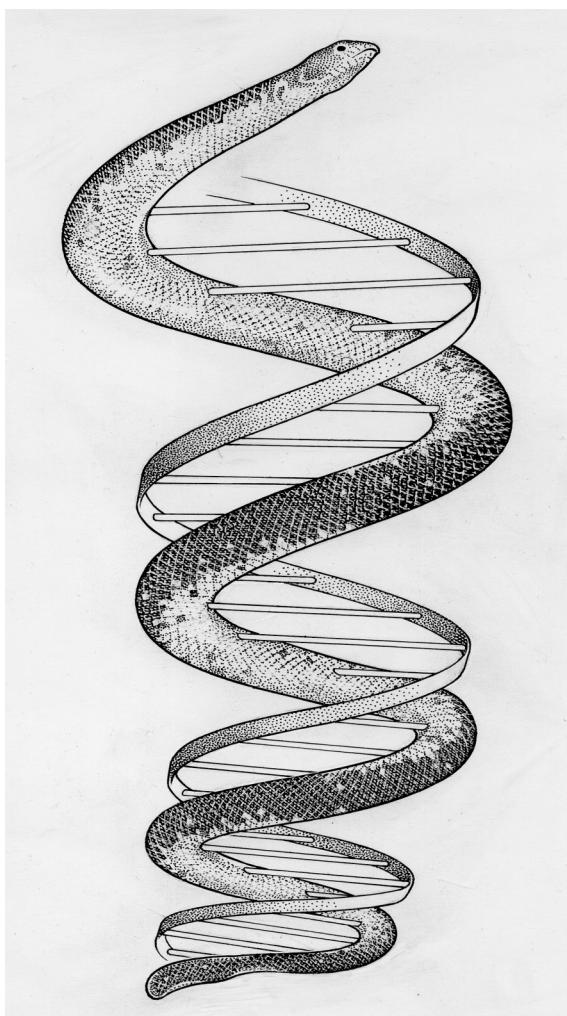


Molecular ecology, evolution and conservation of hydrophiline sea snakes

Thesis submitted by
Ms. Vimoksalehi Lukoschek B.Sc. (Hons.) JCU
August 2007



for the degree of Doctor of Philosophy
School of Marine and Tropical Biology
School of Earth and Environmental Sciences
James Cook University
Townsville, Australia

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Abstract

Hydrophiine sea snakes are the most speciose group of extant marine reptiles. Species occur in a variety of shallow-water habitats throughout the Indo West Pacific with highest species diversity in northern Australia and South East Asia. Each of these regions hosts a suite of endemics but they also share widespread taxa. Viviparity distinguishes hydrophiine sea snakes from many other marine taxa, and reproductive outputs are low and dispersal potentially restricted. These life-history characteristics predict strong population subdivision for marine hydrophiine species. Nonetheless, species preferentially occur in different habitat types that are predicted to restrict dispersal and gene flow to varying degrees and result in divergent genetic signatures among species. These ecological, evolutionary and life-history characteristics make marine hydrophiines an excellent group for comparative study, yet surprisingly little is known about their genetics, evolution and conservation. In this thesis I use molecular genetic techniques to explore the evolutionary history of the marine hydrophiine lineage, and population genetic processes for two sea snake species in northern Australian waters, and discusses the implications of results for conservation.

Evolutionary relationships among marine hydrophiines were first formalised using morphological characters and traditionally three lineages have been recognised: the *Aipysurus* lineage (nine species in two genera), the *Hydrophis* lineage (~40 species: ~27 in the genus *Hydrophis* and the remainder in ten additional genera), and three ‘primitive’ monotypic genera. While taxonomic revisions and controversies abound, none has been based on modern molecular techniques. This study used mitochondrial DNA sequencing to infer evolutionary relationships among representative marine hydrophiine species from the *Aipysurus* lineage (six species), *Hydrophis* lineage (15 species), and two ‘primitive’ genera. Parsimony, likelihood and Bayesian analyses of cytochrome *b* (1080 bp) and 16S rRNA (510 bp) sequences yielded the same optimal tree that grouped *Aipysurus* and *Hydrophis* species into strongly supported reciprocally monophyletic clades. Relationships among species and genera in the *Aipysurus* lineage were well resolved however the same was not true for the *Hydrophis* lineage. The ratio of phenotypic to genetic diversity for the *Hydrophis* lineage was four to seven times greater than for the *Aipysurus* lineage. This evidence, together with the poorly resolved relationships among *Hydrophis* lineage species, suggests that this lineage is a rapidly diverged adaptive radiation, probably driven by sea level fluctuations in South East Asia that promoted vicariance and speciation. In contrast, the *Aipysurus* lineage appears evolutionarily stable.

Sea level perturbations not only influenced speciation patterns in South East Asia and northern Australia, they also left footprints on the population genetic signatures of marine taxa in this region. The cyclic emergence of the Torres Strait land bridge between Australia and New Guinea, repeatedly sundered east and west coast populations in Australia, with subsequent range expansions into new shallow water marine habitats as sea levels rose. These biogeographical perturbations invoke three broad scenarios regarding the directions of inter-glacial range expansion (west to east, east to west, or bi-directional). These hypotheses were evaluated for two widespread and abundant hydrophiine species that differ in their habitat preferences, distributions and evolutionary histories. The olive sea snake, *Aipysurus laevis*, is restricted to Australasian waters where it occurs primarily on reefs, a relatively discontinuous habitat with potential barriers to dispersal. In contrast, the range of the spine-bellied sea snake, *Lapemis curtus*, (*Hydrophis* lineage) extends from Australia to the Arabian Gulf, and this species occupies a variety of shallow-water habitats with fewer barriers to gene flow.

A fragment of the mtDNA ND4-tRNA region (726 bp) was sequenced for *A. laevis* ($n = 354$) from 14 locations in three regions - the Great Barrier Reef (GBR), the Gulf of Carpentaria (GoC) and Western Australia (WA). Forty-one variable sites defined 38 putative haplotypes. Each region was characterised by a unique suite of haplotypes that comprised one or two common and numerous rare haplotypes. There was marked population subdivision ($F_{ST} = 0.61$, $p < 0.001$; $\phi_{ST} = 0.78$, $p < 0.001$) and coalescent analyses revealed no migration between regions. Genetic diversity in the GBR and GoC was low and within-region ‘star’ phylogenies indicated range expansions consistent with recent marine transgressions ~7,000 years ago. In contrast, genetic diversity on most WA reefs was higher with no signal of recent expansion events. Phylogenetic reconstruction indicated that GBR and GoC haplotypes were derived from WA haplotypes, suggesting west to east dispersal. However, network analysis supported range expansion in the GBR and GoC by refugial east coast populations, possibly from the Coral Sea. Patterns of gene flow within regions reflected the potential connectivity among sub-populations afforded by regional habitat types. Taxon-specific microsatellite primers were developed for repeat regions sequenced from positive clones screened from unenriched and enriched genomic libraries. Population genetic analyses of five microsatellite loci revealed weaker population subdivision ($F_{ST} = 0.059$, $p < 0.001$; $R_{ST} = 0.029$, $p < 0.0013$) but similar patterns of population structure. Bayesian clustering analyses identified two populations with individuals from a combined GBR - GoC group distinct from WA.

Sequences of the ND4-tRNA region (706 bp) for *L. curtus* ($n = 62$) revealed 20 variable sites and 17 haplotypes from seven Australian locations and Thailand ($n = 2$). Six locations in the GBR and GoC shared one common haplotype, however the 14 rare haplotypes were not shared between regions and generally not shared among locations. Phylogenetic and network analyses revealed a classical ‘star’ phylogeny with rare haplotypes arranged around the widely distributed common haplotype, and genetic diversity was low, suggesting that Australian waters were colonised recently by one *L. curtus* matriline, probably from South East Asia. Two divergent haplotypes sampled in Thailand indicated strong population subdivision at larger spatial scales. Population subdivision in Australia was low ($F_{ST} = 0.097$, $p < 0.032$; $\phi_{ST} = 0.039$, $p > 0.05$), yet the relationship between genetic and geographical distance revealed a pattern of isolation-by-distance along a geographical gradient consistent with the continuous habitat types used by *L. curtus*. This pattern of isolation-by-distance was not found for *A. laevis* suggesting that habitat discontinuities between reefs within regions act as barriers to gene flow, particularly in WA and the GBR. Microsatellite loci for Australian *L. curtus* individuals were either monomorphic or had very low allelic diversity. By contrast, the two individuals from Thailand had private alleles at several loci.

The conservation status of hydrophiine sea snakes is poorly known, however, recent reports suggest declining abundances and loss of endemic aipysurids on protected Australian reefs. Threatening processes for reef-associated species, such as *A. laevis*, are unclear but must include habitat degradation and loss. *Aipysurus laevis* typically occurs at discrete reefs and may be influenced by metapopulation dynamics. Factors driving spatial and temporal stability of local populations are poorly understood and it is not clear whether specific conservation strategies, such as networks of marine protected areas, will ensure the persistence of this species. Classification tree analyses of distribution data of *A. laevis* on 90 reefs in the southern GBR (surveyed 1 to 11 times over 35 years) indicated that its status on reefs (present = 38, absent = 38, changed = 14) was related to reef location, exposure, and area (data on potential biotic factors were not available). Perhaps more importantly, the prior protection status of reefs did not account for *A. laevis*’ distribution. Despite the ability of *A. laevis* to expand its range into new marine habitats, contemporary gene flow appears restricted. The regional distinctiveness of mtDNA lineages suggests that the GBR, GoC and WA comprise separate management units warranting independent conservation strategies for this species. *Lapemis curtus* had higher levels of gene flow; however, this species accounts for > 50% of sea snake by-catch in Australian trawl fisheries, with tens of thousands caught annually. The direct impact on populations is unknown but reduction in by-catch is undoubtedly desirable.

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