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Phylogenetic Relationships of Terrestrial Australo-Papuan Elapid Snakes (Subfamily Hydrophiinae) Based on Cytochrome *b* and 16S rRNA Sequences

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Phylogenetic relationships among the venomous Australo-Papuan elapid snake radiation remain poorly resolved, despite the application of diverse data sets. To examine phylogenetic relationships among this enigmatic group, portions of the cytochrome *b* and 16S rRNA mitochondrial DNA genes were sequenced from 19 of the 20 terrestrial Australian genera and 6 of the 7 terrestrial Melanesian genera, plus a sea krait (*Laticauda*) and a true sea snake (*Hydrelaps*). These data clarify several significant issues in elapid phylogeny. First, Melanesian elapids form sister groups to Australian species, indicating that the ancestors of the Australian radiation came via Asia, rather than representing a relict Gondwanan radiation. Second, the two major groups of sea snakes (sea kraits and true sea snakes) represent independent invasions of the marine environment. Third, the radiation of viviparous Australian elapids is much older than has been suggested from immunological data. Parsimony analyses were unable to resolve relationships among the Australian radiation, a problem previously encountered with analyses of other (morphological, electrophoretic, karyotypic, immunological) data sets on these species. These data suggest that the reason for this continued difficulty lies in the timing of speciation events: the elapids apparently underwent a spectacular adaptive radiation soon after reaching Australia, such that divergences are ancient even within genera. Indeed, intrageneric divergences are almost as large as intergeneric divergences. Although this timing means that our sequence data cannot fully resolve phylogenetic relationships among the Australian elapids, the data suggest a close relationship of the following clades: *Pseudonaja* with *Oxyuranus; Ogmodon* with *Toxicocalamus; Demansia* with *Aspidomorphus; Echiopsis* with *Denisonia;* the "*Notechis*" lineage with *Drysdalia coronoides;* and *Rhinoplocephalus* and *Suta* with *Drysdalia coronata.* At least two of the Australian genera (*Drysdalia* and *Simoselaps*) appear to be paraphyletic. These sequence data support many of the conclusions reached by earlier studies using other types of data, but additional information will be needed before the phylogeny of the Australian elapids can be fully resolved.

Key Words: **mitochondrial DNA; cytochrome *b;* 16S rRNA; reptile; snake; elapid; sea snake; Australia; New Guinea; Pacific; Asia; biogeography.**

INTRODUCTION

The diverse, cosmopolitan, and medically important elapid snakes are a monophyletic clade of approximately 300 species and 61 genera (Golay *et al.*, 1993) primarily defined by their unique venom delivery system (two permanently erect canaliculate fangs at the end of the maxilla; McDowell, 1968; McCarthy, 1985). Relationships both among and within major elapid clades have been the subject of much debate, but most authorities divide the elapid snakes into two major lineages based on morphological characters associated with cranial kinesis (McDowell, 1970): the "palatine erectors" (Afro-Asian cobras, Asian kraits, Asian and American coral snakes, the sea krait *Laticauda,* and the Bougainville Island *Parapistocalamus*) and the "palatine draggers" (terrestrial Australo-Papuan elapids except *Parapistocalamus,* plus hydrophiid sea snakes). This division largely corresponds to the commonly used families Elapidae and Hydrophiidae (or subfamilies Elapinae and Hydrophiinae) for "elapines" and "hydrophiines" (Smith *et al.*, 1977), except that recent studies have supported the affinity of *Laticauda* with hydrophiines (Cadle and Gorman, 1981; Mao *et al.*, 1983; Schwaner *et al.*, 1985; Slowinski *et al.*, 1997; Keogh, 1997, 1998a). These phylogenetic conclusions have been based on an array of data sets involving external and internal morphology, immunological distances, and biochemical traits. Recently, Keogh (1998a) examined relationships among representatives of the major elapid clades based on mitochondrial DNA se-

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quences. In this paper these data plus homologous sequences gathered from representatives of the major hydrophiine lineages are used to present a more detailed analysis of phylogenetic relationships among hydrophiine snakes.

McDowell's (1970) strong evidence for hydrophiine monophyly has been accepted in recent elapid classifications (Smith *et al.*, 1977; Golay *et al.*, 1993). With the addition of *Laticauda*, hydrophiine monophyly has been well supported by other diverse data sets including immunological distance (Cadle and Gorman, 1981), venom protein sequences (Slowinski *et al.*, 1997), and mitochondrial DNA sequences (Keogh, 1998a). The terrestrial hydrophiine radiation is entirely distributed in the Australo-Papuan region and comprises 27 genera and 102 species (Table 1). The radiation includes a number of medically important groups such as the viviparous tiger snakes, copperheads, rough-scaled snake, and death adders and the oviparous brown snakes, black snakes, taipans, whip snakes, and New Guinea small-eyed snake. However, most of the radiation is composed of relatively small, primarily fossorial, and innocuous species.

Much of the work devoted to terrestrial hydrophiine relationships has been concerned only with the Australian species, in an attempt to address the high degree of taxonomic instability which has dominated the group (reviewed in Mengden, 1983), despite broad consensus that the endemic Melanesian genera (with the possible exception of *Parapistocalamus*) and 20 Australian genera are members of a single clade (McDowell, 1967, 1969a, 1969b, 1970; Schwaner *et al.*, 1985; Keogh, 1998a). McDowell (1967, 1969a, 1969b, 1970) was the first to address the problem within an evolutionary framework; he identified a number of putative "natural groups" through the descriptive study of cranial osteology, myology, and hemipenis anatomy. McDowell's phylogenetic hypotheses stimulated a series of studies that addressed relationships among Australian elapids with diverse data sets, including (i) soft anatomy and other primarily morphological characters (Wallach, 1985, Fig. 1a); (ii) karyotypes and allozyme electrophoresis (Mengden, 1985a, Fig. 1b); (iii) immunological distance (Schwaner *et al.*, 1985); (iv) ecology and morphology (Shine, 1985); and (v) hemipenis anatomy (Keogh, 1998b, Fig. 1c). The 1985 studies culminated in a well-accepted generic-level classification of Australian elapids (Hutchinson, 1990).

Although the conclusions of these studies differed in several respects, their authors were in broad agreement on some significant points. For example, Shine's (1985) suggestion that the viviparous Australian elapids comprise a monophyletic lineage (with the exception of *Pseudechis porphyriacus*) was supported by evidence from internal and external anatomy, karyology, and electrophoresis (Wallach, 1985; Mengden, 1985a) and is consistent with a more recent data set on hemipenis morphology (Keogh, 1998b). Further, most authorities accept that true sea snakes evolved from within this viviparous lineage (McDowell, 1969b, 1972; Minton and da Costa, 1975; Cadle and Gorman, 1981; Minton, 1981; Schwaner *et al.*, 1985; Gopalakrishnakone and Kochva, 1990; Slowinski *et al.*, 1997; Keogh, 1998a). Nonetheless, a number of conflicts remain, stimulating us to gather sequence data in an attempt to clarify phylogenetic relationships among the Australian and Melanesian proteroglyphs and their marine relatives.

TABLE 1

List of Terrestrial Hydrophiine Genera with Common Names, Number of Species (Primarily after Hutchinson, 1990), and Whether They Are Oviparous or Viviparous

Genus	Common name	Number of species	Oviparous/viviparous
Australian elapids			
Acanthophis	Death adders	3+	V
Austrelaps	Copperheads	3	V
Cacophis	Crowned snakes	4	O
Demansia	Whip snakes	6	O
Denisonia	Ornamental snakes	2	V
Drysdalia	White-lipped snakes	4	V
Echiopsis	Bardick	2	V
Elapognathus	Little brown snake	1	V
Furina	Naped snakes	5	O
Hemiaspis	Swamp snakes	2	V
Hoplocephalus	Broad-headed snakes	3	V
Notechis	Tiger snakes	2	V
Oxyuranus	Taipans	2	O
Pseudechis	Black snakes	6	O
Pseudonaja	Brown snakes	7	O
Rhinoplocephalus	Small-eyed snake	6	V
Simoselaps	Coral snakes	12	O
Suta	Black-headed snakes	10	V
Tropidechis	Rough-scaled snake	1	V
Vermicella	Bandy-bandy snakes	5	O
Melanesian elapids			
Aspidomorphus	NG crowned snakes	3	O
Loveridgelaps	Solomons small-eyed snake	1	O?
Micropechis	NG small-eyed snake	1	O
Ogmodon	Fijian bola	1	O
Parapistocalamus	Hediger's coral snake	1	O?
Salomonelaps	Solomons coral snake	1	O
Toxicocalamus	NG forest snakes	9	O

Note. All terrestrial hydrophiine genera were sampled except *Elapognathus* and *Parapistocalamus* for which no tissue samples were available. The division between Australian and Melanesian genera presented here simply represents major geographic distributions. Some "Australian" species also occur in New Guinea (NG).

MATERIALS AND METHODS

Selection of Taxa

Representative species were chosen to maximize the number of phylogenetic hypotheses we could test. Por-

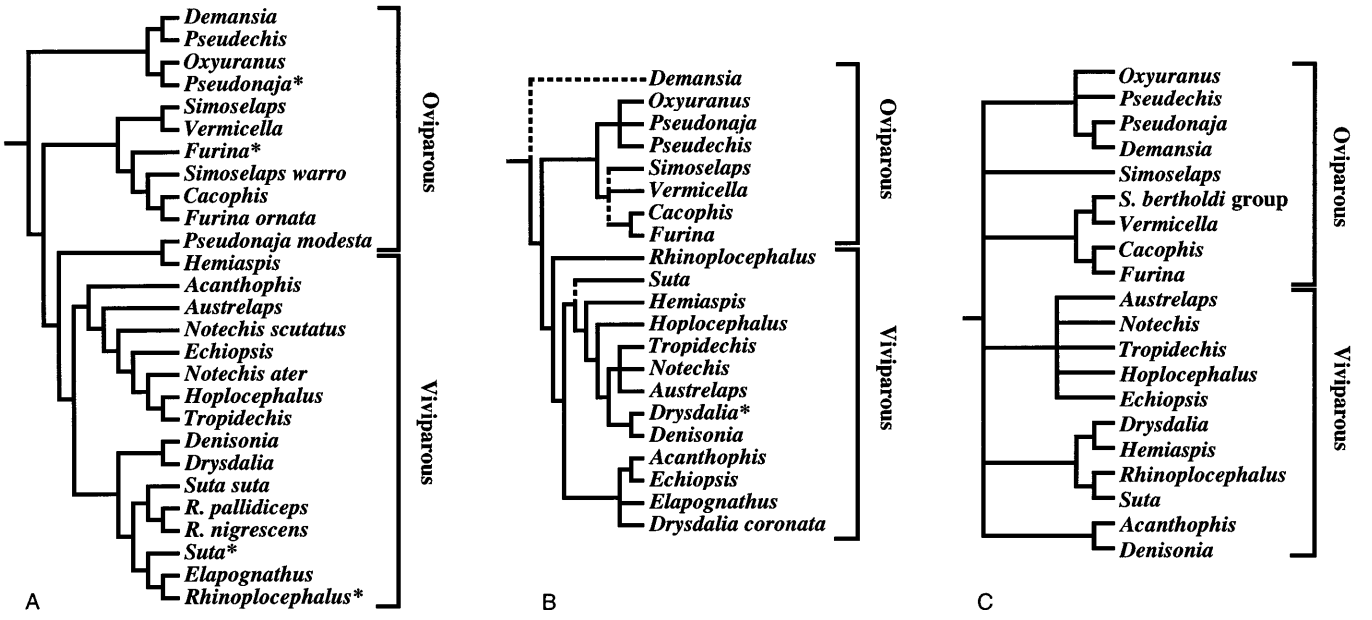

FIG. 1. Phylogenetic hypotheses of relationships for terrestrial Australian elapid snakes. Melanesian elapids have not been incorporated previously into an explicitly drawn phylogenetic tree. (A) Phylogenetic tree redrawn from Wallach (1985) based on a cladistic analysis of 50 primarily morphological characters. (B) Phylogenetic tree redrawn from Mengden (1985) based on a compilation of karyological and electrophoretic data. Species names on Wallach's and Mengden's trees have been updated to be consistent with the recent taxonomy of Hutchinson (1990). Genera marked with an asterisk were found to be polyphyletic by these authors. (C) Phylogenetic tree by Keogh (1998b) based on hemipenial morphology. With the exception of *Pseudonaja modesta* in Wallach's tree, both Wallach and Mengden's analyses support monophyly of the viviparous clade. Keogh's tree is consistent with this hypothesis.

tions of the cytochrome *b* and 16S rRNA mitochondrial genes were sequenced from 64 individuals representing 36 species and 27 genera of terrestrial and marine hydrophiines (Table 2). Of these, 9 species and 9 genera were included as part of a previous study of higher level elapid relationships (Keogh, 1998a). The data set includes 19 of the 20 Australian elapid genera (tissues from the monotypic *Elapognathus minor* were not available) and 6 of the 7 endemic Melanesian elapid genera and species (tissues from the Bougainville Island *Parapistocalamus hedigeri* were not available). A single species was used to represent genera where monophyly is not in question (see Hutchinson, 1990), but multiple congeneric species were examined from a series of genera for which monophyly remains uncertain (*Drysdalia, Pseudechis, Pseudonaja, Rhinoplocephalus,* and *Simoselaps*). Sea kraits (*Laticauda colubrina*) and one true sea snake (*Hydrelaps darwiniensis*) also were included in the analyses to represent the two sea snake radiations.

DNA Sequencing

A 290-bp portion of the cytochrome *b* gene and a 490-bp portion of the 16S rRNA gene were PCR amplified and directly sequenced according to protocols described elsewhere (Keogh, 1998a). Sequences will be deposited in GenBank upon publication.

Testing for Pseudogenes

The amplification of pseudogenes or nuclear paralogues (nonfunctional copies of mitochondrial DNA in the nuclear genome) rather than true mitochondrial DNA can be a significant problem in phylogenetic inference (Zhang and Hewitt, 1996). As obtaining purified mitochondrial DNA from all individuals used in this study was impractical, the presence of pseudogenes was tested for in two ways. Purified mitochondrial DNA was obtained for single individuals of *Pseudonaja textilis* and *Austrelaps superbus* via caesium chloride centrifugation, sequenced for both cytochrome *b* and 16S rRNA, and then compared to sequences obtained via amplifications from salt-extracted total genomic DNA. When tissue sample availability allowed, two individuals of most species also were sequenced for both genes (Table 2). These data on intraspecific variation allowed us to check for sample mixups and PCR contamination and provide a further test of the presence of pseudogenes. Conspecific monophyly in phylogenetic analyses also provided a one-way test of sequence origin.

Phylogenetic Analyses

Cytochrome *b* and 16S rRNA sequences were aligned by eye after initial alignments were made on a restricted number of sequences with the computer pro-

TABLE 2

List of Taxa Sampled in This Study

Taxon	Museum Voucher No. (Tissue No. if different)	Locality
Australian terrestrial elapids		
Acanthophis antarcticus (1)	NTM R17880 (SAM S99)	S Alligator River Floodplains, NT
A. antarcticus (2)	NTM R17881 (SAM T01)	S Alligator River Floodplains, NT
Austrelaps superbus (1)	SAM R19835	Penola S/F, SA
A. superbus (2)	MV D58012 (SAM R58012)	3.5 km SW Mt. Wills, VIC
Cacophis kreffti		
Cacophis squamulosus	SAM R40865 (SAM C02)	Cooranbong, NSW
Demansia atra (1)	(SAM 173)	Jabiru airstrip, NT
D. atra (2)	SAM R29954	Near Humpty Doo, NT
Denisonia devisi (1)	(SAM catalog 5 No. 1)	
D. devisi (2)	(SAM catalog 5 No. 2)	Macquarie Marshes, NSW
Drysdalia coronata (1)	SAM R22966	65 km W. Esperance, WA
D. coronata (2)	SAM R22968	65 km W. Esperance, WA
Drysdalia coronoides (1)	TMH C686 (SAM HM326)	Mt. Rufus, TAS
D. coronoides (2)	TMH C623 (SAM MR184)	Mt. Rufus, TAS
Echiopsis curta (1)	SAM R20837	Carappee Hill Conservation Park, SA
E. curta (2)	SAM RR27494	Hambridge Conservation Park, SA
Furina diadema (1)	SAM R20508	Port Augusta Primary School, SA
F. diadema (2)	SAM R22549	Mambray Creek, Mt. Remarkable National Park, SA
Hemiaspis dameli (1)	SAM catalog 5 No. 4	Dalby, QLD
H. dameli (2)	SAM catalog 5 No. 5	Mullumbimby, NSW
Hemiaspis signata	SAM catalog 5 No. 1	Harrington, NSW
Hoplocephalus bungaroides (1)		
H. bungaroides (2)		
Notechis ater (1)	SAM R31329	Coffin Bay, SA
N. ater (2)	SAM R31604	St. Peter Island, SA
Oxyuranus microlepidotus (1)	SAM R20583	Goyders Lagoon, SA
O. microlepidotus (2)	SAM R26876	Goyders Lagoon, SA
Pseudechis australis (1)	SAM R31703	11 km SSW Maralinga, SA
P. australis (2)	SAM R38475	3 km S NT-SA border, SA
Pseudechis porphyriacus (1)	SAM R25056	5 km E Tungkillo, Harrison Creek, SA
P. porphyriacus (2)	SAM R25297	5 km E Tungkillo, Harrison Creek, SA
Pseudonaja modesta (1)	SAM R21026	Olympic Dam, Roxby Downs, SA
P. modesta (2)	SAM R21028	Olympic Dam, Roxby Downs, SA
Pseudonaja textilis (1)	SAM R25702	7 km NW Nullabor Station, SA
P. textilis (2)	SAM R20756	15 km N Sedan, SA
Rhinoplocephalus bicolor (1)	(SAM catalog 5 No. 1)	
R. bicolor (2)	(SAM catalog 5 No. 2)	
Rhinoplocephalus nigrescens (1)	SAM R44079 (SAM B02)	6.3 km N of Highlands, VIC
R. nigrescens (2)	SAM R21406	Christmas Hills, VIC
Simoselaps bertholdi (1)	SAM R20863	Olympic Dam area, SA
S. bertholdi (2)	SAM R21047	Olympic Dam area, SA
Simoselaps bimaculatus (1)	(SAM catalog 5 No. 1)	
S. bimaculatus (2)	SAM R23353	134 km ENE Laverton, WA
Simoselaps calonotus	SAM R29765	Scarborough Beach, Perth, WA
Simoselaps semifasciatus (1)	SAM R32156 (SAM NP0685)	8 km SW Maralinga, SA
S. semifasciatus (2)	SAM R32157 (SAM NP0686)	8 km SW Maralinga, SA
Suta suta (1)	SAM R20994	5 km NW Billa Kalina Homestead, SA
S. suta (2)	SAM R20995	5 km NW Billa Kalina Homestead, SA
Tropidechis carinatus	SAM R30596	
Vermicella intermedia (1)	SAM R25672	Darwin Area, NT
V. intermedia (2)	SAM R27282	Darwin Area, NT
Melanesian terrestrial elapids		
Aspidomorphus muelleri (1)	SAM CCA0683	Lemkamin, New Ireland Island, PNG
A. muelleri (2)	AM 135504 (SAM 40320)	Parkop Village, West Sepik Province, PNG
Loveridgelaps elapoides	(AM, no number)	
Micropechis ikaheka (1)	SAM 11800	Mt. Menawa, West Sepik Province, PNG
M. ikaheka (2)	AM 135503 (SAM 40306)	Mt. Sumbau, West Sepik Province, PNG
Ogmodon vitianus	(AM, no number)	
Salomonelaps par	(AM, no number)	
Toxicocalamus preussi (1)	AM 136279 (SAM FJ126)	Torricelli Mountains, West Sepik Province, PNG
T. preussi (2)	AM 135505 (SAM40321)	Parkop Village, West Sepik Province, PNG
Sea snake		
Hydrelaps darwiniensis (1)	NTM 16471 (SAM 018)	Home Creek, Bing Bong Station, NT
H. darwiniensis (2)	(SAM S63)	Dinah Beach, NT
Laticauda colubrina (1)	AM 124795 (SAM 4795)	Nagada Harbour (ocean), PNG
L. colubrina (2)	AM 124800 (SAM 4800)	Nagada Harbour (ocean), PNG

Note. Individuals denoted "1" were used in phylogenetic analyses. Museum acronyms are as follows: AM, Australian Museum; SAM, South Australian Museum; MV, Museum of Victoria; NTM, Northern Territory Museum of Arts and Sciences; THM, Tasmanian Museum and Art Gallery, Hobart.

gram CLUSTAL V (Higgins *et al.*, 1991). The 16S rRNA data set contains two unalignable hypervariable regions, one ranging in length from 15 to 35 bp and the other from 6 to 9 bp. These regions were excluded from analyses because site homology could not be confidently ascertained. The data sets were combined to maximize the number of potential synapomorphies available to define nodes and analyzed by maximum parsimony (MP) methods. Because intraspecific variation was negligible (see below), a single individual (denoted "1" in Table 2) was selected as a representative for those species where multiple individuals were sequenced to reduce computational time.

Phylogenetic analyses were performed on two subsets of the combined data because of the large number of taxa included in this study and the consequent enormous number of possible trees. Given the considerable evidence supporting monophyly of the viviparous species (see above), analyses were performed on the following groups: (i) all oviparous species with the addition of *Notechis ater* as a representative of the viviparous lineage and (ii) all viviparous species plus the oviparous *Laticauda*, to test hypotheses of sea snake origins. The outgroup condition was represented by cytochrome *b* and 16S rRNA sequences from *Naja naja* and *Laticauda* for the oviparous and viviparous groups, respectively (Keogh, 1998a).

A multitude of weighting schemes are available for nucleotide sequence data, and there is no clear consensus on which is best. In particular, choosing an overall transition/transversion ratio can be difficult because in general the ratio decreases with increasing genetic distance. Thus, any overall estimate of TI/TV ratio on data sets which include both distantly and closely related species will be wrong for most pairs of taxa and is generally an underestimate (Purvis and Bromham, 1997). Similarly, third-codon positions of protein-coding genes are often phylogenetically informative for closely related taxa, but may amount to random noise in comparisons between distantly related taxa.

Given these difficulties, the following weightings were employed to examine the robustness of tree topology to different schemes. Transversion were weighted over transitions by factors of 2, 5, and 10 (hereafter abbreviated "2TV," "5TV," and "10TV") and third positions of the protein-coding cytochrome *b* gene were subjected to the same weightings as all other characters, or third-position transversions only were used ("3tv only") or were excluded ("-3rd"). Finally, all sites were subjected to transversion parsimony ("TV only"), both including and excluding cytochrome *b* third positions. Thus, a total of 11 separate analyses were performed on each subset of taxa. Analyses were followed by successive approximations (Farris, 1969) based on the rescaled-consistency index (as recommended by Horovitz and Meyer, 1995). All analyses were implemented with the computer program PAUP 3.1.1 using heuristic searches (Swofford, 1993). Because these searches cannot guarantee that the most parsimonious trees will be found, all analyses were replicated 100 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 g_1 statistic (Hillis, 1991; Hillis and Huelsenbeck, 1992), the consistency index (CI, Kluge and Farris, 1969), and 1000 bootstrap pseudo-replicates (Felsenstein, 1985).

RESULTS

Intraspecific Variation and Pseudogenes

Two individuals were sequenced for each of 29 species. In total, 16 species displayed no intraspecific variation in at least one of the two genes (*Acanthophis antarcticus, Denisonia devisi, Drysdalia coronata, Echiopsis curta, Furina diadema, Hoplocephalus bungaroides, Hemiaspis dameli, L. colubrina, Neelaps bimaculatus, N. ater, Oxyuranus microlepidotus, P. porphyriacus, Pseudonaja modesta, Rhinoplocephalus nigrescens, Simoselaps semifasciatus,* and *Suta suta*). Of these, 6 species displayed no intraspecific variation in either gene (*H. bungaroides, H. dameli, P. porphyriacus, P. modesta, R. nigrescens,* and *S. semifasciatus*). Of the remaining 11 species, the maximum cytochrome *b* genetic distance was 2.1% (between *E. curta* specimens) and the maximum 16S rRNA genetic distance was 0.4% (between *N. ater* specimens). In addition, 7 other taxa (*A. superbus, Drysdalia coronoides, Micropechis ikaheka, Pseudechis australis, P. textilis, Toxicocalamus preussi,* and *Vermicella intermedia*) displayed little intraspecific variation in either gene (maximum of 4.9% between *A. superbus* specimens for cytochrome *b* and a maximum of 0.7% between *P. australis* and *P. textilis* specimens for 16S rRNA). Two species displayed larger intraspecific genetic distances in the cytochrome *b* and 16S rRNA data sets, respectively: *D. atra* (10.3 and 1.6%) and *Aspidomorphus muelleri* (15.8 and 2.9%). Nonetheless, all preliminary phylogenetic analyses united the two individuals of these species as sister taxa.

Both light and heavy strands of cytochrome *b* and 16S rRNA *A. superbus* and *P. textilis* sequences amplified from purified mitochondrial DNA and total cellular DNA were identical, providing no evidence of the presence of nuclear pseudogenes in either species. However, the cytochrome *b* sequences from *H. darwiniensis* have been identified as a nuclear pseudogene (Keogh, 1998a) and hence were excluded from all analyses. Given that sequences from the purified and total cellular DNA were identical, and that intraspecific variation was minimal, the null hypothesis that these

sequences are of mitochondrial origin cannot be rejected (Zhang and Hewitt, 1996).

Phylogenetic Information

After the exclusion of the second individual of a species, but including *N. naja* as a representative elapine outgroup, the cytochrome *b* data set was composed of 290 aligned sites, 160 of which were variable and 131 potentially informative under parsimony. Cytochrome *b* sequences could not be obtained from *Ogmodon vitianus* or *Simoselaps bertholdi* despite repeated attempts. The 16S rRNA data set was composed of 443 aligned sites (after the exclusion of the hypervariable regions), 124 of which were variable and 70 potentially informative under parsimony. 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: cytochrome *b* $g_1 = -0.313$ ($P < 0.01$), 16S rRNA $g_1 = -0.665$ ($P < 0.01$), combined $g_1 = -0.531$ ($P < 0.01$). As expected, cytochrome *b* third-codon positions were more variable than first and second positions, but third positions also contained phylogenetic signal (as evidenced by a significant g_1 statistic calculated for 10,000 random trees generated from third-codon positions only: $g_1 = -0.219$, $P < 0.01$).

Maximum Parsimony Analyses

Results of the various analyses for the oviparous and viviparous taxa are shown in Table 3. In evaluating these results, we take topologies which were stable under various weighting schemes as meaningful, even when bootstrap values were low.

Oviparous taxa. The Solomon Islands *Salomonelaps par* formed the sister taxon to other hydrophiines in all analyses (Figs. 2, 3a, and 3b). Other Melanesian elapids (*Loveridgelaps elapoides, M. ikaheka, T. preussi,* and *O. vitianus*) consistently formed sister groups to Australian species (with the exception of *A. muelleri*), although the branch order was unstable (Figs. 2, 3a, and 3b). Among these taxa, *T. preussi* and *O. vitianus* formed sister taxa in most analyses (Figs. 3a and 3b), corroborating morphological data. The fact that successive approximations sometimes united *Ogmodon* with *Demansia atra* (Fig. 2) is probably an artifact, due to the lack of data for *Ogmodon* cytochrome *b* sequences.

Simoselaps semifasciatus and *S. bertholdi* consistently formed a well-supported clade in all analyses (Figs. 3a and 3b), but the relationship of *Simoselaps* "*Neelaps*" *calonotus* and *Simoselaps* "*Neelaps*" *bimaculatus* was unstable under different weighting schemes. "*Neelaps*" *bimaculatus* formed a monophyletic clade with *S. semifasciatus* and *S. bertholdi* in most analyses which included third positions, including the transversion-only analysis (2TV, 3tv only; 5TV; 10TV; TV only, Fig. 3a). "*Neelaps*" *calonotus* was never associated with these species, and instead formed a group with the Fijian *O. vitianus* (10TV, 3tv only; 10TV, -3rd; TV only; TV only, -3rd), the Australian *V. intermedia* (5TV; 10TV; TV only; TV only, -3rd), or the New Guinea *M. ikaheka* (2TV, 3tv only; 2TV, -3rd; 5TV, 3tv only; 5TV, -3rd; Fig. 3b). However, only one of these relationships (that between "*Neelaps*" *calonotus* and *M. ikaheka*) was supported by bootstrap values.

Demansia atra and the New Guinea *A. muelleri* were united as sister taxa in some analyses (2TV; 2TV, 3tv only; 10TV), and successive approximations of the majority of analyses united these species. Together, these species shared various close associations with *Cacophis, Furina,* and *Vermicella* (2TV, -3rd; 5TV; 5TV, 3tv only; 5TV, -3rd; 10TV, 3tv only; 10TV, -3rd), and these last genera were linked with one another. However, the topology within this group was highly unstable. Successive approximations always united at

TABLE 3

Summary of Phylogenetic Analyses on the Oviparous and Viviparous Taxa Showing Number of Most Parsimonious Trees, Tree Lengths, and Individual Tree Consistency Indices (CI) Obtained under Various Weighting Schemes

	Number of trees	Length	CI
Oviparous taxa			
All sites weighted equal			
2TV	15 (1)	1,087 (278)	0.47 (0.74)
5TV	1 (1)	1,784 (412)	0.42 (0.82)
10TV	2 (1)	2,933 (707)	0.42 (0.81)
TV only	19 (7)	228 (65)	0.42 (0.77)
Third-position transversions only			
2TV	11 (1)	679 (192)	0.43 (0.79)
5TV	1 (1)	1,017 (310)	0.42 (0.83)
10TV	3 (1)	1,576 (501)	0.42 (0.83)
Third positions excluded			
2TV	14 (1)	554 (177)	0.49 (0.83)
5TV	6 (1)	893 (299)	0.48 (0.84)
10TV	17 (1)	451 (506)	0.48 (0.84)
TV only	3200*	111	
Viviparous taxa			
All sites weighted equal			
2TV	2 (1)	616 (177)	0.52 (0.83)
5TV	4 (1)	958 (316)	0.52 (0.83)
10TV	2 (1)	1,523 (522)	0.52 (0.83)
TV only	59 (1)	113 (506)	0.51 (0.76)
Third-position transversions only			
2TV	38 (3)	364 (137)	0.52 (0.80)
5TV	6 (1)	554 (210)	0.52 (0.79)
10TV	2 (1)	864 (331)	0.52 (0.83)
Third positions excluded			
2TV	4 (3)	309 (115)	0.60 (0.88)
5TV	1 (1)	498 (184)	0.58 (0.90)
10TV	1 (1)	808 (315)	0.58 (0.90)
TV only	277 (2)	62 (248)	0.59 (0.91)

Note. Tree lengths and consistency indices after successive approximations are shown in parentheses. The transversions-only analysis without cytochrome *b* third positions of the oviparous taxa had to be stopped at 3,200 trees due to computer limitations.

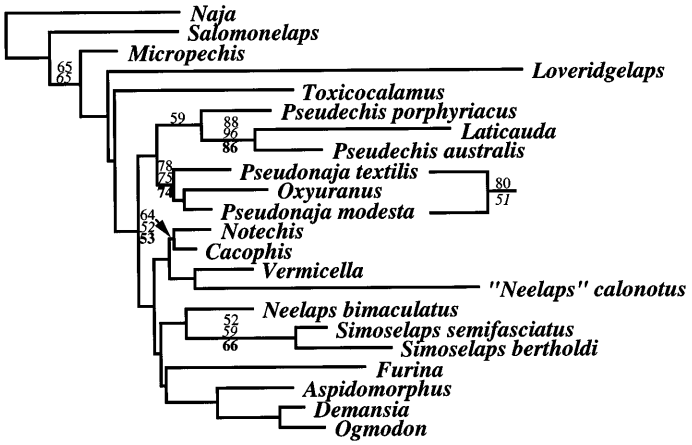

FIG. 2. Phylogenetic relationships among oviparous hydrophiine elapid snakes plus the viviparous *Notechis ater* based on the combined cytochrome *b* and 16S rRNA data sets when cytochrome *b* third positions are weighted the same as all other variable sites. The tree is the single most parsimonious tree generated after one round of successive approximations in the 10TV analysis. Numbers represent bootstrap values from 1000 pseudoreplicates for 2TV (plain type, above the node), 5TV (italics, above the node), and 10TV (boldface type, below the node) analyses.

least three of the genera (in various combinations) and when transversions only were considered, a monophyletic clade was formed by *Aspidomorphus, Cacophis, Furina,* and *Demansia.* In the transversion-only analyses, *N. ater* (included to represent the viviparous lineage) grouped with "*Neelaps*" *calonotus, O. vitianus,* and *Vermicella.* However, many other analyses grouped *Notechis* with *Cacophis* (2TV; 2TV, 3tv only; 5TV; 5TV, 3tv only; 5TV, -3rd; 10TV).

These data suggest that the genus *Pseudechis,* as represented by *P. australis* and *P. porphyriacus,* is paraphyletic with respect to *Laticauda.* The sea krait consistently grouped with the *Pseudechis* species in all analyses (Figs. 2, 3a, and 3b). The relationship of *P. australis* with *Laticauda* was consistently supported in the analyses, regardless of whether cytochrome *b* third positions were included, and under all weighting schemes (including transversions only). However, it is worth noting that *Pseudechis* emerged as monophyletic when *Laticauda* were excluded from the analyses. Further, when *Pseudechis* species were removed, *Laticauda* consistently grouped with the Melanesian elapids. The close relationship of *P. textilis, P. modesta,* and *O. microlepidotus* was evident in all analyses and was supported by moderate to high bootstrap values. However, the *Pseudonaja* species were not consistently monophyletic with respect to *Oxyuranus,* with successive approximations tending to unite *P. textilis* and *Oxyuranus* (Figs. 2, 3a, and 3b).

Viviparous taxa. The close relationships of the taxa comprising the "*Notechis*" lineage (*Austrelaps, Hoplocephalus, Notechis,* and *Tropidechis*) were well supported in virtually all analyses, as was the association of *D. coronoides* with these species. In particular, the sister-group relationship of *Notechis* and *Tropidechis* was supported by very high bootstrap values. However, the relationships of these genera to the other three depended upon the weighting scheme used, with *Hoplocephalus* forming the sister to *Notechis* and *Tropidechis* (2TV, 5TV; 10TV; Fig. 4a), *Austrelaps* forming the sister group to these three genera (2TV, 3tv only; 2TV, -3rd; 5TV, 3tv only; 5TV, -3rd; 10TV, 3tv only; 10TV, -3rd; TV only, -3rd; Figs. 4b and 4c), or *Hoplocephalus* and *D. coronoides* united as a sister clade to the other three genera (2TV, 3tv only; 5TV, 3tv only; 10TV, 3tv only; 10TV, -3rd; Figs. 4b and 4c). None of the analyses suggested that the two *Drysdalia* species sampled are monophyletic.

Monophyly of *Rhinoplocephalus* was well supported by the sequence data in all analyses except one which united *Rhinocephalus bicolor* with *D. coronata* (2TV, 3tv only) and another which united *R. nigrescens* and *Suta* (TV only; TV only, -3rd). *Suta* and *D. coronata* formed a sister clade to the *Rhinoplocephalus* species (2TV; 5TV; 10TV; Fig. 4a) or *D. coronata* and *Suta* formed respective sister groups to the *Rhinoplocephalus* (5TV, 3tv only; 5TV, -3rd; 10TV, 3tv only; 10TV, -3rd; Figs. 4b and 4c). Neither of these alternative topologies was strongly supported.

Hemiaspis monophyly was supported by high bootstrap values in virtually all analyses. Only one analysis produced trees that separated the two *Hemiaspis* species (2TV, -3rd). However, the sister-group relationships of *Hemiaspis* were inconsistent under different weighting schemes. *Hemiaspis* variously formed the sister group to the *Notechis* lineage (2TV), to the other viviparous taxa except the *Notechis* lineage (5TV; 10TV; TV only; TV only, -3rd; Fig. 4a), to a clade formed by *Echiopsis, Denisonia,* and *Acanthophis* (5TV, 3tv only; 5TV, -3rd; 10TV, 3tv only; Fig. 4b), or to *Echiopsis* only

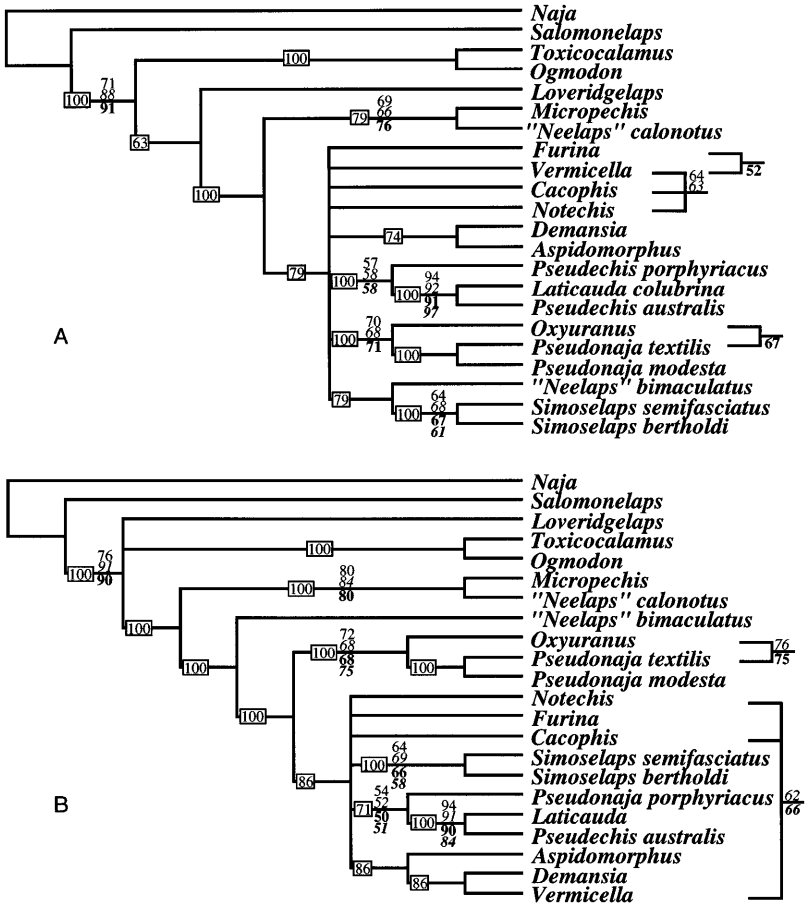

FIG. 3. Phylogenetic relationships among oviparous hydrophiine elapid snakes plus the viviparous *Notechis ater* based on the combined cytochrome *b* and 16S rRNA data sets when cytochrome *b* third positions are subjected to transversion-only parsimony ("3tv only") or were excluded ("−3rd"). (A) Majority rule consensus (proportions in boxes) of 11 trees generated from the 2TV, 3tv only analysis. (B) Majority rule consensus of 14 trees generated from the 2TV, −3rd analysis. Numbers represent bootstrap values from 1000 pseudoreplicates when cytochrome *b* third positions are treated as above and other sites are weighted 2TV (plain text, above the node), 5TV (italics, above the node), 10TV (boldface type, below the node), and TV only (boldface italics, below the node).

(5TV, -3rd; 10TV, -3rd; Fig. 4c). In additional analyses that included the true sea snake *H. darwiniensis*, the sea snake always formed a sister clade to the two *Hemiaspis* species. *Hydrelaps* was never associated with *Laticauda* in these analyses.

Echiopsis and *Denisonia* formed another well-supported clade in many analyses, including the transversions-only analyses (2TV; 2TV, 3tv only; 5TV; 10TV; TV only; Fig. 4a). *Denisonia* and *Acanthophis* formed a weakly supported clade; their sister group was generally either *Echiopsis* (5TV, 3tv only; 10TV, 3tv only; Fig. 4b), or *Echiopsis* plus *Hemiaspis* (5TV, -3rd; 10TV, -3rd; Fig. 4c). However, other analyses placed *Acanthophis* as the sister taxa to all other viviparous elapids (2TV; 2TV, 3tv only; 2TV, -3rd; 5TV) or the sister taxa to all the viviparous species except the five genera associated with the *Notechis* lineage (10TV; Fig. 4a). However, the latter relationships were not supported by bootstrap values.

DISCUSSION

Although our results do not fully resolve Australo-Papuan elapid phylogeny, they do shed light on several significant issues concerning the evolutionary history of the proteroglyphs. The first of these findings concerns methodology, in that the data suggest a plausible reason for the long history of difficulties experienced by workers who have attempted to assess evolutionary relationships among the Australo-Papuan elapid snakes. Despite considerable effort over a long period of time, involving the application of diverse data sets (including information on internal and external anatomy, karyology, electrophoresis, venom proteins, molecular sequences, and ecological traits), the continued instability in elapid taxonomy (Mengden, 1983) bears strong witness to the magnitude of the problems that all workers in this field have encountered. Why should this be so? Plausibly, the answer lies in the time

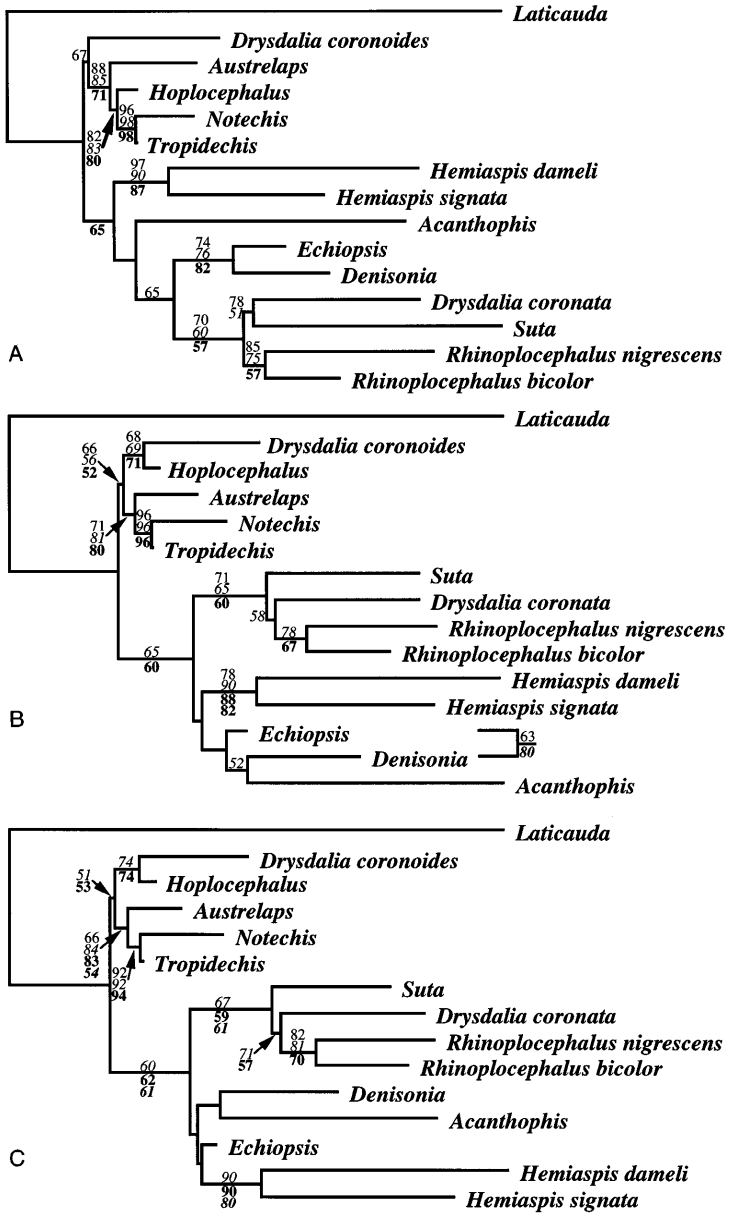

FIG. 4. Phylogenetic relationships among viviparous hydrophiine elapid snakes based on the combined cytochrome *b* and 16S rRNA data sets when cytochrome *b* third positions were treated the same as all other sites, subjected to transversion-only parsimony ("3tv only") or excluded ("−3rd"). (A) Single most parsimonious tree generated after one round of successive approximations in the 10TV analysis. (B) Single most parsimonious tree generated after one round of successive approximations in both the 5TV, 3tv only and the 10TV, 3tv only analyses. (C) Single most parsimonious tree generated after one round of successive approximations in both the 5TV, −3rd and the 10TV, −3rd analyses. Numbers represent bootstrap values from 1000 pseudoreplicates when cytochrome *b* third positions are treated as above and other sites are weighted 2TV (plain type, above the node), 5TV (italics, above the node), 10TV (boldface type, below the node), and TV only (boldface italics, below the node).

course of evolutionary diversification within the members of this group. We would expect great difficulties to arise in phylogenetic reconstruction (regardless of the data set used) if most divergence events in the Australo-Papuan elapids occurred long ago and within a relatively brief period. Our data suggest that this is exactly what has happened. All of the analyses (for both the individual cytochrome *b* and the 16S rRNA data sets, as well as for the combined data set) consistently produced phylogenetic trees characterized by very long terminal branches, but quite short internal branches (e.g., see Figs. 2 and 4). Long branches were evident even between members of the same genus, and intrageneric genetic distances were only marginally smaller

than intergeneric distances for most of the taxa studied (*Cacophis, Hemiaspis, Pseudechis, Pseudonaja, Rhinoplocephalus,* and *Simoselaps*). Together, these patterns suggest that divergences within genera are nearly as old as those between putative major lineages. Thus, these lineages apparently diverged from one another at about the same time and long ago. These ancient splits are supported by fossil evidence (Scanlon, 1996).

This concentration in the timing of divergence confounds our efforts to reconstruct phylogenies, because relatively few retained synapomorphic changes would have occurred over the short time periods between divergence events. Nonetheless, our results clarify several important aspects of proteroglyph phylogeny, and we proceed to discuss some of the implications of the analyses. Figure 5 displays conservative summaries of results obtained from phylogenetic analyses of the oviparous and viviparous taxa, respectively.

Are the Australian Elapid Snakes Relicts of the Gondwanan Fauna or Derived from a More Recent Asian Invasion?

Because of the prolonged physical separation of Australia from other continents, endemic terrestrial Australian fauna basically fall into two groups: those that reached Australia prior to the continent breaking off from the Gondwanan landmass approximately 160 million years ago (Veevers, 1991) and those that reached Australia more recently, presumably via migration from the north when the Australian plate collided with the Asian plate approximately 15 million years ago (Audley-Charles, 1987, 1991) (see Tyler, 1979; Cogger and Heatwole, 1981; Schwaner *et al.*, 1985; reviewed in Keogh, 1998a). The topologies from all analyses presented here strongly support a "stepping-stone" invasion into the Australian region from the north (Schwaner *et al.*, 1985; Cadle, 1987, 1988), with five of the six included Melanesian genera consistently forming sister clades to the rest of the radiation (Figs. 2 and 3). This result supports McDowell's (1970) conclusion that the Melanesian *Loveridgelaps, Ogmodon,* and *Salomonelaps* (plus the Australian *Vermicella*) comprise the most basal branches of the Australo-Papuan radiation.

Further, while the data presented here do not firmly resolve relationships among Melanesian elapids, the data support a close relationship between the rare Fijian *O. vitianus* and the New Guinea *Toxicocalamus* (as suggested by Bogert and Matalas, 1945), rather than a closer relationship between *Toxicocalamus* and Australian *Simoselaps* species (as suggested by McDowell, 1969a, 1970). The similarities between *Ogmodon* and *Toxicocalamus* extend to ecological traits as well as morphology and molecular features. For example, these two genera comprise the only hydrophiines known to feed primarily or entirely on earthworms (Zug and Ineich, 1993; Shine and Keogh, 1996). All other Australo-Papuan elapid species feed almost entirely on vertebrate prey (Shine, 1991).

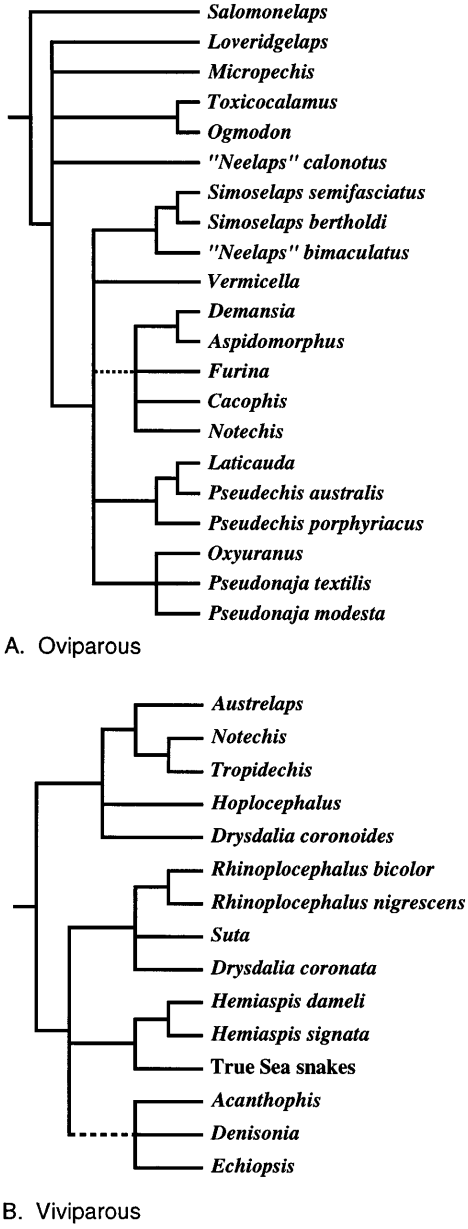

FIG. 5. (A) Conservative summary of phylogenetic relationships obtained from the combined analyses of the cytochrome *b* and 16S rRNA data for (A) the oviparous hydrophiines plus *Notechis ater* and (B) the viviparous hydrophiines.

Do the Banded Burrowing Elapids of Australia Comprise a Monophyletic Lineage?

The genera *Vermicella, Simoselaps,* and "*Neelaps*" (synonymized with *Simoselaps* in Hutchinson, 1990, and Cogger, 1992) have had a complicated and unstable taxonomic history, with widely differing opinions on generic content (see Keogh and Smith, 1996 and Keogh,

1998b). In particular, the "*Neelaps*" species have been variously associated with *Vermicella* (Wallach, 1985; Scanlon, 1985) or *Simoselaps* (Storr, 1967, 1979; Hutchinson, 1990; Cogger, 1992). At present an expanded *Simoselaps* is recognized (Hutchinson, 1990; Cogger, 1992), containing three subgroups that differ in morphological and ecological characters (Shine, 1984; Scanlon and Shine, 1988): the sand-swimming *S. semifasciatus* group, the heavy-bodied *S. bertholdi* group, and the slender "*Neelaps*" species ("*N.*" *bimaculatus* plus "*N.*" *calonotus*). The sequence data presented here suggest that *S. semifasciatus* is closely related to *S. bertholdi* and that these species are closely linked to "*Neelaps*" *bimaculatus*. However, neither "*Neelaps*" *calonotus* nor *V. annulata* associated with the *Simoselaps* species in the analyses. These results are incongruent with those based on hemipenial morphology, because "*Neelaps*" *calonotus* and the *S. semifasciatus* group share a unique hemipenis type, very different than that shared by the *S. bertholdi* group and *Vermicella* (Keogh, 1998b).

Relationships among Other Small Oviparous Elapids

Most of the analyses presented here suggest that the endemic New Guinea genus *Aspidomorphus* (composed of three small cryptozoic species) is closely related to the primarily Australian genus *Demansia* (composed of six slender, fast-moving, diurnal species). A close relationship between these two genera has been suggested on morphological grounds (McDowell, 1967), but is not supported by immunological distance data (Schwaner *et al.*, 1985). Indeed, *Demansia* is immunologically distant from all other Australo-Papuan species (Cadle and Gorman, 1981; Mao *et al.*, 1983). Prior to McDowell's (1967) study, *Aspidomorphus* was thought to be most closely related to the Australian genera *Cacophis* and *Furina* (superficially very similar cryptozoic species). Studies of Australian species have supported the close relationship between *Cacophis* and *Furina* (Wallach, 1985; Mengden, 1985a), and the genera share a unique hemipenis type (Keogh, 1998b) as well as other morphological features (McDowell, 1967). Although no previous studies have united *Demansia* with either *Cacophis* or *Furina* (*Aspidomorphus* was not considered), our analyses consistently grouped these four genera in various combinations. When transversions only were considered, *Cacophis, Furina, Aspidomorphus,* and *Demansia* formed a monophyletic clade. Given this result, it is interesting to note that *Demansia psammophis* was much closer immunologically to *Furina ornata* than to any other species tested by Schwaner *et al.* (1985), although still quite distant from other *Furina* and *Cacophis* species. An examination of cytochrome *b* and 16S rRNA genetic distances shows that the split even between the two *Cacophis* species sampled is of considerable antiquity, despite the morphological similarity between these two species.

Evolutionary Origins of Viviparity

The phylogenetic trees presented here confirm that oviparity is likely to have been the ancestral reproductive condition of the Australo-Papuan elapids (Figs. 2 and 3). This result accords with most published speculations on the polarity of this transition in reproductive modes in reptiles, although some authors have suggested that viviparity may sometimes evolve to oviparity rather than vice versa (e.g., van Wyk and Mouton, 1996; de Fraipont *et al.*, 1996). Viviparity has apparently evolved in two separate lineages; one represented by a single Australian species (*P. porphyriacus*) and the other by the "viviparous lineage" including the true sea snakes (Shine, 1987a) (Figs. 2 and 3). All of the other Australo-Papuan elapids are oviparous, to the best of our knowledge (Shine, 1991; Shine and Keogh, 1996). In the analyses of relationships among the oviparous taxa, *N. ater* was included as a representative of the viviparous lineage to examine the affinities of this lineage. *Notechis* grouped with *Cacophis* in some analyses, but with "*Neelaps*" *calonotus, Vermicella,* and *Ogmodon* in others. Thus, although we cannot identify a specific sister group to the viviparous lineage, the sequence data suggest that this lineage is derived from among the small cryptozoic oviparous taxa. Further, the branch lengths in these trees suggest that the origin of the viviparous lineage is as old as most of the generic-level splits among the terrestrial Australo-Papuan radiation. This conclusion runs counter to published speculations that the viviparous lineage diversified much more recently than the oviparous taxa (Schwaner *et al.*, 1985; Shine, 1985).

Relationships of the Sea Kraits

The analyses presented here strongly suggest that sea kraits (*Laticauda*) are closely related to the large oviparous terrestrial *P. australis* and more distantly related to *P. porphyriacus.* Despite the paraphyletic *Pseudechis* obtained in all of the analyses, the cytochrome *b* and 16S rRNA genetic distances between the *Pseudechis* species were still smaller than their distances from *Laticauda.* However, the distances between the *Pseudechis* species were greater than most of the intergeneric distances observed in the sequence data. The distinctiveness of *P. porphyriacus* is supported by electrophoretic evidence (Mengden *et al.*, 1986), and as noted above, the species also is unique in that it represents a separate evolution of viviparity among the terrestrial Australo-Papuan elapid radiation (Shine, 1987a). Although the great divergence within the genus might suggest that paraphyly with respect to *Laticauda* is not impossible, such a relation-

ship is not corroborated by other data sets and is contradicted by immunological distances (Schwaner *et al.*, 1985). When the *Pseudechis* species were removed from the analyses, *Laticauda* consistently grouped with the Melanesian *Salomonelaps par* and *Loveridgelaps elapoides* (see Keogh, 1998a). A close relationship between *Laticauda* and the Melanesian elapid species is reasonable on both morphological and biogeographic grounds (Keogh, 1998a). Hence, we regard the apparently close association between *Pseudechis* and *Laticauda* with considerable skepticism.

Relationships of the Large Oviparous Elapids

The sequence data strongly support the close relationship of *P. modesta*, *P. textilis*, and *O. microlepidotus*. The relationship between these taxa is corroborated by morphological (Worrell, 1961; Wallach, 1985; Keogh, 1998b), chromosomal, and electrophoretic evidence (Mengden, 1985a, b). Some of the analyses result in a paraphyletic *Pseudonaja* with respect to *Oxyuranus*, reflecting the fact that cytochrome *b* and 16S rRNA genetic distances between the *Pseudonaja* species are only marginally smaller than their distances from *Oxyuranus*. A similar result was obtained by Mengden (1985b). Despite the apparent paraphyly of *Pseudonaja* obtained in some of the analyses, reciprocal monophyly of *Pseudonaja* and *Oxyuranus* is strongly supported by hemipenis morphology (Keogh, 1998b). While the sequence data are equivocal as to the relationship of this clade to other Australian elapids, we note that the relationship of *Pseudonaja* and *Oxyuranus* to *Pseudechis* (Mengden, 1985) or *Pseudechis* plus *Demansia* (Wallach, 1985; Keogh, 1998b) is not supported by the sequence data.

Relationships among the "Notechis" Lineage

Several previous analyses have indicated that a group of large viviparous Australian elapid species constitutes a well-defined clade, comprising *Notechis*, *Austrelaps*, *Hoplocephalus*, and *Tropidechis*. The monophyly of this *Notechis* lineage is corroborated by diverse data sets (Mengden, 1985a; Schwaner *et al.*, 1985; Wallach, 1985). The sequence data suggest that *D. coronoides* also is closely related to these taxa, with the genus *Drysdalia* appearing to be polyphyletic in all of the analyses (see below). The close association of *D. coronoides* with the *Notechis* lineage and *Drysdalia* polyphyly are consistent with electrophoretic data (Mengden, 1985a). The *Notechis* lineage has been interpreted as a relatively recent radiation, based on small immunological distances among the taxa. Schwaner *et al.* (1985) estimated divergences among much of the viviparous lineage to have occurred as recently as 5 million years ago. Our genetic distance data do not support this inference; branch lengths within the *Notechis* lineage are only marginally shorter than are those within most other clades (Fig. 4). Although some studies have united *Echiopsis curta* with the *Notechis* lineage (Storr, 1982; Wallach, 1985; Schwaner *et al.*, 1985; Keogh, 1998b), the analyses presented here associate *Echiopsis* with other viviparous clades (discussed further below).

Relationships among Small Viviparous Australian Elapids

Four small viviparous elapid taxa (the two *Rhinoplocephalus* species, plus *S. suta* and *D. coronata*) consistently formed a clade (Fig. 4). The species currently assigned to *Rhinoplocephalus* and *Suta* have had an unstable taxonomic history, with some of their component taxa assigned to *Denisonia* and to the previously recognized genera *Cryptophis*, *Parasuta*, and *Unechis*. However, the close relationship of these species has been supported by other diverse data sets (Mengden, 1985; Schwaner *et al.*, 1985; Wallach, 1985; Keogh, 1998b). Monophyly of *R. bicolor* and *R. nigrescens* (formerly *Cryptophis*) was supported by the sequence data, but their relationship with *S. suta* and *D. coronata* was less stable. *Drysdalia coronata* either formed a second clade with *S. suta* (Fig. 4a) or was nested within the clade (Figs. 4b and 4c). Paraphyly of *Drysdalia* was strongly supported by the sequence data. While seemingly well defined by external morphological characteristics (Coventry and Rawlinson, 1980), monophyly of the four species currently assigned to *Drysdalia* has been contentious. McDowell (1967) first noted the anatomical distinctiveness of *D. coronata* from its congeners, suggesting that it was more closely related to the *Notechis* lineage. Alternatively, electrophoretic data united *D. coronoides*, *D. mastersi*, and *D. rhodogaster* with the *Notechis* lineage, while *D. coronata* grouped with *E. minor* (Mengden, 1985a, his Fig. 3). *Drysdalia coronata* is also different from its congeners in karyotype (Mengden, 1985a) and diet (Shine, 1981). While uniting the four species with one another (and with *Denisonia*), Wallach (1985) noted numerous internal morphological differences among the species. However, all four *Drysdalia* species share a unique hemipenis type with *Hemiaspis* and other hemipenis similarities with *Rhinoplocephalus* and *Suta* (Keogh, 1998b). The sequence data support the close relationship of *D. coronata* with *Rhinoplocephalus* and *Suta* and the close association of *D. coronoides* with the *Notechis* lineage (contra McDowell, 1967). A sister-group relationship of some *Drysdalia* species with *Denisonia* (as suggested by Wallach, 1985, and Mengden, 1985a) was not supported. *Drysdalia mastersi* and *D. rhodogaster* were not included in this study, but given their small (*D. mastersi*) and large (*D. rhodogaster*) immunological distances from *Notechis* (Schwaner *et al.*, 1985), the affinities of all *Drysdalia* species are likely to be a fruitful area for further research.

Relationships of "Swamp Snakes" and Sea Snakes

The two *Hemiaspis* species (swamp snakes) differ considerably in geographic distributions, diets, and general appearance (e.g., Shine, 1987b; Cogger, 1992). Nonetheless, monophyly of *Hemiaspis* is well supported by the sequence data presented here and is corroborated by morphological (McDowell, 1967; Wallach, 1985; Keogh, 1998b) and molecular studies (Mengden, 1985a). The close association between *Hemiaspis* and *P. modesta* suggested by Wallach (1985) is not supported by these sequence data, nor by other molecular and morphological data sets (Mengden, 1985a, b; Keogh, 1998b). Although the terrestrial sister group of *Hemiaspis* remains obscure, the sea snake *H. darwiniensis* consistently formed the sister group to *Hemiaspis* under virtually all weighting schemes. While not supported by bootstrap values, a close relationship between *Hemiaspis* and the true sea snakes is consistent with morphological data (McDowell, 1969b).

Relationships among the Heavy-Bodied Elapids

Although most elapids are slender in body form, three Australian genera (*Acanthophis, Denisonia,* and *Echiopsis*) consist of heavy-bodied species that are morphologically (and in some respects, ecologically) convergent with viperid snakes on other continents (Cogger, 1992; Shine, 1980). The similarities may reflect adaptations to ambush foraging (Shine, 1980). The affinities of these snakes with one another and with other Australo-Papuan elapids have been the subject of much disagreement. In particular, the affinities of *Acanthophis* have been difficult to ascertain due partly to their highly derived nature not only among hydrophiines, but elapids in general (Shine, 1980; Wallach, 1985; Keogh, 1998b). *Acanthophis* has been associated with *Echiopsis* based on similarities in head scalation (Mengden, 1985a) and venom composition (Marshal and Herrman, 1984) or placed as the sister group to the *Notechis* lineage (including *Echiopsis*) (Wallach, 1985). However, immunological distances suggest that *Acanthophis* is not particularly close to any of a diverse sampling of Australo-Papuan elapids (Schwaner *et al.*, 1985). In addition to its association with *Acanthophis* noted above, *Echiopsis* has been united with the *Notechis* lineage based on morphology (Storr, 1982; Wallach, 1985; Keogh, 1998b) and immunological distance (Schwaner *et al.*, 1985). *Denisonia* has been associated with *Drysdalia* based on morphology (Wallach, 1985) and karyology (Mengden, 1985a, except *D. coronata*), *Acanthophis* based on hemipenis morphology (Keogh, 1998b), and *Echiopsis* based on electrophoretic data (Mengden, 1985a, his Fig. 3). Unfortunately, the contradictory results evident in these studies are echoed by this study. For example, the 16S rRNA genetic distance data suggest that *Echiopsis* is closer to the *Notechis* lineage than either *Acanthophis* or *Denisonia* (corroborating numerous studies), but the cytochrome *b* data do not support this grouping. While the exact nature of the relationship remains equivocal, there appears to be a close association among the three genera. An *Echiopsis–Denisonia* clade is strongly supported in many analyses (Fig. 4a), corroborating electrophoretic data (Mengden, 1985a), while a *Denisonia–Acanthophis* clade is weakly supported in other analyses (Figs. 4b and 4c), corroborating morphological data (Keogh, 1998b). Part of the difficulty in determining relationships of these species probably lies in the age of the divergences between them.

In summary, this study has failed to provide a well-supported dichotomous tree of elapid relationships, but nonetheless has clarified several aspects of proteroglyph phylogeny. The Australo-Papuan elapids are derived from an invasion from Asia, presumably in the Miocene. A large part of the adaptive radiation of these snakes occurred relatively quickly after that initial invasion, resulting in a difficult-to-resolve "star" phylogeny for the major lineages. The radiation of viviparous Australian elapids may be younger than that of the oviparous forms, but not markedly so. The hydrophiine radiation incorporates two independent invasions into the marine habitat, one by the oviparous sea kraits (probably derived from forms similar to the present-day Melanesian elapids) and a more recent origin within the Australian viviparous lineage (probably from forms similar to present-day *Hemiaspis*). At least two genera (*Drysdalia* and *Simoselaps*) may well be paraphyletic and hence deserve further attention.

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