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SYMPOSIUM

Independent Innovation in the Evolution of Paddle-Shaped Tails in Viviparous Sea Snakes (Elapidae: Hydrophiinae)

Kate L. Sanders,[1,*] Arne R. Rasmussen[†] and Johan Elmberg[‡]

*School of Earth and Environmental Sciences, University of Adelaide, South Australia 5000, Australia; [†]The Royal Danish Academy of Fine Arts, Schools of Architecture, Design and Conservation, Esplanaden 34, DK-1263, Copenhagen K., Denmark; [‡]Aquatic Biology and Chemistry, Kristianstad University, SE-291 88 Kristianstad, Sweden

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[1]Email: kate.sanders@adelaide.edu.au

Synopsis The viviparous sea snakes (Hydrophiinae) comprise ~90% of living marine reptiles and display many physical and behavioral adaptations for breathing, diving, and achieving osmotic balance in marine habitats. Among the most important innovations found in marine snakes are their paddle-shaped (dorsoventrally expanded) tails, which provide propulsive thrust in the dense aquatic medium. Here, we reconstruct the evolution of caudal paddles in viviparous sea snakes using a dated molecular phylogeny for all major lineages and computed tomography of internal osteological structures. Bayesian ancestral state reconstructions show that extremely large caudal paddles supported by elongated vertebral processes are unlikely to have been present in the most recent common ancestor of extant sea snakes. Instead, these characters appear to have been acquired independently in two highly marine lineages of relatively recent origin. Both the *Aipysurus* and *Hydrophis* lineages have elongated neural spines that support the dorsal edge of their large paddles. However, whereas in the *Aipysurus* lineage the ventral edge of the paddle is supported by elongated haemapophyses, this support is provided by elongated and ventrally directed pleurapophyses in the *Hydrophis* lineage. Three semi-marine lineages (*Hydrelaps*, *Ephalophis*, and *Parahydrophis*) form the sister group to the *Hydrophis* clade and have small paddles with poorly developed dorsal and ventral supports, consistent with their amphibious lifestyle. Overall, our results suggest that not only are the viviparous hydrophiines the only lineage of marine snakes to have acquired extremely large, skeletally supported caudal paddles but also that this innovation has occurred twice in the group in the past ~2–6 million years.

Introduction

Only ~100 out of at least 9500 extant reptilian species have successfully invaded marine environments and >90% of these are front-fanged snakes in the family Elapidae (Rasmussen et al. 2011). Sea snakes display a remarkable suite of adaptations for breathing, diving, and achieving osmotic balance in marine habitats (Dunson and Dunson 1974; Heatwole and Seymour 1975; Greer 1997). Among the most important challenges imposed by marine life is locomotion: water is a much denser medium than air so that swimming and maneuverability underwater requires efficient modes of dealing with increased drag (Fish 1996, 2001; Pough et al. 2004). Accordingly, many marine snakes have acquired a paddle-shaped (dorsoventrally expanded) tail and dorsoventrally elongated body that generate propulsive thrust during lateral undulations (Heatwole 1999; Aubret and Shine 2008; Brischoux and Shine 2011). In contrast, terrestrial snakes exhibit an ancestral morphology of cross-sectionally relatively circular bodies and tails, and most non-fossorial terrestrial snakes have proportionately longer tails than do marine forms (Brischoux and Shine 2011).

The viviparous or "true" sea snakes (Hydrophiini: Hydrophiinae) are by far the most successful radiation of living marine snakes, with 62 species occupying diverse shallow-water ecosystems throughout the tropical and subtropical Indo-Pacific Oceans (Heatwole 1999; Rasmussen et al. 2011). Two highly

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marine viviparous clades are recognized on the basis of substantial morphological and molecular evidence (Smith 1926; Voris 1977; Rasmussen 1997; Rasmussen 2002; Lukoschek and Keogh 2006; Sanders et al. 2008): an *Aipysurus* lineage of 10 predominantly Australasian species in 2 genera, and a much more speciose *Hydrophis* lineage of 49 species in up to 11 genera (mostly Southeast Asian). In addition, three semi-marine (amphibious) monotypic genera that are endemic to Australo-Papuan coastal habitats appear to form a relatively distant sister lineage to the *Hydrophis* group (*Hydrelaps, Ephalophis*, and *Parahydophis* (McDowell 1969, 1972; Lukoschek and Keogh 2006). The eight species of sea kraits (*Laticauda*) are an older, independently marine lineage of hydrophiine snakes that forage at sea but return to land to digest their prey, mate, and lay eggs (Keogh et al. 1998; Heatwole et al. 2005; Cogger and Heatwole 2006).

Both the viviparous sea snakes and sea kraits have acquired paddle-shaped tails and represent an excellent example of convergent evolution in this respect (Heatwole 1999; Ineich 2004). However, whereas the paddle of the amphibious sea kraits is formed entirely by a cutaneous fold without any modification of the caudal vertebrae, the paddles of most viviparous sea snakes are supported dorsally and ventrally by elongated vertebral processes (Fig. 1) (McDowell 1969, 1972; McCarthy 1987; Rasmussen 1997). The highly marine species in the *Aipysurus* and *Hydrophis* groups share elongated neural spines (dorsal processes) that can reach four times the length of the vertebrae, but exhibit elongation of different ventral caudal processes. *Hydrophis* group species have elongated pleurapophyses (anterior ventral processes likely homologous to the ribs, but fused to the vertebrae) that have become vertically (rather than laterally) directed, whereas *Aipysurus* group species have elongated haemapophyses (posterior ventral processes that either completely or partially enclose the caudal vessels) (McDowell 1972; McCarthy 1987; Rasmussen 2002). The three semi-marine lineages, although phylogenetically nested between the highly marine *Hydrophis* and *Aipysurus* groups, have poorly developed neural spines and ventral caudal processes (equal or shorter than the length of each centrum) (Rasmussen 2002). These observations suggest complex and potentially convergent transitions to paddle-like tails among the major lineages of viviparous sea snakes.

In this article, we (1) reconstruct the first dated molecular phylogeny to include all three monotypic semi-marine genera in addition to the *Hydrophis* and *Aipysurus* groups, (2) generate computed tomography (ct) scans of internal caudal morphology for representatives of each of these lineages, and (3) use Bayesian Markov-chain Monte-Carlo analyses of the phylogenetic and morphological data to provide the first reconstruction of ancestral caudal character states at key nodes in the transition from terrestrial to highly marine forms. By accounting for uncertainty in the phylogeny and in models of changes in character states we provide estimates of confidence levels for the inferred patterns of morphological evolution of the tail.

Methods

Taxon selection, DNA amplification, and sequencing

Twelve hydrophiines in 10 genera were sampled in the present study. This included seven marine species representing both major viviparous clades (the *Aipysurus* and *Hydrophis* groups) and all three semi-marine lineages (*Ephalophis*, *Hydrelaps*, and *Parahydrophis*), plus two terrestrial taxa that form successive outgroups to the marine clade (*Hemiaspis* and *Pseudonaja*) (see Sanders et al. 2008). Vouchers and tissues were obtained by the authors during collecting trips in Southeast Asia and Australia and from specimens on loan from museums. Although this study included only 3 of 10 species in the *Aipysurus* group and 4 of 13 ''genera'' and 47 species recognized in the *Hydrophis* group (Rasmussen et al. 2011), our sampling spanned the basalmost splits in these groups in a larger phylogenetic analysis based on 10 genera and 25 species (Lukoschek and Keogh 2006).

Standard protocols were used to extract genomic DNA (Puregene^{TM} DNA Isolation Tissue Kit, Gentra Systems) from liver and muscle biopsies. PCR was carried out in 25 μL volumes using HotMaster Taq reagents (Perkin Elmer/Applied Biosystems); annealing temperatures varied between 52°C and 58°C depending on the loci and taxa. Three mitochondrial (mt) fragments were sequenced: approximately 840 base pairs (bp) of ND4 (NADH dehydrogenase subunit 4); approximately 1100 bp of cytb (cytochrome b); and approximately 520 bp of 16S rRNA (16S small subunit ribosomal RNA). Double-stranded sequencing was outsourced to the Australian Genome Research Facility Ltd. (AGRF) in Adelaide, Australia. Sequences were checked for ambiguities, and alignments were assembled from consensus sequences of forward and reverse reads using default settings in Geneious Pro v5.1.7 software (Drummond et al. 2010). Twelve additional mitochondrial sequences were obtained from GenBank. Specimen localities, voucher numbers, and GenBank accessions

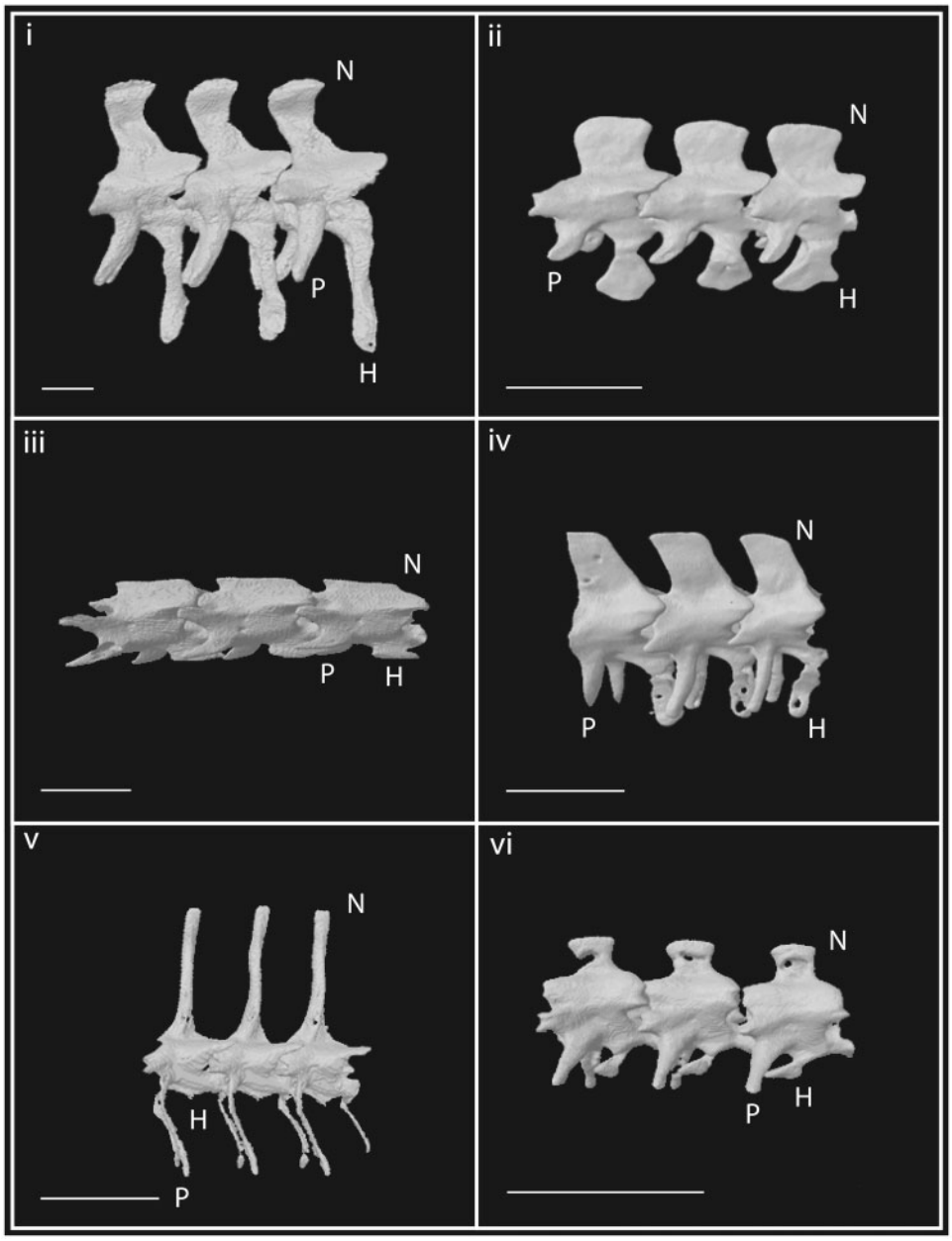

Fig. 1 Ct volume-rendered images of the left lateral view of three mid-tail vertebrae of each major lineage of viviparous sea snakes: (**i**) *Aipysurus eydouxii*, (**ii**) *Ephalophis greyi*, (**iii**) *Hemiaspis dameli*, (**iv**) *Hydrelaps darwiniensis*, (**v**) *Hydrophis elegans*, and (**vi**) *Parahydrophis mertoni*. Note the differences in the modification of the neural spines (labeled N), haemapophyses (H), and pleurapophyses (P) within marine and among marine and terrestrial taxa. Scale bar indicates 2 mm.

are given in Appendix 1; primer information is shown in Appendix 2.

Model selection and data partitioning

Three data partitioning strategies were assessed using Bayesian analyses implemented in BEAST 1.6.1 (Drummond and Rambaut 2007) with an uncorrelated lognormal relaxed clock model and Yule tree prior run for 300,000,000 generations and sampled every 1000 generations. These were: two partitions (mt coding, rRNA), three partitions (mt coding positions 1 + 2, mt coding position 3, rRNA), and four partitions (mt coding position 1, mt coding position 2, mt coding position 3, rRNA). Model selection was performed for each partition using the Akaike information Criterion (AIC) in jModelTest 0.1.1 (Posada 2008). The alternative partitioning schemes were assessed using Bayes factors (Kass and Raferty 1995) calculated in Tracer 1.5 (Rambaut and Drummond 2007) using log-likelihood scores from

the posterior distribution. The optimum strategy was then implemented for all subsequent analyses.

Analyses of phylogeny and times of divergence

Bayesian analyses of the concatenated mitochondrial data were conducted using BEAST 1.6.1 (Drummond and Rambaut 2007) via the Grisu portal on the ARCS Compute Cloud. BEAST analyses were repeated five times with different random starting seeds for each run, unlinked substitution model parameters and clock models for each partition, and estimated base frequencies. A Yule tree model prior with a uniform distribution was used because this is most appropriate for interspecific data (Drummond and Rambaut 2007). A relaxed clock was used with an uncorrelated and log-normally distributed model of rate variation across adjacent branches (Drummond et al. 2006). Separate runs of 300,000,000 generations, sampled every 1000 generations, were combined using LogCombiner 1.6.1 (Rambaut and Drummond 2007). There are no hydrophiine fossils that are sufficiently young to use for calibration of molecular clocks in the present study. To provide a time frame for the inferred transitions in caudal morphology, we used a secondary calibration prior estimated in a previous study (Sanders et al. 2008; see also Sanders and Lee 2008); this corresponded to the posterior distribution for the split between the *Aipysurus* and *Hydrophis* groups (i.e., the basalmost divergence among crown-group sea snakes) and was given a normal distribution with a mean of 6.2 million years ago (Mya) and broad 95% interval of 4.5–8 Mya. Convergence of the MCMC runs was assessed in TRACER v. 1.4.1 (Rambaut and Drummond 2007) by examining likelihood plots and histograms, and effective sample sizes (ESS values) of the estimated parameters. The first 30% of sampled trees of each run were excluded as burn-in, leaving 300,000 trees per run to generate a maximum credibility tree using LogCombiner 1.6.1 and Tree Annotator 1.6.1 (Rambaut and Drummond 2007).

Morphological characters

Micro-ct scanning of one adult male of each of the 12 species included in the molecular analyses was performed to visualize the osteological structure of the tail. Adult specimens were identified by their large, non-flaccid testes. All specimens were scanned on a Skyscan 1076 *in-vivo* X-ray microtomograph at Adelaide Microscopy with the following general settings: resolution 18 μm, rotation step 0.6 degrees, time 295 ms, filter nil. The following settings were optimized depending on the specimen: voltage between 65 and 74 kV, and current between 129 and 139 μA. All reconstructions were performed with Skyscan software (www.skyscan.be/products/downloads.htm). The conversion to cross sections was carried out using NRecon; images were then manipulated and 3D models created using CTAn. The 3D models were viewed and still images made using CTVol. Scale was obtained by measuring the X-ray images in Skyscan's Tview software as well as by measuring some dimensions in the cross-sectional images with CTAn.

We identified four caudal traits that exhibited substantial differences among marine taxa and between marine and terrestrial taxa. These were measured mid-way (50%) along the length of each tail and are shown in Fig. 1. Neural spines were classified as (0) not longer than the centrum, (1) as long as the centrum, and (2) more than the length of the centrum. Shape of tail in transverse section was classified as (0) approximately circular, (1) moderately dorsoventrally expanded (height/width 1.5–3), and (2) extremely dorsoventrally expanded (height/width >6). Pleurapophyses were classified as (0) laterally directed and not longer than the centrum, (1) vertically directed and as long as the centrum, and (2) vertically directed and more than the length of the centrum. Haemapophyses were classified as (0) a low ridge not longer than the centrum, (1) a low keel as long as the centrum, and (2) more than the length of the centrum. For a discussion of these characters and their homologies, see Hoffstetter and Gasc (1969).

Reconstruction of ancestral caudal morphology

To infer ancestral caudal character traits, we used Bayes Multistate implemented in the software BayesTraits (Pagel et al. 2004; Pagel and Meade 2006). Phylogenetic and model uncertainty was accounted for in the analysis using a subsample of 500 trees from the stationary distribution of the combined BEAST analyses and the reversible-jump Markov-chain Monte-Carlo (rj-MCMC) method. The MCMC analyses were repeated six times using a range of ratedev (0.5–100) and both the exponential and gamma hyperpriors to seed the prior distribution of rates. For each run we compared the Markov chains' acceptance values, posterior probabilities, and the stability of the harmonic mean of the likelihoods. The final analysis used an exponential prior (rjhp exp 0.0 30), set acceptance values in the range of 15–25%, and was run for 5 million

iterations with the first 100,000 iterations discarded as burn-in.

The most recent common ancestor (MRCA) method was used to infer ancestral character states for five key nodes: (1) the basal divergence among marine and terrestrial taxa (tree root), (2) the basalmost viviparous sea snake node (i.e., the common ancestor of the *Hydrophis*, *Aipysurus*, and semi-marine lineages (Lukoschek and Keogh 2006), (3) the MRCA of the *Hydrophis* clade plus the three semi-marine intermediate lineages (*Ephalophis*, *Parahydrophis*, and *Hydrelaps*), (4) the MRCA of the sampled *Hydrophis* taxa (*Acalytophis*, *Thalassophina*, and *Hydrophis*), and (5) the MRCA of the *Aipysurus* group (*Aipysurus* and *Emydocephalus*). This approach estimates ancestral states for the clade containing the specified taxa in every sampled tree regardless of whether that clade additionally includes other taxa. The posterior distribution of the ancestral state was then examined across trees to account for uncertainty in the presence and character state of each specified node (Pagel et al. 2004).

Results

Analysis of phylogeny and dating

The final data matrix contained 2426 sites (1762 mitochondrial coding and 664 mitochondrial RNA) of which 872 were polymorphic. All sequences are deposited at GenBank (Accession numbers are given in Appendix 1). Translation of the protein coding regions did not reveal frameshifts, unexpected stop codons, or indels. Bayes factors found strongest support for partitioning the mitochondrial data by coding first vs second vs third codon positions and RNA (all best-fit substitutions models GTRig).

Bayesian analyses of the concatenated dataset using the four-partition strategy yielded ESS values above 200 for all parameters, with similar posteriors, topologies, and branch lengths for separate runs. The final maximum credibility tree from the combined runs (Fig. 3) recovered high support (posterior probabilities [pp] of >0.95) for most internal relationships, including the placement of the terrestrial taxon *Pseudonaja* as sister to a clade comprising *Hemiaspis* (also terrestrial) plus all marine taxa, the reciprocal monophyly of the *Aipysurus* and *Hydrophis* lineages, and the sister-group relationship between *Emydocephalus* and *Aipysurus* (*A. eydouxii* + *A. apraefrontalis*). Within the *Hydrophis* group, *Acalyptophis peronii* + *Hydrophis macdowelli* were strongly supported as sister taxa and formed a polytomy with *Thalassophina viperina* and *Hydrophis elegans*. The three monotypic semi-marine genera form a monophyletic sister lineage to the *Hydrophis* group with moderate support (pp 0.89); *Ephalophis* and *Parahydrophis* are strongly supported as sister taxa (pp 1.0). Mean and 95% HPD (highest posterior distribution) estimates of divergence times for key nodes were 7.8 million years before present (Myr) (95% HPD: 6.0–9.7) between *Hemiaspis* and the marine clade; 6.0 Myr (95% HPD: 4.9–7.0) for basal divergence of the sea snake crown group; 2.8 Myr (95% HPD: 2.2–3.4) for the basal divergence in the *Aipysurus* group (*Emydocephalus* vs *Aipysurus*); 5.1 Myr (95% HPD: 4.1–6.1) for the divergence among the *Hydrophis* group and the three semi-marine lineages (*Ephalophis*, *Parahydrophis*, and *Hydrelaps*); and 1.8 Myr (95% HPD: 1.4–2.3) for the basal divergence between the sampled species of the *Hydrophis* group. These results are consistent with previous studies of hydrophiine phylogeny and divergence times based on larger mitochondrial and concatenated mitochondrial and nuclear datasets (Sanders and Lee 2008; Sanders et al. 2008; Lukoschek et al. 2011).

Comparison of the 95% majority rule consensus tree of all post burn-in trees and the subsample of 500 trees used for reconstructing ancestral character states (see below) demonstrated that the latter was representative of the overall stationary distribution.

Caudal morphology and the reconstruction of ancestral character states

The ct data clearly revealed morphological modifications of the caudal vertebrae that were consistent with published observations (McDowell 1969, 1972; McCarthy 1987; Rasmussen 1997). The character variation found among the scanned specimens is unlikely to be ontogenetic or due to sexual dimorphism because only adult male specimens were used in the final dataset and because species for which both sexes as well as small and large individuals were available showed consistent patterns (data not shown). Classifications of the four caudal traits are shown for each species in Table 1; for one representative of each major lineage, ct images of three mid-tail vertebrae are presented in Fig. 1 and lateral views of the whole tail are presented in Fig. 2.

Two of the character states of interest here are each found only in highly nested, monophyletic lineages and are present in all members of these clades, so that the most likely history for these traits involves a single (and unreversed) origin. First, haemapophyses reach more than the length of the centrum exclusively in the *Aipysurus* lineage, and are present as an indistinct ridge in the *Hydrophis* group and as

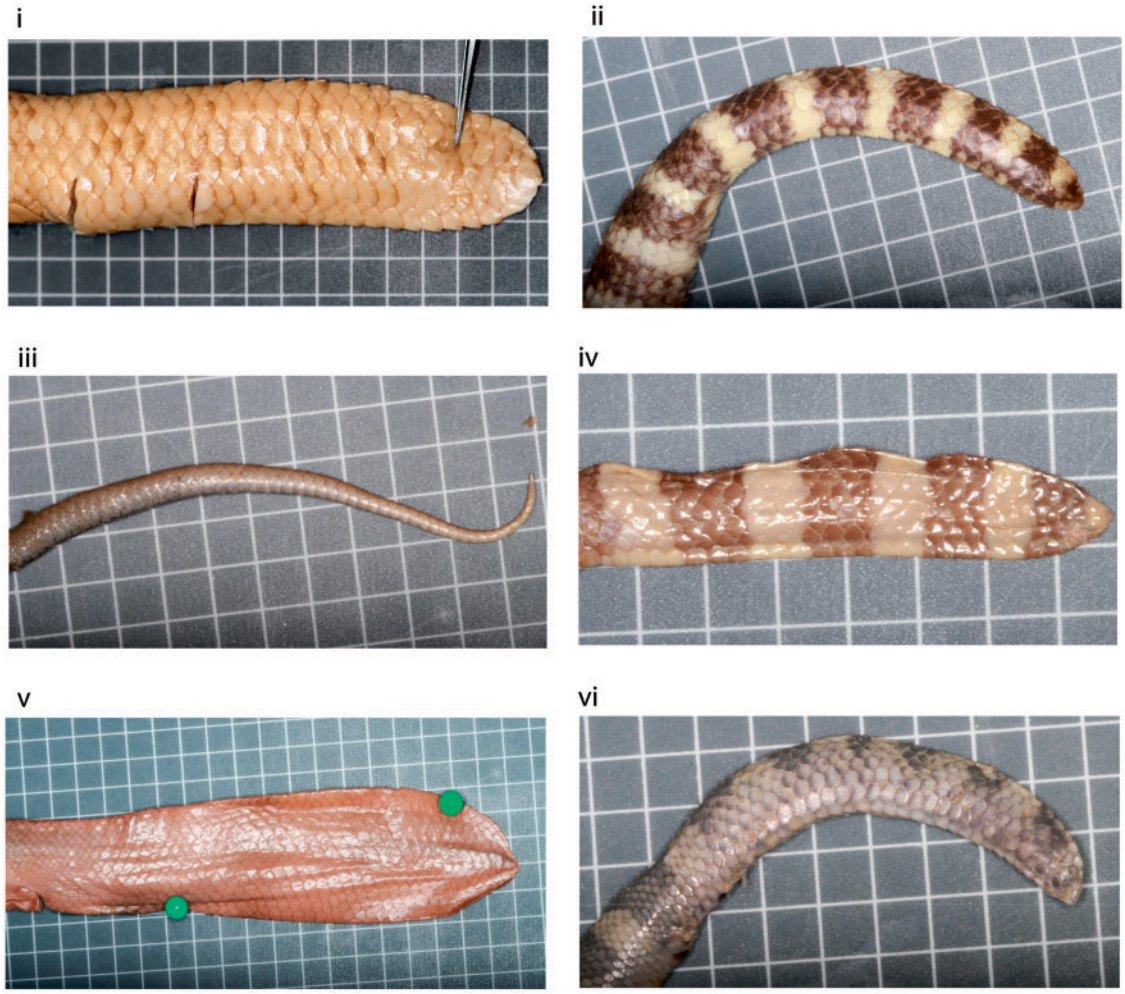

Fig. 2 Photographs showing the left lateral view of the complete tail of each major lineage of viviparous sea snakes: (**i**) *Aipysurus apraefrontalis*, (**ii**) *Ephalophis greyi*, (**iii**) *Hemiaspis dameli*, (**iv**) *Hydrelaps darwiniensis*, (**v**) *Hydrophis macdowelli*, and (**vi**) *Parahydrophis mertoni*. Grid scale represents 1 cm × 1 cm.

a low rectangular keel in the semi-marine *Hydrelaps*, *Ephalophis*, and *Parahydrophis*. Second, only *Hydrophis* group species have extremely elongated pleurapophyses (exceeding the length of the centrum); the pleurapophyses of *Hydrelaps* and the sampled species of the *Aipysurus* group do not exceed the length of the centrum, and in *Ephalophis* and *Parahydrophis* they are considerably shorter than the centrum. Probabilities of reconstructed character states for key nodes are shown in Fig. 3. As expected, reconstructions of ancestral states for these characters recover them as ancestral to their respective clades (elongated pleurapophyses in the *Hydrophis* group; elongated haemapophyses in the *Aipysurus* group) and weakly developed (equal in length or shorter than the centrum) in the MRCA of extant sea snakes and the MRCA of the *Hydrophis* group plus the three semi-marine lineages. Reconstructions of the two remaining characters (elongation of the neural spines and dorsoventral expansion of the tail) suggest that these characters were also weakly developed in both the MRCA of extant sea snakes and the MRCA of the *Hydrophis* group and semi-marine species and were developed independently in the *Aipysurus* and *Hydrophis* groups.

Discussion

The paddle-shaped tail, a key adaptive innovation for locomotion in marine environments, has undergone extensive modification in the major lineages of viviparous sea snakes, confounding previous morphologically based inferences of their interrelationships (McDowell 1972; Rasmussen 2002). Our study provides the first reconstruction of caudal evolution in viviparous sea snakes based on an independent molecular phylogenetic framework. Bayesian reconstructions of ancestral states show that extremely dorsoventrally expanded caudal paddles with well-developed vertebral supports are unlikely to

Table 1 Caudal characters scored using the ct scans generated in this study for 12 marine and terrestrial hydrophiine taxa

Taxon	Neural spines	Tail shape	Pleurapophysis	Haemapophysis	References
Acalyptophis peroni	2	2	2	0	This study
Aipysurus apraefrontalis	2	2	1	2	This study
Aipysurus eydouxii	2	2	1	2	This study; Rasmussen 1997
Emydocephalus annulatus	2	2	1	2	This study
Ephalophis greyi	1	1	0	1	This study; McDowell 1969; McCarthy 1987; Rasmussen 1997
Hemiaspis dameli	0	0	0	0	This study
Hydrelaps darwiniensis	1	1	1	1	This study; Rasmussen 1997
Hydrophis elegans	2	2	2	0	This study
Hydrophis macdowelli	2	2	2	0	This study
Parahydrophis mertoni	0	1	0	0	This study; McDowell 1969
Pseudonaja modesta	0	0	0	0	This study
Thalassophina viperina	2	2	2	0	This study

Notes. Characters were measured mid-way (50%) along the length of each tail and classified as neural spines not longer than centrum (0), as long as centrum (1), more than the length of centrum (2); tail shape approximately circular (mid-tail height roughly equal to mid-tail width) (0), moderately dorsoventrally expanded (height/width 1.5–3) (1), extremely dorsoventrally expanded (height/width >6) (2); pleurapophysis laterally directed and not longer than centrum (0), vertically directed and as long as centrum (1), vertically directed and more than the length of centrum (2); haemapophysis a low ridge, height of ridge not greater than length of centrum (0), a low keel as long as centrum (1), more than the length of centrum (2).

have been present in the common ancestor of extant sea snakes. Instead, these characters appear to have been developed independently in two highly marine lineages of relatively recent origin. Both the *Aipysurus* and *Hydrophis* lineages have elongated neural spines that support the dorsal edge of their strongly dorsoventrally expanded paddles. However, whereas in the *Aipysurus* lineage the ventral edge of the paddle is supported by elongated haemapophyses (median ventral processes), this support is provided by elongated and ventrally directed pleurapophyses (originally lateral processes) in the *Hydrophis* lineage. Three semi-marine species (*Hydrelaps darwiniensis*, *Ephalophis greyi*, and *Parahydrophis mertoni*) form the sister lineage to the *Hydrophis* group and have only moderately dorsoventrally expanded tails with poorly developed neural spines and ventral supports. Given that elongated neural spines are found in both highly marine clades, it is possible that these dorsal supports were present in the common ancestor of viviparous sea snakes and secondarily reduced in the semi-marine taxa. However, it is reasonable to expect that elongation of the neural spines and ventral supports evolved concurrently as paddle size increased—larger paddles would need musculoskeletal support both dorsally and ventrally to overcome greater motion resistance in water. In this case, a more plausible explanation is that dorsal and ventral supports were both elongated independently in the ancestors of the *Aipysurus* and *Hydrophis* lineages. Overall, our results suggest that not only are the viviparous hydrophiines the only lineage of marine snakes to have evolved additional skeletal supports in their paddle tails (including the extinct palaeopheids) (Parmley and Reed 2003) but also that this innovation has occurred twice in the group in the past ~2–6 million years.

The fact that semi-marine taxa *Hydrelaps*, *Ephalophis*, and *Parahydrophis* have only moderately dorsoventrally expanded tails is consistent with experimental evidence that paddles impose a cost to terrestrial locomotion in amphibious lineages (Shine and Shetty 2001; Shine et al. 2003; Aubret and Shine 2008). However, these taxa exhibit notable inter-specific variation, including very broad neural spines in all three species, ventrally broad (keel-like) haemapophyses in *Ephalophis*, and generally more elongated dorsal and ventral supports in *Hydrelaps*. Understanding these differences will require studies of the amount of time that each species spends in water and on land, and the relative importance of each environment for feeding, mating, and escaping predators. The highly marine *Aipysurus* and *Hydrophis* groups also exhibit considerable inter-specific variation in relative paddle size and shape. Some species have vertically asymmetrical paddles that are taller dorsally than ventrally, and/or have paddles with a hard pointed tip formed by a single enlarged scale. This variation might also be explained by differences in ecology (e.g., habitat

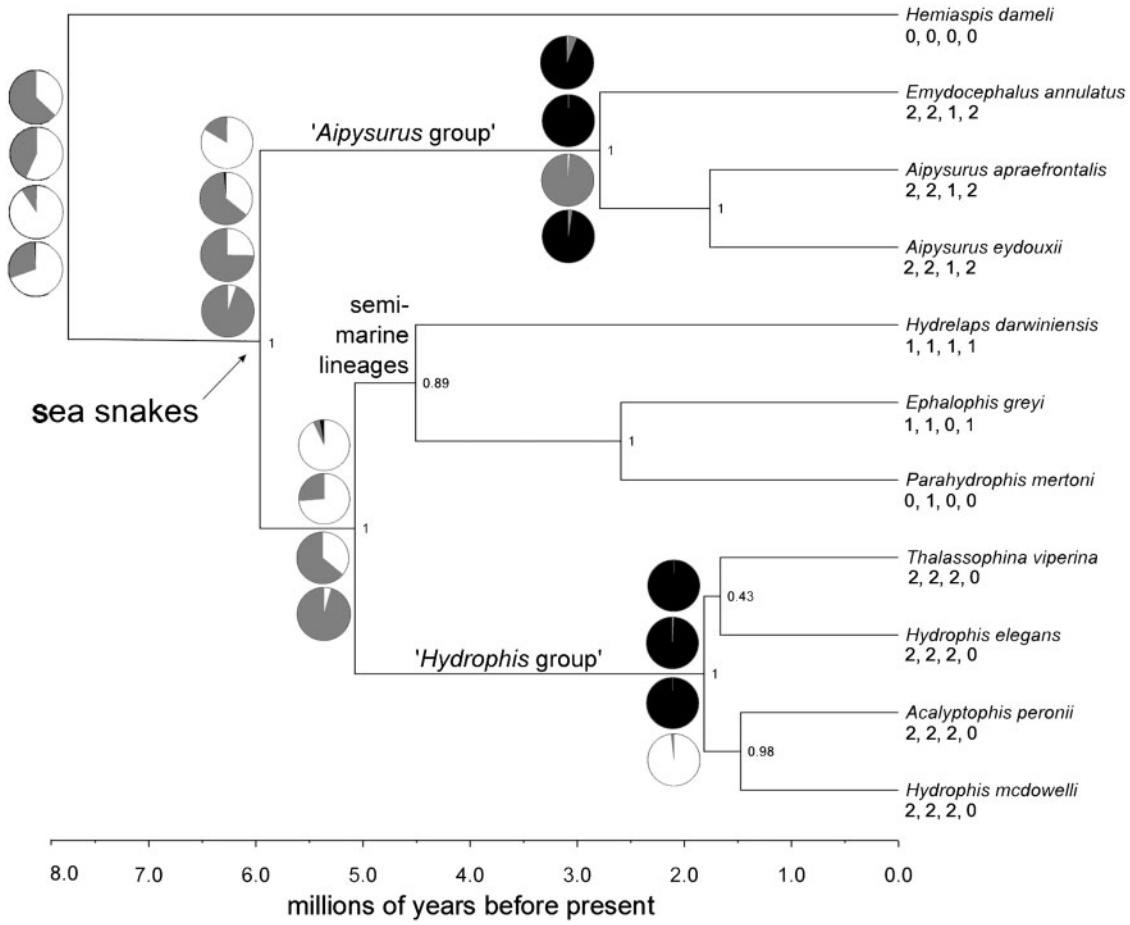

Fig. 3 BEAST maximum credibility ultrametric tree for sampled marine and terrestrial hydrophiine taxa (*Pseudonaja* outgroup not shown). Posterior probability support values are shown for each node. Timescale is in millions of years before present. Pie charts indicate Bayesian posterior probabilities of reconstructed ancestral character states at key nodes: neural spines, tail shape, pleurapophyses, and haemapophyses are shown top to bottom, respectively; colors indicate character classifications (see Table 1 and "Methods" section) and correspond to white = 0; gray = 1; black = 2. Character states for each terminal are shown left to right below taxon labels in the same order as at key nodes.

depth) or behavior (e.g., if the tail is used to provide purchase in benthic microhabitats).

Previous studies on sea kraits have examined the impact of caudal paddle size on locomotor speeds in water and on land (Aubret and Shine 2008). Future work might extend these studies to explore the functional significance of the size and shape of paddles and their vertebral supports in ecologically divergent viviparous lineages, especially the extent to which these enhance maneuverability and locomotor speed and efficiency on land and in water.

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Appendix

Appendix 1 Collection localities and museum and GenBank numbers for the specimens used in this study. ABTC = Australian Biological Tissue Collection; MZB Ophi = Museum of Zoology, Bogor, Indonesia; WAM = Western Australian Museum. Sequences generated in the study are indicated by asterisks (*)

			GenBank accession number		
Taxon	**Locality**	**Museum voucher**	**Cytb**	**ND4**	**16S rRNA**
Acalyptophis peroni	South Sulawesi, Indonesia	MZBOphi4257	JQ217200	JQ217209	JQ217145
Aipysurus apraefrontalis	Exmouth, Western Australia	WAM157818	JX002974*	JX002981*	JX002987*
Aipysurus eydouxii	East Java, Indonesia	MZBOphi4178	JX002975*	JX002982*	JX002988*
Emydocephalus annulatus	Hibernia Reef, Western Australia	ABTC29030	EU547087	EU547038	EU547185
Ephalophis greyi	Yanrey, Western Australia	WAM157940	JX002976*	JX002983*	JX002989*
Hemiaspis dameli	Australia	ABTC06514	EU547073	EU547025	EU547171
Hydrelaps darwiniensis	Bing Bong Station, Northern Territory, Australia	ABTC28875	EU547084	EU547035	EU547182
Hydrophis elegans	Weipa, Queensland, Australia	no voucher	JX002977*	JX002984*	JX002990*
Hydrophis macdowelli	Moondalbee Island, Queensland, Australia	ABTC101326	JX002978*	JX002985*	JX002991*
Parahydrophis mertoni	Palmerston, Northern Territory, Australia	ABTC28239	JX002979*	FJ593201	JX002992*
Pseudonaja modesta	Australia	ABTC56338	EU547049	EU547001	EU547147
Thalassophina viperina	South Sulawesi, Indonesia	MZBOphi4065	JX002980	JX002986	JX002993

Appendix 2 Primer information

Locus	**Primer**	**Reference**
Cytb	L14910 5′-GAC CTG TGA TMT GAA AAA CCA YCG TTG T-3′ H16064 5′-CTT TGG TTT ACA AGA ACA ATG CTT TA-3′	Burbrink et al. (2000)
ND4	M245 5′-TGA CTA CCA AAA GCT CAT GTA GAA GC-3′ M246 5′-TAC TTT TAC TTG GAT TTG CAC CA-3′	Arévalo et al. (1994)
16S rRNA	M1272 5′-CGC CTG TTT ATC AAA AAC AT-3′ M1273 5′-CCG GTC TGA ACT CAG ATC ACG T-3′	Kocher et al. (1989)