Zootaxa 3201: 45–57 (2012)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Hydrophis donaldi (Elapidae, Hydrophiinae), a highly distinctive new species of sea snake from northern Australia

KANISHKA D. B. UKUWELA[1], KATE L. SANDERS[1] & BRYAN G. FRY[2]

[1]*Department of Ecology and Evolutionary Biology, Darling Building, School of Earth and Environmental Sciences, University of Adelaide, Adelaide, SA 5005, Australia*
[2]*Venom Evolution Laboratory, School of Biological Sciences, University of Queensland, Brisbane, QLD 4072, Australia*

Abstract

A new species of viviparous sea snake, *Hydrophis donaldi* **sp. nov.** (Hydrophiinae), is described from the Gulf of Carpentaria, northern Australia. Molecular analyses reveal this species as a deeply divergent lineage within the *Hydrophis* subgroup, and separate it from all other sampled taxa by fixed nucleotide substitutions at three independent mitochondrial and nuclear loci. The new species is assigned to *Hydrophis* based on the current morphological diagnosis of this large but paraphyletic genus, and is distinguished from all other *Hydrophis* species and closely allied genera by a combination of morphological characters relating to scalation, colour pattern and osteology. Using current keys for sea snakes, *H. donaldi* **sp. nov.** might be mistaken for *H. coggeri, H. sibauensis* or *H. torquatus diadema* but it is readily distinguished from these species by a higher number of bands on the body and tail, lower ventral count, strongly spinous body scales, and a wider, more rounded head. Sea snakes have been sampled intensively in the Gulf of Carpentaria due to their vulnerability to bycatch in the region's commercial prawn-trawl fisheries. That this highly distinctive new species has evaded discovery in the region until now is surprising, but might be explained by its habitat preferences. All known specimens of *H. donaldi* **sp. nov.** were found in estuarine habitats that are relatively poorly surveyed and are not targeted by commercial fisheries.

Key words*:* Estuary, Gulf of Carpentaria, *Hydrophis*, phylogenetics, taxonomy

Introduction

The Gulf of Carpentaria (GoC) in northern Australia supports a diverse marine fauna (Mummery & Hardy 1994). Sea snakes are a major component of this fauna and have been sampled intensively due to their vulnerability to bycatch in the region's commercial prawn-trawl fisheries (Redfield *et al.* 1978; Wassenberg *et al.* 1994; Ward 2001). Most sea snake sampling has targeted open (off-shore) habitats, because commercial trawling is restricted to these regions. As a result, coastal habitats such as estuaries, lagoons and tidal creeks have been poorly sampled for sea snakes. Recently, nine specimens of a distinct species of 'viviparous or true' sea snake (Elapidae: Hydrophiinae) were collected (by BGF) from the coastal estuarine habitats of Weipa on the Queensland coast of the GoC. In this paper we describe this new species using morphological and molecular data. Currently 60 species of viviparous sea snakes are recognised (Rasmussen *et al.* 2011). These occur in a wide range of mostly coastal habitats throughout the Indian and western Pacific oceans, but reach their highest diversity in the Indo-Australian region (Rasmussen *et al*. 2011). Viviparous sea snakes form two morphologically (Smith 1926; Voris 1977; Rasmussen 1997; 2002) and genetically (Lukoschek & Keogh 2006) distinct lineages - the *Aipysurus* and *Hydrophis* groups. The *Hydrophis* group is the most diverse with thirteen morphologically distinct genera: *Acalyptophis, Astrotia, Enhydrina, Ephalophis, Hydrelaps, Hydrophis, Kerilia, Kolpophis, Lapemis, Pelamis, Parahydrophis, Thalassophina, Thalassophis. Hydrophis* (Latreille in Sonnini & Latreille, 1801) is the largest of these, comprising 36 nominal species (Rasmussen 2001; Rasmussen *et al.* 2001, 2007) including most of the marine snake species that have been described in the last 10 years (Rasmussen & Ineich 2000; Rasmussen *et al.* 2001, 2007). However, phylogenetic evidence shows *Hydrophis* to be paraphyletic with respect to at least eight other genera and therefore much in need of taxonomic revision (Cadle & Gorman 1981; Lukoschek & Keogh 2006; Sanders *et al.* unpublished data). In

Accepted by D. Gower: 3 Jan. 2012; published: 21 Feb. 2012

northern Australian waters, 31 species of viviparous sea snakes have been recorded, of which 14 are currently classified as *Hydrophis* (Cogger 2000; Wilson & Swan 2008). The position of the viviparous sea snakes within elapid phylogeny is relatively well resolved (Voris 1977; Keogh 1998; Keogh *et al.* 1998 ; Sanders & Lee 2008; Sanders *et al.* 2008), but many species and genus level relationships remain poorly understood despite several studies using morphological characters (e.g. Voris 1977; Rasmussen 1997; 2002) and a molecular analysis using sequence data for most genera (Lukoschek & Keogh 2006). Here we add a distinctive new species to *Hydrophis* and attempt to determine the phylogenetic position of this species using three mitochondrial molecular markers and two nuclear loci from 20 taxa.

Material and methods

Taxon sampling. Nine specimens of the new species were collected by BGF from Albatross Bay (mouths of Hay's Creek and Mission River) in Weipa, Queensland by spot-lighting in early evening from boats cruising at low speeds in shallow (< 10 m) water over sea-grass beds. Tissues (liver samples and tail clippings) were preserved in 90% ethanol; voucher specimens were fixed in formalin and accessioned in the South Australian Museum and the Queensland Museum. A further 16 sea snakes representing 16 species collected by the authors during field trips were sampled for molecular analyses, and an additional 57 mitochondrial sequences were obtained from GenBank. Specimen details are given in Appendix 1.

Morphology. A total of 27 morphological characters were examined in the new species. The following scale counts were taken in all eight specimens: number of ventral scales from neck to cloaca following Dowling (1951); number of caudal scales, counted from the anus/vent to the tip of the tail; and number of costal scale rows (around neck and mid body) following Rasmussen *et al.* (2001). Costal scale row counts around the neck and body were repeated four times to obtain minimum and maximum counts, respectively. Morphometric data taken from the specimens in the type series were: snout to vent length (SVL), length from the tip of snout to posterior margin of anal plate; tail length (TAL), length from posterior margin of anal plate to tip of tail; interorbital distance (IOD), distance between the posterior outer edges of the supraoculars taken dorsally; head width (HW), widest distance between lateral margins of the quadrates; head length (HL), length between anterior medial margin of the rostral plate and posterior margin of the parietals; gape length (GL), distance from the anterior medial margin of the rostral plate to one corner of the mouth, snout to eye length (SEL), length between anterior medial margin of the rostral plate to anterior margin of the eye; snout to nostril length (SNL), length between the anterior medial margin of the rostral plate to the anterior margin of the nostril; nostril to eye (NED), shortest distance between nostril and eye; eye length (EL), maximum horizontal length of the eye; greatest tail height (TH). The sum of SVL and TAL was used to obtain total length (TOTL) and TAL was also expressed as a ratio of SVL. Measurements were taken using a Mitutoyo digital vernier caliper to 0.01 mm. SVL and TAL were measured using a measuring tape. Sex was determined by dissecting specimens to examine the reproductive organs. Institutional codes follow (Leviton *et al.* 1985): Museum of Zoology, Bogor, Indonesia (MZB); Museum of Zoology, University of Copenhagen, Denmark (ZMUC); Museum and Art Gallery of the Northern Territory, Darwin, Australia (NTM); Queensland Museum, Brisbane, Australia (QM); South Australian Museum, Adelaide, Australia (SAMA); Western Australian Museum, Perth, Australia (WAM).

To visualise skull morphology, micro-CT scanning was performed on paratype SAMA R65216 using a Skyscan 1076 in-vivo X-ray microtomograph at Adelaide Microscopy, Adelaide, Australia with the following general settings: resolution 18µm, rotation step 0.6 degrees, time 295 msec, filter nil, voltage 60kV, and current 120µA. All reconstructions were performed with Skyscan software (www.skyscan.be/products/downloads.htm). 3D models were created using CTAn and still images were made using CTVol, with scale obtained by measuring the X-ray images in tview as well as measuring some dimensions in the cross sectional images with CTAn.

Molecular analysis. Molecular analyses included 21 species of sea snakes representing all major lineages within the *Hydrophis* group sensu Smith (1926) (Lukoschek & Keogh 2006). Outgroups used were *Aipysurus laevis* in the mtDNA analysis, and *Parahydrophis mertoni* in the analysis of the anonymous nuclear markers; the reciprocal monophyly of *Aipysurus* and the *Hydrophis* group plus successive sister lineages *Hydrelaps* and *Parahydrophis* is strongly supported based on morphological and molecular analyses (Rasmussen 2002; Lukoschek & Keogh 2006; Sanders *et al.* 2008) and preliminary analyses recovered the new species within the *Hydrophis* group.

The majority of the mitochondrial sequences were downloaded from GenBank (see Appendix 1) but mitochondrial data for the new species and five additional taxa were generated in this study; nuclear sequences were generated for all the taxa sampled in this study. Whole genomic DNA from liver and muscle tissue was extracted using standard Proteinase K protocols. Three mitochondrial markers were used in species delimitation and inferring the phylogenetic position of the new species; these were an ~1032 base pairs (bp) fragment of *cytochrome b* (*Cytb*), ~841 bp of *NADH dehydrogenase subunit 4* (*ND4*) and the adjacent *tRNA* regions and ~522 bp of *16S ribosomal RNA*. Two anonymous nuclear markers (*G1888* and *G1894*) developed using 454 sequencing (Bertozzi *et al*. submitted) were also used in species delimitation. The *G1888* and *G1894* loci consisted of 428 and 422 bp, respectively. Primers are given in Table 1. All markers were amplified using Standard PCR protocols and HotMaster Taq reagents (Applied Biosystems) with 34 cycles; annealing temperatures were 52ºC for mitochondrial markers, and 59ºC for both nuclear markers. PCR products were cleaned by vacuum filtration and the cleaned PCR products were sequenced in both directions (forward and reverse) using automated sequencing machines at the Australian Genome Research facility (AGRF) in Adelaide. Consensus (forward and reverse) sequences were aligned with settings of 65% similarity, Gap opening penalty = 12 and Gap extension penalty = 3 using the Geneious Pro 5.4 alignment software (Drummond *et al.* 2009) and then manually edited and refined by eye. After alignment, *Cytb* and *ND4* sequences were translated into amino acid sequences starting from three different reading frames to check for premature stop codons that might indicate amplification of pseudogenes. The program PHASE v. 2.1.1 (Stephens *et al*. 2001; Stephens & Donnelly 2003) was used to assign single nucleotide polymorphisms (SNPs) derived from the two nuclear markers to a single allelic copy.

TABLE 1. Primer sequences for the molecular markers used in this study.

Gene/Locus	Name	Primer sequence 5'>3'	Source
Cytochrome b	Forward (L14910)	GAC CTG TGA TMT GAA AAA CCA YCG TTG T	Burbrink *et al.* 2000
	Reverse (H16064)	CTT TGG TTT ACA AGA ACA ATG CTT TA	
ND4 and adjacent *tRNA* region	Forward (M245)	TGA CTA CCA AAA GCT CAT GTA GAA GC	Arevalo *et al.* 1994
	Reverse (M246)	TAC TTT TACC TTG GAT TTG CAC CA	
16S rRNA	Forward (M1272)	CGC CTG TTT ATC AAA AAC AT	Kocher *et al.* 1989
	Reverse (M1273)	CCG GTC TGA ACT CAG ATC ACG T	
G1888	Forward (G1888)	CAG GGC CTT GCC TTG TGC CA	Bertozzi *et al.* (submitted)
	Reverse (G1889)	ACC TCT GCG CAC TAT GAC TCT TGA	
G1894	Forward (G1894)	ACC CTT TCA GTC ACA GGT CTG CT	Bertozzi *et al.* (submitted)
	Reverse (G1895)	GAG CGA AAC AGG GAG TTA TCC AAG C	

Sequences for the three mitochondrial markers were concatenated and analyzed using Bayesian and maximum likelihood methods. Bayesian analysis was conducted using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). Bayes factors (Kelchner and Thomas, 2007) were used to assess two alternative partitioning strategies: a three-partition strategy included coding gene by codon (1^{st} + 2^{nd} vs 3^{rd}) and rRNA, and a five-partition strategy included each coding gene separately by codon (1^{st} + 2^{nd} vs 3^{rd}) and rRNA. The two analyses resulted in the same topology and similar branch lengths. However, Bayes factors (Kelchner and Thomas, 2007) and likelihood scores of the two analyses favoured the simpler model and hence only the results of the analysis with three partitions are discussed here. The Akaike information criterion (AIC) was implemented in jModelTest v0.1.1 (Guindon & Gascuel 2003; Posada 2008) to determine the best fitting substitution model for each partition. The analysis was run with model parameters unlinked using default priors for two million generations with two independent runs and two chains sampling every 1000 generations. The first 25% of sampled trees were discarded as burn-in and convergence was assessed by examining effective sample sizes (ESS values), split frequencies of clades across runs and likelihood plots through time in TRACER v1.4.1 (Rambaut & Drummond 2007). Partitioned (coding 1^{st} + 2^{nd} vs 3^{rd} codon positions and rRNA, as in the Bayesian analysis) maximum likelihood (ML) analysis was performed using RAxML v7.1.0 (Stamatakis 2006). The GTR+g substitution model was used as recommended (Stamatakis 2006) to

perform 200 independent ML searches with 1000 non-parametric bootstrap replicates. The two nuclear loci provide low power for model-based (i.e., likelihood or Bayesian) tree inference due to very few variable sites (see Results). Therefore, heuristic parsimony searches were implemented in the dnapars program in PHYLIP v3.69 (Felsenstein 1989), performing 10000 replicates to find the most parsimonious tree(s) for these loci. DNA polymorphism statistics were calculated using DnaSP v5 (Librado & Rozas 2009) and corrected genetic distances between taxa were calculated using the species delimitation plugin (Masters *et al.* 2010) in Geneious Pro 5.4 software.

Results

***Hydrophis donaldi* sp. nov.**
(Figs. 1 and 2, Table 2)

Holotype. QM J90700 (Figs. 1A–C and 2A–B), an adult male from the Gulf of Carpentaria, Weipa, Queensland, Australia (12°35'10.88" S, 141°57'47.21" E) by B. G. Fry on 15th October, 2000. Liver tissue stored in ethanol in the QM (Accession no: 007903).

Paratypes (n = 7). SAMA R65215 (juvenile), SAMA R65216 adult male and SAMA R66274 adult male, all collected from the Gulf of Carpentaria, Weipa, Queensland, Australia (12°35'10.88" S, 141°57'47.21" E) collected by B. G. Fry between the 15-20 October, 2000. Tail tissue stored in ethanol in the ABTC-SAM. QM J63802, QM J63803, QM J63809 and QM J63810 (all adults, sex undetermined), all collected from the Gulf of Carpentaria, Weipa, Queensland, Australia (12°45'4.03" S, 141°53'58.10" E) by B. G. Fry on 1st March, 1997.

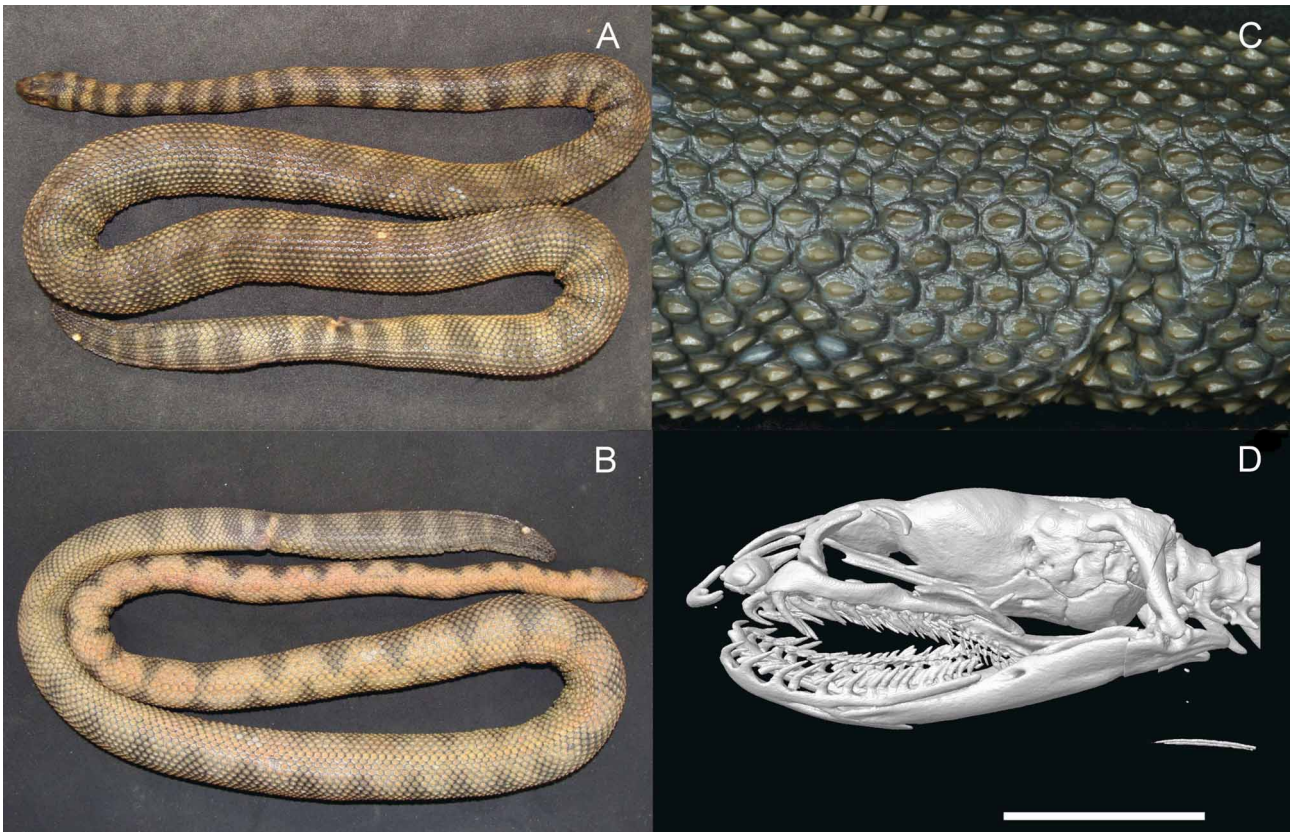

FIGURE 1. *Hydrophis donaldi* **sp. nov.** (A) Dorsal aspect of holotype QM J90700 (before preservation), (B) Ventral aspect of holotype QM J90700 (before preservation), (C) strongly spinous dorsal scales, (D) micro-CT scan of the lateral view of the head of SAMA R65216 (Scale = 10 mm). Note that the maxillary bone does not extend forward far beyond the palatine and that the fang is followed by a diastema. both character states were used by Smith (1926) to diagnose *Hydrophis*.

Diagnosis. *Hydrophis donaldi* **sp. nov.** is distinguished from all other *Hydrophis* species except *H. coggeri, H. sibauensis* and *H. torquatus diadema* by the following combination of characters: ventrals not divided by a longitudinal furrow, 29–30 costal scale rows around neck, 33–35 costal scales around body, 6–7 maxillary teeth behind fang on each side, 246–288 ventrals (Rasmussen *et al.* 2001; Smith 1926). The new species differs from *H. coggeri* by having 47–56 (vs 30–42) bands on the body and tail, strongly spinous (vs feebly carinate) body scales, 246–288 (vs 280–360) ventrals, relatively larger and rounded (vs smaller, elongate) head, and anterior part of the maxilla not arched upwards and the tip of the fang projecting below the level of the maxillary teeth (Fig 1D) (vs anterior part of the maxilla arched upwards and tip of fang not projecting below the level of the maxillary teeth) (Cogger 2000). The new species differs from *H. sibauensis* by a higher number of scale rows around the neck 29–30 (vs 25–26 in *H. sibauensis*) and strongly spinous (vs feebly carinate) body scales (Rasmussen *et al.* 2001). *Hydrophis donaldi* **sp. nov.** differs from *H. torquatus diadema* by a lower midbody scale count (33–35 vs 35–42 in *H. torquatus diadema)* and strongly spinous (vs feebly carinate) body scales (Smith 1926).

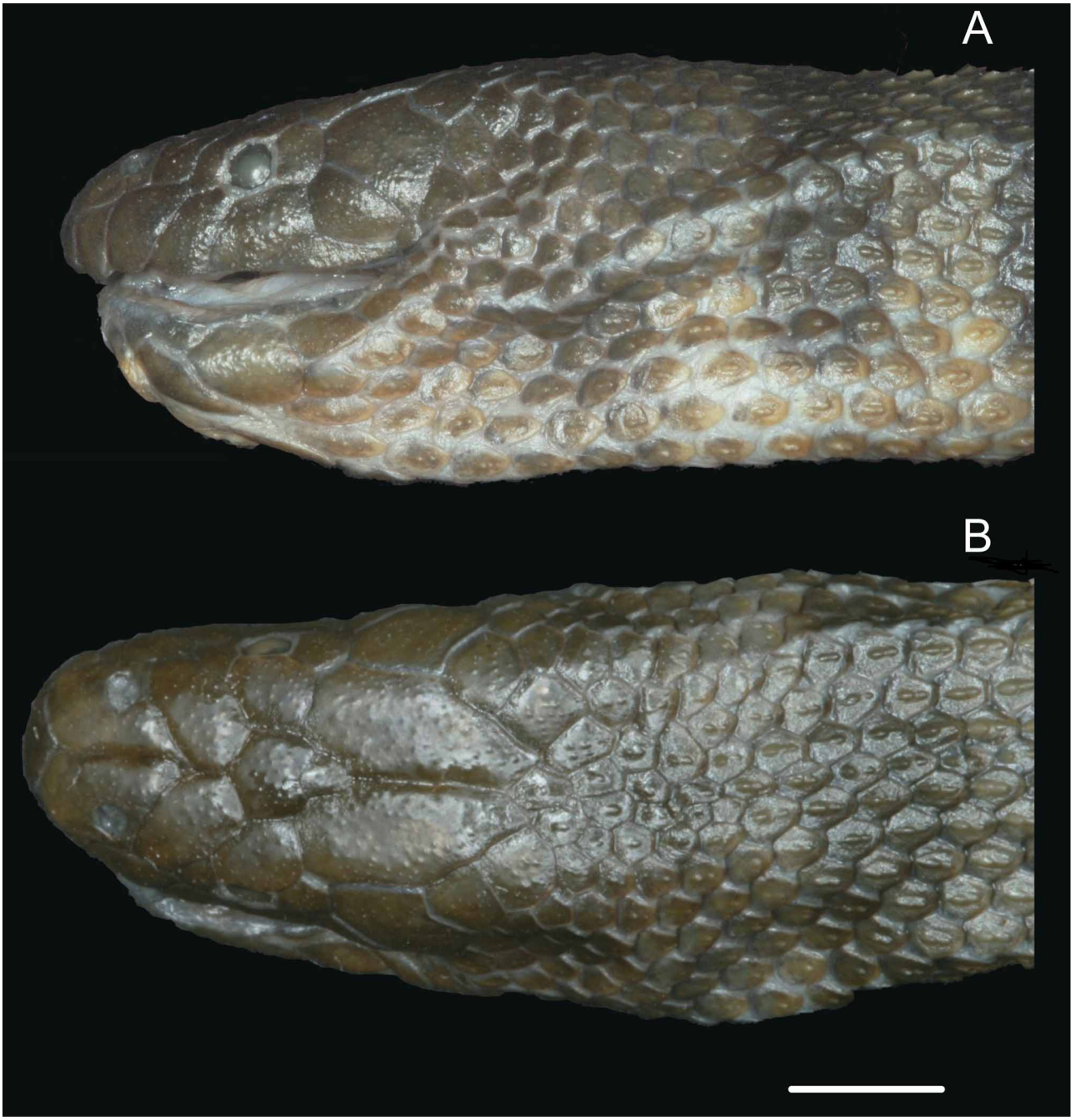

FIGURE 2. *Hydrophis donaldi* **sp. nov.**, holotype QM J90700 (A) lateral aspect of head, (B) Dorsal aspect of head. Scale =10 mm.

TABLE 2. Morphological and morphometric data for the type series of *Hydrophis donaldi* **sp. nov.** Measurements (mm) and scale counts of the head are expressed for the left/right sides. See Methods for explanation of abbreviations.

	QM J90700 (Holotype)	SAMA R65215	SAMA R65216	SAMA R66274	QM J63802	QM J63803	QM J63809	QM J63810
Costals: Neck	29	30	30	29	29	30	29	29
Costals:Mid-body	33	34	34	33	35	34	34	35
Ventral scales	269	246+	268+	278	288	274	273	279
Subcaudal scales	48	42+	21+	44	51	45	46	48
Supralabials	7/7	7/7	7/7	7/7	7/7	7/7	7/7	7/7
Infralabials	7/7	7/7	7/7	7/7	7/7	8/8	8/8	8/8
Preoculars	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Postoculars	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
Ant. Temporals	2/2	1/1	1/1	1/2	2/2	2/2	2/2	2/2
Post. Temporals	2/3	3/2	3/3	2/2	2/2	2/2	2/2	2/2
Maxillary teeth	7/7	-	6/7	7/7	6/6	6/6	6/6	6/6
SVL	756	382	678	649	681	703	692	777
TAL	105	41	95	97	92	94	90	101
IOD	5.78	3.27	5.68	5.97	5.22	5.92	-	5.95
HW	6.90	4.48	6.80	7.63	7.02	8.55	-	9.63
HL	13.78	10.11	13.15	13.76	13.37	15.98	14.11	17.81
GL	13.70/12.36	9.05/8.64	11.10/10.75	12.73/11.69	10.05/10.19	10.56/10.19	11.26/10.04	12.01/12.02
SEL	6.01/5.65	4.11/4.23	5.69/5.50	6.02/6.24	5.16/5.10	4.93/4.99	5.69/5.51	6.68/6.23
SNL	3.26/3.03	2.47/2.75	2.94/2.88	2.91/2.75	2.62/2.42	2.76/3.15	3.14/2.45	3.36/3.13
NED	2.98/2.81	2.60/2.15	3.18/2.98	2.70/2.82	2.83/2.76	2.78/2.79	2.66/2.83	3.02/3.00
EL	1.45/1.54	1.03/1.09	1.75/1.68	1.63/1.73	1.43/1.36	1.22/1.71	1.52/1.56	1.43/1.53
TH	16.71	5.27	16.39	15.31	14.18	14.94	13.02	16.69
TOTL	861	423	773	746	773	797	782	878
TAL/SVL	0.14	0.11	0.14	0.15	0.14	0.13	0.13	0.13

Description of the Holotype. Six maxillary teeth are present behind the poison fang. One pre-ocular and post-ocular scale on each side. Seven supra-labials on each side. First and second supralabials touch the nasal scale and the 3rd and 4th touch the eye. Two anterior and posterior temporals on each side. Seven infralabials on each side. The 1st, 2nd and 3rd infralabials are in contact with the anterior chin-shield and the 4th contacts the posterior chin-shield. Costal scale rows around neck 29; around midbody 33. Dorsal scales strongly spinous. Ventral scales bear two strong spines. Ventrals 269; subcaudals 48; ventrals 1.5 times as wide as adjacent scales. SVL 756 mm; TAL 105 mm; TH 16.71 mm. The dorsal head scales have a rough and warty appearance.

Variation (n = 7). Only differences to the holotype are noted. The number of maxillary teeth behind the poison fang is 6 or 7. Two anterior temporals are present in most of the paratypes, but some specimens have one anterior temporal (Table 2) and SAMA R66274 has one anterior temporal on the left and two on the right. Costal scale rows around the neck 29–30 and around midbody range 33–35. Ventral scales bear two strong spines in all paratypes except in the juvenile SAMA R65215. Ventrals range from 246–288; subcaudals +42–51. SVL in mature individuals 649–777 mm; TL 92-101 mm; TH 13.02–16.69 mm (see Table 2).

TABLE 3. Summary of genetic data. The minimum and maximum fixed nucleotide differences were calculated between *Hydrophis donaldi* **sp. nov.** and the species with which it shares the lowest and highest number of nucleotide differences, respectively.

Gene/Locus	Marker type	Size (bp)	Substitution Model	Total polymorphic sites	Min. fixed nucleotide differences	Max. fixed nucleotide differences
Concatenated mtDNA	mt.DNA	2407	HKY	316	13	151
G1888	anon. nuclear	409	HKY	19	1	7
G1894	anon. nuclear	431	HKY	17	3	9

Colour pattern. Before preservation in the holotype, the background colour was yellowish brown with 56 brownish bands from neck to tail. Bands are broader dorsally, narrow laterally and taper without connecting ventrally. They are wider than the paler interspaces. Bands in first third of body are darker in colour than rest of the body. Head is pale brown in colour. In preserved specimens bands are brownish grey with paler interspaces. The single juvenile specimen (SAMA R65215) has a brighter colouration with a blackish head and bands with turquoise blue interspaces. Number of bands on body and tail in the type series range from 47 (SAMA R65216) to 64 (holotype). See Fig 1 A and B.

Etymology. The species is named to honour Dave Donald, the skipper of the boat who worked tirelessly with us and whose local knowledge facilitated the discovery of this species. We propose the common name 'rough scaled sea snake' for this species due to its strongly keeled body scales.

Distribution. Collection localities for *H. donaldi* **sp. nov.** are restricted to coastal regions of Weipa, Queensland, northern Australia. The specimens were collected from shallow (< 10 m) estuarine habitats (with shale, mud and sea-grass on the bottom) at the mouths of the Mission River and Hey Creek where they connect to Albatross Bay in Weipa, Queensland.

Remarks. *Hydrophis donaldi* **sp. nov.** can be assigned to the genus *Hydrophis* (Smith 1926; McDowell 1972; Cogger 2000) based on the following characters: fewer than 73 scale rows around body, single rostral shield, nasals not separated from internasals, more than four supralabials, ventrals small and not broader anteriorly than posteriorly, mental shield broader than long, shorter head without a bill like snout, shorter gape, ventrals entire, no spines on head shields, preocular scales present, maxillary bone not extending forward beyond the palatine, fang followed by a diastema (see Fig 1D), ventrals distinct throughout the body and not enlarged compared to the dorsal scale rows, ventral scales not broader than twice the adjacent body scales and more than 24 scales around the thickest part of the body.

Genetics. The final concatenated mitochondrial alignment consisted of 2704 bp for 25 individuals. Under AIC GTR+i+g is the best-fit model for each partition. There were 1237 invariable, 571 polymorphic and 362 parsimony informative sites and 599 sites with alignment gaps or missing data. The phased *G1888* nuclear DNA alignment contained 367 invariable, 12 polymorphic , six parsimony informative sites and 49 sites with alignment gaps/missing data. In the phased *G1894* nuclear DNA alignment, 385 sites were invariable, 14 polymorphic, 9 parsimony informative and 23 sites with alignment gaps/missing data.

Maximum likelihood and Bayesian analysis of the mitochondrial alignment yielded similar branch lengths and topologies that differed only in the position of *Astrotia stokesii* (sister to a *Hydrophis caerulescens+H. brooki* clade under ML; sister to *H. elegans* under Bayesian analysis). In the results of both analyses, *H. donaldi* **sp. nov.** forms a well-supported monophyletic sister lineage to all the other sampled ingroup taxa except *Hydrelaps darwiniensis* and *Parahydrophis mertonii*, which formed successive sister lineages to the *Hydrophis* clade (Fig. 3). *Hydrophis donaldi* **sp. nov.** was closest to *H. czeblukovi* , with a mean pairwise corrected (Hasegawa-Kishino-Yano (HKY) correction method) mitochondrial distance of 4.46% and 13 fixed substitutions separating these taxa. The greatest distance among ingroup taxa was between *H. donaldi* **sp. nov.** and *P. mertoni* (14.64%), with 140 fixed nucleotide differences. Pairwise distances between *H. donaldi* **sp. nov.** and *H. coggeri* (the species it most closely resembles morphologically) ranged from 7.1–8.0% with 71 fixed substitutions.

For each nuclear locus *Hydrophis donaldi* **sp. nov.** is represented by a single haplotype that is not shared with any other sampled taxon (parsimony trees are shown in Fig. 4 A and B). For *G1888* there was one fixed nucleotide difference between *H. donaldi* **sp. nov.** and both *H. coggeri* and *Lapemis curtus*, and 7 fixed nucleotide differences between *H. donaldi* sp. nov and *Parahydrophis mertoni*. For *G1894* there was one fixed difference between *H. donaldi* **sp. nov.** and *Acalyptophis peronii*, three between *H. donaldi* **sp. nov.** and *H. coggeri*, and 9 between *H. donaldi* **sp. nov.** and *P. mertoni*. The summary of genetic data from the analysis is shown in Table 3.

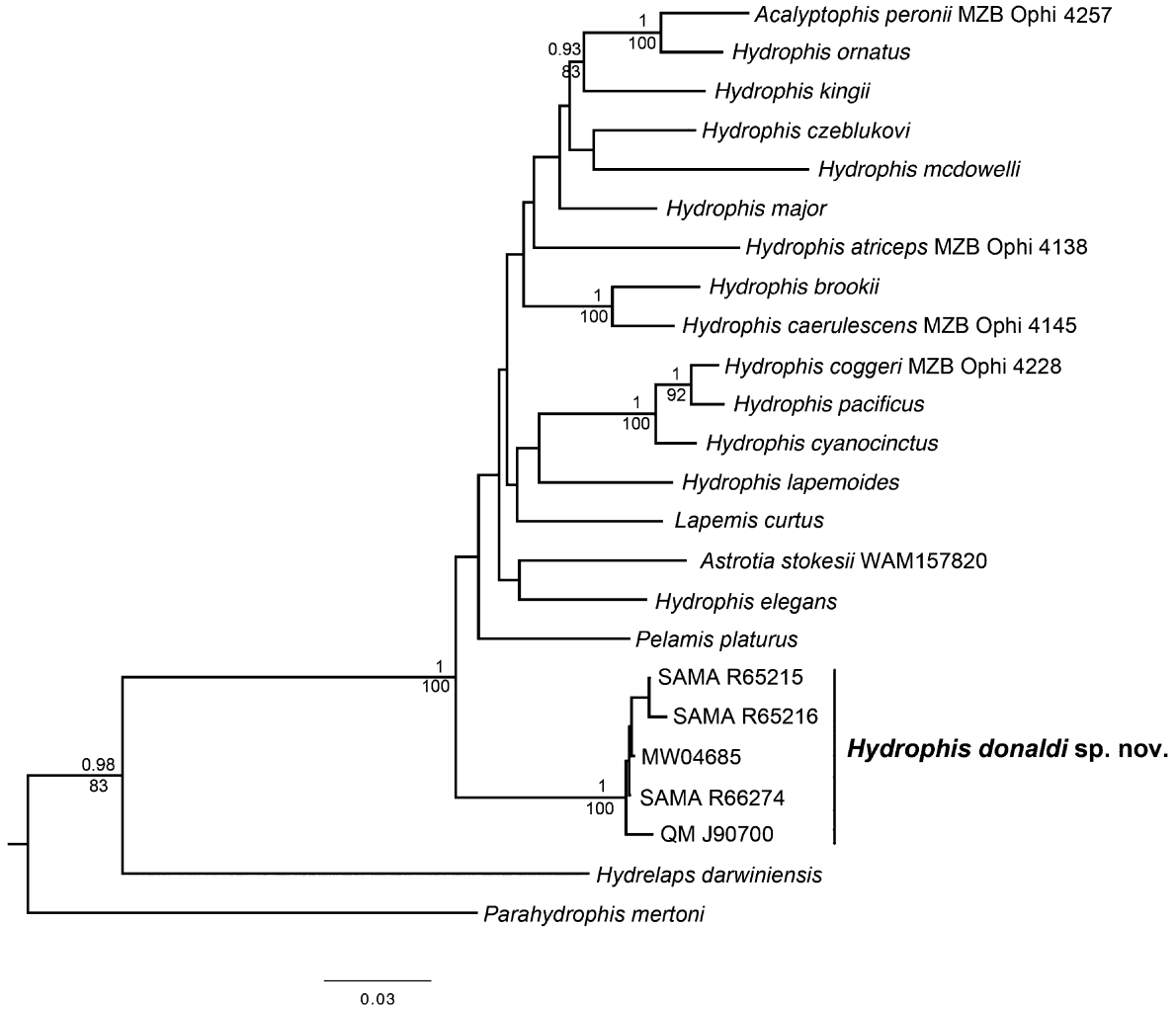

FIGURE 3. Bayesian majority rule consensus tree of the sea snakes sampled in this study based on the concatenated mitochondrial alignment. *Hydrophis donaldi* **sp. nov.** forms a highly divergent lineage although its phylogentic position remains poorly resolved. Nodes with maximum likelihood bootstrap support >70 (below) and Bayesian posterior probability >0.8 (above) are indicated. The outgroup *Aipysurus laevis* is not shown. Scale bar indicates the number of nucleotide substitutions per site.

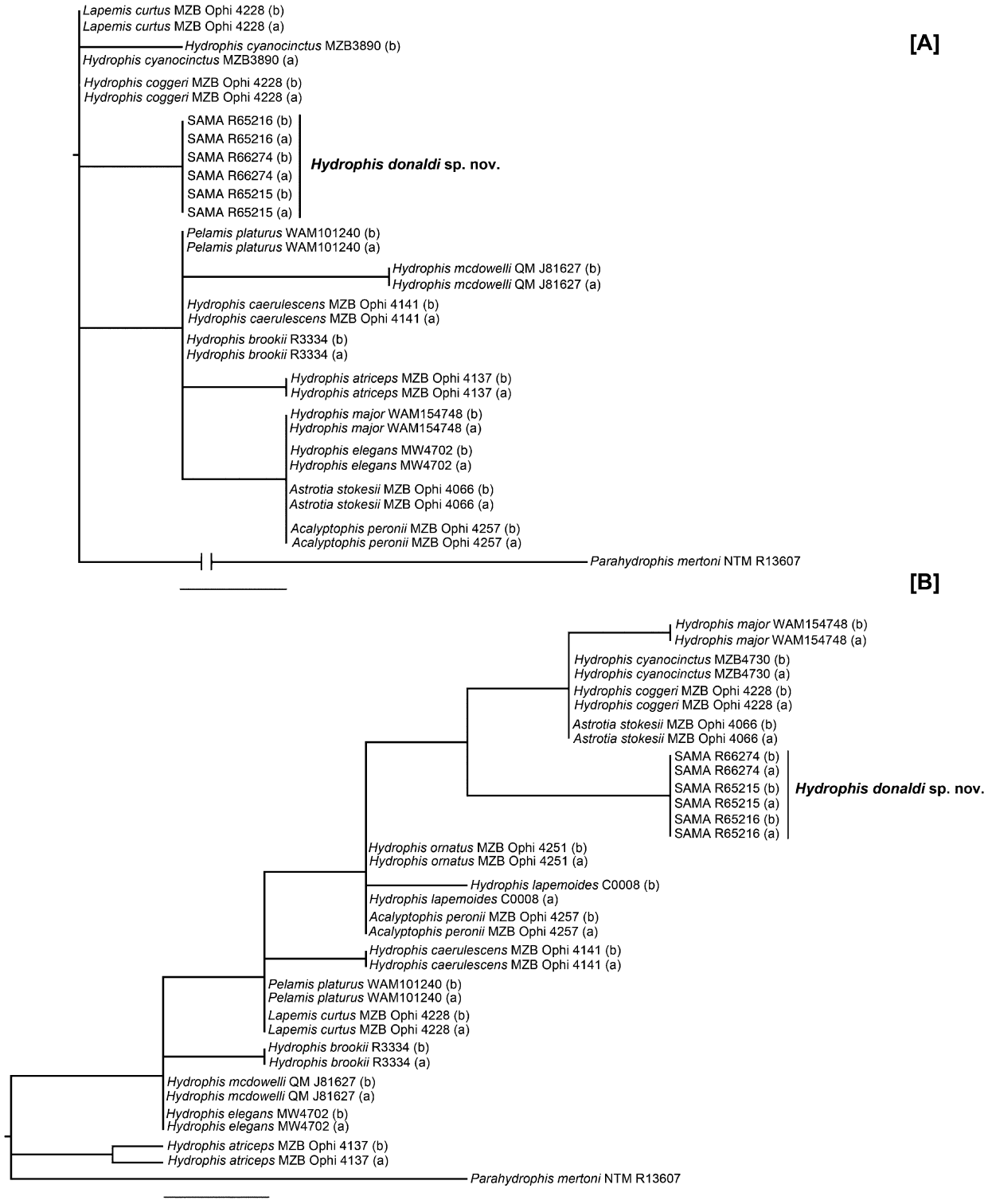

FIGURE 4. The single most parsimonious trees for phased haplotypes of the nuclear loci (A) *G1888* (B) *G1894*. Scale bars indicate branch lengths corresponding to 1 step.

Discussion

Our morphological and genetic data reveal a highly distinct new species. *Hydrophis donaldi* **sp. nov.** forms a deeply divergent mitochondrial lineage in the *Hydrophis* subgroup with unresolved affinities to other sampled species, and the few variable sites across both nuclear loci showed a single unique haplotype in the new species. Based

on current diagnostic characters for Australian (Cogger 2000) and Southeast Asian (Rasmussen 2001) sea snakes, *H. donaldi* **sp. nov.** could be misidentified as *H. coggeri, H. sibauensis or H. torquatus diadema*. However, these four species are readily distinguished by scale morphology, numbers of dark bands and numbers of ventral scales. Our mitochondrial phylogeny contains only 19 of the 50 currently known species of the *Hydrophis* lineage, but it includes one of the most morphologically similar species (*H. coggeri*). There is low node support for most interspecies and generic level relationships, possibly due to the low taxon sampling (Zwickl & Hillis 2002) and short internal branch lengths associated with the exceptionally rapid radiation of the *Hydrophis* clade (Sanders *et al*. 2010). However, the position of the new species within the *Hydrophis* group is well supported.

Given the morphological distinctiveness of *H. donaldi* **sp. nov.**, it is perhaps surprising that it has evaded discovery until now. Sea snakes have been relatively intensively sampled in the Gulf of Carpentaria through bycatch of commercial prawn-trawl fisheries. However, all known specimens of *H. donaldi* **sp. nov.** were found in relatively poorly known estuarine and tidal creek habitats, whereas most biological surveys and fisheries target open-water areas. A priority for future sampling efforts should be to investigate the occurrence of the new species in shallow-marine habitats elsewhere in the GoC and along the New Guinea and northwestern Australian coasts. It is also possible that misidentified specimens of the new species are accessioned in other museums in Australia. Porter *et al*. (1997) report a sea snake collected from the Weipa region with prominently keeled scales, which they doubtfully assigned to *H. coggeri*. Although we were unable to examine this specimen, based on the description and collection locality we expect that it could be *H. donaldi* **sp. nov.**

Most of the more recently described sea snake species are morphologically cryptic or previously unsampled allopatric lineages within an already recognized species (Rasmussen & Ineich 2000; Rasmussen *et al.* 2001); it is very unusual to discover a morphologically and phylogenetically distinct species with uncertain affinities within sea snakes. Our discovery of such a species highlights a need for ongoing investigation on the systematics and evolution of this group of reptiles. That viviparous sea snakes are of medical importance adds further significance to our discovery because proper identification is an essential step in snake-bite treatment. Although the toxicity of the new species is not known, all viviparous sea snakes (except a few fish egg eating species in the *Aipysurus* group) are venomous and potentially dangerous to humans.

Acknowledgements

This work was supported by an Australian Research Council (ARC) Discovery grant to K. L. Sanders, and ARC, Australia & Pacific Science Foundation and University of Queensland funding to B.G. Fry. We thank Peter Blias (South Australian Museum) for micro-CT scanning the specimen and Mike Lee (SAM) for constructive comments on the manuscript. Carolyn Kovach (SAM), Andrew Amey (Queensland Museum) and Patrick Couper (QM) are gratefully acknowledged for the assistance given during the examination of specimens in their care. Constructive criticism by David Gower, Arne Rasmussen and Colin McCarthy is highly appreciated.

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APPENDIX 1. Museum numbers of the specimens, collection localities and the respective Genbank accession numbers used in the molecular phylogenetic analyses. Specimens with two haplotypes for the anonymous nuclear markers are denoted with 'Ha' and 'Hb'. Specimens with identical sequences for a locus have a single Genbank accession number.

Species	Source/Locality	Museum voucher	*Cytb*	*16S rRNA*	*ND4*	*G1888*	*G1894*
Acalyptophis peroni	Sulawesi, Indonesia	MZB Ophi 4257	JQ217200	JQ217145	JQ217209	JQ217156	Ha-JQ217176 Hb-JQ217177
Aipysurus laevis	Genbank	-	DQ233919	DQ233998	EF506675	-	-
Astrotia stokesii	Exmouth bay, WA, Australia	WAM157820	JQ217201	JQ217146	JQ217210	Ha-JQ217157 Hb-JQ217158	-
Astrotia stokesii	Sulawesi, Indonesia	MZB Ophi 4066	-	-	-	-	JQ217178
Hydrelaps darwiniensis	Genbank	-	DQ233947	DQ234046	FJ593200	-	-
Hydrophis atriceps	East Java, Indonesia	MZB Ophi 4138	JQ217206	JQ217152	JQ217216	-	-
Hydrophis atriceps	East Java, Indonesia	MZB Ophi 4137	-	-	-	JQ217162	Ha-JQ217182 Hb-JQ217183
Hydrophis brookii	Genbank	-	DQ233943	DQ234028	FJ593212	-	-
Hydrophis brookii	Thale Luang,Thailand	ZMUC R3334	-	-	-	JQ217163	Ha-JQ217184 Hb-JQ217185
Hydrophis caerulescens	East Java, Indonesia	MZB Ophi 4145	JQ217208	JQ217154	JQ217218	-	-
Hydrophis caerulescens	East Java, Indonesia	MZB Ophi 4141	-	-	-	JQ217164	Ha-JQ217186 Hb-JQ217187
Hydrophis coggeri	Sulawesi, Indonesia	MZB Ophi 4228	JQ217207	JQ217153	JQ217217	JQ217165	JQ217188
Hydrophis cyanocinctus	Genbank	-	DQ233945	DQ234031	FJ593213	-	-
Hydrophis cyanocinctus	West Java, Indonesia	MZB3890	-	-	-	Ha-JQ217166 Hb-JQ217167	JQ217189
Hydrophis czeblukovi	Genbank	-	DQ233944	DQ234019	-	-	-
Hydrophis elegans	Genbank	-	DQ233950	DQ234021	FJ593216	-	-
Hydrophis elegans	Weipa,Qld,Australia	MW04792 (No voucher)	-	-	-	JQ217168	JQ217190

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APPENDIX 1. (continued)

Species	Source/Locality	Museum voucher	*Cytb*	*16S rRNA*	*ND4*	*G1888*	*G1894*
Hydrophis kingii	Genbank	-	DQ233933	DQ234014	FJ593208	-	-
Hydrophis lapemoides	Genbank	-	DQ233954	DQ234033	FJ593218	-	-
Hydrophis lapemoides	Phuket, Thailand	ZMUC C0008	-	-	-	-	Ha-JQ217191 Hb-JQ217192
Hydrophis major	Genbank	-	DQ233937	DQ234018	FJ593210	-	-
Hydrophis major	Shark Bay, WA, Australia	WAM154748	-	-	-	Ha-JQ217169 Hb-JQ217170	JQ217193
Hydrophis mcdowelli	Genbank	-	DQ233956	DQ234029	FJ593221	-	-
Hydrophis mcdowelli	Moondalbee Island, Qld. Australia	QM J81627	-	-	-	JQ217171	JQ217194
Hydrophis ornatus	Genbank	-	DQ233962	DQ234027	FJ593223	-	-
Hydrophis ornatus	Sulawesi, Indonesia	MZB Ophi 4251	-	-	-	-	Ha-JQ217195 Hb-JQ217196
Hydrophis pacificus	Genbank	-	DQ233963	DQ234035	FJ593226	-	-
Lapemis curtus	Genbank	-	DQ233969	DQ234041	FJ593228	-	-
Lapemis curtus	Sulawesi, Indonesia	MZB Ophi 4195	-	-	-	Ha-JQ217172 Hb-JQ217173	Ha-JQ217197 Hb-JQ217198
Parahydrophis mertoni	Genbank	-	DQ233974	DQ234048	FJ593201	-	-
Parahydrophis mertoni	Palmerston, NT, Australia	NTM R13607	-	-	-	JQ217155	JQ217175
Pelamis platurus	Genbank	-	DQ233978	DQ234052	FJ593234	-	-
Pelamis platurus	Floreat beach, WA, Australia	WAM101240	-	-	-	JQ217174	JQ217199
Hydrophis donaldi sp. nov.	Weipa, Qld, Australia	SAMA R65215	JQ217202	JQ217147	JQ217211	JQ217159	JQ217180
Hydrophis donaldi sp. nov.	Weipa, Qld, Australia	SAMA R65216	-	JQ217149	JQ217213	JQ217161	JQ217179
Hydrophis donaldi sp. nov.	Weipa, Qld, Australia	SAMA R66274	JQ217203	JQ217148	JQ217212	JQ217160	JQ217181
Hydrophis donaldi sp. nov.	Weipa, Qld, Australia	QM J90700	JQ217205	JQ217151	JQ217215	-	-
Hydrophis donaldi sp. nov.	Weipa, Qld, Australia	MW04685 (No voucher)	JQ217204	JQ217150	JQ217214	-	-