

Genetic and taxonomic relations of the short-tailed snakes, genus *Stilosoma*¹

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(With 2 figures in the text)

Both morphological and immunological investigations show that *Stilosoma* is related to the American kingsnakes (*Lampropeltis*) and their allies. Immunological tests show an unexpectedly close relationship, indicating its derivation in the Pliocene.

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Introduction

The addition of biochemical data to standard morphological techniques has provided new—and sometimes very different—insights into snake phylogeny (Dowling *et al.*, 1983; Dessauer, Cadle & Lawson, 1987). In particular, the immunological technique of micro-complement fixation (MCF) is highly sensitive to sequence changes in proteins and provides a quantitative scale for evaluating molecular evolutionary change (Maxson & Maxson, 1986). Because of the time-related (stochastic) differentiation of macromolecules within phyletic lines, this method also allows the depiction of a phylogenetic tree with both cladistic and temporal information (Wilson, Carlson & White, 1977).

Immunological distance data allow us to compare the rates of morphological and molecular evolution in related groups of animals, and thus are especially valuable in identifying members of a phyletic line that have experienced rapid morphological change. Such taxa are characterized by a

¹ This paper is Number VI in Dowling's 'Classification of the Serpentes' series

wide morphological gap from their apparent relatives, but display only small immunological distances from them. In our previous studies using MCF analyses of snake albumins, we found three examples of such morphologically divergent taxa among Nearctic snakes (Dowling *et al.*, 1983).

The three examples included the watersnake genus *Nerodia*, which proved to be more closely related (immunologically) to the *elegans-sirtalis* cluster of *Thamnophis* than did the congeneric ribbonsnakes, *T. proximus* and *T. sauritus*. Thus, *Nerodia* is seen as an aquatic specialization of the generalized terrestrial members of *Thamnophis*, making this latter genus 'paraphyletic' in immunological relationships.

A second example was the specialized semifossorial ratsnake *Pituophis*, which, when tested immunologically with *Elaphe obsoleta* antiserum, proved to have evolved somewhere between the divergence times of *E. vulpina* and *E. guttata*. *Pituophis* appears to be derived from one of the generalized terrestrial/arboreal ratsnakes (*Elaphe*), with morphological and behavioural adaptations to pursue and efficiently kill pocket gophers (*Geomys*) in their underground burrows.

The third example involved the specialized ovivorous genus *Cemophora*, whose genetic relations appeared to fall between *Lampropeltis getulus* and *L. triangulum* on the one hand, and *L. calligaster* and *L. mexicanum* on the other. *Cemophora coccinea*, morphologically the most specialized of the three, is a semi-fossorial lizard- and snake-egg eating specialist that is derived from a generalized, basically terrestrial stock of kingsnakes (*Lampropeltis*).

All of these specialized forms appear to have evolved, during Pliocene time or more recently, from their generalized ancestral stocks that date from the mid-Miocene or earlier (Dowling *et al.*, 1983). That these sudden divergences in habits and morphology make their ancestral ('sister') genera paraphyletic should not be surprising, inasmuch as paraphyly appears to be the norm in observable evolutionary history. As stated by Carroll (1988: 13-14), 'The existence of paraphyletic groups is an inevitable result of the process of evolution' and 'Approximately half the recognized taxonomic groups, ranging from species to classes, are paraphyletic.' Thus, in spite of the objections of cladists, species with unusual habits, morphology and physiology will continue to emerge from the ranks of more conservative taxa. They will continue to be recognized taxonomically in order to preserve the classification of (morphologically) definable entities.

We present additional MCF data on the genetic relationships of another morphologically unique and little-known genus of snakes, the American short-tailed snakes (*Stilosoma*). The recent species of this genus, *S. extenuatum*, has a small geographic range in central and northern peninsular Florida which is typical of such sand-dwelling 'Florida Island' species as *Rhineura* and *Neoseps*. Auffenberg (1963) found, '... among North American colubrid snakes, *Stilosoma* appears unique in vertebral form', but the most recent summary of its features (Highton, 1976) gave no further information upon its relationships.

Materials and methods

The morphological data utilized have been obtained from the literature, from the files of Herpetological Information Search Systems (HISS), New York, and from the examination of living, preserved, and especially-prepared specimens (e.g. hemipenes, skeletons). Living representatives of all genera discussed here have been examined (by HGD).

The immunological distance (ID) data were obtained through use of the quantitative MCF technique (Champion *et al.*, 1974; Maxson & Maxson, 1986). Four of the albumin antisera and their preparation have been described earlier (Dowling *et al.*, 1983) and the fifth, to *Coluber constrictor*, was prepared by

M. Hutchinson (at the Academy of Natural Sciences, Philadelphia) according to a similar protocol. Blood samples were available for all species compared to the 5 antisera.

Voucher specimens of all snakes (other than the individuals of *Stilosoma*, which were released at their original localities after sampling) are preserved in the HISS collections or the National Museum of Natural History, Washington. All ultimately will be deposited in the latter institution. Additional data on the specimens will be found in the **Appendix**. Acronyms used for museums are those suggested by Leviton *et al.* (1985).

Albumin ID provides a quantitative estimate of albumin sequence evolution, with each unit of ID representing approximately 1 amino acid difference between the albumins compared (Maxson & Maxson, 1986). It is estimated that 1.7 ID units accumulate for every million years that the 2 compared species have been separated (Wilson *et al.*, 1977; Carlson, Wilson & Maxson, 1978).

Figure 2 was prepared by a modification of our previous methods. We demonstrated (Dowling *et al.*, 1983) that 2 major clades among Nearctic colubrine snakes may be identified as racers, *s.l.* (*Coluber*, *Masticophis* and *Ophedrys*) and as ratsnakes, *s.l.* (*Elaphe* and relatives). We also showed that the latter can be further divided into ratsnakes, *s.s.* (Elaphini: *Arizona*, *Elaphe* and *Pituophis*) and kingsnakes, *s.s.* (*Lampropeltis* and *Cemophora*). All of these colubrines are adequately distinguished from natricines (e.g. *Thamnophis*) and other subfamilies of the Colubridae.

We suggest that if each of these is a real entity (a true clade), then any comparison between a member of one clade with a representative of another clade represents an estimate of the time of the single speciation event that separated those clades. Thus, the different ID measurements between members of these clades represent 3 possible variables: differences in molecular evolutionary rates in some species, differences due to experimental

TABLE I
Albumin immunological distances between selected species of
colubrid snakes

Sera	Antisera				
	TS	CO	MF	EO	LG
Natricinae					
<i>Thamnophis sirtalis</i> (TS)	0	61	58	74	—
Colubrinae: Racers					
<i>Coluber constrictor</i> (CO)	67	0	9	47	32
<i>Masticophis flagellum</i> (MF)	71	13	0	46	43
Colubrinae: Ratsnakes					
Elaphins					
<i>Elaphe obsoleta</i> (EO)	54	38	32	0	34
<i>Pituophis melanoleucus</i> (PM)	—	—	32	8	20
<i>Arizona elegans</i> (AE)	—	—	20	9	26
Lampropeltiins					
<i>Lampropeltis getulus</i> (LG)	62	32	31	23	0
<i>L. triangulum</i> (LT)	—	—	28	15	1
<i>Stilosoma extenuatum</i> (SE)	—	—	21	18	6
<i>Cemophora coccinea</i> (CC)	—	—	22	24	9
<i>Rhinocheilus lecontei</i> (RL)	—	—	23	23	29 ^a
<i>Lampropeltis mexicanum</i> (LM)	—	—	21	23	11
<i>L. calligaster</i> (LC)	—	—	18	19	11

Some of these data appeared in Dowling *et al.* (1983).

^a This single ID comparison suggests that *Rhinocheilus* may be a member of a different clade, and is omitted from the preparation of Fig. 2.