Biological Journal of the Linnean Society, 2006, **89**, 523–539. With 3 figures

Molecular phylogeny of sea snakes reveals a rapidly diverged adaptive radiation

VIMOKSALEHI LUKOSCHEK[1]* and J. SCOTT KEOGH[2]

[1]*School of Tropical Environment Studies and Geography, James Cook University, Townsville, Qld 4811, Australia*
[2]*School of Botany and Zoology, The Australian National University, Canberra, ACT 0200, Australia*

Received 13 December 2004; accepted for publication 15 January 2006

Evolutionary relationships within and between the marine hydrophiine sea snake groups have been inferred primarily using morphological characters, and two major groups traditionally are recognized. The *Aipysurus* group comprises nine species in two genera, and the taxonomically chaotic *Hydrophis* group comprises as many as 40 species, of which 27 are generally allocated to the genus *Hydrophis* and 13 to ten additional genera. In addition to these two major groups are three putatively 'primitive' monotypic genera, *Hydrelaps darwiniensis*, *Ephalophis greyi* and *Parahydrophis mertoni*. The present study investigated the evolutionary relationships of 23 representative species of marine hydrophiines, comprising 15 species from the *Hydrophis* group, six species from the *Aipysurus* group, and *H. darwiniensis* and *P. mertoni*, to address two broad aims. First, the aim was to provide a robust phylogeny for sea snakes to test previous phylogenetic hypotheses based on morphology, and thus provide some taxonomic stability to the group. Second, there was interest in evaluating the hypothesis that the *Hydrophis* group might represent a rapidly diverged adaptive radiation. A large mitochondrial DNA data set based on the cytochrome *b* gene (1080 bp, 401 parsimony informative) and the 16S rRNA gene (510 bp, 57 parsimony informative) was assembled and these data were analysed using parsimony, maximum-likelihood and Bayesian approaches. All analyses yielded virtually the same optimal tree, confirming that hydrophiine sea snakes comprise at least three lineages. The *Aipysurus* group formed a strongly supported and well-resolved monophyletic clade. The *Hydrophis* group also formed a strongly supported clade; however, resolution among the genera and species was very poor. *Hydrelaps darwiniensis* and *P. mertoni* formed a sister clade to the *Hydrophis* lineage. Our phylogeny was used to test the validity of previous taxonomic and phylogenetic hypotheses, and to demonstrate that the genus *Hydrophis* is not monophyletic. Genetic diversity relative to phenotypic diversity is four to seven times greater in the *Hydrophis* lineage compared with the *Aipysurus* lineage. The topology of our phylogenetic hypothesis, combined with the levels of genetic divergence relative to morphological diversity, demonstrate that the *Hydrophis* lineage represents a rapidly diverged adaptive radiation. The data are consistent with the hypothesis that this adaptive radiation may be due to historical sea level fluctuations that have isolated populations and promoted speciation. *Biological Journal of the Linnean Society*, 2006, **89**, 523–539.

ADDITIONAL KEYWORDS: cytochrome *b* – elapid – hydrophiine – rapid evolution – 16S rRNA – speciation – tropical marine.

INTRODUCTION

Hydrophiine or 'true' sea snakes are a diverse radiation of fully marine venomous species that belong to the same evolutionary lineage as venomous terrestrial elapids. Elapid snakes are a monophyletic clade of approximately 300 species in 61 genera (Golay, 1985), and they are defined primarily by their unique venom delivery system of two permanently erect canaliculate fangs at the end of the maxilla (a 'proteroglyphous' condition; McCarthy, 1985). Relationships both among and within major elapid clades have been the subject of considerable discussion, with a focus on the rela-

*Corresponding author. E-mail: vimoksalehi.lukoschek@jcu.edu.au

tionships between sea snakes and terrestrial elapids. Detailed morphological appraisals (McDowell, 1969, 1970, 1972, 1974) resulted in the division of the elapid snakes into two major lineages based on cranial kinesis: the 'palatine draggers' comprising the terrestrial Australo-Papuan elapids (except *Parapistocalamus*) plus the 'true' sea snakes; and the 'palatine erectors' comprising all Asian, African and American terrestrial elapids (and *Parapistocalamus*) and the sea kraits *Laticauda*. This division was formalized when the 'palatine draggers' and 'palatine erectors' were elevated to the status of families: Hydrophiidae and Elapidae, respectively (Smith, Smith & Sawin, 1977). This taxonomic arrangement has been largely supported by more recent molecular data (Keogh, 1998; Keogh, Shine & Donnellan, 1998; Keogh, Scott & Scanlon, 2000; Slowinski & Keogh, 2000); however, most authors retain the family Elapidae for all elapids, and place the 'palatine draggers' and 'palatine erectors' into the subfamilies Hydrophiinae and Elapinae, respectively.

The relationship between the fully marine hydrophiine sea snakes (a diverse group comprising 16 genera and as many as 53 species) and the partially terrestrial *Laticauda* (comprising five species) has been the subject of considerable debate (Rasmussen, 1997). Boulenger (1896) included *Laticauda* in his initial description of the hydrophiine sea snakes as a cohesive group, and Smith (1926) placed both the 'true' sea snakes and *Laticauda* into the Hydrophiidae. This taxonomic arrangement was popular for many years. However, most studies subsequent to the major revisions of McDowell (1970) and Smith *et al*. (1977) have recognized *Laticauda* as a distinct evolutionary lineage based on data from both morphology (Voris, 1977; McCarthy, 1986) and molecular studies (Minton & da Costa, 1975; Cadle & Gorman, 1981; Cadle & Sarich, 1981; Schwaner *et al.*, 1985; Slowinski, Knight & Rooney, 1997; Keogh, 1998; Keogh *et al*., 1998; Slowinski & Keogh, 2000). This arrangement implies two separate invasions of the marine environment (Keogh, 1998). Most studies also support the 'palatine dragger' and 'palatine erector' lineages proposed by McDowell (1970), but the close affinity of *Laticauda* with the Asian, African, and American terrestrial elapids has not been supported (Cadle & Gorman, 1981; Cadle & Sarich, 1981; Mao *et al.*, 1983; Slowinski *et al*., 1997; Keogh, 1998; Keogh *et al*., 1998; Slowinski & Keogh, 2000) and Slowinski *et al*. (1997) formally moved *Laticauda* from the elapine to the hydrophiine lineage.

Although the relationships between the hydrophiine sea snakes and *Laticauda* have received considerable attention, relationships within the marine hydrophiine lineage are poorly understood. Hydrophiine sea snakes occur exclusively in tropical and subtropical waters throughout the Indo-West Pacific region. Species diversity is highest in the tropical coastal waters of Australia (30 species in 12 genera; Cogger, 1996) and Malaysia and the Indonesian archipelago (27 species in ten genera; Heatwole, 1999). A monograph by Smith (1926) on sea snakes included descriptions of 45 hydrophiine species in 14 genera, and this represented almost all currently known sea snake species. Based on detailed morphology of skull osteology, Smith (1926) classified the sea snakes into two subfamilies: Laticaudinae (including the genera *Laticauda*, *Aipysurus* with seven species, and *Emydocephalus* with two species) and Hydrophiinae (12 genera including the species rich *Hydrophis* with 23 species). Most of the remaining Hydrophiinae genera were monotypic. Although it is now clear that *Laticauda* and the 'true' sea snakes do not comprise a monophyletic group, most authors agree that the 'true' sea snakes are monophyletic based on morphological (Voris, 1977; Gopalakrishnakone & Kochva, 1990) and molecular evidence (Cadle & Gorman, 1981; Minton & da Costa, 1975; Schwaner *et al*., 1985; Slowinski *et al*., 1997; Keogh, 1998; Keogh *et al*., 1998).

There is strong evidence to suggest that hydrophiine sea snakes originated from a single invasion of the marine environment by an ancestral Australian-Papuan terrestrial elapid, probably from within the viviparous lineage that also gave rise to the swamp snakes *Hemiaspis* (Keogh, 1998; McDowell, 1969; Keogh *et al*., 1998); however, one challenge to monophyly of the marine hydrophiines has been raised. Based on morphological data, Rasmussen (2002) argued that hydrophiine sea snakes are paraphyletic, and the two subfamilies recognized by Smith (1926) represent separate invasions of the marine environment. This conclusion has not been supported by two molecular studies. Based, respectively, on 344 bp of 12S rRNA sequence and more than 3500 bp of five mitochondrial loci, it has been demonstrated that the hydrophiine sea snakes form a well-supported monophyletic clade within the Australio-Papuan radiation (Nock, 2001; J. S. Keogh, unpubl. data).

The robust molecular phylogeny generated in this study has been used to address two primary issues concerning the evolutionary history of hydrophiine sea snakes. First, an independent test of previous hypotheses of phylogenetic relationships between and within the major marine hydrophiine clades based on morphology is provided. Second, the hypothesis of Burger & Natsuno (1974) and Voris (1977), proposing that the genus *Hydrophis* and allied genera represent a rapidly diverged adaptive radiation, is tested. Because this hypothesis is supported, some of the factors that may account for the rapid speciation of this lineage in the tropical waters of the Indo-West Pacific, and the wide range of morphological adaptations found in this group, are evaluated.

MATERIAL AND METHODS

Taxonomic sampling

Numerous classification systems have been proposed for the 'true' sea snakes at all taxonomic levels, and some contemporary authors (Cogger & Heatwole, 1981; Heatwole & Cogger, 1994; Cogger, 1996; Heatwole, 1999) still follow traditional classification of the family Hydrophiidae by Smith (1926). However, the present study follows the more widely accepted subfamily status of the hydrophiine sea snakes. The present study follows the terminology proposed by McDowell (1969) for the two main marine hydrophiine lineages: the *Aipysurus* group for Smith's (1926) Laticaudinae, excluding *Laticauda*, and the *Hydrophis* group for Smith's (1926) Hydrophiinae, excluding *Hydrelaps*.

Tissue samples were obtained from 74 individuals that comprised 25 species and represented ten hydrophiine sea snake genera and two outgroup species (for sampling locations, see Table 1, Fig. 1). The *Aipysurus* group was represented by five species of *Aipysurus* and *Emydocephalus annulatus*. The *Hydrophis* group was represented by 15 species from six genera. These comprised nine species of *Hydrophis* and four monotypic genera represented by *Acalyptophis peroni* (Boulenger, 1896), *Astrotia stokesi* (Fisher, 1856), *Lapemis curtus* (Gray, 1835) and *Pelamis platurus* (Daudin, 1803). Two species of *Disteira* were also included: *Disteira major* (Shaw, 1802) and *Disteira kingii* (Boulenger, 1896). Most *Hydrophis* species included in the study occur in Australian waters, but the study was able to include *Hydrophis brookii* (Guenther, 1872), *Hydrophis spiralis* (Shaw, 1802), *Hydrophis lapemoides* (Gray, 1894) and *Hydrophis cyanocinctus* (Daudin, 1803) that occur exclusively in south-east Asian waters (Fig. 1). Three of the five species of McDowell's (1972) *Leioselasma* (subgenus of *Hydrophis*) were included: *H. cyanocinctus*, *H. spiralis* and *Hydrophis elegans* (Gray, 1842). *Hydrophis pacificus* (Boulenger, 1896), previously placed in synonymy with *H. elegans* by McDowell (1972), was also included. *Hydrelaps darwiniensis* (Boulenger, 1896) and *Parahydrophis mertoni* (Roux, 1910) represented the 'primitive' sea snakes. *Hemiaspis signata* (Jan, 1859) and *Hemiaspis damelii* (Guenther, 1876) were used as outgroup species, based on their close phylogenetic relationship to hydrophiine sea snakes (Keogh *et al*., 1998; Keogh, 1998).

Tissue samples were obtained from live sea snakes, museum collections and a trawl fishery by-catch study conducted by the Commonwealth Scientific and Industrial Research Organization (CSIRO) in 1996, 1997, and 2001 (Table 1). Live sea snakes were caught in fish catch bags, on scuba or on snorkel, and swum to a tender where they were placed in a large container of seawater. Each snake was removed from the seawater to obtain a small sample of muscle tissue from the flattened ventral surface of the tail and to verify the species' identity. Where possible, only museum samples of entire specimens with vouchers were included. All tissue samples were stored at room temperature in 70% ethanol or in a solution of 20% dimethylsulphoxide saturated with sodium chloride.

DNA extraction and mtDNA amplification

Tissue samples were digested with proteinase K in a CTAB buffer [100 mM Tris-HCl, pH 8, 1.4 M NaCl, 20 mM EDTA, 2% CTAB (hexadecyl-trimethyl-ammonium bromide), 0.2% 2-mercaptoethanol]. Total cellular DNA was purified by extraction with neutralized chloroform-isoamyl-alcohol (24 : 1), precipitated with ethanol and dissolved in TE buffer. The cytochrome *b* and the 16S rRNA mitochondrial genes were targeted because they have provided good resolution for similar studies of other elapids (Keogh, 1998; Keogh *et al*., 1998; Keogh, Scott & Hayes, 2005). Primers used to amplify and sequence cytochrome *b* (1150 bases) and 16S rRNA (530 bases), are shown in Table 2. Target fragments were amplified using a polymerase chain reaction (PCR) comprising 10 ng template DNA, 2 units Taq-polymerase (Qiagen), 4 μL of 10 × Qiagen reaction buffer, 100 mM $MgCl_2$, 1.0 mM dNTPs and 2 pmol of each primer in 40 μL total volume. PCR amplification of double-stranded product was performed using a MJ Research Peltier Thermal Cycler 2000 using a step-down cycling profile that consisted of an initial denaturing step of 94 °C for 5 min followed by one cycle of 94 °C for 30 s, annealing at 70 °C for 15 s, and extension at 72 °C for 90 s. During each subsequent cycle, the annealing temperature was dropped by 2.5 °C until the annealing temperature reached 50 °C (i.e. eight cycles). This was followed by 32 cycles at 50 °C. A final extension step at 72 °C was peformed for 7 min.

DNA sequencing

PCR products were gel purified using the UltraClean 15 DNA purification kit (Geneworks) and both complimentary strands were cycle sequenced using ABI PRISM BigDye (Perkin Elmer) cycle sequencing reaction kit. Due to the length of the cytochrome *b* gene, internal primers were used to obtain reliable sequence from both complimentary strands (Table 2). Reactions were conducted using 4 μL of reaction premix, 2 pmol of amplification primer, and approximately 50–80 ng purified PCR product as template. Cycle sequencing was performed using a MJ Research Peltier Thermal Cycler 2000 and the following profile: 96 °C for 1 min followed by 24 cycles of 96 °C for 30 s, 50 °C for 15 s,

Table 1. Details of specimens and sequences used in the present study

Taxon	Museum Voucher No/source	Sample no.	Location	Latitude	Longitude	GenBank Accession Nos.	
						Cytochrome *b*	16S rRNA
*Acalyptophis peronii**	CSIRO 1203	Ap001	Vanderlin Island, Gulf, NT, Australia	15.20	137.49	DQ233923	DQ234004
Acalyptophis peronii (2)	CSIRO 2580	Ap005	Mornington Island, NT, Australia	16.90	140.20	DQ233924	DQ234005
Acalyptophis peronii (3)	Collected by V. Lukoschek	Ap007	Ashmore Reef, WA, Australia	12.10	123.00	DQ233925	DQ234006
Aipysurus apraefrontalis	CSIRO 1155	Aa001	Ashmore Reef, WA, Australia	19.68	116.70	DQ233905	DQ233981
Aipysurus duboisii	CSIRO 1012	Ad004	Vanderlin Island, NT, Australia	15.13	137.35	DQ233906	DQ233982
Aipysurus duboisii (2)*	Collected by V. Lukoschek	Ad016	Scott Reef, WA, Australia	14.00	121.50	DQ233907	DQ233983
*Aipysurus eydouxi**	CSIRO 1000	Ae001	Groote Eylandt, NT, Australia	13.28	136.47	DQ233908	DQ233984
Aipysurus eydouxi (2)	CSIRO 1014	Ae005	Bundaberg, Qld, Australia	24.68	152.25	DQ233909	DQ233985
Aipysurus eydouxi (3)	CSIRO 2512	Ae008	Groote Eylandt, NT, Australia	13.29	136.64	DQ233910	DQ233986
*Aipysurus fuscus**	Collected by V. Lukoschek	Af003	Ashmore Reef, WA, Australia	12.10	123.00	DQ233911	DQ233987
Aipysurus fuscus (2)	Collected by V. Lukoschek	Af032	Scott Reef, WA, Australia	14.00	121.50	DQ233912	DQ233988
Aipysurus laevis	Collected by V. Lukoschek	Al003	Ashmore Reef, WA, Australia	12.31	123.29	DQ233913	DQ233989
Aipysurus laevis (2)*	Collected by V. Lukoschek	Al008	Townsville, Qld, Australia	18.75	146.98	DQ233914	DQ233990
Aipysurus laevis (3)	Collected by V. Lukoschek	Al009	Townsville, Qld, Australia	18.76	146.02	DQ233915	DQ233991
Aipysurus laevis (4)	Collected by V. Lukoschek	Al067	Swain Reefs #21–441, Qld, Australia	21.55	151.75	DQ233916	DQ233992
Aipysurus laevis (5)	CSIRO 2319	Al219	Vanderlin Island, NT, Australia	15.69	137.93	DQ233917	DQ233993
Aipysurus laevis (6)	CSIRO 2358	Al223	Vanderlin Island, NT, Australia	15.37	137.70	DQ233918	DQ233994
Aipysurus laevis (7)	NTM 17775	Al226	Cartier Islet, WA, Australia	12.31	123.29	DQ233919	DQ233995
Aipysurus laevis (8)	CSIRO 2439	Al229	Vanderlin Island, NT, Australia	15.28	137.53	DQ233920	DQ233996
Aipysurus laevis (9)	CSIRO 2444	Al230	Vanderlin Island, NT, Australia	15.60	137.94	DQ233921	DQ233997
Aipysurus laevis (10)	CSIRO 2461	Al233	Mornington Island, NT, Australia	16.35	138.67	DQ233922	DQ233998
Astrotia stokesii	CSIRO 1042	As002	Mornington Island, NT, Australia	15.98	139.62	DQ233926	DQ234007
Astrotia stokesii (2)*	CSIRO 1227	As004	Groote Eylandt, NT, Australia	12.52	136.90	DQ233927	DQ234008
Astrotia stokesii (3)	CSIRO 2530	As007	Vanderlin Island, NT, Australia	15.53	137.71	DQ233928	DQ234009
Astrotia stokesii (4)	Collected by V. Lukoschek	As008	Ashmore Reef, WA, Australia	12.10	123.00	DQ233929	DQ234010
Disteria kingii	CSIRO 1141	Dk001	Mornington Island, NT, Australia	16.05	139.70	DQ233930	DQ234011
Disteria kingii (2)*	CSIRO 1109	Dk002	Townsville, Qld, Australia	19.28	147.40	DQ233931	DQ234012
Disteria kingii (3)	CSIRO 1035	Dk003	Mornington Island, NT, Australia	16.10	139.73	DQ233932	DQ234013
Disteria kingii (4)	CSIRO 1151	Dk004	Weipa, Qld, Australia	12.45	141.56	DQ233933	DQ234014
Disteria major	CSIRO 1032	Dm002	Mornington Island, NT, Australia	15.82	139.73	DQ233934	DQ234015
Disteria major (2)*	CSIRO 1008	Dm008	Weipa, Qld, Australia	12.52	141.52	DQ233935	DQ234016
Disteria major (3)	CSIRO 1116	Dm020	Mackay, Qld, Australia	20.83	148.97	DQ233936	DQ234017
Disteria major (4)	CSIRO 2527	Dm024	Mornington Island, NT, Australia	15.98	138.32	DQ233937	DQ234018
*Emydocephalus annulatus**	Collected by V. Lukoschek	Ea002	Swain Reefs #21–104, Qld, Australia	21.10	151.30	DQ233938	DQ233999
Emydocephalus annulatus (2)	Collected by V. Lukoschek	Ea003	Swain Reefs #21–104, Qld, Australia	21.10	151.30	DQ233939	DQ234000
Emydocephalus annulatus (3)	NTM 17784 – ABRC 29030	Ea007	Cartier Islet, WA, Australia	12.31	123.29	DQ233940	DQ234001

Emydocephalus annulatus (4)	Collected by V. Lukoschek	Ea009	Ashmore Reef, WA, Australia	12.10	123.00	DQ233941	DQ234002
Emydocephalus annulatus (5)	Collected by V. Lukoschek	Ea088	Scott Reef, WA, Australia	14.00	121.50	DQ233942	DQ234003
Parahydrophis mertoni	NTM 13607 – ABRC 28239	Pm001	Palmerston boat ramp, NT, Australia			DQ233974	DQ234048
Hydrelaps darwiniensis	SAM No # – ABRC 29188	Hd001	Dinal Beach, NT, Australia			DQ233947	DQ234046
Hydrelaps darwiniensis (2)	NTM 16471 – ABRC 28875	Hd002	Bing Bong Station, NT			DQ233948	DQ234047
Hydrophis brookii	FMNH 252511	Hb001	Songkhla, Thailand	7.13 N	100.37	DQ233943	DQ234028
Hydrophis cyanocinctus	FMNH 249391	Hcy001	Phuket, Thailand	8.00 N	98.28	DQ233945	DQ234031
Hydrelaps cyanocinctus (2)*	FMNH 249392	Hcy002	Phuket, Thailand	8.00 N	98.28	DQ233946	DQ234032
Hydrophis czeblukovi	CSIRO 1158	Hc001	North-west Shelf, WA, Australia	20.20	116.85	DQ233944	DQ234019
Hydrophis elegans	CSIRO 1117	He004	Mackay, Qld, Australia	20.88	149.05	DQ233949	DQ234020
Hydrelaps elegans (2)	CSIRO 1083	He005	Townsville, Qld, Australia	19.32	147.07	DQ233950	DQ234021
Hydrelaps elegans (3)*	CSIRO 1160	He010	Weipa, Qld, Australia	12.41	141.56	DQ233951	DQ234022
*Hydrophis lapemoides**	FMNH 249584	Hl001	Phuket, Thailand	8.00 N	98.28	DQ233954	DQ234033
Hydrelaps lapemoides (2)	FMNH 249585	Hl002	Phuket, Thailand	8.00 N	98.28	DQ233955	DQ234034
Hydrophis mcdowelli	CSIRO 1157	Hm001	North-west Shelf, WA, Australia	19.38	118.53	DQ233956	DQ234029
Hydrelaps mcdowelli (2)*	CSIRO 2628	Hm002	Mornington Island, NT, Australia	15.62	137.95	DQ233957	DQ234030
Hydrophis ornatus	CSIRO 1190	Ho002	Groote Eylandt, NT, Australia	14.46	136.47	DQ233958	DQ234023
Hydrelaps ornatus (2)	CSIRO 2357	Ho010	Groote Eylandt, NT, Australia	15.37	137.70	DQ233959	DQ234024
Hydrelaps ornatus (3)	CSIRO 1153	Ho011	North-west Shelf, WA, Australia	19.93	116.00	DQ233960	DQ234025
Hydrelaps ornatus (4)	SAM 23496 – ABRC 55606	Ho013	Gulf of Carpentaria, NT, Australia	14.80	138.00	DQ233961	DQ234026
Hydrelaps ornatus (5)*	CSIRO 2529	Ho014	Mornington Island, NT, Australia	15.98	138.32	DQ233962	DQ234027
Hydrophis pacificus	CSIRO 1149	Hp001	Mornington Island, NT, Australia	16.07	139.70	DQ233963	DQ234035
Hydrophis pacificus (2)*	CSIRO 2581	Hp006	Mornington Island, NT, Australia	16.45	138.79	DQ233964	DQ234036
Hydrophis pacificus (3)	CSIRO 2589	Hp007	Groote Eylandt, NT, Australia	14.61	137.07	DQ233965	DQ234037
Hydrophis spiralis	FMNH 249656	Hsp001	Phuket, Thailand	8.00 N	98.28	DQ233966	DQ234038
*Lapemis curtus**	FMNH 249631	Lc001	Phuket, Thailand	8.00 N	98.28	DQ233967	DQ234039
Hydrelaps curtus (2)	Reef HQ Townsville	Lc002	Yongala, Qld, Australia	19.29	147.59	DQ233968	DQ234040
Hydrelaps curtus (3)	SAM 23494 – ABRC 55605	Lc003	Gulf of Carpentaria, NT, Australia	14.80	138.00	DQ233969	DQ234041
Hydrelaps curtus (4)	CSIRO 1265	Lc089	North-west Shelf, WA, Australia	11.08	130.63	DQ233970	DQ234042
Hydrelaps curtus (5)	CSIRO 2282	Lc111	Groote Eylandt, NT, Australia	14.49	136.33	DQ233971	DQ234043
Hydrelaps curtus (6)	CSIRO 2284	Lc112	Groote Eylandt, NT, Australia	14.45	136.30	DQ233972	DQ234044
Hydrelaps curtus (7)	CSIRO 2328	Lc129	Groote Eylandt, NT, Australia	14.48	136.01	DQ233973	DQ234045
Pelamis platurus	CSIRO – no number	Pp001	Gulf of Carpentaria, NT, Australia	14.80	138.00	DQ233975	DQ234049
Pelamis platurus (2)	SAM 55127	Pp002	Broken Head, NSW, Australia	28.80	153.55	DQ233976	DQ234050
Pelamis platurus (3)*	SAM No # – NR8918	Pp003	Richmond River, NSW, Australia	28.80	153.55	DQ233977	DQ234051
Pelamis platurus (4)	SAM No # – NR8922	Pp004	Sth Ballina, NSW, Australia	28.80	153.55	DQ233978	DQ234052
Hemiaspis damelii	SAM catalogue 5 No.4		Dalby, Qld, Australia			DQ233952	DQ233979
Hemiaspis signata	SAM catalogue 5 No.1		Harrington, NSW, Australia			DQ233953	DQ233980

CSIRO, Commonwealth Scientific & Industrial Research Organization (Fish Number); FMNH, Field Museum of Natural History; NTM, Northern Territory Museum; SAM, South Australian Museum. Individuals denoted by an asterisk were used to estimate intergeneric genetic distances (also shown in Fig. 3).

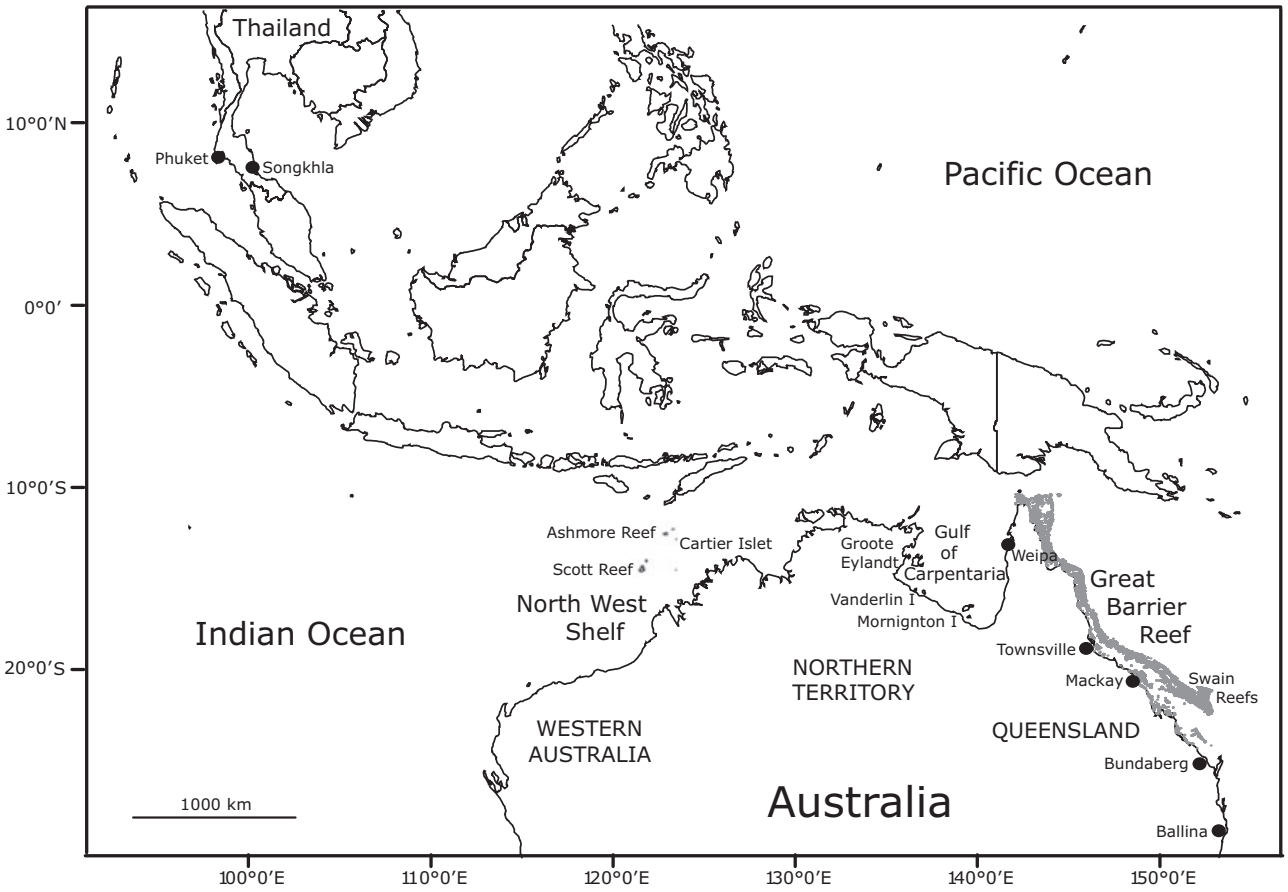

Figure 1. Sampling locations of species included in this study.

Table 2. Primers used to generate polymerase chain reaction products and DNA sequences

Region	Name	Sequence: 5′ > 3′	3′ position	Source
Cytochrome b	tRNA-Glu	TGATMTGAAAACCACCGTTG	14 909	J. S. Keogh (unpubl. data)
	L14968	CCTTATTATTCTCCAACCTTCTAC	14 968	Present study
	Elapid Cytb Lb	GGACAAATATCATTCTGAGCAGCAACAG	15 337	J. S. Keogh (unpubl. data)
	Elapid Cytb H	TTGTAGGAGTGATAGGGATGAAATGG	15 559	J. S. Keogh (unpubl. data)
	H15951	TGATTGAGGCTGTTTGACTGATT	15 951	Present study
	tRNA-ThrA	CCRTCTTTGGTTTACAAYAACAATG	16 070	J. S. Keogh (unpubl. data)
16S rRNA	L2510	CGCCTGTTTATCAAAAACAT	1 847	Palumbi (1996)
	H3056	CTCCGGTCTGAACTCAGATCACGTAGG	2 351	Palumbi (1996)

The letters 'L' and 'H' refer to the light and heavy strands. tRNA-Glu is a light strand primer and tRNA-ThrA is a heavy strand primer. Elapid Cyt *b* H and Lb are internal primers used only for sequencing. Values in the 3 ′ position refer to the position of the 3 ′ base of the primer in the complete Dinodon mtDNA sequence (Kumazawa *et al*., 1998).

60 °C for 4 min. Ramping was set at 1 °C s^{-1}. Extension products were purified using isopropanol precipitation and dried. Sequencing products were electrophoresed on one of the following automated DNA sequencers: ABI Prism 310, ABI Prism 377 or MegaBACE 1000 (Amersham Biosciences).

PHYLOGENETIC ANALYSES

Sequence data were edited using Sequencher 4.1 (Gene Codes Corporation) and provisionally aligned with ClustalX (Thompson *et al*., 1997) and then refined by eye. Following alignment, cytochrome *b* sequences were translated into amino acid sequences

using the vertebrate mitochondrial genetic code. No premature stop codons were observed, and it was concluded that the cytochrome *b* sequences obtained were mitochondrial in origin. The 16S rRNA sequences also displayed no obvious signs of nuclear copies.

Prior to phylogenetic analyses, a partition homogeneity test was performed in PAUP* 4.0b10 (Swofford, 2000) to test whether the individual data sets were heterogeneous with regard to phylogenetic signal. The null hypothesis that the data were homogeneous ($P > 0.05$) could not be rejected, and the data from both genes were combined for all phylogenetic analyses. The 16S rRNA data set contained two small adjacent hyper-variable regions, totalling 15 bp in length, for which it was not possible to align sites or identify site homologies across all taxa, and these were excluded from our analyses.

The complete combined data set was analysed using unweighted parsimony, maximum-likelihood (ML) and Bayesian approaches. The objective criteria provided by the computer program ModelTest 3.06 (Posada & Crandall, 1998) were used to select the most appropriate model of molecular evolution for the combined data set. The estimates of the empirical nucleotide frequencies, substitution rates, gamma distribution (Γ), and proportion of invariant sites (I) provided by ModelTest were used in the ML analyses implemented in PAUP* (Swofford, 2000). The ModelTest was also used to select the most appropriate models for the separate cytochrome *b* and 16S rRNA data sets to estimate interspecific and intraspecific ML genetic distances (for species represented by more than one individual).

Two unweighted parsimony analyses also were performed using PAUP*. In the first, all sites were included and, in the second, the third codon position was excluded from the cytochrome *b* data set (343 characters) to test for saturation and evaluate the effect on tree topology. A strict consensus tree was constructed for each analysis.

The Bayesian analyses were implemented using the same combined data set as the ML analyses and the computer program MrBayes (version 3.0b4) (Huelsenbeck & Ronquist, 2001). Two strategies were used for these analyses. In the first, the substitution rates for the General Time Reversal (GTR) model, gamma distribution, proportion of invariant sites and character state frequencies were estimated for all the data combined. The default value of four Markov chains per run was used and the analysis was run five times to ensure that overall tree-space was well sampled and to avoid being trapped in local optima. Each analysis was run for 1 000 000 generations and sampled every 100 generations, resulting in 10 000 sampled trees. The Markov chain reached stationarity after approximately 100 000 generations (1000 sampled trees), so the first 2000 trees were discarded as the burn-in phase and the remaining 8000 trees were used to construct a 50% majority rule consensus tree and estimate Bayesian posterior probabilities. In the second approach, the data were partitioned into four character sets: the 16S rRNA gene and one for each of the codon positions for the cytochrome *b* gene. The substitution rates for the GTR model, gamma distribution, proportion of invariant sites, and character state frequencies were unlinked and estimated independently for each data partition. Four Markov chains were ran for 7 000 000 generations, sampled every 100 generations and the first 10 000 sampled trees were discarded as the burn-in phase. This analysis was run twice and posterior probabilities for clades were plotted against one another. Low variance was found in estimated posteriors probabilities for focal clades, suggesting that chains had reached stationarity.

Bootstrap values (both parsimony and nonparametric ML) and Bayesian posterior probabilities were used to evaluate branch support. Two unweighted parsimony bootstrap tests, comprising 10 000 replicates each, were performed. The first was on the entire combined data set and the second with cytochrome *b* third codon position excluded. Two maximum likelihood bootstrap tests also were performed. The first comprised one or two individuals to represent each species (detailed in Table 1) and 500 nonparametric ML bootstrap replicates. The second included all 74 individuals, and 100 nonparametric ML bootstrap replicates were performed. In addition, Bayesian posterior probabilities provided a third measure of branch support and may represent a better estimate of phylogenetic accuracy than bootstrap values (Wilcox *et al*., 2002; Reeder, 2003). A conservative statistical approach was taken and a branch was considered to be supported only if it received bootstrap values = 70% (Hillis & Bull, 1993) and posterior probabilities = 95% (Wilcox *et al*., 2002).

Hypothesis testing

The significance of log-likelihood differences was tested between the optimal ML tree and a number of alternative topologies (listed below) representing various alternative hypotheses suggested previously by authors based on morphology. Maximum likelihood trees, constrained to represent each of the alternative hypotheses, were built in PAUP* using the same settings and model of evolution as in the previous searches for optimal trees. Constrained and unconstrained trees were compared using the Shimodaira-Hasegawa test in PAUP* (Shimodaira & Hasegawa, 1999; Goldman, Anderson & Rodrigo, 2000) using full optimization and 10 000 bootstrap replicates. This tests whether the optimal tree is significantly better than each of the alternative hypotheses.

Phylogenetic affinities of Emydocephalus

McDowell (1972: 207–208) questioned the validity of the genus *Emydocephalus*, and suggested that it was related more closely to *Aipysurus eydouxi* than *A. eydouxi* was related to its congeners. He concluded that *Emydocephalus* should be reduced to a subgenus of *Aipysurus*. To test this hypothesis, our tree was compared with an alternative topology where *Emydocephalus* and *A. eydouxi* together form a sister clade to the remaining *Aipysurus* (Fig. 2A).

Phylogenetic affinities of Hydrelaps and Parahydrophis

It is generally agreed that *Hydrelaps* and *Parahydrophis* represent 'primitive' lineages; however, their evolutionary relationships to the *Aipysurus* or *Hydrophis* groups remain unclear (Burger & Natsuno, 1974; McDowell, 1969, 1972, 1974; Voris, 1977). Indeed, they have been variously placed, separately or together, basal to the *Aipysurus* or *Hydrophis* groups, or to both major lineages (McDowell, 1969, 1972, 1974; Burger & Natsuno, 1974; Voris, 1977).

Our tree was compared with a number of alternative topologies. First, the hypotheses was tested that the combined *Hydrelaps*/*Parahydrophis* clade was either a sister taxon to all remaining sea snakes (Fig. 2B) or a sister taxon to the *Aipysurus* group (Fig. 2C). Next, the following hypotheses were tested: *Hydrelaps* was a sister taxon to the *Hydrophis* group, and *Parahydrophis* was a sister taxon to the *Aipysurus* group and vice versa, and *Hydrelaps* was basal to all other sea snakes and *Parahydrophis* was monophyletic with either the *Aipysurus* group or the *Hydrophis* group and vice versa.

Monophyly of Hydrophis

To address questions regarding monophyly of *Hydrophis* and one of its subgenera, *Leioselasma* (McDowell, 1972), our tree was compared with three alternative topologies. In the first, all of our surveyed *Hydrophis* species were forced into monophyly. (Fig. 2D). In the

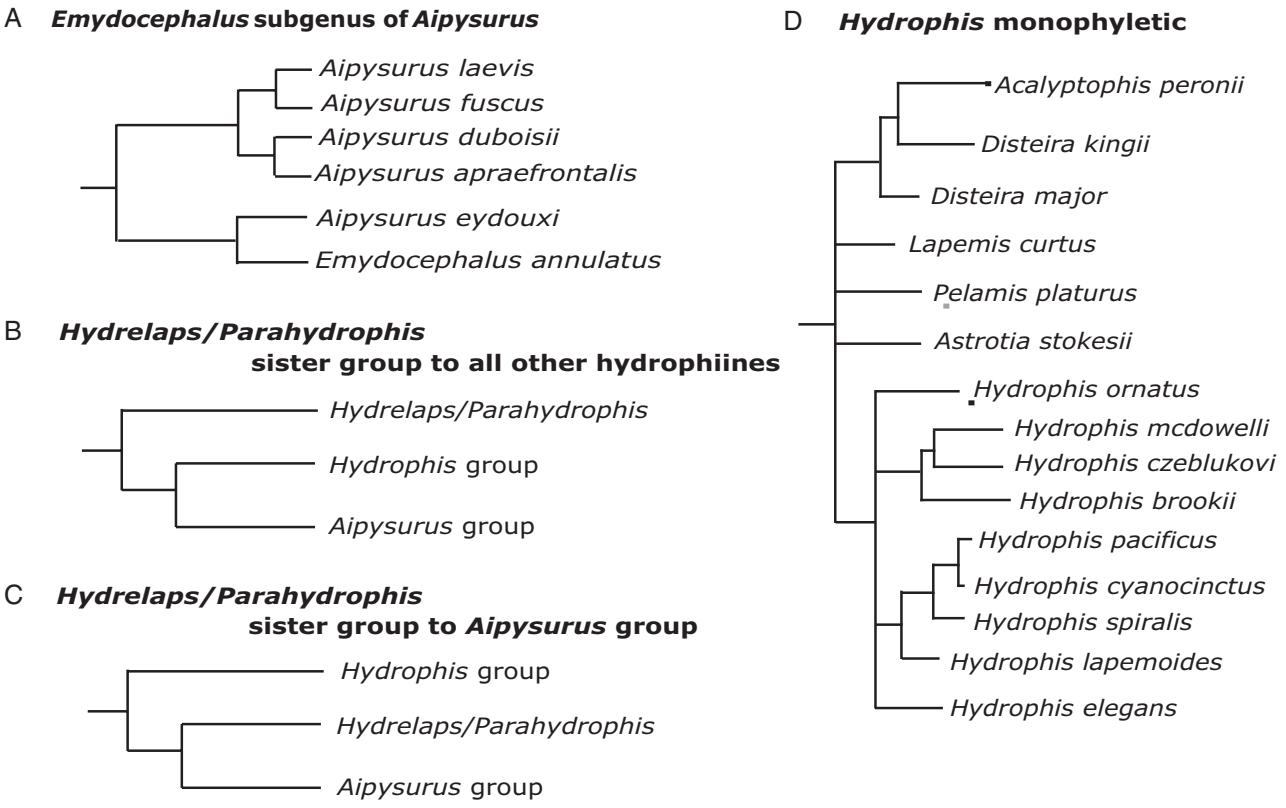

Figure 2. Diagrammatic representations of hypothesized evolutionary relationships based on morphology. Shimodaira–Hasegawa tests were conducted on trees that reflected these hypotheses and included all specimens used in this study. A, phylogenetic affinities of *Emydocephalus*; B, C, phylogenetic affinities of *Hydrelaps* and *Parahydrophis*; D, monophyly of *Hydrophis*. See text for details.

second, all species of the subgenus *Leioselasma* included in our study (*H. spiralis*, *H. cyanocinctus*, *H. pacificus*, and *H. elegans*) were forced into monophyly. Finally, McDowell's (1972) synonymy of *H. pacificus* with *H. elegans* was tested by forcing these two species into monophyly.

Monophyly, composition and phylogenetic affinities of Disteira

Disteira was resurrected by McDowell (1972) to include *Hydrophis major*, *H. kingii*, *Astrotia stokesii* and *Enhydrina schistosa* (Daudin, 1803). However, most subsequent classification schemes retain the monotypic genera *Astrotia* and *Enydrina* and recognize *Disteira* and assign to it *D. major* and *D. kingii* (Cogger, 1975; Minton, 1975; Heatwole & Cogger, 1994; Cogger, 1996). These hypotheses were tested by comparing our tree with two alternative topologies. In the first, both *Disteira* species sampled were forced into monophyly and, in the second, *Disteira* and *Astrotia* were forced into monophyly.

RESULTS

The edited alignment comprised 1518 characters of which 1503 were suitable for phylogenetic analyses. Of these, 544 nucleotide sites (36%) were variable (82 in 16S rRNA, 462 in cytochrome *b*) and 458 nucleotide sites (84%) were informative under parsimony (57 in 16S rRNA, 401 in cytochrome *b*). For the ingroup alone, 496 characters were variable of which 445 were informative under parsimony.

Genetic distance

ModelTest supported the GTR model with gamma distributed rate variation (Γ) and proportion of invariant sites (I) as the best-fit substitution model for each individual data set. These parameters were used to estimate maximum likelihood (ML) genetic distances. Interspecific ML genetic distances for cytochrome *b* ranged widely (Table 3, Appendix 1). The largest distances were in comparisons between species of the *Aipysurus* and *Hydrophis* lineages (~39–55%) whereas the lowest genetic distances were among species within the *Aipysurus* lineage (~3.3–17.6%) and within the *Hydrophis* lineage (~1.4–12.2%). Genetic distances between the *Hydrelaps*/*Parahydrophis* clade and the *Aipysurus* lineage were higher (39–51%) than those between the *Hydrelaps*/*Parahydrophis* clade and the *Hydrophis* lineage (25–35%) (Table 3, Appendix 1). *Hydrelaps* and *Parahydrophis* differed by approximately 25%. As expected, interspecific ML genetic distances for 16S rRNA were considerably smaller but proportional to the cytochrome *b* distances (Table 3, Appendix 1).

Phylogenetic relationships

ModelTest supported GTR + Γ +I as the best-fit substitution model for the combined data set, and these parameters were used for the ML analysis in PAUP*. The optimal ML tree (–ln L = 8684.3) was identical in topology to the five Bayesian consensus trees recovered from estimating one set of parameters for the combined data. The two Bayesian analyses based on the fully partitioned data set also produced virtually identical consensus trees to the ML phylogeny except for minor branch swapping in the *Hydrophis* lineage. Posterior probabilities were similar for the seven consensus trees produced using both Bayesian approaches. The unweighted parsimony analysis resulted in 769 most parsimonious trees and the unweighted parsimony analysis excluding nucleotides at the third codon positions of cytochome *b* resulted in 788 most parsimonious trees. The strict consensus of these trees for each of these analyses again were virtually identical to the optimal ML tree with only minor branch swapping in the *Hydrophis* lineage. The minor topology differences between the analyses occurred only at nodes with no bootstrap support and low posterior probabilities.

The results of our phylogenetic analyses are summarized in Figure 3 where the ML/Bayesian phylogram is presented and two parsimony bootstrap values (first for the entire data set and second for the data set with cytochrome *b* third codon positions excluded; also reported in this order in the text), the nonparametric ML bootstrap values, and the Bayesian posterior probabilities from the combined data set are also shown for each node. For nodes where all four measures of support were 100, this value is summarized by one number on the tree for the sake of simplicity.

The hydrophiine sea snakes comprised two strongly supported monophyletic lineages (Fig. 3). Monophyly of the *Aipysurus* lineage was strongly supported by high bootstrap values and a high Bayesian posterior probability. Within the *Aipysurus* lineage, *E. annulatus* formed a strongly supported sister group to *Aipysurus*. *Aipysurus eydouxi* formed the sister species to its four congeners, and the hypothesis that *A. eydouxi* is related more closely to *Emydocephalus* than to all other *Aipysurus* could be rejected ($P = 0.036$) (Fig. 2A). The remaining four *Aipysurus* species formed two well-supported sister groups, one comprising *A. laevis* and *Aipysurus fuscus*, the other comprising *Aipysurus duboisii* and *Aipysurus apraefrontalis* (Fig. 3). The *Aipysurus* lineage as a whole formed a well-supported sister group to the clade comprising the *Hydrophis* lineage plus *H. darwiniensis* and *P. mertoni*.

Athough monophyly of the clade comprising the Hydrophis lineage plus the *Hydrelaps*/*Parahydrophis*

Table 3. Percent sequence divergences (ranges) for within and between lineage divergences, and intraspecific genetic distances for species represented by more than one individual

Taxon	16S rRNA	Cytochrome *b*
Within lineage divergences		
Aipysurus group	0.39–4.37	3.29–17.58
Hydrophis group	0.19–4.32	1.36–12.23
Hydrelaps/*Parahydrophis* clade	6.51–10.83	25.25–25.96
Between lineage divergences		
Hydrophis and *Aipysurus* groups	5.01–9.65	39.68–54.57
Hydrelaps/*Parahydrophis* and *Aipysurus* group	4.77–8.62	39.03–50.58
Hydrelaps/*Parahydrophis* and *Hydrophis* group	4.90–7.52	24.62–34.57
Intraspecific genetic distances		
Acalyptophis peronii	0.0000	0.0009–0.0066
Aipysurus duboisii	0.0000	0.0063
Aipysurus eydouxi	0.0000–0.0020	0.0010–0.0020
Aipysurus fuscus	0.0000	0.0000
Aipysurus laevis	0.0000–0.0021	0.0000–0.0029
Astrotia stokesii	0.0000–0.0062	0.0009–0.0076
Disteria kingii	0.0000	0.0000–0.0028
Disteria major	0.0000	0.0000–0.0011
Emydocephalus annulatus GBR	0.0000	0.0000
Emydocephalus annulatus NW Shelf	0.0000–0.0021	0.0000–0.0048
Emydocephalus annulatus btw regions	0.0084–0.0110	0.0058–0.0067
Hydrelaps darwiniensis	0.0344	0.0211
Hydrophis cyanocinctus	0.0147	0.0342
Hydrophis elegans	0.0000	0.0019–0.0048
Hydrophis lapemoides	0.0000	0.0009
Hydrophis mcdowelli	0.0020	0.0037
Hydrophis ornatus	0.0000–0.0081	0.0000–0.0028
Hydrophis pacificus	0.0000–0.0039	0.0000–0.0019
Lapemis curtus Australia	0.0000	0.0000–0.0028
Lapemis curtus btw countries	0.0019–0.0021	0.0081–0.0092
Pelamis platurus	0.0000	0.0000–0.0028

Genetic distances are given as ranges and correspond to the model of best fit indicated by ModelTest 3.0 for each data set (GTR + I + Γ for each gene). Note that, for *E. annulatus* and *L. curtus*, genetic distances within and between regions are also presented. Further details are provided in the text.

clade was strongly supported by bootstrap values and posterior probabilities, it was not possible to reject the alternative topologies where the *H. darwiniensis*/*P. mertoni* group formed a sister clade to either the *Aipysurus* lineage ($P = 0.135$) (Fig. 2B), or to both the *Aipysurus* and *Hydrophis* lineages ($P = 0.113$) (Fig. 2C). Monophyly of the *H. darwiniensis*/*P. mertoni* clade itself was poorly supported (parsimony bootstrap values 65% and 68%, respectively; ML bootstrap 52%; Bayesian posterior probability 69%) and it was not possible to reject the alternative hypotheses that place *Hydrelaps* as a sister taxon to the *Hydrophis* lineage and *Parahydrophis* as a sister taxon to the *Aipysurus* lineage ($P = 0.159$) or basal to both lineages ($P = 0.184$). By contrast, the ability to reject the hypotheses of *Parahydrophis* as a sister taxon to the *Hydrophis* lineage and *Hydrelaps* as a sister taxon to the *Aipysurus* lineage ($P = 0.062$) or basal to both lineages ($P = 0.074$) was stronger. Finally, although it was not possible to reject the hypothesis that *Parahydrophis* is basal to both lineages and *Hydrelaps* monophyletic with the *Aipysurus* lineage ($P = 0.099$), based on our data, the hypothesis that *Hydrelaps* is basal to all sea snakes and *Parahydrophis* is monophyletic with the *Aipysurus* lineage ($P = 0.053$) is unlikely.

Monophyly of the *Hydrophis* lineage was very strongly supported; however, most species were characterized by very long branches, and species level and intergeneric relationships were poorly resolved (Fig. 3). Moreover, it is clear that the genus *Hydrophis*, as currently understood, is not monophyletic ($P = 0.0001$) (Fig. 2D). Only two well-supported clades were identified within the *Hydrophis* group, one

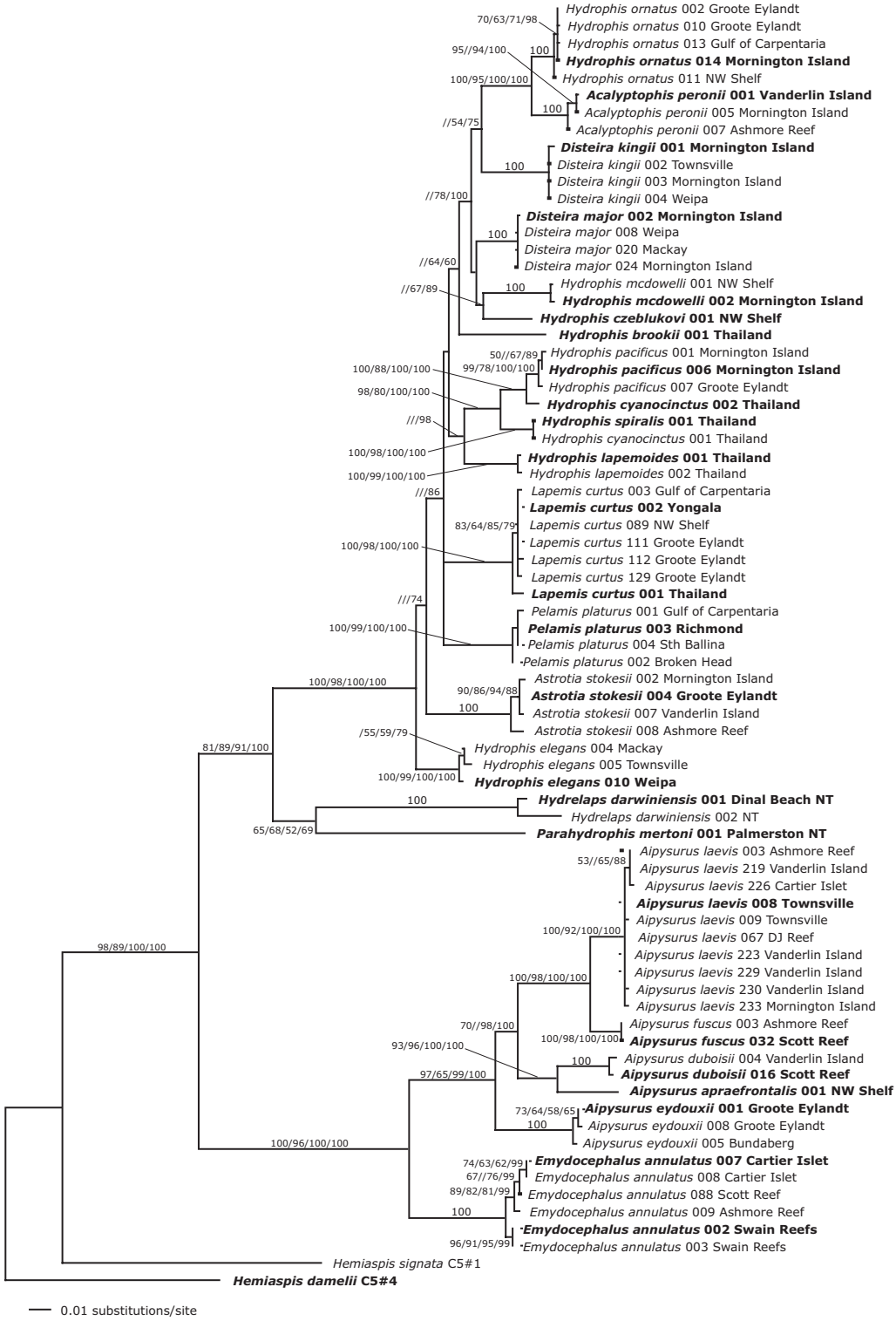

Figure 3. Maximum likelihood/Bayesian tree from combined cytochrome *b* and 16S rRNA data under the best-fitting model GTR + I + Γ. Numbers above or below branches represent parsimony bootstrap values for all characters included/with cytochrome *b* third codon position excluded/nonparametric maximum likelihood bootstrap values/Bayesian posterior probabilities. Nodes for which all three bootstrap measures were 100% and that had Bayesian posterior probabilities of 100 are indicated by 100 on the corresponding branch. Branches with one or more bootstrap values missing indicate that bootstrap values were below 50% for the corresponding analyses. The sampling locations indicated are shown in Figure 1.

comprising *Hydrophis ornatus* and *Acalyptophis peronii* and the other comprising *Hydrophis pacificus*, *H. cyanocinctus* and *H. spiralis* (Fig. 3). *Hydrophis cyanocinctus*, *H. spiralis* and *H. pacificus* all belong to McDowell's (1972) subgenus *Leioselasma*, and although *H. elegans* (also ascribed to the subgenus *Leioselasma*) was not included in this clade in our tree, it was not possible to reject the hypothesis that the subgenus *Leioselasma* comprises a monophyletic group ($P = 0.285$). However, it was possible to reject McDowell's (1972) synonymy of *H. pacificus* with *H. elegans* ($P = 0.0001$). *Disteira kingii* and *D. major* did not form a monophyletic group (Fig. 3) but it was not possible to reject the hypothesis that *Disteira* could be monophyletic ($P = 0.214$). Based on our data, it was possible to reject the hypothesis that *Disteira* and *Astrotia* form a monophyletic group ($P = 0.039$). The very poor resolution among species and genera within the *Hydrophis* lineage, combined with the long branches that characterize most species, suggests that the *Hydrophis* lineage represents a rapidly diverged adaptive radiation.

Intraspecific phylogeography

With only one exception, all branches leading to conspecific individuals were supported by Bayesian posterior probabilities of 100% and bootstrap values ranging from 78% to 100% (Fig. 3). For several species, specimens were obtained from multiple parts of their range and, in most cases, the intraspecific tree topologies reflected these geographical distributions (Figs 1, 3). In particular, *H. ornatus*, *A. peronii*, *H. pacificus*, and *A. stokesii* from the Gulf of Carpentaria formed sister groups to conspecifics from the North-west Shelf. In addition, *A. eydouxi* from the Gulf of Carpentaria formed a sister group to a conspecific individual from the Great Barrier Reef. *Hydrophis elegans* from the Great Barrier Reef formed a sister group to an individual from Weipa in the Gulf of Carpentaria, and *L. curtus* from Australia also formed a sister group to a conspecific individual from Thailand. *Emydocephalus annulatus* also divided into two groups, the north-west Shelf and Great Barrier Reef, and individuals from the north-west Shelf separated out according to the reef from which they were sampled (Figs 1, 3). In addition, intraspecific genetic distances for *L. curtus* and *E. annulatus* were much lower within regions than between regions (Table 3).

DISCUSSION

Our robust molecular phylogeny of the hydrophiine sea snakes has revealed a number of strongly supported matrilineal clades. The topology of the phylogenetic gene-tree corroborates the existence of the long-recognized *Aipysurus* and *Hydrophis* lineages, provides resolution among the *Aipysurus* group species included in our study, and demonstrates that genus *Hydrophis*, as currently understood, is not a monophyletic clade (McDowell, 1969, 1972, 1974; Burger & Natsuno, 1974; Voris, 1977; Rasmussen, 1994). Although the evolutionary relationships of *H. darwiniensis* and *P. mertoni* remain unclear, our topology suggests that *Hydrelaps* represents a sister taxon to the *Hydrophis* group (McDowell, 1972). Finally, our molecular phylogeny strongly supports the hypothesis that the *Hydrophis* lineage represents a rapidly diverged adaptive radiation (Burger & Natsuno, 1974; Voris, 1977). Each of these issues is considered in turn below.

Aipysurus lineage

The *Aipysurus* lineage, comprising nine species, has been taxonomically stable subsequent to Smith's (1926) description of *Aipysurus* and *Emydocephalus*. Our molecular data strongly support monophyly of this group (Fig. 3) and corroborate the distinctive morphological features of the *Aipysurus* lineage that are not found in species of the *Hydrophis* lineage. These include broad ventral scales in 1 : 1 correspondence in number with vertebral number, a median keel, caudal vertebrae with haemapophyses meeting to form complete haemal arches, and a thick cylindrical body (Smith, 1926; McDowell, 1969, 1972; Burger & Natsuno, 1974; Rasmussen, 1997, 2002). These are all primitive characters, however there are very few uniquely derived characters in the sea snakes in general, and none that unite the *Aipysurus* lineage (Voris, 1977).

Smith (1926) recognized two species of *Emydocephalus* and seven species of *Aipysurus*. Our phylogeny grouped *A. laevis* with *A. fuscus*, and *A. duboisii* with *A. apraefrontalis*, into two reciprocally monophyletic and well-supported clades, with *Aipysurus eydouxi* as a sister group (Fig. 3). Although previous studies have not resolved relationship among *Aipysurus* species (McDowell, 1969, 1972, 1974; Burger & Natsuno, 1974; Voris, 1977; Rasmussen, 2002), our results agree with both McDowell (1972) and Voris (1977) who suggested that *A. eydouxi* could represent a stem lineage. *Aipysurus eydouxi* occurs in turbid deeper waters (30–50 m) throughout northern Australia and the Indo-Malay archipelago, and it is the only member of this genus that feeds almost exclusively on fish eggs (Voris & Voris, 1983) and is not endemic to the coral reefs of Australia (Cogger, 1996). *Emydocephalus* species also feed exclusively on fish eggs (Voris, 1966) and *Emydocephalus* species and *Aipysurus eydouxi* share morphological specializations for egg eating, such as extreme fang and venom apparatus reduction (Voris,

1977; Voris & Voris, 1983). Despite this, our data do not support McDowell's (1972) proposed sister group relationship between *Emydocephalus* and *Aipysurus eydouxi* to the exclusion of other *Aipysurus* nor the allocation of *Emydocephalus* to a subgenus of *Aipysurus* (Fig. 2A). Our tree topology does suggest that egg eating may be the ancestral condition in the *Aipysurus* group; however, it is also plausible that the specialization for exclusive egg eating evolved twice in this group, particularly considering the extreme fang and venom apparatus reduction associated with this dietary specialization, and that all members of the *Aipysurus* group include fish eggs in their diets to some extent (Voris, 1972; Voris & Voris, 1983).

Hydrophis lineage

Our phylogeny strongly demonstrates that the *Hydrophis* lineage is monophyletic. However, unlike the *Aipysurus* lineage, the evolutionary relationships among species and genera in this much larger lineage remain poorly understood (Fig. 3). Similarly, although the *Aipysurus* lineage has been taxonomically stable subsequent to Smith (1926), the genera that comprise the *Hydrophis* lineage have been revised numerous times (Smith, 1926; McDowell, 1969, 1972, 1974; Burger & Natsuno, 1974; Voris, 1977; Rasmussen, 1997). This somewhat chaotic taxonomic history almost certainly is due in part to the difficulties in identifying phylogenetically useful morphological and molecular characters for groups that represent rapidly diverged adaptive radiations (Schluter, 2000).

The genus *Hydrophis* represents a good example of the difficulties associated with the taxonomy of this radiation. In an effort to resolve the evolutionary relationships within this genus, McDowell (1972) grouped its 21 species into three subgenera: *Leioselasma*, *Aturia* (*Chitula*) and *Hydrophis*. Cogger (1975) subsequently regarded these as natural groups and Kharin (1984) suggested they be elevated to genera. Although the present study supports the natural grouping of McDowell's smallest subgenus *Leioselasma* (even with the inclusion of *H. elegans*, *Leioselasma* could not be rejected as a natural group), Rasmussen (1994) showed that McDowell's subgenus *Aturia* (nine species) was paraphyletic based on cladistic analysis of the characters used by McDowell to define the subgenera. Our topology demonstrates clearly that the genus *Hydrophis*, as currently understood, is not monophyleic and our result corroborates others who have come to the same conclusion based on other types of data (Cadle & Gorman, 1981; Rasmussen, 1994). Indeed, *Hydrophis* has been described as 'a taxonomic parking place for species whose relationships are not yet understood' (Greer, 1997), and probably comprises all the species in the *Hydrophis* lineage included in this study. Nevertheless, it seems premature to propose yet another taxonomic revision of the genus at this stage. Instead, a taxonomic revision of the *Hydrophis* lineage should be delayed until a molecular phylogeny is available that includes all currently recognized genera (if not species) in this lineage. Moreover, additional mitochondrial and nuclear loci will be needed to resolve the evolutionary relationships within this challenging group.

There are only two well-supported clades within the *Hydrophis* group that can be noted in our phylogeny. The first is the clade comprising *H. ornatus* and *A. peronii* to the exclusion of other *Hydrophis* species, a close relationship that has not been identified in previous studies. The second is the group comprising *H. cyanocinctus*, *H. spiralis* and *H. pacificus* from McDowell's (1972) subgenus *Leioselasma* (Fig. 3). *Hydrophis pacificus* was placed in synonymy with *Hydrophis belcheri* by Smith (1926), moved to the synonymy of *H. elegans* by McDowell (1972), and resurrected by Cogger (1975). Based on our data, *H. pacificus* and *H. elegans*, both of which are Australian endemics with overlapping distributions, are distinct species. However the relationships among *H. pacificus*, *H. cyanocinctus* and *H. spiralis* are less clear. Within this clade, *H. spiralis* (represented by individual Hsp001) and one individual of *H. cyanocinctus* (Hcy001) formed a well-supported group, whereas the second individual of *H. cyanocinctus* (Hcy002) formed a well-supported clade with *H. pacificus* (Fig. 3). Intraspecific genetic distances for *H. cyanocinctus* (1.5% for 16S rRNA and 3.4% for cytochrome *b*) also were higher than those between most other species (Table 3). The most probable explanation of this incongruous grouping is incorrect identification, as there is no agreement on the distinguishing features of *H. cyanocinctus* and *H. spiralis* (McDowell, 1972). Unfortunately, only dried heads remain of the specimens used in this study (H. Voris, pers. comm.) and more work needs to be carried out to clarify the relationships among these closely related species.

Although some monotypic genera such as *Acalyptophis*, *Astrotia*, and *Pelamis* have been recognized since they were first described, the status and/or species composition of other genera such as *Disteira* and *Lapemis* have been more controversial. McDowell (1972) resurrected *Disteira* and assigned to it four species (*D. major*, *D. kingii*, *A. stokesii*, and *E. schistosa*) based on the common possession of an 'adductor mandibulae externus superficialus muscle with very broad dorsal portion which completely conceals the adductor externus medialis' (McDowell, 1972). Burger & Natsuno (1974) agreed that *Disteira*, including *Astrotia* and *Enhydrina*, comprised a natural group but, because it was only distinguished from *Hydrophis* by

one character, they reduced *Disteira* to a fourth subgenus of *Hydrophis*. Most subsequent classifications have retained *Disteira* but only assign to it *D. major* and *D. kingii*. Based on our data, it was not possible to reject monophyly of *D. major* and *D. kingii*. However, if *Disteira* does represent a natural group, our data does demonstrate that *Astrotia* is more distantly related.

The phylogenetic affinities of *H. darwiniensis*, *E. greyi* and *P. mertoni* have been problematical because they display a series of morphological attributes that have been interpreted as 'primitive' among sea snakes (McDowell, 1969, 1972, 1974). McDowell (1969) considered *Ephalophis* as basal to the *Aipysurus* group, and considered *Hydrelaps* basal to either the *Hydrophis* group or to both major lineages. Voris's (1977) phylogenetic analysis of detailed morphological data found that both *Ephalophis* and *Hydrelaps* were basal to the *Hydrophis* group. By contrast Burger & Natsuno (1974) placed *Hydrelaps*, *Ephalophis*, and their newly created genus, *Parahydrophis* (previously *Ephalophis mertoni*), as basal to the *Aipysurus* group that together formed a new subfamily Ephalophiinae or 'Thick Sea Snakes', which was placed basal to their other newly created subfamily Hydrophiinae or 'Flat Sea snakes' (our *Hydrophis* group). Although the *Hydrelaps*/*Parahydrophis* clade formed a distant sister taxon to the *Hydrophis* group in our phylogeny, it was not possible to exclude the alternative hypotheses. However some topologies, in particular those that placed *Hydrelaps* either basal to all sea snakes or monophyletic with the *Aipysurus* group, were in the boarderline rejection region. The evolutionary relationships of these two species may be clarified by including *Ephalophis* in the phylogeny, and/or with additional mitochondrial or nuclear loci.

The *Hydrophis* lineage as a rapidly diverged adaptive radiation

An adaptive radiation comprises a group of species that inhabit a variety of environments, differ in morphological and other traits important in utilizing these environments, and are descended from a common ancestor that rapidly speciated over a short period of time (Schluter, 2000). The *Hydrophis* lineage satisfies all four criteria set out by Schluter (2000) to identify an adaptive radiation including common ancestry (Keogh, 1998), phenotype–environment correlation and trait utility (Voris, 1977; Voris & Voris, 1983), and rapid speciation as demonstrated by our phylogeny. The topology of our tree, lack of resolution between species, nonmonophyly of genera, similar levels of genetic divergence between taxa, and short internodes all support a rapid speciation model.

This is perhaps best exemplified by examining genetic diversity relative to phenotypic diversity (Schluter, 2000). Even though overall genetic diversity is similar for the *Aipysurus* and *Hydrophis* lineages (Table 3), phenotypic diversity relative to genetic diversity is much greater in the *Hydrophis* lineage. For example, mean ML interspecific genetic distance estimates for representatives of the *Hydrophis* and *Aipysurus* lineages for 16S rRNA were 2.37% and 2.73%, and for cytochrome *b* were 8.60% and 11.86%, respectively. The *Aipysurus* lineage comprises two genera and nine species, whereas the *Hydrophis* lineage comprises 11 genera (mostly monotypic) and 23 species. If the number of genera within each lineage is used as a proxy for the levels of phenotypic diversity within each lineage (Schluter, 2000), and it is assumed that the genetic diversity of the *Hydrophis* lineage is well sampled in the present study, then the ratio of phenotypic diversity to genetic diversity of *Hydrophis* lineage is approximately seven-fold that of the *Aipysurus* lineage. A more conservative approach is to consider only the genera sampled in our phylogeny (six genera from the *Hydrophis* lineage, two from the *Aipysurus* lineage); however, the level of phenotypic divergence relative to genetic divergence is still approximately four-fold higher in the *Hydrophis* lineage. A similar result is obtained if one uses the number of species in each lineage as a proxy for phenotypic diversity.

Burger & Natsuno (1974) and Voris (1977) noted the possibility of an adaptive radiation of the *Hydrophis* lineage in the tropical waters of northern Australian and South-east Asia, where they now occur in a wide range of habitats and forage on a wide range of prey items (Voris & Voris, 1983). Voris (1977) hypothesized that ancestral *Hydrophis* populations were isolated repeatedly due to fluctuating sea levels associated with Pleistocene glaciation cycles, promoting speciation. Our data are consistent with Voris's (1977) hypothesis, which is also supported by distributional data. Most members of the *Aipysurus* lineage are endemic to the coral reefs of Australia and New Guinea and, although Voris (1977) suggested that early widespread representatives of *Aipysurus eydouxi* and *Emydocephalus ijimae* experienced the same repeated isolations as the *Hydrophis* lineage, it is more plausible that the *Aipysurus*/*Emydocephalus* complex represents an endemic Australian element (Cogger, 1975) and that *A. eydouxi* and *Emydocephalus* later dispersed into south-east Asian waters. Alternatively, species of the *Aipysurus* lineage may be relics of an earlier adaptive radiation (Burger & Natsuno, 1974).

Although fluctuating sea levels and associated repeated isolation of populations over long periods together comprise a convincing hypothesis to explain

the rapid diversification of the *Hydrophis* lineage, our molecular data also are fully consistent with the phenotypic and ecological diversity displayed within this lineage, particularly with regard to diet specialization and habitat preference. In particular, the *Hydrophis* lineage displays a high level of specialization with respect to prey size and shape, and this is reflected in associated morphological specializations (Voris & Voris, 1983). For example, a suite of long-necked microcephalic *Hydrophis* species feed exclusively on burrowing eels (McCosker, 1975; Voris & Voris, 1983). These ecological factors, combined with sea level fluctuations acting to isolate populations, together represent the most likely explanation for the diversity displayed in this radiation.

ACKNOWLEDGEMENTS

This work was funded by the CRC Reef Research Centre (V. L.) and the Australian Research Council (J. S. K.). Tissue samples were provided by the South Australia Museum, the Commonwealth Scientific and Industrial Research Organization, and the Field Museum of Natural History. We thank John Avise, Helene Marsh, and Michelle Waycott for helpful comments on the manuscript and Harold Voris, Arne Rasmussen, and Harold Heatwole for discussion that helped us improve our work.

REFERENCES

Boulenger GA. 1896. *Catalogue of snakes of the British Museum.* London: British Museum of Natural History.

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

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

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

Cogger H. 1975. Sea snakes of Australia and New Guinea. In: Dunson WA, ed. *The biology of sea snakes*. Baltimore, MD: University Park Press, 59–140.

Cogger H. 1996. *Reptiles and amphibians of Australia*. Melbourne: Reed Books Australia.

Cogger H, Heatwole H. 1981. The Australian reptiles: origins, biogeography, distribution patterns and island evolution. In: Keast A, ed. *Ecological biogeography of Australia*. The Hague: Junk Publications, 1331–1373.

Golay P. 1985. *Checklist and keys to the terrestrial proteroglyphs of the world.* Geneva: Elapsoidea Herpetological Data Centre.

Goldman N, Anderson JP, Rodrigo AG. 2000. Likelihood-based tests of topologies in phylogenetics. *Systematic Biology* **49:** 652–670.

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

Greer AE. 1997. *The biology and evolution of Australian snakes*. Chipping Norton: Beatty & Sons Pty Ltd.

Heatwole H. 1999. *Sea snakes.* Sydney: University of New South Wales Press.

Heatwole H, Cogger H. 1994. Sea snakes of Australia. In: Gopalakrishnakone P, ed. *Sea snake toxinology*. Singapore: Singapore University Press, 167–205.

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

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Keogh JS. 1998. Molecular phylogeny of elapid snakes and a consideration of their biogeographic history. *Biological Journal of the Linnean Society* **63:** 177–203.

Keogh JS, Scott IAW, Hayes C. 2005. Rapid and repeated origin of insular gigantism and dwarfism in Australian tiger snakes. *Evolution* **59:** 226–233.

Keogh JS, Scott IAW, Scanlon JD. 2000. Molecular phylogeny of viviparous Australian elapid snakes: Affinities of 'Echiopsis' atriceps (Storr, 1980) and 'Drysdalia' coronata (Schlegel, 1837), with description of a new genus. *Journal of Zoology, London* **252:** 317–326.

Keogh JS, Shine R, Donnellan S. 1998. Phylogenetic relationships of terrestrial Australo-Papuan elapid snakes (Subfamily Hydrophiinae) based on cytochrome b and 16S r RNA sequences. *Molecular Phylogenetics and Evolution* **10:** 67–81.

Kharin VE. 1984. A review of sea snakes of the group *Hydrophis sensu* lato (Serpentes: Hydrophiidae). 3. The genus *Leioselasma*. *Zoologicheskij Zhurnal* **63:** 1535–1546.

Kumazawa Y, Ota H, Nishida M, Ozawa T. 1998. The complete nucleotide sequence of a snake (*Dinodon semicarinatus*) mitochondrial genome with two identical contrological regions. *Genetics* **150:** 313–329.

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

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.

McCosker JE. 1975. Feeding behaviour of Indo-Australian Hydrophiidae. In: Dunson WA, ed. *The biology of sea snakes*. Baltimore, MD: University Park Press, 217–232.

McDowell SB. 1969. Notes on the Australian sea-snake *Ephalophis greyi* M. Smith (Serpentes: Elapidae, Hydrophiinae) and the 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* **161:** 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. 1974. Additional notes on the Rare and Primitive Sea-snake, *Ephalophis greyi*. *Journal of Herpetology* **8:** 123–128.

Minton SA. 1975. Geographic distribution of sea snakes. In: Dunson WA, ed. *The biology of sea snakes*. Baltimore, MD: University Park Press, 21–32.

Minton SA, da Costa MS. 1975. Serological relationships of sea snakes and their evolutionary implications. In: Dunson WA, ed. *The biology of sea snakes*. Baltimore, MD: University Park Press, 33–58.

Nock CJ. 2001. Molecular Phylogenetics of the Australian Elapid Snakes. Unpublished MSc Thesis, Southern Cross University.

Palumbi SR. 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, eds. *Molecular systematics*. Sunderland, MA: Sinauer Associates, Inc, 205–247.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitutions. *Bioinformatics* **14:** 817–818.

Rasmussen AR. 1994. A cladistic analysis of Hydrophis subgenus Chitulia (McDowell, 1972) (Serpentes, Hydrophiidae). *Zoological Journal of the Linnean Society* **111:** 161–178.

Rasmussen AR. 1997. Systematics of sea snakes: a critical review. *Symposia of the Zoological Society of London* **70:** 15–30.

Rasmussen AR. 2002. Phylogenetic analysis of the 'true' aquatic elapid snakes Hydrophiinae (sensu Smith *et al.*, 1977) indicates two independent radiations into water. *Steenstrupia* **27:** 47–63.

Reeder TW. 2003. A phylogeny of the Australian Sphenomorphus group (Scincidae: Squamata) and the phylogenetic placement of crocodile skinks (Tribolonotus): Bayesian approaches to assessing congruence and obtaining confidence in maximum likelihood inferred relationships. *Molecular Phylogenetics and Evolution* **27:** 384–397.

Schluter D. 2000. The ecology of adaptive radiation. Oxford: Oxford University Press.

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*. Chipping Norton: Beatty & Sons Pty Ltd., 177–184.

Shimodaira H, Hasegawa M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16:** 1114–1116.

Slowinski JB, Keogh JS. 2000. Phylogenetic relationships of elapid snakes based on cytochrome b mtDNA sequences. *Molecular Phylogenetics and Evolution* **15:** 157–164.

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 MA. 1926. *Monograph of the sea-snakes (Hydrophiidae).* London: Taylor & Francis.

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

Swofford D. 2000. *PAUP*. phylogenetic analysis using parsimony (*and other methods)*, Version 4. Sunderland, MA: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **20:** 4876–4882.

Voris HK. 1966. Fish eggs as the apparent sole food item for a genus of sea snake Emydocephalus (Kreft). *Ecology* **47:** 152–154.

Voris HK. 1972. The role of sea snakes (Hydrophiidae) in the trophic structure of coastal ocean communities. *Journal of the Marine Biology Association of India* **13:** 1–14.

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 Zoology* **23:** 411–425.

Wilcox TP, Zwickl DJ, Heath TA, Hillis DM. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution* **25:** 361–371.

APPENDIX

Interspecific genetic distance matrix based on cytochrome *b* sequences (above diagonal) and 16S rRNA sequences (below diagonal)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	*Hemiaspis damelii* (C5#4)	–	0.4789	0.4509	0.4899	0.4547	0.5192	0.4554	0.4194	0.4623	0.4585	0.4539	0.4696	0.3677	0.4896	0.4336	0.4645	0.4444	0.4352	0.4245	0.4270	0.4428	0.4826	0.4460	0.4051
2	*Aipysurus apraefrontalis* (001)	0.1078	–	0.1062	0.1124	0.0684	0.1108	0.1668	0.4325	0.4917	0.4189	0.4090	0.4299	0.5058	0.4773	0.4195	0.4166	0.4631	0.4318	0.4743	0.4481	0.4563	0.4338	0.4401	0.4043
3	*Aipysurus laevis* (008)	0.0831	0.0168	–	0.0330	0.1098	0.1098	0. 1754	0.5346	0.5457	0.4757	0.4785	0.4862	0.4882	0.4967	0.4823	0.5142	0.5088	0.4882	0.5219	0.5115	0.5090	0.5111	0.4898	0.4788
4	Aipysurus fuscus (032)	0.0870	0.0219	0.0040	–	0.1044	0.1033	0. 1758	0.5353	0.5385	0.4634	0.4826	0.4874	0.4847	0.4902	0.4832	0.5140	0.4872	0.4545	0.5085	0.5064	0.4977	0.5272	0.4667	0.4931
5	*Aipysurus duboisii* (016)	0.1055	0.0101	0.0258	0.0294	–	0.0950	0.1566	0.4600	0.5074	0.4453	0.4391	0.4310	0.4756	0.4624	0.4424	0.4549	0.4763	0.4289	0.4904	0.4674	0.4641	0.4816	0.4670	0.4204
6	*Aipysurus eydouxii* (001)	0.1090	0.0287	0.0235	0.0289	0.0335	–	0.1519	0.4828	0.5422	0.4343	0.4612	0.4473	0.4597	0.4544	0.4642	0.4980	0.5073	0.4853	0.5188	0.4996	0.4815	0.4982	0.4501	0.4325
7	*Emydocephalus annulatus* (002)	0.0861	0.0389	0.0329	0.0355	0.0438	0.0352	–	0.4175	0.4804	0.4187	0.4327	0.3971	0.4591	0.3903	0.4031	0.4253	0.4260	0.3968	0.4407	0.4529	0.4500	0.4198	0.4209	0.4153
8	*Astrotia stokesii* (004)	0.1175	0.0715	0.0569	0.0601	0.0796	0.0710	0.0677	–	0.1119	0.1032	0.0904	0.1096	0.3240	0.2953	0.0726	0.0834	0.1001	0.0847	0.0925	0.1089	0.1018	0.1081	0.0954	0.1010
9	*Acalyptophis peronii* (001)	0.1169	0.0756	0.0610	0.0642	0.0836	0.0834	0.0710	0.0271	–	0.0990	0.0734	0.1181	0.3408	0.3498	0.0948	0.1037	0.1077	0.0891	0.0982	0.0851	0.0383	0.1076	0.0797	0.1223
10	*Disteira kingii* (002)	0.1134	0.0719	0.0576	0.0608	0.0799	0.0798	0.0722	0.0222	0.0164	–	0.0701	0.1064	0.3100	0.2773	0.0811	0.0792	0.0990	0.0942	0.0910	0.0803	0.0830	0.0889	0.0792	0.0912
11	*Disteira major* (008)	0.1092	0.0642	0.0565	0.0595	0.0719	0.0719	0.0658	0.0245	0.0188	0.0187	–	0.0819	0.2919	0.2812	0.0646	0.0712	0.0786	0.0726	0.0737	0.0649	0.0592	0.0690	0.0534	0.0782
12	*Lapemis curtus* (001)	0.1153	0.0669	0.0532	0.0562	0.0744	0.0624	0.0669	0.0244	0.0234	0.0188	0.0121	–	0.3457	0.2737	0.0821	0.0904	0.0988	0.0884	0.0946	0.0971	0.1094	0.1023	0.0785	0.0953
13	*Parahydrophis mertoni* (001)	0.1123	0.0477	0.0556	0.0635	0.0553	0.0677	0.0721	0.0631	0.0526	0.0635	0.0494	0.0545	–	0.2525	0.2746	0.3243	0.2681	0.2775	0.2462	0.3006	0.3203	0.3040	0.3106	0.2540
14	*Hydrelaps darwiniensis* (001)	0.1115	0.0702	0.0662	0.0695	0.0862	0.0795	0.0695	0.0650	0.0582	0.0636	0.0666	0.0601	0.0651	–	0.2510	0.3059	0.2711	0.2695	0.2872	0.3109	0.3038	0.3253	0.3257	0.2678
15	*Hydrophis elegans* (010)	0.1018	0.0700	0.0562	0.0592	0.0777	0.0776	0.0658	0.0279	0.0244	0.0267	0.0266	0.0241	0.0563	0.0682	–	0.0669	0.0866	0.0722	0.0843	0.0780	0.0858	0.0821	0.0738	0.0804
16	*Hydrophis lapemoides* (001)	0.0903	0.0603	0.0504	0.0534	0.0679	0.0680	0.0525	0.0297	0.0190	0.0167	0.0165	0.0188	0.0584	0.0583	0.0170	–	0.0757	0.0632	0.0695	0.0915	0.0899	0.0769	0.0822	0.0837
17	*Hydrophis pacificus* (006)	0.1110	0.0685	0.0545	0.0576	0.0765	0.0764	0.0621	0.0297	0.0239	0.0215	0.0211	0.0166	0.0585	0.0555	0.0221	0.0125	–	0.0317	0.0136	0.1024	0.0989	0.0988	0.0972	0.0985
18	*Hydrophis spiralis* (001)	0.0999	0.0601	0.0465	0.0496	0.0651	0.0684	0.0594	0.0276	0.0220	0.0242	0.0238	0.0191	0.0564	0.0594	0.0250	0.0150	0.0103	–	0.0331	0.0915	0.0893	0.0881	0.0774	0.0876
19	*Hydrophis cyanocinctus* (002)	0.1059	0.0651	0.0515	0.0545	0.0728	0.0728	0.0587	0.0271	0.0264	0.0240	0.0236	0.0189	0.0552	0.0521	0.0247	0.0148	0.0020	0.0125	–	0.0961	0.1024	0.0876	0.0873	0.0903
20	*Hydrophis mcdowelli* (002)	0.1340	0.0838	0.0749	0.0783	0.0919	0.0832	0.0775	0.0371	0.0362	0.0263	0.0236	0.0238	0.0752	0.0707	0.0432	0.0262	0.0315	0.0347	0.0343	–	0.0861	0.0876	0.0703	0.0987
21	*Hydrophis ornatus* (014)	0.1214	0.0902	0.0740	0.0775	0.0916	0.0965	0.0815	0.0350	0.0233	0.0215	0.0266	0.0341	0.0715	0.0750	0.0352	0.0268	0.0274	0.0356	0.0301	0.0312	–	0.0948	0.0780	0.0950
22	*Hydrophis brookii* (001)	0.1244	0.0812	0.0634	0.0666	0.0890	0.0825	0.0621	0.0275	0.0367	0.0219	0.0340	0.0288	0.0729	0.0734	0.0379	0.0319	0.0320	0.0352	0.0293	0.0379	0.0437	–	0.0825	0.0928
23	*Hydrophis czeblukovi* (001)	0.1192	0.0751	0.0607	0.0638	0.0766	0.0765	0.0622	0.0298	0.0236	0.0145	0.0165	0.0166	0.0675	0.0678	0.0295	0.0145	0.0192	0.0219	0.0216	0.0239	0.0294	0.0295	–	0.0999
24	*Pelamis platurus* (003)	0.1038	0.0636	0.0501	0.0531	0.0713	0.0712	0.0589	0.0151	0.0145	0.0144	0.0080	0.0079	0.0490	0.0542	0.0171	0.0123	0.0123	0.0147	0.0145	0.0238	0.0268	0.0241	0.0123	–

Genetic distances estimated using model of best fit selected by ModelTest 3.0 for each data set (GTR + I + Γ). Individuals used as indicated in Table 1, Figure 3.