Biological Journal of the Linnean Society (1998), *63:* 177–203. With 4 figures

Molecular phylogeny of elapid snakes and a consideration of their biogeographic history

J. SCOTT KEOGH[1]

School of Biological Sciences A08, University of Sydney, Sydney, NSW 2006, Australia

Received 27 February 1997; accepted for publication 28 August 1997

Evolutionary relationships among the major elapid clades, particularly the taxonomic position of the partially aquatic sea kraits (*Laticauda*) and the fully aquatic true sea snakes have been the subject of much debate. To discriminate among existing phylogenetic and biogeographic hypotheses, portions of both the 16S rRNA and cytochrome *b* mitochondrial DNA genes were sequenced from 16 genera and 17 species representing all major elapid snake clades from throughout the world and two non-elapid outgroups. This sequence data yielded 181 informative sites under parsimony. Parsimony analyses of the separate data sets produced trees of broad agreement although less well supported than the single most parsimonious tree resulting from the combined analyses. These results support the following hypotheses: (1) the Afro-Asian cobra radiation forms one or more sister groups to other elapids, (2) American and Asian coral snakes form a clade, corroborating morphological studies, (3) *Bungarus* forms a sister group to the hydrophiines comprised of *Laticauda*, terrestrial Australo-Papuan elapids and true sea snakes, (4) *Laticauda* and true sea snakes do not form a monophyletic group but instead each group shares an independent history with terrestrial Australo-Papuan elapids, corroborating previous studies, (5) a lineage of Melanesian elapids forms the sister group to *Laticauda*, terrestrial Australian species and true sea snakes. In agreement with previous morphologically based studies, the sequence data suggests that *Bungarus* and *Laticauda* represent transitional clades between the elapine 'palatine erectors' and hydrophiine 'palatine draggers'. Both intra and inter-clade genetic distances are considerable, implying that each of the major radiations have had long independent histories. I suggest an African, Asian, or Afro-Asian origin for elapids as a group, with independent Asian origins for American coral snakes and the hydrophiines.

ADDITIONAL KEY WORDS:—mitochondrial DNA – cytochrome *b* – 16S rRNA – biogeography – evolution – phylogenetic systematics – reptile – snake – elapid.

CONTENTS

[1] Current address: Division of Botany and Zoology, Australian National University, Canberra, ACT 0200, Australia.

0024–4066/98/020177+27 $25.00/0/bj970178

INTRODUCTION

Snakes rank among the most successful of extant vertebrate radiations both in terms of species number (approximately 2750) and geographic distribution (Cadle, 1987). Unfortunately, inferring evolutionary relationships both within and between snake lineages is often difficult because snakes are structurally homogeneous. Many of the morphological adaptations of snakes are the result of reduction and simplification of structures found in their saurian ancestors (Bellairs & Underwood, 1951; Underwood, 1967; Cadle, 1994). This simplification has translated into high levels of parallelisms in many aspects of snake morphology, and the resultant homoplasy makes it difficult to confidently polarize characters (Underwood, 1967; Rabb & Marx, 1973; Cadle, 1982, 1988, 1994). For these same reasons, it is often difficult to identify appropriate outgroups in snakes. As these types of problems plague numerous groups of organisms, many workers have turned to molecular techniques to elucidate relationships among difficult groups. For example, recent years have seen a series of phylogenetic studies on snakes based on diverse molecular data sets including immunological distance (e.g. Schwaner *et al.*, 1985; Cadle, 1988, 1994), allozyme electrophoresis (e.g. Mengden, 1985; Dessauer, Cadle & Lawson, 1987) and DNA sequences (e.g. Knight & Mindell, 1993, 1994; de Queiroz & Lawson, 1994; Forstner, Davis & Arévalo, 1995; Heise *et al.*, 1995; Wüster *et al.*, 1995; Lopez & Maxson, 1996; Kraus, Mink & Brown, 1996).

In this paper I examine higher level relationships within the elapid snakes. The elapid clade is of considerable interest as (i) it displays great morphological diversity and a wide geographic distribution, (ii) there has been substantial disagreement concerning relationships among elapid clades, and (iii) the consequent lack of higher level phylogenetic resolution has impeded our understanding of the biogeographic history of these animals. My goals were twofold: (i) to test among contradictory hypotheses of higher level elapid relationships by inferring relationships among key taxa through phylogenetic analyses of mitochondrial DNA sequences; and (ii) based on these results, to discriminate among various biogeographic hypotheses.

Introduction to elapid snakes

Elapid snakes are members of the Infraorder Caenophidia or 'advanced snakes', a diverse assemblage containing more than 80% of the world's snake species. The Caenophidia is comprised of four major groups; the primarily non-venomous

TABLE 1. Taxonomic summary of the major elapid snake radiations and their distributions. Key species were chosen to represent each group in this study

Elapid Group	Genera/Species	Species sampled	Distribution
Elapines			
Cobras	10/37	4	Africa, Middle East, Asia
American coral snakes	2/61	1	North, Central, and South America
Asian coral snakes	2/16	1	Asia
Terrestrial kraits	1/12	1	Asia
Hydrophiines			
Melanesian elapids	7/17	3	New Guinea, Solomon Islands, Fiji
Australian elapids	20/88	4	Australia, New Guinea (some)
Sea kraits	1/5	1	Asia
True sea snakes	16/57	2	Equatorial waters around the world but most in SE Asia and the Australo-Papuan region.

colubrids, the venomous atractaspids, and two independently evolved venomous groups, the Viperidae (for vipers, rattlesnakes, and their allies) and the elapids (for coral snakes, cobras, sea snakes and their allies). Elapids are variously placed in the family Elapidae or families Elapidae and Hydrophiidae (see below), but this taxonomic distinction is irrelevant for the purposes of this paper as the monophyly of this lineage is not in question (McCarthy, 1985). Elapids are defined primarily by the unique presence of two permanently erect canaliculate front fangs, termed the proteroglyphous condition (McCarthy, 1985). Elapids number approximately 300 species in 61 genera, and are distributed across much of the tropical and subtropical world including the Americas, Africa, Asia, Melanesia, Australia, and the Indian and Pacific oceans (Golay, 1985; Golay *et al.*, 1993). Elapids are comprised of a number of distinct putatively monophyletic lineages, with a very uneven spread of species and generic level diversity among lineages and geographic regions (Table 1). Of the terrestrial groups, the Australian species are most diverse at both the generic and specific level (Mengden, 1983; Hutchinson, 1990). The African elapids are intermediate in diversity, comprising mostly cobras, with allies which range through tropical and sub-tropical Asia. The Melanesian elapids inhabiting New Guinea, the Solomon Islands and Fiji also are intermediate in diversity and highly endemic. In comparison, the American coral snake radiation is much less diverse at the generic level with only two closely related genera, but highly diverse at the species level with 60 *Micrurus* species and one *Micruroides* species (Slowinski, 1995). The continental Asian elapid radiation is diverse with cobras (*Naja* and *Ophiophagus*), Asian coral snakes (*Calliophis* and *Maticora*), as well as the terrestrial kraits (*Bungarus*) and sea kraits (*Laticauda*). The sea snakes are comprised of at least two groups: (1) *Laticauda*, which spend much of their life at sea but come on land to lay their eggs, and like terrestrial snakes have fully developed ventral scales, and (2) the viviparous and fully aquatic hydrophiid or 'true' sea snakes, which have many morphological adaptations to a fully marine life.

As a convenient abbreviation I will refer to the Australian and Melanesian terrestrial elapids as the Australo-Papuan radiation, and these species together with the true sea snakes and *Laticauda* as the hydrophiines. The endemic African elapids and their close Asian cobra relatives (*Naja*, *Ophiophagus* and *Walterinnesia*) will be referred to as the cobras, and these species together with *Bungarus* and the American and Asian coral snakes as the elapines. These groupings are discussed below.

Relationships among elapid snakes

The morphological data

Much of the morphological work designed to clarify higher-level phylogenetic relationships of elapids has been concerned with elucidating sea snake relationships. Boulenger's (1896) division between terrestrial elapid species on the one hand and true sea snakes and *Laticauda* on the other (resulting in the widely accepted Families Elapidae and Hydrophiidae respectively) remained popular for many years (Smith, 1926; Romer, 1956; Dowling 1959, 1967, 1974; Underwood, 1967). However, McDowell's (1967, 1968, 1969a, b, 1970, 1972, 1986) detailed morphological studies suggested that the split between terrestrial and ocean-going species does not represent the most basal split among elapids. McDowell (1970) identified a major subdivision in elapid snakes based on "the kinesis of the palatine bone and the structural peculiarities of that bone associated with kinesis". In McDowell's 'palatine erector' group, which includes all terrestrial elapids occurring in the New World, Africa, Asia, as well as *Laticauda* and the Bougainville Island *Parapistocalamus*, "the palatine is erected along with the maxilla during maximum protraction of the palate" (McDowell, 1970: 147). In contrast, in the members of McDowell's 'palatine dragger' group (which includes all terrestrial Australo-Papuan elapids and true sea snakes), "the palatine functions as an anterior extension of the pterygoid and remains horizontal, even when the maxilla is highly erectile" (McDowell, 1970:148). McDowell (1970) suggested that *Laticauda* is more closely allied with Asian and American coral snakes and *Parapistocalamus* than with true sea snakes, and that true sea snakes were derived from within the terrestrial Australo-Papuan elapid radiation.

In their influential classification, Smith, Smith & Sawin (1977) relied heavily on McDowell's results and erected a Tribe Laticaudini for *Laticauda* within the Family Elapidae. Smith *et al.* (1977) relegated the entire terrestrial Australo-Papuan elapid radiation to a new subfamily Oxyuraninae within the Family Hydrophiidae, while maintaining the subfamily Hydrophiinae for the true sea snakes. The separation of *Laticauda* from true sea snakes has been well accepted in other recent classification schemes (e.g. Burger & Natsuno, 1974; Underwood, 1979). However, further intensive morphological studies on sea snakes and their relatives were inconclusive. Voris (1977) suggested that *Laticauda* either evolved independently from terrestrial elapids or was a very early offshoot of true sea snakes, while McCarthy (1986) supported a closer association between *Laticauda*, true sea snakes and the terrestrial Australian species. No morphological studies have re-examined the close relationship between *Laticauda* and Asian coral snakes suggested by McDowell (1970). Relationships within the Afro-Asian cobra radiation received cursory attention since Bogert's (1943) work, but recent analyses by Wüster & Thorpe (1989, 1990, 1992a,b) have done much work to unravel the taxonomy of Asian *Naja*.

The molecular data

A considerable amount of molecular systematic work has considered relationships among elapid clades, but as with the morphological studies, much of the attention has centred on the affinities of sea snakes. A number of studies have concluded that true sea snakes and terrestrial Australian elapids share a close relationship (Minton & da Costa, 1975; Mao, Chen & Chang, 1977; Mao, Dessauer & Chen, 1978; Cadle & Gorman, 1981; Minton, 1981; Mao *et al.*, 1983; Schwaner *et al.*, 1985; Slowinski,

Knight & Rooney, 1997) supporting the studies of McDowell. However, contrary to McDowell, Mao and colleagues in a series of molecular studies on transferrin immunological distance (Mao *et al.*, 1977), peptide fingerprinting of haemoglobins (Mao *et al.*, 1978), and protein albumin immunological distance (Mao *et al.*, 1983; Guo, Mao & Yin, 1987), concluded that *Laticauda* and true sea snakes form a 'natural group'. Mao *et al.*, (1983) implied a natural grouping of all sea snakes plus terrestrial Australo-Papuan elapids. Minton (1981) and Schwaner *et al.* (1985) also suggested this higher level grouping, though noting a distant relationship between *Laticauda* and true sea snakes, and stated that these sea snake groups had probably evolved independently from terrestrial Australian stock. Similarly, Cadle & Gorman (1981) suggested that the terrestrial Australian elapids were close to both *Laticauda* and true sea snakes based on immunological data, and that all of these lineages were distant to other elapid groups.

The recent venom protein sequence study of Slowinski *et al.* (1997) clearly unites *Laticauda* with the terrestrial Australian elapids and sea snakes to the exclusion of African, Asian and American elapids, although *Laticauda* and true sea snakes did not emerge as a monophyletic lineage in their analyses. Their phylogenetic analyses result in two sister clades, an elapine lineage comprised of Afro-Asian species including eight *Naja* species plus *Aspidelaps scutatus*, *Hemachatus haemachatus*, and *Bungarus*, and a hydrophiine clade comprised of four terrestrial Australian species, four *Laticauda* species and the true sea snakes *Aipysurus laevis* and *Enhydrina schistosa*. Based on their results, Slowinski *et al.* (1997) transferred *Laticauda* to the subfamily Hydrophiinae of Smith *et al.* (1977). Thus, virtually all molecular work on these taxa has come to the conclusion that *Laticauda* and true sea snakes share a closer relationship with terrestrial Australo-Papuan species than with other elapid groups, but that they have evolved independently from within this group. Further, neither Cadle & Gorman (1981), Cadle & Sarich (1981), Mao *et al.* (1983) nor Slowinski *et al.* (1997) supported the close relationship between *Laticauda* and New World coral snakes suggested by McDowell (1970).

Hence, although McDowell's basal split between the palatine erector elapine lineage and the palatine dragger hydrophiine lineage seems well supported, there is still no strong resolution of higher level relationships among elapid clades. This lack of resolution has confounded understanding the biogeographic history behind the virtually world-wide tropical and subtropical distribution of elapids. While present-day elapids are primarily distributed on Gondwanan elements, most authors have supported the hypothesis that elapids are of Old World origin with dispersal to the New World via the Bering Land Strait (Hoffstetter, 1939; Bogert, 1943; Darlington, 1957; Underwood, 1967; Cadle & Sarich, 1981) and Australia via south-east Asia after the Miocene collision of the Australian and Asian tectonic plates (Tyler, 1979; Cogger & Heatwole, 1981; Schwaner *et al.*, 1985; Dessauer *et al.*, 1987; Cadle 1987, 1988). However, no previous studies have included the taxa necessary to test these biogeographic hypotheses.

MATERIAL AND METHODS

Selection of representative taxa

Portions of the cytochrome *b* and 16S rRNA mitochondrial genes were sequenced from 26 individuals representing at least six putative monophyletic elapid clades, 16

TABLE 2. Taxonomic sampling used in this study. Each individual was sequenced for portions of both the 16S rRNA and cytochrome *b* mitochondrial genes. Two individuals were sequenced when tissue availability permitted to test for mis-identifications and pseudogenes. However, only a single individual was used in the phylogenetic analyses presented (number '1' as indicated) because substitution of the second individual results did not change results. Museum acronyms are as follows: AM - Australian Museum, LSUMZ - Louisiana State University Museum of Natural Science (USA), PEM - Port Elizabeth Museum (South Africa), SAM - South Australian Museum, NTM - Northern Territory Museum of Arts and Sciences (Australia)

Taxon	Museum tissue #	Voucher #	Locality
Cobras:			
Hemachatus haemachatus	LSUMZ H-2732	PEM R88890	Summerstrand, Port Elizabeth, Cape Province, South Africa
Naja melanoleuca	LSUMZ H-8630		Africa
Naja naja	SAM CM47		Sri Lanka
Walterinnesia aegyptia	LSUMZ H-2731		Sinai, Egypt
American coral snake:			
Micrurus fulvius	LSUMZ H-7353		Tampa vicinity, Hillsborough County, Florida, USA
Asian coral snake:			
Maticora bivirgata	LSUMZ H-6523		Bangkok vicinity, Thailand
Terrestrial krait:			
Bungarus fasciatus	LSUMZ H-4845		Zoo captive - Thailand
Melanesian Elapids:			
Loveridgelaps elapoides	AM NO #		
Micropechis ikaheka (1)	SAM 11800		Mt. Menawa, West Sepik Province, Papua New Guinea
Micropechis ikaheka (2)	SAM 40306		
Salomonelaps par	AM NO #		
Australian Elapids:			
Acanthophis antarcticus (1)	SAM S99	NTM 17880	S Alligator River Floodplains, NT, Australia
Acanthophis antarcticus (2)	SAM T01	NTM 17881	S Alligator River Floodplains, NT, Australia
Demansia atra (1)	SAM I73		Jabiru air strip, NT, Australia
Demansia atra (2)	SAM 29954	SAM 29954	Near Humpty Doo, NT, Australia
Pseudechis porphyriacus (1)	SAM 25056	SAM 25056	5 km E Tungkillo, Harrison Creek, SA, Australia
Pseudechis porphyriacus (2)	SAM 25297	SAM 25297	5 km E Tungkillo, Harrison Creek, SA, Australia
Tropidechis carinatus	SAM 30596	SAM 30596	
Sea Kraits:			
Laticauda colubrina (1)	SAM 4795	124795	Nagada Harbour (ocean), Papua New Guinea
Laticauda colubrina (2)	SAM 4800	AM 124800	Nagada Harbour (ocean), Papua New Guinea
True Sea Snakes:			
Aipysurus laevis (1)	SAM C010	NTM 17775	Cartier Islet, Sahul Banks, WA, Australia
Aipysurus laevis (2)	SAM C011	NTM 17776	Cartier Islet, Sahul Banks, WA, Australia
Hydrelaps darwiniensis (1)	SAM 018	NTM 16471	Home Creek, Bing Bong Station, NT, Australia
Hydrelaps darwiniensis (2)	SAM S63		Dinah Beach, NT, Australia
Outgroups:			
Morelia viridis (python)	SAM 13080		Wau, Papua New Guinea
Boiga irregularis (colubrid)	SAM Bi84		NT, Australia

elapid genera, 17 elapid species, and two non-elapid outgroups (Table 2). Taxa were sampled from representatives of all major elapid radiations from around the world, but my samples did not include the problematical African *Homoroselaps* and *Atractaspis* whose affinities to elapid snakes have been the subject of continual debate (McDowell, 1968, 1986, 1987; McCarthy, 1985; Underwood & Kochva, 1993; Cadle, 1982, 1988, 1994; Zaher, 1994). Instead I concentrate on those species that are clearly elapids, and the relationships among these taxa.

The terrestrial krait (*Bungarus*), sea krait (*Laticauda*), American coral snake (*Micrurus*), and Asian coral snake (*Maticora*) radiations are each morphologically cohesive and

monophyly of each of these genera is well established (Leviton, 1964; McCarthy, 1986; Slowinski, 1989, 1994a, 1995) so single species were used to represent each clade. Monophyly has been assumed but not well established for other elapid radiations so multiple representative species were chosen carefully to provide a wide sampling of putative clades. Australian elapids are morphologically diverse, as evidenced in the high number of genera (Hutchinson, 1990). Several lines of evidence suggest that Australian elapids comprise at least two major groups, oviparous species comprised of approximately half the Australian species and a viviparous lineage comprised of the other half (Mengden, 1985; Shine, 1985; Wallach, 1985). I have included representatives from both groups (*Pseudechis porphyriacus* and *Tropidechis carinatus*). In addition, I also have included representatives of *Acanthophis* and *Demansia*, two highly derived Australian elapid genera whose relationships to other Australian elapids have been problematical (Keogh, 1997, 1998). Australian death adders (*Acanthophis*) are highly morphologically distinct and convergent upon the Viperidae in their morphology and ecology (Shine, 1980a), clouding understanding of their affinities, while the Australian whip snakes (*Demansia*) are immunologically distinct from other elapids (Cadle & Gorman, 1981; Mao *et al.*, 1983; Schwaner *et al.*, 1985). Melanesian elapids are thought to be part of an Australo-Papuan elapid radiation based on morphology (McDowell, 1967, 1970) and immunological distance data (Schwaner *et al.*, 1985). However, while retaining Melanesian elapids in the Oxyuraninae with the Australian terrestrial elapids and true sea snakes, Wallach & Jones (1992) relegated Melanesian elapids to their own tribe, implying monophyly. I included representatives of three Melanesian genera to test this hypothesis: the Solomon Island *Loveridgelaps elapoides* and *Salomonelaps par* and the New Guinea *Micropechis ikaheka*. I also included taxa from two true sea snake species, *Hydrelaps darwiniensis* and *Aipysurus laevis*. These species were included because they are morphologically distinct from other true sea snakes (McDowell, 1972; Gopalakrishnakone & Kochva, 1990), and the latter is also immunologically distinct (Cadle & Gorman, 1981). Monophyly of Afro-Asian cobras has been questioned by Cadle (1987) based on unpublished biochemical data, so representatives were chosen from three genera including both African and Asian *Naja*.

Non-elapid outgroups were sought at two levels. The colubrid *Boiga irregularis* was used to represent a non-elapid member of the 'advanced snake' or Caenophidian radiation to which the elapids belong, and the python *Morelia viridis* was used to represent an ancestor to the advanced snakes (Heise *et al.*, 1995).

DNA extraction, amplification, and sequencing

Total cellular DNA was obtained from stored frozen (−80°C) or ethanol preserved liver tissues (except for *Salomonelaps par* for which only blood was available) via salt extraction. Double-stranded portions of both the cytochrome *b* and 16S rRNA mitochondrial genes (290 and 490 base pairs respectively, not including primers) were amplified with standard 50 μL polymerase chain reactions (PCR) with the following conditions and primers (1 μL template DNA, 1 unit *Taq* polymerase, 4 mM $MgCl_2$, 5.75 μL 10 X reaction buffer, 1.0 mM dNTPs, 0.25 μM primers [cytochrome *b* – (L) 5′-AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA-3′, (H) 5′-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3′; 16S rRNA - (L) 5′-CGC CTG TTT ATC AAA AAC AT-3′, (H) 5′-CCG GTC TGA ACT CAG ATC ACG T-3′ – universal primers from Kocher *et al.*, 1989]). PCR amplification was

carried out on a Corbett Research FTS-320 Thermal Cycler and consisted of 1 cycle of 94°C for 1 min, 48°C for 1 min and 72°C for 1 min, then 34 cycles of 94°C for 1 min, 48°C for 45 sec and 72°C for 1 min. The program ended with a single step of 72°C for 6 min and then 26°C for 10 sec. PCR product was purified with BresaClean (Bresatec Ltd.), after which both complimentary strands were cycle sequenced for each gene on a Corbett FTS-1 Thermal Sequencer using ABI PRISM (Perkin Elmer) and the same primers as above. Sequencing product was run on an automated Applied Biosystems Model 373A Sequencing System. Sequences will be deposited in GENBANK upon publication.

Testing for pseudogenes

Pseudogenes or nuclear paralogues, non-functional copies of mitochondrial DNA in the nuclear genome, have been discovered in a number of vertebrate and invertebrate species (i.e. Lopez *et al.*, 1994; Arctander, 1995; Collura & Stewart, 1995). Pseudogenes typically display much higher substitution rates due to the loss of functional constraint, confounding homology assessment, and their undetected presence among true mitochondrial sequences can contribute to the generation of incorrect phylogenies (Zhang & Hewitt, 1996). Obtaining purified mitochondrial DNA from all individuals used in this study was impractical, however, I tested for pseudogenes in two ways. Purified mitochondrial DNA was obtained for the elapid *Naja naja* via caesium chloride centrifugation, amplified and sequenced for both cytochrome *b* and 16S rRNA, and then compared to sequences obtained via amplifications from salt extracted total cellular DNA. When tissue sample availability allowed, I also sequenced both genes for two individuals of some species (*Micropechis ikaheka*, *Acanthophis antarcticus*, *Demansia atra*, *Pseudechis australis*, *Aipysurus laevis*, *Hydrelaps darwiniensis*, and *Laticauda colubrina*). The examination of intra-specific variation allowed me to simultaneously check for sample mix-ups and PCR contamination, as well as providing a further test for the presence of pseudogenes.

Phylogenetic analyses

Sequences were aligned by eye after initial alignments were made on a restricted number of sequences with the computer program CLUSTAL V (Higgins, Bleasby & Fuchs, 1991). The 16S rRNA data set contains a hyper-variable region ranging in length from 15 to 35 base pairs. This region was unalignable across all taxa and thus excluded from analyses because site homology could not be confidently ascertained. The resulting data matrices were analysed by maximum parsimony (MP) methods. Given the higher rate of substitution for cytochrome *b* and the conservative nature of 16S rRNA variation, the colubrid *Boiga irregularis* was used as the outgroup for cytochrome *b* analyses and the python *Morelia viridis* was used as the outgroup for 16S rRNA analyses. To maintain consistency in the combined analyses, a single outgroup taxon was created whereby *B. irregularis* and *M. viridis* sequences represented the outgroup condition for the cytochrome *b* and 16S rRNA data sets respectively. Because intraspecific variation was negligible, a single individual was used as a representative (marked as '1' in Table 2) for those species where multiple individuals were sequenced.

MP analyses were implemented with the computer program PAUP 3.1.1 (Swofford, 1993). MP analyses on the individual cytochrome *b* and 16S rRNA data sets were implemented with all variable sites weighted equally and with successive approximations (Farris, 1969) based on the rescaled-consistency index as recommended by Horovitz & Meyer (1995). To examine the effect of transition to transversion weighting schemes on tree topology, further analyses were run with transitions down-weighted relative to transversions by a factor of two to compensate for the observed transitional bias. Transitional bias in each data set was estimated in two ways: (i) by mapping the relative number of unambiguous transitions and transversions onto trees generated from the unweighted analysis with the computer program MacClade 3.04 (Maddison & Maddison, 1992), and (ii) by calculating the range and means of pairwise TI/TV ratios with the computer program MEGA 1.01 (Kumar, Tamura & Nei, 1993). The above phylogenetic analyses also were conducted on a combined data set comprised of both genes. While the debate continues about which types of data are appropriate to combine (de Queiroz, Donoghue & Kim, 1995; Huelsenbeck, Bull & Cunningham, 1996), a combined analysis of these mitochondrial genes is appropriate as sequences from both genes were obtained from the same individuals, mitochondrial genes do not recombine, and the mitochondrial genome acts as a single hereditary unit. Further, Sullivan (1996) found that combining data sets from different mitochondrial genes was highly beneficial in terms of phylogenetic resolution and robust to differing rates. Because of the large number of taxa and consequent large number of possible trees, heuristic searches were used for all MP analyses and replicated 30 times with the random-stepwise-addition and tree-bisection-reconnection branch swapping options of PAUP to increase the chance of finding globally rather than locally most parsimonious trees (Maddison, 1991).

The amount of phylogenetic information in the data sets was estimated with the consistency index (CI, Kluge & Farris, 1969), bootstrap replicates (Felsenstein, 1985), and the g_1 statistic (Hillis, 1991; Hillis & Huelsenbeck, 1992). Relative branch support in each phylogenetic analysis was evaluated with 300 bootstrap pseudoreplicates with 10 random-stepwise-addition searches for each replicate. The g_1 statistic is a measure of non-random phylogenetic structure in the data set and was estimated by examining the tree length distribution of 10 000 randomly generated parsimony trees using PAUP's random trees function. In evaluating the results of these analyses, I regard bootstrap values of >70% as strong evidence of branch support (Hillis & Bull, 1993). However, I take the corroboration of results between data types (i.e. morphology, karyology, allozymes, immunological distance, protein and DNA sequences, etc.) as the strongest evidence of relationship.

RESULTS

Pseudogenes

Both light and heavy strands of cytochrome *b* and 16S rRNA *Naja naja* sequences amplified from purified mitochondrial DNA and total cellular DNA were identical, providing no evidence of the presence of nuclear pseudogenes in *Naja naja*. In those species where two individuals were sequenced, intraspecific cytochrome *b* and 16S rRNA variation was negligible except for the cytochrome *b* sequences obtained from

the sea snake *Hydrelaps darwiniensis* (13.57%). These sequences, as well as the *Aipysurus laevis* cytochrome *b* sequences, displayed particularly high genetic distances from other elapid clades (34.94–43.06% and 22.33–34.94%), from other elapids respectively, much greater than between any other elapid clade. Indeed, maximum cytochrome *b* genetic distances were found between these sea snakes and other elapids rather than between the sea snakes and either the python or colubrid outgroup. These sequences almost certainly represent mitochondrial pseudogenes (Zhang & Hewitt, 1996). Thus, they were excluded from the analyses presented below.

Variation and phylogenetic information

The cytochrome *b* data set comprised 290 aligned sites of which 144 were variable and 110 potentially informative under parsimony after exclusion of *Morelia*, *Aipysurus*, and *Hydrelaps*. After exclusion of the hyper-variable region, the 16S rRNA data set comprised 453 aligned sites of which 120 were variable and 71 potentially informative under parsimony. Jukes & Cantor (1969) intra and inter-clade genetic distances are presented in Table 3. These DNA sequence data contain a significant amount of phylogenetic signal. The distributions of 10 000 randomly generated trees from each of the cytochrome *b*, 16S rRNA, and combined data sets were left-skewed indicating strong phylogenetic signal in the data (Hillis, 1991; Hillis & Huelsenbeck, 1992): cytochrome *b* $g_1 = -0.798$ ($P < 0.01$), 16S rRNA $g_1 = -0.285$ ($P < 0.01$), combined $g_1 = -0.509$ ($P < 0.01$). As expected, cytochrome *b* third codon positions were more variable than first and second positions, however third positions contain phylogenetic signal as evidenced by a significant g_1 statistic calculated for 10 000 random trees generated from third codon positions only ($g_1 = 0.241$, $P < 0.01$). Further, consistency indices generated from the phylogenetic analyses exceeded the 95% confidence limits for random data calculated by Klassen, Moor & Locke (1991).

Some transitional bias was evident in both the cytochrome *b* and 16S rRNA data sets. Pairwise TI/TV ratios for the cytochrome *b* data set ranged from 1.27 (between *Maticora* and *Loveridgelaps*) – 5.86 (between *Acanthophis* and *Pseudechis*) with a mean of 2.30. Pairwise TI/TV ratios for the 16S rRNA data set ranged from 0.73 (between *Walterinnesia* and *Loveridgelaps*) to 9.50 (between *Tropidechis* and *Hydrelaps*) with a mean of 1.95. When the data sets were mapped onto their respective single most parsimonious trees generated from the unweighted analyses of the individual data sets (Figs 2A and 3A – see below) the cytochrome *b* data set was comprised of 252 unambiguous transitions and 116 unambiguous transversions (TI/TV = 2.17) while the 16S rRNA data set was comprised of 139 unambiguous transitions and 73 unambiguous transversion (TI/TV = 1.90). Both estimates of transitional bias are consistent in that they identify TI/TV ratios for both the cytochrome *b* and 16S rRNA data sets of approximately two, thus this TI/TV ratio was used in the weighting schemes below.

Phylogenetic analyses

MP analyses of the unweighted cytochrome *b* data set produced a single most parsimonious tree (Fig. 1A; 498 steps, CI = 0.44), the topology of which did not

TABLE 3. Comparison of mean Jukes-Cantor (1969) genetic distances with and between putative elapid snake clades. Higher taxon names used correspond to those in Tables 1 and 2. 'Cobra' refers to the African elapids and their close Asian relatives, 'coral' refers to the American - Asian coral snake clade, 'Australian' refers to the Australian species, 'Melanesian' to the Papuan and Solomon Island species, and Australo-Papuan to the Australian and Melanesian species as a group. Comparisons between true sea snakes and other elapids could not be made with the cytochrome *b* data, see text for details. The N numbers correspond to the number of genetic distance comparisons between members of different clades

	Cytochrome b			16S rRNA		
	N	Range	Mean	N	Range	Mean
Intra-clade						
Cobra	6	14.82–17.82	16.39	6	4.38–5.40	4.81
Coral	1	—	21.40	1	—	5.67
Australian	6	16.52–20.95	18.80	6	3.34–8.26	5.51
Melanesian	3	19.15–24.69	21.60	3	6.23–6.69	6.30
Australo-Papuan	21	14.82–27.12	20.74	21	3.34–8.20	5.88
True sea snakes				1	—	4.67
Inter-clade						
Cobra - Coral	8	20.49–26.63	22.36	8	4.64–7.49	5.93
Cobra - Australian	16	18.26–29.13	22.15	16	4.90–8.81	6.59
Cobra - Melanesian	12	19.15–26.14	23.26	12	5.91–6.96	6.37
Cobra - Australo-Papuan	28	18.26–29.13	24.60	28	4.90–8.81	6.50
Cobra - *Laticauda*	4	21.40–28.12	24.37	4	5.67–8.03	7.24
Cobra - *Bungarus*	4	20.95–25.65	23.40	4	6.43–7.21	6.89
Cobra - True sea snakes				8	5.66–9.64	7.50
Coral - Australian	8	21.40–29.13	26.89	8	4.66–8.28	6.55
Coral - Melanesian	6	26.14–28.62	27.71	6	4.71–7.73	6.34
Coral - Australo-Papuan	14	21.40–29.13	26.88	14	4.66–8.28	6.46
Coral - *Laticauda*	2	26.14–27.12	26.63	2	6.72–8.57	7.64
Coral - *Bungarus*	2	29.13–30.15	29.64	2	5.90–7.75	6.83
Coral - True sea snakes				4	5.42–8.57	7.51
Australian - *Laticauda*	4	22.33–29.13	26.06	4	6.19–8.81	6.91
Australian - *Bungarus*	4	21.86–29.13	26.43	4	5.91–8.26	7.21
Australian - True sea snakes				8	2.41–7.49	4.89
Melanesian - *Laticauda*	3	22.79–28.62	25.05	3	7.32–7.75	7.52
Melanesian - *Bungarus*	3	24.69–27.62	25.82	3	6.95–8.26	7.37
Melanesian - True sea snakes				6	4.66–8.28	6.35
Laticauda - Australo-Papuan	7	22.37–29.13	25.62	7	6.19–8.81	7.17
Laticauda - Bungarus	1	—	27.62	1	—	7.23
Laticauda - True sea snakes				2	6.20–8.30	7.25
Bungarus - Australo-Papuan	7	21.86–29.13	26.17	7	5.91–8.26	7.37
Bungarus - True sea snakes				2	7.00–7.75	7.36

change after one or more rounds of successive approximations (CI = 0.73). African elapids form a series of paraphyletic sister clades to other elapids in the shortest tree although bootstrap values indicate little support for this resolution among African elapids or between African elapids and other elapid groups. A clade formed by the American-Asian coral snakes was strongly supported with a bootstrap value of 92%. A primarily hydrophiine clade comprised of Australo-Papuan terrestrial elapids and *Laticauda* was weakly supported with a bootstrap value of 55%. Within this group *Bungarus* and a clade comprised of *Laticauda* and the Solomon Island *Salomonelaps* formed sister groups to the rest of the Australo-Papuan elapids. When I weighted transitions relative to transversions by a factor of two, I obtained a single most

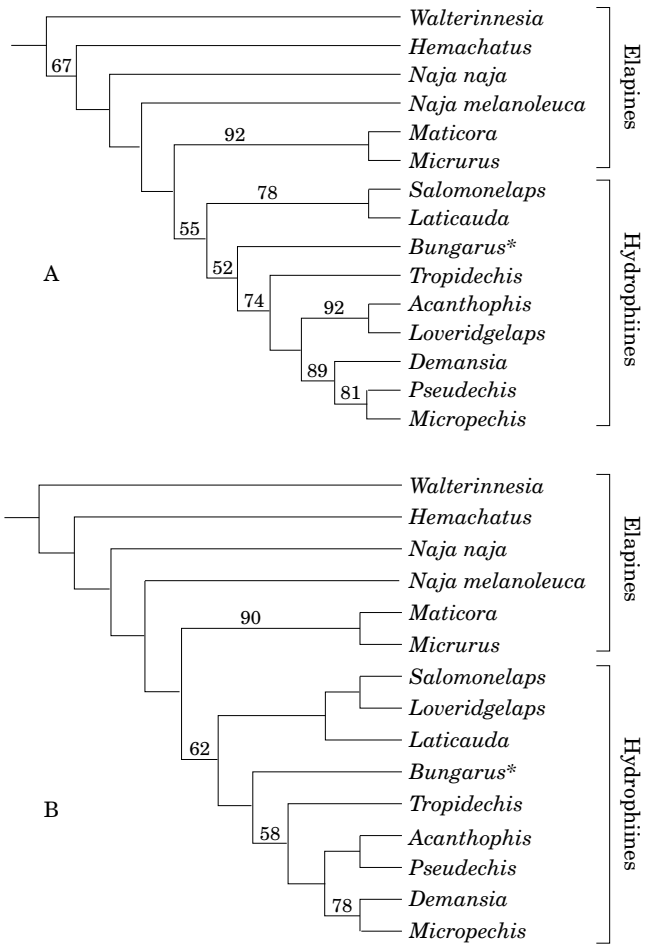

Figure 1. A, single most parsimonious tree generated from the unweighted analysis and successive approximations of the cytochrome *b* data. B, single most parsimonious tree generated with transitions down-weighted relative to transversions by a factor of two. Numbers represent bootstrap values from 300 replicates. The outgroup is not shown. *Bungarus* is formally classified with elapines, but the cytochrome *b* data set consistently placed *Bungarus* within hydrophiines, *contra* the 16S rRNA data set and numerous other studies of relationship.

parsimonious tree (Fig. 1B; 649 steps, CI = 0.44), the topology of which did not change after one or more rounds of successive approximations (CI = 0.72). The tree is very similar to that resulting from the unweighted analyses except that the Melanesian *Loveridgelaps* forms a clade with *Salomonelaps* and *Laticauda*, and *Pseudechis* formed a sister clade to *Acanthophis*. This analysis also placed *Bungarus* within the hydrophiine lineage.

MP analyses of the unweighted 16S rRNA data set produced a single parsimonious tree (Fig. 2; 280 steps, CI = 0.53), the topology of which did not change after one or more rounds of successive approximations (CI = 0.87). As with the cytochrome *b* analyses, African elapids formed a series of paraphyletic sister clades to other elapids in the shortest tree and resolution among African elapids and between

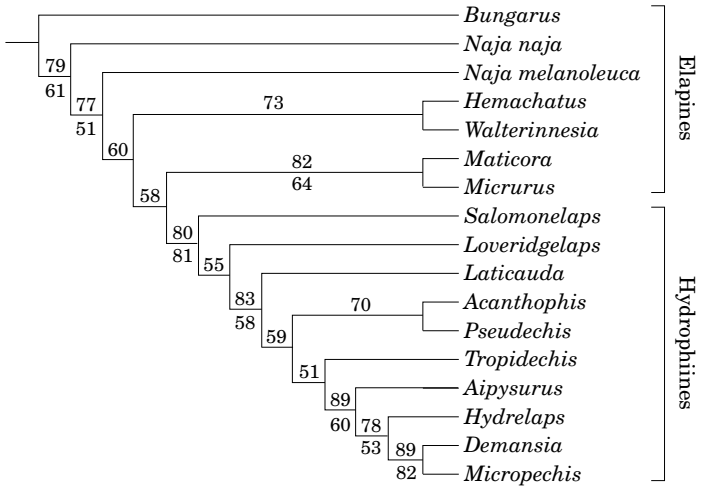

Figure 2. Single most parsimonious tree generated from the unweighted analysis and successive approximations of the 16S rRNA data (bootstrap values from 300 replicates above the nodes). Transitions down-weighted relative to transversions by a factor of two resulted in the identical tree (bootstrap values from 300 replicates below the nodes). The outgroup is not shown.

African elapids and other elapid groups is weak. The 16S rRNA data set, like the cytochrome *b* data set, strongly supported an American-Asian coral snake clade with a bootstrap value of 82%. In the most parsimonious tree, the coral snake clade formed the sister group to the strongly supported (80%) hydrophiine clade comprised of terrestrial Australo-Papuan elapids and *Laticauda* as well as the true sea snakes. Within this hydrophiine lineage, *Salomonelaps* and *Loveridgelaps* formed the sister group to the rest of Australo-Papuan elapids, *Laticauda* and true sea snakes. The true sea snakes *Aipysurus* and *Hydrelaps* did not form a monophyletic clade in these analyses but instead two paraphyletic sister groups to the terrestrial elapids *Demansia* and *Micropechis*. Down-weighting transitions relative to transversions by a factor of two resulted in the same single most parsimonious tree as the above unweighted analysis (Fig. 2; 386 steps, CI = 0.52), the topology of which did not change after one or more rounds of successive approximations (CI = 0.79).

MP analyses of the unweighted combined data set produced three most parsimonious trees (795 steps, CI = 0.46). One round of successive approximations resulted in a single most parsimonious tree (Fig. 3A; CI = 0.78), the topology of which did not change after additional rounds. In agreement with the individual data sets, in the combined analysis African elapids formed a series of paraphyletic clades, the coral snake clade is very strongly supported (95%), as is the hydrophiine clade (95%) comprised of *Laticauda*, Australo-Papuan elapids and true sea snakes. Unlike either individual data set, the shortest tree from the combined analyses placed the coral snake clade as the sister group to other elapids and *Bungarus* as sister group to the hydrophiine lineage. Within the hydrophiines, the Asiatic *Laticauda* and the Melanesian *Salomonelaps* and *Loveridgelaps* formed the sister group to Australian elapids and true sea snakes. Transitions down-weighted relative to transversions by a factor of two resulted in two equally parsimonious trees (1051 steps, CI = 0.46).

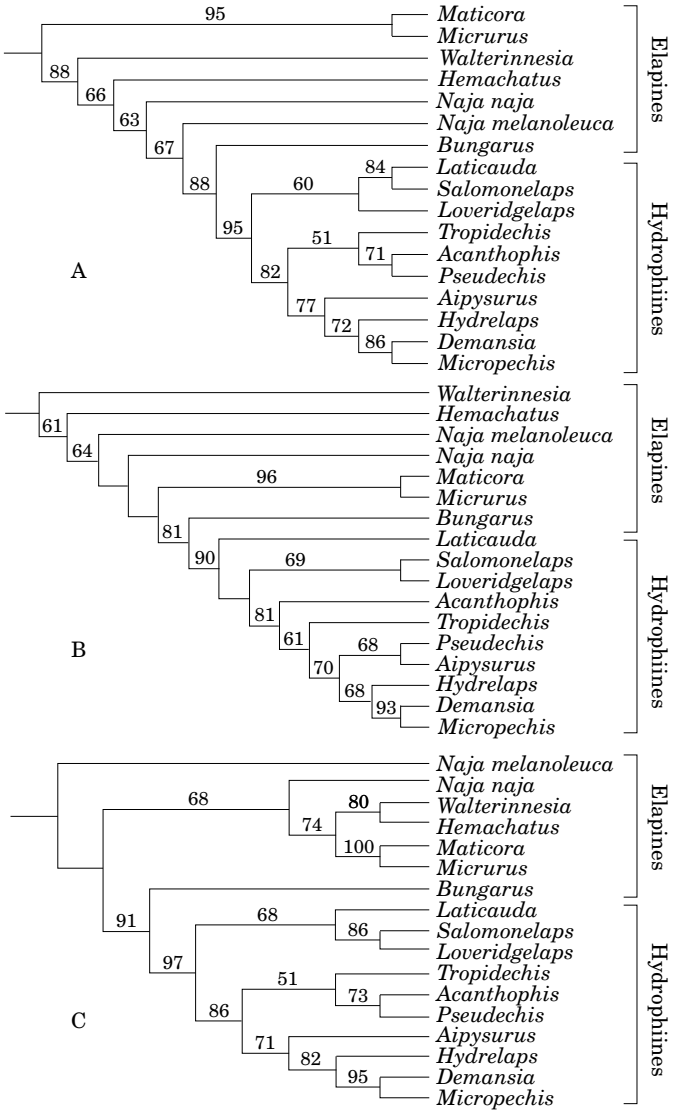

Figure 3. A, single most parsimonious tree generated after one round of successive approximations based on the three most parsimonious trees produced from the combined unweighted cytochrome *b* and 16S rRNA data. B, single most parsimonious tree generated with transitions down-weighted relative to transversions by a factor of two. C, single most parsimonious tree generated after one round of successive approximations based on three most parsimonious trees produced from the combined cytochrome *b* and 16S rRNA data sets when *Naja melanoleuca* was used as the outgroup (outgroups are not shown in A or B). Numbers represent bootstrap values from 300 replicates.

One round of successive approximations resulted in a single most parsimonious tree (Fig. 3B; CI = 0.78), the topology of which did not change after additional rounds. This tree is very similar to that resulting from the unweighted analyses except that the coral snake clade again forms the sister group to the hydrophiines plus *Bungarus* and *Laticauda* forms the sister group to other hydrophiines.

Unfortunately, the sequence data were not adequate to resolve relationships at the base of the elapid tree. The difference in placement of the American-Asian coral snake clade and the African cobra species relative to each other and other elapid groups in the unweighted, successively re-weighted, and 'transitions down-weighted' analyses reflects the very weak support for either alternative hypothesis. Making the coral snake clade the sister group to other elapids in the 'transitions down-weighted' analysis required the addition of only a single extra step. Figure 3C displays the results when *Naja melanoleuca* was used as the outgroup in an unweighted and then successively re-weighted analysis. These analyses nested the coral snake clade within the other three cobra species which together form a clade relative to *Bungarus* and hydrophiines. In these analyses, an elapine clade comprised of cobras and coral snakes is well supported relative to a hydrophiine clade which is highly supported with a bootstrap value of 97%. *Bungarus* is identified as the sister group to the hydrophiines with a 91% bootstrap value. In Figure 4A I present a conservative overall summary of clades that are well supported by these data and the inter-relationships among them. All biogeographic discussion is based on this tree.

DISCUSSION

Phylogenetic relationships

The branching order between trees produced from the individual cytochrome *b* and 16S rRNA analyses differ to varying degrees. However, analyses of the individual data sets are in broad agreement on several issues: (i) African elapids form a series of paraphyletic sister groups to the rest of elapids in the shortest trees, but a series of equivalent (unresolved) sister groups to other elapids in bootstrap consensus trees. (ii) American and Asian coral snakes form a strongly supported clade. (iii) The coral snake clade forms the sister group to the hydrophiines in the shortest trees, but this sister group relationship is weakly supported in both data sets. (iv) The hydrophiine lineage comprised of the terrestrial Australo-Papuan elapids, *Laticauda*, and true sea snakes (16S rRNA only) form a derived monophyletic clade with both data sets. (v) Within the hydrophiine lineage, both data sets identify the Asiatic *Laticauda* and the Melanesian *Loveridgelaps* and *Salomonelaps* as basal or sister groups to the rest of the radiation. Results from analyses of the combined data set are in agreement with each of these points. However, they differ slightly from the individual analyses in the placement of the American-Asian coral snake clade and *Bungarus*. Taken in total, phylogenetic analyses of the individual and combined data sets are largely supportive of a similar topology and consistent with inferences on elapid phylogeny based on morphological and molecular data.

The basal clade

Both the individual and combined analyses consistently place either the Afro-Asian cobras or the American-Asian coral snakes as the sister clade to other elapids. Unfortunately, my data do not resolve clearly whether the cobras or coral snakes are basal to other elapids, or if in fact they together form a sister clade relative to hydrophiines. Similarly, other studies of relationships among these groups have not been conclusive. McDowell (1969a) suggested that the African *Elapsoidea*, *Boulengerina*,

and *Paranaja* probably represent the most primitive of living elapids, although their relationships to other African elapids were not discussed. A study of higher level snake relationships based on 12S and 16S rRNA sequences found that the New World *Micrurus* and *Micruroides* formed a sister clade to the Afro-Asian cobras *Naja* and *Ophiophagus* (Heise *et al.*, 1995). However, neither Asian coral snakes nor hydrophiine representatives were included in this study. Phylogenetic analyses of venom protein sequences identify an elapine clade comprised of the Asian coral snake *Maticora* and various cobras which together form a sister group to various hydrophiines (Slowinski *et al.*, 1997). Given the uncertain nature of relationship between these putative clades based on both morphological and molecular grounds, the cobras and coral snakes may best be considered as equivalent sister groups to other elapids. Despite the ambiguity as to which clade might represent the ancestral condition, the major split identified by McDowell (1970) between elapines and hydrophiines is very well supported, with the possible exception of *Bungarus* (see below).

Cobras

The primarily African cobra radiation is comprised of 10 genera (*Aspidelaps*, *Boulengerina*, *Dendroaspis*, *Elapsoidea*, *Hemachatus*, *Naja*, *Ophiophagus*, *Paranaja*, *Pseudohaje*, *Walterinnesia*), all of which are endemic to that continent except *Naja*, which has numerous species ranging into southern Asia (see Wüster & Thorpe, 1989, 1990, 1992a, b; Wüster *et al.*, 1995), *Walterinnesia* ranging into Iran, and *Ophiophagus* with a fully Asian distribution. Phylogenetic relationships within this radiation have received little attention. Some cobras recently have been incorporated into phylogenetic analyses as outgroups to other snakes (Wallach, 1985; Schwaner *et al.*, 1985; Underwood & Kochva, 1993) or as representatives of major snake clades (Cadle, 1994), but current understanding of relationships within the cobra radiation has not changed significantly since Bogert's (1940, 1943) classic works on African snakes.

Unfortunately, the DNA data sets presented here do not resolve relationships among the primarily African species sampled. The individual DNA data sets agree in that the Afro-Asian cobras form a series of paraphyletic clades relative to other elapids instead of the expected monophyletic clade, but differ in the order in which these species are placed. The combined data set displays the same topology as that from the individual cytochrome *b* analyses, probably reflecting the greater number of variable sites. These results are interesting because the cobra radiation appears to be a rather well defined and distinctive group based on morphological criteria. However, the results from the molecular data may reflect reality as genetic distances presented here support the notion that cobras are comprised of clades which represent ancient divergences (Table 3). Cadle (1987), citing unpublished biochemical data, stated that African elapids do not form a single monophyletic group relative to unspecified Asian elapids. He noted that the cobra-like forms including *Naja*, *Aspidelaps*, *Hemachatus*, *Pseudohaje*, and perhaps *Boulengerina* are most likely monophyletic while *Elapsoidea*, *Paranaja*, *Dendroaspis*, and *Homoroselaps* "have complex relationships to one another and to Asian elapids" (Cadle, 1987: 92). Based primarily on cranial osteology, Underwood & Kochva (1993) found that *Walterinnesia* and *Elapsoidea* could be sister taxa relative to a grouping formed by *Aspidelaps*, *Boulengerina*, *Paranaja*, and *Pseudohaje*. A phylogenetic analysis of venom proteins suggested that *Naja* is not

monophyletic with respect to *Boulengerina* or *Aspidelaps* (Slowinski *et al.*, 1997). Clearly, relationships within the cobra radiation are complex and presently available data sets have not satisfactorily resolved higher level relationships within the group.

American-Asian coral snakes

A close relationship between the Asiatic coral snakes (*Calliophis* and *Maticora*) and the New World coral snakes (*Micrurus* and *Micruroides*) has been suggested based on morphological criteria. McDowell (1967: 530) identified two morphological features that unite the American micrurine radiation with the Asiatic coral snakes *Calliophis* and *Maticora* as well as *Laticauda* and the Bougainville Island *Parapistocalamus*: "the venom gland has its posterior end bent downward behind the corner of the mouth, and the maxilla and ectopterygoid meet in an end-to-end joint that is capable of motion in all planes". McDowell (1969a) identified the second character, what he called a 'universal joint', as the most distinctive character of this group. He also noted that the *Calliophis–Micrurus* group have a much shortened palatine that they share with *Laticauda*. However, McDowell (1969a, 1986) outlined a great deal of variation within *Calliophis* (see also Savitzky, 1979) which lead him to later split the genus among three elapid subfamilies in his classification scheme (McDowell, 1987).

No previous molecular studies have included representatives of both the American and Asiatic coral snake radiations to test the relationship between them. Both the individual and combined data sets presented here very strongly support monophyly of a *Micrurus–Maticora* coral snake clade, thus partially corroborating McDowell's morphological data. Genetic distances between *Micrurus* and *Maticora* are smaller than between the coral snakes as a group and any other elapid group. However, the Jukes & Cantor (1969) genetic distance between the coral snakes is still considerable (21.40 and 5.67% for cytochrome *b* and 16S rRNA, respectively) supporting the notion that divergences within the coral snake lineage are probably quite ancient (Cadle & Sarich, 1981). Unfortunately, I was unable to include representatives of the Asian coral snake genus *Calliophis*, but given the sister group relationship between *Micrurus* and *Maticora* shown here and the close morphological similarity between *Maticora* and at least some *Calliophis* species (McDowell, 1969a, 1986, 1987), my results do not support the claim by McDowell (1986, 1987) that *Maticora* and *Calliophis* are 'transitional' between Atractaspids and his 'euproteroglyphs'. A detailed study of cephalic musculature by Zaher (1994) similarly did not support this 'transitional' hypothesis.

The relationships of Bungarus

The Asiatic terrestrial kraits of the genus *Bungarus* are a morphologically cohesive and highly derived group comprised of 12 species (McDowell, 1970, 1987; Slowinski, 1994a). Based on morphological criteria, *Bungarus* has been considered part of the primarily African cobra radiation, the group with which they always have been classified (i.e. Romer, 1956; Underwood, 1967; 1979; Dowling, 1974; Smith *et al.*, 1977; McDowell, 1987). Thus, *Bungarus* forms part of McDowell's (1970) palatine erector group which also includes the Asian and American coral snakes. However, *Bungarus* is morphologically distinct among elapids. McDowell (1987: 33) stated in reference to Afro-Asian elapines "species diversity is greatest in Africa, but the Asiatic *Bungarus* and *Ophiophagus* are each so peculiar in anatomy as to suggest an ancient divergence". Keeping this in mind, the sister group relationship of *Bungarus*

to the hydrophiines presented here in the combined analysis is interesting in light of peculiarities *Bungarus* displays in the very structures McDowell used to divide the elapids. McDowell (1970: 149) found that *Bungarus* (and *Elapsoidea*) "seem to be palatine erectors in the structure of the palatine-pterygoid joint, but perhaps palatine draggers in function because although the palatine moves parallel to the maxilla, the maxilla is almost completely bound to the horizontal plane." Further, McDowell (1967: 532) identified characteristics of the palatine that *Bungarus* shares with Australo-Papuan elapids and true sea snakes, and pointed out that *Bungarus* and *Laticauda* (plus the African *Elapsoidea* and *Dendroaspis* and the Bougainville Island *Parapistocalamus*) are exceptions among the palatine erectors in that they lack a well developed medial process of the palatine bone (McDowell, 1970).

The sister-group relationship of *Bungarus* to hydrophiines indicated by my data, together with McDowell's findings, suggests that *Bungarus* might represent a transitional elapid clade between the elapine palatine erectors and hydrophiine palatine draggers. The relationship of *Bungarus* to the hydrophiine Melanesian genera *Loveridgelaps* and *Salomonelaps* presented here supports this 'transitional elapid' hypothesis, again corroborated by morphological data. McDowell (1970) considered *Bungarus* closely related to both *Loveridgelaps* and *Salomonelaps*, and identified a number of features that *Bungarus* shares with these species. These features include the indistinguishable nature of head scalation of *Bungarus* and *Loveridgelaps* and their very close general resemblance, hemipenis and subcaudal scale similarities between *Bungarus* and *Salomonelaps*, shared palatine characteristics between *Bungarus* and his '*Vermicella*' group comprised of *Loveridgelaps*, *Salomonelaps*, *Ogmodon*, and *Vermicella* (McDowell, 1967, 1969a, 1970), and shared defensive behaviour characteristics between *Bungarus* and *Vermicella* (McDowell, 1970). *Laticauda* too might be considered transitional between elapines and hydrophiines because while it is a palatine erector according to McDowell (1970), *Laticauda* is a part of the hydrophiine palatine dragger lineage according to a large number of molecular studies including my own (Minton, 1981; Cadle & Gorman, 1981; Mao *et al.*, 1977, 1978, 1983; Schwaner *et al.*, 1985; Guo *et al.*, 1987; Slowinski *et al.*, 1997). In addition to an obvious superficial morphological resemblance between *Bungarus* and *Laticauda*, McDowell (1970: 150) noted that his *Vermicella* group, is "... essentially intermediate between the Afro-Asiatic *Bungarus-Elapsoidea* group and the great majority of Australasian Elapidae." Further, there are other characteristics this group shares that in the future may be identified as synapomorphies for this clade: (i) *Bungarus*, *Laticauda*, *Loveridgelaps* and *Vermicella* are all strongly cross-banded snakes, a feature they share with virtually all Asian and American coral snakes, and (ii) all members of these genera specialise on elongate prey items—most *Bungarus* species feed primarily on other snakes (Slowinski, 1994b), *Laticauda* species feed primarily on eels (Voris & Voris, 1983), while both *Loveridgelaps elapoides* (Shine & Keogh, 1996) and *Vermicella* species specialise on typhlopid snakes (Shine, 1980b; Keogh & Smith, 1996).

Molecular data applied to *Bungarus* relationships have not been conclusive but lend support to this 'transitional elapid' hypothesis. Some researchers have found a closer association between *Bungarus* and Afro-Asian cobras than between other elapid groups (Minton, 1981; Schwaner *et al.*, 1985; Slowinski *et al.*, 1997). Mao *et al.* (1983) and Guo *et al.* (1987) presented phenograms based on immunological distance and plasma albumin comparisons, respectively, that suggested a sister group relationship between *Bungarus* and *Naja* species on the one hand and *Laticauda* and true sea snake species on the other. However these authors considered the split between *Bungarus*

and *Naja* species to be deeper (and thus, perhaps older) than the split between *Laticauda* and true sea snakes. The distance tables presented in both Mao *et al.* (1983) and Guo *et al.* (1987) suggest that the relationship of *Bungarus* and other elapids is obscure, although neither paper discussed the problem. Examination of table 1 in Mao *et al.* (1983) shows that *Bungarus multicinctus* is immunologically much more similar to *Laticauda*, terrestrial Australian elapids, and true sea snakes than it is to *Elapsoidea sundevalli*, *Naja naja* or two *Micrurus* species. A similar result was obtained by Guo *et al.* (1987) for comparisons of *Bungarus multicinctus* to *Laticauda semifasciata* and *Naja naja*, and Cadle & Sarich (1981) found that *Bungarus fasciatus* and *Laticauda* are closer immunologically than *Bungarus* is to a *Micrurus*. *Bungarus* also is quite distinct from other elapids in venom protein sequences (Tamiya, 1985). In a phylogenetic analysis of venom protein sequences, Slowinski *et al.* (1997) found that *Bungarus* species tended to group with numerous members of the Afro-Asian elapine radiation but as a weakly supported sister group to it rather than nested within it. Thus, taking the summation of the placement of *Bungarus* and *Laticauda* in the phylogenetic hypothesis presented here, the well established basal nature of the relationship between *Laticauda* and other hydrophiines (see below), the apparently ancient association of *Bungarus* and other elapines, and the strong morphological evidence of a close relationship of *Bungarus* and some Melanesian elapids into consideration, I suggest that *Bungarus* and *Laticauda* may represent morphologically transitional or intermediate forms between the elapine palatine erector and the hydrophiine palatine dragger lineages.

Australo-Papuan elapids and sea snakes

My phylogenetic analyses clearly support the monophyly and derived nature of McDowell's hydrophiine palatine dragger lineage (McDowell, 1967, 1969a, b, 1970, 1972, 1986, ref,1987). *Laticauda* is part of this lineage, strongly corroborating numerous molecular studies (Minton & da Costa, 1975; Minton, 1978; Mao *et al.*, 1977, 1978, 1983; Cadle & Gorman, 1981; Schwaner *et al.*, 1985; Tamiya, 1985; Guo *et al.*, 1987; Slowinski *et al.*, 1997). Further, the results indicate two independent invasions of the aquatic environment, one by *Laticauda* and another by the true hydrophiid sea snakes, corroborating both morphological (McDowell, 1967, 1969a; Voris, 1977; McCarthy, 1986) and molecular studies (Minton & da Costa, 1975; Minton, 1981; Schwaner *et al.*, 1985; Slowinski *et al.*, 1997). McDowell's (1967, 1969a, 1970, 1972) hypothesized close relationship between *Laticauda* and the Asian and American coral snakes is not supported by my data, nor by other molecular studies (Cadle & Gorman, 1981; Cadle & Sarich, 1981; Mao *et al.*, 1983; Slowinski *et al.*, 1997). However, it does appear that *Laticauda* shares a closer relationship with Asiatic elapids than at least some other Australo-Papuan elapids. Slowinski *et al.* (1997) formally moved *Laticauda* from the elapine lineage to the hydrophiine lineage based on their analyses of venom protein sequences. My results support this move.

Loveridgelaps and *Salomonelaps* have been identified as the most primitive of the Australo-Papuan radiation by McDowell (1970) who considered them more closely related to each other and to *Vermicella*, *Ogmodon* and some *Toxicocalamus*, than to other Australo-Papuan species. I have not included members of these latter three genera in the analyses presented here (see Keogh, Shine & Donnellan, 1998), but the basal nature of the relationship between *Loveridgelaps* and *Salomonelaps* relative to other Australo-Papuan elapids is strongly supported by my data. Further, these species

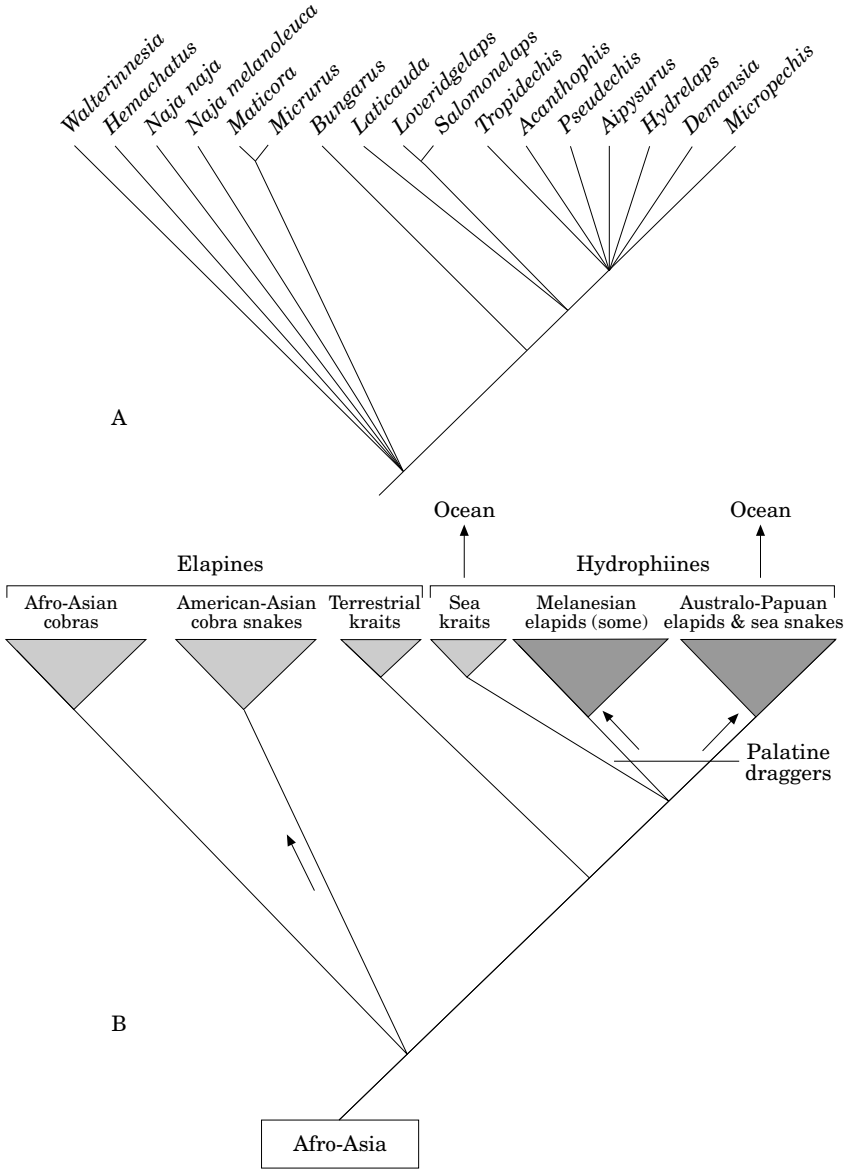

Figure 4. A, consensus of phylogenetic results obtained from analyses of the individual cytochrome *b* and 16S rRNA data sets and the combined data set illustrating only strongly supported nodes. B, area cladogram illustrating the hypothesized biogeographic relationships of the major elapid clades based on the hypothesis of relationship presented above. The traditional split between the elapines and hydrophiines is noted. Elapids are hypothesized to have an African, Asian or Afro-Asian origin with subsequent dispersal to the New World by the American micrurine radiation, and Melanesia and then Australia by the Australo-Papuan elapids and true hydrophiid sea snakes. Arrows denote dispersal from the hypothesized Afro-Asian origin. The data support two independent origins of invasion of the aquatic environment, one in the partially aquatic sea kraits and another in the diverse radiation of fully aquatic true sea snakes, strongly supporting phylogenetic hypotheses of others. The light and dark-shaded clades represent the 'palatine erector' and 'palatine dragger' lineages, respectively, identified by McDowell (1970).

consistently group with *Laticauda*. Schwaner *et al.* (1985) postulated that *Laticauda* probably originated at approximately the same time as the Australo-Papuan elapids differentiated from their Asian ancestors, a hypothesis consistent with my results. Together these Asiatic and Melanesian taxa form the sister group to other Australo-Papuan elapids and sea snakes sampled here. However, the three Melanesian elapids sampled do not appear to be monophyletic as implied in the classification scheme of Wallach & Jones (1992). Thus at least with respect to the three Melanesian elapids represented here, reciprocal monophyly of the Melanesian and Australian terrestrial elapid species cannot be supported.

Resolution among the terrestrial Australian species sampled here is weak, but it is worth mentioning the position of *Acanthophis* and *Demansia* relative to other Australo-Papuan elapid species. Despite the derived nature of both genera and the immunological distinctiveness of *Demansia* (Cadle & Gorman, 1981; Mao *et al.*, 1983; Schwaner *et al.*, 1985), both genera are certainly part of the Australo-Papuan radiation based on the data presented here. Hence, they do not appear to represent separate invasions of the region from different elapid stock. The immunological distinctiveness of *Demansia* may be attributable to accelerated rates of albumin molecular evolution in this genus, as has been found in *Naja* (Mao *et al.*, 1983, Guo *et al.*, 1987). Among the terrestrial Australian elapids, the diverse viviparous species have been hypothesised to be a more recent monophyletic radiation within the terrestrial Australian elapids (Shine, 1985). The phylogenetic hypotheses for Australian elapids presented by Mengden (1985) and Wallach (1985) appear to support this hypothesis. Furthermore, the true hydrophiid sea snakes, all of which are viviparous, are thought to be derived from this lineage (Schwaner *et al.*, 1985; Gopalakrishnakone & Kochva, 1990; Slowinski *et al.*, 1997). The phylogenetic structure evident in the trees presented here appears not to support this hypothesis, however, as both the oviparous *Demansia* and *Micropechis* are derived relative to *Acanthophis*, *Tropidechis*, and the true sea snakes *Aipysurus* and *Hydrelaps* (which are all viviparous). I note, nonetheless, that the support for these nodes is relatively weak. Relationships among the terrestrial Australo-Papuan elapids based on DNA sequences is considered elsewhere (Keogh, 1997; Keogh *et al.*, 1998).

Biogeography

Few authors have speculated on which modern day elapids most closely resemble the ancestral stock or where these ancestors might have lived. Despite the high level of endemicity of African elapids, Hoffstetter (1939) argued, based on fossil evidence, that perhaps a proto-*Naja* stock invaded Europe and Africa independently from Asia during the Miocene. Similarly, Bogert (1943) thought Asia was probably the centre of origin for elapids with dispersal to Africa and the Americas. However, Cadle (1987: 92) pointed out that Africa cannot be discounted as the source of European Miocene *Naja*-like forms. McDowell (1969a) suggested that the African *Elapsoidea*, *Boulengerina*, and *Paranaja* probably represent the most primitive of living elapids. As a basal elapid species or clade is not well resolved with my combined data set, the biogeographic region in which elapids originated is difficult to determine unambiguously due in part to the present-day distribution of the Afro-Asian cobras and American and Asian coral snakes. Present-day elapids are primarily distributed on Gondwanan elements with only *Naja*, *Ophiophagus*, *Laticauda*, *Bungarus* and Asian

coral snakes found on Laurasian elements. Given the lack of resolution among the Afro-Asian cobra and American-Asian coral snake clades in this study and the lack of any strongly supported estimates of elapid age, a Gondwanan origin for elapids (including American coral snakes) cannot be discounted as an explanation for their present-day distribution (by vicariance), as an invasion of Asia by American coral snakes is just as likely as the inverse. However, my data indicate that Asian species represent the sister groups to representative African, American, and Australo-Papuan elapid radiations. Cadle (1987) pointed out that the Asian elapid fauna is comprised of representatives from all six subfamilies recognized by McDowell (1987). Thus, it would appear that Asia is the most likely centre of origin for elapids.

Notwithstanding this inference, I am unable to discriminate between an African or Asian origin for the cobras despite the high level of African endemicity. However, an Asian origin seems the most likely for the American micrurine radiation. A number of authors have suggested that New World coral snakes invaded North America via a land connection (the Bering Land Strait) implying an Old World origin (Hoffstetter, 1939; Bogert, 1943; Darlington, 1957; Underwood, 1967). Cadle & Sarich (1981) argued that their immunological time-of-divergence estimates of New World coral snakes from their Old World relatives were consistent with suggested land connections between Eurasia and North America. However, they could not resolve if this migration actually happened as they were unable to discount hypotheses that the isolation of New World coral snakes was due to the breakup of Gondwanaland, that coral snakes reached the New World by over-water dispersal, or that elapids were once widespread in Laurasia. Despite these alternative hypotheses, an Asian origin of American coral snakes seems most likely, given the well supported nature of the land-bridge connection between Eurasia and the New World which has acted as a dispersal corridor between the continents for many different plants and animals.

The terrestrial Australo-Papuan elapid radiation is the most taxonomically diverse of the various elapid clades at the generic level, with 27 currently recognized genera. Based on this diversity, Storr (1964) assumed the history of elapids in this region to be ancient. Despite this morphological diversity, however, most recent workers have supported the hypothesis that the highly endemic terrestrial Australo-Papuan elapid snakes plus the true sea snakes are a comparatively recent radiation derived from Asian elapid stock following the collision of the Australian and Asian tectonic plates in the mid-Miocene (Underwood, 1967; Tyler, 1979; Cogger & Heatwole, 1981; Schwaner *et al.*, 1985; Dessauer *et al.*, 1987; Cadle, 1987, 1988). Based on their immunological data, Schwaner *et al.* (1985) estimated that the Australo-Papuan elapids last shared a common ancestor with Afro-Asian elapids approximately 20 Mya, near the time when the Australian plate was nearing the Asian plate. These authors then went on to speculate that the *Notechis* lineage (which included true sea snakes) might have arisen only 5 Mya. Cadle & Gorman (1981), using a molecular clock calibration of other terrestrial vertebrates, suggested that the *Hydrophis* sea snake group was less than 5 million years old. As noted by Cadle, (1987, 1988), the published immunological distance data support an Asian origin for Australo-Papuan elapids, corroborating the morphological studies of McDowell (1967, 1969a, 1970).

My results also support an Asian origin for the Australo-Papuan elapids and sea snakes, discounting a Gondwanaland origin for at least this elapid radiation. The phylogenetic tree generated from the combined data set shows strong phylogeography with the terrestrial Australo-Papuan elapids plus sea snakes clearly displaying a

'stepping-stone' pattern of invasion from Asian ancestors. Both Schwaner *et al.* (1985) and Cadle (1988) predicted that this radiation should show relatively little genetic distances from their Asian sister groups. Their prediction of small genetic distances between Australo-Papuan elapids and their Asian relatives was not realized with the two genes and representative taxa sampled here. However, parsimony analyses support their hypothesis of an Asian origin. Cytochrome *b* and 16S rRNA genetic distances indicate that Australo-Papuan elapids plus sea snakes are quite distant from any other elapid group, including the Asian sister groups. However, this may not be too surprising given that the Afro-Asian elapid lineages are likely to be quite ancient.

In addition to the considerable phylogenetic support for an Asian origin of Australo-Papuan elapids and true sea snakes, there is another compelling factor which lends support to this hypothesis. In addition to a very diverse elapid radiation, the Australo-Papuan snake fauna also is comprised of diverse pythonine and typhlopid radiations. However, this region is unusual with respect to other major zoogeographic regions in that it completely lacks a viperid snake fauna, and the Australian colubrid fauna is comprised of only eight genera (10 species), all of which are aquatic or arboreal and shared with New Guinea (genera and species) or Asia (genera). New Guinea has several additional colubrid genera, but again, these are shared with Asia. The advanced Caenophidian snake fauna of the world (comprised of colubrids, viperids, elapids, and atractaspids) is thought to have evolved during the Cenozoic (Cadle, 1987, 1988). Thus, Australia already had separated from South America and was drifting toward Asia during the time when these major snake radiations originated, offering an explanation as to why Australia lacks an ancient advanced snake fauna (Cadle, 1987).

Clearly, the phylogenetic and biogeographic history of elapid snakes is complicated. While the present data support the major division between elapines and hydrophiines, with the exception of *Laticauda*, and an Asian origin for American coral snakes and Australo-Papuan elapids, there is still much work to be done elucidating the fine details of relationship among all the major elapid radiations and their biogeographic histories. Also, more work is needed on which of the elapine radiations represents the most basal of the elapid clade. Future studies might best turn to other genes, perhaps nuclear genes, to address these problems.

ACKNOWLEDGEMENTS

Staff and biologists at the Evolutionary Biology Unit of the South Australian Museum (SAM) went out of their way to make me feel at home during the duration of this and other projects. In particular I thank Steve Donnellan, Jan Birrell, and Steve Cooper. For help in obtaining tissues I am indebted to Steve Donnellan, Greg Mengden, Joe Slowinski, Don Colgon, Fred Sheldon, and Donna Dittmann. My ideas and their presentation were improved by Chris Austin, Bill Branch, Steve Donnellan, Paul Doughty, Mark Hutchinson, Hamlet Giragossyan, Michael Mohony, Rick Shine, Joe Slowinski, Van Wallach, and the EBU crew. This research was supported by grants from the Mark Mitchell Foundation and the University of Sydney Research Grants Scheme. It was conducted while I was supported by a

Fulbright Fellowship from the American-Australian Education Foundation and an Overseas Postgraduate Research Fellowship and University Postgraduate Research Award from the University of Sydney.

REFERENCES

Arctander P. 1995. Comparison of a mitochondrial gene and a corresponding nuclear pseudogene. *Proceedings of the Royal Society of London B* **262:** 13–19.

Bellairs AdA, Underwood G. 1951. The origin of snakes. *Biological Review* **26:** 193–237.

Bogert CM. 1940. Herpetological results of the Vernay Angola Expedition, with notes on African reptiles in other collections. Part 1. Snakes, including an arrangement of African Colubridae. *Bulletin of the American Museum of Natural History* **77:** 1–107.

Bogert CM. 1943. Dentitional phenomena in cobras and other elapids with notes on adaptive modifications of fangs. *Bulletin of the American Museum of Natural History* **81:** 285–360.

Boulenger GA. 1896. *Catalogue of Snakes of the British Museum (Natural History)*. London: British Museum of Natural History.

Burger WL, Natsuno T. 1974. A new genus for the Arafura smooth seasnake and redefinitions of other seasnake genera. *The Snake* **6:** 61–75.

Cadle JE. 1982. Problems and approaches in the interpretation of the evolutionary history of venomous snakes. *Memorias del Instituto Butantan* **46:** 255–274.

Cadle JE. 1987. Geographic distribution: Problems in phylogeny and zoogeography. In: Siegel RA, Collins JT, Novak SS, eds. *Snakes, Ecology and Evolutionary Biology*. New York: McGraw-Hill, 77–105.

Cadle JE. 1988. Phylogenetic relationships among advanced snakes. *University of California Publications in Zoology* **119:** 1–77.

Cadle JE. 1994. The colubrid radiation in Africa (Serpentes: Colubridae): Phylogenetic relationships and evolutionary patterns based on immunological data. *Zoological Journal of the Linnean Society* **110:** 103–140.

Cadle JE, Gorman GC. 1981. Albumin immunological evidence and the relationships of sea snakes. *Journal of Herpetology* **15:** 329–334.

Cadle J, Sarich V. 1981. An immunological assessment of the phylogenetic position of New World coral snakes. *Journal of Zoology (London)* **195:** 157–167.

Cogger HG, Heatwole H. 1981. The Australian reptiles: Origins, biogeography, distribution patterns and island evolution. In: Keast A, ed. *Monographiae Biologicae Vol 41. Ecological Biogeography of Australia Vol 2*. The Hague: Junk, 1331–1374.

Collura RV, Stewart CB. 1995. Insertions and duplications of mtDNA in the nuclear genomes of Old World monkeys and hominids. *Nature* **378:** 485–489.

Darlington PJ. 1957. *Zoogeography: The Geographical Distribution of Animals*. New York: John Wiley and Sons.

Dessauer HC, Cadle JE, Lawson R. 1987. Patterns of snake evolution suggested by their proteins. *Fieldiana, Zoology Series No. 34*: 1–34.

de Queiroz A, Donoghue MJ, Kim J. 1995. Separate versus combined analysis of phylogenetic evidence. *Annual Review of Ecology and Systematics* **26:** 657–681.

de Queiroz A, Lawson R. 1994. Phylogenetic relationships of the garter snakes based on DNA sequence and allozyme variation. *Biological Journal of the Linnean Society* **53:** 209–229.

Dowling HG. 1959. Classification of the serpents: A critical review. *Copeia* **1959:** 38–52.

Dowling HG. 1967. Hemipenes and other characters in colubrid classifications. *Herpetologica* **23:** 138–142.

Dowling HG. 1974. A provisional classification of snakes. In: Dowling HG, ed. *1974 Yearbook of Herpetology*. New York: HISS Publications, 167–170.

Farris JS. 1969. A successive approximations approach to character weighting. Systematic Zoology **18:** 374–385.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39:** 783–791.

Forstner MRJ, Davis SK, Arévalo E. 1995. Support for the hypothesis of Anguimorph ancestry for the suborder Serpentes from phylogenetic analysis of mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **4:** 93–102.

Golay P. 1985. *Checklist and Keys to the Terrestrial Proteroglyphs of the World*. Geneva: Elapsoidea.

Golay P, Smith HM, Broadley DG, Dixon JR, McCarthy C, Rage JC, Schatti B, Toriba M. 1993. *Endoglyphs and other major venomous snakes of the world. A checklist*. S. A. Switzerland: Azemiops.

Gopalakrishnakone P, Kochva E. 1990. Venom glands and some associated muscles in sea snakes. *Journal of Morphology* **205:** 85–96.

Guo YW, Mao SH, Yin FY. 1987. Comparison of cobra plasma albumins with those of banded krait and sea snake. *Comparative Biochemistry and Physiology* **87B:** 559–566.

Heise PJ, Maxson LR, Dowling HG, Hedges SB. 1995. Higher-level snake phylogeny inferred from mitochondrial DNA sequences of 12S rRNA and 16S rRNA genes. *Molecular Biology and Evolution* **12:** 259–265.

Higgins DG, Bleasby AJ, Fuchs R. 1991. Clustal V. Improved software for multiple sequence alignment. *Cabios* **8:** 189–191.

Hillis D. 1991. Discriminating between phylogenetic signal and random noise in DNA sequences. In: Miyamoto MM, Cracraft J, eds. *Phylogenetic Analysis of DNA Sequences*. New York: Oxford University Press, Inc., 278–294.

Hillis DM, Huelsenbeck JP. 19926. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* **83:** 189–195.

Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42:** 182–192.

Hoffstetter R. 1939. Contribution a l'etude des Elapidae actuels et fossiles et de l'osteologie des ophidiens. *Archives der Muséum d'Histoire Naturelle de Lyon* **15:** 1–78.

Horovitz I, Meyer A. 1995. Systematics of New World (Platyrrhini, Primates) based on 16s mitochondrial DNA sequences: A comparative analysis of different weighting methods in cladistic analysis. *Molecular Phylogenetics and Evolution* **4:** 448–456.

Huelsenbeck JP, Bull JJ, Cunningham CW. 1996. Combining data in phylogenetic analysis. *Trends in Ecology and Systematics* **11:** 152–158.

Hutchinson MN. 1990. The generic classification of the Australian terrestrial elapid snakes. *Memoirs of the Queensland Museum* **28:** 397–405.

Jukes TH, Cantor CR. 1969. Evolution of protein molecules. In: Munro HN, ed. *Mammalian Protein Metabolism*. New York: Academic Press, 21–132.

Keogh JS. 1997. Phylogenetic studies on Australo-Papuan elapid snakes based on morphology and DNA. Unpublished Ph.D Thesis, University of Sydney, Australia.

Keogh JS. 1998. Evolutionary implications of hemipenial morphology in the terrestrial Australian elapid snakes. *Zoological Journal of the Linnean Society*. In Press.

Keogh JS, Shine R, Donnellan S. 1998. Phylogenetic relationships of terrestrial Australo-Papuan elapid snakes based on cytochrome *b* and 16S rRNA sequences. *Molecular Phylogenetics and Evolution. In Press*.

Keogh JS, Smith SA. 1996. Taxonomy and natural history of the Australian bandy-bandy snakes (Elapidae: *Vermicella*) with the description of two new species. *Journal of Zoology (London)* **240:** 677–701.

Klassen GJ, Moor RD, Locke A. 1991. Consistency indices and random data. *Systematic Zoology* **40:** 446–457.

Kluge AG, Farris JS. 1969. Quantitative phylogenetics and the evolution of anurans. *Systematic Zoology* **18:** 1–32.

Knight A, Mindell DP. 1993. Substitution bias, weighting of DNA sequence evolution, and the phylogenetic position of Fea's viper. *Systematic Biology* **42:** 18–31.

Knight A, Mindell DP. 1994. On the phylogenetic relationships of Colubrinae, Elapidae, and Viperidae and the evolution of front-fanged venom systems in snakes. *Copeia* **1994:** 1–9.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Science USA* **86:** 6196–6200.

Kraus F, Mink DG, Brown WM. 1996. Crotaline intergeneric relationships based on mitochondrial DNA sequence data. *Copeia* **1996:** 763–773.

Kumar S, Tamura K, Nei M. 1993. *MEGA: Molecular Evolutionary Genetics Analysis, Version 1.01*. Pennsylvania State University, University Park, PA 16802.

Leviton AE. 1964. Contributions to a review of Philippine snakes, III. The genera *Maticora* and *Calliophis*. *Philippine Journal of Science* **92:** 523–550.

Lopez JV, Yuhki N, Masuda R, Modi V, O'Brian SJ. 1994. *Numt*, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. *Journal of Molecular Evolution* **39:** 174–190.

Lopez TJ, Maxson LR. 1996. Albumin and mitochondrial DNA evolution: Phylogenetic implications for colubrine snakes (Colubridae: Colubrinae). *Amphibia-Reptilia* **17:** 247–259.

Maddison DR. 1991. The discovery and importance of multiple islands of most-parsimonious trees. *Systematic Zoology* **40:** 315–328.

Maddison WP, Maddison DR. 1992. *MacClade: Analysis of phylogeny and character evolution. Version 3.04*. Sunderland, Massachusetts: Sinnauer Associates.

Mao SH, Chen BY, Chang HM. 1977. The evolutionary relationships of sea snakes suggested by immunological cross-reactivity of transferrin. *Comparative Biochemistry and Physiology* **57A:** 403–406.

Mao SH, Chen BY, Yin FY, Guo YW. 1983. Immunotaxonomic relationships of sea snakes and terrestrial elapids. *Comparative Biochemistry and Physiology* **74A:** 869–872.

Mao SH, Dessauer HC, Chen BY. 1978. Fingerprint correspondence of haemoglobins and the relationships of sea snakes. *Comparative Biochemistry and Physiology* **59B:** 353–361.

McCarthy CJ. 1985. Monophyly of the elapid snakes (Serpentes: Reptilia). An assessment of the evidence. *Zoological Journal of the Linnean Society* **83:** 79–93.

McCarthy CJ. 1986. Relationships of the laticaudine sea snakes (Serpentes: Elapidae: Laticaudinae). *Bulletin of the British Museum of Natural History (Zoology)* **50:** 127–161.

McDowell SB. 1967. *Aspidomorphus*, a genus of New Guinea snakes of the family Elapidae, with notes on related genera. *Journal of Zoology (London)* **151:** 497–543.

McDowell SB. 1968. Affinities of the snakes usually called *Elaps lacteus* and *E. dorsalis*. Zoological Journal of the Linnean Society **47:** 561–578.

McDowell SB. 1969a. *Toxicocalamus*, a New Guinea genus of snakes of the family Elapidae. *Journal of Zoology (London)* **159:** 443–511.

McDowell SB. 1969b. Notes on the Australian sea-snake *Ephalophis greyi* M. Smith (Serpentes: Elapidae, Hydrophiinae) and origin and classification of sea-snakes. *Zoological Journal of the Linnean Society* **48:** 333–349.

McDowell SB. 1970. On the status and relationships of the Solomon Island elapid snakes. *Journal of Zoology (London)* **151:** 145–190.

McDowell SB. 1972. The genera of sea-snakes of the *Hydrophis* group (Serpentes: Elapidae). *Transactions of the Zoological Society of London* **32:** 189–247.

McDowell SB. 1986. The architecture of the corner of the mouth of colubroid snakes. *Journal of Herpetology* **20:** 353–407.

McDowell SB. 1987. Systematics. In: Siegel RA, Collins JT, Novak SS, eds. *Snakes: Ecology and Evolutionary Biology*. New York: McGraw Hill, 3–50.

Mengden GA. 1983. The taxonomy of Australian elapid snakes, a review. *Records of the Australian Museum* **35:** 195–222.

Mengden GA. 1985. Australian elapid phylogeny: A summary of the chromosomal and electrophoretic data. In: Grigg G, Shine R, Ehmann H, eds. *Biology of Australasian Frogs and Reptiles*. Sydney: Surrey Beatty and Sons, 185–192.

Minton SA. 1978. Serological relationships of some Philippine sea snakes. Copeia **1978:** 151–154.

Minton SA. 1981. Evolution and distribution of venomous snakes. In: Banks CB, Martin AA, eds. *Proceedings of the Melbourne Herpetological Symposium*. Melbourne, 55–59.

Minton SA, da Costa MS. 1975. Serological relationships of sea snakes and their evolutionary implications. In: Dunson W, ed. *The Biology of Sea Snakes*. Baltimore: University Park Press, 33–55.

Rabb GB, Marx H. 1973. Major ecological and geographical patterns in the evolution of colubroid snakes. *Evolution* **27:** 69–83.

Romer AS. 1956. *Osteology of the Reptiles*. Chicago: University of Chicago Press.

Savitzky AH. 1979. The origin of New World proteroglyphous snakes and its bearing on the study of venom delivery systems in snakes. Unpublished Ph.D Thesis, University of Kansas, Lawrence.

Schwaner TD, Baverstock PR, Dessauer HC, Mengden GA. 1985. Immunological evidence for the phylogenetic relationships of Australian elapid snakes. In: Grigg G, Shine R, Ehmann H, eds. *Biology of Australasian Frogs and Reptiles*. Sydney: Surrey Beatty and Sons, 177–184.

Shine R. 1980a. Ecology of the Australian death adder *Acanthophis antarcticus* (Elapidae): Evidence for convergence with the Viperidae. *Herpetologica* **36:** 281–289.

Shine R. 1980b. Reproduction, feeding and growth in the Australian burrowing snake *Vermicella annulata*. *Journal of Herpetology* **14:** 71–77.

Shine R. 1985. Ecological evidence on the phylogeny of Australian elapid snakes. In: Grigg G, Shine R, Ehmann H, eds. *Biology of the Australasian Frogs and Reptiles*. Sydney: Surrey Beatty and Sons, 255–260.

Shine R, Keogh JS. 1996. Food habits and reproductive biology of the endemic Melanesian elapids: Are tropical snakes really different? *Journal of Herpetology* **30:** 238–247.

Slowinski JB. 1989. The interrelationships of Laticaudine sea snakes based on the amino acid sequences of short-chain neurotoxins. *Copeia* **1989:** 783–788.

Slowinski JB. 1994a. A phylogenetic analysis of *Bungarus* (Elapidae) based on morphological characters. *Journal of Herpetology* **28:** 440–446.

Slowinski JB. 1994b. The diet of kraits (Elapidae: *Bungarus*). *Herpetological Review* **25:** 51–53.

Slowinski JB. 1995. A phylogenetic analysis of the New World coral snakes (Elapidae: *Leptomicrurus*, *Micruroides*, and *Micrurus*) based on allozyme and morphological characters. *Journal of Herpetology* **29:** 325–338.

Slowinski JB, Knight A, Rooney AP. 1997. Inferring species trees from gene trees: A phylogenetic analysis of the Elapidae (Serpentes) based on the amino acid sequences of venom proteins. *Molecular Phylogenetics and Evolution* **8:** 349–362.

Smith HM, Smith RB, Sawin HL. 1977. A summary of snake classification (Reptilia: Serpentes). *Journal of Herpetology* **11:** 115–121.

Smith M. 1926. *Monograph of the Sea-Snakes*. London: Oxford University Press.

Storr GM. 1964. Some aspects of the geography of Australian reptiles. *Senckenbergiana Biologica* **45:** 577–589.

Sullivan J. 1996. Combining data with different distributions of among-site rate variation. *Systematic Biology* **45:** 375–380.

Swofford DL. 1993. *PAUP: Phylogenetic Analysis Using Parsimony. Version 3.1.1*. Champaign: Illinois Nat. Hist. Survey.

Tamiya N. 1985. A comparison of amino acid sequences of neurotoxins and of phospholipases of some Australian elapid snakes with those of other proteroglyphous snakes. In: Grigg G, Shine R, Ehmann H, eds. *Biology of Australasian Frogs and Reptiles*. Sydney: Surrey Beatty and Sons, 209–219.

Tyler MJ. 1979. Herpetofaunal relationships of South America with Australia. In: Duellman WE, ed. *The South American Herpetofauna: Its Origins, Evolution and Dispersal*. Lawrence: University of Kansas, 73–106.

Underwood G. 1967. *A Contribution to the Classification of Snakes*. London: Trustees British Museum (Natural History).

Underwood G. 1979. Classification and distribution of venomous snakes in the world. In: Lee CY, ed. *Handbook of Experimental Pharmacology. Vol. 52. Snake Venoms*. Berlin: Springer-Verlag, 16–40.

Underwood G, Kochva E. 1993. On the affinities of the burrowing asps *Atractaspis* (Serpentes: Atractaspididae). *Zoological Journal of the Linnean Society* **107:** 3–64.

Voris HK. 1977. A phylogeny of the sea snakes (Hydrophiidae). *Fieldiana, Zoology* **70:** 79–166.

Voris HK, Voris HH. 1983. Feeding strategies in marine snakes: An analysis of evolutionary, morphological, behavioral and ecological relationships. *American Zoologist* **23:** 411–425.

Wallach V. 1985. A cladistic analysis of the terrestrial Australian elapidae. In: Grigg G, Shine R, Ehmann H, eds. *Biology of the Australasian Frogs and Reptiles*. Sydney: Surrey Beatty and Sons, 223–253.

Wallach V, Jones GS. 1992. *Cryptophidion annamense*, a new genus and species of cryptozoic snake from Vietnam (Reptilia: Serpentes). *Cryptozoology* **11:** 1–37.

Wüster W, Thorpe RS. 1989. Population affinities of the asiatic cobra (*Naja naja*) species complex in south-east Asia: Reliability and random resampling. *Biological Journal of the Linnean Society* **36:** 391–409.

Wüster W, Thorpe RS. 1990. Systematics and biogeography of the Asiatic cobra (*Naja naja*) species complex in the Philippine Islands. In: Peters G, Hutterer R, eds. *Vertebrates in the Tropics*. Bonn: Museum Alexander Koenig, 333–344.

Wüster W, Thorpe RS. 1992a. Dentitional phenomena in cobras revisited: Spitting and fang structure in the asiatic species of *Naja* (Serpentes: Elapidae). *Herpetologica* **48:** 424–434.

Wüster W, Thorpe RS. 1992b. Asiatic cobras: Population systematics of the *Naja naja* species complex (Serpentes: Elapidae) in India and central Asia. *Herpetologica* **48:** 69–85.

Wüster W, Thorpe RS, Cox MJ, Pintakune P, Nabhitabhata J. 1995. Population systematics of the snake genus *Naja* (Reptilia: Serpentes: Elapidae) in Indochina: Multivariate morphometrics and comparative mitochondrial DNA sequencing (cytochrome oxidase I). *Journal of Evolutionary Biology* **8:** 493–510.

Zaher H. 1994. Comments of the evolution of the jaw adductor musculature of snakes. *Zoological Journal of the Linnean Society* **111:** 339–384.

Zhang DX, Hewitt GM. 1996. Nuclear integrations: Challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution* **11:** 247–251.