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Molecular evidence that the deadliest sea snake *Enhydrina schistosa* (Elapidae: Hydrophiinae) consists of two convergent species

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ABSTRACT

We present a striking case of phenotypic convergence within the speciose and taxonomically unstable *Hydrophis* group of viviparous sea snakes. *Enhydrina schistosa*, the 'beaked sea snake', is abundant in coastal and inshore habitats throughout the Asian and Australian regions, where it is responsible for the large majority of recorded deaths and injuries from sea snake bites. Analyses of five independent mitochondrial and nuclear loci for populations spanning Australia, Indonesia and Sri Lanka indicate that this 'species' actually consists of two distinct lineages in Asia and Australia that are not closest relatives. As a result, Australian "*E. schistosa*" are elevated to species status and provisionally referred to *Enhydrina zweifeli*. Convergence in the characteristic 'beaked' morphology of these species is probably associated with the wide gape required to accommodate their spiny prey. Our findings have important implications for snake bite management in light of the medical importance of beaked sea snakes and the fact that the only sea snake anti-venom available is raised against Malaysian *E. schistosa*.

1. Introduction

Convergent evolution in organisms experiencing similar environments is pervasive across taxonomic groups and underlines the importance of adaptive processes in diversification and its potential to confound phylogenetic analysis (Futuyama, 1998). Viviparous sea snakes of the *Hydrophis* lineage (Hydrophiinae) provide a promising model for studies of phenotypic convergence and adaptive radiation in the marine environment (Lukoschek and Keogh, 2006; Sanders et al., 2010; Voris, 1977). The 47 recognised *Hydrophis* group species share a common ancestor only ~5–8 million years ago, yet are ecomorphologically very diverse (Rasmussen et al., 2011; Sanders and Lee, 2008). However, lack of a phylogenetic framework for these species has hindered attempts to assess their patterns of diversification, and they have variously been classified in 10–16 often monotypic or paraphyletic genera (Kharin, 2004; McDowell, 1972; Rasmussen, 1997; Smith, 1926; Voris, 1977).

The 'beaked' or 'hook-nosed' sea snakes in the genus *Enhydrina* are among the most distinctive and widely distributed viviparous sea snakes. They are distinguished from all other sea snakes by an extremely long and narrow (vs triangular) mental (chin) scale that is largely concealed in a deep notch between the lower jaws (Fig. 1). *Enhydrina schistosa* (Daudin, 1803) as currently recognised is found from the Arabian Gulf to Sri Lanka, Southeast Asia, Australia and New Guinea (Heatwole, 1999). This species primarily inhabits coastal and inshore areas and can be abundant in estuaries and lagoons, where it poses a significant risk to fishermen handling nets (Heatwole, 1999; Valenta, 2010). A second species in the genus, *E. zweifeli* (Kharin, 1985), was described from a single male specimen collected in Papua New Guinea (Kharin, 1985) but its validity is not widely accepted.

The authors' recent collection of *Enhydrina schistosa* from Australia and Asia allowed the first comparisons of DNA sequences for this taxon across its range. Because introgression and stochastic lineage sorting can disconnect any individual gene tree from the true species tree (e.g. Seehausen, 2004) we analysed four nuclear DNA loci in addition to mtDNA to better characterise relationships among *E. schistosa* populations. A coalescent species tree, phylogenetic analyses of single-locus and concatenated datasets, and statistical tests of monophyly, together indicate that this single 'species' actually consists of two distinct lineages in Australia and Asia that are not even closest relatives. Understanding the evolutionary relationships of beaked sea snakes is especially important in light of the fact that they are responsible for the large majority

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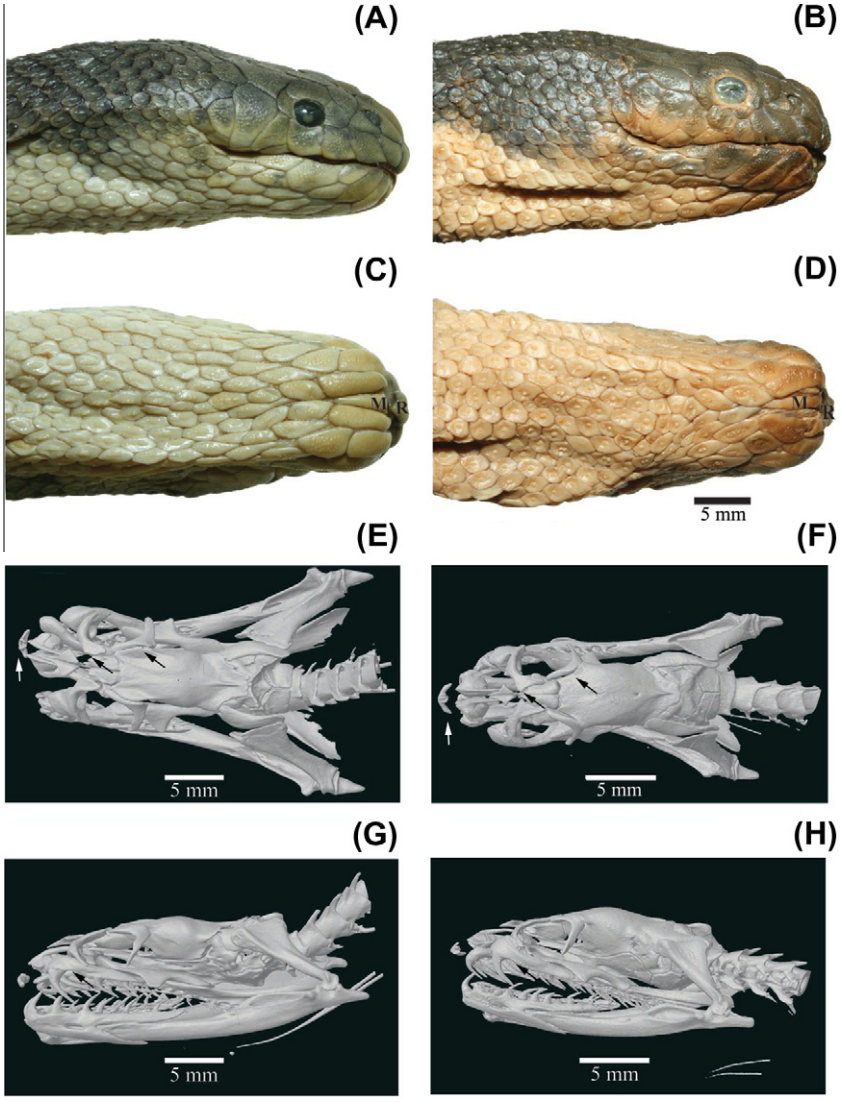

Fig. 1. Diagnostic characters of the genus *Enhydrina* and a comparison of Osteological differences between Asian and Australian *E. schistosa:* (A and C): QM J41880 from Malaysia, (B and D) QM J61632 from Australia. Note the narrow elongated mental scale (M) and pronounced rostral scale (R) in both specimens and split 4th Supralabial in QM J61632 (B). Micro-CT image of the crania of *E. schistosa* from (E and G) Indonesia MZB Ophi 12126 and (F and H) Australia SAMA R65220. Note the convex flange over orbit, anteriorly shallow maxilla, smaller premaxilla and the absence of an anteromedial process in the prefrontal of MZB Ophi 12126.

of recorded sea snake bites (Heatwole, 1999; Reid, 1956; Valenta, 2010).

2. Materials and methods

2.1. Taxon sampling and laboratory protocols

We sampled 11 specimens that are currently recognised as *Enhydrina schistosa* from Asia and Australia. Liver and muscle tissues were mostly obtained from voucher specimens collected by us during field trips in northeast Australia, Indonesia and Sri Lanka, and additional tissues were loaned from museum collections. Specimen localities and museum voucher numbers are provided in the online Supplementary Appendices A and B.

Whole genomic DNA was extracted from liver and muscle tissues using standard Proteinase K protocols (Puregene™ DNA Isolation Tissue Kit, Gentra Systems). We amplified and sequenced a total of 5243 base pairs (bp) from three mitochondrial and four nuclear markers to reconstruct the sea snake phylogeny. Mitochondrial markers were a 1033 bp fragment of *Cytochrome b* (*Cytb*), 839 bp of *NADH dehydrogenase subunit 4* (*ND4*) and the adjacent *tRNA* region, and 532 bp of *16S small subunit ribosomal RNA* (*16S rRNA*). The two nuclear protein coding genes were a 915 bp fragment of *c-mos* (*oocyte maturation factor*) and 1074 bp of *RAG*1 (*recombination activation gene*); and the two anonymous nuclear markers were *G*1888 (428 bp) and *G*1894 (422 bp) isolated from a 454 shotgun sequencing run (Bertozzi et al., 2012). The primer specifications used in the analyses have been published elsewhere (Arevalo et al., 1994; Burbrink et al., 2000; Groth and Barrowclough, 1999; Hugall et al., 2008; Kocher et al., 1989; Saint et al., 1998; Ukuwela et al., 2012). All markers were amplified using standard PCR protocols with HotMaster Taq reagents (Applied Biosystems, Foster city, CA, USA) and 34 cycles; annealing temperatures were 52 °C for mitochondrial markers, 57 °C for *c-mos*, 56 °C for *RAG*1 and 59 °C for the two anonymous nuclear markers. Sequencing of the PCR products was outsourced to the Australian Genome Research Facility (AGRF) in Adelaide.

Consensus sequences of forward and reverse reads were aligned using the Geneious Pro 5.4 software (Drummond et al., 2009) and then manually edited and refined by eye. Additional mitochondrial and nuclear sequences that were generated in previous studies

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(Lukoschek and Keogh, 2006) were downloaded from Genbank (http://www.ncbi.nlm.nih.gov/genbank/). Aligned sequences of the protein coding genes were translated into amino acid sequences to determine the correct reading frame and check for premature stop codons that might indicate amplification of pseudogenes. The program PHASE v. 2.1.1 (Stephens and Donnelly, 2003; Stephens et al., 2001) was used to assign single nucleotide polymorphisms (SNPs) derived from the two anonymous nuclear markers to a single allelic copy. The sequences generated in this study are deposited in the Genbank under the accession numbers JX987138-JX987186 (see Appendix A).

2.2. Phylogenetic analyses

Phylogenetic relationships were inferred by reconstructing gene trees for the mitochondrial data, each nuclear locus, the concatenated nuclear alignment, and a multi-locus coalescent (MC) analysis of all mitochondrial and nuclear loci (to reconstruct the species tree while accounting for gene tree discordance due to coalescent stochasticity). All phylogenetic analyses were run via the GRISU portal on the ARCS Compute Cloud (www.arcs.org.au/).

2.2.1. Single-locus and concatenated analyses

Phylogenies were reconstructed for the single-locus and concatenated alignments using Bayesian inference implemented in the software BEAST v1.6.2 (Drummond and Rambaut, 2007) and partitioned maximum likelihood (ML) analyses using RAxML v7.2.6 (Stamatakis, 2006). Concatenating nuclear loci does not accommodate the potentially confounding effects of stochastic lineage sorting (in contrast, mitochondrial markers are inherited as a single locus). However, due to low numbers of variable sites, single-locus nuclear trees used in MC analyses can suffer from sampling errors. Concatenation provides an opportunity to potentially amplify the underlying signal in the nuclear data, independent of the mitochondrial locus.

Alternative partitioning schemes were assessed for each dataset using best-fit substitution models for each partition determined using the Akaike information criterion (AIC) implemented in jModelTest v0.1.1 (Guindon and Gascuel, 2003; Posada, 2008); these were GTR + i + g for each mitochondrial partition and HKY + i for all the nuclear partitions. Partitioning schemes for the mitochondrial data were a three-partition strategy by codon position (1st + 2nd vs 3rd) and rRNA, and a five-partition strategy of the two coding genes separately by codon (1st + 2nd vs 3rd) and rRNA. The nuclear loci were not partitioned by codon due to very few variable sites. The alternative mitochondrial partitioning schemes were assessed using Bayesian analysis implemented in BEAST with an uncorrelated lognormal relaxed clock and a Yule tree model prior with 10 million generations sampling every 1000 iterations. The three-partition strategy of coding gene by codon position (1st + 2nd vs 3rd) and rRNA was selected as optimum for the mitochondrial alignment using Bayes factors (Kass and Raftery, 1995) calculated in TRACER v1.5 (Suchard et al., 2001).

BEAST analysis of the concatenated mitochondrial alignment was then run for 10 million generations with sampling every 1000 generations, and the concatenated nuclear alignment was run for 8 million generations with sampling every 1000 generations. The individual nuclear loci were run for 4 million generations with sampling every 1000 generations. All analyses used an uncorrelated lognormal relaxed clock model of branch rate variation and a Yule tree model prior. Substitution parameters and clock models were unlinked (i.e. allowed to vary) across partitions. Convergence was assessed by examining effective sample sizes (ESS values >100 are recommended) and likelihood plots through time in TRACER v1.5 (Rambaut and Drummond, 2007), with the first 25% of trees discarded from each run as burn-in. The BEAST maximum credibility trees were summarised in TreeAnnotator v1.6.2 (distributed with BEAST package). All analyses were repeated four times to test the consistency of the outcome of the analyses. All analyses yielded identical or very similar topologies.

Maximum Likelihood analyses were implemented in RAxML v7.2.6 (Stamatakis, 2006) by applying the GTR + g substitution model to the same partitions as for the Bayesian analyses, and performing 200 independent ML searches. Branch support was estimated using 1000 bootstrap pseudoreplicates. *Hemiaspis damielli* and *Aipysurus laevis* were used as outgroups in the mitochondrial and nuclear ML analyses, respectively, because there is strong molecular and morphological evidence that *Hemiaspis* is the closest terrestrial relative to the viviparous sea snakes and that the latter comprises two reciprocally monophyletic *Aipysurus* and *Hydrophis* groups (Lukoschek and Keogh, 2006; Rasmussen, 2002; Sanders et al., 2008).

DNA polymorphism (variability) statistics were calculated using DnaSP 5.0 (Librado and Rozas, 2009) and corrected (HKY) pairwise genetic distances were calculated using the species delimitation plugin (Masters et al., 2010) in Geneious Pro 5.4 (Drummond et al., 2009).

2.2.2. Multi-locus species tree analysis

A Bayesian multi-locus coalescent species tree was then reconstructed using all five loci in ∗BEAST (Heled and Drummond, 2010). This method explicitly accounts for coalescent stochasticity in individual loci by jointly estimating the posterior distributions of species trees and multiple embedded gene trees (Heled and Drummond, 2010). ∗BEAST requires *a priori* assignment of putative species (i.e. divergent reproductively isolated groups). Treating all *E. schistosa* as a single species in a multispecies coalescent analysis cannot test the assumption that the Australasian and Asian populations are separate species. Thus, we provisionally treated each *E. schistosa* individual as a putatively separate species. If all *E. schistosa* subsequently formed a monophyletic group, or at least clustered relatively closely together on the tree, then the single-species hypothesis cannot to be refuted. If, however, *E. schistosa* formed two (or more) groups that are widely separated on the tree, with concordant support from multiple loci for this wide separation, this would form strong evidence for more than one species. We also note that in the concatenated mtDNA tree, all sampled *E. schistosa* was strongly non-monophyletic, suggesting that the assumption of a single species should be tested.

Mitochondrial genes were partitioned by coding codon position (1st + 2nd vs 3rd) and rRNA, and nuclear loci were not partitioned due to their low variability. 100 million generations were performed with sampling every 10,000 generations. Substitution parameters and clock models were unlinked across partitions, but trees were linked for the mitochondrial partitions because these evolve as a single unit. An uncorrelated lognormal relaxed clock model of branch rate variation and a Yule tree model prior were used. Convergence was assessed as for the single-locus and concatenated analyses (see Section 2.2.1 above), by examining effective sample sizes and likelihood plots through time in TRACER v1.5 (Rambaut and Drummond, 2007), with the first 25% of trees discarded from each run as burn-in. Again, BEAST maximum credibility trees were generated using TreeAnnotator v1.6.2 (distributed with BEAST package). MC analyses were repeated four times to test the consistency of the outcome of the analyses. All analyses yielded identical topologies and very similar parameter values, suggesting that they were sampling the same pool of post-burnin trees.

2.3. Monophyly tests

Because our phylogenetic analyses failed to recover Asian and Australian *Enhydrina schistosa* as monophyletic (see Sections 3.1.1 and 3.1.2 below), strength for the alternative hypothesis (that all

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sampled *E. schistosa* form a clade) was statistically tested for each concatenated and single-locus tree and the coalescent species tree by enforcing a monophyly constraint on Asian and Australian *E. schistosa* lineages. The best scoring ML trees from the constrained and unconstrained analyses were compared using S–H tests (Shimodaira and Hasegawa, 1999) in RAxML v7.2.6. Bayesian tests used Bayes factors calculated in TRACER v1.5 (Suchard et al., 2001) using the log files of the unconstrained and constrained analyses. A $\log_{10}$ Bayes factor >3 was considered as evidence against the alternative hypothesis (constrained tree) based on the guidelines of Kass and Raftery (1995).

2.4. Morphological comparisons

External morphological data were collected for 30 museum and field collected specimens of *Enhydrina schistosa* (18 from Asia and 12 from Australia). A total of 13 characters relating to scalation and colour pattern were recorded for each specimen. Scale counts were the number of supralabial scales, number of infralabial scales, number of preocular scales, number of loreal scales, number of anterior temporal scales, number of posterior temporal scales, number of ventral scales, taken from neck to cloaca (Dowling, 1951); number of caudal scales, counted from the vent to the tip of the tail; and number of costal scales (around neck and mid body) (Rasmussen et al., 2001). Costal scale counts around the neck and body were repeated four times to obtain minimum and maximum counts respectively. Snout to vent length (SVL) was measured using as the length from the tip of snout to the posterior margin of anal plate, and tail length (TAL) was the length from posterior margin of anal plate to the tip of tail.

To compare cranial osteology of Asian and Australian *E. schistosa*, micro-CT scanning was performed for one individual each of Asian and Australian *E. schistosa* on a Skyscan 1076 in vivo X-ray microtomograph at Adelaide Microscopy with the following general settings: resolution 18 μm, rotation step 0.6°, time 295 ms, filter nil. Reconstructions were performed with Skyscan software (www.skyscan.be/products/downloads.htm) and the conversion to cross sections was done 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 measuring some dimensions in the cross sectional images with CTAn. Because of size constraints in the micro-CT equipment, the two scanned specimens were sub-adults, and their sex was not reliably determined. The characters identified as different are, however, unlikely to be influenced by ontogenetic stage (see Section 3.2).

3. Results

3.1. Phylogenetic analyses and monophyly tests

3.1.1. Single-locus and concatenated analyses

Bayesian analysis of the mitochondrial data recovered effective samples sizes above 100 for all parameters indicating adequate mixing, and likelihood analysis yielded a log likelihood score of −12128.41 for the best scoring ML tree. Both mitochondrial analyses recovered Australian *Enhydrina schistosa* specimens as a monophyletic sister lineage to the Australian taxon *Hydrophis major* with a posterior probability (pp) of 0.98 and bootstrap support of 70 (Fig. 2). Asian *E. schistosa* specimens were recovered as the sister lineage to the primarily Asian taxa *Hydrophis caerulescens* + *H. brooki* although this relationship was not strongly supported. Bayesian ($\log_{10}$ Bayes factor >3) and ML ($P < 0.05$) analyses strongly rejected the monophyly of Asian and Australian *E. schistosa* for mtDNA (Table 1). The average pairwise corrected (HKY) divergence between Australian and Asian *E. schistosa* for the concatenated mitochondrial alignment was 7.04% (range 6.01–8.12%) and 99 fixed nucleotide differences were found between these taxa. The mean corrected (HKY) pairwise mitochondrial distance between Australian *E. schistosa* and *H. major* was 4.81% (range: 4.37–5.48%) and these taxa had 74 fixed substitutions.

The individual nuclear gene trees recovered much weaker support than the mitochondrial tree, but each locus recovered Asian and Australian *E. schistosa* as non-monophyletic (Supplementary Fig. S1). S–H tests rejected ($P < 0.05$) the monophyly of Asian and Australian *E. schistosa* for *c-mos* and *G*1894 ML trees (Table 1). The *c-mos* result is surprising given the very small log-likelihood difference (Table 1). The standard deviation of the test was also very small. This might be due to low signal in this gene for or against the hypothesis, resulting in even a small difference being significant. For this reason, we suggest that the significance of this result be treated with skepticism. In the Bayesian tests, monophyly of Asian and Australian *E. schistosa* was rejected ($\log_{10}$ Bayes factor >3) for *G*1888 and *G*1894 trees, and the remaining two nuclear loci did not strongly favour either hypothesis (Table 1).

Concatenated nuclear trees yielded low support for most nodes, but both ML and Bayesian trees recovered Asian and Australian *E. schistosa* as non-monophyletic (Fig. 3). In both trees, Asian *E. schistosa* was monophyletic, while *H. major* was nested inside the Australian *E. schistosa*. Monophyly of Asian and Australian *E. schistosa* was strongly rejected ($\log_{10}$ Bayes factor >3) for the Bayesian analysis, but not for the ML analysis (S–H test $P > 0.05$).

3.1.2. Multi-locus species tree analysis

The ∗BEAST coalescent species tree analysis yielded ESS values above 100 for all parameters, and recovered relationships that were congruent with supported nodes in our mitochondrial and concatenated nuclear trees. Our ∗BEAST tree was also very consistent with a larger multi-locus coalescent analysis of sea snake phylogeny (Sanders et al., in press), indicating that our treatment of individual *E. schistosa* specimens as separate species did not greatly impact inference of branch lengths and topology (see Section 2.2.2). Again, Asian and Australian *E. schistosa* were not monophyletic; Australian *E. schistosa* specimens formed the monophyletic sister lineage to *H. major* with a posterior probability (pp) of 0.97. Asian *E. schistosa* were also recovered as monophyletic but were the sister lineage to the *Hydrophis caerulescens* + *H. brooki* lineage (Fig. 4). Bayesian analyses strongly rejected ($\log_{10}$ Bayes factor >3) the monophyly of Asian and Australian *E. schistosa* (Table 1).

3.2. Morphology

Asian and Australian *Enhydrina schistosa* showed several differences in morphological traits relating to scalation, colour pattern and skull osteology. Australian *E. schistosa* has fewer bands on the body (38–49 vs 46–59 in Asian *E. schistosa*), a lower but broadly overlapping ventral scale count (261–313 vs 286–335), and a lower costal scale count (37–46 vs 39–55) (Table 2). The most distinct difference is the presence of a split 4th supralabial scale in Australian *E. schistosa* (Fig. 1A and B). Micro-CT scan reconstructions of the skulls of Australian and Asian *E. schistosa* show that the latter has a convex flange over the orbit, an anteriorly shallow maxilla and a smaller (narrower) premaxilla (Fig. 1E–H). Further, the Asian *E. schistosa* lacks an anteromedial process in the prefrontal (Fig. 1E–H).

4. Discussion

The five independent molecular loci indicate that Asian and Australian *Enhydrina schistosa* consist of two distinct lineages that

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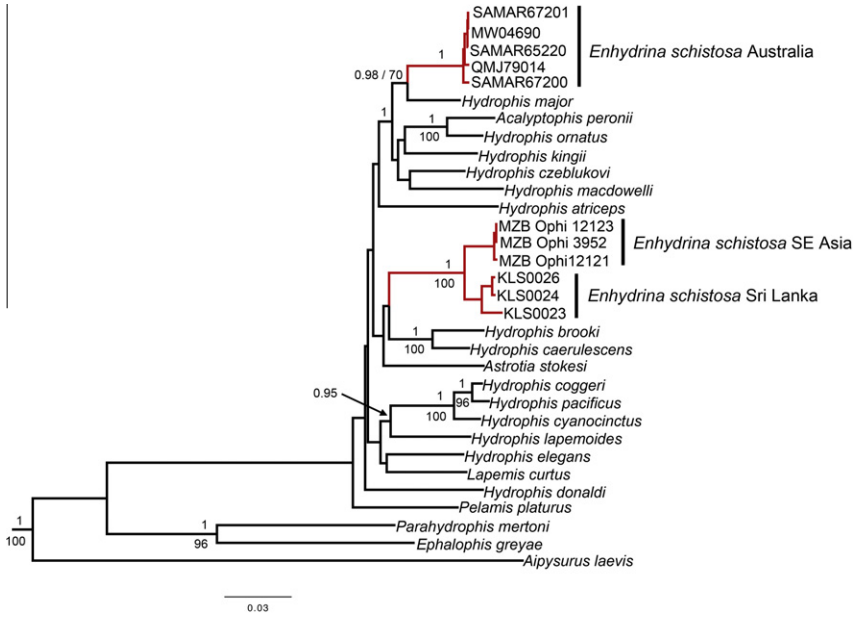

Fig. 2. BEAST maximum credibility tree of three concatenated mitochondrial genes showing the non-monophyly of Asian and Australian *Enhydrina schistosa* lineages. Nodes with maximum likelihood bootstrap support >70 (below) and Bayesian posterior probability >0.9 (above) are indicated. Outgroup *Hemiaspis damelii* is not shown. Scale bar indicates the number of nucleotide substitutions per site.

Table 1
Hypothesis testing. ΔLH = difference of the likelihood scores of best unconstrained and constrained ML trees. ML = maximum likelihood, BI = Bayesian Inference,' + ' indicates strong rejection of *E. schistosa* monophyly, '–' indicates that monophyly of *E. schistosa* is neither strongly supported or rejected.

Analysis	S–H test (ML)		$\log_{10}$ Bayes factors (BI)	Result	
	ΔLH	*P* value		ML	BI
Multilocus Coalescent	n/a	n/a	37.582	n/a	+
Concatenated mtDNA	−33.0228	$P < 0.01$	12.649	+	+
Concatenated nuDNA	−17.356	$P > 0.05$	11.116	–	+
c-mos	−00.0027	$P < 0.01$	00.502	+	–
*RAG*1	−0.32540	$P > 0.05$	−00.459	–	–
*G*1888	−09.6770	$P > 0.05$	06.563	–	+
*G*1894	−16.3151	$P < 0.05$	09.722	+	+

are not closest relatives, suggesting that the 'beaked sea snake' represents an extreme case of convergent phenotypic evolution. The alternative scenario that the specialised *Enhydrina* morphotype is primitive for a large section of the *Hydrophis* group and has been lost repeatedly is far less parsimonious. Our results might also be explained by a failure of our mitochondrial and nuclear markers, which have different strengths and weaknesses, to accurately represent species relationships among the beaked sea snakes. Mitochondrial data (due to maternal inheritance and lack of recombination) are fast-sorting but are susceptible to introgressive hybridisation, which can result in species paraphyly if the mitochondrial genome in one species is replaced by that of another. Slowly evolving nuclear loci are less prone to introgression but are susceptible to stochastic lineage sorting (retention of ancestral polymorphisms across speciation events), which can also cause a lack of within-species monophyly (Meyer and Paulay, 2005).

Consistent with short speciation intervals and slow rates of lineage sorting, our four nuclear loci yielded discordant gene trees and provided very few variable sites, many of which were shared among species that were clearly not closely related in the mitochondrial trees. However, if lineage sorting of nuclear markers and introgression of mtDNA together explained the non-monophyly of Asian and Australian *E. schistosa*, we would not expect to find congruent signal across these independently segregating markers. Conversely, Australian *E. schistosa* and *Hydrophis major* (1) showed close relationships and shared several haplotypes in individual nuclear gene trees, (2) formed a clade in the concatenated nuclear tree and MC coalescent tree, and also in (3) the mitochondrial trees. These concordant results might be explained by historical introgression of both nuclear and mitochondrial DNA from *H. major* into *E. schistosa* in Australia. However, although this scenario could be tested using deep sequencing of candidate taxa, it is inconsistent with the observation that Asian and Australian *E. schistosa* populations shared no substitutions at any nuclear locus, while both shared such substitutions with several other sampled species. Finally, geographic concordance further supports or at least is consistent with independent origins: the coalescent species tree recovered the Asian and Australian *E. schistosa* lineages as sister taxa to respectively Asian (*H. caerulescens* + *H. brooki*) and Australian (*H. major*) lineages.

Enhydrina schistosa is unique among sea snakes in feeding almost exclusively on sea-floor active spiny catfishes and puffer fishes (Voris and Voris, 1983). Selection pressures related to this highly specialist diet might explain the putative convergence of 'beaked' snouts in Asian and Australian forms. The deep median notch between the lower jaws (created by a reduction in the mental scale, the main character used to diagnose the genus) is assumed to facilitate a wider gape for accommodating spiny prey by increasing the area of extensile tissue (McDowell, 1972). This functional hypothesis requires testing, and additional work is

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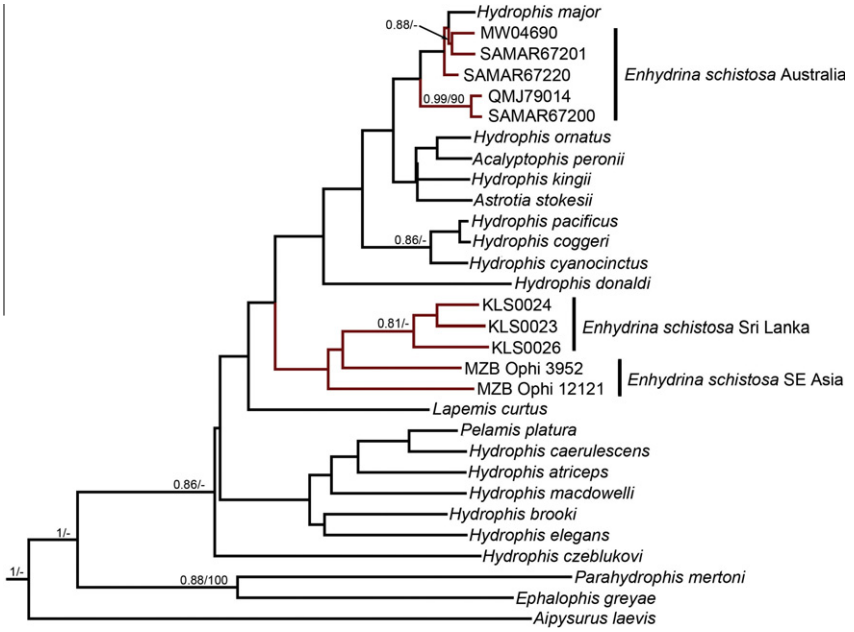

Fig. 3. BEAST maximum credibility tree of four concatenated nuclear markers showing the non-monophyly of Asian and Australian *Enhydrina schistosa* lineages. Nodes with maximum likelihood bootstrap support >70 (below) and Bayesian posterior probability >0.8 (above) are indicated. Outgroup *Hemiaspis damelii* is not shown. Scale bar indicates the number of nucleotide substitutions per site.

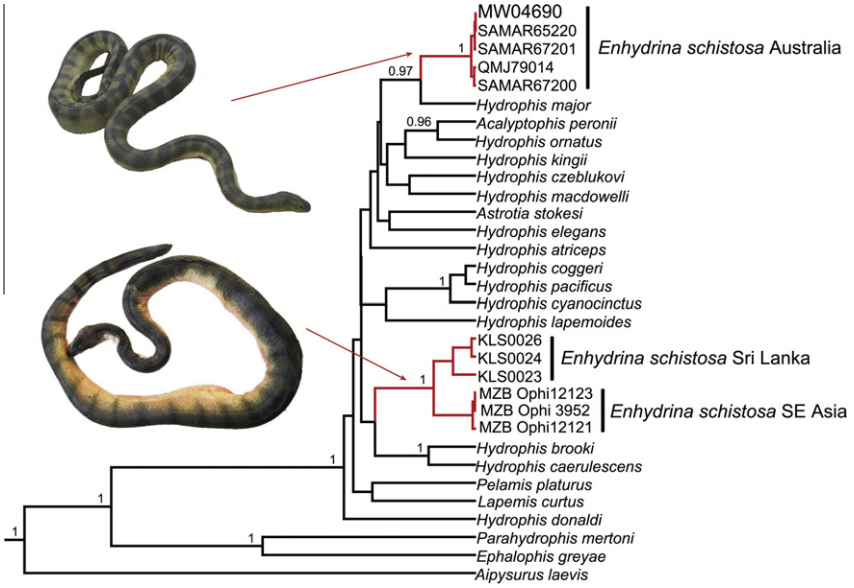

Fig. 4. Bayesian multi-locus coalescent species tree. Asian and Australian *Enhydrina schistosa* lineages form separate and distantly-related clades (each with affinities to geographically proximate taxa). Nodes with Bayesian posterior probability >0.9 are indicated. Outgroup *Hemiaspis damelii* is not shown. (Scale bar = substitutions per site).

Table 2
Comparison of the morphological characteristics of *Enhydrina schistosa* from Australia and Asia and *E. zweifeli*. (Data for *E. zweifeli* are from Kharin (1985).

Character	*E. zweifeli* (Holotype – AMNH 104340)	*E. schistosa* Australia ($n = 12$)	*E. schistosa* Asia ($n = 18$)
4th Supralabial split	Yes	Yes	No
Costals: neck	39	37–46	39–55
Costals: Midbody	48	48–56	42–63
Ventrals	271	261–313	286–335
Subcaudals	?	43–58	32–56
Preoculars	Absent (fused with supraocular)	1–2	1–2
Bands on body	36	38–49	46–59
Maxillary teeth	3	3	3

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needed to better characterise the diet of *E. schistosa* in Australia – the only diet record currently available is of a sea perch (but this is also armored with spines). Notably, the closest relative of Australian *E. schistosa, H. major,* also has a deep chin notch intermediate between *Enhydrina* and typical *Hydrophis*. *Hydrophis major* has a broadly similar diet to *Enhydrina,* feeding mostly on spiny catfishes (Fry et al., 2001) in addition to goby and eel-like fishes (Fry et al., 2001; Voris and Voris, 1983).

Our finding that 'beaked sea snakes' have independent origins in Asia and Australia has important implications for snake bite treatment and management. *Enhydrina schistosa* is responsible for the majority of deaths and injuries from sea snake bites (Heatwole, 1999) and the only sea snake anti-venom available is raised against Malaysian *E. schistosa* (CSL Limited, Australia). Snake venom has a strong phylogenetic component, and the venom of the two *Enhydrina* populations has been shown to have a similar overall composition but different specific isoforms (Fry et al., 2003). Nevertheless, both *E. schistosa* groups (Asian and Australian) have very similar LD50s values, with both being extremely toxic (0.15 mg/kg for the Asian: Minton and Minton, 1969; and 0.164 mg/kg for the Australian: Broad et al., 1979). However, a key aspect of sea snake venom evolution, that has prevented this misidentification from having catastrophic medical implications, is that all sea snake venoms are very streamlined due to feeding on a single higher taxon (bony fish) (Fry et al., 2003). Consequently all sea snake venoms tested to-date have been well-neutralised by the only available antivenom (Chetty et al., 2004).

4.1. Taxonomy

Our results show that allopatric populations in Asia and Australia which were formerly all termed *Enhydrina schistosa* should be recognised as separate species. The type locality of *E. schistosa* is Tranquebar, South India (Holotype: BMNH 1946.1.10.7), we therefore keep the original name for populations distributed in Asia. *Enhydrina zweifeli* was described based on a single specimen from New Guinea but without reference to other *Enhydrina* specimens from the Australo-Papuan region. It differs from *E. schistosa* by having fewer bands on the body, lower but overlapping costal counts, a split 4th supralabial, no preocular, and a more weakly bilobed hemipenis (Kharin, 1985). It is unclear whether *E. schistosa* from the Australian region should be designated as *E. zweifeli*, given the large and overlapping range of scale counts and colour pattern characters in Asian and Australian *E. schistosa* and *E. zweifeli* (Table 2). However, the Australian *E. schistosa* and *E. zweifeli* share the split 4th supralabial; furthermore, *E. zweifeli* has low ventral counts and band counts, which are similar to those in Australian *E. schistosa* but well below those of Asian *E. schistosa* (Table 2 and Fig. 1). Thus, we tentatively refer Australian *E. schistosa* to *E. zweifeli*. Morphological and molecular data for additional specimens from New Guinea are needed to confirm this arrangement and more clearly delimit the distributions of these species. *Enhydrina schistosa* and *E. zweifeli* are both deeply nested in the *Hydrophis* group, hence we follow Sanders et al. (in press) in recognising the genus *Hydrophis* for both *E. schistosa* and *E. zweifeli*.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.09.031.

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