluster of colubrids and elapids is a clade (Group VIII) of the two pythons and a clade (Group IX) of the two boine boas examined.

Basal to these is another unresolved, heterogeneous group (X) containing the Ethiopian *Atractaspis* and two species of the Nearctic genus *Heterodon*, an apparently misplaced Neotropical woodsnake *Tropidophis canus*, and a small subclade made up of the Oriental genus *Oligodon* and the Nearctic genus *Phyllorhynchus*.

The basal section of the tree (Fig. 5) contains a large sample of vipers (Group XI) that are separated into three well-distinguished subclades. The majority of species sampled are crotalines (A) from both Asia and the Americas. Most of the species of rattlesnakes (both *Crotalus* and *Sistrurus*) have identical alleles at all four loci (Table V). A separate subclade (B) contains the Palaearctic and Oriental viperines *Cerastes, Pseudocerastes*, and *Daboia*, and a third subclade (C) is composed of the Ethiopian viperines *Atheris* and *Causus*. The distinctive Oriental viper, *Calloselasma rhodostoma* is distantly joined to the Oriental viperine clade. A misplaced Neotropical dipsadine, *Atractus trilineatus*, forms a separate basal branch (A').

## TABLE III

*Alleles in tree-racers, relict colubrids, colubrine boigins/philothamnins, and Oriental rear-fanged watersnakes*

| | Acp | Ldh | Mdh | Pgm |
|---|---|---|---|---|
| **III** | | | | |
| *Chrysopelea ornata* | 33 | 25 | 02 | 08 |
| *Dendrelaphis caudolineata* | 39 | 25 | 01 | 08 |
| **IV** | | | | |
| *Calamaria gervasii* | 41 | 40 | 06 | 03/08 |
| *Rhamphiophis oxyrhynchus* | 26 | 38 | 06 | 08 |
| *Psammophis condenarus* | 26 | 38 | 06 | 08 |
| *Carphophis amoena* | 31 | 33 | 07 | 08/22 |
| *Diadophis punctatus* | 24 | 33 | 07 | 08/20 |
| *Farancia abacura* | 24 | 33 | 06 | 08 |
| *Farancia erytrogramma* | 24 | 33 | 06 | 08 |
| **V** | | | | |
| *Psammodynastes pulverensis* | 29 | 26 | 16 | 08 |
| *Boiga multimaculata* | 29 | 32 | 16 | 08 |
| *Telescopus* sp. | 29 | 28 | 16 | 08 |
| *Boiga nigriceps* | 29 | 28 | 16 | 03 |
| *Oxybelis fulgidus* | 29 | 28 | 07 | 08 |
| *Philothamnus* sp. | 33 | 11 | 15 | 08 |
| *Dinodon semicarinatum* | 33 | 28 | 20 | 08 |
| *Dryocalamus trivirgatus* | 33 | 28 | 05 | 08 |
| *Ahaetulla prasina* | 33 | 28 | 16 | 08 |
| *Boiga cyanea* | 33 | 28 | 16 | 08 |
| *Boiga cynodon* | 33 | 28 | 16 | 08 |
| *Boiga dendrophila* | 33 | 28 | 16 | 08 |
| *Boiga drapiezii* | 33 | 28 | 16 | 08 |
| *Boiga jaspida* | 23 | 28 | 16 | 10 |
| *Lycodon laoensis* | 22 | 28 | 16 | 10 |
| **VI (A)** | | | | |
| *Acrochordus javanicus* | 18 | 36 | 29 | 08 |
| **VI (B)** | | | | |
| *Enhydris bocourti* | 10 | 36 | 05 | 08 |
| *Enhydris enhydris* | 10 | 36 | 15 | 08 |
| *Enhydris jagori* | 10 | 36 | 09 | 08 |
| *Enhydris plumbea* | 10 | 36 | 09 | 08 |
| *Enhydris chinensis* | 13 | 36 | 15 | 08 |
| *Erpeton tentaculatum* | 19 | 36 | 15 | 08 |
| *Homalopsis buccata* | 10 | 36 | 15 | 15 |
| *Bitis arietans* | 10 | 36 | 15 | 23[1] |

[1] Unique to viperids

The basal taxa (Group XII) among primitive snakes (henophidians) include the Nearctic erycine boa *Charina bottae*, and a second species of the West Indian woodsnakes, *Tropidophis haetianus*. One (of five) species of the West Indian xenodontine genus *Arrhyton* (*A. funereus*) is also placed here owing to its three unique alleles. Its single non-unique allele ($Acp^{22}$), however, is shared with other West Indian xenodontines.

At the very base of the tree is a clade (Group XIII) of the four species of typhlopid blindsnakes, genus *Typhlops*, which differ from all other snakes at all four loci. This is a highly compact and distinctive clade, with one species differing from the remainder by a single allele.

TABLE IV

*Alleles in xenodontines (Colubridae: Xenodontinae), elapids, pythonids, and boids*

| | Acp | Ldh | Mdh | Pgm |
|---|---|---|---|---|
| **VII (A)** | | | | |
| Lamprophis fuliginosus | 24 | 28 | 18 | 08 |
| Alsophis cantherigerus | 24 | 36 | 18 | 08 |
| Antillophis parvifrons | 24 | 16 | 18 | 08 |
| Arrhyton exiguum | 24 | 05 | 18 | 08 |
| Helicops angulatus | 15 | 36 | 18 | 08 |
| Helicops leopardinus | 15 | 36 | 18 | 08 |
| Hydrodynastes gigas | 07 | 36 | 18 | 08 |
| Alsophis portoricensis | 22 | 36 | 18 | 08 |
| Arrhyton callilaemum | 22 | 05 | 18 | 08 |
| Arrhyton landoi | 22 | 05 | 18 | 08 |
| Arrhyton taeniatum | 22 | 03 | 18 | 08 |
| Philodryas burmeisteri | 38 | 31 | 18 | 08 |
| Philodryas viridis | 26 | 37 | 18 | 08 |
| Dipsas catesbyi | 20 | 27 | 18 | 08 |
| Rhadinaea flavilata | 12 | 27 | 18 | 08 |
| Liophis miliaris | 08 | 27 | 18 | 08 |
| Lystrophis dorbingi | 08 | 17 | 18 | 08 |
| Xenodon severus | 08 | 17 | 18 | 08 |
| Waglerophis merrami | 08 | 17 | 18 | 10 |
| Thamnodynastes strigilis | 01 | 02 | 18 | 08/16 |
| Clelia rustica | 36 | 28 | 18 | 08 |
| Liophis viridis | 36 | 28 | 18 | 09 |
| Liophis poecilogyrus | 08 | 28 | 18 | 06 |
| Hypsirhynchus ferox | 03 | 15 | 18 | 08 |
| Uromacer oxyrhynchus | 03 | 07 | 18 | 08 |
| Uromacer frenatus | 03 | 07 | 18 | 15 |
| Ialtris dorsalis | 03 | 23 | 04/18 | 06 |
| Uromacer catesbyi | 03 | 23 | 04/18 | 08 |
| **VII (A')** | | | | |
| Darlingtonia haetiana | 14 | 09 | 18 | 25 |
| **VII (B)** | | | | |
| Naja naja | 29 | 31 | 18 | 07 |
| Ophiophagus hannah | 29 | 31 | 18 | 12 |
| Bungarus fasciatus | 29 | 31 | 18 | 12 |
| Micrurus annellatus | 29 | 31 | 18 | 12 |
| Micrurus diastema | 29 | 31 | 18 | 12 |
| Micruroides euryxanthus | 29 | 20 | 18 | 12 |
| **VIII** | | | | |
| Python regius | 32 | 41 | 25 | 13 |
| Python reticulatus | 32 | 34 | 25 | 08 |
| **IX** | | | | |
| Boa constrictor | 16 | 35 | 23 | 08/16 |
| Epicrates striatus | 34 | 35 | 23 | 20 |
| **X** | | | | |
| Atractaspis corpulentus | 27 | 39 | 16 | 03 |
| Heterodon platirhinos | 24 | 06 | 16 | 04 |
| Heterodon simus | 24 | 30 | 16 | 04 |
| Tropidophis canus | 03 | 10 | 28 | 04 |
| Oligodon modestus | 40 | 32 | 16 | 19 |
| Phyllorhynchus decurtatus | 40 | 32 | 06 | 17 |

## TABLE V

*Alleles in viperids, henophidians, and typhlopids*

| | *Acp* | *Ldh* | *Mdh* | *Pgm* |
|---|---|---|---|---|
| **XI (A)** | | | | |
| *Atractus trilineatus* | 11 | 27 | 07 | 16 |
| *Agkistrodon piscivorus* | 25 | 27 | 17 | 15 |
| *Agkistrodon contortrix* | 25 | 27 | 17 | 15 |
| *Agkistrodon bilineatus* | 25 | 27 | 17 | 15/23[1] |
| *Hypnale hypnale* | 17 | **01** | 02 | 15 |
| *Trimeresurus elegans* | 17 | 30 | 02 | 15 |
| *Trimeresurus albolabris* | 17 | 30 | 02 | 21 |
| *Tropidolaemus wagleri* | 17 | 30 | **24** | 15 |
| *Trimeresurus tokarensis* | 17 | 27 | 02 | 15 |
| *Trimeresurus flavoviridis* | 17 | 27 | 02 | 15 |
| *Trimeresurus kanburiensis* | 17 | 27 | 02 | 15/21 |
| *Atropoides nummifer* | 17 | 24/27 | 02/22 | 15 |
| *Bothrops atrox* | 17 | 24/27 | 22 | 15 |
| *Crotalus scutellatus* | 17 | 27 | 22 | 15 |
| *Trimeresurus okinavensis* | 17 | 27 | 21 | 15 |
| *Sistrurus ravus* | 17 | 27 | 08 | 15 |
| *Sistrurus miliarius* | 17 | 27 | 08 | 15 |
| *Crotalus* (10 species)[2] | 17 | 27 | 08 | 15 |
| *Sistrurus catenatus* | 17 | 27 | 08 | 15 |
| *Crotalus lepidus* | 17 | 27 | 08 | 15/22 |
| *Crotalus cerastes* | 17 | 27 | 08 | 15/23 |
| *Crotalus viridis* | 17 | 27 | 08 | 23 |
| **XI (B)** | | | | |
| *Calloselasma rhodostoma* | 04 | 27 | 02 | 09 |
| *Pseudocerastes persicus* | 10 | 42 | 02 | 24 |
| *Cerastes vipera* | 10 | 27 | 02 | 24 |
| *Daboia russelii* | 10 | 42 | 02 | 14 |
| **XI (C)** | | | | |
| *Atheris squamigera* | 02 | **43** | 15 | 23 |
| *Causus rhombeatus* | 02 | 30 | 15 | 24 |
| **XII** | | | | |
| *Arrhyton funereus* | 22 | **04** | **11** | **05** |
| *Charina bottae* | 37 | 21 | 27 | 07 |
| *Tropidophis haetianus* | 19 | 07 | 26 | 11 |
| **XIII** | | | | |
| *Typhlops jamaicensis* | 30 | 22 | 10 | **02** |
| *Typhlops richardi* | 30 | 22 | 10 | 01 |
| *Typhlops platycephalus* | 30 | 22 | 10 | 01 |
| *Typhlops hypomethes* | 30 | 22 | 10 | 01 |

[1] *Pgm*[23] is found in both causines and crotalines

[2] *Crotalus adamanteus, atrox, catalinensis, durissus, horridus, mitchelli, molossus, pusillus, ruber, willardi*

The Cavalli-Sforza & Edwards D (1967) phenogram differs from the Nei's I (1972) phenogram in the following respects. Groups I and II, the colubrine and natricine colubrids cluster together, but in turn cluster with group IV. Group III contains *Philothamnus* sp., which was placed within Group V together with *Chrysopelea ornata* and *Dendrelaphis_caudolineata* in the Nei's I phenogram. Group III clusters with group V, then joins the cluster of groups I, II, and IV. The only other differences are three minor branch reversals of terminal taxa.
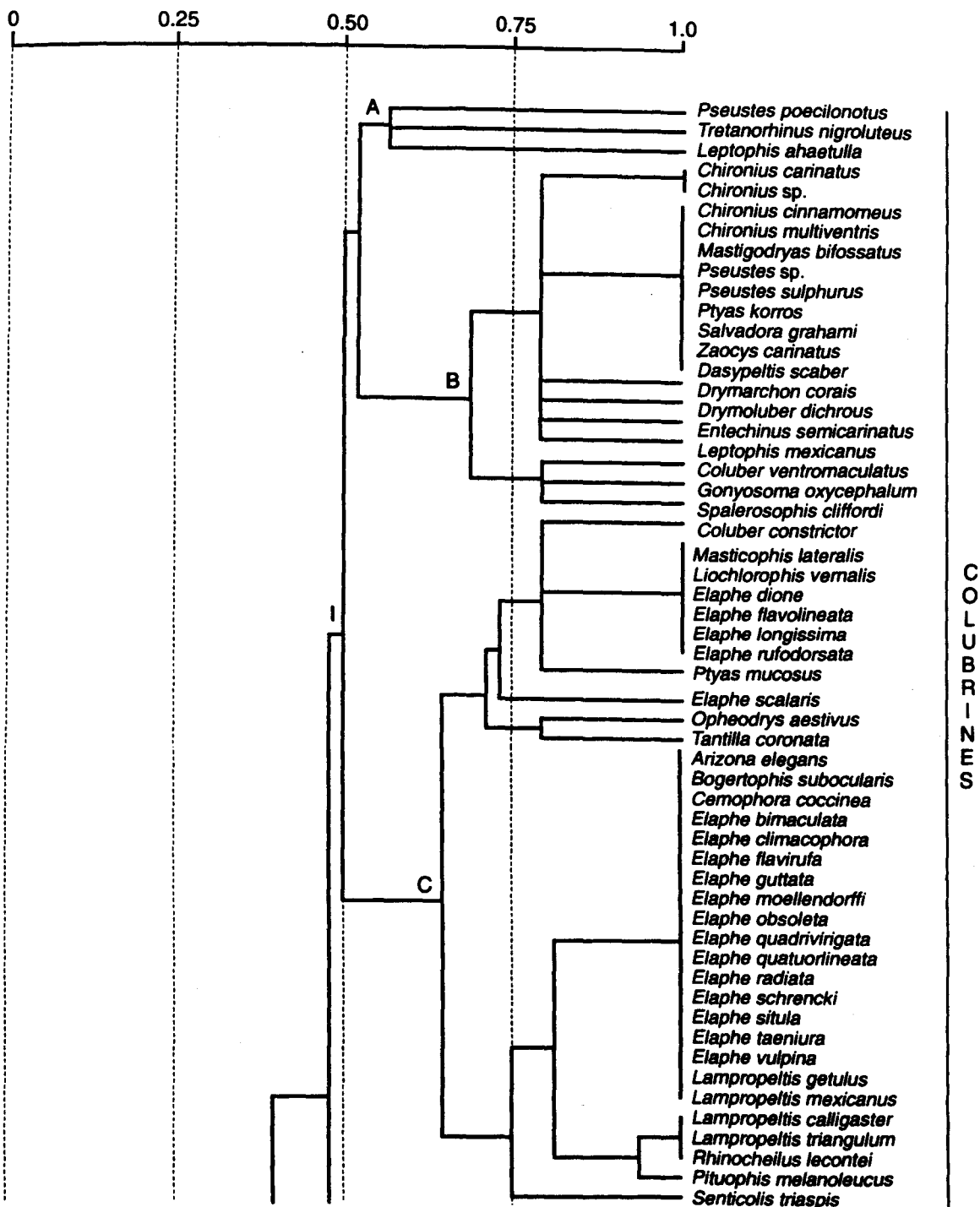
0        0.25              0.50              0.75        1.0

A ─ Pseustes poecilonotus
Tretanorhinus nigroluteus
Leptophis ahaetulla
Chironius carinatus
Chironius sp.
Chironius cinnamomeus
Chironius multiventris
Mastigodryas bifossatus
Pseustes sp.
Pseustes sulphurus
Ptyas korros
Salvadora grahami
Zaocys carinatus
B ─ Dasypeltis scaber
Drymarchon corais
Drymoluber dichrous
Entechinus semicarinatus
Leptophis mexicanus
Coluber ventromaculatus
Gonyosoma oxycephalum
Spalerosophis cliffordi
Coluber constrictor
Masticophis lateralis
Liochlorophis vernalis
Elaphe dione
Elaphe flavolineata
Elaphe longissima
Elaphe rufodorsata
Ptyas mucosus
Elaphe scalaris
Opheodrys aestivus
Tantilla coronata
Arizona elegans
Bogertophis subocularis
Cemophora coccinea
Elaphe bimaculata
Elaphe climacophora
Elaphe flavirufa
Elaphe guttata
Elaphe moellendorffi
Elaphe obsoleta
Elaphe quadrivirigata
Elaphe quatuorlineata
Elaphe radiata
Elaphe schrencki
Elaphe situla
Elaphe taeniura
Elaphe vulpina
Lampropeltis getulus
Lampropeltis mexicanus
Lampropeltis calligaster
Lampropeltis triangulum
Rhinocheilus lecontei
Pituophis melanoleucus
Senticolis triaspis

I

C

C O L U B R I N E S

FIG. 1. Group I, the racers and ratsnakes and their allies (Colubrinae: Colubrini). Two Neotropical racers (plus, *Tretanorhinus*, a misplaced Neotropical dipsadine) with an unusual combination of alleles are separated (A) from the other racers. Notable here are the distinctions of tropical (Oriental and Neotropical) racers (B) from Holarctic ratsnakes and Nearctic racers (C). (The scale on Figs 1–5 is Nei's I (1972) values.)
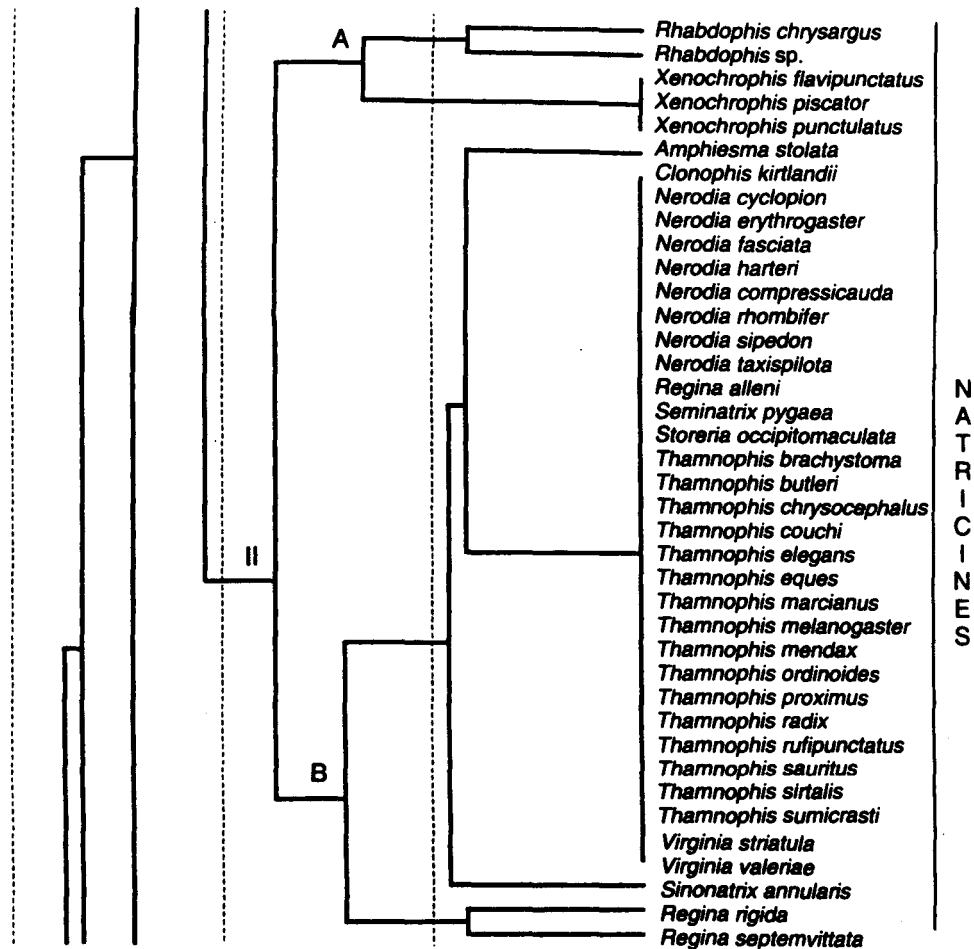
A

*Rhabdophis chrysargus*
*Rhabdophis sp.*
*Xenochrophis flavipunctatus*
*Xenochrophis piscator*
*Xenochrophis punctulatus*
*Amphiesma stolata*
*Clonophis kirtlandii*
*Nerodia cyclopion*
*Nerodia erythrogaster*
*Nerodia fasciata*
*Nerodia harteri*
*Nerodia compressicauda*
*Nerodia rhombifer*
*Nerodia sipedon*
*Nerodia taxispilota*
*Regina alleni*
*Seminatrix pygaea*
*Storeria occipitomaculata*
*Thamnophis brachystoma*
*Thamnophis butleri*
*Thamnophis chrysocephalus*
*Thamnophis couchi*
*Thamnophis elegans*
*Thamnophis eques*
*Thamnophis marcianus*
*Thamnophis melanogaster*
*Thamnophis mendax*
*Thamnophis ordinoides*
*Thamnophis proximus*
*Thamnophis radix*
*Thamnophis rufipunctatus*
*Thamnophis sauritus*
*Thamnophis sirtalis*
*Thamnophis sumicrasti*
*Virginia striatula*
*Virginia valeriae*
*Sinonatrix annularis*
*Regina rigida*
*Regina septemvittata*

II

B

N
A
T
R
I
C
I
N
E
S

FIG. 2. Group II, the watersnakes (Colubridae: Natricinae), are subdivided into primitive Oriental genera (A) and advanced genera of both Oriental and Nearctic regions (B).

## Discussion

We recognize that our study was of uneven coverage, both taxonomically and geographically. We had few Ethiopian snakes and none from Australia. We lacked representatives of Anomalepididae, Leptotyphlopidae, Uropeltiidae, Loxocemidae, Xenopeltiidae, Aniliidae, Bolyeriidae, and Hydrophiidae, and our coverage of the remaining families (with only 215 species of some 2700 known) is spotty. Nevertheless, with our representation of 110 genera from nine families, this is the largest and most diverse sample of snakes to be compared in any single molecular study, and the data provided by this study have made it possible to address a number of areas in the relationships and classification of these animals.

The great majority of the 215 species examined were placed in clades that had been previously defined by morphological (Malnate, 1960; Rossman & Eberle, 1977; Dowling & Duellman, 1978; Jenner, 1981; McDowell, 1987), immunological (Mao & Dessauer, 1971; Dowling *et al.*, 1983; Cadle, 1984*a*, *b*, 1988), and/or other biochemical data (Lawson & Dessauer, 1981; Lawson,
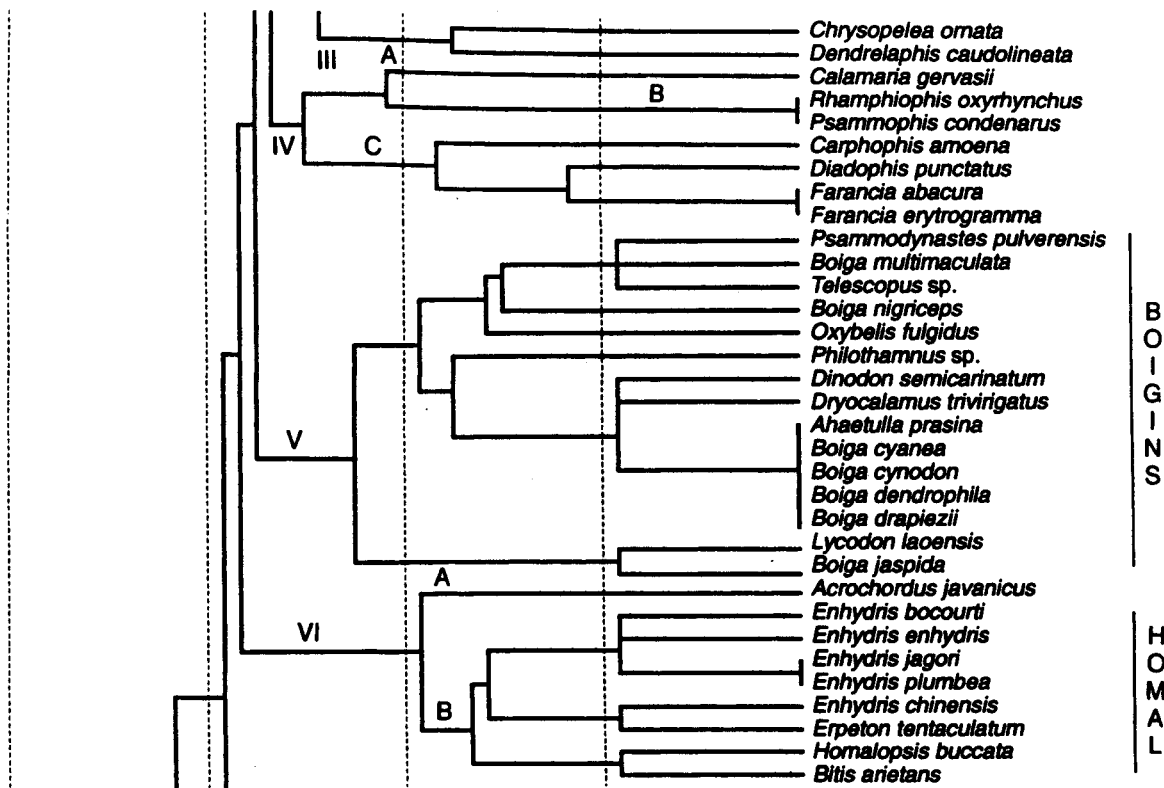
FIG. 3. Group III (Colubrinae: ?Philothamnini) comprises two Oriental tree-snakes with an unusual mixture of alleles that prevents a definitive tribal allocation.

Group IV consists of three quite separate clades: (A) the lone representative of a large Oriental group of fossorial snakes (Calamariinae); (B) two members of an Ethiopian group of sand-racers (Psammophiinae); and (C) three of the "North American Relict" genera.

Group V includes an apparently related cluster of Ethiopian, Oriental, and Nearctic treesnakes (Boigini/Philothamnini).

Group VI comprises (B) the well-defined Oriental rear-fanged watersnakes, Homalopsinae, with a suggestion of distant relationship to (A) an aglyphous Oriental wart snake (Acrochordidae).

1987). All of the families that commonly are recognized on the basis of morphology—other than the Colubridae—were clearly distinguished by using these four electrophoretic loci. The Colubridae, in contrast, was divided into clades at several different levels, concordant with previous studies suggesting paraphyly or polyphyly (Dowling & Duellman, 1978; Lawson & Dessauer, 1981; Dowling et al., 1983).

A dramatic example of supported clades is that showing the separation of colubrines and natricines. In spite of these being among the most recently evolved of major clades, their distinction found here is supported by consistent differences in hemipenial and vertebral morphology, as well as by immunological comparisons, which show a mean albumin immunological distance (ID) of about 60 between the two subfamilies (Dowling et al., 1983). A somewhat greater distance (about 70 ID) was found between colubrines and xenodontines (Cadle, 1984a).
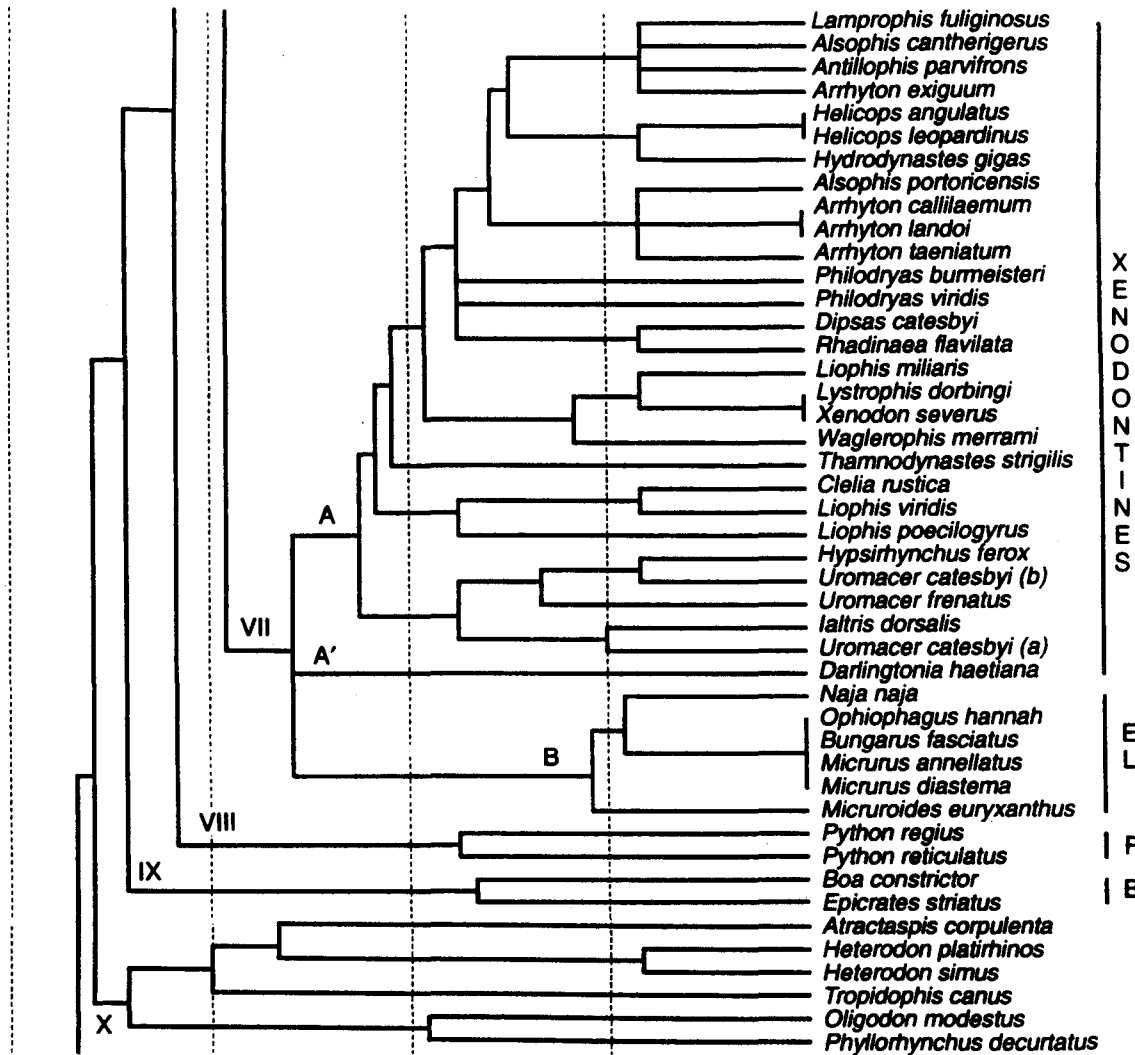
FIG. 4. Group VII comprises (A) the Neotropical (primarily South American) snakes (Xenodontinae) along with two species of (primarily Central American) snakes (Dipsadinae) of the genera *Dipsas* and *Rhadinaea*, (A') a xenodontine (*Darlingtonia*) with divergent alleles, and (B) six elapids (Elapidae).

Group VIII includes the two pythons (Pythonidae), and Group IX the two tropical boas (Boidae: Boinae).

Group X is a miscellaneous cluster including *Atractaspis*, the Ethiopian stiletto snake (Atractaspidinae), two species of *Heterodon* (a divergent North American Relict; see Group IV), and a highly divergent pair (*Oligodon* and *Phyllorhynchus*) of 'Oriental egg-eaters' (Colubrinae: Oligodontini).

The phenogram (Figs 1–5) shows 13 groups (I–XIII), including three unresolved 'clades' consisting of geographically mixed groups of distantly related taxa (IV, X, and XII). These last groups have either a preponderance of unique alleles, too many common (uninformative) alleles, or a combination of alleles that appear to give conflicting phylogenetic information. Most of the clades are clearly distinguished, however, and most have been recognized previously by one or more workers. We offer clarification of the content of recognized clades, support for relationships among them, and comments on problem taxa.
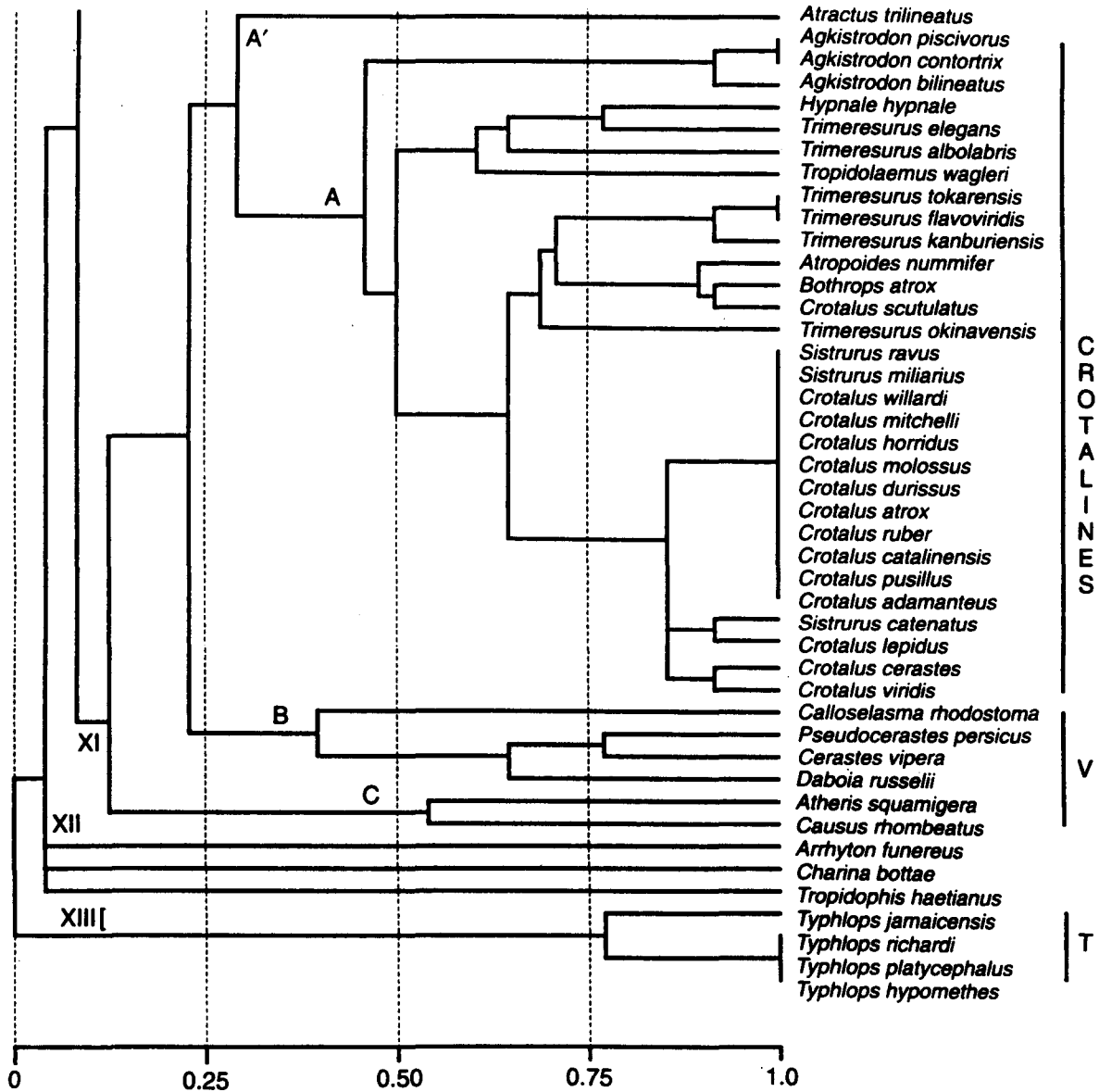
FIG. 5. This basal portion of the tree includes three highly divergent groups. Group XI is a viperid cluster (except (A'), a misplaced Neotropical dipsadid). The pitviper (Crotalinae) branch (A) shows the divergence of the genus *Agkistrodon* from other American and Oriental pitvipers and the uniformity of alleles among American rattlesnakes (*Crotalus* and *Sistrurus*). (B) comprises Palaearctic and Oriental vipers (Viperinae), plus the pitviper *Calloselasma*, which are distinguished from the Ethiopian genera (C) *Atheris* and *Causus* (Causinae).

Group XII includes three distinct members, the misplaced xenodontine, *Arrhyton* (see Discussion), the lone representative of the sandboas, *Charina* (Boidae: Erycinae), and a West Indian woodsnake, *Tropidophis* (Tropidophiidae).

Group XIII, at the base of the tree, comprises a very homogeneous cluster of blindsnakes (Typhlopidae), which share no alleles with any other snake.

TABLE VI

*Identification of groups*

| Fig. 1, Table I | |
|---|---|
| | I. Ratsnakes, racers, and allies (Colubrinae) |
| |     A. Neotropical racers. [Also *Tretanorhinus* a dipsadine] |
| |     B. Palaearctic, Oriental, and Neotropical racers |
| |     C. Holarctic ratsnakes, Nearctic racers, and allies |
| Fig. 2, Table II | |
| | II. Watersnakes, gartersnakes, and allies (Natricinae) |
| |     A. Oriental watersnakes |
| |     B. Holarctic watersnakes, gartersnakes, and allies |
| Fig. 3, Table III | |
| | III. Distinctive Oriental tree-racers |
| | IV. Relict colubrids from Oriental, Ethiopian, and Nearctic regions |
| | V. Oriental treesnakes and tree-racers (Boigini/ Philothamnini). [Also *Psammodynastes pulverensis*] |
| | VI.      A. An Oriental wartsnake, *Acrochordus* (Acrochordidae) |
| |         B. Oriental rear-fanged watersnakes (Homalopsinae). [Also *Bitis arietans*, a viperine] |
| Fig. 4, Table IV | |
| | VII.    A. Primitive South American snakes (Xenodontinae). [Also an Ethiopian lamprophiine, *Lamprophis fuliginosus*, and two dipsadines, *Dipsas* and *Rhadinaea*] |
| |       A'. *Darlingtonia*: A xenodontine |
| |       B. Cobras, kraits, and coral snakes (Elapidae) |
| | VIII. Pythons (Pythonidae) |
| | IX. Tropical Boas (Boinae) |
| | X. Miscellaneous group of primitive snakes (the Ethiopian *Atractaspis*, the Nearctic *Heterodon*, and a West Indian woodsnake, *Tropidophis canus*) and a clade consisting of the Oriental *Oligodon modestus* and the Nearctic *Phyllorhynchus decurtatus* |
| Fig. 5, Table V | |
| | XI. Vipers (Viperidae) |
| |     A. American and Oriental pitvipers (Crotalinae) |
| |     A'. A dipsadine colubrid, *Atractus* |
| |     B. Palaearctic and Oriental vipers (Viperinae). [Also *Calloselasma rhodostoma*] |
| |     C. Ethiopian vipers (Causinae) |
| | XII. Three unresolved taxa: *Arrhyton funereus*, a West Indian xenodontine, *Charina bottae*, a Nearctic erycine boid, and *Tropidophis haetiana*, a Neotropical woodsnake |
| | XIII. Blindsnakes (Typhlopidae) |

## *Colubridae: Colubrinae: Colubrini*

The 54 species of colubrins (Fig. 1; Table I) were distinguished (Group I) from all other snakes. No species from other recognized taxa were included in the two major clades (B, C), although a small clade (A) of two unusual tree-racers was poorly resolved because of their unusual combinations of alleles and the inclusion of a misplaced dipsadine, *Tretanorhinus nigroluteus*. With few exceptions, colubrines are distinguished by alleles $Acp^{38}$ (Neotropical and Oriental treesnakes and racers) and $Acp^{40}$ (North American and Palaearctic ratsnakes and racers). The close relationship found between Neotropical and Oriental colubrines (Group B) was wholly unexpected.

This study also supports the allocation by immunological studies of the African egg-eating snake, *Dasypeltis scabra*, to the colubrines (Lopez, Maxson & Dowling, 1993), and specifically to
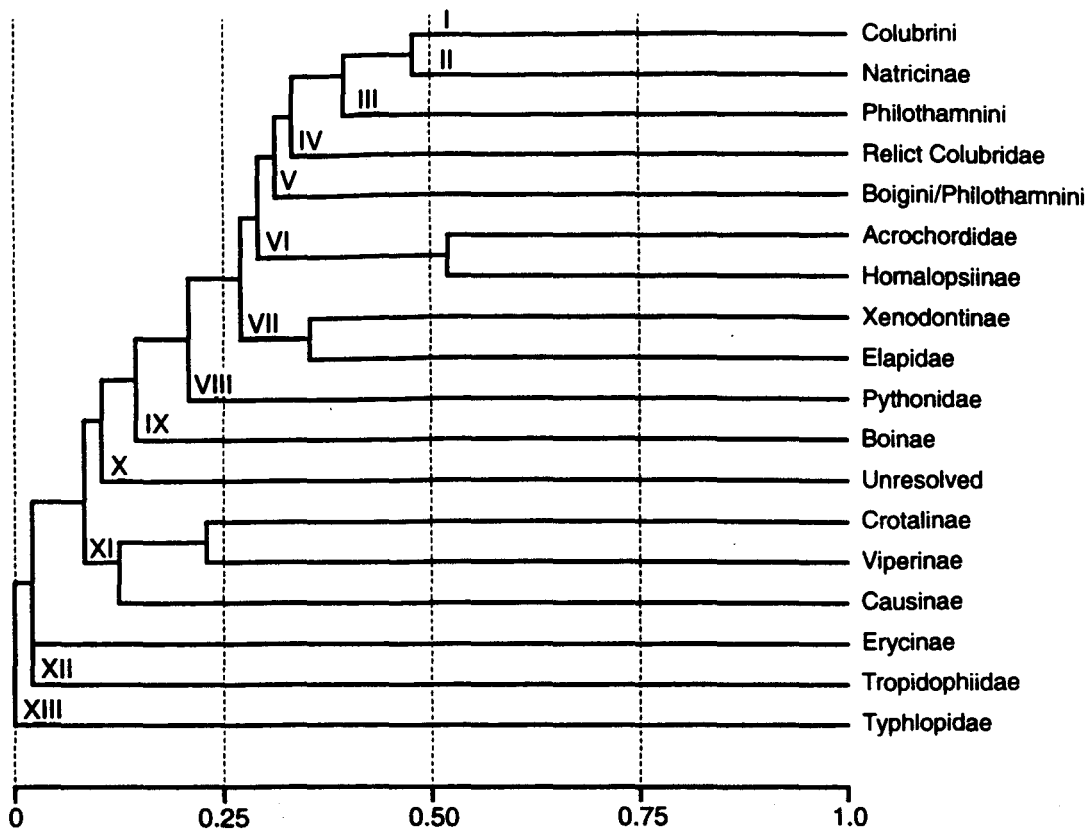
Fig. 6. Summary tree of the 18 major clusters of snakes found in this study (Figs 1–5). The distinction of clades and their contents are strongly supported except where noted in the text. Their relative positions on the tree, however, are not well supported.

a close relationship with Old World racers. The alleles of *Dasypeltis* are identical with those of many racers, differing from *Spalerosophis* (with which it was immunologically compared) only by a unique $Mdh^{03}$ allele in the latter species. The colubrine alliance with natricines is based upon the common possession (with rare exceptions) of the allele $Ldh^{29}$.

### Colubridae: Natricinae

The North American natricines [Fig. 2 (B); Table II], as has previously been indicated on the bases of morphology and chromosome data (Rossman & Eberle, 1977), are a very recent group. Almost all, 30 of the 32 species compared, have the same alleles at all four loci. *Regina rigida* and *R. septemvittata*, however, differ in sharing a unique allele ($Mdh^{14}$). On the other hand, *Regina alleni* possesses the common *Mdh* allele, and clusters with the other North American water-snakes. This supports Price's (1983) contention that *Regina*, as recognized by Rossman (1963), is polyphyletic, although Rossman (1985) disputed this.

Dowling *et al.* (1983) found in repeated immunological comparisons that *Thamnophis mendax*

differed greatly from other members of this genus, showing an ID of 46 from *T. sirtalis*, in contrast to the other 12 species of *Thamnophis*, which had IDs of 1–12. This ID of 46 equalled the greatest distance obtained within the Natricinae, that to the Ethiopian genus *Natriciteres* (Dowling *et al.*, 1983). The same specimen of *T. mendax* tested here proved to have an allelic composition identical to other American natricines (Table II), suggesting that, rarely, immuno-logical comparisons of occasional specimens or species may give anomalous IDs (Ibrahimi *et al.*, 1980).

The few Asian watersnakes examined are much more divergent, with some (*Amphiesma stolata* and *Sinonatrix annularis*) differing from members of the American group, each by only a single unique allele, while others [*Xenochrophis* and *Rhabdophis* (A)] differ from all other natricines by two distinctive alleles ($Acp^{04}$ and $Acp^{05}$, respectively). Morphological differences between these latter and other natricines were noted previously by Malnate (1960), and by Mahendra (1984), who erected a separate Oriental subfamily, Rhabdophiinae, for them.

### Colubridae: Colubrinae: Boigini/Philothamnini

The rear-fanged boigins (Fig. 3, Group V; Table III) show a greater degree of allelic differentiation than do the previous groups. They appear to be the result of an older radiation and may represent two or more phyletic lines. They are recognized here as a tribe of Colubrinae to indicate their close morphological relationship (other than their grooved rear teeth) to the other members of that subfamily (Smith, 1943). This group contains *Boiga, Dinodon, Dryoca-lamus*, and *Lycodon*, which are terrestrial or arboreal nocturnal snakes (with vertically-elliptical pupils), with their distributions centred in the Oriental region. The primarily Ethiopian genus *Telescopus* appears to belong to this group, differing by no more than a single allele from any of the other members.

The group of diurnal tree-racers (with round or horizontally-elliptical pupils) is not distinguished from the nocturnal boigins in this study. It contains *Ahaetulla, Oxybelis*, and *Philothamnus* which are (respectively) Oriental, Neotropical, and Ethiopian in distribution, but none differs from the members of the nocturnal group by more than a single allele. The Ethiopian species in this group were recognized as the Philothamninae by Bourgeois (1968), and these tree-racers are included here as a diurnal tribe Philothamnini, which is closely allied with the nocturnal boigins. The other (Group III) Oriental tree-racers, *Chrysopelea* and *Dendrelaphis*, also may belong with this group but they differ by two or more alleles from those included, and cannot be firmly associated with them by these data.

One species that the allozyme data place with the boigin colubrids is the Oriental 'false viper' *Psammodynastes*, which is very divergent morphologically from boigins and all other colubrines. It retains hypapophyses throughout the body vertebrae and has a hemipenis with a bifurcate sulcus and spines arranged in chevrons. Together with its vertically-elliptical pupils and rear fangs, these features suggest a lamprophiine rather than a colubrine/boigin relationship. *Psammodynastes* was recognized as a unique Ethiopian entry into the Oriental region by Smith (1943: 139), and Parker (1949) suggested that its relations were with an African/ Madagascan 'lycodontine' group including *Geodipsas, Pythonodipsas*, and *Ditypophis*. Unfortunately, none of these was available for our study and we are unable to test this hypothesis.

## Colubridae: Homalopsinae

This distinctive clade [Fig. 3, Group VI (B)] is made up of mainly Oriental rear-fanged watersnakes, some of whose members range south-eastward to Australia. With their specialized aquatic adaptations, they were recognized long ago as a distinct subfamily of the Colubridae. Distantly associated with them is the Oriental Wartsnake, *Acrochordus javanicus*. Although the acrochordids have geographic distributions and aquatic adaptations similar to those of the homalopsines, they are aglyphodont and their morphology is so peculiar that McDowell (1975, 1979, 1987) placed them in a separate superfamily of snakes. A recent DNA analysis placed them outside the Caenophidia (Heise *et al.*, 1995). Thus, with only a single distinctive allele in common, its association here is suggestive rather than definitive. The assignment of *Bitis arietans* (an Ethiopian viperid) to this clade is clearly in error (Ashe & Marx, 1987; Heise *et al.*, 1995).

## Colubridae: Xenodontinae

This South American group of snakes has considerable morphological variability and also is genetically diverse [Fig. 4, Group VII (A, A'); Table IV]. The allele that mainly indicates the association of its members is $Mdh^{18}$, which otherwise is found only in elapids and some dipsadines, neither of which shares any other alleles with this taxon other than the presence of $Ldh^{27}$ in dipsadines (see below). The separation of *Darlingtonia* (A') from the remainder of the xenodontines is due to its unique alleles at three loci; it does retain the distinctive xenodontine allele $Mdh^{18}$ and possesses none of those alleles characteristic of dipsadines or elapids. *Arrhyton funereus* also possesses unique alleles at three loci, and is placed at the base of the tree (Fig. 5, Group XII); however, it retains the $Acp^{22}$ allele that is found in other members of its genus (but not the $Mdh^{18}$ characteristic of xenodontines).

The morphologically distinct group of Middle American snakes, usually known as dipsadines (Dowling & Duellman, 1978), is not defined by our data. This is due, at least in part, to each of the four species having a unique allele at the *Acp* locus. In addition, two of the members placed in the dipsadinae by morphological (Jenner, 1981) and immunological (Cadle, 1984*b*) data (*Dipsas* and *Rhadinaea*) share an *Mdh* allele typical of xenodontines (with which they cluster, Fig. 5), whereas the other two (*Atractus* and *Tretanorhinus*) share an *Mdh* allele more characteristic of colubrines. All four taxa have an allele ($Ldh^{27}$), found otherwise only in one xenodontine (*Liophis miliaris*) and most viperids (Table V); this may represent a convergence in electromorph mobility. The unusual combination of alleles, along with other unique alleles, has resulted in these two latter members being allocated to different parts of the phenogram, *Atractus* to the viperid clade [Fig. 5, Group XI (A')] and *Tretanorhinus* to the colubrines [Fig. 1, Group I (A)]. Although *Atractus* is an unusual dipsidine in some morphological features, *Tretanorhinus* appears to be a rather typical dipsadine (Pinou & Dowling, 1994).

An Ethiopian genus, *Lamprophis*, has alleles that suggest a close relationship with West Indian xenodontines. It is morphologically distinct from these snakes, however, possessing posterior body vertebrae with hypapophyses and a hemipenis with typical lamprophiine spines arranged in chevrons. We had none of its presumed Ethiopian relatives for comparison and are unable to resolve these conflicting indications of relationships with our data.

## Elapidae

Although a morphological study (Savitzky, 1978) indicated that coral snakes were derived from South American xenodontines, analyses of immunological data (Cadle & Sarich, 1981) and DNA sequence data (Heise *et al.*, 1995) did not support that theory. Our electrophoretic data (Table IV) also support the monophyly of elapid snakes.

The Ethiopian genus *Atractaspis* [Fig. 4, Group X (A)] has been tentatively associated with elapids (Cadle, 1988; Dessauer *et al.*, 1987), and a recent analysis of mitochondrial DNA sequence data showed statistically significant support for this association (Heise *et al.*, 1995). In this study, *Atractaspis* clustered (apparently in error) with two species of the North American relict genus *Heterodon*.

## Pythonidae

The two species of the genus *Python* formed a distinct clade (Fig. 4, Group VIII), although *P. regius* is Ethiopian and *P. reticulatus* is Oriental. They share two distinctive alleles at the *Acp* and *Mdh* loci. Both pythons possess individually unique alleles at the *Ldh* locus, and *P. regius* also has a unique allele at the *Pgm* locus. *Python reticulatus*, in contrast, possesses the most common allele, $Pgm^{08}$, which is found in more than 100 species across diverse taxa. This allele also is seen in *Boa* (as a heterozygote), and is the only allele in common between boids and pythonids.

## Boidae

Three boids were included in the study. *Boa* and *Epicrates*, both boines, cluster to form a distinct clade (Group IX) sharing uniquely derived alleles at *Ldh* and *Mdh* loci (Fig. 4, Table IV). The third member of this family, *Charina*, an erycine boa, possesses individually unique alleles at three loci and shares the fourth (*Pgm*) only with the elapid *Naja* (this almost surely represents a convergent electromorph). This combination of alleles resulted in *Charina* being allocated near the base of the phenogram (Fig. 5, 'Group' XII; Table V).

## Viperidae

It may be a surprise to some that the viperids occupy a position near the base of caenophidian snakes (Fig. 5, Group XI; Table V), well below that of the colubrids. This position, suggested long ago by Mosauer (1935) on the evidence of body muscles, was also supported immunologically by Cadle (1988), and recently by Heise *et al.* (1995) on the basis of mitochondrial DNA sequences. It has been noted for a long time that the deeply divided hemipenis and bifurcate sulcus of viperids (similar in that way to those of boas and pythons) probably was not derived from the single hemipenis and simple sulcus of typical colubrids.

In our study [Fig. 5, Group XI (A)], the American members of the genus *Agkistrodon* represent a basal group within the crotalines. Also, the greater allelic diversity among Asian crotalines, and the considerable difference between American *Agkistrodon* and other American crotalines, suggests that Asia has provided at least two ancestral species to the American radiation.

The Oriental genus *Calloselasma* was placed as a distant viperine in our phenogram because at two loci the alleles it has are found in both crotalines and viperines and the other two loci are not informative, so it might be placed in either clade. Morphologically, its distinctive loreal pit (and

the associated excavated maxilla) place it unambiguously as a crotaline. Its position among crotalines, however, is still undetermined (see Gloyd & Conant, 1990).

## Tropidophiidae

We examined two species of this West Indian group (*T. haetianus* and *T. canus*) and found them to differ greatly from other snakes, as well as from one another. Together, they possessed five unique alleles, with no alleles in common between the two species. Because the non-unique alleles gave conflicting indications of relationship (possibly due to convergent electromorphs), *T. haetianus* falls at the base of the tree, and *T. canus* is 'lost' among some unresolved 'colubrids' (Fig. 4, Group X). That the basal position is probably correct is indicated by the data presented in Heise *et al.* (1995), which placed *Tropidophis wrighti* near the base of their tree with *Python* and *Loxocemus*.

## Typhlopidae

The basal clade of the tree (Fig. 5, Group XIII) contains the four species of blindsnakes, *Typhlops*. All the alleles in these four species are unique to this taxon. This agrees with recent evidence from DNA sequence data (Heise *et al.*, 1995) that the Scolecophidia represent an ancient, basal taxon. It is also true, however, that these are all West Indian species; typhlopids from other regions might show a greater diversity of alleles, and members of the other two scolecophidian families (Anomalepididae and Leptotyphlopidae) might demonstrate equally distinct allelic combinations.

## Unresolved clades

In some respects, the 'unresolved' taxa are the most interesting. In general, they are snakes of isolated clades that have no other related species among those examined, thus, they give conflicting information and cannot be allocated to a recognized clade.

The first of these (Fig. 3, Group IV) consists of species usually placed among the colubrids. Three subclades are seen:

(A) an Oriental Reedsnake, *Calamaria gervasii*, is the lone representative of a large clade of primitive burrowing snakes of that region (Inger & Marx, 1965). They have no other known close relatives and are often placed in a separate subfamily (Colubridae: Calamariinae).

(B) Two sandracers of Ethiopian relationships, *Rhamphiophis oxyrhynchus* is South African and *Psammophis condenarus* is from Thailand (although most of the species in this genus are African). In spite of their different generic status and their distant geographic locales, they have identical alleles at all four loci, two of which are unique to these two snakes (Table III). There are several other Ethiopian genera of this clade (often recognized as the Psammophiinae), which are easily identified by their unusual unornamented hemipenes (Bogert, 1940; Dowling & Duellman, 1978; Broadley, 1983).

(C) Four primitive Nearctic snakes in three genera (*Carphophis*, *Diadophis*, and *Farancia*) of unknown relationships. These 'North American Relicts' recently were reviewed by Pinou (1993). The similarity of alleles found here (especially at the *Acp* and *Ldh* loci) is the clearest indication of their close relationship found to date. The $Acp^{24}$ allele is otherwise found only in the North American genus *Heterodon* and some West Indian xenodontines.

The second 'unresolved' clade consists of six species in five diverse genera. Several clades are suggested (Fig. 4, Group X), but in only two of these are the species sufficiently closely associated to be worthy of consideration. The misplaced species of *Tropidophis*, which most likely belongs with its congener at the base of the tree (Fig. 5, 'Group' XII) was mentioned previously. Similarly, the Ethiopian snake *Atractaspis* also appears to be misplaced (see discussion under Elapidae). The two species of the Nearctic genus *Heterodon* differ from one another by a single allele, but the genus appears to be an isolated member of the 'North American Relicts' (Pinou, 1993), sharing with them the $Acp^{24}$ allele found in three of the other members of that group and in West Indian xenodontines.

The other clade is perhaps the most interesting, in that it suggests another Oriental-American relationship. The Nearctic leaf-nosed snakes *Phyllorhynchus* were seen as an isolated genus among North American snakes by Dowling & Duellman (1978: 112b.2,3) because of their unusual hemipenial morphology. Although the hemipenial structure of *Phyllorhynchus* suggested a relationship to the Oriental genus *Oligodon*, both genera had hemipenes that were unique within the Colubrinae, and because of this these genera were tentatively allocated as a tribe (Oligodontini) of the 'Lycodontinae'.

Later, Cadle (1984c) published a single immunological measurement (*Oligodon* sp. to *Trimorphodon biscutatus* antiserum) of 27 ID, which strongly suggested that *Oligodon* was a colubrine. We can now substantiate the colubrine relationship of both by the possession of $Acp^{40}$, which is characteristic of colubrines and the *Phyllorhynchus–Oligodon* relationship by their common possession of a rare allele, $Ldh^{32}$ (otherwise found only in *Boiga multimaculata*). This allocation is supported as well by common elements of morphology. The two snake genera have similar habitus, similar tooth structure, and similar head patterns, thus, their recognition as a colubrine tribe (Oligodontini) appears reasonable. They are off-set from the colubrine clade in the tree because each species has a (different) unique *Pgm* allele and the *Mdh* alleles are uninformative, giving mixed indications of relationship.

## Comment

In this study, there are a few apparently distantly related taxa sharing an electrophoretic allele that is otherwise not common. For example, *Bitis arietans* has allele $Ldh^{36}$, which it shares with some colubrines, but which is not found in other viperids. This is probably an example of convergence in electrophoretic mobility for two different alleles of a protein. It has been shown that protein gel electrophoresis distinguishes different forms (alleles) of the same protein through differences in size, conformation, and charge (Ramshaw, Coyne & Lewontin, 1979). In that study, 8 of 20 (40%) known variants in human haemoglobin were distinguished using a single condition in starch gel electrophoresis. A comparison of actual amino acid (AA) sequences with electrophoretic mobility for the enzyme malate dehydrogenase (*Mdh*, a protein used in this study), showed that 12 of 21 (57%) unique AA sequences were detected using a single condition (Boyd *et al.*, 1994). For *Salmonella enterica*, in those cases where mobility did not change even though there was an AA substitution, the net charge of the protein had not changed (e.g. allele k). However, 'allele' b appears to have arisen twice from the same 'ancestral' sequence through different substitutions, which resulted in a more negative net charge for the protein and convergence in band mobility on the gel.

McLellan (1984) showed that, for cetacean myoglobins, 93% of the unique AA sequences (13 of the 14 species examined) could be distinguished by running the samples at five different pH

values (range 4.8 to 10.4). This is based on the observation that the charge of a protein changes as the pH of the solution changes (Johnson, 1974). By employing a series of different electrophoretic conditions in succession [sequential electrophoresis (Singh, Lewontin & Felton, 1976; Coyne, 1982)], a researcher can detect almost all of the variants in a single protein. This technique has been employed in a number of electrophoretic studies (Coyne *et al.*, 1979; Hedges, 1989), and it has been shown that additional electrophoretic conditions do, indeed, reveal hidden variation. Because of the number of taxa included in this study, sequential electrophoresis was not performed; therefore, there is a possibility that some electromorphs represent protein variants with different AA sequences.

TABLE VII

*System of classification adopted for taxa included in this study[1]*

### SUBORDER SERPENTES — SNAKES

Infraorder SCOLECOPHIDIA — Blindsnakes
      Typhlopidae — Typical Blindsnakes
Infraorder HENOPHIDIA — Primitive Snakes
      Pythonidae — Pythons
      Boidae — Boas
         Boinae — Tropical Boas
         Erycinae — Holarctic Sandboas
      Tropidophiidae — Neotropical Woodsnakes
Infraorder CAENOPHIDIA — Advanced Snakes
      Acrochordidae — Oriental Wartsnakes
      Viperidae — Vipers
         Viperinae — Palaearctic and Oriental Vipers
         Causinae — Ethiopian Vipers
         Crotalinae — Pitvipers
      Elapidae — Cobras and Allies
      Colubridae (*sensu lato*) — Modern Snakes[2]
         Lamprophiinae — Ethiopian Housesnakes and Allies[3]
         Atractaspidinae — Ethiopian Stiletto Snakes
         Psammophiinae — Ethiopian Sand-racers
         Homalopsiinae — Oriental Rearfanged Watersnakes
         Calamariinae — Oriental Reed Snakes
         Xenodontinae — South American Snakes
         Dipsadinae — Central American Snakes
         Colubrinae — Ratsnakes, Racers, and Allies
            Colubrini — Ratsnakes and Racers
            Boigini — Oriental Treesnakes and Allies
            Philothamnini — Tropical Tree-racers
            Oligodontini — Oriental Eggeaters
        Natricinae — Modern Watersnakes and Allies
            Rhabdophiini — Oriental Watersnakes
            Natricini — Holarctic Watersnakes

[1] Modified from McDowell (1987); infraorders from Underwood (1967); some subfamilies added from Smith, Smith & Sawin (1977); some subfamilies reduced to tribal level to indicate closer relationships (see **Discussion**)

[2] Some workers (including HGD) would consider several of the included subfamilies as distinct families, thus returning some taxa here indicated as tribes to their former status as subfamilies

[3] This distinctive Ethiopian clade has been confused with Oriental "Lycodontines" ( = Boigins) because of similar tooth arrangement, and has received several other names (e.g. Boaedontines, Boodontines). We here apply the oldest available name, Lamprophiinae (from Lamprophes, Fitzinger, 1843; type genus *Lamprophis* Fitzinger, 1843).

## Summary and Conclusions

In spite of the small number of loci (four) and the sampling of only one representative of each species, this broad survey of protein variation among snakes shows remarkable concordance with current taxonomy and provides additional illumination of some controversial aspects of snake taxonomy.

That we distinguished well-recognized clades (families, subfamilies, tribes) of snakes, ranging from blindsnakes and pythons to racers and watersnakes, supports this approach (see Table VII for taxonomic identification of clades). The most interesting of the findings, and perhaps the most important, however, are in the indications of relationship (and non-relationship) among less well-established clades.

The precise delineation of the subfamilies Colubrinae and Natricinae, the two most derived of major clades, is significant. Of even greater interest are the numerous indications of inter-hemispheric and inter-continental relationships among these and other subfamilies. That almost all American species of natricines (30 of 32) have identical alleles at all four loci and are closer to some Old World genera (*Amphiesma* and *Sinonatrix*) than to others supports the idea (Rossman & Eberle, 1977) that this is a recent radiation from a single Old World ancestral stock. Conversely, inasmuch as *Rhabdophis* and *Xenochrophis* differ from both of these other Oriental genera, as well as from their American relatives, Mahendra's (1984) recognition of a separate subfamily Rhabdophiinae for these two genera is supported (here considered a tribe, Rhabdophiini).

The pattern of relationships found among natricines is repeated in a more complex fashion among colubrines. The New World representatives of this taxon, however, rather than indicating a single Oriental entry as in natricines, suggest that several Oriental colubrine taxa have migrated from eastern Asia to colonize the New World.

That the American ratsnakes (*Elaphe*) and their relatives are associated with some (but not all) Palaearctic members of that genus was to be expected. The Old World species of '*Elaphe*' have been known to be polyphyletic for many years (Lawson & Dessauer, 1981; Dowling & Fries, 1987), as were the American species until recently, when the genera *Senticolis* and *Bogertophis* were recognized (Dowling & Fries, 1987; Dowling & Price, 1988). The situation with Holarctic racers (*Coluber, Masticophis*), which appear to derive from another ancestral stock, is not as clear, but it is notable that the American species do not cluster with the single Old World representative of *Coluber* (*C. ventromaculatus*) that was included.

Wholly unexpected, however, was the great similarity between Neotropical and Oriental colubrines, many of which have identical alleles at all four loci (e.g. the Neotropical *Mastigodryas, Pseustes*, and two species of *Chironius*; and the Oriental *Ptyas* and *Zaocys*). This suggests a recent Oriental-derived radiation of racers in Central and South America similar to the pattern seen in elapids.

A somewhat more extensive geographic relationship is less clearly indicated by the distribution of certain diurnal tree-racers. Members of the Ethiopian genus *Philothamnus*, the Oriental genera *Ahaetulla, Chrysopelea*, and *Dendrelaphis*, and *Oxybelis* of the Neotropics, have generally similar, though not identical, alleles. These snakes have been believed previously to be independently-derived arboreal snakes from regional radiations. Inasmuch as the snakes suggested by corresponding alleles are all tree-racers with a common suite of morphological characters, and their combination of alleles differs from those of the other racers in those regions, this suggests that Bourgeois's (1968) Ethiopian 'Philothamninae' (Colubridae: Colubrinae:

Philothamnini, as recognized here) may be pantropical in distribution. These several 'unresolved' genera may represent a few members of this clade in each of three continents.

Our study provides further support for the utility of slow-evolving protein loci in systematic studies involving large numbers of taxa (Hedges, 1989; Burnell & Hedges, 1990; Hass, 1991). With our restrictions of a single specimen per species and only four loci, we were unable to distinguish relationships at the species level or at superfamilial levels. At the levels of genera, tribes, subfamilies, and families, however, this approach provided some valuable information on the content of supergeneric clades, their geographic distributions, and their relationships. Inasmuch as the separation of clades ranging from the Cretaceous (e.g. Typhlopidae) to the Miocene or later (Asian-American colubrid relations) were distinguished, this technique has the potential to examine the relationships among all living snakes. At most levels, these new data have proved to be complementary to those obtained in recent molecular studies using other techniques (e.g. immunology, nucleotide sequences).

Although not conceived as a taxonomic study, these data have clarified a significant number of supergeneric relationships, and have supported the monophyly of taxa previously described on the basis of morphological characteristics. These include the Boidae, Elapidae, Pythonidae, Typhlopidae, and Viperidae. Within the large family Colubridae, these include the Colubrinae, Homalopsinae, Natricinae, and Xenodontinae.

This study adds to a growing database on snake systematics. Information from morphology (e.g. hemipenes, vertebrae, scale ultrastructure), protein electrophoresis, immunology (micro-complement fixation), and DNA sequences will continue to refine our knowledge of the phylogeny of the Serpentes.

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## Appendix

List of specimens examined. (RH = Richard Highton frozen collection, Department of Zoology, University of Maryland; SBH = S. Blair Hedges frozen collection, Department of Biology, Pennsylvania State University; USNM = US National Museum of Natural History.)

N,S,E,W = cardinal points of the compass; US = United States of America; VI = Virgin Islands, West Indies; WI = West Indies.)

*Acrochordus javanicus*, RH 65022, "Thailand"; *Agkistrodon bilineatus*, RH 68179, "Mexico"; *Agkistrodon contortrix*, RH 54411, US: N. Carolina; *Agkistrodon piscivorus*, RH 53668, US: Florida; *Ahaetulla prasina*, RH 56803, "Thailand"; *Alsophis cantherigerus*, USNM 335885, WI: Cuba; *Alsophis portoricensis*, USNM 327163, WI: Puerto Rico; *Amphiesma stolata*, RH 65049, Pakistan; *Antillophis parvifrons*, USNM 329379, WI: Dominican Republic; *Arizona elegans*, RH

57389, "W US"; *Arrhyton callilaemum*, RH 54948, WI: Jamaica; *Arrhyton exiguum*, RH 59393, WI: Puerto Rico; *Arrhyton funereus*, USNM 328399, WI: Jamaica; *Arrhyton landoi*, USNM 335888, WI: Cuba; *Arrhyton taeniatum*, USNM 335892, WI: Cuba; *Atheris squamigera*, RH 63870, "Africa"; *Atractaspis corpulenta*, RH 65710, "Africa"; *Atropoides nummifer*, RH 68180, "C America".

*Bitis arietans*, RH 58157, "Africa"; *Boa constrictor*, RH 55193, "S America"; *Bogertophis subocularis*, RH 50765, US: Texas; *Boiga cyanea*, RH 65040, "Thailand"; *Boiga cynodon*, RH 57241, "Thailand"; *Boiga dendrophila*, RH 53011, "Thailand"; *Boiga drapiezii*, RH 65028, "Thailand"; *Boiga jaspida*, RH 65032, "Thailand"; *Boiga multimaculata*, RH 65017, "Thailand"; *Boiga nigriceps*, RH 56805, "Thailand"; *Bothrops atrox*, RH 68182, "S America"; *Bungarus fasciatus*, RH 63881, "SE Asia".

*Calamaria gervasii*, RH 68177, Philippines: Negros Id.; *Calloselasma rhodostoma*, RH 56348, "SE Asia"; *Carphophis amoena*, RH 53027, US: Alabama; *Causus rhombeatus*, RH 65707, "Africa"; *Cemophora coccinea*, RH 54131, US: S Carolina; *Cerastes vipera*, RH 58104, "Africa"; *Charina bottae*, RH 54514, US: Oregon; *Chironius carinatus*, RH 68227, "S America"; *Chironius cinnamomeus*, RH 59706, "S America"; *Chironius multiventris*, RH 59707, "S America"; *Chironius* sp., RH 68228, "S America"; *Chrysopelea ornata*, RH 57234, "Thailand"; *Clelia rustica*, RH 59778, "Argentina"; *Clonophis kirtlandii*, RH 49910, US: Ohio; *Coluber constrictor*, RH 53305, US: Maryland; *Coluber ventromaculatus*, RH 50767, "Afghanistan"; *Crotalus adamanteus*, RH 53010, US: Florida; *Crotalus atrox*, RH 61042, US: Arizona; *Crotalus catalinensis*, RH 52540, Mexico: Sta. Catalina Id.; *Crotalus cerastes*, RH 59785, US: California; *Crotalus durissus*, RH 52560, Paraguay; *Crotalus horridus*, RH 60210, US: New Jersey; *Crotalus lepidus*, RH 52541, US: Texas; *Crotalus mitchelli*, RH 52544, US: Nevada; *Crotalus molossus*, RH 52553, Mexico: Veracruz; *Crotalus pusillus*, RH 52538, Mexico: Michoacan; *Crotalus ruber*, RH 52548, Mexico; *Crotalus scutulatus*, RH 52558, US: Nevada; *Crotalus viridis*, RH 52542, US: Arizona; *Crotalus willardi*, RH 52550, Mexico: Sonora.

*Darlingtonia haetiana*, USNM 329423, WI: Haiti; *Dasypeltis scaber*, RH 64432, "Africa"; *Dendrelaphis caudolineata*, RH 56536, "Thailand"; *Diadophis punctatus*, RH 52696, US: Kentucky; *Dinodon semicarinatum*, RH 63884, "Asia"; *Dipsas catesbyi*, SBH 171139, Peru; *Drymarchon corais*, RH 53659, "SE US"; *Drymoluber dichrous*, RH 59704, "S America"; *Dryocalamus trivirgatus*, RH 57766, "Thailand".

*Elaphe bimaculata*, RH 61041, "China"; *Elaphe climacophora*, RH 62685, "Japan"; *Elaphe dione*, RH 58863, "Yugoslavia"; *Elaphe flavirufa*, RH 52534, "Guatemala"; *Elaphe flavolineata*, RH 65011, "Thailand"; *Elaphe guttata*, RH 59788, US: N Carolina; *Elaphe longissima*, RH 68191, "Europe"; *Elaphe moellendorffi*, RH 66366, "China"; *Elaphe obsoleta*, RH 60213, US: Maryland; *Elaphe quadrivirgata*, RH 62686, "Japan"; *Elaphe quatuorlineata*, RH 58864, "Yugoslavia"; *Elaphe radiata*, RH 56596, "Thailand"; *Elaphe rufodorsata*, RH 57758, "Eurasia"; *Elaphe scalaris*, RH 57751, "Europe"; *Elaphe schrencki*, RH 61052, "China"; *Elaphe situla*, RH 57757, "Europe"; *Elaphe taeniura*, RH 58140, "Thailand"; *Elaphe vulpina*, RH 58141, US: Wisconsin; *Enhydris bocourti*, RH 65031, "Thailand"; *Enhydris chinensis*, RH 61226, "Asia"; *Enhydris enhydris*, RH 65033, "Thailand"; *Enhydris jagori*, RH 65027, "Thailand"; *Enhydris plumbea*, RH 65037, "Thailand"; *Entechinus semicarinatus*, RH 62675, "Asia"; *Epicrates striatus*, USNM 329372, WI: Dominican Republic; *Erpeton tentaculatum*, RH 65029, "Thailand".

*Farancia abacura*, RH 53660, US: Georgia; *Farancia erytrogramma*, RH 53661, US: Georgia; *Gonyosoma oxycephalum*, RH 56349, "Thailand"; *Helicops angulatus*, RH 68190, "S America";

*Helicops leopardinus*, RH 59714, "Argentina"; *Heterodon platirhinos*, RH 53519, US: Georgia; *Heterodon simus*, RH 56143, US: Florida; *Homalopsis buccata*, RH 65043, "Thailand"; *Hydrodynastes gigas*, RH 64031, "Argentina"; *Hypsirhynchus ferox*, USNM 329438, WI: Dominican Republic; *Hypnale hypnale*, RH 66577, "Ceylon"; *Ialtris dorsalis*, USNM 329439, WI: Haiti.

*Lampropeltis calligaster*, RH 54397, US: Maryland; *Lampropeltis getulus*, RH 61349, US: S Carolina; *Lampropeltis mexicanus*, RH 52887, US: Texas; *Lampropeltis triangulum*, RH 58860, US: Pennsylvania; *Lamprophis fuliginosus*, RH 62688, "Africa"; *Leptophis ahaetulla*, RH 59703, "Argentina"; *Leptophis mexicanus*, RH 61941, Belize; *Liochlorophis vernalis*, RH 58867, US: Pennsylvania; *Liophis miliaris*, RH 59712, "Argentina"; *Liophis poecilogyrus*, RH 59702, "Argentina"; *Liophis viridis*, RH 59710, "S America"; *Lycodon laoensis*, RH 65038, "Thailand"; *Lystrophis dorbingi*, RH 56145, "Argentina".

*Masticophis lateralis*, RH 58862, "W US"; *Mastigodryas bifossatus*, RH 59169, "Argentina"; *Micruroides euryxanthus*, RH 52532, "US: Arizona"; *Micrurus annellatus*, SBH 171123, Peru; *Micrurus diastema*, RH 52446, Mexico: Quintana Roo. *Naja naja*, RH 58101, "SE Asia"; *Nerodia compressicauda*, RH 56648, US: Florida; *Nerodia cyclopion*, RH 57873, US: Florida; *Nerodia erythrogaster*, RH 57752, US: Maryland; *Nerodia fasciata*, RH 54405, US: N Carolina; *Nerodia harteri*, RH 48473, US: Texas; *Nerodia rhombifera*, RH 63454, US: Oklahoma; *Nerodia sipedon*, RH 57762, US: Maryland; *Nerodia taxispilota*, RH 56011, US: S Carolina; *Oligodon modestus*, RH 68173, Philippines: Negros Id.; *Opheodrys aestivus*, RH 61940, US: Alabama; *Ophiophagus hannah*, RH 60814, "SE Asia"; *Oxybelis fulgidus*, RH 59705, "S America".

*Philodryas burmeisteri*, RH 59711, "Argentina"; *Philodryas viridis*, RH 68184, "S. America"; *Philothamnus* sp., RH 61045, "Africa"; *Phyllorhynchus decurtatus*, RH 60813, "W US"; *Pituophis melanoleucus*, RH 50769, US: Arizona; *Psammodynastes pulverensis*, RH 57759, "Thailand"; *Psammophis condenarus*, RH 56013, "Thailand"; *Pseudocerastes persicus*, RH 63871, "SW Asia"; *Pseustes poecilonotus*, RH 59708, "Argentina"; *Pseustes* sp., RH 62677, "S America"; *Pseustes sulphurus*, RH 68186; *Ptyas korros*, RH 56535, "Thailand"; *Ptyas mucrosus*, RH 56036, "Asia"; *Python regius*, RH 56012, "Africa"; *Python reticulatus*, RH 57242, "Thailand".

*Regina alleni*, RH 50531, US: Florida; *Regina rigida*, RH 57872, US: N Carolina; *Regina septemvittata*, RH 54423, US: Maryland; *Rhabdophis chrysargus*, RH 65035, "Thailand"; *Rhabdophis* sp., RH 68176, Philippines: Negros Id.; *Rhadinaea flavilata*, RH 55942, US: N Carolina; *Rhamphiophis oxyrhynchus*, RH 52866, "Africa"; *Rhinocheilus leconti*, RH 58106, "W US".

*Salvadora grahami*, RH 56651, "W US"; *Seminatrix pygaea*, RH 50933, S Carolina; *Senticolis triaspis*, RH 61332, "US: Arizona"; *Sinonatrix annulata*, RH 61058, "Japan"; *Sistrurus catenatus*, RH 60815, "W US"; *Sistrurus miliarius*, RH 53518, US: Florida; *Sistrurus ravus*, RH 56584, "Mexico"; *Spalerosophis cliffordi*, RH 58139, "Israel"; *Storeria occipitomaculata*, RH 57765, US: Wisconsin.

*Tantilla coronata*, RH 55944, US: N Carolina; *Telescopus* sp., RH 63877, "Africa"; *Thamnodynastes strigilis*, RH 56034, "Argentina"; *Thamnophis brachystoma*, RH 49665, US: New York; *Thamnophis butleri*, RH 60355, US: Ohio; *Thamnophis chrysocephalus*, RH 48483. Mexico: Oaxaca; *Thamnophis couchi*, RH 59801, US: California; *Thamnophis elegans*, RH 48477, US: California; *Thamnophis eques*, RH 48472, Mexico: Durango; *Thamnophis marcianus*, RH 58931, US: Texas; *Thamnophis melanogaster*, RH 48480, "Mexico"; *Thamnophis mendax*, RH 49656, Mexico: Tamaulipas; *Thamnophis ordinoides*, RH 48464, US: Washington; *Thamnophis proximus*, RH 48463, US: Texas; *Thamnophis radix*, RH 48471, Canada: Saskatchewan; *Thamnophis*

*rufipunctatus*, RH 48481, US: Arizona; *Thamnophis sauritus*, RH 57763, US: Maryland; *Thamnophis sirtalis*, RH 59804, US: Kentucky; *Thamnophis sumicrasti*. RH 48482, Guatemala; *Tretanorhinus nigroluteus*, RH 63583, Belize; *Trimeresurus albolabris*, RH 65709, "SE Asia"; *Trimeresurus elegans*, RH 63876, "SE Asia"; *Trimeresurus flavoviridis*, RH 61043, "Okinawa"; *Trimeresurus kanburiensis*, RH 55996, "Thailand"; *Trimeresurus okinavensis*, RH 65708, "Okinawa"; *Trimeresurus tokarensis*, RH 63874, "Ryukyu Is."; *Tropidolaemus wagleri*, RH 59028, "Thailand"; *Tropidophis cana*, RH 54404, Bahamas Is.: Andros; *Tropidophis haetianus*, USNM 329447, WI: Dominican Republic; *Typhlops hypomethes*, SBH 161292, US: Puerto Rico; *Typhlops jamaicensis*, USNM 328403, WI: Jamaica; *Typhlops platycephalus*, SBH 161288, US: Puerto Rico; *Typhlops richardi*, USNM 336117, VI: St Thomas.

*Uromacer catesbyi*, SBH 102474, WI: Dominican Republic; *Uromacer frenatus*, USNM 329444, WI: Haiti; *Uromacer oxycephalus*, RH 60820, WI: Dominican Republic.

*Vipera* (= *Daboia*) *russelii*, RH 58102, "SE Asia"; *Virginia striatula*, RH 48468, US: Maryland; *Virginia valeriae*, RH 48467, US: Maryland.

*Waglerophis merrami*, RH 55936, "Argentina".

*Xenochrophis flavipunctatus*, RH 61060, "Asia"; *Xenochrophis piscator*, RH 65036, "Thailand"; *Xenochrophis punctulata*, RH 61049, "Japan"; *Xenodon severus*, RH 68185, "S America."

*Zaocys carinatus*, RH 56807, "Thailand".