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# Phylogenetic relationships and molecular evolution in uropeltid snakes (Serpentes: Uropeltidae): allozymes and albumin immunology

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Multilocus electrophoretic methods and microcomplement fixation comparisons of serum albumin are used to assess phylogenetic relationships among species of uropeltid snakes, to infer aspects of their population biology and biogeography, and to evaluate their relationships to other primitive snakes (Henophidia). There is very good agreement between phylogenetic inferences derived from the electrophoretic data and those derived from the albumin immunological data. Protein variation detected by electrophoresis is relatively high among 17 operational taxonomic units (OTUs) examined. The mean number of alleles per locus (5.1 across all OTUs), levels of polymorphism (25% of loci), and heterozygosity (4–6%), are typical of, or greater than, values reported for other snakes. Species of uropeltids are genetically highly differentiated, as measured by genetic distances (lowest interspecific Nei's unbiased genetic distances, 0.22–0.27 among several Sri Lankan species; 2.3 between *Teretrurus* of India and other uropeltines). The phylogenetic tree most consistent with both the immunological and electrophoretic data shows uropeltines from Sri Lanka to be monophyletic, but the Indian species are paraphyletic with respect to those from Sri Lanka. *Rhinophis travancoricus* of India is inferred to be the sister taxon to the Sri Lankan radiation. As the genera are presently understood, neither *Rhinophis* nor *Uropeltis* appears to be monophyletic. A biogeographic scenario derived from the phylogenetic hypothesis suggests an early diversification of uropeltids in India, followed by a single invasion into the lowlands of Sri Lanka. Subsequent evolution on Sri Lanka resulted in occupation of montane biotopes. *Cylindrophis* is the sister group to uropeltines and is considered a member of the Uropeltidae. The immunological data indicate no phylogenetic association between uropeltids and other 'anilioid' taxa, specifically *Anilius*, *Loxocemus* or *Xenopeltis*, although we cannot rule out a very remote relationship. We specifically reject the hypothesis that uropeltines and scolecophidians form a clade relative to henophidians. High levels of genetic variation and a trend toward negative $F_{IS}$ values for polymorphic loci in three populations



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suggest generally large effective population sizes and outbreeding in these species. The niche-width variation hypothesis for allozyme loci is not supported by the uropeltid data. In comparison to other vertebrates, the relationship between Nei's genetic distance and albumin immunological distance in uropeltids suggests either conservative albumin evolution or strong differentiation at electrophoretic loci.

KEY WORDS:—Uropeltidae – Aniliidae – Serpentes – phylogeny – allozymes – microcomplement fixation – molecular evolution – biogeography – Sri Lanka – India – systematics.

CONTENTS



## INTRODUCTION

The evolution and biogeography of the biota of India and Sri Lanka has been of long-standing interest in evolutionary biology (e.g. Blanford, 1901). Of the many biotic elements characteristic of this region, one of the more unusual is a group of specialized, but primitive, snakes commonly placed in their own family, the Uropeltidae. Comprising about 30 species in India and 15 in Sri Lanka, there are currently eight recognized genera (Smith, 1943; Gans, 1966; Rajendran, 1985). Two genera (*Rhinophis* and *Uropeltis*) have been recorded from both India and Sri Lanka, one (*Pseudotyphlops*) is endemic to Sri Lanka, and the remaining genera (*Brachyophidium*, *Melanophidium*, *Platyplectrurus*, *Plectrurus*, and *Teretrurus*) are endemic to India. The possible existence of a single species of *Platyplectrurus* in both areas seems to have been based on an erroneous record (De Silva, 1980). Numerous aspects of the morphology and biology of uropeltids are unusual. The structure of the occipito-atlas joint is unique among amniotes (Williams, 1959). They are burrowers, some species with the caudal tip modified into a blunt rough surface that accumulates a plug of soil and hence blocks the tunnel posteriorly (Gans, 1976; Gans & Baic, 1977). The axial musculature has numerous unusual biochemical and morphological properties associated with burrowing (Gans, Dessauer & Baic, 1978). What little is known about the general biology of the various species has been summarized by Gans (1976, 1987) and Rajendran (1985).

Partly because of their morphological peculiarities the relationships and classification of uropeltids have been controversial. Most workers since Romer (1956) have recognized a close relationship between uropeltids and *Cylindrophis*. The latter includes a single species (*C. maculatus*) endemic to Sri Lanka, and

several others distributed from Burma throughout south-east Asia to Malaysia and Indonesia (McDowell, 1975). Other genera sometimes considered as possible relatives of the uropeltids + *Cylindrophis* are *Anomochilus* and *Xenopeltis* of south-east Asia, and *Loxocemus* and *Anilius* of Central America and northern South America, respectively. The relationships of *Anomochilus*, which is known from few specimens, is under study (Wallach, 1988) and will not be considered further in this report. Various combinations of these genera are often grouped together as primitive henophidians (Anilioidea), but specific arrangements and, by implication, the underlying phylogenetic hypotheses, differ.

Four recent classifications of anilioid taxa are summarized in Table 1. Only Dowling & Duellman (1978) and Dowling (1988) place uropeltids *sensu stricto* with the blind snakes (Typhlopoidea; Scolecophidia). Underwood (1967), Rieppel (1979a, 1979b) and McDowell (1987) place uropeltids *sensu lato* with the primitive snakes (Henophidia, Alethinophidia) and suggest varied affinities to *Anilius* of northern South America and to *Anomochilus* and *Cylindrophis* of south-east Asia. As will be seen, our data are most fully consistent with McDowell's (1987) arrangement. Accordingly, throughout the remainder of this paper we include *Cylindrophis* in the subfamily Cylindrophinae of the family Uropeltidae, with the species formerly considered to be uropeltids (Smith, 1943) placed in the subfamily Uropeltinae.

The present report results from a series of trips to India and Sri Lanka (by CG) intended specifically to resolve a number of questions about uropeltid biology and taxonomy. Relationships within the group are controversial and poorly understood, and many aspects of their population biology remain unreported. In the present report we use multilocus electrophoretic studies and immunological studies of serum albumin to gain insight into the relationships of Sri Lankan uropeltids with each other, with the Indian forms and with other anilioid snakes. Although we do not here attempt to place these taxa within the

TABLE 1. Several recent classifications of uropeltids and other primitive snakes considered in this paper

*McDowell (1987)*
Alethinophidia
  Anilioidea
    Aniliidae-*Anilius*
    Uropeltidae
      Cylindrophiinae—*Cylindrophis*
      Uropeltinae—*Uropeltis*, etc.*
    Loxocemidae—*Loxocemus*
    Xenopeltidae—*Xenopeltis*
  Booidae
    Boidae—*Boa*

*Rieppel (1979)*
Alethinophidia
  Anilioidea
    Aniliidae
      Aniliinae—*Anilius*
      Cylindrophinae—*Cylindrophis*
    Uropeltidae—*Uropeltis*, etc.*
  Booidea
    Boidae
      Pythoninae—*Loxocemus*
      Xenopeltinae—*Xenopeltis*
      Boinae—*Boa*

*Dowling & Duellman (1978)*
Typhlopoidea
  Uropeltidae—*Uropeltis*, etc.*
Booidae
  Aniliidae—*Anilius*, *Cylindrophis*
  Boidae—*Loxocemus*, *Xenopeltis*, *Boa*

*Underwood (1967)*
Henophidia
  Aniliidae—*Anilius*, *Cylindrophis*
  Uropeltidae—*Uropeltis*, etc.*
  Boidae—*Boa*

*Includes the eight genera *Uropeltis*, *Rhinophis*, *Platyplectrurus*, *Pseudotyphlops*, *Melanophidium*, *Brachyophidium*, *Teretrurus* and *Plectrurus* (i.e. Uropeltinae, as used in this paper).

broader phylogenetic context of the Henophidia, our present data are suggestive concerning questions such as the monophyly of the Anilioidea. We infer aspects of uropeltine biogeographic history, and make suggestions for a revised taxonomy which is now in preparation. Finally, we interpret the measures of biochemical polymorphism with respect to aspects of population size and structure (e.g. Wright, 1978), features that have been especially difficult to study in secretive organisms such as uropeltids, and examine some aspects of comparative protein evolution in the group.

## MATERIALS AND METHODS

### *Sampling*

Living uropeltines and *Cylindrophis* were collected in Sri Lanka and India by CG and brought live to the laboratory. Samples of heart, liver, kidney, skeletal muscle and blood were collected from freshly killed snakes. Plasma was separated from blood cells and all tissues were frozen immediately. Until required for experimental work, tissues were maintained in the frozen tissue collection at the L.S.U. Medical Center in New Orleans. Methods of tissue storage and curation have been described (Dessauer & Hafner, 1984). The sample of *Uropeltis ceylanicus* used in the immunological study was whole blood preserved in the field in phenoxyethanol-phosphate-sucrose (Nakanishi *et al.*, 1969). The biochemical analyses were performed blind in that neither localities nor species identifications were initially made available. Specimens utilized, their collecting sites, and voucher numbers are listed in Appendix 1. Two species of *Rhinophis*, designated sp. 1 and sp. 2, will be described in forthcoming systematic papers. Sample sizes are small (1–3) for taxa except *Rhinophis philippinus* (51 individuals of two populations), *R. drummondhayi* (10), and *R. blythii* (6). Because uropeltine species are well-differentiated electrophoretically (see following), the small sample sizes should be adequate for calculating genetic variability and distances, and for estimating phylogenetic relationships (Gorman & Renzi, 1979; see also Hillis, 1987).

Our sampling relative to the diversity of uropeltines was as follows (number of species sampled/total species): *Rhinophis* (India), 1/2; *Rhinophis* (Sri Lanka), 7/12; *Uropeltis* (India), 3/19; *Uropeltis* (Sri Lanka), 2/2; *Pseudotyphlops* (Sri Lanka), 1/1; *Teretrurus* (India), 1/1; *Cylindrophis* (Sri Lanka), 1/1; *Cylindrophis* (mainland Asia), 1/6. We have had no tissue samples of *Brachyophidium*, *Melanophidium*, *Platyplectrurus* or *Plectrurus*, totalling about ten species endemic to India. Our samples therefore include approximately one-third of the known species of uropeltines, one-quarter of the known uropeltids and two-thirds of those occurring in Sri Lanka.

### *Electrophoretic methods*

Tissues were homogenized in two to three volumes of a solution containing 0.25 mol $l^{-1}$ of sucrose and 20 mg $l^{-1}$ of dithiothreitol, and centrifuged at $5000 \times \boldsymbol{g}$ to separate soluble proteins from cell debris. Aliquots of homogenates or of blood plasma were applied to slots in starch gels and subjected to vertical gel electrophoresis (Smithies, 1959) overnight at a potential gradient of 6–8 V $cm^{-1}$

in a cabinet maintained at about 4°C. Details of electrophoretic methods are described in Dessauer & Cole (1984).

Following electrophoresis, enzymes and non-enzymic proteins determined by 28 presumptive loci were localized on gel slices by means of histochemical stains, fluorescence or autoradiography. Localization techniques for the majority of enzymes closely followed descriptions by Harris & Hopkinson (1976). Transferrins were identified by iron-59 binding and autoradiography (Giblett, Hickman & Smithies, 1959). Myoglobins were detected in muscle homogenates by the presence of a light brownish band migrating anodally on unstained gels; its identity was confirmed by the benzidine test (Smithies, 1959).

Peptidase substrates were valyl-leucine for PEP-A, leucyl-glycyl-glycine for PEP-B and phenylalanyl-proline for PEP-D. 4-Methylumbelliferyl acetate was the substrate for ES-D. Identifications of these specific esterases and peptidases follow nomenclature used to describe enzymes of man having similar substrate requirements and subunit numbers (Harris & Hopkinson, 1976). Plasma albumin was localized on gels stained with the non-specific protein dye naphthol blue black and identified by its relatively high concentration and fast migration rate during electrophoresis.

Allozyme data were analysed using the BIOSYS-1 program package of Swofford & Selander (1981). Genetic variability at allozyme loci was assessed by direct counts of percentage loci polymorphic ($P$) and percentage loci heterozygous per individual (H). Heterozygote deficiencies and deviations from Hardy-Weinberg equilibrium were evaluated by chi-square goodness-of-fit tests and by calculation of $F_{IS}$, a measure of deviation from random mating within sub-populations (Wright, 1978; calculated only for populations with sample sizes $\geq 10$). Significance of the $F_{IS}$ values was tested by the statistic $(F_{IS})^2N$, where $N$ is the sample size, with one degree of freedom in a chi-square distribution (Li & Horvitz, 1953). We used Wright's (1978) modification of Rodgers' (1972) genetic distance ($D_R$) and Nei's (1978) unbiased genetic distance ($D_m$), which corrects for small sample sizes, for expressing the degree of divergence among populations. The standard errors of these distance estimates are large because heterozygosities are also high for at least some species. For these reasons, our estimate of relationships among these species based on genetic distances should be considered provisional. We are encouraged, however, by the general congruence between the pattern of relationships indicated by the analysis of genetic distances, by a qualitative character analysis of the allozyme data, and by the analysis of the albumin immunological data.

Genetic relationships among species were estimated by UPGMA clustering of Nei's $D_m$ and by a Distance Wagner analysis of Rogers' $D$ (as modified by Wright, 1978) using the Multiple Addition Criterion option (Swofford & Selander, 1981) of BIOSYS-1. Because we lacked data for albumin and transferrin in *Rhinophis travancoricus*, we analysed the genetic distance data in two ways, first by deleting these two loci from the data matrix and including all taxa in the analysis; second, by deleting *R. travancoricus* from the analysis and analysing the complete set of loci for other OTUs. The results of both analyses were very similar. The trees were rooted using *Cylindrophis maculatus* as an outgroup (confirmed by both morphological studies and by the immunological data of this study).

We also constructed parsimony trees from the gene frequency data by the

method of Swofford & Berlocher (1987) using the FREQPARS program provided by D. L. Swofford. Since the current version of that program does not guarantee finding the most parsimonious solution, we used input trees derived from several types of distance and character state analyses of our allozyme data.

### *Immunological methods*

Antisera to albumins were produced for the scolecophidian, *Leptotyphlops humilis*, and the following henophidian species: *Rhinophis philippinus* (albumin isolated from pooled plasma of available specimens), *Cylindrophis rufus*, *Anilius scytale*, and *Boa constrictor* (Table 7). Except for *R. philippinus*, albumins were isolated by their precipitation from plasma or muscle (*Leptotyphlops humilis* only) using rivanol (6,9-diamino-2-ethoxyacridine lactate) followed by vertical slab polyacrylamide gel electrophoresis (PAGE) as detailed by Cadle (1988). Albumin of *R. philippinus* was isolated directly from plasma by PAGE. Albumins were identified on gels by their fluorescence in the presence of 8-anilino-1-naphthalene sulphonate. Fluorescing bands were cut from the gel and eluted in isotris buffer (Champion *et al.*, 1974). Antisera were induced in Dutch Belted rabbits (three per albumin) over a 13-week-period following the schedule of Maxson, Highton & Wake (1979). Individual antisera were pooled in inverse proportion to their microcomplement fixation (MC'F) titers; titers for these pooled antisera ranged from 1800 (*Rhinophis*) to 6000 (*Boa*), with an average of 4000. In immunodiffusion tests against whole plasma, pooled antisera except that for *Rhinophis* showed single precipitin arcs. The antiserum to *Rhinophis* showed a minor secondary arc in addition to the major albumin arc. However, in MC'F experiments against whole plasma and purified albumins, no differences in titration curves were observed for plasma and albumins, indicating that the secondary component in this antiserum had no measurable effect on the albumin immunological distances (AIDs) obtained. With few exceptions noted in Table 7, immunodiffusion and MC'F experiments were carried out using pooled antisera according to protocols outlined in Champion *et al.* (1974).

In analysing our data from the immunological comparisons, we used *Leptotyphlops* as an outgroup in assessing rates of albumin evolution among henophidians, and *Boa* as an outgroup in more detailed assessments of rates among uropeltids.

## RESULTS

### *Electrophoresis*

Table 2 summarizes allozyme data for the 15 species of uropeltids and *Cylindrophis maculatus*. Alleles are listed in order of their increasing distance from the anode. Genetic variability parameters and calculations of $F_{IS}$ values for variable loci in each of three populations where $N \geq 10$ (*R. drummondhayi* and two populations of *R. philippinus*) are given in Tables 3 and 4, respectively. Estimates of genetic relatedness among the species are presented as Nei's (1978) unbiased genetic distance, $D_m$, and Rogers' (1972) distance, $D_R$ (Table 5). The Nei distance measures are summarized in a UPGMA phenogram (Fig. 1).

Patterns of variation for polymorphic proteins are consistent with the inference

that they are inherited as codominant alleles, as in other snakes (Dessauer, Cadle & Lawson 1987). Protein variation detectable by electrophoresis was extensive. The number of alleles/locus across all species ranged from three to nine (mean = $5.1 \pm 1.9$). No alleles were fixed identically in all species, although nearly half of the loci were fixed or nearly fixed in each OTU (ADH-1, LDH-1, LDH-2, MDH-1, MDH-2, ICD-1, ICD-2, SOD-1, GOT-1, CK-2, AP, MYG, GPD). Given the high degree of polymorphism observed in those species for which we have adequate samples ($N \geq 10$), the apparent fixation of nearly half the loci within other species undoubtedly reflects the small sample sizes for these. The most variable loci were GOT-2, GPI, TF, and ACON, each with seven alleles; GPD and PEP-B, with eight alleles; and PGD with nine alleles. *Cylindrophis maculatus* was the most differentiated species, with unique alleles at 18 of the 28 loci.

Sample sizes ($N \geq 10$) are adequate for an assessment of intrapopulation variability only for the two populations of *Rhinophis philippinus* and one of *R. drummondhayi* (Table 3). The percentage of polymorphic loci ($P$) is close to the maximum values reported for snakes (approximately 30%), whereas the heterozygosities ($H$) are typical or perhaps somewhat higher than values reported for other snakes (Table 1 of Dessauer *et al.*, 1987). Despite the small sample sizes for the other species, levels of heterozygosity and polymorphism are quite high in some. For example, in both *Rhinophis dorsimaculatus* ($N = 2$) and *Uropeltis phipsoni* ($N = 1$) the direct count estimates of $H$ are about 11%. The percentage of loci polymorphic was greater than 17% in four species (*Rhinophis* sp. 1, *R. dorsimaculatus*, *R. blythii*, and *U. melanogaster*; $N = 2$–6). The only species for which no variation was detected was *Teretrurus rhodogaster* ($N = 2$). Together these data suggest that some species of uropeltids maintain high levels of intrapopulation variation in allozyme loci, which may be interpreted with respect to aspects of the population biology of these species (see following).

Two measures indicate no significant departures from Hardy-Weinberg expectations for heterozygote proportions in any populations of uropeltids. Based on the exact significance probabilities for small samples (Elston & Forthofer, 1977) no populations had significant heterozygote deficiencies. Moreover, the $F_{IS}$ values calculated for three populations (Table 4), although non-significant because of small sample sizes, tend toward negative values, indicating a general lack of inbreeding and heterozygote deficiency.

The most striking overall trend apparent from the measures of genetic relatedness among species (Table 5) is the very great genetic distances ($D_m$) separating species of uropeltids. The lowest interspecific comparisons are among *Rhinophis drummondhayi*, *Rhinophis* sp. 1 and *R. blythii* ($D_m = 0.22$–$0.27$). The two populations of *R. phillippinus* appear closely related ($D_m = 0.06$), and no fixed allelic differences between them are observed at any loci. At the other extreme, the average $D_m$ between *Teretrurus rhodogaster* and other uropeltines is $2.3 \pm 0.24$. *Cylindrophis maculatus* is the most differentiated species, averaging $D_m = 2.5 \pm 0.11$ from the uropeltines.

The UPGMA phenogram (Fig. 1) summarizes the genetic differentiation among the species examined, and suggests three major clusters of species: (1) *Cylindrophis maculatus*; (2) the Indian species *Teretrurus rhodogaster*, *Uropeltis liura*, *U. phipsoni*; and (3) *Rhinophis travancoricus* (India) and all species from Sri Lanka. The Wagner analysis of Rogers' distance (Fig. 2) indicates these same three

TABLE 2. Alleles detected at 28 loci in 17 taxa of uropeltid snakes. Frequencies are given for alleles at polymorphic loci

| | Population or taxon* | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| ADH-1 | D | D | C | B | B | E | C | B | B | B | C | C | B | A | B | C | E |
| GPD | C | B 0.042<br>C 0.958 | C | D | E | B | F | B | B | B | G | G | A | B | A | A | H |
| LDH-1 | A | A | A | F | F | A | A | A | F | B | A | A | A | A | D | E | C |
| LDH-2 | A | A | C | A | A | A | A | A | A | A | A | B | A 0.5<br>B 0.5 | B | B | B | C |
| MDH-1 | B | B | B | D | B | B | C | B | D | B | B | D | B | D | D | C | A |
| ICD-1 | A | A | A | A | A | A | A | A | A | A | B | B | A | C | B | B | C |
| ICD-2 | B | B | B | B | B | A | C | A | B | B | A | A | C | C | C | C | D |
| PGD | A 0.211<br>G 0.789 | A 0.833<br>G 0.083<br>H 0.083 | G | H 0.286<br>I 0.714 | B | F 0.75<br>H 0.25 | F | H | C | F | A 0.167<br>C 0.667<br>F 0.167 | E 0.5<br>G 0.5 | F | F | F 0.75<br>H 0.25 | D | E 0.5<br>F 0.5 |
| SOD-1 | B | B | C | C | C | B | B | C | C | A | B | C | B | B | B | B | A |
| SOD-2 | C | C | B | C | C | C | B 0.25<br>C 0.75 | B 0.25<br>C 0.75 | C | C | C | C | C | C | C | B | A |
| GOT-1 | C | C | B | C | C | C | C | C | C | C | A | A | C | B | B | B | B |
| GOT-2 | B 0.026<br>C 0.974 | C | D | C 0.15<br>E 0.859 | E | F | F 0.75<br>G 0.25 | F | C | F | C | C | F | E | E | D | A |
| PGM-1 | B 0.914<br>C 0.086 | A 0.042<br>B 0.958 | B | B | B | B | B | B | B | B | B | B | B | A 0.5<br>B 0.5 | A | A | B |
| AK | B | B | A | A 0.778<br>B 0.222 | A 0.5<br>B 0.5 | A | A | A | A 0.8<br>B 0.2 | A | A 0.75<br>B 0.25 | A | A | A | A | A | C |
| CK-2 | E | E | E | A | A | E | E | A | A | E | E | E | C | D | D | D | B |
| AP | C 0.013<br>D 0.987 | D | D | D | D | C | C | D | D | D | D | D | D | D | D | B | A |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ACON-1 | A | A | B | B | B | D | B 0.75<br>D 0.25 | B 0.5<br>D 0.5 | B 0.9<br>C 0.1 | D | D | D | F | G | G | E | D |
| ES-D | B 0.208<br>D 0.5<br>E 0.208<br>F 0.083 | D 0.375<br>F 0.625 | D | C 0.05<br>D 0.95 | D | D | D | F | D | D | C | C | D | C | C | C | A |
| PEP-A | C | C | E | E | C 0.5<br>E 0.5 | C | A | C | E 0.5<br>F 0.5 | C | C 0.5<br>D 0.5 | E | F | D | F | D | B |
| PEP-B | C | C | B | E | B | A | C | A 0.25<br>B 0.75 | B 0.8<br>E 0.2 | F | B | B | E | B 0.5<br>C 0.5 | G | H | D |
| PEP-D | B 0.947<br>D 0.053 | B 0.042<br>D 0.958 | G | B 0.05<br>D 0.95 | E 0.75<br>F 0.25 | D 0.5<br>E 0.5 | D | D | D | B | D | A 0.25<br>B 0.5<br>D 0.25 | E | E | C | E | D |
| ADA | D | D 0.958<br>E 0.042 | D | D 0.95<br>E 0.05 | D 0.5<br>E 0.5 | D | D 0.25<br>E 0.75 | D | D | C 0.25<br>D 0.75 | E | F | E | A | A | A | B |
| MPI | A 0.111<br>B 0.889 | B 0.958<br>C 0.042 | B 0.75<br>C 0.25 | B | A 0.25<br>B 0.75 | B | B | A | B | B | D | B | B | D | C | E | F |
| GPI | F | F | F | F 0.7<br>G 0.3 | F | F | F | F | F | C | F 0.833<br>G 0.167 | G | B | E | E | D | A |
| TF | E | E | G | E | D | D | C 0.5<br>D 0.5 | G | C 0.1<br>D 0.8<br>F 0.1 | C | E 0.25<br>F 0.75 | C | — | C | A | D | B |
| ALB | B | B | B | B | B | C | C | C | C | B | A | C | — | B 0.5<br>C 0.5 | A | C | A |
| MYG | D | D | D | D | D | D | D | D | D | D | D | D | D | B | B | C | A |

1, *Rhinophis philippinus*, Palatene. 2, *R. philippinus*, Matalapitiya. 3, *R. trevelyanus*. 4, *R. drummondhayi*. 5, *Rhinophis* sp. 1. 6, *R. oxyrhynchus*. 7, *R. dorsimaculatus*. 8, *Rhinophis* sp. 2. 9, *R. blythii*. 10, *Pseudotyphlops philippinus*. 11, *Uropeltis melanogaster*. 12, *U. phillipsi*. 13, *Rhinophis travancoricus*. 14, *U. phipsoni*, 15, *U. liura*. 16, *Teretrurus rhodogaster*. 17, *Cylindrophis maculatus*.

TABLE 3. Measures of intrapopulation variability for three populations of uropeltids. $P$ = Percent polymorphism considering all alleles; $P_{0.95}$ = percent polymorphism where the frequency of the most common allele does not exceed 0.95; $H$ = direct count estimate of heterozygosity; $A$ = number of alleles per locus. Standard errors are given in parentheses

| | $N$ | $P$ | $P_{0.95}$ | $H$ | $A$ |
|---|---|---|---|---|---|
| *Rhinophis philippinus* (Palatene) | 39 | 25 | 17.9 | 0.056 (0.027) | 1.32 (0.13) |
| *Rhinophis philippinus* (Matalapitiya) | 12 | 25 | 7.14 | 0.036 (0.015) | 1.29 (0.10) |
| *Rhinophis drummondhayi* | 10 | 25 | 25.0 | 0.062 (0.024) | 1.25 (0.08) |

groupings, although there are differences in the arrangement of taxa within group (3). In both analyses, however, three clusters of OTUs within group (3) appear consistently: the two populations of *Rhinophis philippinus*, *Uropeltis melanogaster–U. phillipsi*, and *R. drummondhayi–R. blythii-Rhinophis* sp. 1–*Rhinophis* sp. 2. With the exception of these OTU clusters we do not consider relationships among group (3) species resolvable by these data. Clearly, most of the differentiation occurs among individual species and not among successive clusters of species (note basal branch lengths as compared to tip lengths in Fig. 2). After optimization of branch lengths the distance Wagner tree had a low percent standard deviation (Fitch & Margoliash, 1967) of 5.42 (cophenetic correlation = 0.955) and a single very small (0.001) negative branch. These measures indicate a very good overall fit of the tree to the original data.

The trees we evaluated using FREQPARS ranged in length from 283.6 to 315. Three trees (lengths 283.6–285.3) were separated by a gap of seven steps

TABLE 4. Summary of $F_{IS}$, a measure of deviation from random mating within populations, for variable allozyme loci in two populations of *Rhinophis philippinus* and one of *Rhinophis drummondhayi*. Two alleles are present at each locus except for PGD in *R. philippinus* from Matalapitiya (three alleles), and ES-D in *R. philippinus* from Palatene (four alleles). Invariant loci are left blank. Although non-significant, the trend toward negative values is evident

| | *Rhinophis philippinus* (Palatene) ($N$ = 39) | *Rhinophis philippinus* (Matalapitiya) ($N$ = 12) | *Rhinophis drummondhayi* ($N$ = 10) |
|---|---|---|---|
| GPD | — | −0.043 | — |
| PGD | −0.267 | −0.143 | 0.300 |
| GOT-2 | −0.026 | — | −0.176 |
| PGM-1 | −0.094 | −0.043 | — |
| AK | — | — | −0.286 |
| AP | −0.013 | — | — |
| ES-D | 0.026 | 0.467 | −0.053 |
| PEP-D | −0.056 | −0.043 | −0.053 |
| ADA | — | −0.043 | −0.053 |
| MPI | 0.156 | −0.043 | — |
| GPI | — | — | 0.048 |
| Mean | −0.039 | 0.109 | −0.039 |

TABLE 5. Nei's (1978) unbiased genetic distance ($D_m$) above the diagonal, and Roger's genetic distance ($D_R$), as modified by Wright (1978) below the diagonal for 17 uropeltid OTUs. In comparisons involving heterozygous loci in which only a single individual was examined, Nei's unbiased genetic distance is replaced with the standard measure (Nei, 1972)

0.674

| | Taxa | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxa | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| 1. *R. philippinus* (Palatene) | — | 0.060 | 0.589 | 0.675 | 0.557 | 0.477 | 0.595 | 0.641 | 0.595 | 0.511 | 0.657 | 0.931 | 0.751 | 1.426 | 2.214 | 3.228 | 3.252 |
| 2. *R. philippinus* (Matalapitiya) | 0.239 | — | 0.655 | 0.618 | 0.566 | 0.455 | 0.539 | 0.534 | 0.579 | 0.577 | 0.983 | 0.753 | 0.753 | 1.416 | 2.092 | 3.193 | 2.574 |
| 3. *R. trevelyanus* | 0.656 | 0.684 | — | 0.637 | 0.544 | 0.765 | 0.723 | 0.693 | 0.599 | 0.796 | 0.848 | 0.713 | 0.961 | 1.437 | 2.060 | 1.639 | 2.140 |
| 4. *R. drummondhayi* | 0.679 | 0.661 | 0.674 | — | 0.229 | 0.724 | 0.684 | 0.490 | 0.150 | 0.610 | 1.044 | 0.837 | 0.704 | 1.381 | 1.463 | 3.411 | 2.544 |
| 5. *Rhinophis* sp. 1 | 0.636 | 0.643 | 0.639 | 0.450 | — | 0.642 | 0.736 | 0.410 | 0.239 | 0.539 | 0.845 | 0.998 | 0.622 | 1.345 | 1.683 | 2.974 | 3.187 |
| 6. *R. oxyrhynchus* | 0.636 | 0.595 | 0.724 | 0.701 | 0.674 | — | 0.351 | 0.361 | 0.614 | 0.367 | 0.580 | 0.894 | 0.538 | 1.171 | 1.941 | 2.322 | 1.874 |
| 7. *R. dorsimaculatus* | 0.654 | 0.632 | 0.709 | 0.686 | 0.704 | 0.539 | — | 0.693 | 0.664 | 0.648 | 0.652 | 0.964 | 0.517 | 1.151 | 1.704 | 1.560 | 2.197 |
| 8. *Rhinophis* sp. 2 | 0.671 | 0.633 | 0.699 | 0.608 | 0.572 | 0.544 | 0.693 | — | 0.387 | 0.511 | 0.616 | 0.871 | 0.713 | 1.318 | 1.832 | 3.005 | 2.302 |
| 9. *R. blythii* | 0.652 | 0.630 | 0.662 | 0.368 | 0.461 | 0.665 | 0.682 | 0.557 | — | 0.535 | 0.786 | 0.762 | 0.735 | 1.308 | 1.552 | 3.452 | 2.478 |
| 10. *P. philippinus* | 0.622 | 0.655 | 0.737 | 0.664 | 0.637 | 0.550 | 0.683 | 0.626 | 0.636 | — | 0.864 | 0.967 | 0.565 | 1.357 | 1.681 | 3.248 | 1.986 |
| 11. *U. melanogaster* | 0.676 | 0.648 | 0.744 | 0.781 | 0.733 | 0.651 | 0.677 | 0.664 | 0.720 | 0.749 | — | 0.345 | 0.864 | 0.955 | 1.437 | 1.559 | 2.082 |
| 12. *U. phillipsi* | 0.760 | 0.776 | 0.707 | 0.735 | 0.774 | 0.756 | 0.771 | 0.748 | 0.716 | 0.778 | 0.535 | — | 1.079 | 1.012 | 1.273 | 1.619 | 2.302 |
| 13. *R. travancoricus* | 0.713 | 0.716 | 0.780 | 0.697 | 0.669 | 0.637 | 0.628 | 0.704 | 0.710 | 0.652 | 0.747 | 0.801 | — | 0.926 | 1.014 | 1.544 | 2.833 |
| 14. *U. phipsoni* | 0.850 | 0.852 | 0.862 | 0.843 | 0.835 | 0.816 | 0.809 | 0.839 | 0.835 | 0.851 | 0.766 | 0.783 | 0.766 | — | 0.441 | 0.887 | 2.130 |
| 15. *U. liura* | 0.921 | 0.923 | 0.928 | 0.859 | 0.881 | 0.915 | 0.889 | 0.903 | 0.874 | 0.896 | 0.857 | 0.838 | 0.792 | 0.590 | — | 0.850 | 2.920 |
| 16. *T. rhodogaster* | 0.966 | 0.968 | 0.895 | 0.967 | 0.953 | 0.942 | 0.877 | 0.963 | 0.972 | 0.977 | 0.875 | 0.887 | 0.883 | 0.760 | 0.755 | — | 3.248 |
| 17. *C. maculatus* | 0.961 | 0.946 | 0.932 | 0.939 | 0.953 | 0.908 | 0.925 | 0.933 | 0.940 | 0.921 | 0.917 | 0.934 | 0.961 | 0.925 | 0.965 | 0.976 | — |

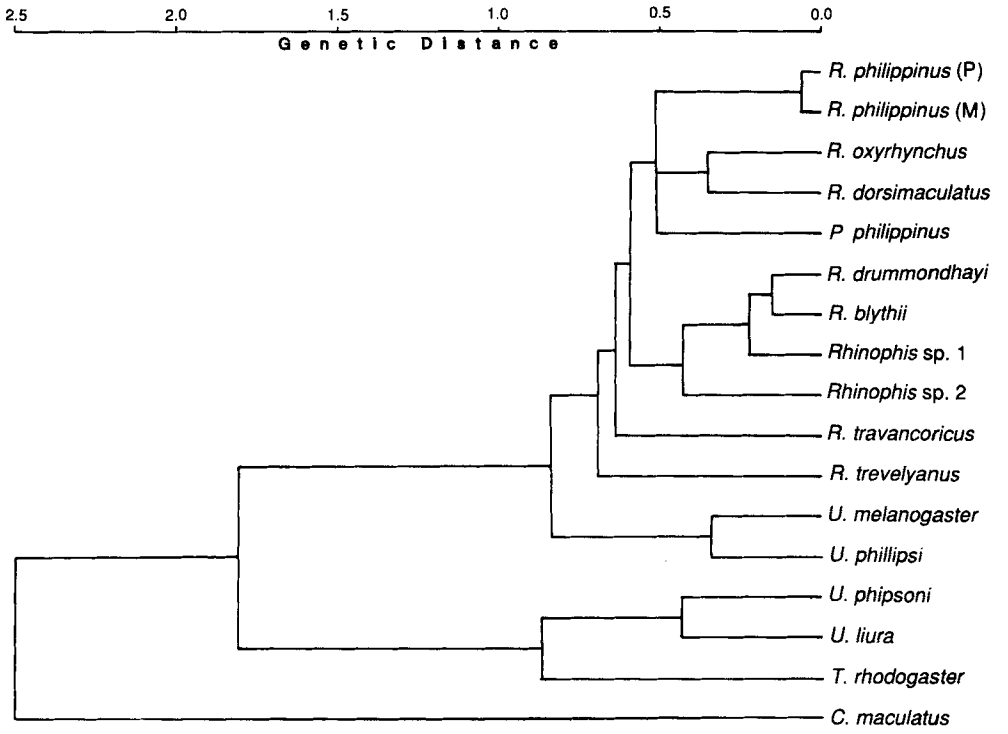


Figure 1. UPGMA phenogram clustering Nei's unbiased genetic distance ($D_m$) among 17 OTUs of uropeltids. The distances are based on the 26 loci scored for all OTUs.

from the next tree examined. The shortest trees included the Distance Wagner topology (Fig. 2; length = 284.4) and two others within one step on either side of that length. These other trees differed substantially in branching structure from the Distance Wagner tree and from each other (Fig. 3). Although one of the more trenchant differences among the trees is in the relative placement of the Sri Lankan uropeltines to those of India (monophyletic in the Distance Wagner topology, paraphyletic in the other two), there are numerous other differences as well. The few similarities among the trees include: (1) The two *Rhinophis philippinus* populations are monophyletic. (2) *R. blythii*, *R. drummondhayi*, and *Rhinophis* sp. 1 form a clade; in the Distance Wagner tree and one of the others (Fig. 3A), *Rhinophis* sp. 2 also joins this cluster. (3) *Teretrurus* and species of *Uropeltis* from India form a clade; in the Distance Wagner tree and Fig. 3A, Indian *Uropeltis* are monophyletic, whereas in Fig. 3B they are not. (4) *R. travancoricus* is not in the clade including other Indian uropeltines. (5) *Rhinophis* is paraphyletic if the Sri Lankan forms of *Uropeltis* and *Pseudotyphlops* are considered to belong to separate genera. (6) *Uropeltis* is not monophyletic if the Sri Lankan species *melanogaster* and *phillipsi* are placed in that genus.

### *Microcomplement fixation*

Our rate tests (Table 6) using *Leptotyphlops* as an outgroup indicate the conservative nature of *Boa* albumin (AID = 150) as compared to other

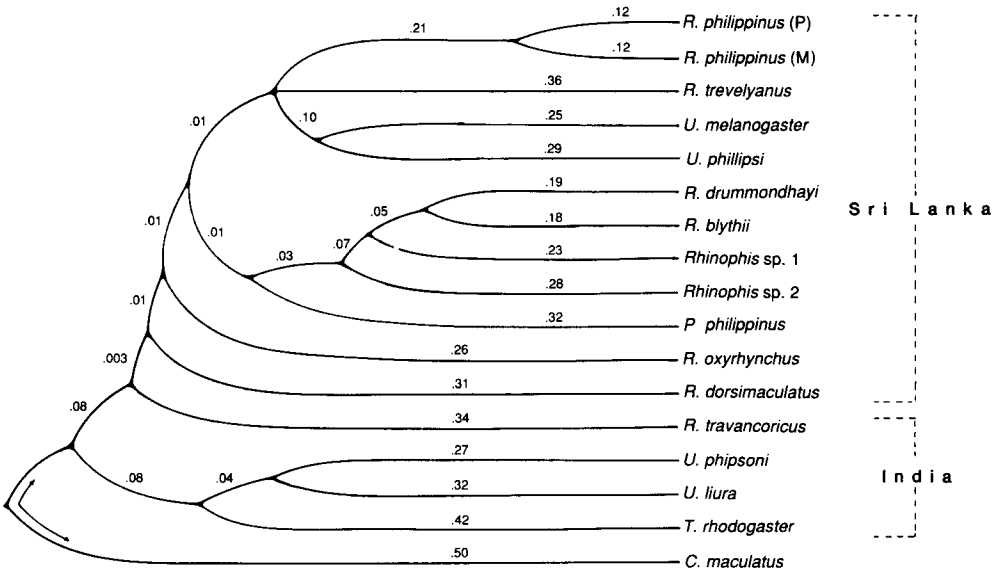


Figure 2. A Distance Wagner analysis of Rogers' genetic distance (as modified by Wright, 1978) among 17 OTUs of uropeltids. This is the optimized network using distances based on the 26 loci scored for all OTUs. The calculated length of each branch is indicated. The % standard deviation (Fitch & Margoliash, 1967) for the tree is 5.04; the cophenetic correlation is 0.955. A small negative branch (−0.001), not shown, connects *R. trevelyanus* to the branch leading to *R. philippinus*. Gene frequencies were also fitted to this topology using the FREQPARS algorithm and the resulting tree (length = 284.3) was the second shortest tree found by this method (see Fig. 3). Branch lengths and hypothetical allele frequencies for the FREQPARS tree are available from JEC upon request.

henophidians (AIDs > 170). This confirms earlier suspicions that such was the case, based on the broad cross-reactivity of antisera to *Boa* albumin with a wide variety of other snake albumins (Dessauer *et al.*, 1987; Cadle, 1988). Because of the conservative nature of *Boa* albumin, low distances bewtween *Boa* and other taxa is not necessarily indicative of close phylogenetic relationships (see Cadle, 1988, for discussion). However, this conservative property makes *Boa* albumin useful as an outgroup for examining rates of albumin evolution in other taxa. Using *Boa* as an outgroup to the uropeltids indicates that rates of albumin evolution among these species is reasonably homogeneous (mean AID = $102 \pm 0.7$; Table 7).

TABLE 6. Albumin immunological distances between *Leptotyphlops humilis* (Scolecophidia) and several henophidians. These distances are estimated using microcomplement fixation supplemented with enhanced Ouchterlony immunodiffusion tests (Dessauer *et al.*, 1987)

| Species tested | Anti-*Leptotyphlops* albumin |
|---|---|
| *Boa constrictor* | 150 |
| *Cylindrophis rufus* | ≥ 170 |
| *Anilius scytale* | ≥ 180 |
| *Xenopeltis unicolor* | ≥ 170 |
| *Loxocemus bicolor* | > 180 |

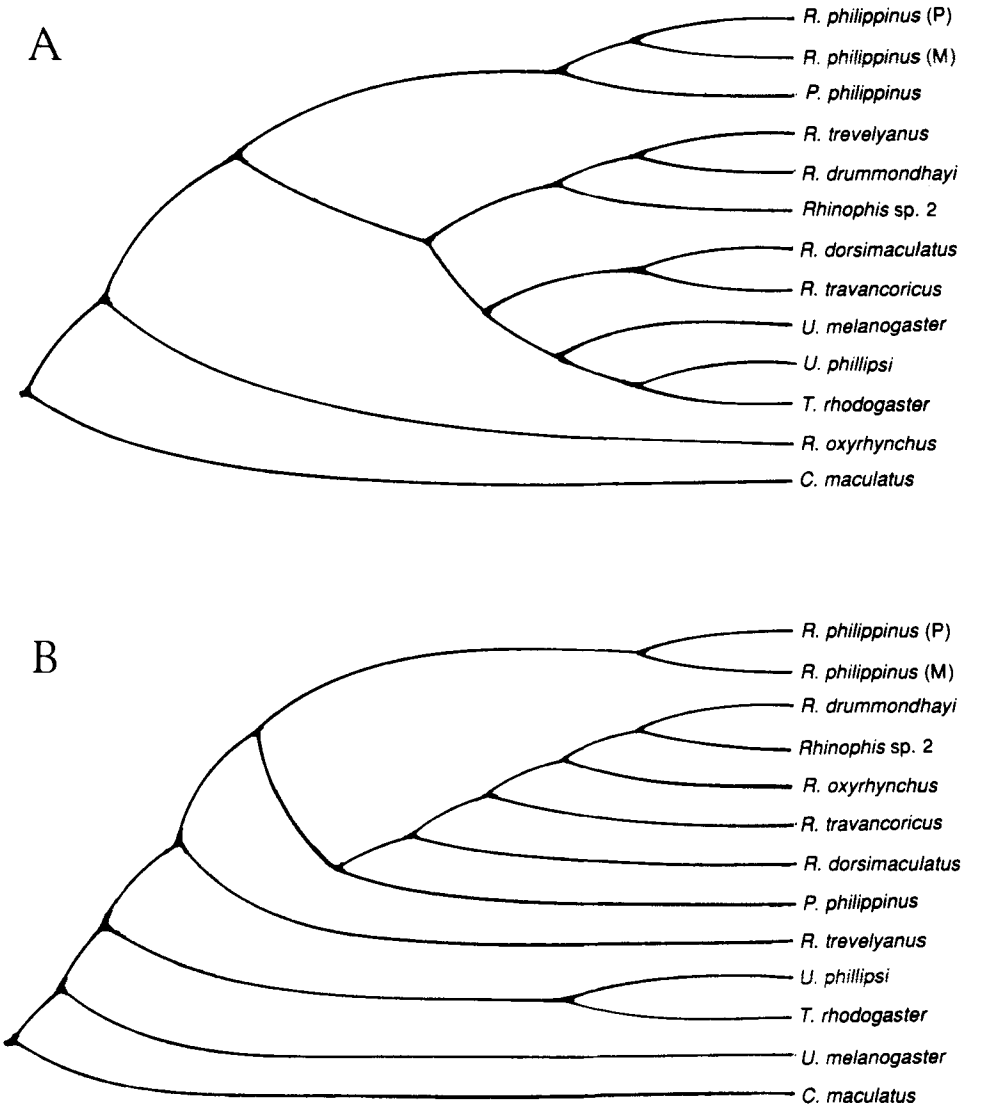


Figure 3. Two of three shortest trees evaluated by FREQPARS optimization of the allozyme data (the third was the Distance Wagner topology; see Fig. 2). These trees are simplified by not indicating the position of *R. blythii* and *Rhinophis* sp. 1, which cluster with *R. drummondhayi* in both trees, and *U. phipsoni* and *U. liura*, which cluster with *T. rhodogaster* in both trees. A, Length = 283.6. B, Length = 285.3. Specifications for each tree, including branch lengths and allele distributions, are available upon request from JEC. Note that these trees differ substantially from one another and from Fig. 2. The tree in Fig. 2 is most consistent with the immunological data (see text).

Table 7 summarizes results of MC'F comparisons of albumins of uropeltines and other henophidians. Reciprocity of those comparisons for which reciprocal measurements are available is reasonably good (% non-reciprocity (Sarich & Cronin, 1976) = 8.24). Immunological distances between the albumins of *Rhinophis philippinus* and those of other Sri Lankan species of uropeltines, including *Pseudotyphlops* and *Uropeltis*, are small, ranging from 8 to 18 (mean = $12 \pm 1.3$). In contrast, immunological distances to species from India are

TABLE 7. Albumin immunological distances involving uropeltid albumins. Antisera are to albumins of *Rhinophis philippinus*, *Cylindrophis rufus*, *Anilius scytale*, and *Boa constrictor*

| Species tested | Antisera to albumins of | | | |
|---|---|---|---|---|
| | *Rhinophis* | *Cylindrophis* | *Anilius* | *Boa* |
| UROPELTINAE (SRI LANKA) | | | | |
| *Rhinophis philippinus* | 0 | 64 | 128 | 103 |
| *R. dorsimaculatus* | 8 | 61 | — | 114 |
| *R. drummondhayi* | 13 | 46 | — | 102 |
| *R.* sp. 2 | 17 | 56 | — | 98 |
| *R. oxyrhynchus* | 10 | 58 | — | 113 |
| *R. blythii* | 10 | 54 | — | — |
| *R. trevelyanus* | 18 | 58 | — | 92 |
| *Pseudotyphlops philippinus* | 10 | 72 | — | 112 |
| *Uropeltis melanogaster* | 8 | 60 | — | 105 |
| *U. phillipsi* | 15 | 63 | — | 116 |
| UROPELTINAE (INDIA) | | | | |
| *U. liura* | 33 | 44 | — | 96 |
| *U. ceylanicus* | 40 | 48 | — | 100 |
| *U. phipsoni* | 26 | 48 | — | — |
| *Rhinophis travancoricus* | 20 | 55 | — | — |
| *Teretrurus rhodogaster* | 54 | 83 | — | — |
| CYLINDROPHINAE | | | | |
| *Cylindrophis rufus* | 78 | 0 | 121 | 88 |
| *C. maculatus* | 88 | 9 | 123 | *c.* 91 |
| OTHER "HENOPHIDIA" | | | | |
| *Anilius scytale* | 135* | 139 | 0 | 93 |
| *Loxocemus bicolor* | 130* | 111 | 133 | 162 |
| *Xenopeltis unicolor* | 144* | 100 | 136 | 130 |
| *Boa constrictor* | 90 | 92 | 126 | 0 |

*Because of the large distances involved in these comparisons and the relatively low titre of the pooled antisera to *Rhinophis* albumin, these distances were determined using the individual antiserum of highest titre.

uniformly higher (range 20–54, mean = 34 ± 5.9). Using only an antiserum to a single species of *Rhinophis* it is not possible to estimate phylogenetic relationships among the Sri Lankan species because distances are uniformly low. However, these data are consistent with the interpretation that some species of *Rhinophis* from Sri Lanka are no more closely related to *R. philippinus* than are *Pseudoptyphlops* and Sri Lankan species of *Uropeltis*, as indicated by our allozyme data. Furthermore, *R. travancoricus* of India shows the lowest immunological distance to *R. philippinus*, in agreement with our allozyme data indicating a phyletic association between *R. travancoricus* and the Sri Lankan radiation (Fig. 2). We interpret the relatively greater albumin distances between *R. philippinus* and the *Teretrurus*/*Uropeltis* from India as indicative of a closer phyletic relationship among the species from Sri Lanka than to these species; we cannot evaluate relationships among Indian species with these data. Although *Teretrurus* is immunologically more distant from *R. philippinus* than are the other Indian uropeltines, the comparisons to *Cylindrophis* (AID = 83) suggest that its albumin is more changed than that of other Indian uropeltines (mean AID = 49 ± 2.3). This suggests that *Teretrurus* may not be more removed phylogenetically from the Sri Lankan clade than are other Indian species, as

would be indicated by a purely phenetic interpretation of the distances (see Cadle, 1988 for discussion of rate tests).

By reciprocal measures and the unidirectional immunological distances between *Cylindrophis rufus* and other species of henophidians, *Cylindrophis* is the closest henophidian to the uropeltines among those tested (mean = $58 \pm 2.6$ using unidirectional comparisons only; mean = $71 \pm 7$ using the reciprocal values between *R. philippinus* and *C. rufus*). Because the rate tests (Tables 6 and 7) suggest that neither *Cylindrophis* nor uropeltine albumins are conservative, the relatively low distances between them is indicative of their close phylogenetic relationship. The immunological distance between the two species of *Cylindrophis* (AID = 9) is about the same magnitude as those separating Sri Lankan species of uropeltines, despite the range disjunction and rather strong morphological differentiation (Williams, 1959) between the two species.

No other henophidians tested are especially close to the uropeltids immunologically (immunological distances between uropeltids and *Boa* are low because of the conservative albumin of the latter). Interpretations of the large distances (Table 7) are made difficult by two factors. First, most comparisons involving *Anilius*, *Loxocemus* and *Xenopeltis* are at the upper limit of resolving power of the MC'F technique. Second, the large distances make it difficult to use outgroups to perform relative rate tests, which are necessary for the phylogenetic interpretation of immunological data (Cadle, 1988; Dessauer *et al.*, 1987). The most appropriate outgroup with which to perform rate tests for these taxa is the Scolecophidia, but these comparisons are rendered very difficult because of the distances involved (Table 6). Lacking a well-corroborated phylogeny of henophidian taxa (itself a paraphyletic group; see, e.g. Groombridge, 1979) makes other possible outgroup comparisons difficult to justify at the present time. However, we have seen no indications in our immunological data thus far that *Anilius*, *Loxocemus* or *Xenopeltis* is especially divergent with regard to its rate of albumin evolution. Thus, we interpret these data as indicating no particular phylogenetic association between uropeltids and other 'anilioid' taxa (*Anilius*, *Loxocemus*, *Xenopeltis*). That is, a clade of anilioid taxa is not suggested by our data, other than the uropeltine-*Cylindrophis* association already mentioned. Some aspects of our data, such as the relatively low immunological distances between *Cylindrophis* and *Loxocemus* and *Xenopeltis* (Table 7) require further investigation once we have a more comprehensive understanding of albumin evolution in henophidians. The great albumin immunological distances involved here suggest that other methods may be more appropriate for developing a molecular phylogeny for these ancient lineages.

We specifically reject the hypothesis that uropeltines are more closely related to the Scolecophidia than to any henophidian lineages (Dowling & Duellman, 1978; Dowling, 1988; see Table 1). As already indicated, our data suggest a sister-group relationship between uropeltines and *Cylindrophis*, as corroborated by numerous morphological features. We did not attempt MC'F comparisons to uropeltines using the antiserum to *Leptotyphlops* albumin because antigen samples were in short supply. However, numerous enhanced Ouchterlony immunodiffusion tests (Dessauer *et al.*, 1987) comparing henophidian albumins suggest that the MC'F immunological distances between *Leptotyphlops* and uropeltines are greater than 150. Thus, we consider *Cylindrophis* and uropeltines

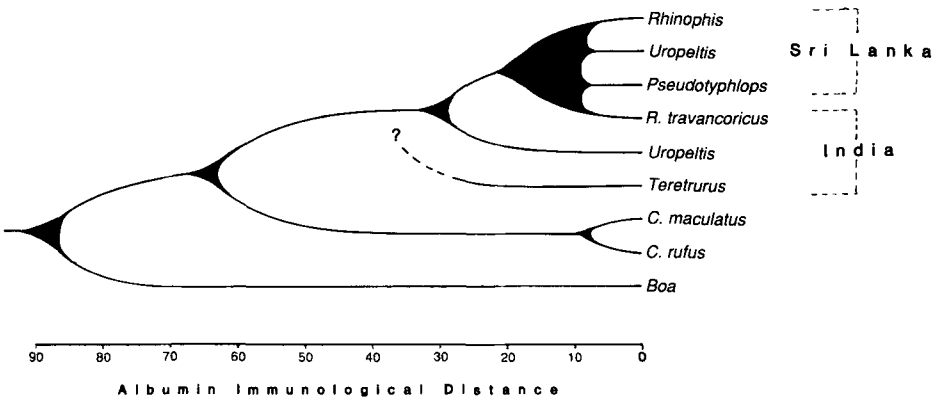


Figure 4. A phylogenetic tree expressing relationships among uropeltids estimated by MC'F comparisons of albumins. As few reciprocal comparisons are available, the tree is left unresolved in detail and branch lengths are not estimated. The scale indicates albumin immunological distance relative to the three taxa for which we have antisera (*Rhinophis philippinus*, *Cylindrophis rufus*, and *Boa constrictor*). The relative rate tests in Table 7 were used to interpret major patterns of branching. The position of *Teretrurus* is left unresolved, as we lack an antiserum to any Indian species and cannot determine whether there is a clade comprising *Teretrurus* and *Uropeltis* from India, or whether *Teretrurus* diverged earlier from the common uropeltine lineage (see text).

as sister taxa, but their relationships to other henophidian taxa unresolved on the basis of present immunological data.

Our phylogenetic conclusions derived from the immunological data are summarized as a branching diagram (Fig. 4). In the absence of reciprocal immunological comparisons, we rely on the rate tests for relative placement of taxa. We restrict our interpretations here to the pattern of relationships within uropeltids, as this is the aspect of the data in which we have the most confidence. Our interpretation of these data is thus consistent with inferences from the phenetic and phylogenetic analyses of the allozyme data (Figs 1, 2) in recognizing a clade including *Rhinophis travancoricus* and all Sri Lankan species as a monophyletic group, in suggesting the early divergences among the Indian forms, and indicating the close relationship between the two species of *Cylindrophis*.

## DISCUSSION

### *Phylogenetic conclusions*

*Uropeltine relationships.* No one has ever seriously questioned the monophyly of the uropeltines, as they share numerous synapomorphies of skeletal structure and soft anatomy (reviews in Gans, 1976 and Rieppel, 1977, 1978, 1979a,b). Our data confirm this hypothesis and demonstrate a great degree of molecular differentiation between the uropeltines and their sister group, *Cylindrophis*. Indeed, the genetic differentiation among species of uropeltines as indicated by both allozymes and albumin immunology suggests an old radiation with extant forms being the result of speciation events extending into the remote past. Under this view some of the morphological peculiarities of uropeltines as a group must be seen as specializations that arose very early in the history of the lineage and

have since undergone little anagenetic change. In this context, it would be most interesting to obtain data bearing on the phylogenetic position of *Melanophidium*, *Platyplectrurus*, *Plectrurus* and *Brachyophidium*, as these are restricted to India where the most ancient divergences among uropeltines possibly are (see below).

Analysis of the allozyme data using FREQPARS resulted in three more or less equally parsimonious trees (Figs 2, 3), but they differ substantially in branching order from one another. Although rigid adherence to the parsimony criterion would result in choosing the tree of Fig. 3A as the best supported (shortest) tree, we do not feel that this is necessarily justified by consideration of the albumin immunological data (Table 7 and Fig. 4), which are more in agreement with the topology of the Distance Wagner tree shown in Fig. 2. Optimization of this tree by the FREQPARS algorithm results in a length only 0·7 steps shorter than the most parsimonious tree examined. If we use the trees in Fig. 3 as predictions concerning albumin evolution in uropeltids, then the albumins of *Uropeltis melanogaster* or *Rhinophis oxyrhynchus* should be very divergent from all other uropeltines. This prediction is not substantiated. Table 7 shows that both of these species are very close to *Rhinophis philippinus* (AIDS 10 and 8), and that neither has especially divergent albumins when compared to the outgroups, *Cylindrophis* and *Boa*. Thus, these rate-controlled immunological data are inconsistent with both of the trees in Fig. 3, but are consistent with the tree in Fig. 2. We therefore consider the tree in Fig. 2 as the best-supported by these criteria, even though slightly longer than the most parsimonious topology discovered. Concerning broad patterns of uropeltine phylogeny this tree (Fig. 2) suggests the monophyly of the Sri Lankan uropeltines, but a more ancient series of cladogenetic events among Indian species.

Details of relationships among species of uropeltines are not fully resolved by these data, as the immunological data involve too few reciprocal comparisons and the allozyme data are equivocal in several respects. However, several clusters of species common to all of the analyses of the allozyme data (Figs 2, 3), enumerated above, reflect strongly-supported components by these data. One such cluster involves *R. blythii–R. drummondhayi–Rhinophis* sp. 1–*Rhinophis* sp. 2 (Figs 2, 3A), with perhaps *R. trevelyanus* also joining this group (Fig. 3B). A clade comprising *Teretrurus* and Indian species of *Uropeltis* is seen in all trees derived from the allozyme data (Figs 1–3). Both the allozyme and immunological data support the inclusion of *R. travancoricus* (from India) in the clade comprising all of the Sri Lankan uropeltines (Figs 2, 4). Otherwise, the trees differ in the relative placement of uropeltine species.

The pattern of relationships among uropeltines may be summarized as follows. Our data do not indicate a monophyletic clade of Indian species, but rather, they suggest a series of speciation events giving rise to the present diversity in India and a clade which subsequently gave rise to all Sri Lankan species. Among the taxa that we examined, *Rhinophis travancoricus* is the sister taxon to the Sri Lankan radiation. We emphasize that this pattern could be modified upon examination of the other species of Sri Lankan uropeltines, but these comprise only three species of *Rhinophis* and one of *Uropeltis*. All species of these genera from Sri Lanka appear closely related by both allozyme and immunological criteria. A more complex relationship, not evaluated in this study, may also result once the genera endemic to India can be incorporated into the phylogeny. This may not change our inference that the Sri Lankan uropeltines are a clade,

but may result in an expanded view of that clade, and will certainly bear on interpretations of the evolution and biogeography of the Indian forms.

*Comments on the Anilioidea.* The immunological data presented here, unpublished MC'F comparisons, and extensive Ouchterlony immunodiffusion comparisons of snake albumins, do not indicate a clade comprising uropeltids (*sensu lato*) and other taxa often grouped with them as the Anilioidea or the Aniliidae (see Table 1), referred to hereafter as Anilioidea. This is especially true of *Anilius*, which is often viewed as the sister taxon to either *Cylindrophis* or *Cylindrophis*-uropeltines (e.g. Rieppel, 1977, 1979a,b; Groombridge, 1979; Hoffstetter & Rage, 1977). Rieppel (1977, 1979c), Groombridge (1979), and McDowell (1987) pointed out that many of the features characterizing the Anilioidea are primitive, rendering that taxon paraphyletic. Although we have not yet developed a comprehensive phylogeny of henophidians based on the immunological data, our present results lend no support to the concept of a clade comprising uropeltids (*sensu lato*) and any of the other anilioid taxa. Until the relationships of these taxa are further resolved, we suggest that biogeographic and evolutionary syntheses (e.g. Cracraft, 1974; Hoffstetter & Rage, 1977; Rage, 1981; Rieppel, 1979b) that are dependent on a monophyletic Anilioidea (or Aniliidae if uropeltids are considered the sister group to *Anilius*) are premature. Such resolution also will bear ultimately on the systematic placement of a large number of 'aniliid' fossil vertebrae known from North America, South America and Europe (Hoffstetter & Rage, 1977; Rage, 1981, 1987; Bailon, 1988).

## *Biogeography*

*Broader categories.* Our recognition of a clade comprising all Sri Lankan species of uropeltines, and the present range disjunction between the Sri Lankan species, *Cylindrophis maculatus*, and its congeners invite a consideration of the possible biogeographic history of these units. Many Indian species of uropeltines are upland species occurring in moist montane forests (Rajendran, 1985). On Sri Lanka, species of uropeltids occur along the eastern coast and others range into the central highlands, with *Cylindrophis maculatus* ranging across moist lowlands into the low mountains (Bachman, 1985; De Silva, 1980). Sri Lanka is a continental island separated from India by shallow water ( $< 100$ m) and a series of islands. Although its fauna has numerous endemics (Nussbaum & Gans, 1981; Gans & Fetcho, 1982), many of its species are also found in India (review in Darlington, 1957: 491). For a group of burrowers such as uropeltines it is perhaps not surprising that endemism is fairly high at the species level. Several faunal elements of Sri Lanka, including caecilians (Nussbaum & Gans, 1981), some lizards, birds and other snakes, show the pattern exhibited by *C. maculatus*, with Indomalayan rather than Indian affinities (Darlington, 1957).

Eustatic changes in sea level have resulted in direct connections between India and Sri Lanka intermittently since at least the Miocene (Jacob, 1949; Moore, 1960). Our phylogeny for uropeltines would suggest a single origin for the radiation on Sri Lanka. Although we cannot provide a rigorous time framework for this origin, the molecular differentiation between *Rhinophis travancoricus* (the sister species of the Sri Lankan radiation) and the Sri Lankan species is reasonably large ($D_m = 0.733 \pm 0.17$; AID = 20). If a calibration of the immunological data for colubrid albumins (Cadle, 1988) is also applicable to uropeltid

albumins, this suggests a minimum of 10–15 million years of separation. Significantly, *R. travancoricus* is a low- to mid-elevation species occurring in southern India (Rajendran, 1985). Thus, its distribution is such that a sister relationship between it and the Sri Lankan radiation is not unlikely.

Given the apparently numerous connections between India and Sri Lanka over the past few million years (and the caveat that several Indian species remain to be examined) one might have expected a more complex biogeographic relationship between the two areas. That we do not see one is perhaps one indication of the limited dispersal capabilities of these fossorial snakes. It may also reflect the situation that the eastern plains of south India are wide and relatively dry; certainly at present they lack uropeltid populations or rivers that might facilitate cross-oceanic transport by rafting. The pattern of relationships of the Indian species and their molecular differentiation from one another and from the Sri Lankan species is an indication that uropeltines has a long period of differentiation in India before their entry into Sri Lanka. If our hypothesis concerning the monophyly of the Sri Lankan uropeltines is correct, then it suggests that vicariance of a fauna generally distributed in southern India and Sri Lanka was not the mode of differentiation of the extant radiations in that area.

The disjunction of *Cylindrophis maculatus* from its Indomalayan relatives poses a somewhat different biogeographic pattern that could, nevertheless, be due to historical events similar to those isolating the uropeltines in Sri Lanka. Such a view would entail the assumption that sister taxa of *C. maculatus* on the Indian mainland have become extinct (perhaps as a result of the present aridity of south India just mentioned). As measured immunologically, the molecular differentiation between the two species of *Cylindrophis* (AID = 9) is only about half that between *Rhinophis travancoricus* (India) and the Sri Lankan uropeltines (AID = 20) (unpublished electrophoretic comparisons of *C. maculatus* and *C. rufus* show a similar disparity as compared to the differentiation between Sri Lankan uropeltines and *R. travancoricus*). Since our rate tests (Table 7) indicate no clear differences in rates of albumin evolution in *Cylindrophis* and the uropeltines, this suggests that the speciation of the two *Cylindrophis* occurred much later than the separation of the Sri Lankan clade of uropeltines from its mainland sister clade.

*Uropeltine radiation in Sri Lanka.* Accepting the tree in Fig. 2 as the best-supported hypothesis of uropeltine relationships suggests a general correspondence to their distributions on the islands. *Rhinophis oxyrhynchus* and *R. dorsimaculatus* (northern and western plains), and *Pseudotyphlops philippinus* (northern, western and southern plains up to about 400 m) are lowland species. The remaining species either occupy mid-levels mountains or the very highest ones. Thus, *R. philippinus* and *R. trevelyanus* occur at elevations between 400 and 800 m in a central belt of mountains, replacing each other in a zone from the Gammaduwa area south to above Balangoda. *Uropeltis phillipsi* is endemic to the mountains of the Gammaduwa area, whereas the locally parapatric *U. melanogaster* ranges from there to south of Kandy, with another mid-elevation population found far to the south. These mid-elevation species form a clade in Fig. 2. The high mountain species, *Rhinophis* sp. 1, *R. drummondhayi*, *R. blythii* and *Rhinophis* sp. 2 also form a natural group (Fig. 2) that occupies contiguous areas above 1000 m in the south-central mountain plateau.

If these relationships can be corroborated with other character sets, then it

suggests that on Sri Lanka at most there have been two radiations of uropeltines into montane biotopes. The relationships here postulated are broadly concordant with present distribution patterns of the species at mid- and high elevations. Further investigation may confirm the hypothesis suggested by the distance Wagner analysis (Fig. 2), and supported by FREQPARS optimization of this tree, that the lowland species, *Rhinophis dorsimaculatus*, *R. oxyrhynchus* and *Pseudotyphlops philippinus*, are early derivatives of the Sri Lankan clade. Since the Indian sister taxon to the Sri Lankan uropeltines (*R. travancoricus*) is also a lowland species, we hypothesize that the radiation of uropeltines on Sri Lanka was initially by occupation of the lowland biotopes and subsequent adaptation to montane environments.

### *Comments on taxonomy*

Our finding that neither *Rhinophis* (in the sense of Smith, 1943) nor *Uropeltis* is monophyletic with respect to other uropeltine genera (regardless of which tree of Figs 2, 3 is accepted) raises questions concerning the definition of genera within this group. Most genera are defined using a combination of external scale features and particularly the structure of the terminal shield (Smith, 1943). Although we do not suggest specific taxonomic rearrangements at this time, our data do indicate that such revision (which is now underway) is warranted.

### *Genetic variation and population biology*

Aspects of genetic structure and population biology of secretive species such as uropeltids are difficult to evaluate directly. A well-developed theoretical and empirical framework relating variation detected by electrophoresis within populations to genetic structure (e.g. Wright, 1978) allows us to make inferences concerning these aspects of their biology. The high levels of polymorphism within populations and heterozygosity within individuals (Table 3) are both often associated with large effective population sizes (Nei, 1975; Nei, 1983), and indicate that these species maintain relatively large, outbreeding populations. Similarly, the lack of any indication of heterozygote deficiencies and the tendency toward negative $F_{IS}$ values (Table 4) are suggestive of populations in which matings between related individuals are uncommon and in which population substructuring (deme formation) does not occur. We infer from these data that despite the apparent fragmentary distribution and small range size of many species of uropeltids (Rajendran, 1985; De Silva, 1980; personal observations) some populations can be large and outcrossing. Anecdotal observations are in agreement with this inference, in that at least some species are locally abundant and may be the most common snakes where they are found.

There are further evolutionary implications of the levels of genetic variation we detected in uropeltids. Their average to high levels of genetic variation is contrary to some expectations based on the 'niche-width variation hypothesis' (e.g. Nevo, 1978, 1982). According to this hypothesis, greater environmental heterogeneity should select for greater variation at allozyme loci, and conversely, greater environmental homogeneity should result in less genetic variability (reduced levels of polymorphism and heterozygosity). This hypothesis has been used to explain patterns of allozyme variability in some groups (e.g. McDonald

& Ayala, 1974; Nevo, 1982), but has received little credence from other studies (Gooch & Schopf, 1973; Mitter & Futuyma, 1979; Schnell & Selander, 1981; Patton, 1984; Futuyma & Peterson, 1985). We do not consider the theoretical and conceptual difficulties with the niche-variation hypothesis as it pertains to allozyme loci (reviewed in Lewontin, 1974; Soule, 1976; Kimura, 1983), but here concentrate on the empirical evidence for such an association in uropeltids, a group inhabiting subterranean environments which are often interpreted as prime examples of relative stability, predictability and simplicity (e.g. Nevo, 1982).

By either percentage polymorphism or heterozygosity estimates (Table 3), the three uropeltid populations for which we have adequate sample sizes show levels of genetic variation that are equivalent to or higher than estimates for a wide variety of other snakes (summarized in Dessauer *et al.*, 1987). Even other species of uropeltids for which our sample sizes are small apparently have relatively high levels of genetic variation (see Results). Thus, we do not see in uropeltids the expected decrease in variation that would be predicted by the niche-width variation hypothesis. We echo earlier comments (Dessauer *et al.*, 1987) that any observed relationship between levels of genetic diversity and ecological diversity is very tenuous, and suggest that most empirical evidence favours aspects of population genetic structure and history as major determinants of the genetic variation reflected in allozyme polymorphisms.

### *Molecular evolution*

The general correspondence between measures of genetic divergence using MC'F comparisons of single proteins and those based on multilocus genetic distance estimates using electrophoresis is well known (Sarich & Cronin, 1976; Sarich, 1977; Maxson & Maxson, 1979; Wyles & Gorman, 1980). The correlation between the two measures and the slope of the relationship varies among taxonomic groups, and also depends on the proportion of 'rapidly-evolving' and more 'slowly-evolving' loci used in the electrophoretic studies (Sarich, 1977). For example, in plots of albumin immunological distances against Nei's standard genetic distance ($D$; Nei, 1972) the slopes of the regressions vary from about 22 in some salamanders and mammals (Maxson & Maxson, 1979; Wyles & Gorman, 1980) to about 38 in some mammals and reptiles (Sarich, 1977; Wyles & Gorman, 1980). No previous studies have investigated this relationship in any groups of snakes.

The relationship between albumin immunological distance and Nei's standard genetic distance for uropeltids is shown in Fig. 5. Considering only points in which $D \leq 2.2$, the slope of the line passing through the origin is 17.5 and the correlation between the two measures is highly significant ($r = 0.94$, $P < 0.01$). Maxson & Maxson (1979) noted that electrophoretic measures of divergence tend to asymptote at values of $D$ greater than about 2. We see this effect in our data for the two points with values of $D > 3$ (Fig. 5), and therefore do not include them in this discussion.

Our slope of 17.5 is lower than any previously reported slopes for the relationship between albumin immunological distance and electrophoretic genetic distance in other taxa (Sarich, 1977; Maxson & Maxson, 1979; Wyles & Gorman, 1980). Although a lower slope could be explained by inclusion of a

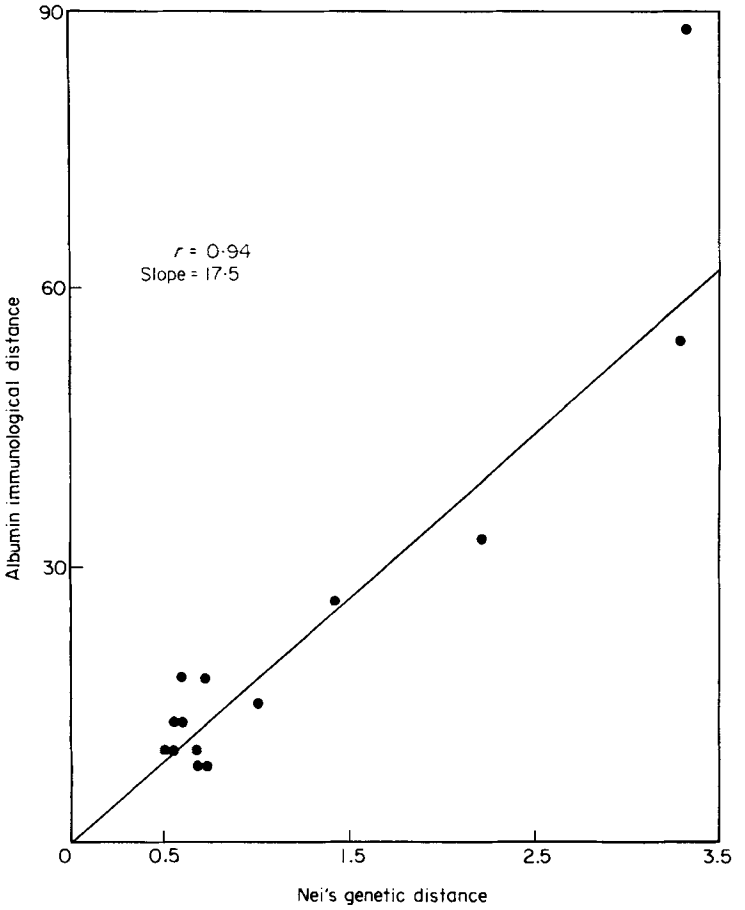


Figure 5. Microcomplement fixation immunological distances among albumins plotted against the Nei standard genetic distances ($D$) for species pairs of uropeltids, $r = 0.94$.

higher proportion of rapidly-evolving loci in our electrophoretic study (Sarich, 1977), thereby inflating estimates of $D$, this is unlikely to explain our results since only about 10% of the loci we included in our calculations were rapidly-evolving (these generally include secreted proteins, such as albumin and transferrin, as well as some enzymes such as esterases). Another possibility, which we are unable to evaluate with present data, is a bias toward low values in the MC'F distances, as shown for some primate comparisons (Cronin, Sarich & Ryder, 1984). We are inclined to attribute the lower slope in part to increased resolution of allelic differences in our electrophoretic study (hence yielding greater $D$ values) due to the use of the vertical gel method, long gel-running times (17 to 20 hours) and exhaustive testing of running conditions to discriminate maximally among taxa. These are not the standard conditions used in most electrophoretic surveys from which the $D$ values used by Sarich (1977) and subsequent workers were calculated.

The indication of rather high $D$ values for given AID values in uropeltids is also seen by expressing one in terms of the other. For uropeltids AID/D averages $19.8 \pm 0.4$. In two groups of colubrid snakes, natricines and colubrines, electro-

phoretic genetic distances average about 0.48 between Old and New World species (Dessauer *et al.*, 1987), and the corresponding albumin immunological distances generally range from 30–50 (Dowling *et al.*, 1983; Cadle, 1984; Cadle, unpublished data). For these colubrids, AID/D is on the order of 60–100, or three to five times the value for uropeltids. It should be noted that this latter value is minimally twice the expectation based on the original calibration of these two measures (Sarich, 1977). Thus, in comparison to other vertebrates, the relationship between protein evolution as measured electrophoretically and by albumin immunology in uropeltids indicates relatively great differentiation at electrophoretic loci, or, conversely, rather conservative albumin evolution. Our present data do not distinguish between these alternatives.

Because calculations of genetic distances from electrophoretic data are very dependent on the choice of loci examined (Sarich, 1977), and on the discriminatory power of the electrophoretic techniques used, we attribute part of our 'high' *D* values to these factors. However, there is now ample evidence that rates of albumin evolution differ among taxonomic groups and this possibility should not be discounted in the uropeltid case. Early work on birds (Prager *et al.*, 1974) and more recent investigations of turtles (Rainey, 1983) showed that the rate of change of their albumins is about one-third to one-fifth that of some mammalian albumins (e.g. ungulates and anthropoids). More detailed consideration of rates within mammals clearly shows rate variation among major groups. For example, phyllostomatoid bat albumins have changed at a rate about twice that for ungulate and anthropoid albumins (Cronin & Sarich, 1980; V. M. Sarich, personal communication), whereas carnivore albumins in general are slower (Sarich, 1985). Among snakes, some viperid albumins, especially among pit vipers, appear to change at a rate about 50% slower than that typical of colubrids (Dessauer *et al.*, 1987). Thus, we should expect the relationship between albumin immunological distances and Nei's genetic distances to vary among taxonomic groups. These considerations do not obviate the use of these data for dating evolutionary events, but they do argue for caution in the application of 'standard' calibrations for deriving divergence times from either genetic or immunological distances. We urge molecular workers to use rate tests and develop taxon-specific calibrations for these measures.

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## APPENDIX

Vouchers for specimens used in the electrophoretic and immunological studies. AL numbers are in the collection of Gans. HCD numbers are frozen tissue samples in the collection of Dessauer; voucher specimens are in the Gans collection. Other specimens are in the Carnegie Museum of Natural History, Pittsburgh, Pennsylvania (CM); Museum of Zoology, Louisiana State University, Baton Rouge, Louisiana (LSUMZ); Field Museum of Natural History, Chicago, Illinois (FMNH); Academy of Natural Sciences, Philadelphia (ANSP); and the Museum of Vertebrate Zoology, University of California, Berkeley, California (MVZ). Sample sizes are given in parentheses following the species names.

*Anilius scytale* (2) PERU: Depto. Cuzco, 4 km SW by road Pilcopata, 570 m elev. (MVZ 197123); SURINAM: locality unknown (LSUMZ 40625). *Boa constrictor* (1) MEXICO: Michoacán, Rio Tepalcatepeque at Capirio (MVZ 172374). *Cylindrophis maculatus* (1) SRI LANKA: Biyagama, Kelaniya (CM 93661). *Cylindrophis rufus* (1) Locality unknown (MVZ 176553). *Leptotyphlops humilis* (6) USA: California, San Diego Co., Sentenac Canyon (ANSP 31215–31220). *Loxocemus bicolor* (1) MEXICO: Oaxaca, 3 miles S Tehuantepec on road to Salinas Cruz (MVZ 143487). *Pseudotyphlops philippinus* (2) SRI LANKA: Dewatura lines below Namunukula (AL 210, AL 286). *Rhinophis blythii* (6) SRI LANKA: Talawakele (AL 226, AL 232, AL 241). *Rhinophis dorsimaculatus* (2) SRI LANKA: Marichchukkaddi, Murunga PO (AL 290, AL 291). *Rhinophis drummondhayi* (10) SRI LANKA: Pindarawatta, north of Namunukula (AL 076, AL 295, AL 296); SRI LANKA: Pingarawa estate, below Namunukula (AL

196). *Rhinophis oxyrhynchus* (2) SRI LANKA: Polonnaruwa (AL 601). *Rhinophis philippinus* (12) SRI LANKA: Matalapitiya, 3.5 mi WNW Nikakotua (AL 497, AL 521). *Rhinophis philippinus* (39) SRI LANKA: Palatenne (Opalgalla group), below Pride's Gap (AL 533, AL 458, AL 517, AL 280, AL 473, AL 519, AL 455). *Rhinophis travancoricus* (1) INDIA: Ambadi Rubber Plant, near Pechiparai Dam, Kanyakumari District, Tamil Nadu (HCD 5875). *Rhinophis trevelyanus* (3) SRI LANKA: Gampola (Illawatura) (AL 024, AL 301, AL 604). *Rhinophis* sp. 1 (2) SRI LANKA: Harasbedda division (Liddesdale group) (AL 214). *Rhinophis* sp. 2 (2) SRI LANKA: Bibilegama road, N of Namunukula (AL 100, AL 200). *Teretrurus rhodogaster* (2) INDIA: Mandjolai (AL 094, AL 095). *Uropeltis ceylanicus* (1) INDIA: Kerala State, Trivandrum District, Ponmudi (FMNH 217698). *Uropeltis liura* (2) INDIA: Mandjolai, Tamil Nadu (AL 091, AL 095). *Uropeltis melanogaster* (3) SRI LANKA: Kandy area (AL 233); Nicapota, near Lemastota (AL 181); Lemastota (AL 164). *Uropeltis phipsoni* (1) INDIA: small mountain road, Reman, opposite Taylor LIT, Tamil Nadu. (HCD 5876). *Uropeltis phillipsi* (3) SRI LANKA: Gammaduwa lines (AL 251, AL 479). *Xenopeltis unicolor* (1) received from Bangkok, Thailand (LSUMZ 35262).