The diversification of the Australo-Papuan elapid radiation with a focus on sea snakes (Hydrophiinae)

James Henry Nankivell

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Thesis Abstract

The front-fanged elapids (Elapidae: Hydrophiinae) are the dominant radiation of terrestrial snakes on the Australian continent, and within the last 10 million years have invaded tropical marine habitats to become the most species rich clade of marine reptiles alive today. My thesis had two broad aims in relation to the Hydrophiinae radiation: 1) Investigating the hidden diversity within the subfamily and 2) Why do some groups diversify rapidly and others do not?

Numerous species of the sea snake genus *Aipysurus* have suffered declines in their former stronghold – the offshore reefs of the Timor Sea. However, the discovery of previously overlooked populations on the Western Australian Coast has highlighted critical gaps in our knowledge of these species. I used thousands of nuclear SNPs to show that, in four of five codistributed *Aipysurus* species, deep genetic divergences separate coastal Western Australian from offshore Timor Sea populations. I used morphological and colour pattern traits to characterise differences in these lineages and found convergent changes towards larger and longer bodies and paler body colouration in the Western Australian Coast compared to the Timor Sea lineages. I suggested that these remarkably parallel transitions reflect adaptations relating to locomotion and crypsis in the deep soft sediment habitats of the Western Australian Coast versus the complex and shallow coral reefs of the Timor Sea. I recommended recognition of these historically isolated lineages as Evolutionary Significant Units (ESUs) for management purposes. These results also provide a valuable framework for investigating the roles of biogeography and adaptation in lineage diversification.

I also investigated lineage formation in the sister group to *Aipysurus*, *Emydocephalus,* using nuclear SNPs combined with morphological and ecological data. *Emydocephalus* is a small fish-egg eating specialist with a patchy geographic distribution in the Indo-West Pacific. Within this species, I found very similar morphological and biogeographic patterns to those that I uncovered in *Aipysurus*, but divergence was much greater and I described the coastal Western Australian population as a new species, *Emydocephalus orarius*. I also reconstructed a phylogeny of *Demansia*, the most species rich terrestrial elapid genus in Australia. I found that current species boundaries in the widespread *D. psammophis* group inadequately represented the lineage diversity in this clade, and described a morphologically distinct new species, *Demansia cyanochasma*, from the deserts of Southern Australia.

Finally, I investigated the evolution of the rapidly radiating *Hydrophis* group in a macroevolutionary context. I reconstructed a phylogeny using thousands of nuclear SNPs,

which provided substantially higher resolution of relationships than previous studies have produced using mitochondrial and low-variation nuclear sequences. For most species, I then used three ecologically relevant morphological characters combined with diet data to define five broad ecomorphs. Ancestral state reconstruction using these data showed that burrowing prey specialisations are the likely ancestral state for *Hydrophis*. I also uncovered a strong pattern of rapid ecomorphological diversification within major ocean basins, highlighting the clade as an important system for testing hypotheses of adaptive radiation.

This thesis provides a basis for understanding the process of lineage diversification, ranging from incipient speciation in *Aipysurus* through to the ~50 species radiation in *Hydrophis*. I integrated a combination of new molecular techniques with traditional museum-based data collection and worked with industry partners to improve sampling of molecular and ecological data. This integrative approach has provided a novel perspective on the evolution of the Australian elapid fauna.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree. The author acknowledges that copyright of published works contained within the thesis resides with the copyright holder(s) of those works. I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time. I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

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Contributions

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Authors: James H. Nankivell[1 2], Arne R. Rasmussen[7], Emma Sherratt[1 2], Claire Goiran[3], Kate L. Sanders[1 2]
Status: Unsubmitted, prepared in publication format.

Co-author affiliations

[1]School of Biological Sciences, University of Adelaide, Adelaide, Adelaide SA 5005, Australia
[2]South Australian Museum, North Terrace, Adelaide SA 5000, Australia
[3]Labex Corail & ISEA, Université de la Nouvelle-Calédonie, Nouméa, New Caledonia
[4]Department of Primary Industries and Regional Development, Perth, Western Australia, Perth
[5]Department of Biological Sciences, Macquarie University, NSW, Australia
[6]School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia
[7]The Royal Danish Academy of Fine Arts, Schools of Architecture, Design and Conservation (KADK), Copenhagen, Denmark
[8]Institute for Applied Ecology, University of Canberra, Canberra, ACT, Australia
[9]Research Associate, Collections & Research, Western Australian Museum, 49 Kew Street, Welshpool, Western Australia, 6106
[10]Snakes Harmful & Harmless, 9 Birch Place, Stoneville, Western Australia, 6081, Australia
[11]Faculty of Science & Engineering, Flinders University, Bedford Park, South Australia, 5042, Australia

Chapter 1
Introduction

Introduction

Investigating biological diversity: Species and species diversification

The diversity of life on earth is distributed unevenly. This is clear when comparing a relatively depauperate desert with the spectacular diversity of a lowland tropical rainforest (Wallace, 1878). This observation is equally apparent when comparing across the tree of life; for example, in Australia marsupials are far more diverse than monotremes but these groups have inhabited the same range of environments over a similar timeframe (Upham et al., 2019). Diversity is fundamentally controlled by rates of speciation minus extinction. In the tropics, for example, high diversity may due to long term stability and therefore low rates of extinction (Mortiz et al., 2000). Many of the examples that best encapsulate diversification and the factors that shape it are adaptive radiations, and for this reason it is no surprise that examples such as the Galapagos Finches inspired Darwin's theory of evolution (Darwin, 1859).

Adaptive radiations occur when a single lineage diversifies into a broad ecological niche space, often forming an assemblage of closely related sym- or parapatric species. This process is often accompanied by rapid species diversification, but not all rapid diversification events are adaptive (Givnish, 2015). Ecological opportunity and opening of new niche space is thought to play an integral role in this process and has been studied extensively in insular systems such as freshwater lakes and islands. In the absence of competition, the initial invasion of these environments is expected to trigger rapid speciation initially, which will then slow down as available niche space becomes saturated (the 'early burst' model) (Morlon et al., 2010). An alternate avenue to the opening up of ecological opportunity is the development of a certain trait or aspect of life history, which may leave an early burst phylogenetic signal similar to that of classic island radiations (Yoder et al., 2015; Givnish et al., 2015). For example, in East African Cichlids a combination of extrinsic (development of new rift lakes) and intrinsic factors (low dispersal, evolution of specialised pharyngeal jaw) have caused numerous parallel adaptive radiations (Seehausen, 2006). Additionally, aspects of genomic architecture and existing genetic diversity may precipitate rapid radiation in some groups by providing the genetic variation that underpins adaptations. Hybridisation among divergent lineages can further facilitate adaptation and speciation via the reassembly of existing genetic variation into novel combinations (Brawand et al., 2014; Meier et al., 2017).

Speciation is the evolution of reproductive isolation. A dominant mode of diversification therefore involves biogeographic processes that disrupt gene flow ('allopatric speciation'). This particularly prevalent in highly heterogeneous environments with high topographic or bathymetric complexity such as mountain ranges or islands (Smith et al., 2014). Shared biogeographic barriers generate and maintain diversity in many groups of organisms and allopatric speciation is one of the biggest contributors to overall diversity (Chesser and Zink, 1994). Biogeographic barriers may be effective at preventing contemporary gene flow, or may represent historical barriers that enable the accumulation of genetic incompatibilities (postzygotic isolation), which is then reinforced by pre-zygotic mechanisms such as assortative mating that further prevent gene flow (Schneider and Moritz, 1999). In large complex environments such as continental landmasses or ocean basins the drivers of diversity may be difficult to interpret due to the large number of interconnected causative factors. In some systems such as the East African cichlids speciation is both para/sympatric (due to both ecological and sexual selection) and allopatric, leading to prodigious rates of species accumulation (Kocher, 2004). The vast majority of studies on adaptive radiations occur in insular systems (e.g. islands, lakes, mountaintops). As noted in Soulebeau et al. (2015), studies on adaptive radiations within ocean basins are rare.

The typical unit measured in studies of diversification is the species, however definitions of what constitutes a species vary widely. Traditional definitions such as the biological species concept usually assume reproductive isolation as the primary indication of species boundaries (Mayr, 2000). However, these definitions are problematic for a variety of reasons such as speciation with gene flow, parthenogenic species and the practical difficulty in measuring reproductive isolation. Many alternative definitions exist, and the species definition typically used in herpetology is the general lineage concept (De Queiroz, 1998). The general lineage concept states that a species is a separately evolving metapopulation, and that many the previous definitions represent steps along a speciation continuum, however, reproductive isolation still remains a key determinant of species status (De Queiroz, 2007).

Elapid Snakes

Elapids are a diverse family (~400 species) (Uetz et al., 2023) of advanced snakes (Colubroidea) that are primarily defined by the presence of fixed front fangs (McCarthy, 1985). Throughout much of their range in Afro-Eurasia and South America elapids are familiar components of the snake fauna but generally make up a small fraction of the local species richness compared to other colubroid families. In these regions, elapids are broadly

either large generalists (i.e. *Naja*, *Dendroaspis*) or ophiophagous specialists (*Bungarus, Calliophis*, *Micrurus*). However, in Australia, elapids are the dominant component of the snake fauna, particularly in the temperate and arid zones (Cogger, 2014). The Australo-Papuan elapid radiation (Hydrophiinae) makes up approximately half of all elapids with 206 described species with an origin 25 Mya (Lee et al., 2016). Hydrophiines in Australia fill many of the ecological niches that are filled by other colubroid snakes elsewhere. These include active diurnal predators (*Demansia*), sit-and-wait ambush predators (*Acanthophis*), small fossorial predators (*Neelaps*, *Simoselaps*), and large generalists similar to cobras elsewhere (*Notechis, Pseudechis, Pseudonaja*). Among the Hydrophiines, viviparity arose repeatedly, likely as an adaption to inhabiting the cooler climates of southern Australia (Shine, 1987; Sanders et al., 2008). The sister group to the Hydrophiinae are the amphibious marine sea kraits (*Laticauda*) that are found throughout the Indo-West Pacific including New Guinea, although curiously not modern Australia (Heatwole et al., 2017). While it was generally assumed that *Laticauda* and Hydrophiinae share a common terrestrial ancestor, recent research examining the marine origin of horizontally transferred transposable genetic elements (Galbraith et al., 2022) suggests that this ancestor may have already transitioned to the marine environment. A marine ancestor followed by a secondary reversal back to terrestrial environments may explain the early arrival of elapids in Australia (as opposed to various other colubroid snake families that arrived later and are represented by fewer species). Despite an early arrival in Australia and subsequent expansion into greater ecomorphological space, speciation rates among terrestrial elapids in Australia are not significantly higher than the background rates in Afro-Asian and New World elapids (Lee et al., 2016).

Within the Hydrophiinae are the viviparous sea snakes, the only extant group of fully marine reptiles as well as the most diverse, with approximately 60 recognised species (Rasmussen *et al.* 2011b; Uetz et al., 2023). This transition from the terrestrial to marine environment has led to a wide suite of evolutionary traits related to survival in the marine environment including paddle-shaped tails, cutaneous respiration, and valved nostrils among other adaptations (Heatwole 1978; Brischoux and Shine 2011; Palci et al., 2019). The sensory systems of sea snakes have also diverged from those of their terrestrial counterparts: the visual pigments of many sea snakes have spectral sensitivities that are shifted towards longer wavelengths (Simões et al., 2020), and some species have evolved an entirely novel ability to withdraw their vulnerable tail paddle in response to light (Crowe-Riddell et al., 2019).

The two main clades within the viviparous sea snakes are the *Aipysurus* (*Aipysurus* and *Emydocephalus*) and *Hydrophis* (*Ephalophis*, *Hydrelaps*, *Hydrophis*, *Microcephalophis* and *Parahydrophis*) groups. Despite superficial similarities, before molecular analysis became available it remained unclear whether the *Hydrophis* and *Aipysurus* groups were monophyletic (Smith, 1926; Rasmussen, 2002, but see McDowell, 1972). While the *Aipysurus* and *Hydrophis* groups are now known to be sister groups, many of their morphological marine adaptations suggest that they likely had an amphibous marine ancestor, fully marine habits evolved independently in the two groups (Rasmussen, 2002; Sanders et al., 2012).

Invasion of novel habitats and geographical regions is often associated with rapid adaptive radiations as species diversify in response to new ecological opportunities. However, in sea snakes the initial invasion of marine habitats is not correlated with a rapid increase in speciation rates (Lee et al., 2016). Both the fully marine *Aipysurus* group (12 species) and the three monotypic semi-aquatic genera (*Ephalophis*, *Hydrelaps* and *Parahydrophis*) have speciation rates similar to background rates in Hydrophiinae. In contrast, the nested *Hydrophis* clade has speciation rates three-fold faster than other elapids with ~50 species arising in as little as 3 Mya. This rapid speciation is also accompanied by extreme ecomorphological diversity and the evolution highly specialised forms with up to ~25 species with overlapping ranges in Southeast Asia (Voris, 1977; Elfes et al., 2013). In contrast, the *Aipysurus* group is relatively conservative in morphology and the majority of species are restricted to the Australian region.

The striking contrast between *Hydrophis* and *Aipysurus* in both speciation rates and ecomorphological diversity cannot easily be explained by the classic adaptive radiation model of invasion into a new habitat. Both species groups broadly overlap in geographic distribution, and current phylogenies suggest the *Aipysurus* group invaded fully marine habitats around the same time as *Hydrophis* (Sanders et al., 2013). An alternative model for an adaptive radiation is the key innovation hypothesis, where a phenotypic innovation arises that allows a lineage to escape from previous evolutionary constraints (Simpson, 1953; Schluter, 2000). Both *Aipysurus* and *Hydrophis* are superbly adapted to marine environments, and existing evidence indicates that they are comparable in traits such as swimming efficiency, osmoregulation and sensory acuity (Rasmussen et al., 2011). The most striking difference between the groups is the increased morphological diversity in *Hydrophis*, particularly the convergent evolution of microcephalic species which has not occurred in *Aipysurus*. Microcephalic sea snakes are defined not just by small heads, but by slender forebodies that are frequently only a third of the circumference of the hindbody,

and dietary data suggests that they use this unusual morphology to feed on eels in their burrows (Voris and Voris, 1983).

Sherratt et al., (2018) showed that in most species microcephaly is achieved by substantially increasing the number of vertebrae and as a result the number of vertebrae in *Hydrophis,* particularly in the forebody, is extraordinarily variable compared to *Aipysurus*. In microcephalic sea snakes there is an increase in number of small pre-atrial vertebrae that lead the forebody and head being small and highly mobile, which probably allows greater access to burrowing prey (Sherratt et al., 2022). The microcephalic *Hydrophis* species which feed exclusively on burrowing eels have been relatively well characterised, but so far less is known about other ecomorphological groupings. Previous research on microcephalic sea snakes has generated an abundance of morphological data (Sherratt et al., 2019; Sherratt et al., 2020) and dietary data is available for many species (Voris and Voris, 1983; Fry et al., 2001); however, the lack of a well resolved phylogeny currently hinders understanding of ecomorphological diversification in the group.

Relevance for Conservation

Sea snakes have a turbulent taxonomic history, particularly those now in the genus *Hydrophis*, with up to 16 genera and subgenera previously recognised for the ~50 species (reviewed by Rasmussen, 1997). Identification of species has often proved challenging and many of the resources for doing so are inaccurate or require use of difficult and unusual characters such as counts of maxillary teeth (Rasmussen et al., 2014). Species boundaries are often poorly defined (Ukuwela et al., 2013) and sea snakes are frequently misidentified (e.g. Kim et al., 2016; Weinstein et al., 2021; Speed et al., 2022). In the rapidly radiating *Hydrophis* sea snakes, single marker sanger sequencing is frequently inadequate for untangling species boundaries, in part due to incomplete lineage sorting or mitochondrial capture (Sanders et al., 2013).

High throughput sequencing molecular methods are a useful and affordable tool for accurately determining species boundaries with far more power than single locus sanger sequencing. Methods such as double-digest RADseq allow for the generation of thousands of unlinked SNPs in non-model species and have rapidly been taken up as an important tool in speciation biology and population genetics (Peterson et al., 2012). This suite of methods is particularly useful in rapid radiations such as sea snakes where short intervals between cladogenesis can confound analyses using longer more conserved genomic regions.

Sea snakes have undergone severe declines in parts of their range, particularly in their former strongholds in the Timor Sea. Complete collapse and local extinctions of nearly all resident species on Ashmore Reef was recorded in the early 2000s, including two species which at the time were only known from the region and were feared extinct (Guinea, 2006; Guinea, 2013; Lukoschek et al., 2013). Similar although less dramatic declines have been recorded at other Timor Sea reefs, as well as on the Swain Reefs in Southern Queensland (Lukocheck et al., 2007). Disturbingly the cause for these declines is still unclear despite being in isolated locations where human impact is likely low (Somaweera et al., 2021). Recent surveys have found small numbers of sea snakes in the mesophotic zone of Ashmore Reef, but the population likely remains substantially lower than before crash in the early 2000s (Speed et al., 2022). Sea snakes are also frequently caught by prawn trawlers as bycatch in Northern Australia and surveys show that for some species this fishing pressure is potentially unsustainable (Milton et al., 2008). In Southeast Asia, particularly Vietnam and China, sea snakes are deliberately targeted as a food source from the Gulf of Thailand (Cao et al., 2014). This trade is essentially unmonitored, and the most recent survey was conducted in 2010-2012 (Cao et al., 2014). Anecdotal reports (Nankivell & Rossetto pers. obs. 2022) suggest that the trade has collapsed in recent years but it is unclear whether this is due to population declines or socio-economic market factors. Sea snakes are frequent bycatch in other parts of the Indo-West Pacific and are a common hazard for artisanal fishers (Rao et al., 2021).

Monitoring of potentially threatened sea snakes is contingent on accurate species identification, as in most cases a whole slew of species may be caught in the same fishery. Much of the survey data collected for Australian fisheries is from non-specialist crew member observers, who struggle to identify bycatch from many different taxa. Identification of sea snakes is reliant on material that is often outdated and there is frequent mismatch between trawl data collected by crew member observers and that collected by specialists on research trawls (Hourston pers. comm.). Sea snakes have variable life history traits and this leads to variable responses to threats such as trawling and climate change (Fry et al., 2001; Milton, 2001). More reliable identification materials are required to generate a better understanding of the status of the world's sea snakes, however for this taxonomy must be accurate and useable by non-specialists. Even researchers frequently misidentify sea snakes, and this has potential consequences for understanding population health and trends of individual species (Guinea, 2022).

Broad Aims

The overarching aim of this thesis was to investigate patterns of diversity and diversification in Australian elapid snakes. My first broad aim was to uncover hidden lineage and species diversity within the terrestrial and marine elapid radiations, which is vital not only for answering blue skies evolutionary questions but also for providing a scientific basis for conservation and environmental management in a changing world. My second major aim was to explore the roles that ecological and geographic factors have played in shaping sea snake diversity at both population and macroevolutionary scales. I addressed these aims using a comparative phylogenetic framework and by combining new and existing molecular, morphological, geographic and ecological data.

1) Uncovering hidden diversity in Australian elapids

Despite being a familiar and conspicuous part of the Australian fauna, new species of elapid continue to be discovered and described (Derez et al., 2018; Maryan et al., 2020). Sea snakes remain relatively poorly known taxonomically and are frequently misidentified even by specialists (Guinea 2022). This issue is partly due to poorly defined species boundaries and operational difficulties in applying the characters used to distinguish sea snakes. Correct identification is vital for management and conservation. Sea snakes have become a particular priority for conservation managers because they have been impacted by regional extinctions and are a conspicuous component of bycatch in non-selective trawl fisheries. High-throughout sequencing techniques now allow more accurate species boundaries to be determined which have previously proved intractable using sanger sequencing and morphology alone.

I integrated nuclear SNP and mitochondrial datasets combined with morphology to investigate species boundaries in the terrestrial elapid genus *Demansia* and the sea snake *Emydocephalus*. This resulted in published descriptions of two new species. Additionally, I defined geographically concordant Evolutionary Significant Units in a suite of four codistributed and regionally threatened *Aipysurus* species.

2) Investigating patterns and processes of diversification in marine elapids

In Hydrophiinae there are two obvious events that provide ecological opportunity, firstly the original colonisation of the colubroid free Australian landmass and secondly the later marine colonisation with a the pre adaptation of live bearing young. Yet phylogenetic data does not support either of these events leading to rapid diversification, instead the major rapid diversification occurs in *Hydrophis* sea snakes, and this occurred several million years after the invasion of fully marine habitats. Determining the geographical setting of the *Hydrophis*

radiation is currently limited by poor phylogenetic resolution and a lack of ecomorphological characterisation.

To address this, I used thousands of SNPs to generate a well-resolved phylogeny for ~70% of *Hydrophis* species. Additionally, I used to a combination of morphological and life history traits to define ecomorphs and used trait mapping and ancestral reconstruction to understand their evolution. I reconstructed the biogeographical history of history across the major ocean basis of the Indo-West Pacific.

Thesis Outline

Chapter 2

Molecular and morphological data identify geographically concordant ESUs in endangered *Aipysurus* sea snakes

I analysed genome-wide SNPs and ecomorphological traits to define units for the conservation management of *Aipysurus* sea snakes. This group contains nine fully-marine species distributed primarily in the northern Australian region, including two critically endangered listed and one endangered listed species on Australia's EPBC Act 1999. Our results show convergent patterns of genetic and morphological divergence between coastal and offshore populations in four (of five) co-distributed *Aipysurus* lineages. Coastal populations in all four lineages have longer bodies, higher vertebrate counts, and lighter colour patterns compared to offshore-reef populations. This evidence of historical isolation and divergent adaptive evolution underpins our recommendation that coastal and offshore-reef populations of *A. duboisii*, *A. apraefrontalis* and *A. foliosquama* be recognised as ESUs, while the current taxonomy (including the species status of *fuscus* and *A. tenuis*) be retained.

Chapter 3

A new species of turtle-headed sea snake (Emydocephalus: Elapidae) endemic to Western Australia

I described a new species of turtle-headed sea snake *Emydocephalus orarius* sp. nov. (Elapidae) from Western Australia's Coral Coast, Pilbara and Kimberley regions. Phylogenetic analysis of mitochondrial markers places the new species as the sister lineage to the two currently recognised species in *Emydocephalus*: *E. annulatus* from the Timor Sea reefs and Coral Sea, and *E. ijimae* from the Ryukyu Islands. Analysis of nuclear SNP data from the new species and *E. annulatus* from Australia and New Caledonia provides additional independent evidence of their evolutionary distinctiveness. The new taxon appears to reach greater total lengths (> 1 m in the new species versus typically~ 80

cm in *E. annulatus*/*E. ijimae*). The new species is known largely from soft-bottomed trawl grounds, unlike *E. annulatus* and *E. ijimae* which usually inhabit coral reefs. The discovery of this new species brings the number of sea snake species endemic to Western Australia to six. This chapter was developed in my honours but new samples were added to the molecular and morphological datasets, as well as significant reanalysis rewriting to prepare the chapter for publication.

Chapter 4

Whip it into shape: Revision of the *Demansia psammophis* (Schelgel, 1837) complex (Squamata: Elapidae), with a description of a new species from central Australia

I used a combination of mitochondrial and SNP data to reconstruct the phylogenetic tree of *Demansia.* I intensively sampled *Demansia psammophis* group, which has been recognised as problematic for a significant amount time, across the Northern Territory, South Australia and Western Australia. These data revealed a hitherto undescribed species of *Demansia* with a vast range across the central deserts of Australia. Extensive morphological data showed that the new species was morphologically diagnosable using colour pattern characteristics from congeners. I also placed the subspecies *Demansia reticulata cupreiceps* into junior synonymy of *D. reticulata*.

Chapter 5

SNP-based analyses reveal phylogenetic relationships and ecomorph evolution in rapidly radiating *Hydrophis* sea snakes

I used thousands of nuclear SNPs to reconstruct the *Hydrophis* phylogeny and resolve the previously intractable backbone. Strong concordant support was found across most of the tree and new relationships were uncovered between clades. I used ABBA-BABA to and found two candidate introgression events between non-sister lineages. I built a framework to for identifying ecomorphs in *Hydrophis* and assigned most species to one of five broad ecomorphological groupings using morphological and dietary data. I used likelihood and Bayesian methods to reconstruct the ancestral ecomorphs and geographical range of *Hydrophis* sea snakes with much greater fidelity than was possible previously.

Chapter 6

Thesis summary and discussion

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By signing the Statement of Authorship, each author certifies that:

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Chapter 2

Molecular and morphological data identify geographically concordant ESUs in endangered *Aipysurus* sea snakes

Molecular and morphological data identify geographically concordant ESUs in endangered *Aipysurus* sea snakes

James Nankivell, Kate Sanders

Abstract

Identifying genetically and ecologically distinct populations (evolutionarily significant units, ESUs) is a key focus of efforts to conserve species' potential to respond to environmental change. We analysed genome-wide SNPs and ecomorphological traits to define units for the conservation management of *Aipysurus* sea snakes. This group contains nine fully-marine taxa distributed primarily in the northern Australian region, including two critically endangered listed and one endangered listed species on Australia's EPBC Act 1999. Our results show convergent patterns of genetic and morphological divergence between coastal and offshore populations in four (of five) co-distributed *Aipysurus* lineages. In *A. apraefrontalis*, *A. duboisii*, *A. foliosquama*, and the *A. fuscus-A. tenuis* complex, individuals from the Western Australian Coast and remote Timor Sea reefs form genetically distinct genetic clusters with gene-tree based divergence estimates of five hundred to seven hundred thousand years. Genetic patterns are matched by convergent differences in morphology. Coastal populations in all four lineages have longer bodies, higher vertebrate counts, and lighter colour patterns compared to offshore-reef populations. This evidence of historical isolation and divergent adaptive evolution underpins our recommendation that coastal and offshore-reef populations of *A. duboisii*, *A. apraefrontalis* and *A. foliosquama* be recognised as ESUs, while the current taxonomy (including the species status of *A. fuscus* and *A. tenuis*) be retained. In *A. laevis* (our fifth co-distributed lineage), body length and colour pattern variation mirrored the coastal-offshore divisions seen in the other *Aipysurus*, but this species showed different patterns of geographic genetic structure that are incongruent with current subspecies boundaries. Finally, the remarkable convergence in most species' diversity patterns implicates shared geographic and ecological mechanisms of lineage formation. We suggest that biogeographic isolation has facilitated local adaptation related to locomotory performance and crypsis in open soft-bottomed coastal habitats versus structurally complex reefs.

Introduction

Identifying genetically and ecomorphologically divergent populations within species provides valuable information for biodiversity conservation. Where such studies uncover common patterns of diversity among species, this is an important step in understanding how geographic and ecological factors shape speciation.

The nine species (Uetz et al., 2023) of *Aipysurus* sea snakes are highly marine adapted and often thought to be primarily coral reef dwellers (Cogger, 2014). They are primarily Australian in distribution with eight out of nine taxa found in the waters surrounding the continent, of which five are endemic to Western Australia and the Timor Sea reefs of the North-west Shelf. Two of these species (*A. apraefrontalis* and *A. foliosquama*) were only known from Ashmore and Cartier Reefs, however unexplained and rapid declines led to their extinction from their entire known distribution by 2006 (Guinea, 2006; Guinea, 2012, Lukoschek et al., 2013). Examination of museum material and further collecting on Western Australian Coast showed that these species have a significantly larger distribution and were not extinct as previously feared (Smith, 1974; Sanders et al., 2015; D'Anastasi et al., 2016). These species are listed as Data Deficient on the IUCN red list and critically endangered by Australia's EPBC Act 1999. The remaining Timor Sea endemic, *Aipysurus fuscus*, is more widespread on various reefs in the Timor Sea but declines and contemporary hybridisation with the more common *A. laevis* (Sanders et al., 2014) have led to its listing as endangered. The Shark Bay endemic *A. laevis pooleorum* was described as subspecies of *A. laevis* (Smith, 1974) although it was raised without justification to full species by Wells and Wellington (1983) and is listed as Data Deficient on the IUCN red list.

Previous analysis of mitochondrial markers (Nitschke et al., 2018) showed significant population structure between Western Australian Coast (Western Australian Coast) and Timor Sea lineages (Timor Sea) in all species of *Aipysurus* examined. Timor Sea populations were closer to those in the east coast of Australia than the geographically proximate Western Australian Coast region. The estimated divergence time was significantly deeper in closely related *Emydocephalus* which led to the description of a new species based on morphological and molecular characters (Nankivell et al., 2020). The new species, *Emydocephalus orarius,* was distinguished by its significantly greater size and a complex colour pattern that was never

melanistic, unlike *E. annulatus* which is frequently melanistic across Australia and New Caledonia (Goiran et al., 2017).

The North-west coast of Australia is a vast and shallowly sloping region of continental shelf between Exmouth and the Bonaparte Gulf. The region is divided into 11 marine bioregions from west to east: Ningaloo, Pilbara (Nearshore and Inshore), Eighty Mile Beach, Canning, Kings Sound, Kimberley and Bonaparte Gulf with the Northwest Shelf and Oceanic Shoals bordering these regions further offshore. The border between the Canning and Kimberley basins lies approximately north of Cape Leveque. There is no contemporary deep-water barrier between these basins to limit dispersal for shallow water taxa (Wilson, 2013); however, the extreme tidal regime in the region, and the outflow from King's Sound, likely plays an important role in preventing gene flow between taxa on either side (Travers et al., 2010; West et al., 2021).

The strength of this barrier varies considerably among taxa, with life history traits associated with dispersal and site attachment likely influencing the degree of population structure (Berry et al., 2017; DiBattista et al., 2017). The geomorphology and therefore breadth of available habitats differ considerably between the Canning and Kimberley. The Canning basin is underlain by Cretaceous sedimentary rocks and gently slopes towards the continental shelf with relatively featureless flats of soft sediment (Brocx and Semeniuk, 2010; Wilson, 2013). No large permanent rivers or estuaries drain into the Canning Basin meaning sediment outflow and turbidity is low. The Kimberley basin has a more complex geomorphology, with older proterozoic metamorphic structures that have been subjected to extensive periods of terrestrial erosion (Wilson, 2014). This is reflected in the coastline with complex rias, and many offshore islands that represent high points in the landscape during lower sea level stands. These hard rocky surfaces provide anchoring for extensive coral reef communities throughout the coastal Kimberley (Tiechert and Fairbridge, 1948; Richards et al., 2018). Some of the largest tides in the world are present in this region, with tidal scouring and strong currents remodelling the seafloor. In addition, rainfall is significantly higher in the Kimberley and large drainages such as the Fitzroy River dump great quantities of sediment into the coastal regions, leaving it rich in nutrients with high turbidity. Offshore of the Kimberley, tides and tidal outflow are significantly lower but the underlying geomorphology remains complex. In deep regions soft sediments predominate but along the edge of the shelf banks rise subaerially to within ~20 metres of the surface, most of these are dominated by

Halimeda. However, the largest of these banks are formed of coral and large reefs around low islands on the Ashmore platform are present (Gunn, 1988; Wilson, 2013).

In fish contrasting habitat requirements between reef and open water habitats have driven changes in body shape. In coral reef fishes a less elongate and deeper body is favoured as this improves ability to manoeuvre and accelerate quickly in a cluttered environment (Larouche et al., 2020). In contrast fishes found in open habitats tend to be elongated with a thinner body width to improve swimming efficiency over extended distances. This is frequently described as fineness, which is the ratio between body length and body width (Ahlborn et al., 2009). Sea snakes are subjected to similar environmental and hydrodynamic pressures as fishes but their response to these have not been previously investigated in an evolutionary context. *Aipysurus* sea snakes in Western Australia offer an ideal system to investigate this relationship due to the parallel population structure between coral reef dwelling Timor Sea populations and the (soft bottom) dwelling populations off the Western Australian Coast for five species groups.

Colour pattern is important in sea snakes for predator avoidance through misdirection, crypsis and aposematism (Rasmussen and Elmberg, 2009; Goiran et al., 2022; Shine et al., 2022). Industrial melanism has been cited as the primary driver of melanic *Emydocephalus* in heavy metal rich waters in New Caledonia (Goiran et al., 2017), however melanic snakes are present in relatively pristine offshore reefs in Australia. An alternative hypothesis is that non-mimetic (eg. melanic in New Caledonia *Emydocephalus*) colour is primarily driven by crypsis and colour matching the environment, such as in shadowed coral crevices. Numerous reef fishes have multiple stable colour pattern morphs, with different distinct preferences related to background colour matching, although other factors such as sexual selection are undoubtedly at play (Cortesi et al., 2017; Whitney et al., 2018).

Here we aimed to combine SNPs and morphology to define ESUs for conservation. We analysed thousands of SNPs from 54 individuals using phylogenetic and clustering methods. We measured a small number of ecologically relevant morphological traits from 201 specimens and used photographs of specimens and live individuals to characterise colour patterns.

Methods

Sampling

Tail and liver samples were obtained for 54 individuals of 8 *Aipysurus* species. Samples were gathered from museum collections (Museum and Art Gallery of the

Northern Territory (MAGNT), South Australian Museum (SAMA) and Western Australian Museum (WAM). For phylogenetic analysis we used one sample from each population identified in Nitschke et al., (2018) and Lukoschek (2018). The phylogenetic dataset contained sixteen samples from seven described *Aipysurus* species with just *A. eydouxii* missing, as well as a sample each for *Emydocephalus annulatus* and *Emydocephalus orarius* for use as outgroups. For population-level analyses, we targeted five species groups, three of which are endemic to Western Australia and the Timor Sea (*A. apraefrontalis*, *A. foliosquama* and a combined *A. fuscus*/*A. tenuis* dataset) and two are widespread from Australia to New Caledonia (*A. duboisii and A. laevis*).

Tissues were extracted using a salting out method and quantified using a Quantus fluorometer to obtain >500ng of DNA at concentrations of 50ng/ml. Samples were concentrated using a Centrivap DNA concentrator if required and checked for quality using gel electrophoresis. Samples were sent to Diversity Arrays Technology Pty Ltd (DArTseq, Canberra, Australia) where SNP genotyping was conducted using a proprietary genome complexity reduction pipeline using a pair of restriction enzymes (PstI and HpaII) (Kilian *et al.* 2012; Georges *et al.* 2018). After initial digestion/ligation reactions and amplification samples are sequenced on an Illumina Hiseq 2500.

We obtained raw demultiplexed reads from DArTseq which we checked for quality using FASTQC (Andrews, 2010) and then filtered out adaptors and quality trimmed using BBduk (Bushnell, 2014). We called SNPs using iPYRAD v.0.9.85 (Eaton and Overcast, 2020) run on the University of Adelaide Phoenix HPC. Filtered and demultiplexed reads were assembled de novo, setting the cluster threshold to 0.85, mindepth (statistical and majrule) to 5 and maxdepth to 10000. For the phylogenetic analysis the min samples per locus was set to 2 in order to maximise SNPs called, as overly stringent filtering can cause serious allelic dropout at deeper phylogenetic scales (Eaton et al., 2017). The phylogenetic SNP dataset contained 33888 SNPs with 54.31% missing data, with no samples containing greater than 66% missing data. For the species level datasets, we applied a more stringent filter, aiming for min samples per locus to be close to 90% for each SNP dataset. For *A. apraefrontalis* we obtained 6124 SNPs (0.28% missing, min samples per locus 3), *A. duboisii* we obtained 3042 SNPs (9.31% missing, min samples per locus 15), *A. foliosquama* we obtained 883 SNPs (0.23% miss, min samples per locus 3), *A.*

fuscus/tenuis we obtained 1352 SNPs (0.18% miss, min samples per locus 4), *A. laevis* we obtained 9302 SNPs (5.16 miss, min samples per locus 22).

For phylogenetic tree reconstruction we used ModelFinder (Kalyaanamoorthy et al*.,* 2017) implemented in IQTree v. 2.0.3 (Nguyen *et al.* 2015) to select the best fit codon partitioning scheme of TVM+F using Bayesian Information Criterion (BIC). Maximum likelihood tree reconstruction was implemented in IQTree and support was assessed using 1000 ultrafast bootstrap replicates. The SNP tree was rooted using the *Emydocephalus annulatus* and *E. orarius* lineage, as previous work has recovered this as the well supported sister to *Aipysurus* (Sanders et al., 2008), and visualised in Figtree v.1.4.4 (Rambaut, 2012). We used the chronotree function in phytools (Revell et al., 2012) to date the phylogenetic tree using the secondary calibration of 9 Mya for the root node (i.e. *Emydocephalus* versus *Aipysurus*) (Lee et al., 2016).

As an exploratory step we generated Principal Component Analysis (PCA) plots using the SNP data for each species and species complex separately. We generated the plots using the iPYRAD v.0.9.85 python pipeline with specimens assigned to populations found in Nitschke et al., (2018), with SNPs kept if they're present in greater than 75% of populations using the *mincov* option. We used the *sample* option to impute missing data based on the allele frequencies of populations defined a priori. We randomly subsampled unlinked SNPs, running 25 replicates.

Population structure was evaluated using the Bayesian approach implemented in Structure 2.3.4 (Pritchard et al. 2000) for large SNP genotype data. We used the species level datasets for each species of *Aipysurus*. Based on a model of allele frequencies under Hardy-Weinberg equilibrium (HWE) and assuming linkage disequilibrium, Structure estimates the probability that each individual belongs to an ancestral cluster (K), recording posterior probability distributions for admixture proportion (Q) in each cluster. For each dataset, Structure was rerun for 10 seeds ranging from K=1 to K=10 under the admixture model with independent allele frequences. Each run used a burn-in step of 50,000 followed by 50,000 Markov Chain Monte Carlo (MCMC) iterations. The best supported number of ancestral clusters (K) was determined by examining posterior probability distributions and using log-likelihood ratio tests (Pritchard et al. 2000). Runs for optimum K were then summarised using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and Q values were plotted. All analyses were run in the ipyrad analysis toolkit.

Very small sample sizes (n = 1 to 3 per population) were used for population genetic analyses of *A. apraefrontali*s, *A. foliosquama* and *A. fuscus-A. tenuis*. Simulation studies have shown that when large numbers of SNPs are used, population genetic estimates such as Fst can be calculated from as few as two individuals (Nazareno et al., 2017; Qu et al., 2019). In this paper, however, we limit our analyses to differentiating populations of divergent lineages (Figure 2) based on similarities in allele frequencies across large numbers of SNPs. For the three species pairs with five or less individuals we used a simple measure of SNP distances to compare geographic lineages (Tables 1 – 3).

Table 1 – Matrix of SNP distances between *A. apraefrontalis* samples, lower left are raw SNP distances while top right are percentage of SNP distances to total SNPs.

	Aaprae4_12_01 (Timor Sea)	**KLS0834 (Western Australian Coast)**	**KLS1001 (Western Australian Coast)**	**SS171013-03 (Western Australian Coast)**
Aaprae4_12_01 (Timor Sea)	N/A	12.90%	11.85%	12.02%
KLS0834 (Western Australian Coast)	790	N/A	7.50%	7.51%
KLS1001 (Western Australian Coast	726	459	N/A	7.64%
SS171013-03 (Western Australian Coast)	736	460	468	N/A

Table 2 – Matrix of SNP distances between *A. foliosquama* samples, lower left are raw SNP distances while top right are percentage of SNP distances to total SNPs.

	Afo1 (Timor Sea)	**Afo8 (Timor Sea)**	**SS171014-02 (Western Australian Coast)**
Afo1 (Timor Sea)	N/A	6.34%	15.86%
Afo8 (Timor Sea)	56	N/A	18.12%
SS171014-02 (Western Australian Coast)	140	160	N/A

Table 3 – Matrix of SNP distances between *A. fuscus/tenuis* samples, lower left are raw SNP distances while top right is percentage of SNP distances to total SNPs.

	HybFa01 (tenuis)	**Afu2 (fuscus)**	**SAMA R68141 (fuscus)**	**VU140 (fuscus)**
HybFa01 (tenuis)	N/A	18.12%	17.31%	14.42%
Afu2 (fuscus)	245	N/A	8.88%	7.32%
SAMA R68141 (fuscus)	234	120	N/A	6.73%
VU140 (fuscus)	195	99	91	N/A

Morphological analysis

We gathered morphological data from 201 sea snakes in the genus *Aipysurus* from seven nominal species from localities in the North-west of Australia from Shark Bay to the Timor Sea. Specimens were identified to species using the key in Rasmussen et al., (2014). Specimens were held in the following museum: Australian Museum (AMS); Museum and Art Gallery of the Northern Territory (MAGNT); South Australian Museum (SAMA); Western Australian Museum (WAM). We collected data for seven ecologically relevant morphological characters: Ventral Scale counts; Snout to Vent Length (SVL); Tail Length (TailL); Forebody circumference (Fore); Hindbody Circumference (Hind); Head Length (HeadL); Head Width (HeadW).

We pooled sexes and scaled morphometric measurements by SVL to get relative measurements. We analysed morphological characters within each species to measure variation between populations. Firstly, to measure whether increased number of vertebrae was correlated with increased body size we plotted SVL against vertebrae. Secondly to determine whether an increase in vertebrae was positively correlated with an increase in fineness we plotted hindbody width against vertebrae. We divided SVL by hindbody to measure fineness, although we note as we measured the circumference of the body rather than the point-to-point width it is not directly comparable to fineness. We measured the circumference rather than width because formalin fixed sea snakes usually lose their laterally compressed shape due to gravity, hence body width would give widely varying values. To visualise the datasets, we plotted them using ggplot2 (Wickham, 2022). Guided by the phylogenetic dataset, we analysed *A. fuscus* and *A. tenuis* together as well as *A. l. laevis* and *A. l. pooleorum.*

We used photographs from museum specimens and field-caught individuals to assess colour patterns. We used the same three broad colour pattern morphs used by Shine et al., (2022) for *Emydocephalus* with a modified approach to scoring: melanic, intermediate and high contrast banded/light. To determine colour pattern morph we counted the ratio of light to dark scales across a single unit of bands at midbody. We used the midlateral scale row, for example in a snake with 19 midbody scale rows we would count scales at row 5. We counted scales midlaterally as most *Aipysurus* sea snakes are counter shaded with a dark dorsum and pale venter although there are exceptions. The 'melanic' morph was defined as those which were monochromatically dark with no obvious bands or pattern, 'intermediate' were those with a low contrast pattern or a ratio of dark:light scales >0.5 and 'light' were defined by a ratio of dark:light scales <0.5. The same observer (JHN) scored each specimen. Bar plots for colour pattern were produced in ggplot2 (Wickham, 2022).

Results

Molecular analysis

The final alignment contained 33388 SNPs with 54.31% missing data. The phylogenetic tree topology had largely strong support with bootstrap values of 100% for most nodes excluding two nodes among *A. laevis* which had support values between 70% and 85% (Figure 1). At the species level the topology of our SNP tree was consistent with the mitochondrial tree previously recovered by Nitschke et al., (2018). We obtained conflicting results to those of Lukoschek et al., (2018) in which A. tenuis samples are misidentified as A.fuscus, and Western Australian Coast A. laevis are misidentified as A. tenuis (D'Anastasi, in prep.).

Within *Aipysurus, A. mosaicus* was the well supported sister to the remaining species, which were divided into two clades. First, the *Aipysurus laevis* clade consisted of *A. laevis, A. fuscus, A. pooleorum* and *A. tenuis*, with a close sister relationship between the allopatrically distributed *A. fuscus* and *A. tenuis*. We recovered the Timor Sea population as sister to the remaining populations, however support was equivocal for this relationship (BS = 85%). We found *A. pooleorum* was nested within *A. laevis* as sister to the Western Australian Coast population, while the New Caledonian population was recovered as well supported sister to the east coast population. The other clade consisted of *Aipysurus duboisii* versus *A. apraefrontalis* plus *A. foliosquama*. The relationships among internal branches of *Aipysurus duboisii* were consistent with previous phylogenetic trees, with the Western Australian Coast population as sister to the remaining populations. The New Caledonian population

was recovered as sister to the east coast population represented by a specimen from the Gulf of Carpentaria (MAGNT R36638).

PCAs showed similar patterns of divergence in all three of the Timor Sea/Western Australian endemics with a clear separation on PC0 (51.6% in *A. apraefrontalis*; 65.7% in *A. foliosquama*; 56.1% in *A. fuscus*/*tenuis*) and then PC1 explaining variation between individuals within the population with the most individuals (25.1% in *A. apraefrontalis*; 34.3% in *A. foliosquama*; 24.0% in *A. fuscus*/*tenuis*) (Figure 2). In *A. duboisii* PC0 explained 29.1% of the variation largely separating the Western Australian Coast population from others, while PC1 explained 10.7% of variation separated out the Arafura, New Caledonia and Timor Sea populations, with Arafura and New Caledonia in close proximity. In *A. laevis* PC0 explained 17.7% of the variation with New Caledonia on one end and then a combination of Western Australian Coast, Shark Bay and Timor Sea on the other with Arafura in the middle. PC1 explained 8.1% of variation and primarily separated Arafura out from all other populations.

Structure runs of the well sampled populations revealed contrasting patterns of inferred ancestry. In *A. duboisii* the evanno method supported K=3, with the three clusters consisting of Western Australian Coast, Timor Sea and East Coast/New Caledonia. The Timor Sea population was inferred as partially admixed, although sample size was only two. *A. laevis* was also recovered with K=3 but with contrasting geographic patterns of ancestry to *A. duboisii*. All geographic populations of *A. laevis* had Q > 0.5 for one of the inferred ancestral populations, which had the high Q value in a largely homogenous Western Australian Coast/Timor Sea/Shark Bay cluster. New Caledonia and Eastern Australia had similar patterns of inferred ancestry but differed in the degree of admixture. The remaining three species all had similar patterns of inferred ancestry with separate Western Australian Coast and Timor Sea clusters, although sample sizes were low for all species. SNP distances within populations for the three NW Australia endemic *Aipysurus* were all between six and eight percent. The between population SNP distances ranged from approximately 12% in *A. apraefrontalis* through to 18% in *A. foliosquama* as well as between *A. fuscus* and *A. tenuis*.

Morphological Analysis

We sampled 25 *Aipysurus apraefrontalis* for morphological data, with 11 from the Timor Sea and 14 from the Western Australian Coast. *A. apraefrontalis* from the

Timor Sea had a lower SVL (345–810) than those from the Western Australian Coast (670–1050) and lower but just overlapping vertebral counts (139–152 vs. 147–157). The Western Australian Coast population of *A. apraefrontalis* had a marginally higher fineness value than the Timor Sea (9.65 vs. 10.45) (Table 4), but this was not significant. In *A. apraefrontalis* an increased SVL of the Western Australian Coast population is associated with a roughly proportional increase in both vertebrae and hindbody circumference to the Timor Sea population. Notably the Western Australian Coast *A. apraefrontalis* population also has a relatively smaller head than (both HeadW and HeadL) the Timor Sea population (Supp. Figure 1).

We sampled 33 *Aipysurus duboisii* for morphometric and vertebral data, with 23 from the Timor Sea and 20 from the Western Australian Coast. *A. duboisii* from the Timor Sea had a lower SVL (420–1020) than those from the Western Australian Coast (850–1340) as well as lower and non-overlapping vertebral counts (149–167 vs. 171–188). The Western Australian Coast population of *A. duboisii* had significantly higher fineness than the Timor Sea population, which was the highest of any species measured (12.41 vs. 10.44). In *Aipysurus duboisii* greater SVL is strongly correlated with both an increase in vertebrae number and fineness.

We sampled 46 *Aipysurus foliosquama* for morphometric and vertebral data, with 41 from the Timor Sea and five from the Western Australian Coast. *A. foliosquama* from the Timor Sea and Western Australian Coast broadly overlapped in all measurements taken although there were some trends. Generally the Timor Sea population had a lower (but broadly overlapping) SVL, vertebral count and fineness. Western Australian Coast *A. foliosquama* reach greater sizes but this is less pronounced than in the preceding species.

We sampled 41 *Aipysurus fuscus* and 5 *A. tenuis. Aipysurus fuscus* had a significantly lower SVL, with no overlap among sampled specimens and a lower and non-overlapping number of vertebrae. *A. tenuis* had a higher mean fineness than *A. fuscus* (12.41 vs. 9.25) although this was not significant, likely due to small sample sizes of *A. tenuis*. This species complex followed the same trends as *A. duboisii* and had the greatest difference in mean fineness between any of the codistributed species measured.

We sampled 14 *Aipysurus laevis* from the Timor Sea and 28 from the Western Australian Coast as well 10 *A. laevis pooleorum* from Shark Bay. There was no correlation between increased vertebrae and SVL in any *A. laevis* populations, however the Western Australian Coast population reached a greater SVL than the

other populations sampled. Mean fineness was nearly the same between Timor Sea and Western Australian Coast populations of *A. laevis* and slightly higher in *A. laevis pooleorum* but not significantly so.

Table 4 - Mean fineness in *Aipysurus* species comparing the Timor Sea to Western Australian Coast populations, calculated by dividing SVL by hindbody. Bolded values were significant at $p < 0.05$ using a student's t-test. Not shown is *A. laevis pooleorum* which had a mean fineness of 8.06.

	Timor Sea	**WA Coast**
Aipysurus apraefrontalis	9.65	10.45
Aipysurus duboisii	**10.44**	**12.41**
Aipysurus foliosquama	9.32	9.81
Aipysurus fuscus/tenuis	9.25	12.04
Aipysurus laevis	7.47	7.26

All *Aipysurus* species sampled showed a trend towards lighter colour patterns in Western Australian Coast populations compared with those from the Timor Sea (Figures 5 – 6). Of the eleven *Aipysurus apraefrontalis* examined from the Timor Sea three (27%) were dark brown with no discernible pattern, while the remaining eight (73%) were of the intermediate morph. The intermediate morph had a simple banded colour pattern with a dark brown base colour and approximately equally sized mid brown bands, typically specimens of this morph were smaller individuals. Ten of the eleven (91%) specimens examined from the Western Australian Coast belonged to the light morph, with the ground colour being bright yellow. Dark brown bands that tapered to a point were formed by dark triangular centres of scales, giving the snakes of this morph a reticulated appearance. In live photos the pale edges of scales were orange, but this has likely faded from preserved specimens.

A total of 7 from 25 (28%) *Aipysurus duboisii* examined from the Timor Sea were melanistic, which were typically larger adult individuals. In preservative these specimens were dark brown but photos from live individuals show these were likely jet black in life, with some indistinct pale scale margins in some cases. The intermediate morph made up the majority of the remaining Timor Sea specimens (17,

68%) and typically had a dark brown ground colour with thin white bands being found on the lower half of the flank before tapering to a point, most of these specimens were juveniles and smaller individuals. Western Australian Coast *Aipysurus duboisii* were largely of the light morph (7 of 9, 78%) which consisted of a cream ground colour with black triangles centred on individual scales forming bands that tapered finer down the flanks. The two intermediate individuals had a similar colour pattern to the light morph however the black bands were more extensive. As opposed to the Timor Sea populations, the larger individuals tended to be lighter and juveniles darker.

A total of 42 *A. foliosquama* specimens were examined for colour pattern, of which 36 were from the Timor Sea and only 6 were from the Western Australian Coast population. In the Timor Sea population 23 were melanistic and had no discernible pattern, with a dark brown ground colour in preservative. The remainder belonged to the intermediate morph which had a simple banded pattern with a dark brown ground colour and brown bands, nearly identical to the equivalent *A. apraefrontalis* morph. The Western Australian Coast population of *A. foliosquama* was evenly split between the intermediate and light morph, the light morph was banded like the intermediate morph but with a light red or orange ground colour. No specimens from Shark Bay were available but low-resolution photos from trawlers suggest they largely belong to the intermediate morph (Nankivell, pers obs).

A total of 39 specimens of *A. fuscus* and *A. tenuis* were examined for colour pattern, of which 29 were *A. fuscus* from the Timor Sea and 10 were *A. tenuis* from the Western Australian Coast. All *A. fuscus* specimens examined belonged to the melanistic morph and were uniform dark brown with no discernable pattern. However some photos of live specimens show that at least some have a faint pattern but this was not visible in preserved specimens examined, most of which were from Ashmore Reef. *A. tenuis* were by and large light morph, with a cream ground colour, brown head and a varying degree of black scales predominantly on the dorsum. In some *A. tenuis* the black scales formed tapering bands and in two the body colour was predominantly black with thick cream bands.

A total of 29 *A. laevis* specimens were examined from Timor Sea (8) and the Western Australian Coast (21). All of the preserved specimens examined from the Timor Sea were melanistic with no discernible pattern, however live photos of this population show that it is typically counter shaded in life and the lack of pattern may be an artefact of preservation. The majority of *A. laevis* from the Western Australian

Coast were assigned to the light morph (20 of 21), large individuals had an immaculate cream body colour with a striking dark brown head. In smaller individuals some dorsal scales were dark brown and in smaller juveniles these formed stripes down the body. No preserved specimens of the Shark Bay population (currently classified as *A. pooleorum*) were available, however their pattern was previously described by Smith (1974) as sexually dimorphic. Males typically are counter shaded with a ragged delineation between a dark brown dorsum and cream venter, while females are dark brown with thin greyish flank bands which are typically only a single scale wide.

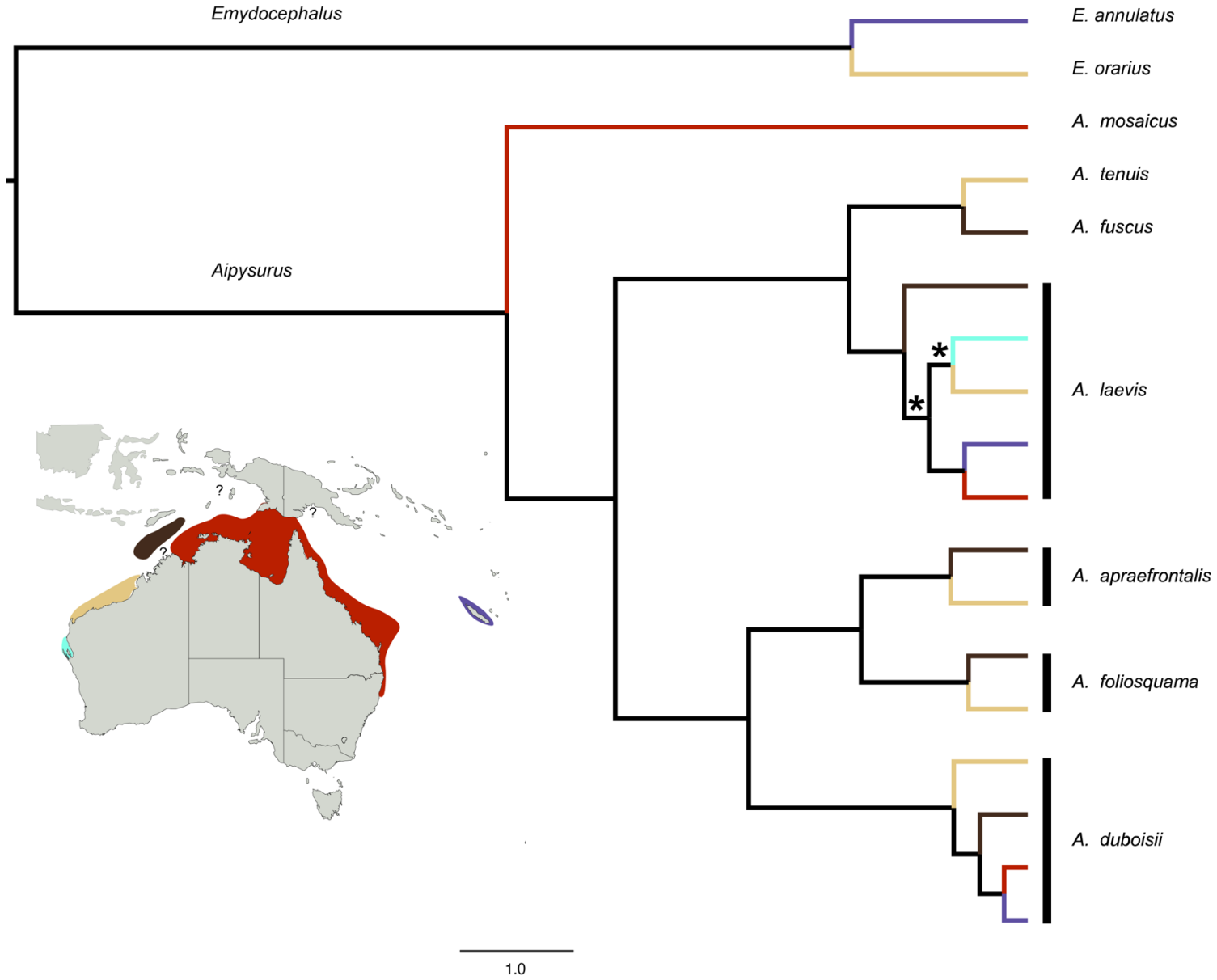
Emydocephalus
Aipysurus
E. annulatus
E. orarius
A. mosaicus
A. tenuis
A. fuscus
A. laevis
A. apraefrontalis
A. foliosquama
A. duboisii
*
*
?
?
?
1.0

Figure 1 - Maximum likelihood consensus tree of *Aipysurus* reconstructed from 33388 unlinked SNPs. Rooted using *Emydocephalus*. All support values are 100% except for two marked with an asterisk, which were two of the internal nodes within *Aipysurus laevis*. Areas where *Aipysurus* species are known or expected to occur but population assignment is uncertain are marked with a "?" on the map. Scale bar is in Mya. Colours as follows: cyan = Shark Bay, Yellow = Western Australian Coast, Brown = Timor Sea, Red = Arafura, Purple = New Caledonia.

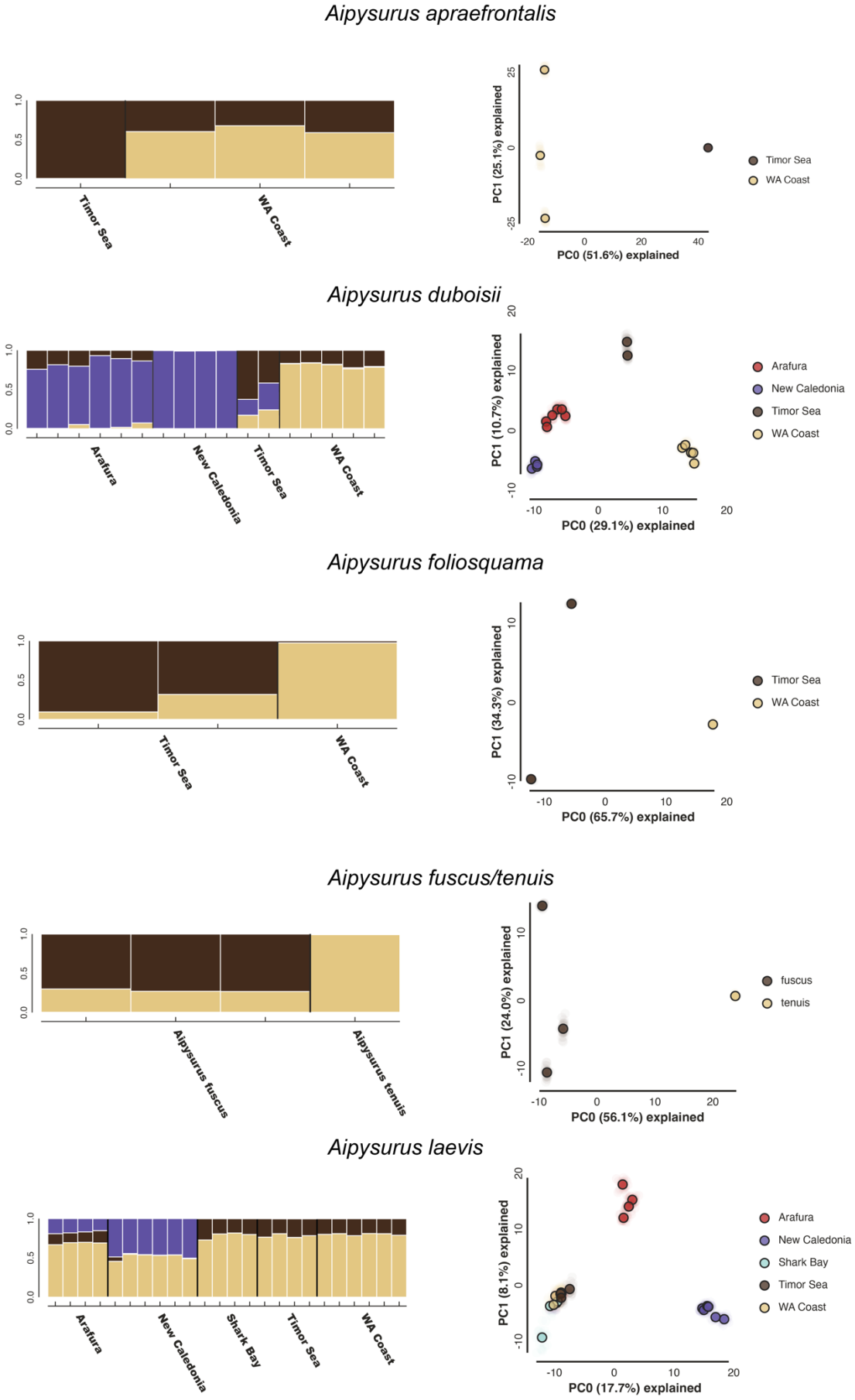

Figure 2 – Structure plots (left) and PCAs (Right) for five codistributed *Aipysurus* lineages. Colours follow map Figure 1.

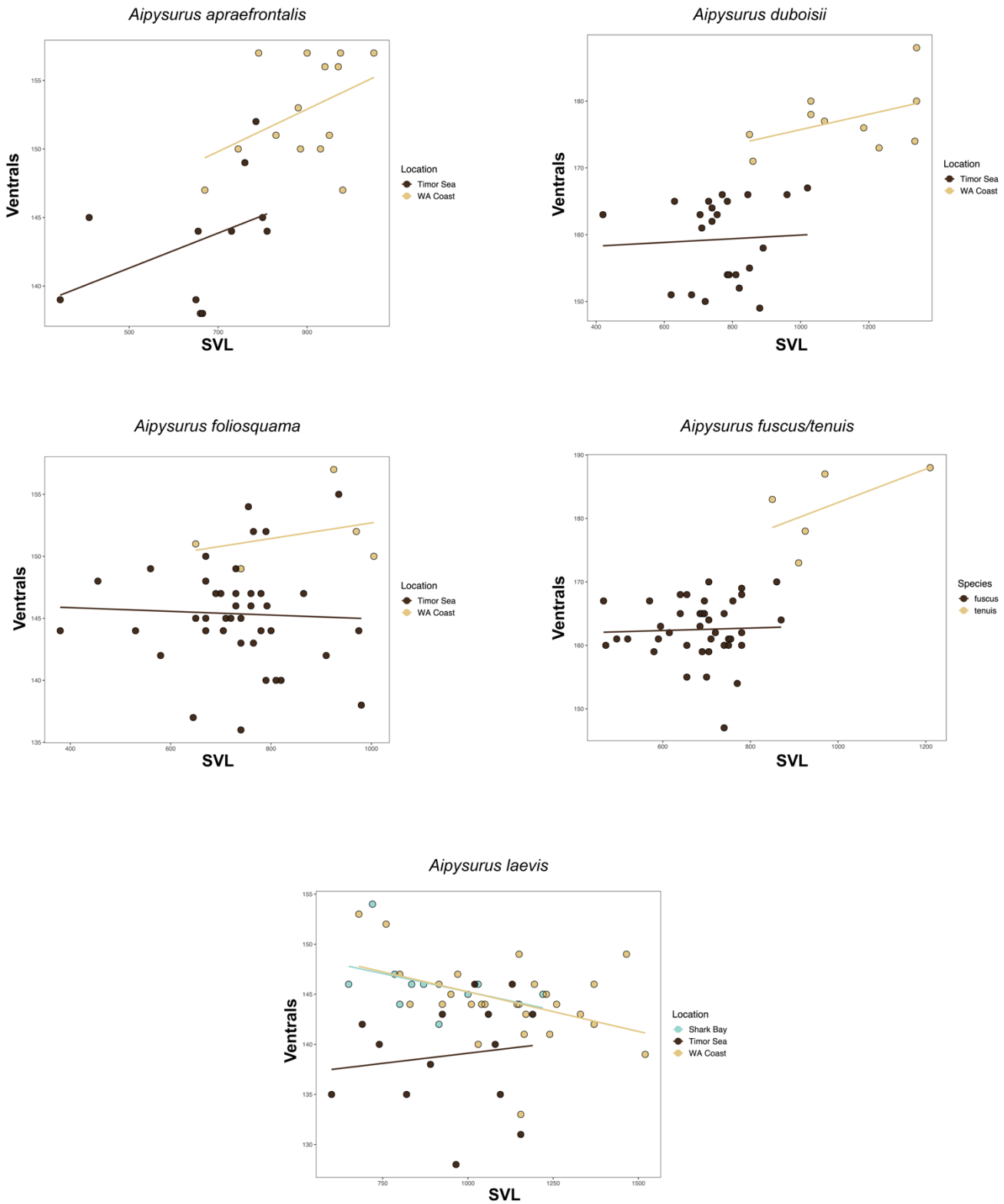

Figure 3 - Scatterplots of Ventral scales (y axis) versus SVL (x axis) in five codistributed *Aipysurus* lineages.

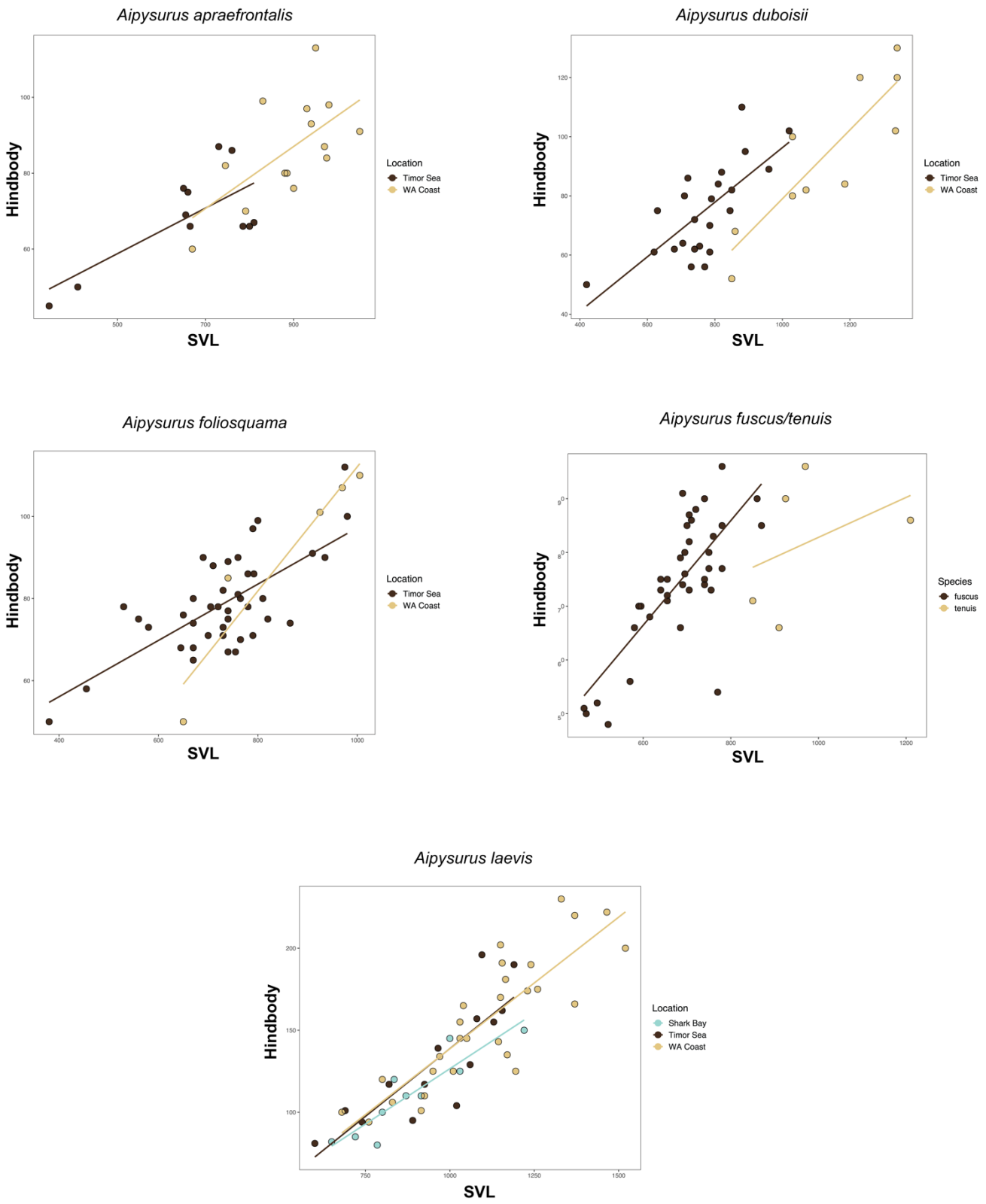

Figure 4 - Scatterplots of hindbody circumference (y axis) versus SVL (x axis) in five codistributed *Aipysurus* lineages.

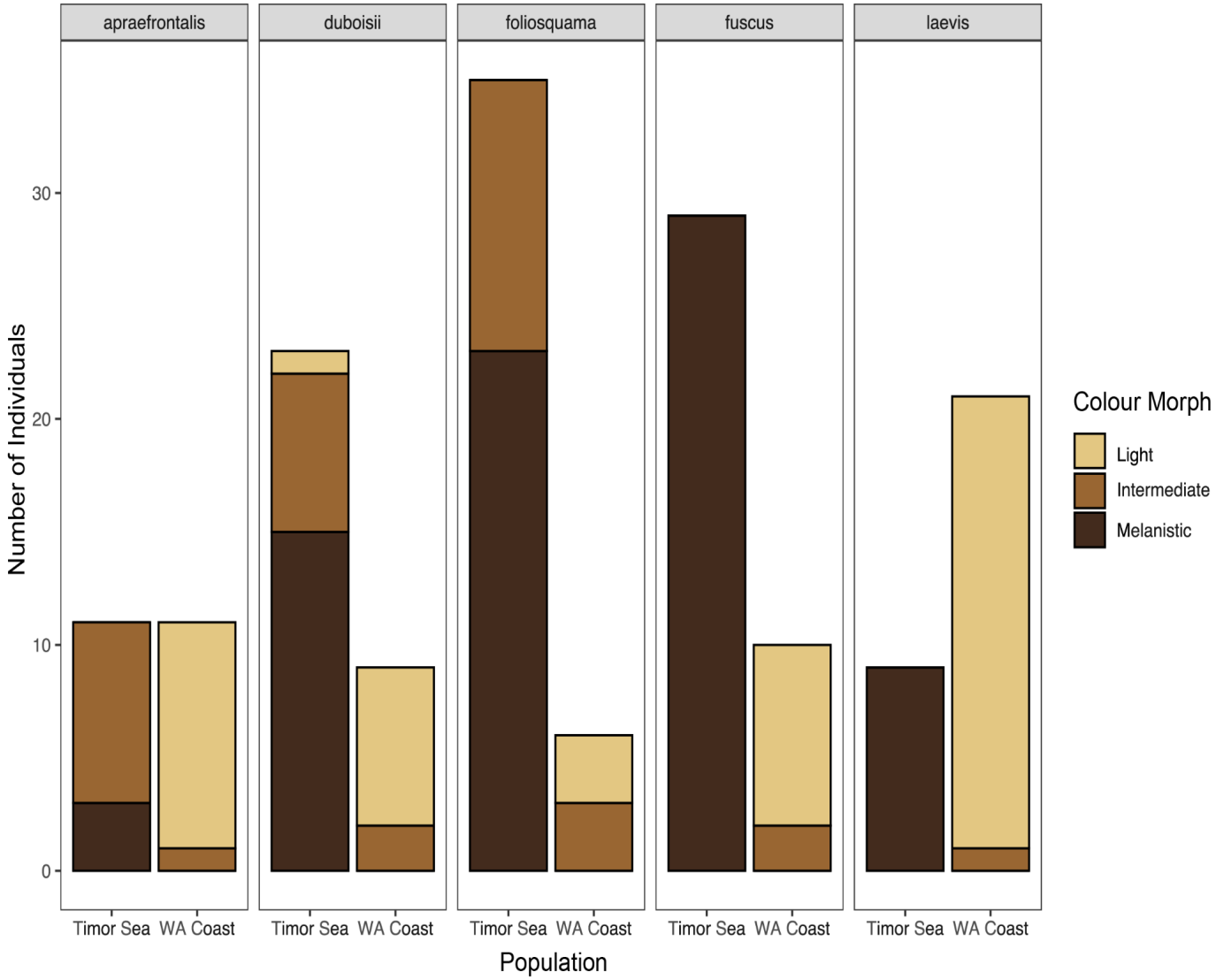

Figure 5 – Barplots showing colour pattern (defined in methods) distribution in five codistributed *Aipysurus* lineages.

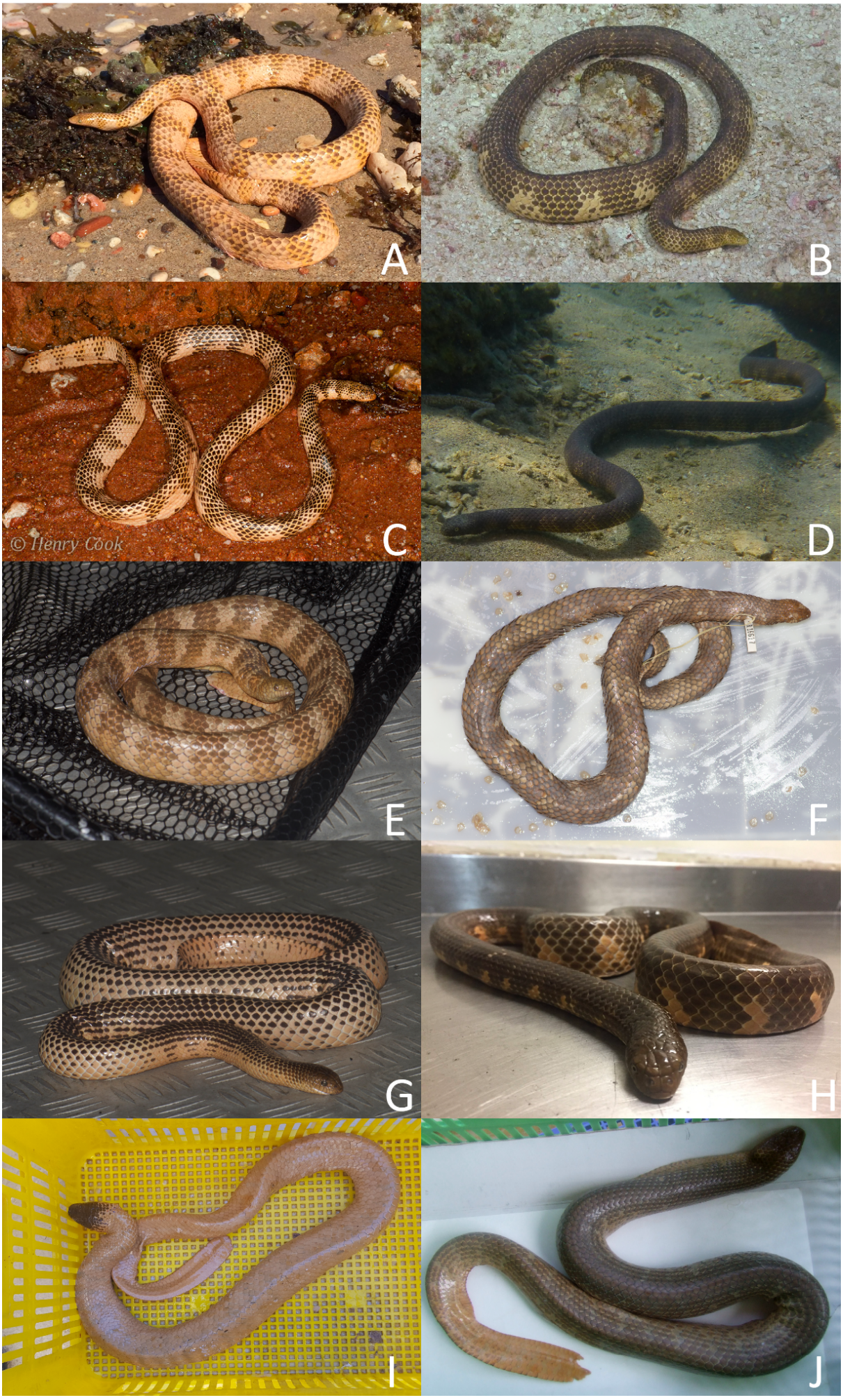
A
B
© Henry Cook
C
D
E
F
G
H
I
J

Figure 5. - Representatives of light (left) and dark (right) morphs for each species: A) *Aipysurus apraefrontalis,* Exmouth Gulf (Vinay Udyawer); B) *Aipysurus apraefrontalis*, Ashmore Reef (RV Falkor); C) *Aipysurus duboisii,* Port Hedland (Henry Cook); D) *Aipysurus duboisii*, Noumea (Claire Goiran); E) *Aipysurus foliosquama*, Pilbara (James Nankivell); F) *Aipysurus foliosquama*, Ashmore Reef; G) *Aipysurus tenuis*, Pilbara (James Nankivell); H) *Aipysurus fuscus*, Heywood Shoals (Vinay Udyawer); I) *Aipysurus laevis*, Pilbara (SS Investigator); J) *Aipysurus laevis*, Gulf of Carpentaria (Mahree Dee-White).

Discussion

Our study reveals common patterns of biogeographic and adaptive evolution in four of five co-distributed *Aipysurus* lineages. In the discussion below, we use these results to delimit units for conservation management, and identify the geographic and ecological mechanisms by which these distinct populations may have arisen.

Identifying phylogenetically and ecologically significant units for conservation

Aipysurus apraefrontalis, *A. duboisii*, *A. foliosquama*, and the *A. fuscus-A. tenuis* complex all show convergent morphological variation in genetically differentiated population pairs from coastal versus offshore habitats. The SNP phylogeny was time-scaled using a secondary calibration; hence our splitting times are very approximate. Nonetheless, minimum divergence estimates of ~500,000 years, together with the presence of distinct genetic clusters in Bayesian and ordination analyses, indicate limited historical and contemporary gene flow between coastal and offshore populations. These genetic divisions are mirrored by quantitative differences in morphology. Coastal populations in all four lineages have longer bodies, higher vertebrae counts, and lighter colour patterns compared to offshore-reef populations. The adaptive significance of these trait changes is discussed below; however, all are ecologically relevant and their convergence in four population and species pairs provides compelling evidence of divergent local adaptation in coastal versus offshore-reef *Aipysurus*.

The presence of ecologically and genetically distinct populations of sea snakes has important implications for their conservation management. Existing occurrence records show that offshore populations of all four species and species complexes occupy smaller and much more fragmented distributions compared to adjacent coastal populations. Many of the remote offshore reefs and shoals occupied by *Aipysurus* are within marine protected areas. However, unexplained population declines at some of these protected reefs have led

to EPBC Act 1999-listing of *A. fuscus* as endangered, and *A. apraefrontalis* and *A. foliosquama* as critically endangered. Surveys at Ashmore prior to 2013 indicated population declines of *Aipysurus apraefrontalis* and *A. foliosquama* of at least 90% over 15 years (three generation lengths) and dedicated searches of both reefs failed to sight either species in 2005, 2007, 2012 and 2013 (Guinea et al. 2005; Guinea 2012; Lukoschek et al. 2013; Somaweera et al 2021). Speed et al (2022) reported a single individual *A. apraefrontalis* at 60-100m at Ashmore Reef, indicating that the species might be retained in (or have migrated to) deeper habitats than those surveyed during previous expeditions. Coastal *Aipysurus* populations may be larger and more continuous than their offshore counterparts given that they occupy extensive habitats along Western Australia's shallow continental shelf. However, *Aipysurus* are a significant component of the bycatch of multiple coastal trawl fisheries (Kangas, 2007; Udyawer et al., 2020), and may also be vulnerable to habitat loss as a result of major infrastructure developments in this region. Fine-scale genetic and habitat analyses within coastal and offshore populations are needed to quantify connectivity, and assess the scale of threats to Aipysurus in these regions.

It is clear from our results that coastal and offshore populations of *Aipysurus apraefrontalis*, *A. duboisii*, *A. foliosquama* and *A. fuscus-A. tenuis* have undergone divergent adaptive evolution over a substantial period of historical isolation. *A. fuscus* and *A. tenuis* are already recognised as separate species based on non-overlapping patterns of variation in vertebral counts (Smith, 1974; Kharin and Czeblukov, 2006; and supported here). We suggest the current taxonomy for *A. duboisii*, *A. apraefrontalis* and *A. foliosquama* be retained, but recommend that the coastal and offshore-reef populations of these species be recognised as Evolutionarily Significant Units (sensu Moritz, 1994). Separate management of these functionally discrete units is likely to be the surest strategy for maximising their evolutionary potential to respond to future change. These sea snake ESUs should also be considered in the planning of regional protected areas networks for the preservation of functionally distinct communities.

The fifth co-distributed lineage, *Aipysurus laevis*, presents substantially different genetic and morphological boundaries compared to the patterns shared by its congeners. Populations of this species from the Western Australian Coast and offshore-reefs form a single cluster in the SNP analyses, which contrast with mitochondrial markers which show clear separation between Western Australian Coast and Timor Sea populations. Lack of nuclear population structure compared with mitochondrial may reflect a historical signal subsequently obliterated by contemporary migration, or alternatively be related to life history traits (i.e. male biased dispersal). Morphological variation does not follow

geographic patterns to the extent of the other codistributed *Aipysurus* species, although on the Western Australian Coast *A. laevis laevis* do tend to be larger and paler but without corresponding increases in vertebrae or fineness. Two subspecies are currently recognised in A. laevis: A. l. pooleorum from Shark Bay at the south-western limits of the range, and A. l. laevis from Exmouth to New Guinea and New Caledonia. Notably *A. laevis pooleorum* from Shark Bay are nearly identical in morphological characters to those from the Timor Sea despite being separated by >1000 km, possibly reflecting shared environmental pressures. Our results show that revision is needed but refrain here.

Ecological and geographic mechanisms of lineage formation

Similar levels of divergence between coastal and offshore lineages are indicative of shared responses to biogeographic history in four co-distributed *Aipysurus* species. Our approximate date estimates, from 700k years ago in *Aipysurus duboisii* to 530k years ago in *A. foliosquama* and *A. fuscus-tenuis*, a period which coincides with numerous cycles of sea level change in the region. During peak glacial periods much of the North-west shelf was exposed and shallow water habitats used by sea snakes would have been less extensive. The barrier between the Canning and Kimberley bioregions likely coincided with a narrow constriction in the shelf where there was little shallow water habitat before the drop off from the continental slope (Wilson et al., 2013). The Fitzroy River formed an estuary at this narrow point, and it may have been a combination of extensive freshwater outflows, large tidal regime and a lack of shallow water habitats that maintained this biogeographic barrier. The continuing existence of this barrier through high sea level interglacials in four of the five species warrants further investigation.

Restricted gene flow likely facilitated the morphological divergence of coastal versus offshore-reef populations. Convergent shifts in body length, vertebral count and colour pattern characters in four co-distributed lineages provides compelling evidence of local adaptation. Selection pressures relating to diet and habitat are dominant drivers of body shape variation in snakes. Dietary specialisation in particular has been linked to body length, girth, and vertebral counts in *Hydrophis* sea snakes (Voris and Voris, 1983; Sherratt et al., 2013; Chapter 5, this thesis). *Hydrophis* species with thicker, shorter bodies and low-moderate vertebral counts typically have generalist diets or specialise on toxic-spinous prey. Those using mostly elongate (especially eel) prey have longer bodies and higher vertebral counts (but to greater extremes than seen in coastal *Aipysurus;* e.g. ~280 vertebrae in *H. kingii* versus ~190 in *A. tenuis*). It is likely that differences in the diets of coastal and offshore *Aipsyurus* have contributed to their body shape differences to some

degree. However, almost all available diet records for *Aipysurus* indicate a generalist diet for most species with a wide variety of fish taken (mostly fusiform and goby-like), suggesting a minor role for diet in driving body shape changes in most of these species. One notable exception might be Western Australian Coast *A. apraefrontalis* who's only diet record is of a burrowing eel and which have a significantly smaller head than the Timor Sea population. This hypothesis is consistent with the parallel patterns of diet, habitat and body shape evolution reported for *Emydocephalus*, which is the sister lineage to *Aipysurus* (Nankivell et al., 2020; this thesis chapter 3). The two *Emydocephalus* share adaptations to a highly specialist diet of fish eggs, but the coastal species, *E. orarius,* is longer bodied and has higher ventral counts than its offshore sister species, *E. annulatus*. The deep divergence (~ two million years) separating *E. orarius* and E. *annulatus* might indicate that coastal and offshore *Aipysurus* lineages are also on independent evolutionary trajectories and perhaps represent incipient species.

Hydrodynamic adaptations to open coastal versus structured offshore-reef habitats might also explain the morphological differences between these lineages. Sea snakes are anguilliform (eel-like) swimmers that propel themselves through water using lateral deformation waves that move backwards along the full length of their bodies (Graham et al., 1987). The kinematics of anguilliform locomotion has been studied extensively in teleosts. In elongate, finless species, there are strong causal relationships between body size and shape and swimming performance. Body length is a major determinant of critical swimming capacity (Wang et al., 2013; Tack et al., 2021), and fineness ratio (body length relative to cross-sectional width) is related to endurance capacity, with greater fineness providing higher sustained swimming efficiency via reduced drag (Walker et al., 2013). Comparative studies in fish and cetaceans have linked optimised fineness ratios with the locomotory requirements of different habitat types; species that occupy open habitats or rely on patchily dispersed resources have higher fineness ratios than those in structurally complex reef or coastal habitats (Woodward et al., 2006). The coastal habitats of *Aipysurus* are relatively simple and open, consisting largely of sand flats. In contrast, their offshore-reef habitats in the Timor Sea are structurally complex with thick coral growth. Coastal populations of all species had longer bodies compared to offshore populations, but some of these (*A. duboisii* and *A. fuscus/tenuis*) exhibited higher fineness, while in others (*A. apraefrontalis, foliosquama* and *A. laevis*) body length increased in proportion to hindbody girth. Coastal sea snakes might experience selection for increased endurance-swimming performance, or critical-swimming performance, or both. Recent telemetry studies are beginning to shed light on individual movements in sea snakes and these may provide greater insight into

their movement and use of habitat (Udyawer et al., 2015; Udyawer et al., 2020b). While there must be many functional demands on snake body shape, we suggest that selection on locomotory efficiency is a major driver of change in *Aipysurus* (and *Emydocephalus*)

Finally, colour pattern is an important and often highly labile trait that is subject to strong evolutionary pressure in nearly all photic environments. A number of sea snake species are regarded as aposematic, although it is possible in some environments bands may effectively function as crypsis through flicker fusion (Goiran et al., 2022). In our study, coastal populations of *Aipysurus* were universally paler in colour than those from the Timor Sea, frequently with cream bodies with some darker markings and bands as well as often a dark brown head. In contrast Timor Sea *Aipysurus* were generally dark brown or black, frequently with no discernible bands, or when present they are often low in contrast to the ground colour. We consider it highly unlikely that this is driven by industrial melanism in this case as these reefs are some of the least impacted by human activity in the world. We suggest that instead the darker colour patterns may function to hide snakes sheltering in crevices in reef habitats. Notably all of the species in our study are either confirmed or suspected to have phototactic tails, which is suggested to aid in helping snakes shelter effectively (Crowe-Riddell et a., 2018). Coastal *Aipysurus* are unlikely to have access to dark crevices to hide, and instead may shelter on the seafloor where pale sandy colours may offer some degree of crypisis which may be further enhanced by a dark head if the snake is foraging in burrows.

Conclusions

Identifying genetically and ecologically unique lineages within species is of key conservation concern. We showed in a codistributed and threatened genus of Australian sea snakes that common biogeographical and ecological factors have led to the formation of parallel lineages in four species which we designate Evolutionarily Significant Units (ESUs). Given their different use of habitat and response to threats, treating the ESUs as separate management units will likely lead to better conservation outcomes. Further investigation of potential contact or hybrid zones between these ESUs north of the Dampier Peninsula as well as the inshore Kimberley where sea snake collections have been limited, will shed further light on their evolutionary history and taxonomic status.

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Supplementary Material

Supplementary data is available at https://figshare.com/s/64646eb0a098d6e7c8b9

Supplementary Methods

We pooled sexes and scaled morphometric measurements by SVL to get relative measurements. We analysed morphological characters within each species to measure variation between ESUs. To visualise the datasets we created boxplots for ventral scale measurements and generated PCAs of morphometric data using the prcomp function in R and plotted them using ggplot2.

Supplementary Results

The first two PC axes in our principal component analyses explained between 67.9% and 75.9% of variation in the data.

For *Aipysurus apraefrontalis* PC1 (51.9% variance) was largely explained by SVL and relative Fore, HeadL and HeadW, largely separating the ESUs with no overlap. Western Australian Coast *A. apraefrontalis* were larger in size with relatively small forebodies and heads compared with those from the Timor Sea. The single specimen from the Arafura

ESU collected from Anson Bay is morphologically intermediate between the other ESUs. PC2 (24.7% variance) was explained by TailL and Hind which separated sexes rather than along geographical lines. Western Australian Coast *A. apraefrontalis* had greater numbers of ventral scales than other ESUs with no overlap and a mean difference of ~10. All *A. apraefrontalis* from Western Australian Coast and Arafura we examined were pale in colour versus dark brown in Timor Sea specimens, with only a hint of lighter crossbands in some specimens.

For *Aipysurus duboisii* PC1 (53.25% variance) was explained mostly by Fore, Hind, HeadL and HeadW and partially by SVL. PC2 (22.19%) was explained by TalL and SVL. Western Australian Coast specimens were larger with shorter tails and proportionally smaller heads and bodies than those from Timor Sea. There was no overlap between Western Australian Coast and Timor Sea while Arafura occupied an intermediate position overlapping with the other ESUs. Western Australian Coast *A. duboisii* have an average of ~15 more ventral scales than other ESUs with no overlap, while Arafura and Timor Sea ventral counts were broadly overlapping although a with a mean 5 more in Arafura specimens. Juvenile specimens from all ESUs have largely the same colour patterns with dark dorsal colouration and pale yellowish flank bands that darken towards the dorsal surface. Adult *A. duboisii* differed in colour pattern with those from Western Australian Coast and Arafura being largely cream with darker bands broken up and often forming longitudinal stripes while those from Timor Sea are predominantly black with the only pale colouration being found on scale edges and sometimes dark brown flank bands.

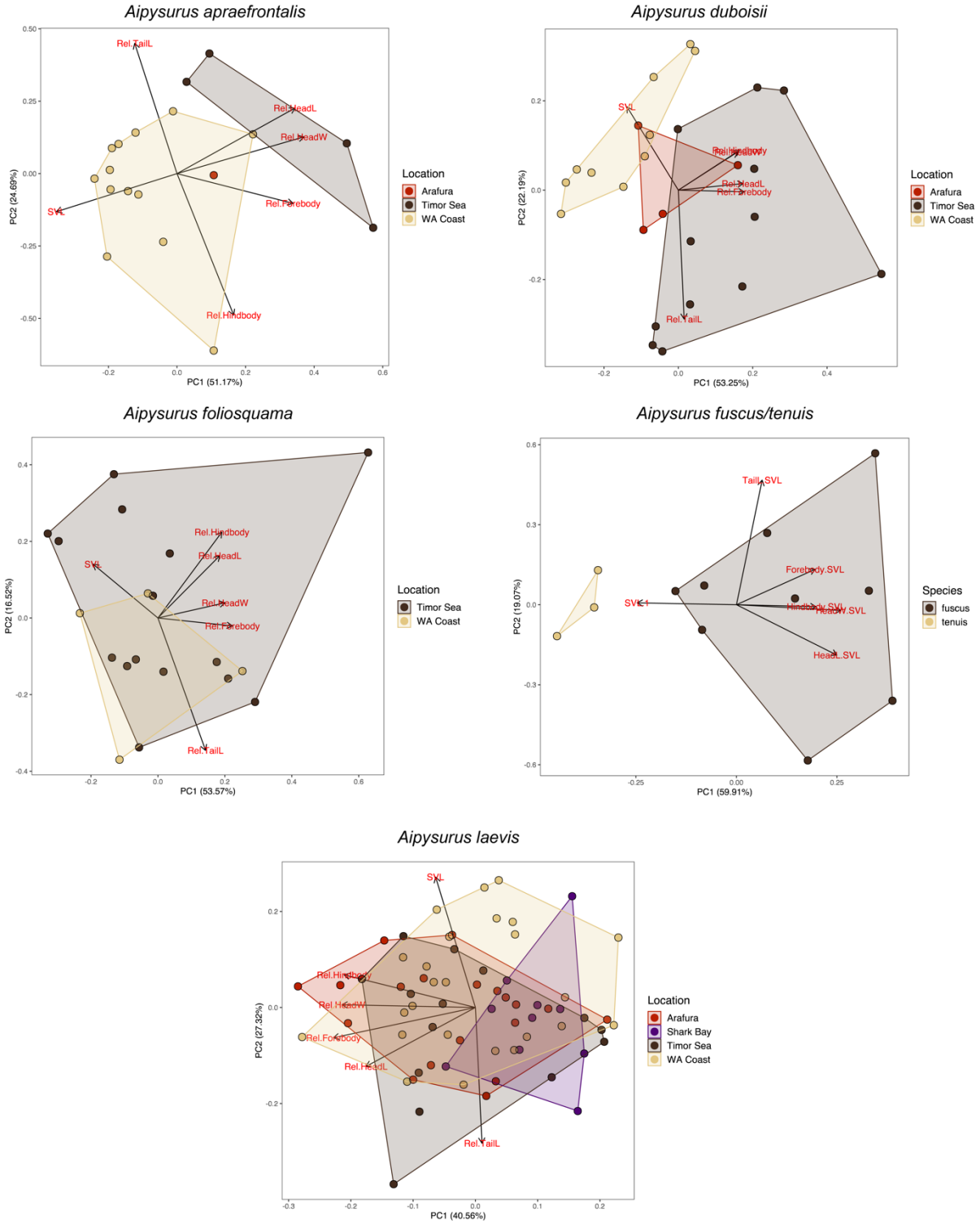
Loadings on the *Aipysurus foliosquama* PCA were similar to the previous species, with PC1 (53.6% variance) explained largely by relative Fore, Hind, HeadL and HeadW as well as partially SVL. PC2 (16.5% variance) was explained mostly by TailL and SVL. Western Australian Coast and Timor Sea specimens overlap broadly, with Timor Sea occupying much greater morphological space. *A. foliosquama* from Western Australian Coast had significantly greater but overlapping ventral counts than those from the Timor Sea. *A. foliosquama* from the Western Australian Coast were typically pale brown in colour with a reddish flush in life which fades in preservative while those from the Timor Sea were usually dark brown, faint bands were present in both populations.

PC1 accounted for 59.9% of variation on the *A. fuscus/tenuis* PCA and was driven primarily by SVL, Fore, Hind and HeadW while PC2 accounted for 19.1% of variation and was explained primarily by TailL. *A. tenuis* were proportionally longer with relatively smaller heads and lower relative girth than *A. fuscus*. Additionally ventral counts did not overlap between species with *A. tenuis* having a mean ~20 more ventral scales with no overlap. All

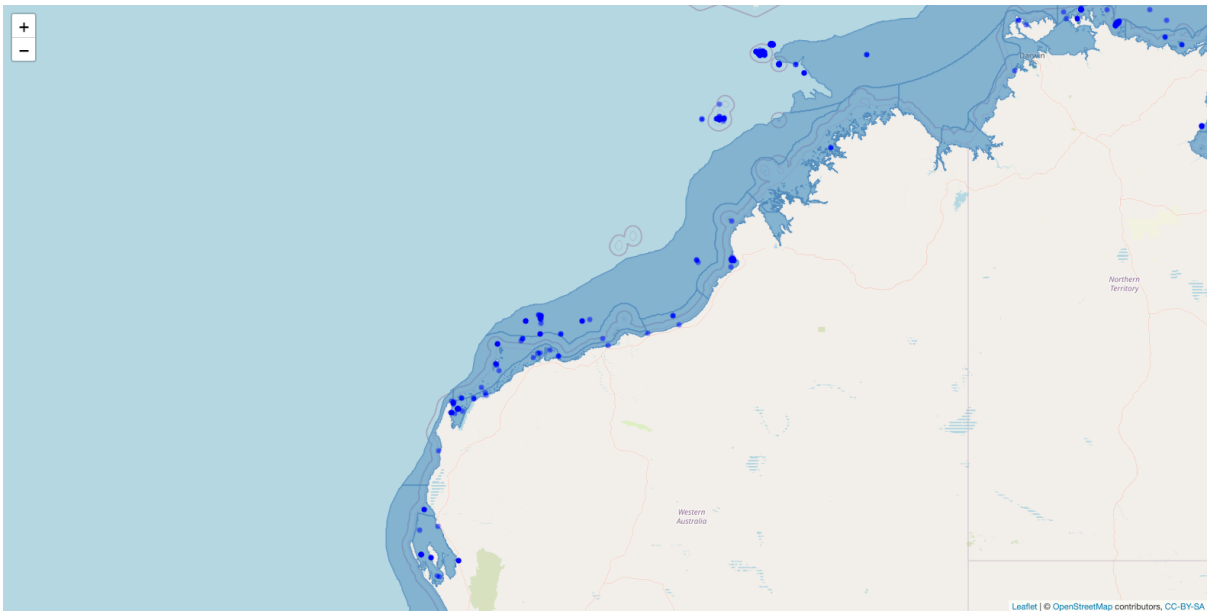
A. tenuis had predominantly pale cream bodies with dark brown heads, with some dark scales forming longitudinal stripes down the body. While there was considerable variation in the degree of dark striping the flanks remained cream in all individuals. In contrast all *A. fuscus* observed were almost entirely uniform brown in preservative, although in life many individuals have either faint pale bars or pale lower flanks.

In the *A. laevis* plot PC1 accounted for 40.6% of variation and was mostly explained by relative Fore, Hind, HeadL and HeadW while PC2 accounted for 27.3% of variation and was explained by TailL and SVL. All *A. laevis* populations broadly overlapped on the PCA although there were a few observable trends. The Western Australian Coast population reached typically larger sizes than other populations, while those from Shark Bay typically had proportionally smaller heads and lower relative girth than other populations. Ventral scales in all populations were broadly overlapping although the Western Australian Coast and Shark Bay populations had significantly higher mean counts than the Arafura and Timor Sea populations. The Western Australian Coast population was highly distinct in colour pattern, with an almost entirely cream body with a dark brown head and dark scales were present on the dorsum of juveniles but were scarce to absent in adults. The remaining populations were extraordinarily variable and were typically darker brown and either monochromatic or with pale bars and flanks. However some individuals among these populations were a lighter yellow often with darker heads but never as highly contrasting as those from the Western Australian Coast.

There was a clear overall trend towards elongation, greater ventral counts and pale body colouration in Western Australian Coast populations in most measured *Aipysurus* taxa although details varied among species. Typically the geographically proximate Timor Sea populations for most species were smaller and darker while those from the Arafura were intermediate in morphology between Timor Sea and Western Australian Coast although sample sizes were smaller.



Supplementary Figure 1 – PCAs of morphological data in five codistributed *Aipysurus* species



Supplementary Figure 2 – *Aipysurus* records from the North-west coast, with IMCRA bioregions plotted.

Appendix

Appendix 1 – Samples used in molecular analyses.

Genus	Species	Sample	Location	Country
Aipysurus	apraefrontalis	Aaprae 4.12.01	Ashmore Reef	Australia
Aipysurus	apraefrontalis	SS171013-03	Pilbara	Australia
Aipysurus	apraefrontalis	KLS1001	Exmouth Gulf	Australia
Aipysurus	apraefrontalis	KLS0834	Exmouth Gulf	Australia
Aipysurus	duboisii	SS171027-01	Pilbara	Australia
Aipysurus	duboisii	SS171026-01	Pilbara	Australia
Aipysurus	duboisii	KLS0825	Exmouth Gulf	Australia
Aipysurus	duboisii	KLS0839	Exmouth Gulf	Australia
Aipysurus	duboisii	KLS0698	Exmouth Gulf	Australia
Aipysurus	duboisii	VU134	Heywood Shoal	Australia
Aipysurus	duboisii	VU124	Heywood Shoal	Australia
Aipysurus	duboisii	QM J80495	Sir Edward Pellow Group, N	Australia
Aipysurus	duboisii	MAGNT R36638	Gulf of Carpentaria	Australia
Aipysurus	duboisii	QM J80683	Waddy Point, Fraser Island	Australia
Aipysurus	duboisii	QM J83742	Pinnacles, Fraser Island	Australia
Aipysurus	duboisii	QM J95847	Peregrian Beach	Australia
Aipysurus	duboisii	NC_Jan2019_281	Noumea	New Caledonia
Aipysurus	duboisii	NC_Jan2019_250	Noumea	New Caledonia
Aipysurus	duboisii	NC_Jan2019_240	Noumea	New Caledonia

Aipysurus	duboisii	NC_Jan2013_190	Noumea	New Caledonia
Aipysurus	foliosquama	Afo1	Ashmore Reef	Australia
Aipysurus	foliosquama	Afo8	Ashmore Reef	Australia
Aipysurus	foliosquama	SS171014-02	Pilbara	Australia
Aipysurus	tenuis	HybFa01	Broome	Australia
Aipysurus	tenuis	SS171020-04	Pilbara	Australia
Aipysurus	fuscus	VU140	Heywood Shoal	Australia
Aipysurus	fuscus	Afu2	Ashmore Reef	Australia
Aipysurus	fuscus	SAMA R68141	Ashmore Reef	Australia
Aipysurus	laevis	KLS0327	Scott Reef	Australia
Aipysurus	laevis	VU132	Ashmore Reef	Australia
Aipysurus	laevis	VU131	Ashmore Reef	Australia
Aipysurus	laevis	VU121	Ashmore Reef	Australia
Aipysurus	laevis	NC_Jan2019_227	Noumea	New Caledonia
Aipysurus	laevis	NC_Jan2019_271	Noumea	New Caledonia
Aipysurus	laevis	NC_Jan2019_217	Noumea	New Caledonia
Aipysurus	laevis	NC_Jan2019_282	Noumea	New Caledonia
Aipysurus	laevis	NC_Jan2019_213	Noumea	New Caledonia
Aipysurus	laevis	NC_Jan2019_247	Noumea	New Caledonia
Aipysurus	laevis	MAGNT R37140	West of of Mornington Island	Australia
Aipysurus	laevis	MAGNT R37101	North of Groote Eylandt	Australia
Aipysurus	laevis	MAGNT R37034	North of Mornington Island	Australia
Aipysurus	laevis	MAGNT R37032	North of Mornington Island	Australia
Aipysurus	laevis	MAGNT R36654	South of Groote Eylandt	Australia
Aipysurus	laevis	VU62	Pilbara	Australia
Aipysurus	laevis	VU21	Broome	Australia
Aipysurus	laevis	SS171106-02	Pilbara	Australia
Aipysurus	laevis	SS170810-01	Pilbara	Australia
Aipysurus	laevis	KLS0796	Exmouth Gulf	Australia
Aipysurus	laevis	KLS1166	Broome	Australia
Aipysurus	pooleorum	BD330	Shark Bay	Australia
Aipysurus	pooleorum	BD304	Shark Bay	Australia
Aipysurus	pooleorum	BD342	Shark Bay	Australia
Aipysurus	pooleorum	BD326	Shark Bay	Australia

Appendix 2 – Specimens used in morphological analyses.

Genus	Species	Specimen No.	Location	Country

Aipysurus	foliosquama	WAM R129802	Ashmore Reef	Australia
Aipysurus	foliosquama	MAGNT R16618		
Aipysurus	foliosquama	MAGNT R17783	Hibernia Reef	Australia
Aipysurus	foliosquama	MAGNT R21271	Hibernia Reef	Australia
Aipysurus	foliosquama	MAGNT R21666	Ashmore Reef	Australia
Aipysurus	foliosquama	SAMA R68146	Ashmore Reef	Australia
Aipysurus	foliosquama	SAMA R68139	Ashmore Reef	Australia
Aipysurus	foliosquama	MAGNT R16619		
Aipysurus	foliosquama	MAGNT R16617		
Aipysurus	foliosquama	MAGNT R17779	Hibernia Reef	Australia
Aipysurus	foliosquama	SAMA R68143	Ashmore Reef	Australia
Aipysurus	foliosquama	SAMA R68144	Ashmore Reef	Australia
Aipysurus	foliosquama	WAM R129806	Ashmore Reef	Australia
Aipysurus	foliosquama	MAGNT R13328	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44646/FMNH 216509	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R37134	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44877	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R130010	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R41542	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R41508	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R40492	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44443	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R41537	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R41521	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R40489	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R40488	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R40483	Ashmore Reef	Australia

Aipysurus	foliosquama	AM R44446	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R42701	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R41516	Hibernia Reef	Australia
Aipysurus	foliosquama	AM R41526	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R37137	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44899	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44890	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44576	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44597	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44619	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44456	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44592	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44896	Ashmore Reef	Australia
Aipysurus	foliosquama	AM R44906	Ashmore Reef	Australia
Aipysurus	foliosquama	WAM R154744		
Aipysurus	foliosquama	WAM R165709	Shark Bay	Australia
Aipysurus	foliosquama	WAM R150365		
Aipysurus	foliosquama	No tag (Barrow Island?)		
Aipysurus	foliosquama	AM R104810	Pilbara	Australia
Aipysurus	fuscus	MAGNT R17786	Hibernia Reef	Australia
Aipysurus	fuscus	Afu#5	Ashmore Reef	Australia
Aipysurus	fuscus	Afu#3	Ashmore Reef	Australia
Aipysurus	fuscus	Afu#4	Ashmore Reef	Australia
Aipysurus	fuscus	SAMA R68141	Ashmore Reef	Australia
Aipysurus	fuscus	Afu#6	Ashmore Reef	Australia
Aipysurus	fuscus	WAM R129846		
Aipysurus	fuscus	WAM R129815	Ashmore Reef	Australia

Aipysurus	fuscus	WAM R129814	Ashmore Reef	Australia
Aipysurus	fuscus	AM R130012	Ashmore Reef	Australia
Aipysurus	fuscus	AM R8995	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44862	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44588	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44865	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44431	Ashmore Reef	Australia
Aipysurus	fuscus	AM R8993	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44580	Ashmore Reef	Australia
Aipysurus	fuscus	AM R37142	Ashmore Reef	Australia
Aipysurus	fuscus	AM R41056	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44441	Ashmore Reef	Australia
Aipysurus	fuscus	AM R41531	Ashmore Reef	Australia
Aipysurus	fuscus	AM R41538	Ashmore Reef	Australia
Aipysurus	fuscus	AM R40482	Ashmore Reef	Australia
Aipysurus	fuscus	AM R41533	Ashmore Reef	Australia
Aipysurus	fuscus	AM R42708	Ashmore Reef	Australia
Aipysurus	fuscus	AM R40498		
Aipysurus	fuscus	AM R40481	Ashmore Reef	Australia
Aipysurus	fuscus	AM R41534	Scott Reef	Australia
Aipysurus	fuscus	AM R41535	Ashmore Reef	Australia
Aipysurus	fuscus	AM R37135	Ashmore Reef	Australia
Aipysurus	fuscus	AM R42697	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44586	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44603	Ashmore Reef	Australia

Aipysurus	fuscus	AM R45052	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44869	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44916	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44880	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44462	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44469	Ashmore Reef	Australia
Aipysurus	fuscus	AM R44879	Ashmore Reef	Australia
Aipysurus	tenuis	tenuis1	Broome	Australia
Aipysurus	tenuis	tenuis2	Broome	Australia
Aipysurus	tenuis	KLS1283	Pilbara	Australia
Aipysurus	tenuis	WAM R73659	Pilbara	Australia
Aipysurus	tenuis	AM R142924	Broome	Australia
Aipysurus	pooleorum	WAM R141327	Shark Bay	Australia
Aipysurus	pooleorum	WAM R21364	Shark Bay	Australia
Aipysurus	pooleorum	WAM R21367	Shark Bay	Australia
Aipysurus	pooleorum	WAM R29931	Shark Bay	Australia
Aipysurus	pooleorum	WAM R29932	Shark Bay	Australia
Aipysurus	pooleorum	WAM R98801	Shark Bay	Australia
Aipysurus	pooleorum	WAM R21365	Shark Bay	Australia
Aipysurus	pooleorum	WAM R22419	Shark Bay	Australia
Aipysurus	pooleorum	WAM R25608	Shark Bay	Australia
Aipysurus	pooleorum	WAM R98799	Shark Bay	Australia
Aipysurus	laevis	MAGNT R17773	Cartier Islet	Australia
Aipysurus	laevis	MAGNT R17774	Cartier Islet	Australia
Aipysurus	laevis	MAGNT R17775	Cartier Islet	Australia
Aipysurus	laevis	MAGNT R21269	Hibernia Reef	Australia
Aipysurus	laevis	MAGNT R21273	Hibernia Reef	Australia
Aipysurus	laevis	WAM R129810	Cartier Islet	Australia
Aipysurus	laevis	WAM R58822	Ashmore Reef	Australia
Aipysurus	laevis	WAM R93500	Scott Reef	Australia
Aipysurus	laevis	WAM R93502	Scott Reef	Australia
Aipysurus	laevis	MAGNT R17788	Hibernia Reef	Australia
Aipysurus	laevis	MAGNT R21249	Hibernia Reef	Australia
Aipysurus	laevis	MAGNT R21270	Hibernia Reef	Australia
Aipysurus	laevis	WAM R129807	Ashmore Reef	Australia
Aipysurus	laevis	WAM R129809	Cartier Islet	Australia

Aipysurus	laevis	WAM R44421	Pilbara	Australia
Aipysurus	laevis	WAM R22385	Onslow	Australia
Aipysurus	laevis	WAM R26414	Exmouth Gulf	Australia
Aipysurus	laevis	WAM R37885	Onslow	Australia
Aipysurus	laevis	WAM R44183		
Aipysurus	laevis	WAM R73649	Pilbara	Australia
Aipysurus	laevis	WAM R73674	Pilbara	Australia
Aipysurus	laevis	WAM R76485	Pilbara	Australia
Aipysurus	laevis	WAM R27206	Onslow	Australia
Aipysurus	laevis	laevisStrippedTail	Broome	Australia
Aipysurus	laevis	KLS1010	Broome	Australia
Aipysurus	laevis	KLS1015	Broome	Australia
Aipysurus	laevis	WAM R174249	Broome	Australia
Aipysurus	laevis	WAM R174255	Broome	Australia
Aipysurus	laevis	WAM R174248	Broome	Australia
Aipysurus	laevis	WAM R174250	Broome	Australia
Aipysurus	laevis	WAM R174245	Broome	Australia
Aipysurus	laevis	WAM R14207	Broome	Australia
Aipysurus	laevis	WAM R64993	Barrow Island	Australia
Aipysurus	laevis	WAM R72672	Dampier	Australia
Aipysurus	laevis	WAM R73650	Dampier	Australia
Aipysurus	laevis	WAM R73685		
Aipysurus	laevis	WAM R84471	Broome	Australia
Aipysurus	laevis	WAM R84472	Roebuck Bay	Australia
Aipysurus	laevis	Dusky#1		
Aipysurus	laevis	Dusky#2		
Aipysurus	laevis	KLS0690		
Aipysurus	laevis	WAM R174246		
Aipysurus	duboisii	WAM R93521	Scott Reef	Australia
Aipysurus	duboisii	WAM R93511	Scott Reef	Australia
Aipysurus	duboisii	WAM R93518	Scott Reef	Australia
Aipysurus	duboisii	WAM R93497	Scott Reef	Australia
Aipysurus	duboisii	WAM R129812	Ashmore Reef	Australia
Aipysurus	duboisii	WAM R129813	Ashmore Reef	Australia
Aipysurus	duboisii	WAM R93520	Scott Reef	Australia
Aipysurus	duboisii	WAM R93517	Scott Reef	Australia
Aipysurus	duboisii	WAM R93519	Scott Reef	Australia
Aipysurus	duboisii	MAGNT R17787	Hibernia Reef	Australia
Aipysurus	duboisii	MAGNT R17781	Hibernia Reef	Australia
Aipysurus	duboisii	MAGNT R21262	Hibernia Reef	Australia
Aipysurus	duboisii	AM R44428	Ashmore Reef	Australia

Aipysurus	duboisii	AM R44457	Ashmore Reef	Australia
Aipysurus	duboisii	AM R37139	Ashmore Reef	Australia
Aipysurus	duboisii	AM R41520	Ashmore Reef	Australia
Aipysurus	duboisii	AM R42024	Ashmore Reef	Australia
Aipysurus	duboisii	AM R44459	Ashmore Reef	Australia
Aipysurus	duboisii	AM R44895	Scott Reef	Australia
Aipysurus	duboisii	AM R44861	Ashmore Reef	Australia
Aipysurus	duboisii	AM R44611	Ashmore Reef	Australia
Aipysurus	duboisii	AM R44631	Ashmore Reef	Australia
Aipysurus	duboisii	AM R44863	Scott Reef	Australia
Aipysurus	duboisii	WAM R73673	Pilbara	Australia
Aipysurus	duboisii	WAM R73653	Dampier	Australia
Aipysurus	duboisii	WAM R73658	Pilbara	Australia
Aipysurus	duboisii	WAM R44182	Exmouth Gulf	Australia
Aipysurus	duboisii	KLS0664	Broome	Australia
Aipysurus	duboisii	WAM R73666	Pilbara	Australia
Aipysurus	duboisii	WAM R154751	Exmouth Gulf	Australia
Aipysurus	duboisii	WAM R26416	Exmouth Gulf	Australia
Aipysurus	duboisii	WAM R156216	Exmouth Gulf	Australia
Aipysurus	duboisii	KLS1294	Pilbara	Australia
Aipysurus	apraefrontalis	WAM R129803	Ashmore Reef	Australia
Aipysurus	apraefrontalis	WAM R129805	Ashmore Reef	Australia
Aipysurus	apraefrontalis	MAGNT R17778	Ashmore Reef	Australia
Aipysurus	apraefrontalis	SAMA R68142	Ashmore Reef	Australia
Aipysurus	apraefrontalis	WAM R73667	Legendre Island	Australia
Aipysurus	apraefrontalis	WAM R14126		
Aipysurus	apraefrontalis	WAM R26415	Exmouth Gulf	Australia
Aipysurus	apraefrontalis	WAM R31443	Exmouth Gulf	Australia
Aipysurus	apraefrontalis	WAM R47748		
Aipysurus	apraefrontalis	Unaccessioned	Exmouth Gulf	Australia
Aipysurus	apraefrontalis	WAM R154750	Exmouth Gulf	Australia
Aipysurus	apraefrontalis	WAM R73668	Legendre Island	Australia

Aipysurus	apraefrontalis	WAM R179591	Exmouth Gulf	Australia
Aipysurus	apraefrontalis	WAM R43897	Exmouth Gulf	Australia
Aipysurus	apraefrontalis	WAM R157818	Exmouth Gulf	Australia
Aipysurus	apraefrontalis	AM R26716		
Aipysurus	apraefrontalis	WAM R22961	Roebourne	Australia
Aipysurus	apraefrontalis	AM R142926*		
Aipysurus	apraefrontalis	AM R44475	Ashmore Reef	Australia
Aipysurus	apraefrontalis	AM R42711	Ashmore Reef	Australia
Aipysurus	apraefrontalis	AM R37141	Ashmore Reef	Australia
Aipysurus	apraefrontalis	AM R40493	Ashmore Reef	Australia
Aipysurus	apraefrontalis	AM R44616	Ashmore Reef	Australia
Aipysurus	apraefrontalis	AM R44878	Ashmore Reef	Australia
Aipysurus	apraefrontalis	AM R37140*	Ashmore Reef	Australia

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Principal Author

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Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	10/03/2023

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Claire Goiran
Contribution to the Paper	Collected tissue samples and contributed to writing and editing of manuscript.

Signature		Date	10/03/2023

Name of Co-Author	Mathew Hourston		
Contribution to the Paper	Facilitated engagement with fisheries for sample collection, assisted with creating figures, contributed to writing and editing of manuscript.		
Signature		Date	14 March 2023

Name of Co-Author	Richard Shine		
Contribution to the Paper Assisted in writing paper	Collected tissue samples, provided morphological data and contributed to writing and editing of manuscript..		
Signature		Date	10 March 2023

Name of Co-Author	Arne R Rasmussen		
Contribution to the Paper	Collected tissue samples, provided morphological data and contributed to writing and editing of manuscript.		
Signature		Date	10 March 2023

Name of Co-Author	Vicki Thomson		
Contribution to the Paper	Assisted with labwork, contributed to writing and editing of manuscript.		
Signature		Date	13 March 2023

Name of Co-Author	Kate L. Sanders

Contribution to the Paper	Conceived of the research, assisted with all aspects of data collection and analysis, contributed to writing and editing of research.		
Signature		Date	15//03/2023

Chapter 3

A new species of turtle-headed sea snake (*Emydocephalus*: Elapidae) endemic to Western Australia

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A new species of turtle-headed sea Snake (*Emydocephalus*: Elapidae) endemic to Western Australia

JAMES H. NANKIVELL[1,6], CLAIRE GOIRAN[2,], MATHEW HOURSTON[3], RICHARD SHINE[4], ARNE R. RASMUSSEN[5], VICKI A. THOMSON[1] & KATE L. SANDERS[1]

[1]*School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia*

[2]*Labex Corail & ISEA, Université de la Nouvelle-Calédonie, Nouméa, New Caledonia*

[3]*Department of Primary Industries and Regional Development, Perth, Western Australia, Australia*

[4]*Department of Biological Sciences, Macquarie University, NSW, Australia; School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia*

[5]*The Royal Danish Academy of Fine Arts, Schools of Archiecture, Design and Conservation, (KADK), Copenhagen, Denmark*

[6]*Corresponding author. E-mail: james.h.nankivell@adelaide.edu.au*

Abstract

We describe a new species of turtle-headed sea snake *Emydocephalus orarius* **sp. nov.** (Elapidae) from Western Australia's Coral Coast, Pilbara and Kimberley regions. Phylogenetic analysis of mitochondrial markers places the new species as the sister lineage to the two currently recognised species in *Emydocephalus*: *E. annulatus* from the Timor Sea reefs and Coral Sea, and *E. ijimae* from the Ryukyu Islands. Analysis of nuclear SNP data from the new species and *E. annulatus* from Australia and New Caledonia provides additional independent evidence of their evolutionary distinctiveness. The new taxon is usually morphologically diagnosable from its congeners using a combination of scalation and colour pattern characters, and appears to reach greater total lengths (>1 m in the new species versus typically ~80 cm in *E. annulatus/E. ijimae*). The new species is known largely from soft-bottomed trawl grounds, unlike *E. annulatus* and *E.ijimae* which usually inhabit coral reefs. The discovery of this new species brings the number of sea snake species endemic to Western Australia to six.

Key words: Hydrophiinae, marine,mitochondrial, RADseq, Australia, systematics

Introduction

Turtle-headed sea snakes in the genus *Emydocephalus* (Elapidae: Hydrophiinae) are fully marine snakes that primarily occupy shallow coral reef habitats in Australia, New Caledonia, the Phillippine Islands, Vietnam (only one specimen collected), and Japan (Minton & Heatwole, 1975; Dotsenko, 1999; Ineich and Laboute 2002; Rasmussen and Ineich 2010). They reach a maximum total length of ~1 m, are diurnal, and have an oophagous diet limited to the eggs of demersal spawning fish. (Guinea 1996; Rasmussen and Ineich 2010; Goiran *et al*. 2013). Specialist oophagy in *Emydocephalus* is linked to a morphologically and biochemically degenerated venom system (Minton, 1983) and a foraging behaviour that, unusually among macrostomate snakes, involves slow and continuous browsing reminiscent of herbivorous mammals (Shine *et al*., 2004).

In his 1926 monograph on sea snake diversity, Smith recognised two species in *Emydocephalus*: *E. annulatus* Krefft, 1896 and *E. ijimae* Stejneger, 1898. *E. annulatus* has a disjunct distribution in the Timor Sea and Coral Sea, and was described in 1869 by Krefft, who gave the type locality as 'probably the Australian seas'. *E. ijimae* was described by Stejneger in 1898 based on specimens from the Ryukyu Islands in Japan, but its distribution also includes the East China Sea off China and Taiwan. Most subsequent authors have followed Smith by recognising only these two species in *Emydocephalus* (e.g. Rasmussen & Ineich 2010; Kharin 2008; Voris 1966). However, Rasmussen & Ineich (2010) reported considerable overlap in the scalation and colour pattern characters used to distinguish *E. annulatus* and *E. ijimae*, and highlighted the difficulties in using these characters to assign populations that are located

between the two type localities in Japan and Australia to either species. Philippine *Emydocephalus* are currently of uncertain status but are at least superficially similar to *E. annulatus* as discussed by Rasmussen and Ineich (2010). In 2011, Dotsenko described the single specimen recorded from Vietnam (Dotsenko 1999) as a new species (*E. szczerbaki*). In an "Addition" to his description of *E. szczerbaki*, Dotsenko (2011b) noted the uncertain status of this species and based on the findings of Rasmussen and Ineich (2010) we regard *E. szczerbaki* as a synonym of *E. annulatus* (Cao *et al*., 2017). Phylogeographic studies of *Emydocephalus* have been limited to *E. annulatus* populations in the Australian region, but these revealed species-level mitochondrial and nuclear divergences between coastal Western Australian versus adjacent Timor Sea and more remote Coral Sea populations (Lukoschek 2018, Nitschke *et al*. 2018). Although no precise type locality has been given for *E. annulatus,* it is reasonable to expect that the type material was collected from the Coral Sea population for two reasons. First, almost all of Krefft's species descriptions are based on specimens collected on the east coast of Australia, and *E. annulatus* are occasionally beach-washed from South East Queensland to Sydney. Secondly, the type material agrees morphologically with specimens from the Coral Sea (M. Guinea, pers. comm.). Hoser (2016) named *Emydocephalus* populations from coastal Western Australia and the Timor Sea as a new species; however we follow the recommendations of Kaiser *et al*. (2013) and consider names published outside of the peer-reviewed literature post 2000 to be unavailable.

Here, we revise species boundaries in *Emydocephalus* by integrating mitochondrial and nuclear genetic data with morphological evidence and information on habitat preferences for populations in coastal Western Australia, the Timor Sea reefs, New Caledonia and Japan. Based on our results, we describe the *Emydocephalus* from coastal Western Australia as a new species, bringing the number of endemic sea snakes in Western Australia to six. We also reveal a close genetic affinity between *E. ijimae* from Japan and *E. annulatus* from the Timor and Coral Seas, and recommend further studies to infer the evolutionary history of these populations.

Materials and methods

Molecular Analysis

Sample collection.
Fifty individuals of *Emydocephalus* were sampled in the molecular analyses. This included 48 tail skin and liver tissues that were collected during fieldwork by the authors and other collaborators and two additional tissues gifted from museum collections. Cytochrome b sequences for 21 of these samples plus representatives of other *Aipysurus* group species were sequenced in Nitchske *et al*. (2018) and used here. No tissue samples were available with which to generate new data for *E.ijimae*, but unpublished mitochondrial NADH dehydrogenase subunit 4 (ND4) sequences were available for this species (see below).

DNA Extraction and quantification.
Whole genomic DNA was extracted from skin and liver samples using a Gentra Puregene kit, following the standard protocol. If DNA yield was expected to be low, 0.5 µL of glycogen was added after the protein was discarded. DNA was quantified using a Quantus fluorometer, operated as per the manufacturer's instructions, to ensure at least 200 ng/µL was available for optimal RADseq library creation. Any samples that did not reach this threshold were excluded from further steps in the RADseq data generation. Samples to be used in RADseq were either diluted or concentrated using a Centrivap DNA concentrator.

Mitochondrial amplification and sequencing.
We generated 14 new *Emydocephalus* cytochrome b (cytb) mitochondrial sequences for this study. Fragments of up to 1,099 base pairs were amplified using forward primer L14910 (M460) (5'GAC CTG TGA TMT GAA AAA CCA YCG TTG T -3') and reverse primer H16064 (M461) (5'-CTT TGG TTT ACA AGA ACA ATG CTT TA -3') (de Queiroz *et al*., 2002). One lower quality tissue that did not successfully amplify for the full cytb fragment was amplified using modified primers which target a ~800 bp fragment of cytb; forward primer L14968 (M1270) (5'CCT TAT TAT TCT CCA ACC TTC TAC -3') and reverse primer H15951 (M1271) (5'-TGA TTG AGG CTG TTT GAC TGA TT -3').

Mitochondrial NADH dehydrogenase subunit 4 (ND4) and neighbouring tRNA-His and tRNA-Ser was sequenced for six samples from the Western Australian Coast, using the forward primer M245 (5'-TGA CTA CCA AAA

GCT CAT GTA GAA GC-3') and reverse M246 (5'-TTT TAC TTG GAT TTG CAC CA-3') (Arévalo *et al*., 1994). These ND4 sequences were aligned with six ND4 sequences available for *E. ijimae* and 12 sequences available for *E. annulatu*s from New Caledonia and the Timor Sea.

For all three mitochondrial fragments, PCR reactions comprised 25 µL: 16.5 µL double distilled water, 0.1 µL Immolase DNA Polymerase, 5 µL 5X MRT buffer, 1.2 µL each of forward and reverse primer and 1 µL of DNA template. All reactions were run on the same thermal cycling program of 10 min at 95 °C for initial denaturation, followed by 37 cycles of 45 s at 94 °C, 45 s at 52 °C for annealing, 1 min at 72 °C for extension with a final extension for 10 min at 72 °C.

PCR products were checked for successful amplification by gel electrophoresis and vacuum cleaned before Sanger sequencing at the Australian Genome Research Facility (AGRF) in Adelaide. Sequences were then checked by visual inspection of chromatograms using Geneious Pro v.11.0.5 (Drummond *et al*., 2010), calling bases as 'N' if ambiguous.

Mitochondrial sequence analysis and tree reconstruction.

Sequences were aligned using MUSCLE 3.8 (Edgar, 2004) in Geneious Pro v.11.0.5 (Drummond *et al*., 2018) using default settings, and then checked by eye. Mitochondrial cytb and ND4 genes were not concatenated because a minority of individuals were sequenced for both genes. Best-fit partitioning schemes and models of sequence evolution were selected using the program PartitionFinder2 (Lanfear *et al*., 2012). Based on the Bayesian Information Crierion (BIC), two partitions (codon positions 1 + 2: TRN+I; position 3: TRN+G) were selected for cytb, and three partitions were selected for ND4 (tRNA codon positions 1+2+3 with ND4 position 1: F81; ND4 position 2: F81; and ND4 position 3: HKY).

Phylogenetic tree reconstruction was carried out using Bayesian analysis in the program BEAST 2 (Bouckaert *et al*., 2014). Clock and tree models were linked with a strict clock selected and a coalescent constant population tree prior. To place an approximate timescale on the tree, a secondary node age calibration was applied to the root node (*Emydocephalus* + *Aipysurus*) using a normal distribution with a mean of 10 million years ago (Mya) and a standard deviation of 2.5 Mya based on the most recent molecular dating study of elapids (Lee *et al*. 2016). The Markov chain Monte Carlo (MCMC) was run for 10,000,000 generations, with trees sampled every 1000 generations. Convergence was checked using Tracer v1.6 (Rambaut *et al*., 2014) and the first 3,000 trees were removed as burn-in. The remaining 7,000 trees were used to generate a maximum clade credibility tree with TreeAnnotater v1.7 (Rambaut & Drummond, 2013). Initial BEAST runs for cytb using the TRN substitution model (selected using PartitionFinder) failed to reach convergence after 10,000,000 generations, the final analysis was run using the simpler HKY model of sequence evolution for both codon partitions. p-distances between lineages were calculated using the species delimitation plugin (Masters *et al*., 2011) in Geneious Pro v.11.0.5 (Drummond *et al*., 2010).

RADseq Library Preparation.

The double digest RADseq (ddRADSeq) library preparation protocol broadly followed Peterson *et al*. (2012). Samples were initially ordered on a 96-well plate according to original DNA yield as a proxy for quality. The restriction enzymes PstI and HpaI were added to each individual sample and incubated at 37 °C for 2 h. These enzymes were selected as they have proven informative for population genetics and phylogenetic analysis in the terrestrial elapid tiger snake, *Notechis scutatus* (Peters, 1861) (Vicki Thomson, unpublished data). In order to demultiplex each sample after pooling, each sample had a pair of adapters ligated to each end (A + B), one of which (A) contained a unique molecular tag that was used to match each fragment to a sample. The second Y-shaped adapter (B) was added to ensure only those samples containing adapters (A + B) were amplified in the next PCR step. In order to be sequenced on an Illumina machine the fragments of DNA were pooled together (as four sets of 24 samples each, along with samples from other studies) and amplified using Illumina primers (matching unique sections of adapters A + B). These pools were then size selected to a range between 100–300 bp using Ampure magnetic beads before being checked using an Agilent tapestation. The pooled library was then outsourced to AGRF for single-end Illumina sequencing on a NextSeq 1 x 75 bp High output run.

RADseq data filtering and assembly.

Raw ddRADseq data were demultiplexed using the program GBSX. Adapters were then removed and all reads were trimmed to 61bp using BBduk (Bushnell, 2014) as the rest of pipeline required all sequences to be of the same length. Only samples with over 100,000 reads were kept in the rest of pipeline, which resulted in a total of nineteen samples, including representatives of each available putative population. The Stacks pipeline (Catchen *et al*., 2013) was used

for the rest of the data processing and catalog creation. It was run on the University of Adelaide Phoenix High performance Computing System (HPC).

We used a *de novo* approach to assemble the RADseq loci and followed the optimisation process of Rochette & Catchen (2017). As different datasets will have different characteristics it is preferable to optimise parameters instead of relying on default settings. The ustacks program takes short-read sequences and aligns them into exactly matching stacks, from which putative RAD loci and their single nucleotide polymorphisms (SNPs) are derived. The maximum distance between stacks (-M) and minimum depth of coverage required for stacks creation (-m) were both set to three, a value that optimised the number of SNPs called. The cstacks module was used to create a catalogue of consensus loci from merged samples, after which we used the sstacks module to match samples back to the catalog. We set the minimum number of ingroup samples with data for a given locus to be retained in the final dataset to 90% of the samples. The output was formatted as a vcf file, which was then converted to other formats using a python script (Ortiz, 2018).

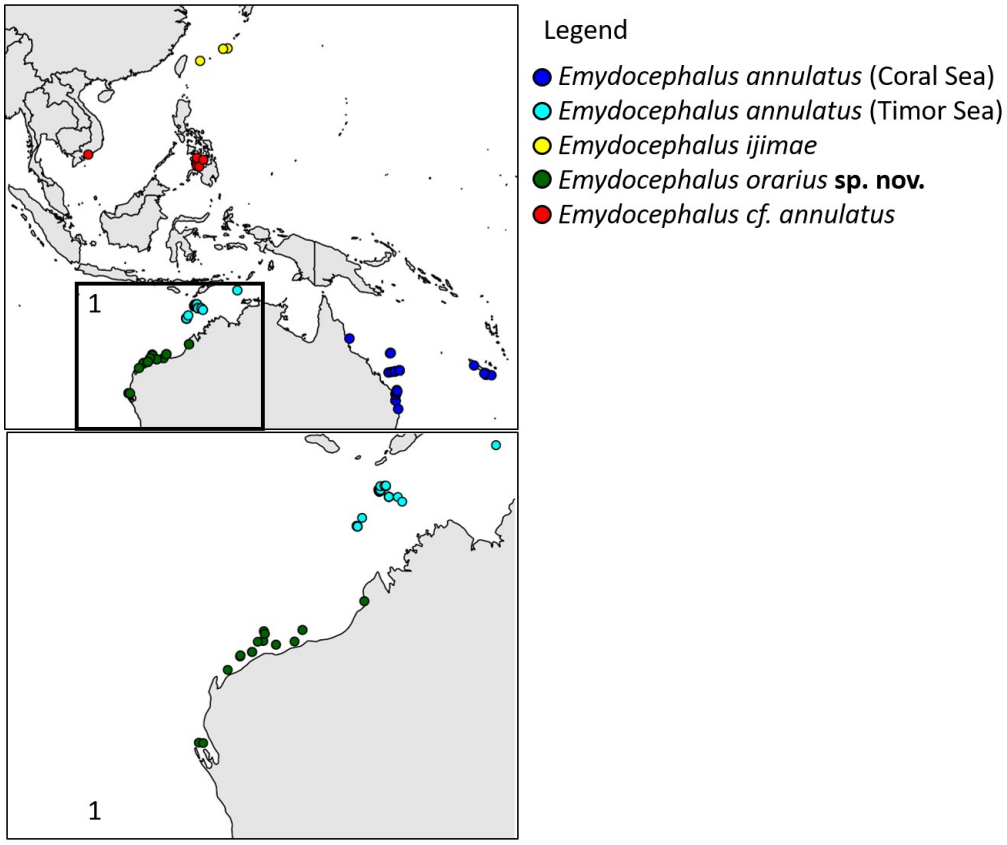

FIgurE 1. Distribution of *Emydocephalus* species. Compiled from tissue samples, museum records and literature (Alcala 2000; Dotsenko 2010; Rasmussen and Ineich, 2010). Green=*E. orarius* **sp. nov.**; yellow=*E.* ijimae; turquoise=*E. annulatus* (Timor Sea); blue=*E. annulatus* (Coral Sea); red=*E. cf. annulatus* (specific identity currently unclear).

RADseq analysis and tree reconstruction.

For phylogenetic tree reconstruction, 1639 biallelic SNPs were concatenated without invariant sites using the program RAxML 8.2.11 (Stamatakis, 2006) in Geneious Pro v.11.0.5 (Drummond *et al*. 2010). Unlike Bayesian analyses in BEAST, maximum likelihood analyses in RAxML can account for ascertainment bias (due to sampling only variable sites), which can otherwise result in incorrect tree topology and branch lengths (Leaché *et al*., 2015). A GTR+Γ site model was used (as has typically been applied in other RADseq studies e.g. Pauquet *et al*., 2018; de Oca *et al*., 2017), with a rapid bootstrapping algorithm and search for the highest likelihood tree algorithm. The number of bootstrap replicates was 1000 and a Lewis (2001) ascertainment bias correction was applied. Trees were visualised using FigTree 1.4.3 (Rambaut, 2012).

Morphological Analysis.

Our definitions of external morphological characters follow Smith (1926) with additional characters from Rasmussen *et al*. (2014). Costal scale counts were measured in a transverse series between (but not including) ventral scales to ensure the most accurate count (Rasmussen *et al*., 2014). The neck and midbody scale counts were each repeated four times to ensure that these represent minimum and maximum counts for neck and midbody scales, respectively.

Generally the minimum scale count is found between two and four times the head length posterior to the neck while the maximum scale count usually occurs just posterior to the midbody. Additional, transverse counts of body scales were taken 10 ventrals anterior to the vent and at the mid tail. Ventral scale counts were obtained from the first enlarged scale on the neck to (but not including) the anal plate; subcaudal counts were obtained from the first scale posterior to the cloaca to the tip of the tail. Cephalic scalation was measured for both left and right sides where appropriate to incorporate variation in individual specimens.

The position of the posterior tip of the heart and the anterior tip of the liver was measured in relation to the number of ventral scales anterior to their position (Rasmussen, 1989). The relative % heart position (%VS-heart) and % liver position (%VS-liver) are given by the formula: organ position/number of ventral scales*100. Snout to vent length (SVL) and tail length (TAL) were measured using a measuring tape and summed to obtain total length in mm (TL). Sex was determined by the examination of sex organs by dissection, or by tail proportions and secondary characteristics (e.g. ventral scale rugosity or the presence of a rostral spine) if dissection was not possible. Additional notable features such as the condition of scales and their rugosity were also recorded.

results

Mitochondrial sequence analysis and tree reconstruction

The maximum clade credibility BEAST tree for cytb (Fig. 2) yielded relationships and branch lengths (between *Emydocephalus* and *Aipysurus* and among *Aipysurus* species) consistent with previous mitochondrial phylogenies (e.g. Lukoschek and Keogh 2006; Sanders *et al*., 2013a). Only *Emydocephalus* lineages were sampled for ND4 (Fig. 3). Both BEAST gene trees recovered a deep divergence between *Emydocephalus* sampled from coastal Western Australia and all other localities, i.e. the Timor (and Coral Seas in the cytb tree, and the Timor and Coral Seas plus *E. ijimae* from Japan rendering *E. annulatus* as previously defined paraphyletic in the ND4 tree (Fig. 3). The coastal Western Australian lineage was separated from the Timor Sea, Coral Sea and *E. ijimae* (Japan) clade by a corrected pairwise divergence of 3.8 % for ND4. For cytb, a pairwise divergence of 4.2 % was calculated between the coastal Western Australian lineage and Timor-Coral Sea clade, with a mean divergence time estimate of 2.1 Mya (1.01, 3.39 95% HPD). Both gene trees recovered closely related clades corresponding to the Timor Sea versus Coral Sea populations (with corrected pairwise divergences of 0.7 % and 0.9 % for cytb and ND4, respectively). In the ND4 tree, *E. ijimae* formed a close sister clade to the Timor Sea plus Coral Sea clade, with a corrected pairwise divergence of 0.9 %. Phylogenetic trees with all tips labelled with specimen numbers are available in the supplementary material.

Phylogenetic analysis of rADseq data

Both Bayesian and maximum likelihood analysis of concatenated nuclear SNP data strongly supported the reciprocal monophyly of *Aipysurus* and *Emydocephalus* (Posterior support=1, Bootstrap support=100). Within *Emydocephalus* the SNP trees topology was broadly consistent with that found using mitochondrial genes (Fig. 4). A sister relationship between the Western Australian Coastal population and the Timor Sea + Coral Sea clade was strongly supported. Some finer-scale population structure was detected in the Coral Sea clade with two moderately supported clades of New Caledonian samples, but these did not correspond to geographic locale. Because the other two populations have small sample sizes we decided not to carry out further analysis of finer-scale geographic population structure.

Morphological analysis

Only seven specimens were available for *Emydocephalus* from the Western Australia coast, yet significant differences between Western Australian Coastal and all other sampled *Emydocephalus* populations were found when each sex was compared separately. In male Western Australian Coastal *Emydocephalus,* mean values for nearly all characters differed significantly from *E. ijimae* despite some overlap in ranges (Table 1). Male *Emydocephalus* from the Western Australian Coast were closer morphologically to *E. annulatus* in the Timor and Coral Seas, but with significant (albeit overlapping) differences in the number of ventral scales. Too few female specimens (N=2) from the Western Australian Coast were examined to test the significance of variation in ventral scale count, but the counts from these animals were higher than the range for other species of *Emydocephalus* (Table 2), as it was for males. The first supralabial scale contacted the pre-ocular scale in 10 of 11 Western Australian Coastal *Emydcephalus* compared with only 4 out of 32 *Emydocephalus* from the Timor and Coral Sea (Fig. 5).

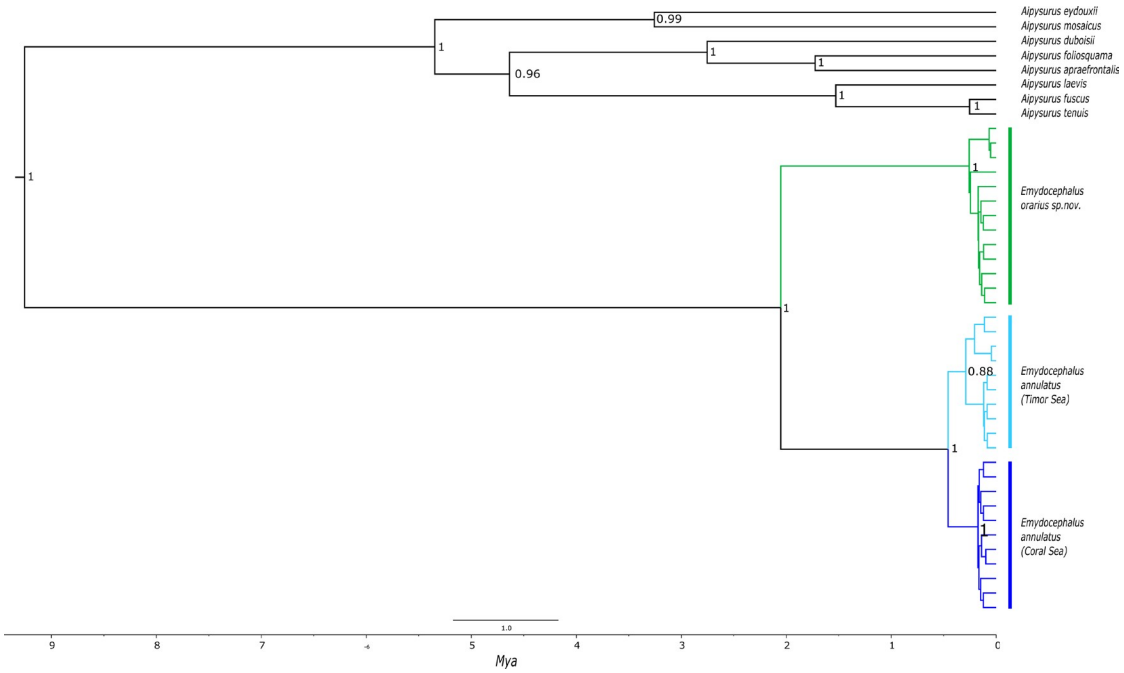

FIgurE 2. Cytochrome b maximum clade credibility BEAST tree for *Emydocephalus*. *Aipysurus* species included for comparison.

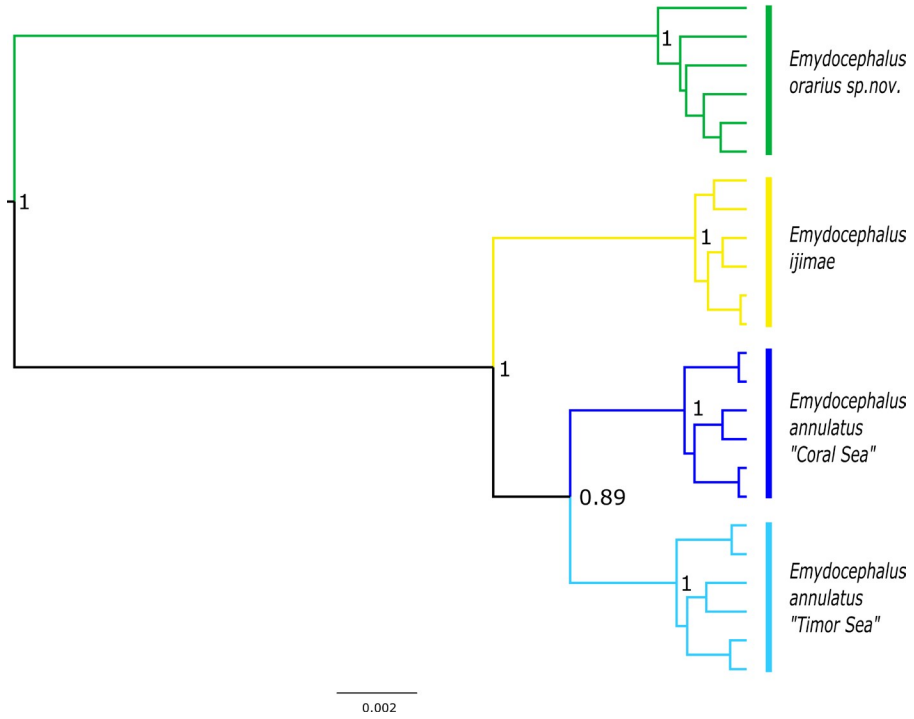

FIgurE 3. ND4 maximum clade credibility species. Scale bar represents nucleotide substitutions per base pair.

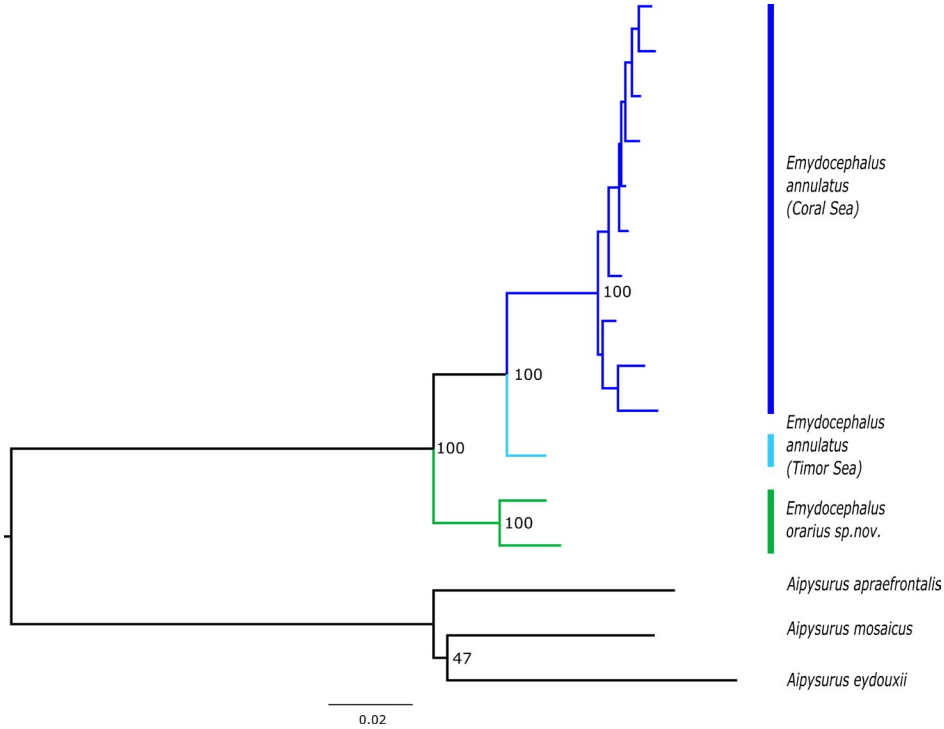

FIgurE 4. Maximum likelihood tree of *Emydocephalus* species using dataset. Scale bar represents nucleotide substitutions per base pair.

Other subtle differences in morphology were noted while examining specimens, including aspects of colour pattern and general size and shape. *Emydocephalus* species are highly variable in colour, but based on museum specimens (N=7) and photographs of live snakes (N=11) from the Department of Primary Industries and Regional Development (DPIRD), specimens from coastal Western Australian fell into two broad groupings based on colour pattern. Individuals from Shark Bay generally had well defined high-contrast white bands on a black background (Fig. 5A–C, 7B), while those from the Pilbara were heavily spotted with poorly defined bands (Fig. 5D, 7A). No completely melanistic individuals from coastal Western Australia were noted in this study, but melanistic individuals accounted for 18 % of Timor Sea *Emydocephalus* (N=114) and 74 % from the Coral Sea (N=1335) (Goiran *et al*., 2017). Western Australian Coastal *Emydocephalus* generally obtain a greater total length than *Emydocephalus* from other regions with a mean of 98 cm and maximum of 116 cm (N=14). Mean total length for Timor Sea *Emydocephalus* was 69 cm with a maximum length of 98 cm (N=7) while those from New Caledonia reached a mean of 61.1 cm with a maximum of 91.7 cm (N=1269) (R. Shine and C. Goiran, unpubl. data).

Taxonomy

On the basis of nuclear DNA, mitochondrial DNA and morphological evidence we regard the coastal Western Australian *E. annulatus* as an evolutionarily distinctive new species with no available name. A mitochondrial phylogeny places the new species as the sister lineage to the two currently recognised species in *Emydocephalus (E. annulatus* and *E. ijimae*), and high-resolution nuclear SNP data provide evidence of a lack of interbreeding between the new species and adjacent allopatric *Emydocephalus* populations in the Australian region. While the new species usually differs in morphology from its congeners there are no unambigous and non-overlapping diagnostic characters, therefore locality or genetic information may be required for positive identification.

Emydocephalus orarius sp. nov.

Fig. 5, 7A–B

Holotype. WAM R165708. Adult male collected in Shark Bay (25°15`38”S, 113°08`19”E), WA on 10/02/2006 by G. Parry.

Paratypes. WAM R73661 (female), Pilbara coast (20°00`00”S, 116°40`00”E); WAM R47852 (male), Barrow Island (20°48`00”S, 115°24`00”E); WAM R73651 (male), Pilbara coast (19°26`00”S, 118°49`00”E); WAM R73662

(male), Legendre Island (19°30`00”S, 116°42`00”E); WAM R174521 (female), Dampier (20°39`00”S, 116°41`00”E).

generic Assignment. *Emydocephalus orarius* **sp. nov.** can be assigned to the genus *Emydocephalus* by possessing three supralabial scales, the second of which is much larger than the others and occupies most of the border of the mouth.

FIgurE 5. Holotype of *Emydocephalus orarius* **sp. nov.** WAM R165708 dorsal (A); Ventral (B); cephalic scalation (C); and paratype WAM R73661 (D)

Diagnosis. A large (maximum recorded 116 cm TL) *Emydocephalus* species that usually differs from its congeners by the possession of two prefrontal scales, first supralabial in contact with preocular scale, enlarged tubercules on ventral scales of adult males, body pattern consisting of 19–21 bands. Genetically diagnosed from all other sequenced *Emydocephalus* by the following ND4 sites: 84(C), 111(A), 177(C), 195(C), 234(C), 261(C), 273(T), 288(C), 294(C), 342(T), 357(C), 396(T), 414(C), 486(G), 510(G), 569(C), 576(C), 609(C), 651(A), 667(G), 669(T).

Comparisons. *Emydocephalus orarius* **sp. nov.** can usually be distinguished from *E. ijimae* in having two prefrontal scales (versus usually three or four pre-frontal scales), an elongated first supralabial scale which contacts the pre-ocular and often in colour pattern with a lower and non-overlapping number of bands when they are present (19–21 versus 24–32 in *E. ijimae*). Male *E. orarius* **sp. nov.** also have higher mean numbers of ventral scales (144.8 versus 141.7 in *E. ijimae*) and subcaudal scales (32.3 versus 27.8 in *E. ijimae*) and lower mean scale rows around the midbody (17 versus 18.1 in *E. ijimae*) and neck (15 versus 16.1 in *E. ijimae*) (Table 1). Mature male *E. orarius* **sp. nov.** also possess tubercules on the body whereas in *E. ijimae* these are absent. The two female *E. orarius* **sp. nov.** available for study also have a higher number of ventral scales (at least 144 versus less than 142 in *E. ijimae*). *Emydocephalus orarius* **sp. nov.** can usually be distinguished *E. annulatus* populations in the Australian region (both

Timor Sea and Coral Sea) in having a higher number of ventral scales (mean 144.7 versus 136.9 in *E. annulatus* (Tables 1 and 2); usually possessing a distinctively elongated first supralabial scale that contacts the pre-ocular scale (10 out of 11 individuals examined) versus a much smaller first supralabial that does not contact the pre-ocular (4 of 32 *E. annulatus* examined did have an enlarged first supralabial contacting the pre-ocular) (Fig. 6).

Description of holotype. Adult male, snout to vent length (SVL) 60.4 cm. Tail length 12.6 cm. In an excellent state of preservation. Body moderately slender, increasingly laterally compressed posteriorly. Head approximately the same width as the neck, short and rounded both from above and in profile. Rostral scale is small, five-sided, with a slight projection pointing anterio-ventrally. Scale sensilla present all over head and particularly numerous on snout. Nasal scales large and symmetrical, with valvular nostrils on the posterior edge of the nasal scales orientated dorsally. Two prefrontals each penetrating shallowly between frontal and supraocular. Frontal rounded, penetrating shallowly between prefrontals. Two parietals, each large and symmetrical. One preocular and two postoculars on each side. Three supralabials on each side. First supralabial contacts nasal and preocular excluding the second supralabial from touching the nasal, second supralabial is larger than other supralabials and contacts the preocular, eye, postocular and lower anterior temporal scale. Three anterior temporals on right side due to fragmentation of the upper, two anterior temporals on the left side. Mental scale small and triangular. Four infralabials on each side. First two infralabials barely contacting the margin of the mouth, while the third infralabial is enlarged and occupies most of the border of the mouth. Two pairs of sublinguals, well developed and broadly in contact with each other, anterior pair of sublinguals in contact with first, second and third infralabials, posterior pair of sublinguals only in contact with the third supralabial.

Body scales large and imbricate, 15 rows near the neck, 17 at midbody and 15 anterior to the vent. Posterior dorsal scales are enlarged from just anterior to the vent until the tip of the tail. Ventrals broad, three to four times the width of adjacent body scales with a slight median keel, numbering 145 excluding the anal plate. Paddle-shaped tail is long (21% of total length) with a highly enlarged terminal scale, with nine scale rows around the mid-tail, 36 subcaudal scales.. Posterior tip of heart and anterior tip of liver are at ventral scale number 44, 30.3 % of the total ventral count.

Colouration in preservative. Head dark brown, with cream markings along the posterior edge of the prefrontal scales and around the postocular and temporal scales. A single thin cream ring encircles the posterior edge of the head. Ground colour is dark brown with 21 white bands. White bands are usually separated by at least one dark scale on the dorsal surface and are usually
offset from each other. Dark centres to white scales on the dorsal three or four scales. Dark brown bands are immaculate. Five offset white bands on the tail. Ventral surface entirely dark brown.

In life. Colour pattern similar but dark brown regions were black resulting in higher contrast pattern.

Variation in *Emydocephalus orarius* sp. nov. The following description refers to all seven *Emydocephalus orarius* **sp. nov.** specimens examined (5 males, 2 females) including the holotype and all paratypes.

External morphological characters. One preocular and two postoculars in all seven specimens. Three supralabials in 7/7. Only the first supralabial in contact with nasal (6/7), first and second supralabials in contact with the nasal (1/7). First and second supralabials in contact with the preocular (6/7), only the second supralabial in contact with nasal in one specimen. Only the second supralabial in contact with the eye (7/7). Two anterior temporals on each side except in the holotype, which has three on the right side. Infralabials 3/3 (left/right) in five specimens, 3/2 in one specimen and 4/4 in the holotype. Two pairs of large well-developed sublinguals in all specimens. Anterior pair of sublinguals in contact with only the first infralabial in three specimens, the first and second in one specimen and the first three infralabials in one specimen. Posterior pair of sublinguals in contact with first and second infralabials in three specimens, the first, second and third infralabials in contact in one specimen and only the second infralabial in contact in one specimen. Fifteen scale rows on the neck (7/7), 17 scale rows near midbody (7/7) and 15 scale rows ten ventral anterior to the vent (7/7). Nine scale rows around the tail (6/7), eight scale rows around the tail in one specimen. Ventrals 144–146, enlarged and three to four the size of adjacent body scales in all specimens. Subcaudals 31–36 in male specimens, unknown in females as neither specimen has a complete tail. Mean SVL in males 69.1 cm (49.5–85.0 cm, N=5), mean tail length 12.2 cm (9–13.8 cm, N=4). SVL of the two females are 85.5 cm and 87 cm. Numerous specimens released following capture in research trawls from the Pilbara coast were in excess of 110 cm total length with a maximum of 116 cm (DPIRD, unpublished data), combined data is presented in results.

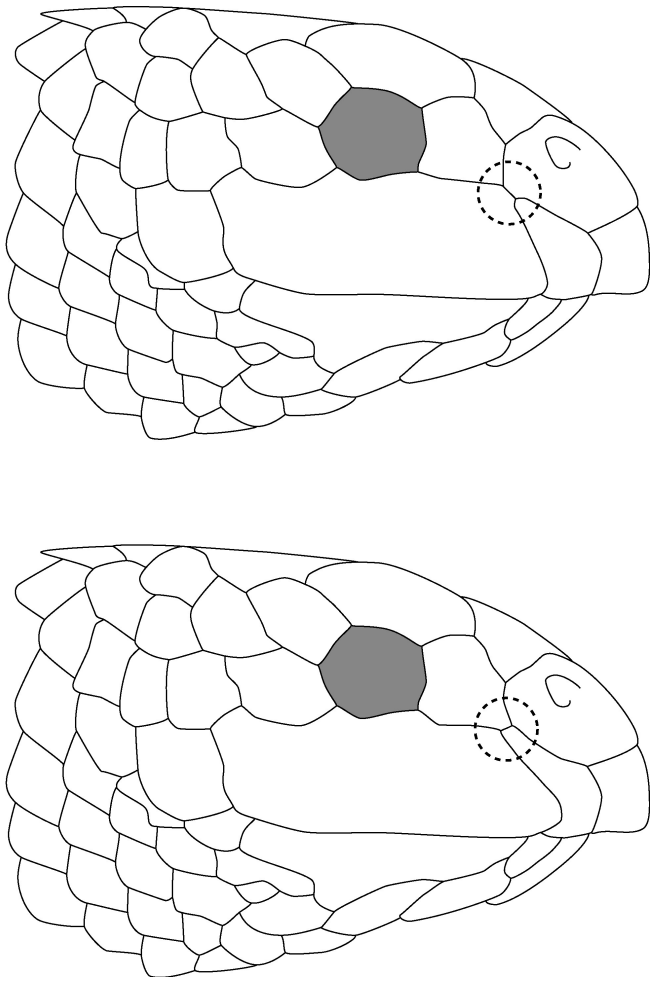

FIgurE 6. Typical cephalic scalation in *Emydocephalus annulatus* (upper) and *Emydocephalus orarius* **sp. nov.** (lower)

Internal morphological characters. In males posterior tip of heart between ventral scale numbers 42–44, %VSheart 29.2%–30.3%, anterior tip of liver at ventral scale 44, heart-liver gap 0–2. In the only female measured posterior tip of heart at ventral scale 47, %VS-heart 32.4 %, liver at anterior tip of liver at ventral scale 48 in female, %VS-liver 33.1 % in female, heart-liver gap of 1.

Colouration. Colour pattern highly variable but appears to consist of two broad geographic variants. (1) a banded form from Shark Bay and (2) a form with indistinct bands and often heavy spotting from the Pilbara coast. Animals from Shark Bay usually have 19–22 highly contrasting black and white bands on the body, although sometimes these do not meet on the dorsal surface, instead forming off-centre bars. Relative width of the bands is variable, with black bands broader in some specimens and white bands broader in others. Black spotting is sometimes present in the white bands on only the dorsal surface, white spotting in the black bands is usually absent. The head ranges from almost entirely white to cream to black with a white bar across the pre-frontal scales. Animals from the Pilbara coast usually have indistinct white bands on a black or dark brown body. Scales in the white bands usually have a black centre, which in some highly contrasting animals forms a densely speckled pattern. Some animals are largely black with only a trace of white bars on the side, but no completely melanistic specimens were recorded. The Pilbara coast animals usually have a black head with white or cream mottling, but it is highly variable among individuals.

Distribution. The new species is endemic to coastal Western Australia, with specimens from Shark Bay, Exmouth Gulf, Pilbara coast and Broome but not from the intervenening Ningaloo Reef..

TAblE 1. Morphological data for male *Emydocephalus.* Significance of differences between mean values was tested between *E. orarius* **sp. nov.** and each remaining *Emydocephalus* population as identified with molecular methods using a two-tailed Students t-test. Characters which differ significantly from *E. orarius* **sp. nov.** are labelled in **bold**.

Characters	***Emydocephalus orarius* sp. nov.**	***Emydocephalus ijimae***	***Emydocephalus annulatus* (CS)**	***Emydocephalus annulatus* (Timor Sea)**
	N=5	N=34	N=16	N=35

SR-neck	15 (15, SD=0)	**16.09 (15–17, SD=0.67)**	15.13 (15–16, SD=0.34)	14.89 (13–15, SD=0.4)
		P<0.001	P=0.43	P=0.5
SR-body	17 (17, SD=0)	**18.12 (16–19, SD=0.91)**	16.94 (16–17, SD=0.25)	16.63 (15–17, SD=0.77)
		P=0.01	P=0.59	P=0.24
SR-ant. vent	15 (15, SD=0)	14.85 (14–15, SD=0.36	15 (14–16, SD=0.41)	14 (13–16, SD=1.05)
		P=0.37	P=1	P=0.176
Body bands	20 (19–21, SD=1.15)	**28.22 (25–32, SD=2.08)**	**25 (25, SD=0)**	23 (19–30, SD=0.70)
		P < 0.001	P < 0.005	P=0.11
Tail bands	4.5 (3–5, SD=1)	**6.56 (5–8, SD=0.7)**	5 (5, SD=0)	5 (4–6, SD=0.7)
		P < 0.001	P=0.54	P=0.32
Ventrals	144.8 (144–146, SD=0.84)	**141.68 (138–148, SD=2.28)**	**139.69 (135–146, SD=2.94)**	**137 (130–144, SD=3.24)**
		P < 0.005	P < 0.005	P < 0.001
Subcaudals	32.25 (31–35, SD=1.89)	**27.77 (25.34, SD=1.76)**	31.13 (29–33, SD=1.09)	**29.71 (26–32, SD=1.31)**
		P < 0.001	P=0.13	P < 0.01
%VS-Heart	29.73 (29.17–30.3, SD=0.8)	**31.39 (29.50–33.33, SD=0.91)**	30.98 (29.08–32.87, SD=1.18)	29.65 (26.62–31.58, SD=1.31)
		P=0.02	P=0.18	P=0.73
%VS-Liver	30.45 (30.35–30.56, SD=0.15)	**32.53 (30.22–34.39, SD=1.00)**	31.57 (29.79–33.57, SD=1.11)	30.07 (26.62–32.64, SD=1.34)
		P < 0.01	P=0.18	P=0.69

Etymology. The species epithet 'orarius' (Latin, 'coastal') refers to the coastal Western Australian distribution of the new species. Other species of *Emydocephalus* are found on coral reefs, typically on clear oceanic reefs some distance away from coastlines of major landmasses.

Natural History. No diet records are reported for *Emydocephalus orarius* **sp. nov.** but it likely feeds exclusively on fish eggs like other members of the genus. Habitat preferences are poorly known but it appears to inhabit soft-bottomed sea floors as the majority of specimens are known from trawls over these substrates. Animals from the Pilbara coast have mostly been caught in waters 40–60 m deep, while those from Shark Bay have been caught in water <20 m deep (DPIRD, unpublished data). Specimens have mostly been collected during the day, indicating that *E. orarius* **sp. nov.** is diurnal like its congeners.

Discussion

In the context of the recent radiation (~ 9–18 Million years ago [Mya]) (Lee *et al*., 2016) of hydrophiine sea snakes, *E. orarius* **sp. nov.** is an evolutionarily distinctive new species. Our approximate divergence timescale (based on a secondary node age calibration of a single mitochondrial gene) suggests that *E. orarius* **sp. nov.** last shared a common ancestor with its congeners ~2 Mya. This timing is broadly coincident with the divergence of morphologically diagnosed sister species in the *Aipysurus-Emydocephalus* clade (e.g. *A. foliosquama-A. apraefrontalis*, *A. mosaicus-A. eydouxii*). Cryptic speciation among allopatric populations of *Emydocephalus* was suspected in light of their strong philopatric behaviour and limited dispersal capabilities (Lukoschek & Shine, 2012). However, the geographical patterns of genetic structure reported here do not conform to the expectations of isolation by distance or marine biogeographic history. Geographically proximate Western Australian Coastal and Timor Sea lineages of *Emydocephalus* are separated by relatively deep mitochondrial divergences (pairwise 3.8 % ND4; 4.1 % cytb), whereas the reciprocally remote Timor Sea and Coral Sea *E. annulatus* and Japanese *E. ijimae* form a shallow clade with less than 1% pairwise ND4 divergence. Nuclear SNP and cytb analyses did not include *E. ijimae* but were

consistent with the ND4 analysis in recovering a Timor Sea-Coral Sea *E. annulatus* clade. These *E. annulatus* populations are separated by ~3000km and a historical land barrier has impeded marine dispersal across the Torres Strait throughout most of the Pleistocene (Wilson, 2013). Shallow mitochondrial and nuclear divergences nonetheless suggest recent connectivity between disjunct *E. annulatus* populations on the oceanic reefs of the Timor and Coral Seas. Denser population sampling is needed to examine the dispersal history of *E. annulatus* in the Australia-Pacific region.

TAblE 2. Morphological data for female *Emydocephalus.* Significance was not tested due to a lack of female *E. orarius* **sp. nov.** specimens.

Characters	***Emydocephalus orarius* sp. nov.**	***Emydocephalus ijimae***	***Emydocephalus annulatus* (CS)**	***Emydocephalus annulatus* (Timor Sea)**
	N=2	N=22	N=10	N=23
SR-neck	15 (15, SD=0)	16.05 (15–19, SD=1.05)	15 (15, SD=0)	14.96 (14–16, SD=0.37)
SR-body	17 (17, SD=0)	17.09 (16–18, SD=0.42)	17 (17, SD=0)	16.91 (16–17, SD=0.29)
SR-ant. vent	15 (15, SD=0)	14.44 (13–15, SD=0.70)	14.88 (13–16, SD=0.83)	14.40 (13–16, SD=1.05)
Body bands	N/A	26.94 (24–29, SD=1.59)	26.33 (26–27, SD=0.58)	23.6 (21–25, SD=1.67)
Tail bands	N/A	5.28 (5–6, SD=0.46)	5 (5, SD=0)	4 (3–5, SD=0.71)
Ventrals	144.5 (144–145, SD=0.7)	**138.17** **(136–142, SD=1.53)**	**137.4** **(133–142, SD=2.76)**	**134.7** **(128–142, SD=3.46)**
Subcaudals	N/A	21.65 (19–25, SD=1.53)	26.1 (25–27, SD=0.74)	24.52 (21–28, SD=1.59)
%VS-Heart	32.4 (32.4, SD=0)	30.82 (28.78–31.89, SD=0.91)	31.22 (29.93–33.09, SD=1.02)	29.72 (28.36–30.66, SD=0.71)
%VS-Liver	33.1 (33.1, SD=0)	31.75 (30.22–33.33, SD=0.98)	31.51 (29.93–33.09, SD=0.96)	29.77 (28.36–30.66, SD=0.77)

The proximity of the continental shelf in Western Australian to the oceanic Timor Sea reefs varied dramatically during the Pleistocene, being closest during periods of low sea levels (Wilson, 2013). Treml *et al*. (2015) identified a semi-permeable barrier to marine dispersal between the Western Australian Coast and the Timor Sea reefs, and sea snakes show shallow genetic structure within several species (and a species divergence, between *A. fuscus* and *A. tenuis*) consistent with restricted gene flow across these regions (Nitschke *et al*., 2018; Lukoschek, 2018). However, preliminary observations of habitat preferences and body size variation suggest an important role for ecological factors in the isolation of *E. orarius* **sp. nov.** from its congeners. *Emydocephalus annulatus* and *E. ijimae* are usually found on coral reefs remote from continental coastlines (Alcala *et al*. 2000; Lukoschek *et al*. 2007; Minton and Dunson, 1985), and are consequently very rare in prawn trawl by-catch (e.g. Wassenberg *et al*. 2001). In contrast, all *E. orarius* **sp. nov.** specimens known to us were collected over soft-bottomed (non-reef) habitats from Shark Bay to Broome, primarily during research trawls and commercial prawn trawls. Further evidence that *E. orarius* **sp. nov.** does not share a strong preference for reef habitats with its congeners is that it has been collected in the relatively turbid Exmouth Gulf but is not known from the adjacent Ningaloo Reef, which offers extensive reef habitat and has a relatively well-documented marine fauna. Given that the earliest branching lineages in *Aipysurus* (the sister clade to *Emydocephalus*) are not reef-associated either, it is possible that the specialisation for reef habitats evolved relatively recently in *Emydocephalus*, i.e. in the approximately 500,000-year-old *E. annulatus-E. ijimae* lineage.

FIgurE 7. *Emydocephalus* species in life: *Emydocephalus orarius* **sp. nov** from Pilbara (A) and Shark Bay (B) (DPIRD), *Emydocephalus annulatus* from the Timor Sea (C) (Jenna Crowe-Riddell) and *Emydocephalus ijimae* from Okinawa (D) (Vladimir Dinets).

In addition to their divergent habitat preferences, available data and observations suggest that *E. orarius* **sp. nov.** is also longer and more heavy-bodied than the primarily reef-dwelling *Emydocephalus* species. Variation in body size is closely linked to numerous life history traits in reptiles, including fecundity and niche occupation (e.g. Shine 1994; Fitzgerald *et al*. 2004), and in sea snakes body length and girth have been correlated with trophic differentiation (Shine *et al*. 2002; Brischoux *et al*. 2007; Sherratt *et al*. 2018). Our study shows that the primary evolutionary divergence in *Emydocephalus* is between a putatively non reef-associated, coastal species restricted to Western Australia, and an adjacently distributed reef-specialist clade (*E. annulatus-E. ijimae*) with a disjunct range that spans much of the Australian-Pacific region. Thus, it seems likely that ecological isolation has been an important driver of speciation in *Emydocephalus* in addition to geographic distance and other extrinsic biogeographic barriers.

While this paper describes a new species of *Emydocephalus* from Western Australia, bringing the number of endemic sea snakes in this region to six**,** it also provides molecular evidence that the Asian taxon *E. ijimae* is very closely affiliated to *E. annulatus* from Australia and New Caledonia. However, although the six *E. ijimae* specimens sequenced were found to be separated from *E. annulatus* (in the Timor and Coral Seas) by less than 1% pairwise mitochondrial ND4 divergence, this species is morphologically distinctive (Rassmusen and Ineich, 2010) and differs from it congeners in traits related to mating behaviour (males lack rugosity on their scales; Wall, 1909). Hence, it is possible that *E. ijimae* and *E. annulatus* are reproductively isolated species with recent divergence times that are comparable to other morphologically diagnosed species pairs such as *Aipysurus fuscus+A. tenuis* (Nitschke *et al*. 2018) and *Hydrophis cyanocinctus+H. melanocephalus* (Sanders *et al*. 2013b). It is also possible that with further molecular and morphological analyses the Timor Sea population will warrant recognition as a separate species, bringing the number of allopatric species in the clade to three. To resolve these questions, future studies should aim to quantify introgression across the region by extending nuclear SNP sampling to *E. ijimae* and geographically intermediate populations in the Philippines and Vietnam.

Data Accessibility

Data associated with this paper has been deposited in the Dryad online data repositroy under doi:10.5061/dryad.v41ns1rrv as follows: Cytochrome b alignment (1) ddRADseq alignment (2) ND4 alignment (3) Cytochrome b maximum clade credibility tree with tips labelled (4) ND4 maximum clade credibility tree with tips labelled (5) ddRADseq maximum likelihood tree with tips labelled (6) List of specimens examined for morphological data (7).

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Name of Co-Author	Brad Maryan
Contribution to the Paper	Conceived of research, collected and analysed morphological data, contributed to the writing and drafting of the manuscript

Signature		Date	10/03/2023

Name of Co-Author	Brian Bush		
Contribution to the Paper	Conceived of research, collected and analysed morphological data, contributed to the writing and drafting of the manuscript		
Signature		Date	10/03/2023

Name of Co-Author	Mark Hutchinson		
Contribution to the Paper	Conception and scope of paper, assistance with writing and editing		
Signature		Date	14/03/2023

Chapter 4

Whip it into shape: Revision of the *Demansia psammophis* (Schlegel, 1837) complex (Squamata: Elapidae), with a description of a new species from central Australia

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Whip it into shape: Revision of the *Demansia psammophis* (Schlegel, 1837) complex (Squamata: Elapidae), with a description of a new species from central Australia

JAMES H. NANKIVELL[1,*], BRAD MARYAN[2], BRIAN G. BUSH[3] & MARK N. HUTCHINSON[1,4,5]

[1]*School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, AUSTRALIA.*

https://orcid.org/0000-0003-0521-7383

[2]*Research Associate, Collections & Research, Western Australian Museum, 49 Kew Street, Welshpool, Western Australia 6106, AUSTRALIA. lerista@bigpond.com; https://orcid.org/0000-0003-0349-0255*

[3]*Snakes Harmful & Harmless, 9 Birch Place, Stoneville, Western Australia 6081, AUSTRALIA.*

bush@iinet.net.au; https://orcid.org/0000-0002-3487-6620

[4]*South Australian Museum, North Terrace, Adelaide, South Australia 5000, AUSTRALIA.*

Mark.Hutchinson@samuseum.sa.gov.au; https://orcid.org/0000-0003-2910-1983

[5]*Faculty of Science & Engineering, Flinders University, Bedford Park, South Australia 5042, AUSTRALIA.*

[*]*Corresponding author. james.h.nankivell@adelaide.edu.au*

Abstract

The genus *Demansia* Günther is the most diverse genus of Australian terrestrial elapids. A phylogenetic framework for the familiar but problematic taxa *D. psammophis* and *D. reticulata* (Gray) has been long overdue to ascertain interspecific relationships and resolve unclear taxonomic issues. Here we present an integrated molecular phylogenetic and morphological analyses to review species delineation, resulting in confirmation that both *D. psammophis* and *D. reticulata* are full species and that some populations referred to *D. r. cupreiceps* Storr are not distinguishable from more typical *D. reticulata*. We also find the widespread central Australian population (treated by most authors as part of *cupreiceps*) to be specifically distinct. We redescribe *D. psammophis* and *D. reticulata* to clarify morphological and geographical boundaries and describe *D. cyanochasma* **sp. nov.** based on a combination of molecular genetic markers, details of colour and pattern, adult total length and a few morphometric attributes. We also designate a lectotype for *D. psammophis* from the original syntype series and comment on the necessity for further taxonomic refinement of this distinctive group.

Key words: *Demansia cyanochasma* **sp. nov.**, morphology, mtDNA, nDNA, phylogeny, Reptilia, synonymy, taxonomy, whip snakes

Introduction

The slender, large-eyed ‘whip snake’ or ‘racer’ morphotype has evolved multiple times within colubroid snakes. Genera such as *Psammophis* Fitzinger (Psammophiidae), *Masticophis* Baird & Girard, *Platyceps* Blyth, *Pytas* Fitzinger (Colubrinae) and *Echinanthera* Cope (Dipsadinae), comprise snakes with this body form adapted for active predation and rapid movement through open terrestrial habitats such as grasslands and shrublands (Shine 1980). Among the Australian elapids, the genus that best exemplifies this morphotype is *Demansia,* a clade of small to moderately large, diurnal, oviparous snakes distributed over much of Australia, with one species extending into southern New Guinea (Storr *et al.* 2002; Shea & Scanlon 2007; Cogger 2018; Wilson & Swan 2021). All species appear to rely on vision and speed to prey on equally fast-moving diurnal lizards (Shine 1980).

The species of *Demansia* form a well-defined clade that has remained stable since being redefined as distinct from *Pseudonaja* Günther (Worrell 1963; McDowell & Cogger 1967). Morphological (McDowell & Cogger 1967) and

molecular (Strickland *et al*. 2016; Lee *et al*. 2016) data show that *Demansia* is phylogenetically closer to the rainforest genus *Aspidomorphus* Fitzinger endemic to New Guinea than to the ecologically similar *Pseudonaja*. Systematic morphological studies have progressively improved our understanding of species boundaries in *Demansia* (Storr 1978; Shea 1998; Shea & Scanlon 2007) but knowledge gaps remain. The work of Shea & Scanlon (2007) was a particularly important contribution as it acknowledged the uniformity of many traditional scalation characters in *Demansia*, a cause of much of the preceding history of "lumping" various populations, and defined new characters based on aspects of colour pattern that revealed strongly corroborated morphospecies. However, even in their study, regional variation in colour pattern characters were difficult to deal with, and it has become clear that a continuing major barrier to resolving difficulties in *Demansia* species definition is a lack of detailed molecular data to resolve phylogenetic affinities of problematic taxa. Perhaps the most glaring unsolved problem is the widespread and historically well-collected *D. psammophis* complex (Shea & Scanlon 2007; Allen & Vogel 2019; Chapple *et al.* 2019), that has defied taxonomic stability ever since the original species descriptions of the core species, *D. psammophis* (Schlegel, 1837) and *D. reticulata* (Gray, 1842), in the 19th century.

The problems of the *D. psammophis* complex became apparent following the revision by Storr (1978) that recognised *D. reticulata* with two subspecies in Western Australia. An olive rather than coppery brown head colour formed the basis for distinguishing the subspecies *D. r. reticulata* from *D. r. cupreiceps* (Storr 1978), the former extending from near Perth to the Edel Land Peninsula and Dirk Hartog Island, while the latter extended north and east from the Shark Bay coast (Wooramel) into the Pilbara region and desert areas of adjacent states of Northern Territory and South Australia. Although the revision by Storr (1978) only dealt with Western Australian species, this subsequently led to the uncertain combining with *D. psammophis*, effectively creating three trinomials of *D. p. cupreiceps*, *D. p. psammophis* and *D. p. reticulata* (e.g. Cogger 1983; Storr *et al.* 1986). A contributing factor to the uncertainty was the presence of *Demansia* individuals from large areas of central Australia with morphological similarities to both *D. psammophis* and *D. reticulata*. Since then, the tendency of classifications to be arbitrarily changed without further analysis is highlighted by the varying treatment of these taxa at both subspecies or full species levels in Wilson & Swan (2013 and all subsequent editions). Hutchinson & Tyler (1996) had noted that specimens identified as both *D. psammophis* and *D. reticulata* are sympatric in the Flinders Ranges of South Australia without any evidence of intergradation, and some authors have mostly treated *D. psammophis* as a species distinct from *D. reticulata*, including *D. r. cupreiceps* (e.g. Ehmann 2005; Allen & Vogel 2019; Chapple *et al*. 2019).

Our principal aim in this study is to provide a robustly supported phylogeny for the *D. psammophis* complex, with an emphasis on molecular coverage throughout central Australia and in areas of inferred sympatry or close parapatry between putative taxa. Our molecular results support recognition of both *D. psammophis* and *D. reticulata* as full species, and that the nominate population of the subspecies *D. r. cupreiceps* is conspecific with *D. reticulata*. In addition, we find that the widespread central Australian population, which incorporates the eastern part of the distribution of *D. r. cupreiceps*, represents a new species which we describe and compare with both of its near relatives.

Material and methods

DNA analysis. Sequence data were obtained from subsampled liver and heart tissues from collections of the Australian Biological Tissue Collection, South Australian Museum, Adelaide (ABTC), Australian Museum, Sydney (AM), Museum and Art Gallery of the Northern Territory, Darwin (MAGNT), Queensland Museum, Brisbane (QM), South Australian Museum, Adelaide (SAMA) and Western Australian Museum, Perth (WAM). We first sampled 14 of 15 recognised taxa in *Demansia*, along with a series of outgroups, using mtDNA, then used this to focus on a smaller sample of tissues from eight *Demansia* taxa that represented the broad pattern of variation across the genus with an emphasis on the putative taxa *D. psammophis*, *D. r. reticulata* and *D. r. cupreiceps* to resolve their problematic relationships, particularly where they come into contact.

Mitochondrial DNA analysis. We used a total of 59 samples, representing the 14 *Demansia* and outgroup taxa plus the problematic central Australian population. We rooted *Demansia* by including *Aspidomorphus schlegelii* (Günther) as the sister clade of *Demansia* based on the phylogenetic trees of Strickland *et al.* (2016). From these we obtained 879 bp NADH dehydrogenase 4 (ND4) and neighbouring tRNA-His and tRNA-Ser for using the forward primer M0245 (5'-TGA CTA CCA AAA GCT GTA GAA GC-3') and reverse primer M0246 (5'-TTT TAC TTG GAT TTG CAC CA-3') (Arévalo *et al.* 1994). PCR reactions comprised 25 µL: 16.5 µL water, 0.1 µL Immolase

DNA polymerase, 5 µL 5X MRT Buffer, 1.2 µL each of forward and reverse primer and 1 µL DNA template. Reactions were run on a thermal cycling program of 10 min at 95 C° for initial denaturation, followed by 37 cycles of 45 s at 94 C°, 45 s at 52 C for annealing, 1 min at 72 C° for extension with a final extension for 10 min at 72 C°. PCR products were checked for successful amplification by gel electrophoresis and purified PCR product was outsourced for Sanger sequencing to the Australian Genome Research Facilities (AGRF) Adelaide node.

Chromatograms were checked by eye in Geneious Prime 2022.0.1 and resulting sequences were aligned using Muscle 3.8.425 (Edgar 2004) on default settings. Resulting alignments were checked by eye and translated to amino acids to check for any premature stop codons. For Maximum Likelihood phylogenetic tree reconstruction, we used IQTree v. 2.0.3 (Nguyen *et al.* 2015), implementing ModelFinder (Kalyaanamoorthy *et al.* 2017), full codon models for partitions are shown in Table 1. Support was assessed using 1000 Ultrafast bootstraps.

TABLE 1. Codon models used for Maximum Likelihood mitochondrial tree estimation (Fig. 1).

Gene	Sites	Model
ND4	Position 1	HKY+F+R2
ND4	Position 2	TIM3+F+G4
ND4	Position 3	HKY+F+G4
tRNA	Position 1	TIM2+F+I+G4
tRNA	Position 2	TNe+I+G4
tRNA	Position 3	JC+I+G4

Nuclear DNA analysis. Guided by mtDNA results we selected 40 samples for nDNA genotyping at Diversity Arrays Technology (DArTseq, Canberra, Australia) who conduct a proprietary genome complexity reduction pipeline using a pair of restriction enzymes (PstI and HpaII) (Kilian *et al.* 2012; Georges *et al.* 2018). After initial digestion/ligation reactions and amplification samples are sequenced on an Illumina Hiseq 2500. DArTseq apply filters to screen for bacterial contamination as well as include a series of technical replicates to ensure accurate SNP calling and deliver a matrix of polymorphic loci which yielded 67,023 SNPs.

We imported sequence data into the R package DArTR (Gruber *et al.* 2018) and applied a series of additional filters including reproducibility (keeping SNPs present in 100% of technical replicates), loci callrate (retaining loci found in > 90% of individuals), read depth (removing loci with a read depth < 5) and removing monomorphic loci. A total of 9,189 loci remained which were exported to an alignment of concatenated SNPs containing a single random SNP per fragment and checked by eye in Geneious Prime 2022.0.1. For phylogenetic tree reconstruction we used ModelFinder (Kalyaanamoorthy *et al.* 2017) implemented in IQTree v. 2.0.3 (Nguyen *et al.* 2015) to select the best fit codon partitioning scheme. Maximum likelihood tree reconstruction was implemented in IQTree and support was assessed using 1000 Ultrafast bootstraps. The SNP tree was rooted using a midpoint root and visualised in Figtree v.1.4.4 (Rambaut 2012).

Morphological analysis. The morphology of the specimens used in the genetic study was examined for characters that robustly correlated with each of the genetically determined species. We then conducted a detailed morphological examination of non-genotyped specimens drawn widely across the central and western parts of Australia, using these empirically determined distinguishing morphological characters to assign specimens to species, enabling us to map the distribution of each of the species under study from the states of Northern Territory, South Australia and Western Australia. Fewer specimens were examined from New South Wales and Queensland and so distribution mapping was largely reliant on publicly accessible databases for these states.

We examined specimens held in collections at the Museum and Art Gallery of the Northern Territory, Darwin (MAGNT), South Australian Museum, Adelaide (SAMA) and Western Australian Museum, Perth (WAM). Type specimens were examined from the Natural History Museum in London, United Kingdom (NHMUK, formerly BMNH) and WAM. We were unable to have the syntypes of *Elaps psammophis* held in the Muséum National d'Histoire Naturelle in Paris, France (MNHN) examined in detail due to closure of the collections for moving, but we examined photographs of the specimens. Our selection of specimens examined for our morphological analysis was usually guided by preservation condition, maturity (e.g. juveniles largely excluded), if a tissue sample had been genotyped and for geographic coverage. We also examined photographs of several key specimens from other collections at the Australian Museum, Sydney (AM), Australian National Wildlife Collection, Canberra (ANWC), Museums Victoria, Melbourne (NMV) and Queensland Museum, Brisbane (QM). Specimens and photographs

examined are listed in the Appendix. We also made use of some photographs from public databases such as iNaturalist to examine colour pattern variation in life.

TABLE 2. Morphological characters measured and counted in this study.

Character	Description
ToL	Total body length, measured from tip of snout to tail tip, including terminal scale.
SVL	Snout-vent length, measured from tip of snout to posterior edge of anal scale.
TailL	Tail length, measured from posterior edge of anal scale to tail tip, including terminal scale.
HeadL	Head length, measured from tip of snout to medial posterior point of parietals on nape.
HeadW	Head width, measured at widest point, immediately posterior to eyes.
HeadD	Head depth, measured at deepest point, immediately posterior to eyes.
SnL	Snout length, measured from tip of snout to anterior margin of eye.
BW	Body width, measured at middle of SVL.
BD	Body depth, measured at middle of SVL.
FrL	Frontal length, measured from anterior to posterior edges.
FrW	Frontal width, measured at anterior widest point.
SupOcL	Supraocular length, measured from anterior to posterior edges.
SupOcW	Supraocular width, measured at middle above eye.
EyeW	Eye width, measured from anterior to posterior edges.
VS	Ventral scales, counted from first broad ventral to, but not including anal scale.
MBSR	Midbody scale rows, counted diagonally at middle of SVL.
AntSR	Anterior scale rows, counted diagonally back from and including dorsal scale contacting first broad ventral immediately posterior to head.
PostSR	Posterior scale rows, counted diagonally forward from and including dorsal scale contacting last broad ventral immediately anterior to anal scale.
ScS	Subcaudal scales, counted from first divided scale posterior to vent but not including terminal scale.
VertSc	Vertebral scales, counted from first scale posterior to parietals on nape to scale immediately above posterior edge of anal scale.
SupLab	Supralabials.
InfLab	Infralabials.

Table 2 shows the morphological characters measured and counted in this study. There were some missing scores due to damage such as broken tails and condition of very old or poorly preserved specimens. Notes were also taken on the size, shape and positioning of head scales of each specimen. Measurements and scale counts largely follow Maryan *et al*. (2020), and morphological definitions follow those used by Storr *et al.* (2002) and Cogger (2018). For this study, ToL, SVL and TailL were measured with a metal ruler to the nearest 1.0 mm. TailL is presented as length (mm) and as a percentage of SVL. Specimens preserved in a circular or twisted position were straightened on a flat surface when measured for ToL, SVL and TailL. All other meristic characters of HeadL, HeadW, HeadD, SnL, BW, BD, FrL, FrW, SupOcL, SupOcW and EyeW were measured with digital calipers to the nearest 0.1 mm under a microscope, with SupOcL, SupOcW and EyeW recorded on the right side only. VS counts were taken from the anteriormost first broad ventral to, but not including the anal scale following the Dowling (1951) method and MBSR counts were taken at middle of the SVL, counts were also taken from posterior to the head counted diagonally back from first broad ventral (AntSR) and anterior to the anal scale counted diagonally forward from last broad ventral (PostSR). VertSc counts were taken from the first scale posterior to parietals on nape to scale immediately above posterior edge of the anal scale marked by the insertion of a pin through the body. Where possible, sex was determined from everted hemipenes or by internal examination to detect either gonads in males and follicles or oviductal eggs in females.

We recorded colour pattern characters in each specimen as follows: head colour and if the same or differs from body colour at midbody and posteriorly, extent of the dark margins on dorsal scales posterior to nape, at midbody and on posterior body, extent of the dark tail of comma-shaped circumorbital marking ventrocaudal to eyes and extent of the dark transrostral line to the nostrils or eyes.

Colour in life is based on illustrated figures and field observations of live individuals.

An index of body robustness (IBR in Table 3) was calculated by dividing the SVL by the average of body width (BW) and body depth (BD), as follows: IBR = (SVL/ [(BW + BD) /2]). We divided morphometric data by SVL and then tested for significance between taxa using Mann-Whitney U-tests due to the likelihood of non-normal distributions, regarding a p-value of < 0.01 as significant. Operations were implemented in The R Project for Statistical Computing version 3.5.3.

Results

DNA analysis. Maximum likelihood analysis of the mitochondrial ND4 gene (Fig. 1) recovers poorly resolved relationships at the base of the tree, with a clade consisting of three eastern and eastern inland species, *D. flagellatio*, Wells & Wellington, *D. rimicola* Scanlon and *D. torquata* (Günther) as sister to the rest of *Demansia*. *Demansia simplex* Storr is recovered as sister to remaining species, but bootstrap support is low (BS = 62). The remaining species are strongly supported as a monophyletic group, which divides into three well-supported clades. One of these comprises the black whip snakes, *D. papuensis* (Macleay) and *D. vestigiata* (De Vis), large tropical savannah species; the second is a trio of smaller species from the north and northwest tropical arid zone, *D. calodera* Storr, *D. quaesitor* Shea and *D. rufescens* Storr. The third clade includes the *D. psammophis* complex, comprising *D. psammophis*, *D. reticulata*, including *D. r. cupreiceps* and the central Australian population. Also in this clade are two species from the monsoonal to arid north and northwest, *D. shinei* Shea and *D. angusticeps* (Macleay).

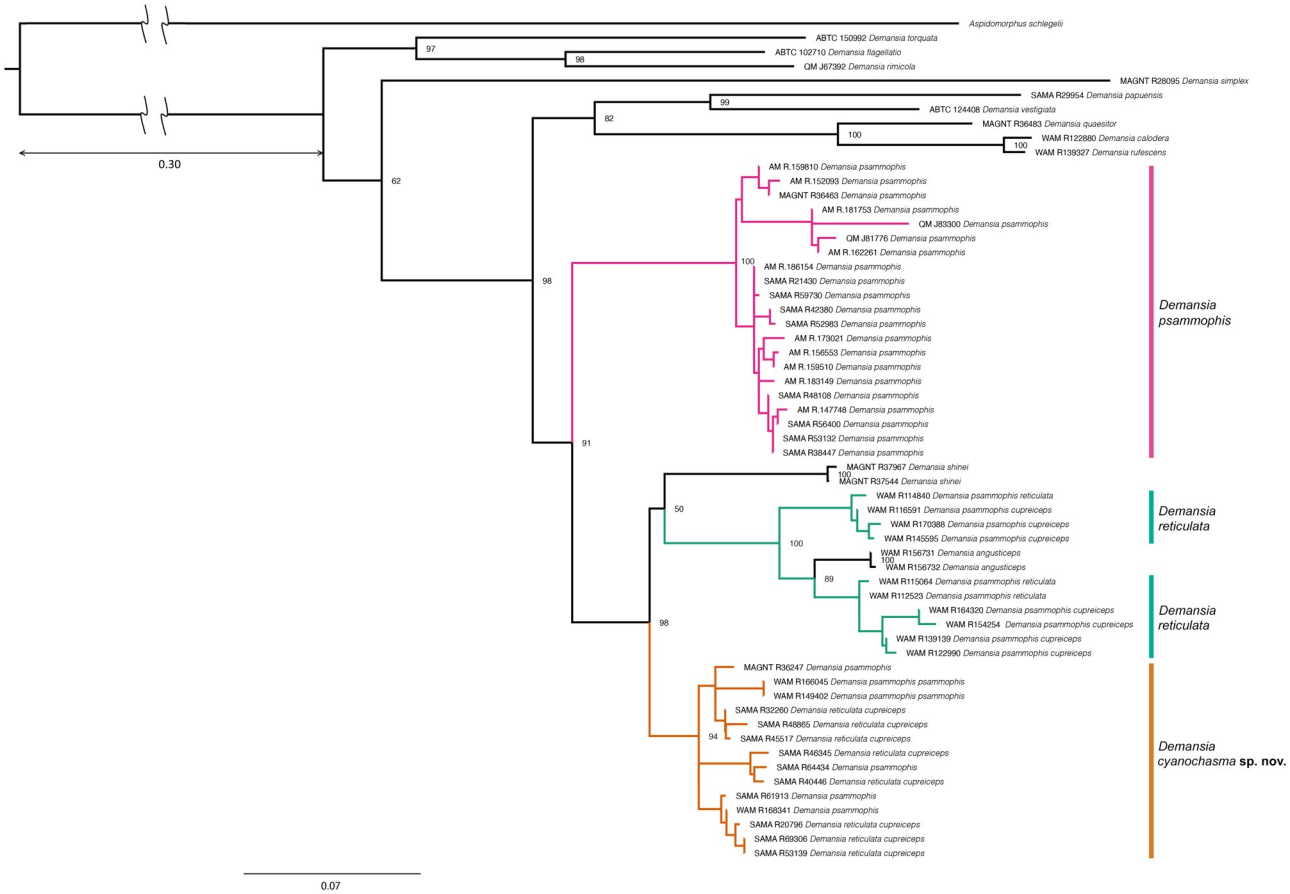

FIGURE 1. Maximum likelihood consensus tree of mitochondrial ND4+tRNA gene. Bootstrap support is shown only for major clades. *Aspidomorphis schlegelii* was selected as outgroup based on Strickland *et al.* 2016. Tip labels correspond to museum registration numbers including previous nomenclatural arrangement. Colours and names on the right of the figure represent the updated nomenclature presented in the current paper.

Within this clade, *D. psammophis* is recovered as the sister of the remaining species (BS = 91), while *D. reticulata* is found to be paraphyletic, including *D. angusticeps*. The results for *D. reticulata* were complex. Our samples fell into two major clades, each well-supported as monophyletic (BS values of 100 and 94). One of these clades, weakly recovered as sister to *D. shinei*, includes samples from the west coast north to Port Hedland and the Pilbara, and as far east as the Canning Stock Route. This clade also included our two specimens of *D. angusticeps.* The two subclades of *D. reticulata* that are formed by the branching of *D. angusticeps* both include specimens from the geographic regions regarded as including the type localities (Perth area and SE of Carnarvon, respectively) of both subspecies, *D. r. reticulata* and *D. r. cupreiceps*. We label them both *D. reticulata* in Fig. 1. The second clade was recovered as the sister of the *D. reticulata–D. angusticeps–D. shinei* clade and variously identified as *reticulata*, *cupreiceps* or *psammophis* from central Australia (southeastern inland of WA through NT to western Qld and SA to Flinders Range). There was little phylogeographic structure within this central Australian population despite occupying a vast range.

Maximum likelihood analysis of DArTseq generated SNPs (Fig. 2) was largely concordant with the ND4 mitochondrial tree, although with some important differences. Midpoint rooting places *D. simplex* as the sister to the remaining species of *Demansia,* while *D. rimicola* is the next earliest diverging lineage. This is followed by *D. vestigiata* as the sister of the *D. psammophis* complex. Monophyly of the *D. psammophis* clade is moderately well-supported (BS = 89) and as with the mitochondrial tree, *D. psammophis* is found as the sister of the remaining species. Within *D. psammophis* some geographic structure was present with one clade consisting of individuals in coastal southeastern Queensland and northeastern New South Wales and the other containing all individuals in South Australia and most of western and southern New South Wales.

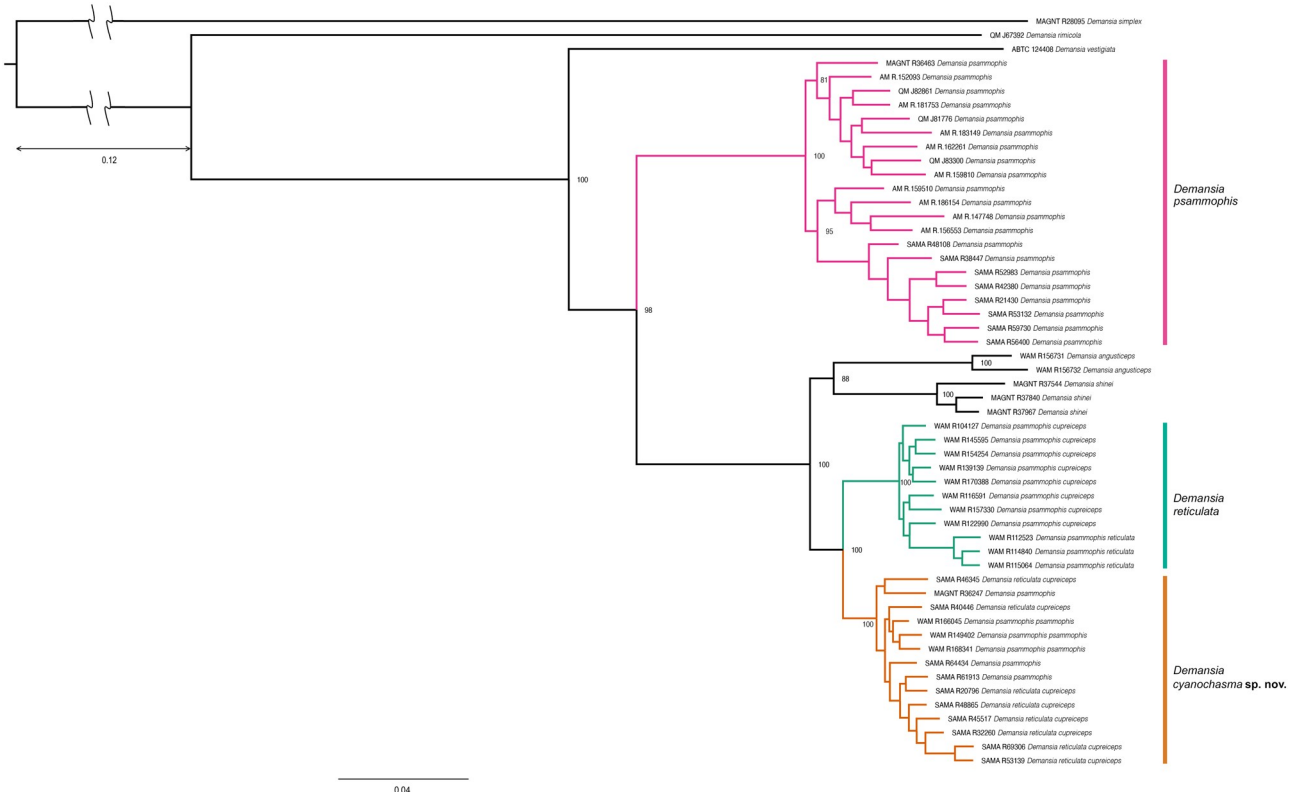

FIGURE 2. Maximum likelihood consensus tree of 6,946 unlinked SNPs. Bootstrap support is shown only for major clades. Tip labels correspond to museum registration numbers including previous nomenclatural arrangement, underlining previous confusion with species boundaries within this complex. Colours and names on the right of the figure represent the updated nomenclature presented in the current paper.

In contrast to the mitochondrial tree, *D. angusticeps* was not embedded within *D. reticulata*, instead branching as a moderately well-supported (BS = 88) sister of *D. shinei*, the two species strongly supported (BS = 100) as belonging to a clade comprising *D. reticulata* and the central Australian population. All the western specimens assigned to *D. r. reticulata* and *D. r. cupreiceps*, the same specimens grouped in the mitochondrial tree, fall into a single well-supported clade labelled *D. reticulata*. The three individuals from southern Western Australia that had

been registered as *D. r. reticulata* form a monophyletic subclade contained within a broader clade including *D. r. cupreiceps* individuals from adjacent areas to their north. The final central Australian population is composed of the

TABLE 3. Summary of statistics and statistical pairwise analysis for measurements (mm) and meristic counts of the *Demansia psammophis* complex. Statistically significant results in bold at the level of p <0.01. Values shown are the mean, ± standard deviation and (range). Sample sizes: N is shown at the head of each column. See Table 2 for abbreviations of measurements and counts. MBSR, SupLab and InfLab are excluded. Unsexed juveniles with SVL < 250 mm are excluded from measurement and ratio summaries but included in meristic counts. Abbreviations are: Dp *Demansia psammophis*, Dr *Demansia reticulata*, Dc *Demansia cyanochasma* **sp. nov.**

Character	*D. psammophis* N:21, + Juv. N:23	Dr *versus* Dp	*D. reticulata* N:66, + Juv. N:67	Dr versus Dc	*D. cyanochasma* **sp. nov.** N:68, + Juv. N:76	Dc *versus* Dp
ToL	724.3±86.5 (530–865)	0.17090	763.6±124 (431–964)	**0.00001**	657.5±132.4 (327–890)	0.05360
SVL	549.6±68.6 (400–655)	0.05490	588.5 ±99.9 (320–754)	**0.00001**	510.0±101.3 (255–700)	0.13888
TailL	171.0±22.3 (130–210)	0.98400	172.1±30.1 (93–230)	**0.00046**	149.5±30.8 (69–194)	**0.00906**
TailL%SVL		0.01428		0.99202		**0.00960**
HeadL	14.7±1.3 (11.6–17.7)	**0.00010**	16.6±2.2 (11.0–20.8)	**0.00001**	14.5±2.0 (10.2–18.6)	0.98404
HeadL/SVL		0.00410		0.10524		**0.00050**
HeadW	8.3±1.1 (6.2–11.7)	**0.00010**	9.8±1.6 (5.8–13.7)	**0.00001**	8.6±1.3 (5.5–11.6)	0.22246
HeadW/SVL		0.00024		0.37886		**0.00006**
HeadD	6.4±1.0 (4.9–9.0)	**0.00034**	7.9±1.4 (5.1–11.0)	**0.00001**	6.6±1.0 (4.2–8.4)	0.17086
HeadD/SVL		0.00001		0.33205		**0.00052**
SnL	5.0±0.5 (3.9–6.6)	**0.00064**	5.8±0.9 (3.7–7.4)	**0.00001**	4.8±0.7 (3.1–5.9)	0.21130
SnL/SVL		0.00374		0.02674		0.15854
BW	9.7±1.8 (6.4–13.9)		11.7±2.2 (5.8–16.8)		9.8±2.1 (5.1–14.5)	
BD	8.2±1.5 (5.7–11.7)		10.2±2.5 (4.6–15.4)		8.6±1.8 (5.1–12.8)	
IBR	15.6±2.0 (10.7–19.2)	**0.00001**	13.6±1.4 (10.7–19.2)	0.62414	13.9±1.6 (11.0–17.4)	**0.00020**
FrL	5.6±0.5 (4.6–6.8)	0.14680	6.0±0.8 (4.2–7.6)	**0.00080**	5.4±0.7 (3.4–6.6)	0.28914

.....continued on the next page

TABLE 3. (Continued)

Character	*D. psammophis* N:21, + Juv. N:23	Dr *versus* Dp	*D. reticulata* N:66, + Juv. N:67	Dr versus Dc	*D. cyanochasma* **sp. nov.** N:68, + Juv. N:76	Dc *versus* Dp
FrL/SVL		0.98404		0.00386		0.07508
FrW	2.7±0.3 (2.3–3.3)	0.72634	2.6±0.4 (1.6–3.4)	**0.00001**	2.3±0.4 (1.5–3.1)	**0.00028**
FrW/SVL		0.09600		0.16452		0.08544
SupOcL	4.7±0.4 (3.6–5.3)	**0.00090**	5.1±0.6 (3.5–6.8)	**0.00001**	4.5±0.7 (2.7–5.7)	0.47152
SupOcL/SVL		0.29372		0.09692		0.03000
SupOcW	2.2±0.2 (1.8–2.5)	**0.00038**	2.5±0.3 (1.6–3.0)	**0.00001**	2.1±0.4 (1.3–3.3)	0.21870
SupOcW/SVL		0.13104		0.87288		0.25848
EyeW	3.5±0.3 (3.0–4.0)	0.35238	3.6±0.4 (2.6–4.5)	**0.00001**	3.2±0.4 (2.1–4.0)	**0.00694**
EyeW/SVL		0.06724		0.00512		1.00000
VS	186.3±6.6 (178–203)	0.01468	189.3±4.8 (180–204)	0.14430	187.9±6.2 (173–203)	0.20054
AntSR	16.2±0.8 (15–17)	**0.00652**	16.8±0.5 (16–18)	0.77182	16.7±0.5 (16–18)	0.84148
PostSR	13.3±0.6 (13–15)	0.13362	13.0±0.3 (13–14)	0.68180	13.0±0.3 (12–14)	0.01828
ScS	80.0±5.4 (69–91)	0.05328	76.5±6.3 (65–90)	0.01278	74.4±4.8 (64–85)	0.08616
VertSc	185.7±2.7 (182–188)	**0.00024**	194.8±5.0 (185–206)	1.00000	194.5±6.3 (179–208)	**0.00152**

same samples that formed a clade in the mitochondrial tree. These specimens show little geographic structure, the basal pair including MAGNT R36247 (Elliott, Northern Territory) as sister to SAMA R46345 (south of William Creek in central South Australia), then a clade including specimens from Western Australia to northern South Australia, and a crown group of specimens from the Great Victoria Desert in southeastern inland of Western Australia east to the Flinders Ranges in South Australia.

Both molecular analyses thus demonstrate the existence of a clade including *D. psammophis* and *D. reticulata* as well as *D. angusticeps* and *D. shinei.* Both data sets also agree in finding that the central Australian population previously confused with either *D. psammophis* or *D. reticulata* form a clade of their own.

Sequence data are available as a digital repository at https://doi.org/10.25909/22045220 including 1) ND4 alignment and 2) Nuclear SNP alignment. Molecular sequences are deposited in GenBank under accession numbers OQ920125–OQ920182 (see Appendix for material sequenced and respective list of numbers).

Morphological analysis. As sexual dimorphism is known to be present in numerous elapids including *Demansia* (Shine 1980; Shea & Scanlon 2007) we initially partitioned morphometric and meristic data by sex. We found that relative TailL (SVL/TailL) and ScS counts for *D. reticulata* and the central Australian population were lower in females than for males, although this relationship was not significant for *D. psammophis*. All other characters measured and counted were not significantly different between sexes and so these were examined with both sexes pooled in subsequent analyses (Table 3).

We found no significant differences in any morphometric or meristic data between the subspecies *D. r. reticulata* and *D. r. cupreiceps* so combined the two respective datasets into one species in Table 3. Among the specimens in the WAM collection (115 registered as *D. r. reticulata* and 85 specimens as *D. r. cupreiceps*), we found that in specimens from within the distribution of *D. r. reticulata* (south of Wooramel), head colour was grey in 36%, brown in 33%, and a mixture of brown anteriorly and grey posteriorly in 29% of specimens. By contrast, the brown head colour was recorded in 84% and grey in 15% of specimens from within the distribution of *D. r. cupreiceps*. Five specimens from across the regions of both subspecies were recorded with a black head colour. Our data together with photographs of live individuals and field observations show that individual variation is such as to make the diagnostic use of head colour unreliable. At most there seems to be an overall trend for brown head colour to become increasingly prevalent along a south to north gradient.

We then compared morphological datasets between three genetic clades in the *D. psammophis* complex, excluding the morphologically divergent *D. angusticeps* and *D. shinei*. We found that *D. reticulata* (both subspecies) had consistently the largest ToL and SVL and that this was significant when compared to the other two species. Significant differences in characters associated with relative head size, such as HeadL, HeadW and HeadD were found between species as well as relative EyeW and IBR (Table 3). It is likely that many of the significant differences in relative body proportions are due to allometry. Comparison of colour patterns revealed differences that were congruent with the genetic clades involving extent of facial markings and dark margins on body scales combined with other elements of colour and pattern.

Taxonomic conclusions. We confirm that both *D. psammophis* and *D. reticulata* are full species and find no support for the existence of subspecies within *D. reticulata* and relegate the taxon *D. r. cupreiceps* as a junior subjective synonym of *D. reticulata*. We also find, based on concordant mitochondrial DNA, nuclear DNA and morphology, that the central Australian population of *Demansia* represents a new species.

Taxonomy

Elapidae Boie, 1827

Hydrophiinae Fitzinger, 1843

Demansia Günther, 1858

Type species. *Lycodon reticulatus* (= *Demansia reticulata*) Gray 1842: 54, by subsequent designation (see Klemmer 1963: 286). For content, see Cogger (2018) and Wilson & Swan (2021).

Diagnosis. Species within the genus *Demansia* are small to moderately large diurnal oviparous snakes (total

lengths to 560 mm in the smallest species *D. simplex* and to 1,650 mm in the largest species *D. papuensis*) with: slender bodies (moderate in *D. simplex*), head moderately long and slightly to moderately wider than neck, prominent canthus rostralis, frontal long and narrow, internasals present and slightly smaller than prefrontals, nasals very wide anteriorly (laterally aligned bell-shape) narrowing to, and then extending from nostril and in point or short contact with preocular (occasionally separated by prefrontals), two postoculars, temporals 2 + 2 with lower primary temporal descending deeply between last two supralabials (occasionally contacting oral margin), supralabials 6, infralabials 7, midbody scales non-glossy in 15 rows, anal and subcaudal scales divided, long tails (up to 40% of snout-vent length in *D. flagellatio*), variable body colour of shades of brown, olive, greyish, greenish, reddish, blue or black with dark pigment on body scales of basal or apex blotch or spot, narrow margins on anterior facets or indistinct to distinct margins on all facets, head colour same as body or clearly demarcated of greyish, bluish grey, orange brown or shades of brown, dark band (typically faded in adults) on nape in some species, consistent colour pattern of pale-edged dark comma-shaped marking encompassing eyes then broadening ventrocaudally of either short abruptly ending or long, but inconsistent in *D. papuensis* and *D. vestigiata*, all species except *D. papuensis* and *D. vestigiata* with pale-edged dark transrostral line between the nostrils and occasionally extending to the eyes, ventral surface white, greenish white, bluish white, brown or greyish with glossy shine (typically dark spots on anterior ventrals in *D. olivacea* (Gray) and *D. rimicola*), large eyes with diameter markedly greater than the distance from the mouth to the eye, with yellowish, golden brown or reddish irides and large round black pupils (Bush 2017). Non-lobate and bulbous hemipenis with uniform parallel rows of spines and enlarged basal hooks (Keogh 1999). Further distinguished by two derived character states that are unique among elapids: a very thin but deep dorsal extension of the parasphenoid process that forms a thin, bony interorbital septum (Greer 1997), and an elevated number of chromosomes 2N = 42 (Mengden 1985).

Demansia psammophis (Schlegel, 1837)

Yellow-faced Whip Snake
Elaps psammophis Schlegel 1837a,b, *Essai sur la physionomie des serpens*, Part I: 182, Part II: 455. (Figs. 3, 4, 10)

Synonymy.
Pseudelaps psammophidius Duméril, Bibron & Duméril 1854, *Erpétologie générale ouhistoire naturelle complète des reptiles Vol. VII*: 1234.
Pseudelaps psammophis Girard 1858, *United States exploring expedition during the years 1838, 1839, 1840, 1841, 1842*: 178.
Demansia psammophis Günther 1858, *Catalogue of colubrine snakes in the collection of the British Museum*: 212.
Pseudoelaps psammophideus Jan 1859, *Prodrome d'une iconographie descriptive des ophidiens*: 17.
Diemenia psammophis Krefft 1866, *Transactions of the Philosophical Society of New South Wales*, 1862–1865: 42. *Demansia psammophis psammophis* Cogger 1979, *Reptiles & Amphibians of Australia*: 608.

Type specimens. Guibé & Roux-Estève (1972: 133), Cogger *et al.* (1983: 222) and Wallach *et al*. (2014: 211) listed two syntypes MNHP 7663A and MNHP 7666, now registered as MNHN-RA-0.7663 and 0.7666, respectively. The basis for this appears to be Schlegel (1837b) providing scale counts for two specimens, however he also provided a third set of counts in the first section of his monograph (Schlegel 1837a). The two parts are considered to both contribute to the original description of the species (e.g. Schatti *et al*. 2010). Duméril *et al.* (1854: 1234) noted and it is currently shown online in the MNHN collection there are three specimens of which MNHN-RA2012.0406 (previously MNHN 7663A) is also a syntype (see https://science.mnhn.fr/institution/mnhn/collection/ra/item/2012.406).

Schlegel (1837a,b) only mentioned New Holland as the source of the specimens of his *Elaps psammophis*, but also stated that Quoy & Gaimard, or Péron & Lesueur were the collectors of these and the specimens of *Elaps (= Elapognathus) coronatus* described at the same time. Duméril *et al.* (1854) recorded that their specimens were collected by Quoy & Gaimard from Port du Roi Georges (= King George Sound, Albany, Western Australia) in July 1827, however this expedition was exploring New Ireland and New Britain in the Bismark Archipelago (Duyker 2014). Regardless of when the specimens were collected, no species of *Demansia* occurs within 250 kilometres of King George Sound, and the syntypes conform to *D. psammophis* in colour and pattern so must have an origin in eastern Australia. Quoy & Gaimard collected twice in Australia, the first time with de Freycinet (Voyage of "*Uranie*",

1817–1820) and the second time with Dumont d'Urville (Voyage of "*Astrolabe*", 1826–1829), but only the first expedition included a visit to an area where *D. psammophis* occurs (Sydney area, New South Wales) and only the second passed through King George Sound (Adler 2012). During the 1801–1804 Baudin expedition, Péron & Lesueur visited and collected at both Sydney and King George Sound (Bonnemains *et al*. 1988). Thus, the data for the date, locality and collectors of the type specimens appear to have become inextricably confused, but the identity of the type specimens is not in doubt, and we agree with previous workers (e.g. Cogger *et al*. 1983; Wallach *et al*. 2014) that the eastern Australian species is *D. psammophis* (Schlegel 1837a,b).

No lectotype designation has yet been proposed for *Elaps psammophis*. We therefore designate specimen MNHN-RA-0.7666 (Fig. 3, reportedly female) as the lectotype to become the sole name-bearing type of this nominal taxon, in accordance with the International Code of Zoological Nomenclature (ICZN) Article 74.7.3, which shows the paired (reddish in life) anterior paravertebral stripes diagnostic for this species. The specimen has had the skull removed, but the external cephalic scalation has been carefully retained (Fig. 3).

Paralectotypes. MNHN-RA-0.7663 and MNHN-RA-2012.406 (formerly MNHN 7663A), reportedly males, same details as lectotype.

FIGURE 3. Preserved lectotype MNHN-RA-0.7666 of *Elaps psammophis* with arrows showing paired (reddish in life) anterior paravertebral stripes diagnostic for *D. psammophis* (photo: https://science.mnhn.fr/institution/mnhn/collection/ra/item/0.7666).

Revised diagnosis. A medium-sized *Demansia* with more than 177 ventrals and with a cream-edged black transrostral line typically only extending to the nostrils, lacking any traces of hood or collar markings, head and body colour of pale to medium olive brown, pale grey or bluish grey, sometimes grading to yellowish on posterior body or only the tail, typically a pair of reddish paravertebral stripes or dorsal zone on the anterior body, body scales lack or have only indistinct dark anterior margins so that a reticulated pattern is absent, a relatively short dark comma-shaped circumorbital marking not extending as far as the fifth supralabial, ventral surface with a yellowish or greenish median stripe.

Description. Mean snout-vent length 550 mm (max. 655 mm) this study, Shine (1980) recorded a maximum SVL of 835 mm from east coast specimens, maximum total length to 865 mm (this study), males and females similar in size (mean SVL males 550 mm, females 550 mm), tail length 26.2% to 34.2% of snout-vent length (mean 30.9%). 15 midbody scale rows, increasing to 16 or 17 posterior to the head and decreasing to 13 or 14 anterior to the vent; 178– 203 ventrals (mean 186); 69–91 subcaudals (mean 80); 182–188 vertebrals (mean 185). No morphological characters were observed as sexually dimorphic in our dataset for *D. psammophis.* Head scalation as for genus diagnosis above.

Colour of body in life variable (Fig. 4), pale to medium olive brown, pale grey or bluish grey sometimes grading to yellowish on posterior body or only the tail, variably expressed but usually poorly defined pair of dull orange to dark reddish paravertebral stripes or dorsal zone on the anterior body, black scale margins absent, or rarely only weakly developed anteriorly, head sometimes with scattered dark speckling and washed with pale yellowish extending as far as the first 4–7 vertebrals; a distinct dark transrostral line with pale margin extending horizontally to nostrils and pale markings in front of and behind the eyes forming the margin to the relatively short dark comma-shaped circumorbital marking, with its tail not extending beyond the middle of the fifth supralabial, only occasionally contacting lower primary temporal; ventral surface glossy white with a yellowish or greenish median stripe appearing anteriorly and becoming increasingly prominent posteriorly, in larger individuals this fades to a narrow greyish line; ventral surface of the tail cream to yellowish, becoming brighter towards the tip; irides with a golden yellow to orange ring bordering the round pupil.

In preservative, all yellowish pigments leach away and body colour fades to a dull shade of grey. The reddish paravertebral stripes often become harder to discern and fade to yellow but generally do not disappear completely, while the dull yellowish or greenish ventral stripe also tends to fade and sometimes vanish.

Variation. There is variation in the reddish dorsal markings in this species. Photographs of live individuals (Fig. 4) show almost complete absence to weak or strong, dull orange to dark reddish paired paravertebral stripes on the anterior body, to a broad reddish dorsal zone with the stripes merged (Fig. 4D). The separation of the two stripes by the vertebral zone is the most common condition with their merging into a single broad marking generally seen only in Queensland. Most specimens have a uniform pale grey body and tail colour, but in some individuals the colour changes gradually from grey to yellowish posteriorly.

FIGURE 4. Adult *Demansia psammophis* photographed in life from (A) Southern Flinders Ranges, South Australia, (B) Hattah, Victoria, (C) Sydney, New South Wales, and (D) Lake Murphy Conservation Park, Queensland (photos A: S. Scott; B: P. Robertson; C: J. Farquhar; D: B. Maryan).

Distribution. *Demansia psammophis* is widespread throughout the temperate, semiarid and subtropical areas of eastern Australia (Fig. 5), from the Flinders Ranges of South Australia south to the northwestern outskirts of Adelaide, then east via the Murray River valley to northwestern Victoria, and then through the Murray-Darling basin to the

Great Dividing Range and coast of New South Wales and Queensland, south as far as Wollongong and north to the Cooktown area on the lower Cape York Peninsula.

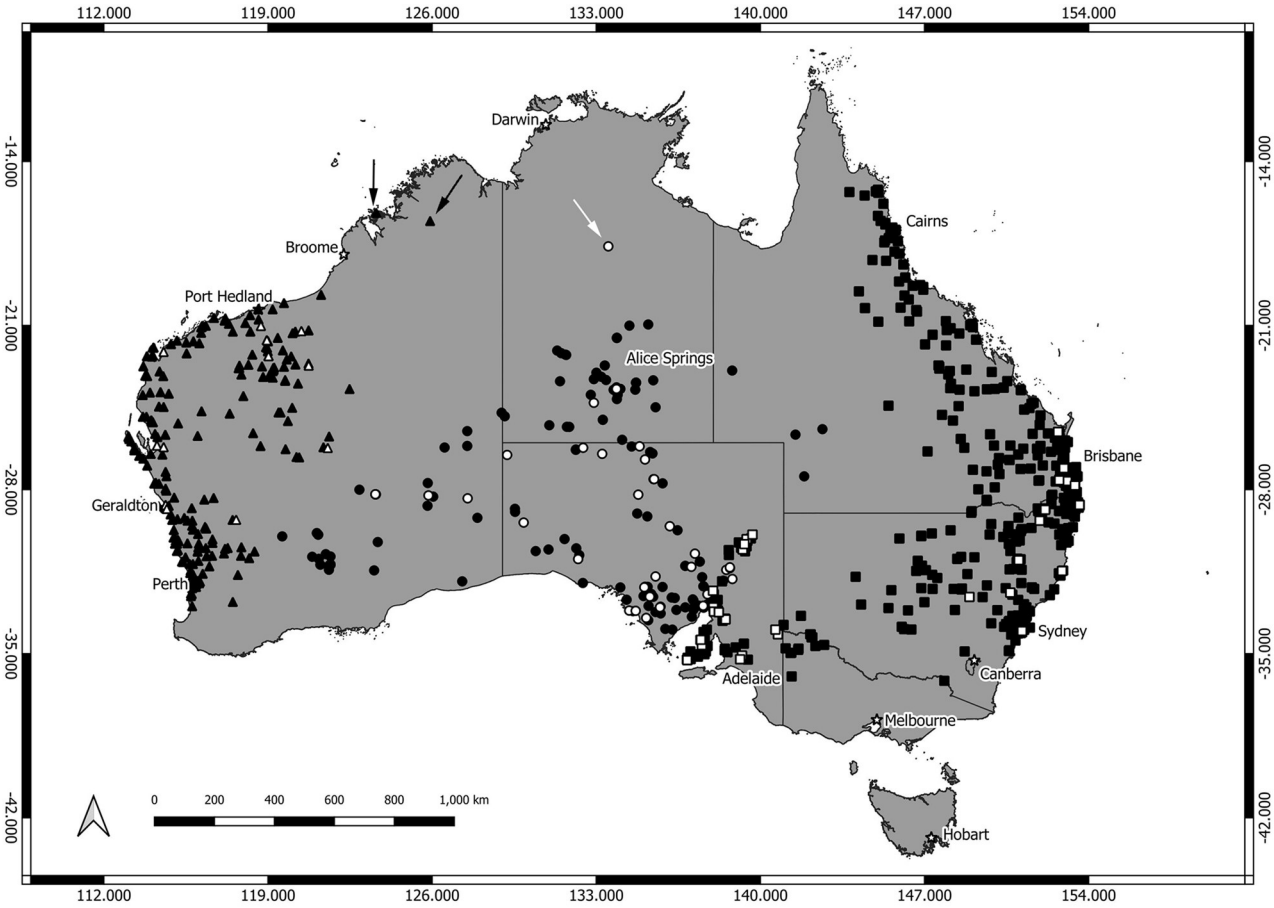

FIGURE 5. Map of Australia showing distribution of *D. psammophis* (squares), *D. reticulata* (triangles) and *D. cyanochasma* **sp. nov.** (circles). White symbols indicate tissues sequenced in DNA analysis. Arrows indicate outlier populations of *D. reticulata* (black) and *D. cyanochasma* **sp. nov.** (white). Collection records are based on OZCAM (2022).

Habitat and ecology. *Demansia psammophis* occupies a variety of temperate, semiarid to subtropical vegetation associations growing on light to heavy, often stony soils, including coastal forests, heathlands, shrublands, dry sclerophyll woodlands of mallee and/or other eucalypts, margins of rainforests, often with dense understorey vegetation (hummock grasslands of the interior), and rocky areas such as along drainage lines, outcrops and hills (Wilson & Knowles 1988; Griffiths 2006; Cogger 2018; Swan *et. al*. 2022; Wilson & Swan 2021; Wilson 2022). This species also occupies disturbed agricultural areas adjacent to uncleared vegetation and gardens in towns and some cities.

Sympatry or close parapatry with other species. Based on collection records the geographic distributions of *D. psammophis* and the new species are parapatric but they may be syntopic in the Flinders Ranges of South Australia. Currently the two species are known to occur within ~ 26 km of each other: SAMA R53132 of *D. psammophis* from Dutchman's Stern *versus* SAMA R70189 of the new species from 1 km NNW of Port Augusta. *Demansia psammophis* is also known in the Gammon Ranges which is approximately 50 km from the nearest record of the new species in Ikara-Flinders Ranges National Park.

Demansia psammophis and *D. reticulata* as redefined herein are widely allopatric with a minimum separation distance of at least 2000 km (Fig. 5). *Demansia psammophis* is sympatric with *D. torquata* across nearly the entirety of the latter species' range and in the north of its range has broad overlap with both *D. papuensis* and *D. vestigiata* (Wilson & Swan 2021).

Comparisons with other species. *Demansia psammophis* differs from all other *Demansia* by its combination of relatively plain body colour without black hood or collar markings on the head and neck, presence of a dark transrostral line not reaching the eyes, relatively short tail on circumorbital marking ventrocaudal to the eyes, the

variable extent of reddish paravertebral stripes on the anterior body and ventral surface with a yellowish or greenish median stripe.

It is most like the parapatric new species but it differs by the absence of dark smudging on margins of body scales and typical presence of both a pair of reddish paravertebral stripes and a median yellowish or greenish stripe or series of blotches on ventral surface. Further distinguished from it on proportionately smaller head size and by head, posterior body and tail colour typically same as body (*versus* brown head, posterior body and tail colour contrasting with bluish grey body) and a larger mean SVL of 550 mm (*versus* 510 mm) (Table 3).

Differs from *D. reticulata* by lack of dark scale margins (Figs. 10E, F *versus* Figs. 10H, I) and dark tail on comma-shaped circumorbital marking typically short, usually terminating on the fifth supralabial, Fig. 10D (*versus* dark tail on comma-shaped circumorbital marking typically long, usually extending across the fifth supralabial to the lower primary temporal, Fig. 10G). Further distinguished by olive brown, pale grey or bluish grey rather than greenish body colour, smaller SVL mean of 550 mm (*versus* 588 mm) and proportionally shorter frontal scale (Table 3).

Differs from the sympatric *D. torquata* by the absence of a dark, pale-edged collar at any age (*versus* collar present, though often fading with age), having a dark transrostral line that typically stops at the nostril (*versus* typically reaches the anterior border of the eye), reddish paravertebral stripes or a reddish dorsal zone anteriorly on olive brown, pale grey or bluish grey body (*versus* uniform olive brown body), comma-shaped circumorbital marking typically terminating on the fifth supralabial (*versus* comma-shaped circumorbital marking typically long and terminating on the lower primary temporal), and ventral surface typically whitish with a median yellowish or greenish stripe or series of blotches (*versus* ventral surface flushed with pink to red).

Remarks. Shine (1980) presented information on body sizes, food habits and reproduction of *D. psammophis*. This species appears to be tolerant of major disturbances, as indicated by its occurrence in agricultural and urbanised areas, including along the inner-city drainage lines of both Sydney and Brisbane. *Demansia psammophis* is of Least Concern under the IUCN criteria owing to its widespread distribution without any perceived threats, occurring in many conservation areas (Shea *et al*. 2017), but in Victoria it is listed as Near Threatened due to a paucity of records (Robertson & Coventry 2019).

Our study focused on the problem of the new species and its relationship to *D. psammophis* towards the south and west of its distribution in South Australia. However, some questions remain regarding the existence of variation along the east coast, particularly in Queensland. The nuclear genetic sampling (Fig. 2) found that east coast specimens formed a moderately well-supported subgroup (BS = 81), and photographs of live individuals show that, especially in northeastern Queensland, the typically paired reddish paravertebral stripes are sometimes replaced by a broad reddish zone on the anterior body, facial comma markings tend to be less extensive and dark anterior margins on body scales more distinct than further south. Additional work on these populations will be needed to determine the significance of these and other variations.

Demansia reticulata (Gray, 1842)

Reticulated Whip Snake
Lycodon reticulatus Gray 1842, *Zoological Miscellany,* 51–57: 54. (Figs. 6, 7, 10)

Synonymy.
Demansia reticulata Günther 1858, *Catalogue of colubrine snakes in the collection of the British Museum*: 212.
Diemenia reticulata Günther 1863, *Annals and magazine of natural history*: 350.
Diemenia psammophis var. *reticulata* Boulenger 1896, *Catalogue of the Snakes in the British Museum (Natural History), Vol III*: 322.
Demansia psammophis reticulata Glauert 1957, *A Handbook of the Snakes of Western Australia 2nd Edition*: 32.
Demansia reticulata cupreiceps Storr 1978, *Records of the Western Australian Museum*, 6: 295 [in part].
Demansia reticulata reticulata Storr 1978, *Records of the Western Australian Museum*, 6: 297.
Demansia psammophis cupreiceps Cogger 1983, *Reptiles & Amphibians of Australia 3rd Edition*: 431 [in part].
Demansia cupreiceps Wells & Wellington 1985, *Australian Journal of Herpetology*, Supplementary Series, 1: 45 [in part].
Demansia reticulatus Wells & Wellington 1985, *Australian Journal of Herpetology*, Supplementary Series, 1: 45.

Lectotype. NHMUK 1946.1.19.79 (Fig. 6A), designation by Storr (1978: 297), male, original type locality "Australia", obtained from W. Buchanan (not G. Krefft as reported by Storr [1978]). No more precise locality is

available for the specimens described by Gray, but Buchanan is also noted in herpetology catalogues as the source of type specimens for other species such as *Acritoscincus trilineatus* (Gray) and *Cryptoblepharus buchananii* (Gray), all of which with a probable origin from southwestern Western Australia, in the vicinity of Perth (G. Shea, pers. comm.). Paralectotype. NHMUK 1946.1.19.78, male, same details as lectotype.

FIGURE 6. (A) Preserved lectotype NHMUK 1946.1.19.79 of *Lycodon reticulatus*, and (B) preserved holotype WAM R34555 of *Demansia reticulata cupreiceps* (photos A: P. Campbell; B: B. Maryan).

Some of the *D. r. cupreiceps* paratypes (Storr 1978: 297) have been identified as specimens of the new species: WAM R15066, WAM R15156, WAM R22072, WAM R34203 (in error, R34303), WAM R36637 (see Appendix). **Revised diagnosis**. A medium-sized *Demansia* with more than 179 ventrals and with a cream-edged black transrostral line typically only extending to the nostrils, lacking any traces of hood or collar markings, and combining a variable body colour of shades of green, olive green, pale or dark green grading to pale, yellowish or dark brown on posterior body and tail, all body scales except on posterior body and tail with black margins forming a distinct reticulated pattern, a moderately long dark tail on comma-shaped circumorbital marking, extending as far as the lower

primary temporal. Ventral surface glossy whitish to pale greenish white, occasionally yellowish anteriorly and on throat, lateroventral edges often with dark smudging.

Description. Mean snout-vent length 588 mm (max. 754 mm), maximum total length to 964 mm, males and females similar in size (mean SVL males 594 mm, females 580 mm), tail length 23.5–34.0% of snout-vent length (mean 29.2%). 15 midbody scale rows, increasing to 16 or 17 (rarely 18) posterior to the head and decreasing to 13 (rarely 14) anterior to the vent; 180–204 ventrals (mean 189); 65–90 subcaudals (mean 76); 185–206 vertebrals (mean 194). Tail length and subcaudal counts were the only significant sexually dimorphic characters, mean male tail length was 30.6% of SVL with a mean of 80.1 subcaudal scales compared with mean female tail length of 27.2% of SVL and a mean of 71.2 subcaudal scales.

Head scalation as for genus diagnosis above.

Colour of body in life variable (Fig. 7), shades of green from olive green, pale or dark green with gradual change on posterior third of body and tail, including the latter's ventral surface, to shades of pale, yellowish or dark brown; posterior to nape, body scales with narrow to wide black margins, clearly widest on base and apex forming distinct reticulated pattern, occasionally with more extensive black margins along the vertebral zone; head and nape varies from grey, pale yellowish or dark brown, with or without darker brown to black spots or smudges on prefrontals, frontal, supraoculars and parietals; occasionally with blackish head (Fig. 7C); demarcated head colour extending on to the nape as far as the first 1–11 vertebrals; a dark transrostral line, sometimes indistinct, with pale lower margin extending horizontally to nostrils, less frequently extending posterior to the nostrils and merging with dark upper posterior margins of first two supralabials and reaching the anterior portion of preocular; pale markings in front of and behind the eyes forming the margin to the dark comma-shaped circumorbital marking, with its ventrocaudal tail moderately long extending from the fourth supralabial across the length of the fifth supralabial to terminate at or on the anterior portion of the lower primary temporal; ventral surface glossy whitish to pale green, lateroventral edges often with dark smudging; occasionally yellowish anteriorly, including under head and on throat; irides with a golden yellow ring bordering the round pupil.

In preservative, body colour fades to a dull pale brown. The dark margins on body scales remain distinct in most specimens but are often less contrasting in juveniles and immatures. Head, nape, posterior body and tail colour are similarly pale brown, except fading to white ventrally under the tail.

Variation. There is significant geographic variation in head colour in this species. Southwestern populations mostly have a greyish head colour (Fig. 7A) while those from further north, including the Pilbara, have the brown head colour (Figs. 7B, D) that led to the description of *D. r. cupreiceps* (Fig. 6B). More extensive collections reveal inconsistency in head colour across the supposed subspecies, with specimens from the south of the range sometimes showing brown head colour and some from the Pilbara lacking it, and specimens from throughout the distribution sometimes having a blackish head (Fig. 7C). In addition, the distribution of colour variation does not match the genetic relationships within the species, as specimens with museum identifications as both subspecies are represented across the full range of mitochondrial variation (Fig. 1) and the nuclear markers show that the '*reticulata'* specimens form a clade with southern '*cupreiceps'* to the exclusion of more northerly '*cupreiceps'* (Fig. 2). Given the genetic relationships among *D. reticulata* populations and the new species, it seems likely the brown head and tail combination was a characteristic of their common ancestor, with a change to a concolorous head then evolving in the southwest.

Lectotype of *Lycodon reticulatus* Gray, 1842. (Fig. 6A). Measurements (in mm) and meristic values, as determined in this study: ToL 960, SVL 754, TailL 206 (27.3%), HeadL 20.8, HeadW 12.7, HeadD 9.5, SnL 7.2, BW 14.0, BD 12.1, FrL 6.8, FrW 3.0, SupOcL 6.6, SupOcW 2.6, EyeW 4.2, VS 191, MBSR 15, AntSR 17, PostSR 13, ScS 72, VertSc 195, SupLab 6, InfLab 7.

Scalation and proportions are as in the diagnosis for the genus and species. Individual characteristics include the asymmetric presence of a small additional supralabial below the lower primary temporal on the right side, and an irregular medial suture between internasals such that left prefrontal contacts the right internasal. Colour dull pale brown on the dorsal surface, except in areas where the outermost layer of the epidermis has detached exposing a pale grey colour beneath, but the dark edges and interspaces between the scales described by Gray (1842: 54) are still present. The dark margins on the body scales are clearly widest on the base and apex and the reticulated pattern is welldeveloped. The head is a similar dull pale brown with darker brown smudges on prefrontals, frontal, supraoculars and parietals. The dark comma-shape ventrocaudal to eyes is long but indistinct, and contacts the lower primary temporal, less discernibly on the right side. The darker transrostral line is indistinct and extends to the nostrils as does the lower white margin. The ventral surface under the head and along the body is slightly lighter than dorsal surface, except in areas where the outermost layer of the epidermis has detached exposing a white colour beneath.

FIGURE 7. Adult *Demansia reticulata* photographed in life from (A) Melaleuca Park, Western Australia, (B) Whim Creek, Western Australia, (C) Yandeyarra Community, Western Australia, and (D) Spinifex Village Yandi, Western Australia (photos A: B. Maryan; B–D: B. Bush).

Distribution. Essentially unchanged from species map of Storr *et al.* (2002: 174, as *D. p. cupreiceps* and *D. p. reticulata*), excluding the distribution of the eastern population listed as *D. p. psammophis* that is now the new species. *Demansia reticulata* is endemic to Western Australia and widespread throughout the dry temperate, semiarid to arid areas of west coastal and adjacent interior and northwestern Western Australia (Fig. 5), including on Dirk Hartog Island. There are apparent outlier populations in the west Kimberley (Fig. 5) at Wotjulum (WAM R12359) and Manning Creek (WAM R32067). The northern range limits of *D. reticulata* are uncertain, possibly an artefact of few collections from poorly surveyed regions such as the Eighty Mile Beach area.

Habitat and ecology. *Demansia reticulata* occupies a variety of dry temperate, semiarid to arid vegetation associations growing on light to loamy, often stony soils, including coastal sand dunes and littoral vegetation on sandy beaches, dry sclerophyll woodlands of banksia, mallee and/or other eucalypts, heathlands, shrublands, hummock grasslands and occasionally rocky areas such as granite outcrops, limestone platforms and elevated rocky ridges (Wilson & Knowles 1988; Maryan 1996; Bush *et al*. 2007; Bush *et al*. 2010; Bush & Maryan 2011; Cogger 2018; Wilson & Swan 2021). Field observations indicate a preference for lighter soils supporting dense low heaths or hummock grasslands, and that it appears to be less prevalent on harder erosional surfaces (B. Bush & B. Maryan, pers. obs.). This species is a frequent visitor to town gardens and around village areas on mine sites in the Pilbara (Bush & Maryan 2011). During summer in the Pilbara, it can occasionally be observed active at night on roads (B. Bush, pers. obs.). Field observations have been made of a *D. reticulata* two metres high in an *Acacia* possibly hunting geckos near Yalgoo (J. Vos, pers. obs.), and examination of a roadkill *D. reticulata* revealed a *D. calodera* inside the stomach on Minilya Station (B. Bush, pers. obs.).

Sympatry or close parapatry with other species. In Western Australia (Fig. 5), the geographic distributions of *D. reticulata* and the new species are known to occur within approximately 140–240 km of each other (*D. reticulata* from Jouerdine Nature Reserve *versus* the new species from Mount Manning Range and Bullabulling), and within

approximately 280 km in the north (*D. reticulata* from Lorna Glen Station *versus* the new species from Cosmo Newberry).

Broad sympatry involving *D. reticulata* occurs throughout large areas with *D. calodera* in the northern Geraldton Sandplains and Carnarvon bioregions (Storr & Harold 1990; Kendrick 1993; McKenzie *et al.* 2000; Bush *et al*. 2007), and with *D. rufescens* in the Pilbara bioregion and an outlying population on the North West Cape (Bush & Maryan 2011).

Demansia reticulata is regionally sympatric with *D. angusticeps* in the Dampierland and Central Kimberley bioregions. *Demansia reticulata* is also sympatric with *D. papuensis* on the Yampi Peninsula, where both species are known from the same location of Wotjulum (Shea 1998: 56).

Comparisons with other species. *Demansia reticulata* differs from *D. psammophis* by distinct dark scale margins (Figs. 10H, I *versus* Figs. 10E, F) and dark tail on comma-shaped circumorbital marking typically long, usually extending across the fifth supralabial to the lower primary temporal, Fig. 10G (*versus* dark tail on commashaped circumorbital marking typically short, usually terminating on the fifth supralabial, Fig. 10D). Further distinguished by greenish rather than olive brown, pale grey or bluish grey body colour, larger SVL mean of 588 mm (*versus* 550 mm) and proportionally longer frontal scale (Table 3).

From *D. psammophis* it is distinguished on distribution being wholly found in WA (*versus* absent from that state) (Fig. 5) and dark tail on comma-shaped circumorbital marking typically long, usually extending across fifth supralabial to the lower primary temporal (Fig. 10G) (*versus* tail of comma-shaped circumorbital marking typically short, usually terminating on fifth supralabial, Fig.10D). Further distinguished by green rather than greyish body colour and proportionally longer frontal scale (Table 3).

Remarks. Historically, *D. reticulata* has been documented as being intolerant of major disturbances (Storr *et al.* 1978: 191–192; Bush *et al.* 2010: 144), as indicated by its disappearance from some remnant bushland areas in the Perth area (e.g. last collected at Kings Park in 1942). This apparent scarcity in heavily modified areas appears to be supported by biological surveys in the Wheatbelt and Perth areas detecting no populations of this species (Chapman & Dell 1985; How & Dell 2000). Away from areas of localised and historical land clearing, *D. reticulata* remains widespread and secure in areas of suitable habitat and is likely considered to be Least Concern under the IUCN conservation criteria following exclusion of the new species, with an EOO (extent of occurrence) of 1,255,938 km^2 and an AOO (area of occupancy) of 1,520 km^2. It occurs in many large, relatively undisturbed nature reserves and national parks (e.g. Yalgorup, Yanchep, Moore River, Badgingarra, Lesueur, Kalbarri, Kennedy Range, Cape Range, Millstream-Chichester and Karijini).

***Demansia cyanochasma* sp. nov.**
Desert Whip Snake (Figs. 8–10)

Chresonymy
Diemenia reticulata Lucas & Frost 1896, *Report on the Work of the Horn Scientific Expedition to Central Australia, Part II, Zoology*: 147.
Demansia psammophis reticulata Kinghorn 1956, *Snakes of Australia 2nd Edition*: 131 [in part].
Demansia psammophis Mitchell 1961, *Harmless or Harmful? 2nd Edition*: 23. *Demansia reticulata cupreiceps* Storr 1978, *Records of the Western Australian Museum*: 295 [in part].
Demansia psammophis cupreiceps Cogger 1983, *Reptiles & Amphibians of Australia 3rd Edition*: 431 [in part].
Demansia reticulata Dell & How 1984, *Records of the Western Australian Museum*: 65.
Demansia psammophis psammophis Storr, Smith & Johnstone 1986, *Snakes of Western Australia*: 69 [in part].

Holotype. SAMA R48865 (Fig. 8), male, Moonaree Station, Gawler Ranges (-31.7017, 135.5319), South Australia, Australia, collected by H. Ehmann, 2 November 1996. Fixed in 10% formalin, stored in 70% ethanol; liver tissue stored at SAMA as ABTC58583.

Paratypes (20). Northern Territory: MAGNT R01371, male, 97.4 km W of Yuendumu Turnoff (-23.283, 132.900); MAGNT R01565, female, Alice Springs (-23.700 133.867); MAGNT R03467, male, Arltunga Goldfields, Arltunga Historical Reserve (-23.433 134.700); MAGNT R16599, female, Elliot (-17.617 133.517); MAGNT R32379, male, Warrabri (-21.000 134.417); MAGNT R36247, female, Alice Springs (23.700 133.867). South Australia: SAMA R32260, female, Scrubby Peak area, Gawler Ranges (-32.5625 135.2875); SAMA R48412, female, 6.1 km S of

Mount Bray, Mount Barry Station (-28.2114 134.7942); SAMA R49048, male, 4.9 km ESE of Welcome Well, Arcoona Station (-31.2908 137.0583); SAMA R53139, male, 4.5 km ESE of Callory Bore, Flinders Ranges (31.8100 138.8081); SAMA R60568, female, 0.9 km ESE of Hamilton Homestead (-26.7108 135.0822); SAMA R64434, female, 12.9 km SSE of Lake Ifould, Great Victoria Desert (-30.9600 132.2297); SAMA R67418, male, 1.8 km WSW of Hiltaba Homestead (-32.1642 135.0525); SAMA R70189, female, 1 km NNW of Port Augusta (-32.4622 137.7448); SAMA R71354, male, Enorama Spring, Flinders Ranges (-31.31311 138.70076). Western Australia: WAM R72500, male, 3 km SW of Buningonia Spring (-31.4430 123.5278); WAM R73340, male, 3.5 km WNW of South East Peak, Mount Manning Range (-29.9916 119.6083); WAM R166045, male, Neale Junction Nature Reserve (-28.2450 125.8458); WAM R168341, male, Great Victoria Desert (-28.3677 127.5161); WAM R169206, female, 7–8 km WNW of Point Salvation (-28.2000 123.6000).

FIGURE 8. Preserved holotype SAMA R48865 of *Demansia cyanochasma* **sp. nov.** (Photo: M. Hutchinson).

Diagnosis. A medium-sized *Demansia* with more than 172 ventrals and with a cream-edged black transrostral line typically only extending to the nostrils, lacking any traces of hood or collar markings, and combining a variable body colour of shades of bluish grey or grey with a brown head and posterior body and tail, indistinct dark margins on the body scales, a relatively short dark tail on comma-shaped circumorbital marking typically extending to the fifth supralabial and occasionally contacting or continuing on to the lower primary temporal, ventral surface white to bluish white.

Description. Mean snout-vent length 510 mm (max. 700 mm), maximum total length to 890 mm, males and females similar in size (mean SVL males 501 mm, females 523 mm); tail length 24.7–38.9% of snout-vent length; 15 midbody scale rows, increasing to 16 or 17 (rarely 18) posterior to the head and decreasing to 13 (rarely 12 or 14) anterior to the vent; 173–203 ventrals (mean 188); 64–85 subcaudals (mean 74); 179–208 vertebrals (mean 195). Tail length and subcaudal counts were the only significant sexually dimorphic characters, mean male tail length was 30.4% of SVL with a mean of 77 subcaudal scales compared with a mean female tail length of 28.0% of SVL and 70 subcaudal scales.

Internasals much wider than long; prefrontals about equal size and much larger than internasals; one preocular, higher than wide in broad contact with prefrontal and second and third supralabials, in short contact with nasal; two postoculars, the upper wider and lower narrower; two large primary temporals, the lower much larger and projecting between fifth and sixth supralabials but not contacting oral margin; two smaller secondary temporals of about equal size above sixth supralabial; six supralabials, fourth and fifth in contact with lower postocular, the fifth and sixth of equal size and much the largest; seven infralabials, fourth the largest, sixth much narrower and slightly wider than fifth, seventh much the smallest; mental broadly triangular; anterior chin shields in broad contact with infralabials one to three, posterior chin shields in narrow contact with third infralabial and in broad contact with fourth infralabial; four rows of intergulars between chin shields and anteriormost broad ventral.

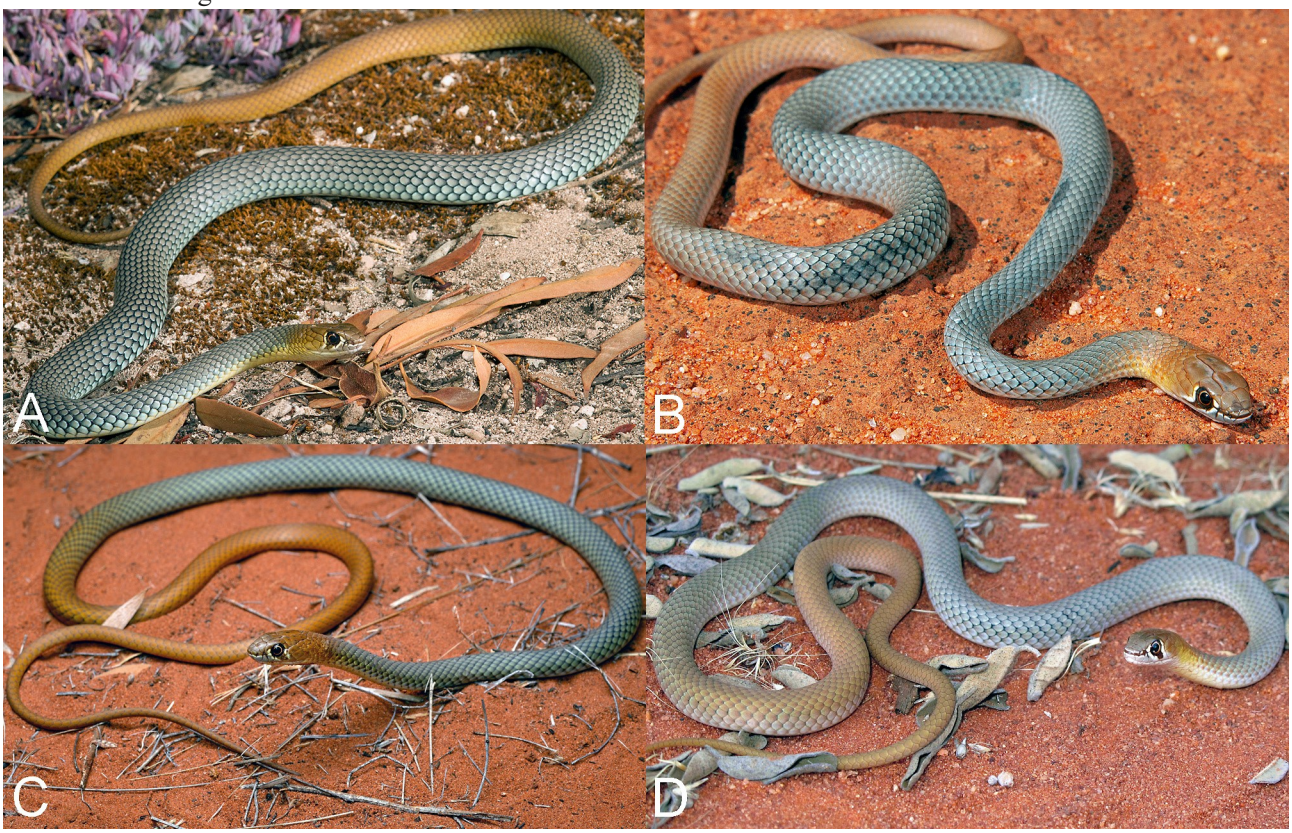

FIGURE 9. Adult *Demansia cyanochasma* **sp. nov.** photographed in life from (A) Streaky Bay, South Australia, (B) MungaThirri (formerly Simpson Desert) National Park, Queensland, (C) Kata Tjuta (the Olgas), Northern Territory, and (D) Tanami Desert, Northern Territory (photos A: B. Schembri; B: M. Sanders; C: M. Summerville; D: R. Lloyd).

Colour of body in life variable (Fig. 9), shades of bluish grey or grey, grading to pale orange or yellowish brown on posterior body and tail; body scales, except distally and on tail, typically with narrow black margins on anterior facets with slight widening at base (mostly concealed by overlapping leading edge of preceding scale), and margins on posterior facets and apex with narrow to wide obscure black smudging forming indistinct reticulated pattern (smudging most reduced lateroventrally and restricted to apex only); dark pigment along the posterior third of body gradually reduces to, at most, a very narrow darker zone on the anterior facets; demarcated head and nape pale brown, orange brown to dark brown, extending on to the nape as far as the first 4–7 vertebrals; a distinct dark transrostral line with pale margin extending horizontally to nostrils and pale markings in front of and behind the eyes forming the margin to the dark comma-shaped circumorbital marking, with its ventrocaudal tail relatively short extending from the fourth supralabial to, but not beyond the middle of the fifth supralabial, only occasionally contacting lower

primary temporal; ventral surface glossy white to pale bluish white, with a yellowish tinge on the anterior ventrals, particularly on throat; irides with a golden yellow ring bordering the round pupil.

In preservative, the body colour fades to a dull shade of grey. Head, nape, posterior body and tail colour fade to a duller brown. Ventral surface white and the yellowish tinge on the anterior ventrals and throat is retained in only the more recently preserved individuals.

Variation. No geographic pattern of variation was evident despite the wide distribution. Individual variation was limited to sometimes weaker expression of the transrostral line or occasional darker smudges on the head or labial scales. One specimen (SAMA R61913) has a concolorous grey head and body, grey smudging on anterior edge of ventrals, more distinct posteriorly with typical pale brown posterior body and tail. Only one specimen (SAMA R1319) has the lower primary temporal descending deeply between last two supralabials contacting the oral margin, and two specimens (SAMA R70149, WAM R97309) have 13 midbody scale rows.

Morphological characters are defined in Table 2 and the data presented in Table 3 for all specimens of *D. cyanochasma* **sp. nov.** listed in the Appendix. Table 4 presents the individual measurements and meristic counts for the paratype series of *D. cyanochasma* **sp. nov.**

Details of holotype (Fig. 8). Measurements (in mm) and meristic values: ToL 621, SVL 488, TailL 133 (27.2%), HeadL 14.7, HeadW 8.6, HeadD 7.0, SnL 5.0, BW 9.1, BD 8.1, FrL 5.2, FrW 2.1, SupOcL 4.5, SupOcW 1.9, EyeW 3.1, VS 178, MBSR 15, AntSR 17, PostSR 13, ScS 67, VertSc 184, SupLab 6, InfLab 7.

Scalation and proportions are as in the diagnosis for the genus and species. Individual characteristics include an offline medial suture between internasals so that right prefrontal contacts left internasal. In preservative, the holotype is a dull pale grey on the dorsal surface of the body, and the clearly demarcated head, posterior third of body and tail are a dull pale brown, fading to white ventrally under the tail, and head with indistinct darker smudging on frontal, supraoculars and parietals, extending back on to first six vertebrals on the nape.

Etymology. The specific name *cyanochasma* is modified from the Greek words *kỳanos* meaning blue and *khásma* meaning space or expanse, alluding to the bluish grey anterior body colour of this species and reference to its distribution filling the geographical space between *D. psammophis* and *D. reticulata*. Used as a noun in apposition.

Distribution. *Demansia cyanochasma* **sp. nov.** is widespread throughout the semiarid and arid areas of central Australia (Fig. 5), from the southeastern inland of Western Australia, much of South Australia, central and southern Northern Territory to southwestern Queensland. There is an apparent outlier population (MAGNT R16599) from Elliot in the Davenport Murchison Ranges bioregion (Fig. 5).

Habitat and ecology. *Demansia cyanochasma* **sp. nov.** appears to be a habitat generalist, occupying a variety of semiarid to arid vegetation associations growing on sandy, loamy and stony soils and rocky hills. Dominant vegetation can be arid woodlands, shrublands, hummock grasslands and chenopod plains (Fig. 11) and it often occupies areas around human dwellings and abandoned ruins. This species is a frequent visitor to town gardens in the Western Australian Goldfields (B. Bush & P. Orange, pers. obs.) and in Alice Springs, where it is regularly caught and relocated by herpetologists (R. Neindorf, pers. comm.). Habitat and ecological notes on the species (variously referred to as *D. reticulata, D. r. cupreiceps, D. psammophis* or *D. p. cupreiceps*) can be found in Dell & How (1984, 1988), Orange (1991, 1994), Reid *et al.* (1993), Burbidge *et al*. (1995), Brandle (2010), Swan & Wilson (2015) and Thompson & Thompson (2007). In hot weather it has been observed active at night on roads (B. Bush & J. Farquhar, pers. obs.).

Sympatry or close parapatry with other species. At the southeastern extremities of its range in South Australia, *D. cyanochasma* **sp. nov.** extends to the middle parts of the Flinders Ranges (e.g. Wilpena Pound – Brachina Gorge areas) where it abuts the range of *D. psammophis* both to the north (Gammon Ranges) and south (Hawker southwards); however the two have not been found in syntopy. At the western extremities of its range in Western Australia it is also parapatric with *D. reticulata.* A field observation of *D. cyanochasma* **sp. nov.** has been

TABLE 4. Individual measurements (mm) and meristic counts for the paratype series of *Demansia cyanochasma* **sp. nov.**

Museum #	MAGNT R01371	MAGNT R01565	MAGNT R03467	MAGNT R16599	MAGNT R32379	MAGNT R36247	SAMA R32260	SAMA R48412	SAMA R49048	SAMA R53139	SAMA R60568	SAMA R64434	SAMA R67418	SAMA R70189	SAMA R71354	WAM R72500	WAM R73340	WAM R166045	WAM R168341	WAM R169206
Sex	M	F	M	F	M	F	F	F	M	M	F	F	M	F	M	M	M	M	M	F
ToL	689	890	677	696	677	686	574	670	758	789	560	604	699	585	571	718	785	791	707	781
SVL	532	700	517	550	525	533	450	525	590	595	438	463	542	458	440	548	600	598	534	600
TailL	157	190	160	146	152	153	124	145	168	194	122	141	157	127	131	170	185	193	173	181
% SVL	29.5	27.1	30.9	26.5	28.9	28.7	27.5	29.3	28.4	32.6	27.8	30.4	28.9	27.7	29.7	31.0	30.8	32.2	32.3	30.1
HeadL	15.3	16.7	13.6	14.6	14.7	14.8	13.6	14.8	16.7	16.9	13.2	13.7	15.6	13.8	13.5	15.2	16.7	17.7	15.7	16.7
HeadW	9.2	9.2	9.7	8.3	9.3	8.0	7.5	9.1	9.5	10.3	8.0	8.4	8.8	8.4	7.3	8.4	9.6	9.7	9.5	10.3
HeadD	7.5	7.8	7.3	7.2	7.3	6.8	6.3	7.1	7.1	7.7	5.8	5.8	7.1	6.2	7.2	7.1	7.0	8.2	7.2	8.0
SnL	4.8	5.0	4.4	5.3	4.8	5.3	4.4	5.0	5.5	5.3	4.1	4.1	5.0	4.8	4.7	5.9	5.1	5.2	5.3	5.8
BW	12.0	12.9	11.1	11.3	11.9	7.8	8.5	9.7	9.6	11.3	7.2	9.4	9.8	9.4	8.5	9.6	10.4	10.7	10.2	14.5
BD	9.5	12.8	9.7	7.4	9.4	8.6	9.1	9.4	8.0	10.9	6.7	7.9	8.8	8.5	6.3	8.3	9.6	9.5	7.7	10.7
FrL	5.3	5.1	5.1	5.7	5.8	5.4	5.1	5.3	6.0	6.4	4.7	5.1	5.8	5.4	4.7	6.1	6.2	6.5	6.3	5.9
FrW	2.6	2.4	2.2	2.0	2.3	2.4	2.3	2.3	2.3	2.7	1.8	2.0	2.4	2.2	2.1	2.6	2.4	2.4	2.5	2.6
SupOcL	4.4	5.2	5.3	5.0	5.0	4.4	4.5	5.1	5.4	4.9	3.8	4.3	4.8	4.1	4.0	4.9	5.2	5.2	4.9	5.0
SupOcW	2.0	2.1	1.9	2.0	1.9	2.1	2.1	2.2	2.3	2.4	2.3	2.0	2.1	1.9	1.8	2.3	2.6	2.6	2.3	2.5
EyeW	3.3	3.4	3.1	3.4	3.4	3.3	3.3	3.5	3.6	3.3	3.1	3.0	3.8	3.1	3.1	3.5	3.6	2.9	3.7	3.1
VS	189	201	185	194	195	198	179	181	188	181	191	183	182	182	182	190	184	189	193	189
MBSR	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
AntSR	17	16	16	17	16	17	18	17	17	17	17	17	17	17	16	17	17	16	16	16
PostSR	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	12	14	13	13
ScS	75	72	75	69	75	76	67	71	75	80	73	70	74	68	72	82	80	79	79	70
VertSc	199	208	193	196	198	202	188	191	194	186	197	189	186	186	186	194	198	204	198	196
SupLab	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
InfLab	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7

made further west at Karalee Rock (M. Sanders, pers. obs.) in closer proximity (~ 150 km) to the Jouerdine Nature Reserve record WAM R136510 of *D. reticulata*.

Based on collection records in Shea & Scanlon (2007) broad sympatry involving *D. cyanochasma* **sp. nov.** possibly occurs throughout large areas with *D. rimicola* in the central Northern Territory and southwestern Queensland and with *D. shinei* in the central and southern Northern Territory. D. *cyanochasma* **sp. nov.** and *D. rimicola* are known from the vicinity of Boulia in Queensland and *D. cyanochasma* **sp. nov.** and *D. shinei* have been recorded from the vicinities of Uluru and Yuendumu in the Northern Territory. All three species are known from the same location of Elliot in the Northern Territory. In southwestern Queensland collection records suggest *D. cyanochasma* **sp. nov.** and *D. quaesitor* possibly occur in close parapatry in the vicinity of Boulia.

Comparisons with other species. *Demansia cyanochasma* **sp. nov.** differs from all other *Demansia* except *D. psammophis* and *D. reticulata* in combining the consistent presence of a brown head, posterior body and tail contrasting with a bluish grey or grey body, each body scale having a typically indistinct dark margin (Fig. 10C shows the extreme condition of the dark margin), absence of any hood or collar markings on head and neck and the consistent presence of a circumorbital comma-shaped marking and transrostral line.

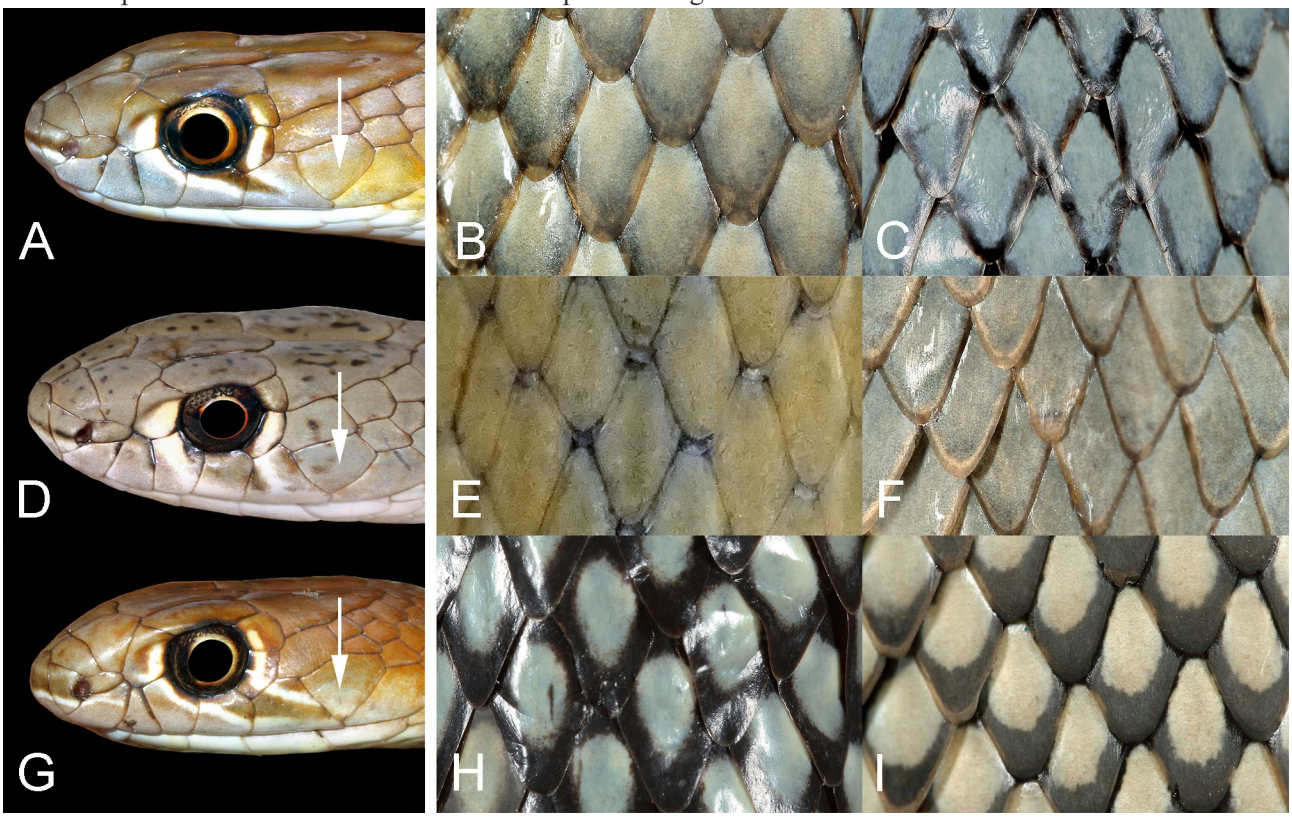

FIGURE 10. The typical extent of dark comma-shaped marking ventrocaudal to eyes in live *Demansia* specimens (A, D, G), and dark margins or otherwise on body scales in preserved *Demansia* specimens (B, C, E, F, H, I). Arrows indicate the lower primary temporal scale. A–C: *D. cyanochasma* **sp. nov.**, D–F: *D. psammophis*, and G–I: *D. reticulata* (photos A, G: B. Bush; B, C, F, H, I: B. Maryan; D: S. Scott; E: M. Hutchinson).

From *D. psammophis* it is distinguished by its demarcated brown head (Fig. 10A), posterior body and tail and the presence of more distinct dark scale margins (Figs. 10B, C) *versus* head (Fig. 10D), posterior body and tail similar colour to body or tail only becoming gradually yellowish towards the tip, dark scale margins not, or only weakly developed anteriorly (Figs. 10E, F); absence of reddish paravertebral stripes or dorsal zone seen in many *D. psammophis* (see above) and ventral surface white to bluish white *versus* whitish with a median yellowish or greenish stripe or series of blotches, fading to grey on large individuals. Further distinguished by a smaller SVL mean of 510 mm (*versus* 550 mm), proportionally larger head and less robust body determined by IBR (Table 3).

From *D. reticulata* it is distinguished by its much less contrasting dark scale margins (Figs. 10B, C *versus* Figs. 10H, I) and dark tail on comma-shaped circumorbital marking typically short, usually terminating on the fifth supralabial Fig. 10A (*versus* tail of comma-shaped circumorbital marking typically long, usually extending across the fifth supralabial to the lower primary temporal, Fig.10G). Further distinguished by the consistent presence of a

brown head colour (*versus* variably present in *D. reticulata*, see above), bluish grey or grey rather than greenish body colour, smaller SVL mean of 510 mm (*versus* 588 mm) and proportionally shorter frontal scale (Table 3).

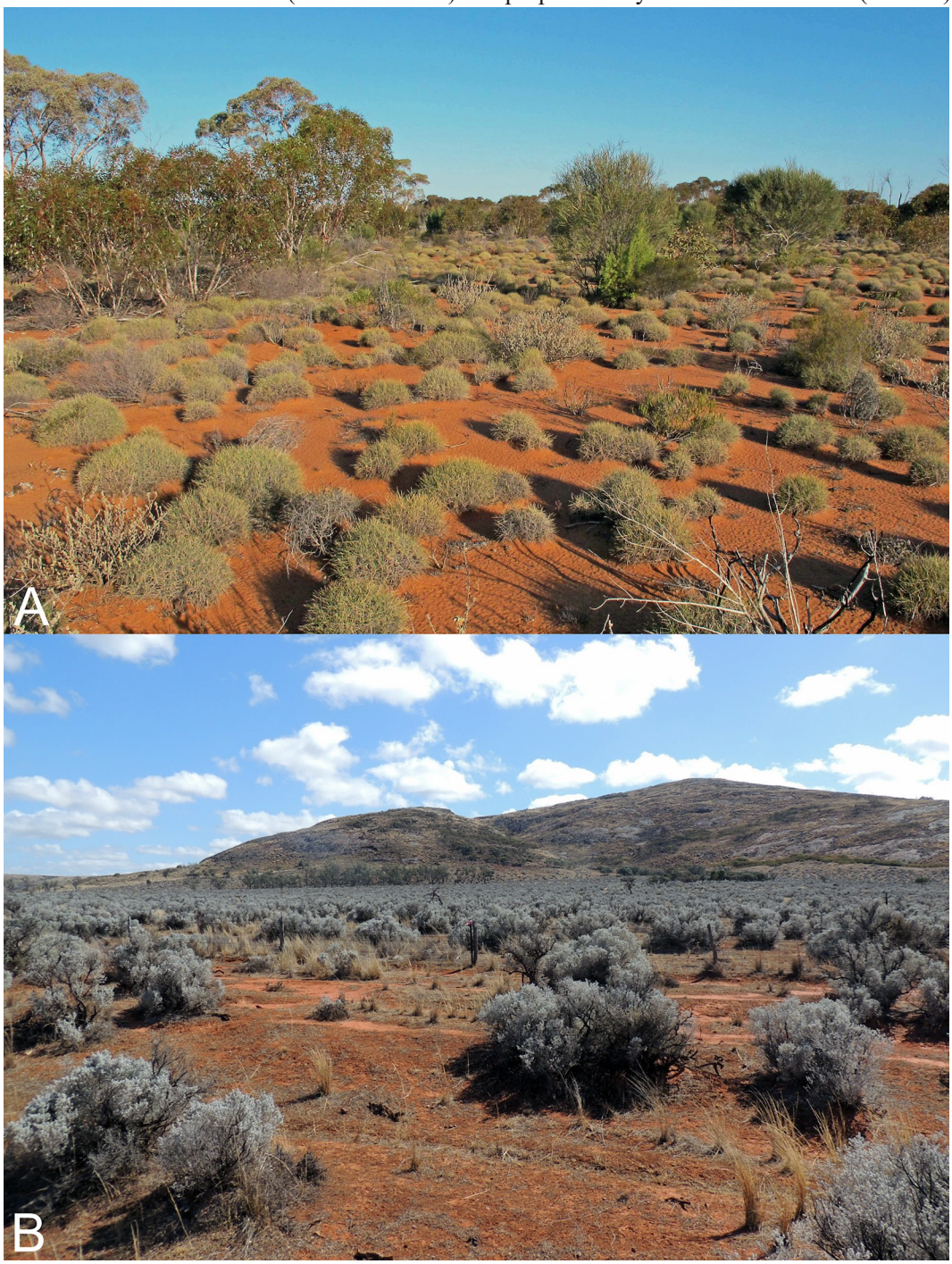

FIGURE 11. Representative habitats of *Demansia cyanochasma* **sp. nov.**: (A) sandplain vegetated with mallee over hummock grassland near Plumridge Lakes, Great Victoria Desert, Western Australia, and (B) chenopod shrubland and stony hills, Hiltaba, South Australia (photos A: B. Maryan; B: M. Hutchinson).

Remarks. *Demansia cyanochasma* **sp. nov.** has been illustrated in numerous publications, always under some combination of the three names *psammophis*, *reticulata* and *cupreiceps*. The earliest illustration of *D. cyanochasma* **sp. nov.** we are aware of is in Cogger (1967: Plate 46) as *D. psammophis* from Coober Pedy in South Australia. Other examples are Weigel (1990: 50), Storr *et al.* (2002: 123, Plate 23), Thompson & Thompson (2006: 117), Bush *et al.* (2007: 247), Eipper (2012: 172), Williams and Maier (2019: 226), Wilson (2022: 282) and Allen & Vogel (2019: 61–62). Additionally, both Storr *et al*. (2002: 171) and Ehmann (2005: 216) refer to the eastern subspecies of *D. p. psammophis* from Western Australia and *D. reticulata* individuals from South Australia, respectively with having

narrow or no black margin to scales and a usually indistinct darker netted body pattern, features consistent with *D. cyanochasma* **sp. nov.**

Demansia cyanochasma **sp. nov.** is secure and widespread spanning four Australian states and is likely considered to be Least Concern under the IUCN conservation criteria, with an EOO (extent of occurrence) of 2,304,774 km^2 and an AOO (area of occupancy) of 1,772 km^2. It occurs in many large, relatively undisturbed aboriginal lands/nature reserves and national parks (e.g. Goongarrie, Queen Victoria Springs, Great Victoria Desert, Uluru-Kata Tjuta, West MacDonnell, Gawler Ranges and Munga-Thirri (Simpson Desert)) that include much of semiarid to arid central Australia.

Discussion

This study has provided the first phylogeny for all currently known species of *Demansia* except *D. olivacea*, emphasising the relationships of populations long referred to two familiar but problematic taxa, *D. psammophis* and *D. reticulata*. Our combined genetic and morphological analyses confirm that both *D. psammophis* and *D. reticulata* are full species and subspecific classification is unwarranted, leading us to synonymise *D. r. cupreiceps* with *D. reticulata*. Much of the taxonomic confusion over the last 40 or more years has involved the uncertain affinities of central Australian populations which we now show belong to a new species, *D. cyanochasma* **sp. nov.**

The description of *D. cyanochasma* **sp. nov.** brings the total number of currently known species of *Demansia* to fifteen, making it the most speciose clade of Australian terrestrial elapids (Cogger 2018; Wilson & Swan 2021). Morphologically, *D. cyanochasma* **sp. nov.** is most like some *D. reticulata* in colour pattern, but it is well supported as genetically distinct and can also be identified purely through morphological features such as its less extensive circumorbital marking, weaker dark scale margins and smaller size. *Demansia cyanochasma* **sp. nov.** appears to be a desert generalist occupying much of Australia's central and western arid zone, particularly in the presence of hummock grasslands, that supports the richest lizard fauna in the world (Powney *et al*. 2010) and provides ample prey for a medium-sized saurophagous snake.

The fact that this widespread and morphologically distinct species has remained unrecognised can be attributed to several factors. *Demansia* species are highly conserved morphologically with very few traditional scale characters distinguishing species (Shea & Scanlon 2007). Instead, most *Demansia* species can be distinguished by colour patterns and by more subtle aspects of morphology such as head and body proportions. However, many species also exhibit varying amounts of geographical variation and with many species being wary inhabitants of remote areas, it has taken a long time to acquire specimens from critical areas of distribution where contact or intergradation between possible species exist. General fauna surveys associated with state driven concerns for biodiversity or associated with mining and agricultural developments have allowed the accumulation of enough specimens as well as coloured photographs of live individuals from across their entire distribution to evaluate their systematic status.

Although not comprehensive in terms of molecular sampling for the *Demansia* species, our study provides a starting point for further work on this speciose genus. Among its findings, the molecular study provided equivocal support for either *D. simplex* or a clade consisting of *D. flagellatio, D. rimicola* and *D. torquata* as being basal to the rest of the genus. *Demansia simplex* has a relatively plesiomorphic unspecialised morphology and may represent a model of the early morphology of the genus as discussed by Scanlon (2003) and Scanlon & Lee (2004: 338). The other early diverging clade, the eastern tropical species *D. flagellatio*, *D. rimicola* and *D. torquata*, suggest that a potential origin of diversification for the genus was in this geographic area. The remaining species form a crown group that divides into three clades, with a western dry tropical to arid clade (*D. calodera*, *D. quaesitor*, *D. rufescens*), the two large, monsoonal black species (*D. papuensis* and *D. vestigiata*), and the clade containing the species under study here plus two additional species, *D. angusticeps* and *D. shinei*. This suggests a picture of tropical monsoon climatic origins for the genus, with subsequent speciation in the east and west, followed by expansion of a more adaptable *psammophis* clade, with southerly invasion of *D. psammophis* into cooler habitats in the southeast of the continent and *D. reticulata* along the west coast. The molecular phylogeny also suggests significant amounts of convergence within the genus, with the more extremely attenuate species, such as *D. flagellatio* and *D. shinei*, not found as close relatives, but instead being closely allied with more robust sister species and contrasting head and collar markings similarly seem to have been gained and/or lost several times across the genus.

Within the *D. psammophis* complex, *D. cyanochasma* **sp. nov.** shares a common ancestry with *D. reticulata*, suggesting invasion of the true arid zone from an ancestry in the semiarid and seasonally dry tropical areas of the west. This appears to reflect a common pattern in Australian squamates that has been well-documented in lizards

(e.g. Ashman *et al*. 2018; Laver *et al*. 2018) where clades originate in the Australian monsoonal tropics before later diversifying into the arid and temperate zones. While *D. psammophis* has penetrated cool temperate regions better than any other *Demansia* species, it appears limited by aridity, tracking the Murray River valley and higher elevation Flinders Ranges within South Australia. Of the remaining species in this clade, *D. angusticeps* may be the species that stays closest to the ancestral climatic niche of the genus, along the margins of the monsoon tropics, while the morphologically distinctive *D. shinei* represents a separate invasion of central Australia.

Despite the progress made in this study; knowledge gaps remain of *Demansia* species-level taxonomy in Australia. Our sampling for *D. psammophis* lacked geographic coverage in New South Wales and Queensland and further investigation is needed into these populations given the morphological variation in such a widespread species. Reflecting this, we are now working towards a more complete sampling across all *Demansia* species with an emphasis on genetically characterising additional specimens from key geographic regions and morphotypic forms (see Shea & Scanlon 2007). This will allow us to generate the detailed genetic framework required to define how many additional candidate species are likely to be present, before ultimately assessing these for morphological diagnosability.

The mito-nuclear discordance between *D. angusticeps* and *D. reticulata* in our study demonstrates the pitfalls of exclusively using mitochondrial genes to infer systematic relationships, and we recommend integrative approaches to taxonomy as best practice.

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APPENDIX. Material (prefixes omitted) examined in this study. Under the species column: H indicates holotype, P paratype, L lectotype, PL paralectotype. Abbreviations are: ABTC Australian Biological Tissue Collection, Adelaide; AM Australian Museum, Sydney; ANWC Australian National Wildlife Collection, Canberra; MAGNT Museum and Art Gallery of the Northern Territory, Darwin; MNHN Muséum National d'Histoire Naturelle, Paris; NHMUK Natural History Museum United Kingdom, London; NMV Museums Victoria, Melbourne; QM Queensland Museum, Brisbane; SAMA South Australian Museum, Adelaide; WAM Western Australian Museum, Perth; NSW New South Wales; NT Northern Territory; QLD Queensland; SA South Australia; WA Western Australia. Mor. indicates specimens examined as follows: *morphology only; **morphology and DNA; ***DNA only and ****photograph only. Under the sex column Juv. indicates unsexed juvenile.

Species	Reg. #	Sex	Locality	State	Lat. (S)	Long. (E)	Mor.
D. angusticeps	WAM 156731–32		Broome	WA	-17.983611	122.214444	***
D. calodera	WAM 122880		10 km NE of Cape Cuvier	WA	-24.138333	113.445833	***
D. cyanochasma	AM 141950		Boulia	QLD	-22.916	138.800	****
D. cyanochasma	ANWC 02720		Cuddapan Station, W of Windorah	QLD	-25.65	141.50	****
D. cyanochasma	MAGNT 00837	F	Alice Springs	NT	-23.7000	133.867	*
D. cyanochasma P	MAGNT 01371	M	97.4 km W of Yuendumu Turnoff	NT	-23.283	132.900	*
D. cyanochasma P	MAGNT 01565	F	Alice Springs	NT	-23.700	133.867	*
D. cyanochasma	MAGNT 02459	F	20 km W of Alice Springs, Honeymoon Gap	NT	-23.7366	133.7417	*
D. cyanochasma	MAGNT 02461	M	Near Alice Springs Airport	NT	-23.750	133.867	*
D. cyanochasma P	MAGNT 03467–69	M,M,M	Arltunga Goldfields, Arltunga Historical Reserve	NT	-23.433	134.700	*
D. cyanochasma	MAGNT 05704	M	7.6 km S of Aileron	NT	-22.700	133.367	*
D. cyanochasma P	MAGNT 16599	F	Elliot	NT	-17.617	133.517	*
D. cyanochasma	MAGNT 17513	Juv.	Uluru National Park	NT	-25.25	131.00	*
D. cyanochasma	MAGNT 23387	M	Winparku, W of Haasts Bluff	NT	-23.373	131.457	*
D. cyanochasma	MAGNT 23687	Juv.	Curtin Springs Station	NT	-25.317	131.750	*
D. cyanochasma	MAGNT 26806	Juv.	Umbeara Station	NT	-25.870	134.107	*
D. cyanochasma	MAGNT 32375	M	50 km S of Alice Springs	NT	-24.133	133.883	*
D. cyanochasma	MAGNT 32378	M	Ernabella	SA	-26.283	132.133	*
D. cyanochasma P	MAGNT 32379	M	Warrabri	NT	-21.000	134.417	*
D. cyanochasma	MAGNT 32380	M	Mount Blatherskite	NT	-23.733	133.883	*

D. cyanochasma	MAGNT 32383	F	Todd River, Simpson Desert	NT	-24.483	135.533	*
D. cyanochasma P	MAGNT 36247	F	Alice Springs	NT	-23.700	133.867	**
D. cyanochasma	MAGNT 37310	M	Alice Springs, 4 Mimosa Court	NT	-23.700	133.867	*
D. cyanochasma	MAGNT 38827	F	Tanami Road Gas Pipeline, Tanami Desert	NT	-22.06366	131.3368	*
D. cyanochasma	MAGNT 38871	M	Tanami Road Gas Pipeline, Tanami Desert	NT	-22.17422	131.50393	*
D. cyanochasma	MAGNT 38872	M	Tanami Road Gas Pipeline, Tanami Desert	NT	-22.23694	131.68618	*
D. cyanochasma	QM 28370		Barcoo	QLD	-25.416667	142.65000	****
D. cyanochasma	QM 91614		Ballera	QLD	-27.430833	141.871944	****
D. cyanochasma	SAMA 1319	M	Wharminda Siding, Eyre Peninsula	SA	-33.97	136.25	*
D. cyanochasma	SAMA 3090	M	Fowlers Bay area	SA	-31.98	132.43	*
D. cyanochasma	SAMA 3633	F	Hatches Creek	NT	-20.95	135.22	*
D. cyanochasma	SAMA 18596	F	Near Oodnadatta	SA	-27.55	135.47	*
D. cyanochasma	SAMA 20796	F	Peela Tank, Eyre Peninsula	SA	-33.02	135.72	**
D. cyanochasma	SAMA 24829	F	Bunyeroo Gorge, Flinders Ranges	SA	-31.42	138.55	*
D. cyanochasma	SAMA 28499	M	102.5 km ENE of Minnipa, Gawler Ranges	SA	-32.60	136.22	*
D. cyanochasma P	SAMA 32260	F	Scrubby Peak area, Gawler Ranges	SA	-32.5625	135.2875	**
D. cyanochasma	SAMA 40446	M	Abminga Ruins	SA	-26.15	134.85	**
D. cyanochasma	SAMA 45517	Juv.	9 km N of Whyalla	SA	-32.9542	137.5622	**
D. cyanochasma	SAMA 46163	Juv.	23 km NW of Kunytjanu	SA	-26.5167	129.2019	*
D. cyanochasma	SAMA 46345	M	8.7 km WSW of Johnson Bore	SA	-29.5581	136.1389	**
D. cyanochasma P	SAMA 48412	F	6.1 km S of Mount Bray, Mount Barry Station	SA	-28.2114	134.7942	*
D. cyanochasma H	SAMA 48865	M	Moonaree Station, Gawler Ranges	SA	-31.7017	135.5319	**
D. cyanochasma P	SAMA 49048	M	4.9 km ESE of Welcome Well, Arcoona Station	SA	-31.2908	137.0583	*
D. cyanochasma	SAMA 50165	M	14.4 km S of Sentinel Hill	SA	-26.2108	132.4428	*
D. cyanochasma P	SAMA 53139	M	4.5 km ESE of Callory Bore, Flinders Ranges	SA	-31.8100	138.8081	**
D. cyanochasma	SAMA 56663	M	5.8 km S of Tyringa Homestead, Eyre Peninsula	SA	-33.1567	134.4075	*
D. cyanochasma	SAMA 58478	F	0.7 km WNW of Mount Wedge, Eyre Peninsula	SA	-33.4725	135.1339	*
D. cyanochasma	SAMA 60410	Juv.	80.8 km W of Oak Valley, Great Victoria Desert	SA	-29.4025	129.9061	*
D. cyanochasma P	SAMA 60568	F	0.9 km ESE of Hamilton Homestead	SA	-26.7108	135.0822	*
D. cyanochasma	SAMA 61913	M	0.3 km ENE of Port Kenny, Eyre Peninsula	SA	-33.1683	134.6836	**
D. cyanochasma	SAMA 61925	F	3 km S of Agnes Creek	SA	-26.4700	133.2556	*

D. cyanochasma P	SAMA 64434	F	12.9 km SSE of Lake Ifould, Great Victoria Desert	SA	-30.9600	132.2297	**
D. cyanochasma P	SAMA 67418	M	1.8 km WSW of Hiltaba Homestead	SA	-32.1642	135.0525	*
D. cyanochasma	SAMA 69306	M	3.2 km NW of Oraparinna	SA	-31.3439	138.7039	**
D. cyanochasma	SAMA 70149	F	1 km E of Andamooka Homestead	SA	-30.7289	137.2110	*
D. cyanochasma P	SAMA 70189	F	1 km NNW of Port Augusta	SA	-32.4622	137.7448	*
D. cyanochasma	SAMA 70731	M	Maralinga Tjarutja, Great Victoria Desert	SA	-28.81552	129.53373	*
D. cyanochasma P	SAMA 71354	M	Enorama Spring, Flinders Ranges	SA	-31.31311	138.70076	*
D. cyanochasma P of *D. r. cupreiceps*	WAM 15066	F	Maloora Rockhole	WA	-29.2000	127.9333	*
D. cyanochasma P of *D. r. cupreiceps*	WAM 15156	F	Warburton Range Mission, Elder Creek	WA	-26.2000	126.5333	*
D. cyanochasma P of *D. r. cupreiceps*	WAM 22072	M	Warburton Range Mission	WA	-26.1333	127.5000	*
D. cyanochasma	WAM 34101	M	Coolgardie	WA	-30.9500	121.1667	*
D. cyanochasma P of *D. r. cupreiceps*	WAM 34203	F	2 km E of Docker River Settlement	NT	-24.8666	129.1000	*
D. cyanochasma P of *D. r. cupreiceps*	WAM 36637	F	37 km NE of Maralinga	SA	-30.1055	131.6500	*
D. cyanochasma P	WAM 72500	M	3 km SW of Buningonia Spring	WA	-31.4430	123.5278	*
D. cyanochasma	WAM 72753	M	3.5 km NE of Comet Vale	WA	-29.9194	121.1417	*
D. cyanochasma P	WAM 73340	M	3.5 km WNW of South East Peak, Mount Manning Range	WA	-29.9916	119.6083	*
D. cyanochasma	WAM 87884	M	Cosmo Newberry	WA	-28.0000	122.9000	*
D. cyanochasma	WAM 97309	F	Kambalda	WA	-31.1833	121.6667	*
D. cyanochasma	WAM 100562	M	20 km S of Menzies	WA	-29.8500	121.0833	*
D. cyanochasma	WAM 100871	F	Kambalda East	WA	-31.2000	121.6667	*
D. cyanochasma	WAM 101489	Juv.	25 km NNE of Queen Victoria Spring	WA	-30.2333	123.6833	*
D. cyanochasma	WAM 126521	M	Kambalda East	WA	-31.2000	121.6667	*
D. cyanochasma	WAM 135110	M	Bullabulling	WA	-30.8613	120.8869	*
D. cyanochasma	WAM 137757	Juv.	45 km E of Madura Roadhouse	WA	-31.9108	127.2861	*
D. cyanochasma	WAM 147004	F	7–8 km WNW of Point Salvation	WA	-28.2000	123.5833	*
D. cyanochasma	WAM 149402		7–8 km WNW of Point Salvation	WA	-28.1944	123.5808	***
D. cyanochasma	WAM 163275	M	Neale Junction Nature Reserve	WA	-28.6858	125.8061	*
D. cyanochasma P	WAM 166045	M	Neale Junction Nature Reserve	WA	-28.2450	125.8458	**
D. cyanochasma P	WAM 168341	M	Great Victoria Desert	WA	-28.3677	127.5161	**
D. cyanochasma P	WAM 169206	F	7–8 km WNW of Point Salvation	WA	-28.2000	123.6000	*
D. flagellatio	ABTC 102710		Boodjamulla (Lawn Hill) National Park	QLD	-18.73947	138.31905	***
D. papuensis	SAMA 29954		Near Humpty Doo	NT	-12.63	131.25	***

D. psammophis	AM 147748		Cronulla Sewage Treatment Plant	NSW	-34.02971	151.16388	***
D. psammophis	AM 152093		Bolivia Hill	NSW	-29.32138	151.91804	***
D. psammophis	AM 156553		Denman	NSW	-32.38999	150.66416	***
D. psammophis	AM 159510		The Tor, Moonbi Gap Road, Moonbi	NSW	-31.00694	151.02527	***
D. psammophis	AM 159810		Morgans Gully, Boonoo Boonoo National Park	NSW	-28.8550	152.1486	***
D. psammophis	AM 162261		Byron Bay	NSW	-28.65	153.61	***
D. psammophis	AM 173021		Pearl Beach	NSW	-33.533	151.300	***
D. psammophis	AM 181753		Karawatha	QLD	-27.6322	153.0830	***
D. psammophis	AM 183149		Lake Innes Estate, Port Macquarie	NSW	-31.46555	152.87062	***
D. psammophis	AM 186154		Mount Arthur SW of Wellington	NSW	-32.57485	148.91835	***
D. psammophis	MAGNT 00301	F	Engadine, Sydney	NSW	-34.067	151.017	*
D. psammophis	MAGNT 01124	M	Heathcote	NSW	-34.133	150.967	*
D. psammophis	MAGNT 05422	M	Chinchilla Rifle Range	QLD	-26.733	150.633	*
D. psammophis	MAGNT 09267	M	Barratta Station, Flinders Ranges	SA	-30.250	139.083	*
D. psammophis	MAGNT 36463	F	Maryborough	QLD	-25.533	152.700	**
D. psammophis PL	MNHN-RA-0.7663	M		NSW			****
D. psammophis PL	MNHN-RA-2012.406	M		NSW			****
D. psammophis L	MNHN-RA-0.7666	F		NSW			****
D. psammophis	QM 81776		Couran Cove Island Resort, South Stradbroke Island	QLD	-27.821111	153.419167	***
D. psammophis	QM 82861		Caboolture area	QLD	-27.083333	152.950000	***
D. psammophis	QM 83300		Ipswich	QLD	-27.598	152.75	***
D. psammophis	SAMA 19587	M	Renmark	SA	-34.17	140.75	*
D. psammophis	SAMA 21430	M	Port Germein Gorge	SA	-33.02	138.00	**
D. psammophis	SAMA 24405	F	Inneston Ruins, Yorke Peninsula	SA	-35.27	136.88	*
D. psammophis	SAMA 25083	M	Port Pirie area	SA	-33.20	138.02	*
D. psammophis	SAMA 38447	F	7 km NW of Murray Bridge	SA	-35.0767	139.2178	**
D. psammophis	SAMA 42380	F	1.1 km W of Teatree Creek, Nepabunna/Balcanoona Roads	SA	-30.55	139.25	**
D. psammophis	SAMA 48108	F	Old Timor Out Station, Calperum Station	SA	-33.9711	140.6456	**
D. psammophis	SAMA 48922	M	Ferries McDonald Road, South End	SA	-35.2343	139.1393	*
D. psammophis	SAMA 50408	F	14.7 km ESE of Mount Coffin, Mooloolloo Station	SA	-30.5764	138.6522	*
D. psammophis	SAMA 50750	M	8 km NE of Yacka, Broughton River Valley	SA	-33.5333	138.5194	*
D. psammophis	SAMA 52983	Juv.	4.6 km NE of Freeling Heights, Arkaroola	SA	-30.1108	139.4158	**
D. psammophis	SAMA 53132	F	Dutchmans Stern	SA	-32.3117	137.9814	**
D. psammophis	SAMA 56400	Juv.	2.6 km NW of White Cliff Hill	SA	-33.2289	138.2292	**

D. psammophis	SAMA 59723	F	8.9 km NNE of Barker Rocks, Yorke Peninsula	SA	-34.63583333	137.5077778	*
D. psammophis	SAMA 59730	M	4.9 km SSW of Chinaman Wells, Yorke Peninsula	SA	-34.4036111	137.4636111	**
D. psammophis	SAMA 64100	F	Terrapina Spring, Hamilton Creek, Moolawatana Station	SA	-29.9153	139.6664	*
D. psammophis	SAMA 64858	M	4.9 km WNW of Arkaroola Homestead	SA	-30.3208	139.3206	*
D. psammophis	WAM 68895	M	Sydney	NSW	-33.8833	151.5000	*
D. quaesitor	MAGNT 36483		Snake Creek Bunkers, Adelaide River Hills	NT	-13.221	131.088	***
D. reticulata	AM 70020		Mukinbudin	WA	-30.916	118.200	****
D. reticulata	ANWC 02114		Wiluna	WA	-26.6000	120.2167	****
D. reticulata	ANWC 02188		16 km E of Wiluna, Millbillillie Station	WA	-26.6167	120.3333	****
D. reticulata	MAGNT 07219	M	Sandfire	WA	-19.683	121.267	*
D. reticulata PL	NHMUK 1946.1.19.78	M	Perth	WA			*
D. reticulata L	NHMUK 1946.1.19.79	M	Perth	WA			*
D. reticulata	NMV 65997		Canning Stock Route, between Wells 1–10	WA	-23.70	122.48	****
D. reticulata	WAM 622	F	Wickepin	WA	-32.7833	117.5000	*
D. reticulata P of *D. r. cupreiceps*	WAM 8283	F	Onslow	WA	-21.6333	115.1167	*
D. reticulata P of *D. r. cupreiceps*	WAM 8284	M	Onslow	WA	-21.6333	115.1167	*
D. reticulata P of *D. r. cupreiceps*	WAM 9412	M	Wandagee Station	WA	-23.8333	114.4167	*
D. reticulata P of *D. r. cupreiceps*	WAM 11491	M	Learmonth area	WA	-22.2500	114.0833	*
D. reticulata P of *D. r. cupreiceps*	WAM 12359	M	Wotjulum	WA	-16.1833	123.6167	*
D. reticulata P of *D. r. cupreiceps*	WAM 13309	F	16 km E of Carnarvon, Gascoyne Research Station	WA	-24.8833	113.8167	*
D. reticulata P of *D. r. cupreiceps*	WAM 15057	M	Mount Edgar	WA	-21.2500	120.1667	*
D. reticulata P of *D. r. cupreiceps*	WAM 15059	M	Mundabullangana	WA	-21.2500	118.2500	*
D. reticulata	WAM 15063	M	Bunjil	WA	-29.6500	116.3500	*
D. reticulata	WAM 21724	M	Whitfords Beach	WA	-31.8083	115.7333	*

D. reticulata P of *D. r. cupreiceps*	WAM 22948	F	Manberry	WA	-23.9583	113.6583	*
D. reticulata P of *D. r. cupreiceps*	WAM 23941	F	32 km N of Beyondie	WA	-24.5000	120.0333	*
D. reticulata P of *D. r. cupreiceps*	WAM 24018	M	Carnarvon	WA	-24.8833	113.6667	*
D. reticulata P of *D. r. cupreiceps*	WAM 30957	M	Spear Hill, Shaw River	WA	-21.5166	119.4167	*
D. reticulata	WAM 31154	M	Bencubbin	WA	-30.8166	117.8500	*
D. reticulata	WAM 31541	F	Cadoux	WA	-30.7144	117.1221	*
D. reticulata P of *D. r. cupreiceps*	WAM 32067	M	Manning Creek	WA	-16.5333	125.9167	*
D. reticulata H of *D. r. cupreiceps*	WAM 34555	M	Callagiddy	WA	-25.0333	114.0167	*
D. reticulata P of *D. r. cupreiceps*	WAM 34705	M	Exmouth	WA	-21.9333	114.1167	*
D. reticulata P of *D. r. cupreiceps*	WAM 37644	M	Callagiddy	WA	-25.0500	114.0333	*
D. reticulata P of *D. r. cupreiceps*	WAM 40676, 40681	F,M	Middle Well, Callagiddy Station	WA	-25.1166	114.0917	*
D. reticulata P of *D. r. cupreiceps*	WAM 41913	F	Hamersley Iron	WA	-22.7000	117.7833	*
D. reticulata	WAM 42377	M	Dirk Hartog Island	WA	-25.7500	113.0500	*
D. reticulata P of *D. r. cupreiceps*	WAM 45888	F	32 km W of Roy Hill	WA	-22.7500	119.6833	*
D. reticulata P of *D. r. cupreiceps*	WAM 52714	M	Marandoo Mine, Mount Bruce	WA	-22.6666	118.1667	*
D. reticulata P of *D. r. cupreiceps*	WAM 54599	M	Wooramel Homestead	WA	-25.6166	114.1833	*
D. reticulata	WAM 57774	M	17 km NW of Harvey	WA	-32.9666	115.7667	*
D. reticulata	WAM 59299	F	Golden Bay	WA	-32.4333	115.7167	*
D. reticulata	WAM 62160	F	Jandakot	WA	-32.1500	115.8333	*
D. reticulata	WAM 72964	M	19 km N of Coolimba	WA	-29.7166	114.9833	*
D. reticulata	WAM 74831	M	Banningarra Spring, Pardoo Station	WA	-20.0333	119.6833	*
D. reticulata	WAM 87533	F	14 km SSW of Gascoyne Junction	WA	-25.1666	115.1667	*
D. reticulata	WAM 88601	M	20 km WSW of Hamelin Homestead	WA	-26.4666	114.0000	*
D. reticulata	WAM 101268	F	Tardun	WA	-28.7833	115.7500	*
D. reticulata	WAM 104127	F	Woodstock Station	WA	-21.6219	118.9531	**
D. reticulata	WAM 112112	M	Chichester Range	WA	-21.9438	118.9628	*
D. reticulata	WAM 112523	F	14.7 km W of Paynes Find	WA	-29.2819	117.6428	**
D. reticulata	WAM 114477	M	Mount Fraser	WA	-25.5833	118.3667	*

D. reticulata	WAM 114840	F	Spalding Park, Geraldton	WA	-28.6500	114.6333	**
D. reticulata	WAM 115064	F	Narngulu	WA	-28.8166	114.6833	**
D. reticulata	WAM 116591	M	11 km NE of Carbla Homestead	WA	-26.1166	114.2667	**
D. reticulata	WAM 119218	M	Wurarga	WA	-28.4166	116.2833	*
D. reticulata	WAM 121557	M	Mardathuna Homestead area	WA	-24.4666	114.5500	*
D. reticulata	WAM 121577	F	Meedo Homestead area	WA	-25.6500	114.6167	*
D. reticulata	WAM 122525	F	1.2 km NE of Meedo Homestead	WA	-25.6538	114.6256	*
D. reticulata	WAM 122990	F	Woodleigh Station	WA	-26.1830	114.5500	**
D. reticulata	WAM 125972	M	Marymia	WA	-25.0666	119.8500	*
D. reticulata	WAM 128258	F	Lesueur National Park	WA	-30.0338	115.0189	*
D. reticulata	WAM 133183	M	Jurien	WA	-30.3000	115.0333	*
D. reticulata	WAM 136510	F	Jouerdine Nature Reserve	WA	-30.6333	118.4272	*
D. reticulata	WAM 139139	F	Meentheena	WA	-21.2288	120.4258	**
D. reticulata	WAM 141186	Juv.	15 km NNE of Lancelin	WA	-30.6083	115.1144	*
D. reticulata	WAM 141400	M	Cape Preston area	WA	-21.0438	116.1872	*
D. reticulata	WAM 145595	M	80 km S of Port Hedland	WA	-21.0100	118.7000	**
D. reticulata	WAM 151463	M	Lorna Glen Station	WA	-26.1861	121.3667	*
D. reticulata	WAM 154254	F	Fortescue Marsh	WA	-22.2991	119.0228	**
D. reticulata	WAM 157330	M	Yanrey Station	WA	-22.1180	114.5475	**
D. reticulata	WAM 160060	M	24 km E of Oakover River	WA	-21.30	121.20	*
D. reticulata	WAM 164320	M	Lorna Glen Station	WA	-26.2166	121.5500	**
D. reticulata	WAM 164372	M	Jandakot Airport	WA	-32.1166	115.8333	*
D. reticulata	WAM 165907	F	Waldburg Station	WA	-24.7500	117.3667	*
D. reticulata	WAM 170388	F	18 km NW of Balfour Downs Homestead	WA	-22.7006	120.7370	**
D. rimicola	QM 67392		Whitehill Station	QLD	-23.591667	144.038889	***
D. rufescens	WAM 139327		Meentheena	WA	-21.281667	120.466111	***
D. shinei	MAGNT 37544		50 km E of Threeways Roadhouse, Barkly Highway	NT	-19.527133	135.570283	***
D. shinei	MAGNT 37840		18 km E of Threeways Roadhouse, Barkly Highway	NT	-19.473250	134.404400	***
D. shinei	MAGNT 37967		22 km N of Tennant Creek, Stuart Highway	NT	-19.444498	134.215275	***
D. simplex	MAGNT 28095		Dorat Road, Adelaide River	NT	-13.354	131.141	***
D. torquata	ABTC 150992		Strathdickie	QLD	-20.32361	148.61739	***
D. vestigiata	ABTC 124408		Saibai Island	QLD	-9.400	142.667	***

TABLE 1. Codon models used for Maximum Likelihood mitochondrial tree estimation (Fig. 1).

Gene	Sites	Model
ND4	Position 1	HKY+F+R2
ND4	Position 2	TIM3+F+G4
ND4	Position 3	HKY+F+G4
tRNA	Position 1	TIM2+F+I+G4
tRNA	Position 2	TNe+I+G4
tRNA	Position 3	JC+I+G4

TABLE 2. Morphological characters measured and counted in this study.

Character	Description
ToL	Total body length, measured from tip of snout to tail tip, including terminal scale.
SVL	Snout-vent length, measured from tip of snout to posterior edge of anal scale.
TailL	Tail length, measured from posterior edge of anal scale to tail tip, including terminal scale.
HeadL	Head length, measured from tip of snout to medial posterior point of parietals on nape.
HeadW	Head width, measured at widest point, immediately posterior to eyes.
HeadD	Head depth, measured at deepest point, immediately posterior to eyes.
SnL	Snout length, measured from tip of snout to anterior margin of eye.
BW	Body width, measured at middle of SVL.
BD	Body depth, measured at middle of SVL.
FrL	Frontal length, measured from anterior to posterior edges.
FrW	Frontal width, measured at anterior widest point.
SupOcL	Supraocular length, measured from anterior to posterior edges.
SupOcW	Supraocular width, measured at middle above eye.
EyeW	Eye width, measured from anterior to posterior edges.
VS	Ventral scales, counted from first broad ventral to, but not including anal scale.

MBSR	Midbody scale rows, counted diagonally at middle of SVL.
AntSR	Anterior scale rows, counted diagonally back from and including dorsal scale contacting first broad ventral immediately posterior to head.
PostSR	Posterior scale rows, counted diagonally forward from and including dorsal scale contacting last broad ventral immediately before anal scale.
ScS	Subcaudal scales, counted from first divided scale posterior to vent but not including terminal scale.
VertSc	Vertebral scales, counted from first scale posterior to parietals on nape to scale immediately above posterior edge of anal scale.
SupLab	Supralabials.
InfLab	Infralabials.

TABLE 3. Summary of statistics and statistical pairwise analysis for measurements (mm) and meristic counts of the *Demansia psammophis* complex. Statistically significant results in bold at the level of $p < 0.01$. Values shown are the mean, ± standard deviation and (range). Sample sizes: N is shown at the head of each column. See Table 2 for abbreviations of measurements and counts. MBSR, SupLab and InfLab are excluded. Unsexed juveniles with SVL < 250 mm are excluded from measurement and ratio summaries but included in meristic counts.

Character	*D. psammophis* (Dp) N:21, + Juv. N:23	Dr vs Dp	*D. reticulata* (Dr) N:66, + Juv. N:67	Dr vs Dc	*D. cyanochasma* **sp. nov.** (Dc) N:68, + Juv. N:76	Dc vs Dp
ToL	724.3±86.5 (530–865)	0.1709	763.6±124 (431–964)	**0.00001**	657.5±132.4 (327–890)	0.05360
SVL	549.6±68.6 (400–655)	0.0549	588.5 ±99.9 (320–754)	**0.00001**	510.0±101.3 (255–700)	0.13888
TailL	171.0±22.3 (130–210)	0.984	172.1±30.1 (93–230)	**0.00046**	149.5±30.8 (69–194)	**0.00906**
TailL%SVL		0.01428		0.99202		**0.00960**
HeadL	14.7±1.3 (11.6–17.7)	**0.0001**	16.6±2.2 (11.0–20.8)	**0.00001**	14.5±2.0 (10.2–18.6)	0.98404
HeadL/SVL		**0.0041**		0.10524		**0.00050**
HeadW	8.3±1.1 (6.2–11.7)	**0.0001**	9.8±1.6 (5.8–13.7)	**0.00001**	8.6±1.3 (5.5–11.6)	0.22246
HeadW/SVL		**0.00024**		0.37886		**0.00006**
HeadD	6.4±1.0 (4.9–9.0)	**0.00034**	7.9±1.4 (5.1–11.0)	**0.00001**	6.6±1.0 (4.2–8.4)	0.17086
HeadD/SVL		**0.00001**		0.33205		**0.00052**
SnL	5.0±0.5 (3.9–6.6)	**0.00064**	5.8±0.9 (3.7–7.4)	**0.00001**	4.8±0.7 (3.1–5.9)	0.21130
SnL/SVL		**0.00374**		0.02674		0.15854

BW	9.7±1.8 (6.4–13.9)		11.7±2.2 (5.8–16.8)		9.8±2.1 (5.1–14.5)	
BD	8.2±1.5 (5.7–11.7)		10.2±2.5 (4.6–15.4)		8.6±1.8 (5.1–12.8)	
IBR	15.6±2.0 (10.7–19.2)	**0.00001**	13.6±1.4 (10.7–19.2)	0.62414	13.9±1.6 (11.0–17.4)	**0.00020**
FrL	5.6±0.5 (4.6–6.8)	0.1468	6.0±0.8 (4.2–7.6)	**0.0008**	5.4±0.7 (3.4–6.6)	0.28914
FrL/SVL		0.98404		**0.00386**		0.07508
FrW	2.7±0.3 (2.3–3.3)	0.72634	2.6±0.4 (1.6–3.4)	**0.00001**	2.3±0.4 (1.5–3.1)	**0.00028**
FrW/SVL		0.096		0.16452		0.08544
SupOcL	4.7±0.4 (3.6–5.3)	**0.0009**	5.1±0.6 (3.5–6.8)	**0.00001**	4.5±0.7 (2.7–5.7)	0.47152
SupOcL/SVL		0.29372		0.09692		0.03000
SupOcW	2.2±0.2 (1.8–2.5)	**0.00038**	2.5±0.3 (1.6–3.0)	**0.00001**	2.1±0.4 (1.3–3.3)	0.21870
SupOcW/SVL		0.13104		0.87288		0.25848
EyeW	3.5±0.3 (3.0–4.0)	0.35238	3.6±0.4 (2.6–4.5)	**0.00001**	3.2±0.4 (2.1–4.0)	**0.00694**
EyeW/SVL		0.06724		**0.00512**		1.00000
VS	186.3±6.6 (178–203)	0.01468	189.3±4.8 (180–204)	0.1443	187.9±6.2 (173–203)	0.20054
AntSR	16.2±0.8 (15–17)	**0.00652**	16.8±0.5 (16–18)	0.77182	16.7±0.5 (16–18)	0.84148
PostSR	13.3±0.6 (13–15)	0.13362	13.0±0.3 (13–14)	0.6818	13.0±0.3 (12–14)	0.01828
ScS	80.0±5.4 (69–91)	0.05328	76.5±6.3 (65–90)	0.01278	74.4±4.8 (64–85)	0.08616
VertSc	185.7±2.7 (182–188)	**0.00024**	194.8±5.0 (185–206)	1	194.5±6.3 (179–208)	**0.00152**

TABLE 4. Individual measurements (mm) and meristic counts for the paratype series of *Demansia cyanochasma* **sp. nov.**.

Museum #	MAGNT R01371	MAGNT R01565	MAGNT R03467	MAGNT R16599	MAGNT R32379	MAGNT R36247	SAMA R32260	SAMA R48412	SAMA R49048	SAMA R53139	SAMA R60568	SAMA R64434	SAMA R67418	SAMA R70189	SAMA R71354	WAM R72500	WAM R73340	WAM R166045	WAM R168341	WAM R169206
Sex	M	F	M	F	M	F	F	F	M	M	F	F	M	F	M	M	M	M	M	F
ToL	689	890	677	696	677	686	574	670	758	789	560	604	699	585	571	718	785	791	707	781
SVL	532	700	517	550	525	533	450	525	590	595	438	463	542	458	440	548	600	598	534	600
TailL	157	190	160	146	152	153	124	145	168	194	122	141	157	127	131	170	185	193	173	181
% SVL	29.5	27.1	30.9	26.5	28.9	28.7	27.5	29.3	28.4	32.6	27.8	30.4	28.9	27.7	29.7	31.0	30.8	32.2	32.3	30.1
HeadL	15.3	16.7	13.6	14.6	14.7	14.8	13.6	14.8	16.7	16.9	13.2	13.7	15.6	13.8	13.5	15.2	16.7	17.7	15.7	16.7
HeadW	9.2	9.2	9.7	8.3	9.3	8.0	7.5	9.1	9.5	10.3	8.0	8.4	8.8	8.4	7.3	8.4	9.6	9.7	9.5	10.3
HeadD	7.5	7.8	7.3	7.2	7.3	6.8	6.3	7.1	7.1	7.7	5.8	5.8	7.1	6.2	7.2	7.1	7.0	8.2	7.2	8.0
SnL	4.8	5.0	4.4	5.3	4.8	5.3	4.4	5.0	5.5	5.3	4.1	4.1	5.0	4.8	4.7	5.9	5.1	5.2	5.3	5.8
BW	12.0	12.9	11.1	11.3	11.9	7.8	8.5	9.7	9.6	11.3	7.2	9.4	9.8	9.4	8.5	9.6	10.4	10.7	10.2	14.5
BD	9.5	12.8	9.7	7.4	9.4	8.6	9.1	9.4	8.0	10.9	6.7	7.9	8.8	8.5	6.3	8.3	9.6	9.5	7.7	10.7
FrL	5.3	5.1	5.1	5.7	5.8	5.4	5.1	5.3	6.0	6.4	4.7	5.1	5.8	5.4	4.7	6.1	6.2	6.5	6.3	5.9
FrW	2.6	2.4	2.2	2.0	2.3	2.4	2.3	2.3	2.3	2.7	1.8	2.0	2.4	2.2	2.1	2.6	2.4	2.4	2.5	2.6
SupOcL	4.4	5.2	5.3	5.0	5.0	4.4	4.5	5.1	5.4	4.9	3.8	4.3	4.8	4.1	4.0	4.9	5.2	5.2	4.9	5.0
SupOcW	2.0	2.1	1.9	2.0	1.9	2.1	2.1	2.2	2.3	2.4	2.3	2.0	2.1	1.9	1.8	2.3	2.6	2.6	2.3	2.5
EyeW	3.3	3.4	3.1	3.4	3.4	3.3	3.3	3.5	3.6	3.3	3.1	3.0	3.8	3.1	3.1	3.5	3.6	2.9	3.7	3.1
VS	189	201	185	194	195	198	179	181	188	181	191	183	182	182	182	190	184	189	193	189
MBSR	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

AntSR	17	16	16	17	16	17	18	17	17	17	17	17	17	17	16	17	17	16	16	16
PostSR	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	12	14	13	13
ScS	75	72	75	69	75	76	67	71	75	80	73	70	74	68	72	82	80	79	79	70
VertSc	199	208	193	196	198	202	188	191	194	186	197	189	186	186	186	194	198	204	198	196
SupLab	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
InfLab	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7

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Principal Author

Name of Principal Author (Candidate)	James Henry Nankivell		
Contribution to the Paper	Conceived of the research, collected tissue samples, extracted DNA, conducted molecular analyses, collected morphological data, wrote the first manuscript and edited all subsequent versions		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	10/03/2023

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Arne Rasmussen		
Contribution to the Paper	Collected tissues and morphological data, contributed to writing and editing of manuscript		
Signature		Date	15/03/2023

Name of Co-Author	Emma Sherratt		
Contribution to the Paper	Collected morphological data, advised on statistical analysis, provided edits to manuscript		
Signature		Date	14/03/2023

Name of Co-Author	Claire Goiran		
Contribution to the Paper	Collected tissue samples, contributed to writing and editing of manuscript.		
Signature		Date	10/03/2023

Name of Co-Author	Kate L. Sanders		
Contribution to the Paper	Conceived of research, collected tissue samples and morphological data, assisted with analyses, contributed to the writing and editing of the manuscript.		
Signature		Date	15/03/2023

Chapter 5

SNP-based analyses reveal phylogenetic relationships and ecomorph evolution in rapidly radiating *Hydrophis* sea snakes

SNP-based analyses reveal phylogenetic relationships and ecomorph evolution in rapidly radiating *Hydrophis* sea snakes

James Nankivell, Arne Rasmussen, Emma Sherratt, Claire Goiran, Kate Sanders

Abstract

Phylogenetic analyses of recent, rapid radiations are often confounded by incomplete lineage sorting and past introgression of genetic markers. Within snakes, the fully marine *Hydrophis* clade is conspicuous in having anomalously high speciation rates, with ~47 species sharing a common ancestor dated at only 5-8 million years ago. *Hydrophis* are also ecologically very diverse, exhibiting great body size and shape variation associated with dietary specialisation. However, lack of phylogenetic resolution, especially along a backbone of short internal branches, has hindered the many previous investigations of *Hydrophis* diversification. To address this limitation, genome-wide SNPs were generated using reduced-representation genome sequencing of ~70% of recognised *Hydrophis* species. These data and a mitochondrial alignment were analysed to infer phylogeny, detect cases of mito-nuclear discordance, and identify putative signatures of past introgression. The SNP trees provided substantially higher resolution of *Hydrophis* phylogeny compared to previous analyses of mitochondrial and low-variation nuclear markers. There were several conflicts between strongly supported nodes in the nuclear versus mitochondrial trees, and significant signals of past introgression were detected in four pairs of lineages. This included geographically overlapping species that formed shallow, unresolved clades in the mitochondrial analyses but were well-resolved by the SNP data. Finally, dietary data and three traits related to body size and shape were used to identify broad ecomorph categories, and reconstruct ancestral patterns of ecomorphological diversification using the new SNP phylogeny. These analyses extended previous studies by defining five distinct ecomorphs, and identifying eel-specialists as the ancestral ecomorphs within *Hydrophis*, followed by a more recent and replicate origins of toxic-spinous and generalist ecomorphs.

Introduction

Rapid adaptive radiations provide important systems for comparative studies of trait evolution and speciation (Seehausen, 2006; Hodges & Derieg, 2009). Yet these are also among the most challenging groups in which to resolve phylogeny. Short intervals between speciation events in rapid radiations can cause individual gene trees to differ from the true species tree. This can be due to low resolution and incomplete lineage sorting of phylogenetic markers (Degnan & Rosenberg, 2009; Meleshko et al. 2021), or introgression among lineages resulting in reticulate (non-bifurcating) relationships (Chen et al, 2009). Recent studies of rapid radiations of non-model organisms have overcome these challenges using reduced-representation genome sequencing to generate thousands of genome-wide markers with which to resolve relationships and detect signatures of past introgression events (e.g. Alexander et al. 2017; MacGuigan & Near, 2019).

Hydrophis sea snakes (Elapidae: Hydrophiinae) are an adaptive radiation of ~47 (Uetz et al., 2022) species that exhibit three-fold faster rates of lineage accumulation than all other elapid snakes (~400 marine, amphibious and terrestrial species) (Lee et al., 2016). *Hydrophis* share a common ancestor dated at only ~5-8 million years old (Lee et al., 2016; Sanders et al., 2013b), and exhibit high sympatric species richness throughout the tropical and subtropical Indo-Pacific (Elfes et al. 2013). *Hydrophis* was classified into 10-14 genera and/or subgenera reflecting ecomorphological diversity that has confounded attempts to resolve phylogeny using morphological characters (Voris, 1977; Rasmussen, 1994). Previous molecular studies using Sanger-sequenced mitochondrial sequences, and nuclear sequences with low-variability, also resulted in conflicting and incompletely resolved relationships. While molecular and morphological support has been found for some inter-specific groupings, many relationships within these groups have remained unclear and the short branches along the 'backbone' of *Hydrophis* phylogeny are almost entirely unresolved (Ukuwela et al., 2016).

A robust phylogeny is needed to support the broad scope of studies on this evolutionarily dynamic clade. Investigations of trait evolution in *Hydrophis* have focused especially on marine adaptations, including diving physiology (Heatwole and Seymour, 1975; Seymour, 1982; Palci et al., 2019), osmoregulation of salt balance (Dunson and Stokes, 1983; Lillywhite et al., 2014), and sensory biology (Crowe-

Riddell et al., 2016; Simões et al., 2020). The *Hydrophis* clade is unusual among other marine (and terrestrial) snakes in exhibiting highly varied body shapes linked to correspondingly diverse dietary specialisations (Voris and Voris 1983; Sanders et al., 2013a; Sherratt et al., 2019b). The most extreme and highly replicated forms are so-called microcephalic species, which have greatly reduced forebody girths and tiny heads that adapt them to a specialist diet of burrowing eels. The convergent evolution of replicated suites of ecologically relevant characters between species has been well documented in taxa such as *Anolis* lizards and cichlid fish, and species in these groups are assigned to "ecomorphs" (Williams, 1972; Huie et al., 2021; Burress et al., 2022). Within sea snakes, several studies have resolved the evolution and developmental basis of the microcephalic ecomorph (Sherratt et al., 2018; Sherratt et al., 2022), but comprehensive analyses of the full array of *Hydrophis* ecomorphs have been hindered by the lack of a robust phylogenetic framework.

Here, we aimed to improve the resolution of the *Hydrophis* phylogeny by generating 9368 SNP loci for 33 (70%) of the 47 *Hydrophis* species using reduced restriction enzyme digestion and short fragment sequencing. We analysed these data to infer phylogeny using likelihood analyses of concatenated loci and species tree methods, and using D-statistics (ABBA-BABA tests) to detect putative introgression events following speciation. Using this new phylogenetic hypothesis, we examined patterns of adaptive evolution. To do this, we used four key traits to define discrete ecomorph categories and mapped these onto our new tree to visualise broadscale adaptive shifts.

Methods

Sampling

We assembled a SNP dataset using the DArTseq (Diversity Arrays Technology Pty Ltd., Canberra, Australia) platform for 38 *Hydrophis* sea snake specimens from 33 species out of 47 (~70%) recognised *Hydrophis* species (Uetz et al., 2022). This sampling included all but two (*Thalassophis* and *Mediohydrophis*) of the previously recognised genera and subgenera. We chose the outgroups *Hydrelaps darwiniensis* and *Microcephalophis gracilis* based on previous molecular phylogenetic analyses, which robustly recovered these taxa as reciprocal sister lineages to *Hydrophis* (e.g. Sanders et al., 2013b). Multiple individuals were included for *Hydrophis*

cyanocinctus, *H. curtus* and *H. ornatus* due to known non-monophyly of mitochondrial markers and/or unresolved species boundaries (Ukuwela et al., 2014, 2016). A sample putatively belonging to the enigmatic taxon *H. vorisi* was also included (MEL-475); this was provisionally identified from a photograph by colour pattern (>70 bands) and head-body shape, however no specimen was available for this individual. We also sequenced a 33 year old museum specimen (MAGNT R16697) which we tentatively keyed to *H. laboutei* (Rasmussen et al., 2014). Details of specimens used in the molecular analyses are provided in tables 1 and 2.

Tissues were extracted using a salting out method and quantified using a Quantus fluorometer to obtain >500ng of DNA at concentrations of 50ng/ml. Samples were concentrated using a Centrivap DNA concentrator if required and checked for quality using gel electrophoresis. Samples were sent to Diversity Arrays Technology Pty Ltd (DArTSeq, Canberra, Australia) where SNP genotyping was conducted using a proprietary genome complexity reduction pipeline using a pair of restriction enzymes (PstI and HpaII) (Kilian *et al.* 2012; Georges *et al.* 2018). After initial digestion/ligation reactions and PCR amplification samples were sequenced on an Illumina Hiseq 2500. DArTseq applies filters to screen for bacterial contamination and includes a series of technical replicates to ensure accurate SNP calling and deliver a matrix of polymorphic loci which yielded 69,096 SNPs. Sequence data was imported into the R package DArTR (Gruber *et al.* 2018) and a series of additional filters was applied, including reproducibility (keeping SNPs present in 99% of technical replicates), loci callrate (retaining loci found in > 70% of individuals), read depth (removing loci with a read depth < 5) and removing monomorphic loci. A total of 9368 loci remained with 19.06% missing; these were exported to an alignment of concatenated SNPs containing a single random SNP per fragment.

The cytochrome b (cytb) mitochondrial alignment was compiled largely from existing samples (Appendix S1 from Ukuwela et. al., 2016). We generated two new cytochrome b sequences for *Hydrophis ornatus* (New Caledonia) and *H. vorisi*. Fragments of up to 1,099 base pairs were amplified using forward primer L14910 (M460) (5'GAC CTG TGA TMT GAA AAA CCA YCG TTG T -3') and reverse primer H16064 (M461) (5'-CTT TGG TTT ACA AGA ACA ATG CTT TA -3') (de Queiroz et al., 2002). PCR reactions comprised 25 µL: 16.5 µL double distilled water, 0.1 µL Immolase DNA Polymerase, 5 µL 5X MRT buffer, 1.2 µL each of forward and reverse primer and 1 µL of DNA template. All reactions were run on the same

thermal cycling program of 10 min at 95 °C for initial denaturation, followed by 37 cycles of 45 s at 94 °C, 45 s at 52 °C for annealing, 1 min at 72 °C for extension with a final extension for 10 min at 72 °C. PCR products were checked for successful amplification by gel electrophoresis and vacuum cleaned before Sanger sequencing at the Australian Genome Research Facility (AGRF) in Adelaide. Sequences were then checked by visual inspection of chromatograms using Geneious Pro v.11.0.5 (Drummond et al., 2010), calling bases as 'N' if ambiguous.

Phylogenetic tree reconstruction

For phylogenetic tree reconstruction we used ModelFinder (Kalyaanamoorthy *et al.* 2017) implemented in IQTree v. 2.0.3 (Nguyen *et al.* 2015) to select the best fit partitioning scheme of TVM+F+R2. Maximum likelihood tree reconstruction using 9368 SNPs was implemented in IQTree and support was assessed using 1000 ultrafast bootstrap replicates. The SNP tree was rooted using the taxon *Hydrelaps darwiniensis* and visualised in Figtree v.1.4.4 (Rambaut, 2012). In addition to IQtree, we used SVDquartets to infer a species tree. Unlike concatenation methods, SVDquartets does not assume concordance between gene trees, so can yield a more accurate species tree under scenarios with high incomplete lineage sorting and low resolution due to short gene sequences (Chou et al., 2015). We used the implementation of SVDquartets in PAUP* v. 4a168 (Swofford, 2002) with the parameters evalQuartets=all, ambigs=distribute and 1000 bootstrap replicates to assess support. A total of 91930 quartets were evaluated. We converted the maximum likelihood tree to a chronogram using the very approximate *Microcephalophis-Hydrophis* node age of 3 Mya from Sanders et al., (2013b) using the chronos function in R package phytools v.1.2 (Revell, 2012) in R Statistical Environment v.4.2.2 (R Core Team, 2022).

For Maximum Likelihood phylogenetic tree reconstruction of the mitochondrial cytochrome b gene, we used IQTree v. 2.0.3 (Nguyen *et al.* 2015). By implementing ModelFinder (Kalyaanamoorthy *et al.* 2017) and using the Bayesian information criterion (BIC), three partitions were selected (cytb site 1: TN+F+I+G4; site 2: TN+F+G4; site 3: HKY+F+I+G4). Support was assessed using 1000 ultrafast bootstrap replicates.

Tests for introgression

We used Dsuite (Malinsky et al., 2021) to estimate D statistics and associated f_4 ratios to test for signals of introgression. The statistics are applied to 4 taxon quartets with assigned branching relationships of (((P1,P2)P3)O) where the alleles are considered ancestral ("A") or derived ("B") and the outgroup is assigned the ancestral allele A. Under a model of no excess gene flow ABBA and BABA site patterns are expected to occur with equal frequency due to incomplete lineage sorting, while an excess of one of these patterns (sharing of derived alleles) indicates greater than expected gene flow between P3 and either P1 or P2 (Durand et al., 2011; Patterson et al., 2012). For introgression tests we removed 3 samples which had significant missing data (*Hydrophis kingii*, *H. cf. laboutei* and *H. pachycercos*) and set *Microcephalis gracilis* as the outgroup rather than the more distantly related *Hydrelaps darwiniensis*. This left a SNP matrix containing 7506 unlinked SNPs and used the svdquartets tree for topology. We calculated Dtrios for all possible combinations of species across the tree. Because it can be difficult to interpret many sets of correlated f_4 ratios, we used the fbranch (f_b) statistic to assign gene flow events to specific taxa or internal branches of the phylogeny (Malinksy et al., 2018). Simulation tests of f_b accuracy showed a good ability to identify correct donor and recipient branches using $>10^5$ SNPs where gene flow was strong (Malinsky et al. 2021) [see Fig 4]. We consider the significant results of our tests to represent candidate introgression events that will provide future targets for further (including model-based) analyses using population sampling to infer allele frequencies instead of the simple site patterns used in our preliminary analyses.

Ecomorph identification and ancestral reconstructions

We obtained information on diet and three morphological traits for each species from the published literature (Glodek & Voris, 1982; Sherratt et al., 2022) and new museum and field collected data (summarised in Appendix/Supplementary material). Morphological traits relate to body shape, size and elongation: relative girth is the ratio of the girth at the neck and girth at 0.75 of snout to vent length; body length is average snout to vent length; vertebral number is the average count of pre-cloacal (body) vertebrae. These ecologically relevant traits are highly variable among *Hydrophis* species (Sherratt et al., 2019b). We designated prey- type ecomorphs by

ordinating the three components of body size and shape variation and identifying distinct morphological groupings that corresponded to dietary categories based on proportions of different prey types (Supplementary Table 1). While our approach will undoubtedly overlook variation within ecomorphs and species, it uses the substantial data available to identify a broader set of ecomorphs than previous studies, which primarily focused on transitions to morphotypes with dietary specialisations on burrowing prey (Sherratt et al., 2018). For each species, intraspecific lineages were treated as the same due to inconsistency in available morphological and dietary data. Ecomorphs were mapped to the maximum likelihood phylogeny, although lineages with unknown ecomorph assignment were dropped for ancestral state reconstruction.

Bayesian stochastic character mapping was performed with the *make.simmap* function implemented in the R package phytools (Revell, 2012) to estimate ancestral state reconstruction of ecomorphs in *Hydrophis*. A phylomorphospace plot was generated using phytools (Revell, 2012) by projecting the maximum likelihood tree into the bivariate morphospace of vertebrae and relative girth. Convergence was assessed using the convevol package (Stayton, 2018) to estimate metrics of convergence. C_1-C_4 are distance-based metrics, with C_1 values between 0 and 1 where 0 represents no convergence (i.e. the morphological pattern seen is entirely driven by phylogenetic signal) and 1 represents maximum convergence (i.e. the phylogenetic signal has no impact on the focal taxa distribution in morphospace). Meanwhile C_2 takes into account the absolute amount of evolution between tips, while C_3 and C_4 are generated as proportions of C_2 to allow comparisons between datasets (Stayton, 2015).

Ancestral area reconstructions

We inferred ancestral geographical distribution probabilities for nodes in the ML SNP tree using the R package BioGeoBEARS (Biogeography with Bayesian Evolutionary Analysis in R Scripts) v.1.1.2 (Matzke, 2013). For this analysis we coded each lineage as being found in one or more of the three major ocean basins which sea snakes inhabit: Sunda shelf (S) (green) including Wallacea and the South China Sea; Indian Ocean (IO) (red) from the Straits of Malacca to the Persian Gulf; Australia (A) (blue) including the Sahul Shelf as well as outlying regions such as Melanesia. *Hydrophis platura platura* was assigned to all three regions, reflecting its

vast range across the Indian and Pacific Oceans. We implemented the likelihood framework to reconstruct ranges under both DEC (dispersal-extinction-cladeogenesis) and DEC+J (which adds the free parameter J, to represent the relative weight of jump dispersal events in cladeogenesis) dispersal models. We compared the statistical fit of these models to our data using their log likelihoods and AIC (Akaike Information Criterion).

Results

Phylogenetic trees + Mitonuclear discordance

The final nuclear alignment contained 9368 SNPs with 19.06% of sites missing. Phylogenetic tree topologies were broadly consistent across maximum likelihood (IQTREE) and species tree (SVDQuartets) analyses of SNP data, with some conflicting nodes along the short backbones of these trees. In contrast, the mitochondrial tree had little support for the backbone of the tree and recovered only three interspecific groups with more than 70% bootstrap support.

Consistent with previous studies (Sanders et al., 2013b), *Microcephalophis gracilis* was unequivocally recovered as sister lineage to *Hydrophis*. The first split within *Hydrophis* involved a clade of two species pairs, *H. atriceps* + *H. fasciatus* and *H. brookii* + *H. caerulescens* as sister group to *H. donaldi* (Clade 1, Fig. 4). These relationships were strongly supported other than the inclusion of *H. donaldi* in the SVDquartets tree, which had only moderate bootstrap support.

Both SNP based phylogenies and the mitochondrial tree recovered a shallow '*Hydrophis cyanocinctus* group' of *H. jerdonii, H. belcheri, H. coggeri, H. parviceps, H. melanocephalus* and *H. cyanocinctus*. The two SNP trees placed *H. elegans* + *H. vorisi and H. spiralis* at the base of this clade and together with the *H. cyanocinctus* group this forms clade 2 (Fig. 5). ML and SVDquartets trees conflict with *H. elegans* and *H. spiralis* each being placed as sister to the remainder of clade 2 respectively. Extensive discordance between well supported mitochondrial versus nuclear relationships is present in this group the *H. cyanocinctus* group. It is notable that mitochondrial markers fail to resolve the *H. cyanocinctus* group but nuclear SNP markers resolve this group well with the exception of close relatives *H. cyanocinctus* and *H. melanocephalus*.

Hydrophis curtus (clade 3) forms a long branch that is sister to the remaining species and consists of deeply divergent Indian Ocean and eastern lineages that were also recovered in the mitochondrial tree. Strong concordant support is also found for a combined Australian and *H. ornatus* group (clades 7+8 along with *H. peronii* and *H. macdowelli*) that has been identified in previous studies (Ukuwela et al., 2016) and this group remains largely the same in terms of species content for the SNP analyses. However, at the species level there is some topological conflict within the SNP trees, as well as extensive mito-nuclear discordance. The placement of *H. peronii* varies considerably between SNP trees, although neither places this species within the *H. ornatus* group as per the mitochondrial phylogeny. *H. macdowelli* shows affinities with *H. czeblukovi* and its relatives in the mitochondrial and SVDquartets analyses but in the ML analysis it is recovered as a poorly supported sister to the *H. ornatus* group. For both SNP and mitochondrial trees, the topological relationships within the *H. ornatus* group are well supported, but they conflict in the placement of either *H. ornatus* (SEA) or *H. ornatus* (IO) as sister to *H. lamberti*. The remaining species form intermediate lineages between *H. curtus* and the Australian/*H. ornatus* group, but are separated by very short branches that show low support and conflict among analyses. Predictably these species are placed chaotically within the mitochondrial tree.

In addition, we placed three taxa on the tree that have not appeared in previous analyses. *Hydrophis vorisi* was found to have close affinities with *H. elegans* in all trees. *H. cf. laboutei* was recovered as sister to the morphologically similar *H. pachycercos,* which conforms to the expectation that these are allopatric sister species (Rasmussen & Ineich, 2001). Finally, *H. ornatus* from New Caledonia was found to be sister to the geographically proximate *H. ocellatus,* rendering *H. ornatus* polyphyletic.

Inspection of the nuclear SNP and mitochondrial trees revealed eight strongly supported topological conflicts (Figure 1). These included differences in the positions of deeply nested lineages, *Hydrophis elegans* and *H. vorisi* as well as *H. peronii*, and among species within the shallow *H. cyanocinctus* and *H. ornatus-lamberti* clades. A number of these topological conflicts appear to consist of a mitochondrial clade largely grouped by geography (e.g. *H. stokesii* - *H. elegans*) while SNP trees recover these species as close to morphologically similar species (*H. stokesii* - *H. major* and *H. elegans* - *H. spiralis*). Similar patterns are apparent in the *H. cyanocinctus* group.

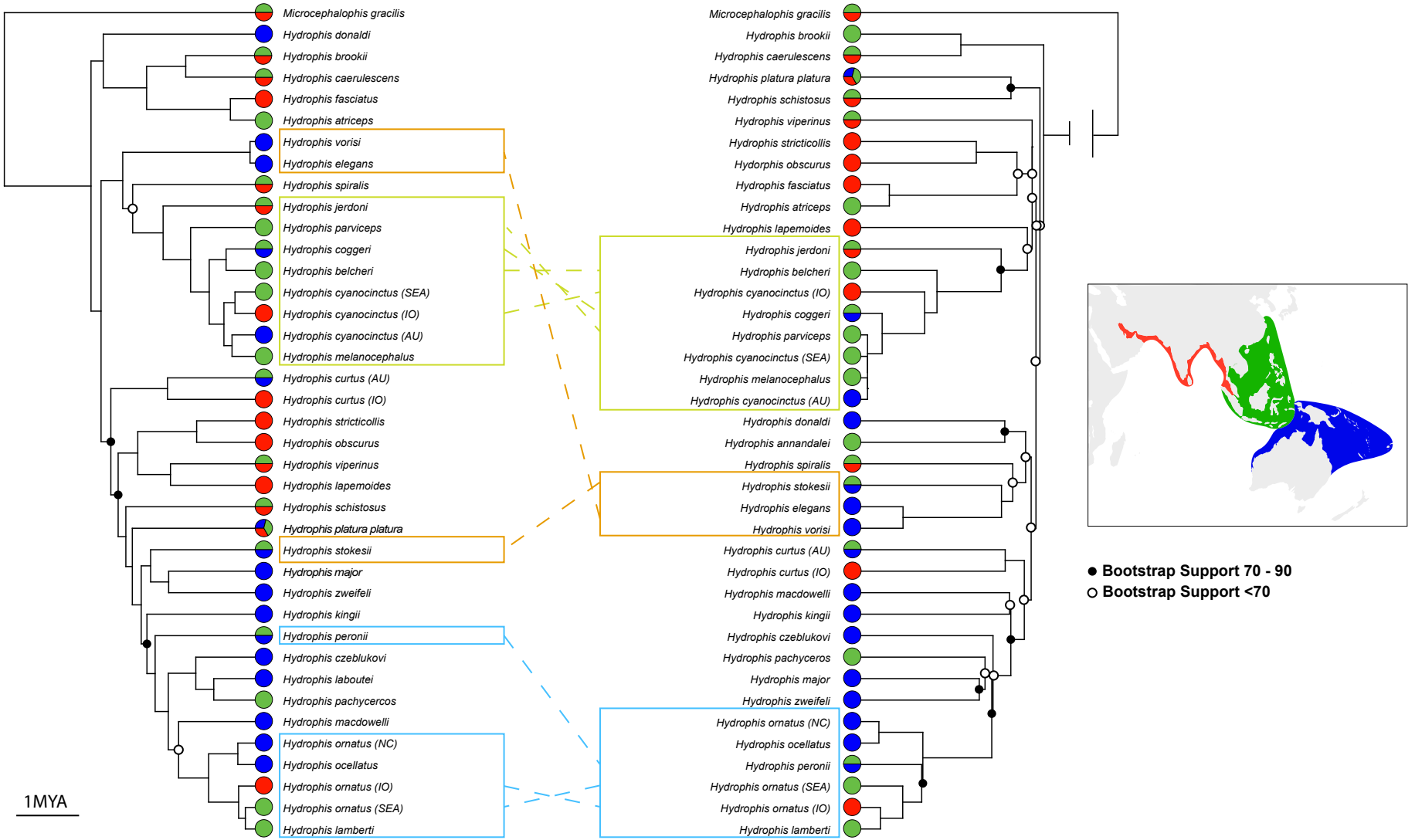
Microcephalophis gracilis
Hydrophis donaldi
Hydrophis brookii
Hydrophis caerulescens
Hydrophis fasciatus
Hydrophis atriceps
Hydrophis vorisi
Hydrophis elegans
Hydrophis spiralis
Hydrophis jerdoni
Hydrophis parviceps
Hydrophis coggeri
Hydrophis belcheri
Hydrophis cyanocinctus (SEA)
Hydrophis cyanocinctus (IO)
Hydrophis cyanocinctus (AU)
Hydrophis melanocephalus
Hydrophis curtus (AU)
Hydrophis curtus (IO)
Hydrophis stricticollis
Hydrophis obscurus
Hydrophis viperinus
Hydrophis lapemoides
Hydrophis schistosus
Hydrophis platura platura
Hydrophis stokesii
Hydrophis major
Hydrophis zweifeli
Hydrophis kingii
Hydrophis peronii
Hydrophis czeblukovi
Hydrophis laboutei
Hydrophis pachycercos
Hydrophis macdowelli
Hydrophis ornatus (NC)
Hydrophis ocellatus
Hydrophis ornatus (IO)
Hydrophis ornatus (SEA)
Hydrophis lamberti
1MYA
Microcephalophis gracilis
Hydrophis brookii
Hydrophis caerulescens
Hydrophis platura platura
Hydrophis schistosus
Hydrophis viperinus
Hydrophis stricticollis
Hydorphis obscurus
Hydrophis fasciatus
Hydrophis atriceps
Hydrophis lapemoides
Hydrophis jerdoni
Hydrophis belcheri
Hydrophis cyanocinctus (IO)
Hydrophis coggeri
Hydrophis parviceps
Hydrophis cyanocinctus (SEA)
Hydrophis melanocephalus
Hydrophis cyanocinctus (AU)
Hydrophis donaldi
Hydrophis annandalei
Hydrophis spiralis
Hydrophis stokesii
Hydrophis elegans
Hydrophis vorisi
Hydrophis curtus (AU)
Hydrophis curtus (IO)
Hydrophis macdowelli
Hydrophis kingii
Hydrophis czeblukovi
Hydrophis pachyceros
Hydrophis major
Hydrophis zweifeli
Hydrophis ornatus (NC)
Hydrophis ocellatus
Hydrophis peronii
Hydrophis ornatus (SEA)
Hydrophis ornatus (IO)
Hydrophis lamberti
● Bootstrap Support 70 - 90
○ Bootstrap Support <70

Figure 1. - Maximum likelihood trees obtained with IQ-TREE for 9,368 nuclear SNPs (left) and maximum likelihood analysis of 1,053 base pairs of mitochondrial cytochrome b (right). Nodes with bootstrap support of 70-90% have closed circles and those with lower than 70% have open circles, those without circles had >90% support. Discordant phylogenetic relationships are shown using lines between trees, for lineages that are resolved in both trees (highlighted using boxes). Only well supported groups in the mitochondrial dataset are highlighted and compared with the SNP dataset. Root not shown.

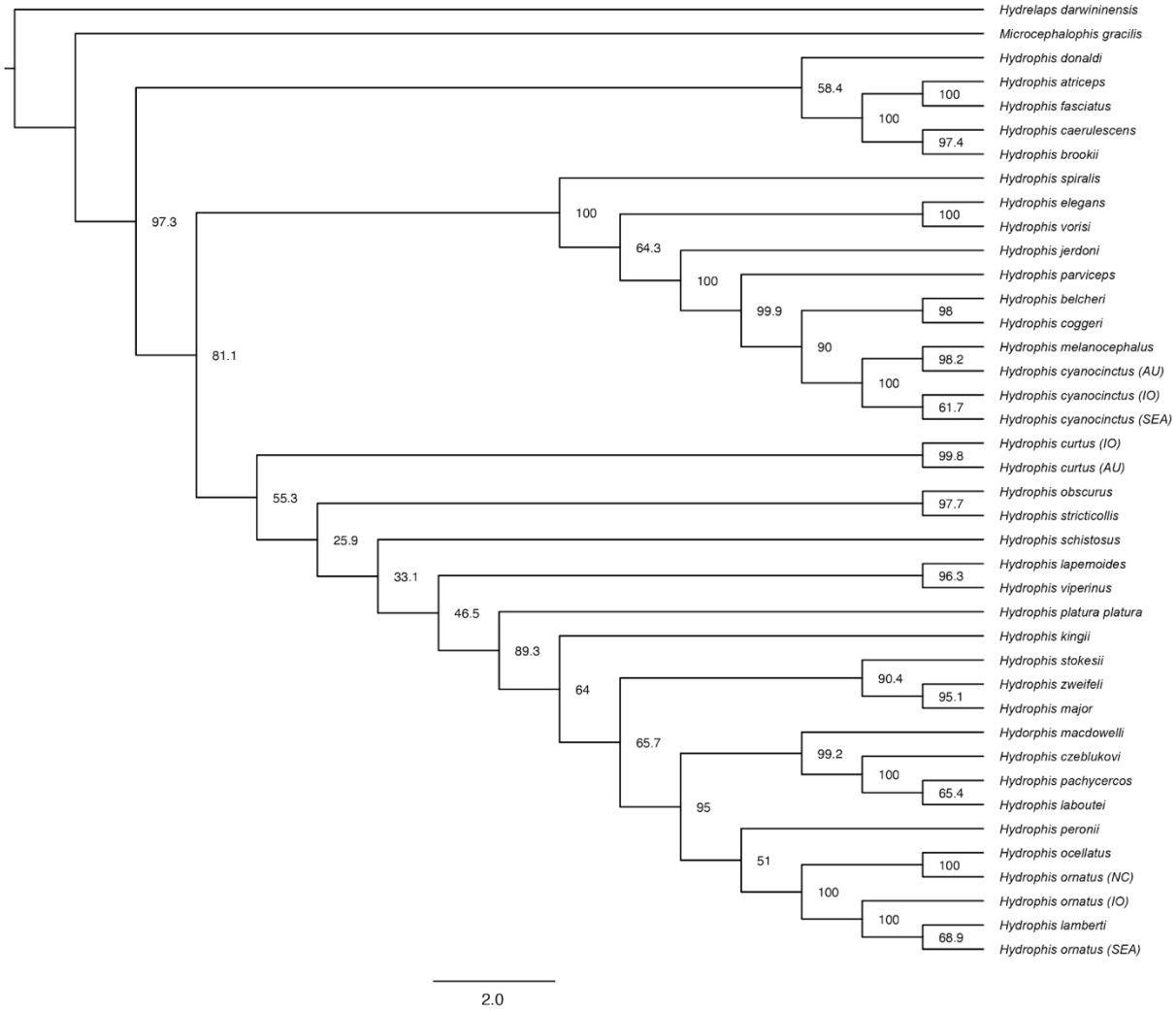

Figure 2. - Cladogram reconstructed from 9368 SNPs using SVDquartets. Branch support assessed using 1000 bootstrap replicates.

ABBA-BABA tests of introgression

Using the alignment of 7506 SNPs, 7140 trios of taxa were investigated in Dsuite for signs of introgression. Of these, 72 (1%) had a Z score above 3 indicating support for gene flow. The f_b values derived from correlated f_4 values highlighted 12 potential introgression events of which four had a f_b above 5%. The strongest signals of introgression were detected between *H. coggeri* and the paraphyletic *H. cyanocinctus* + *H. melanocephalus* lineage (f_b = 22.3%), and between *H. lamberti* and the Indian Ocean lineage of *H. ornatus* (f_b = 21.6%). High f_b values were also found between New Caledonian *H. ornatus* and Southeast Asian *H. ornatus* (f_b = 8.9%). A signal of introgression was also found between *H. obscurus* and the lineage

containing *H. viperinus* and *H. lapemoides* (f_b = 13.1%); however, this should be treated with caution due to the as the result may be confounded by incorrect topology.

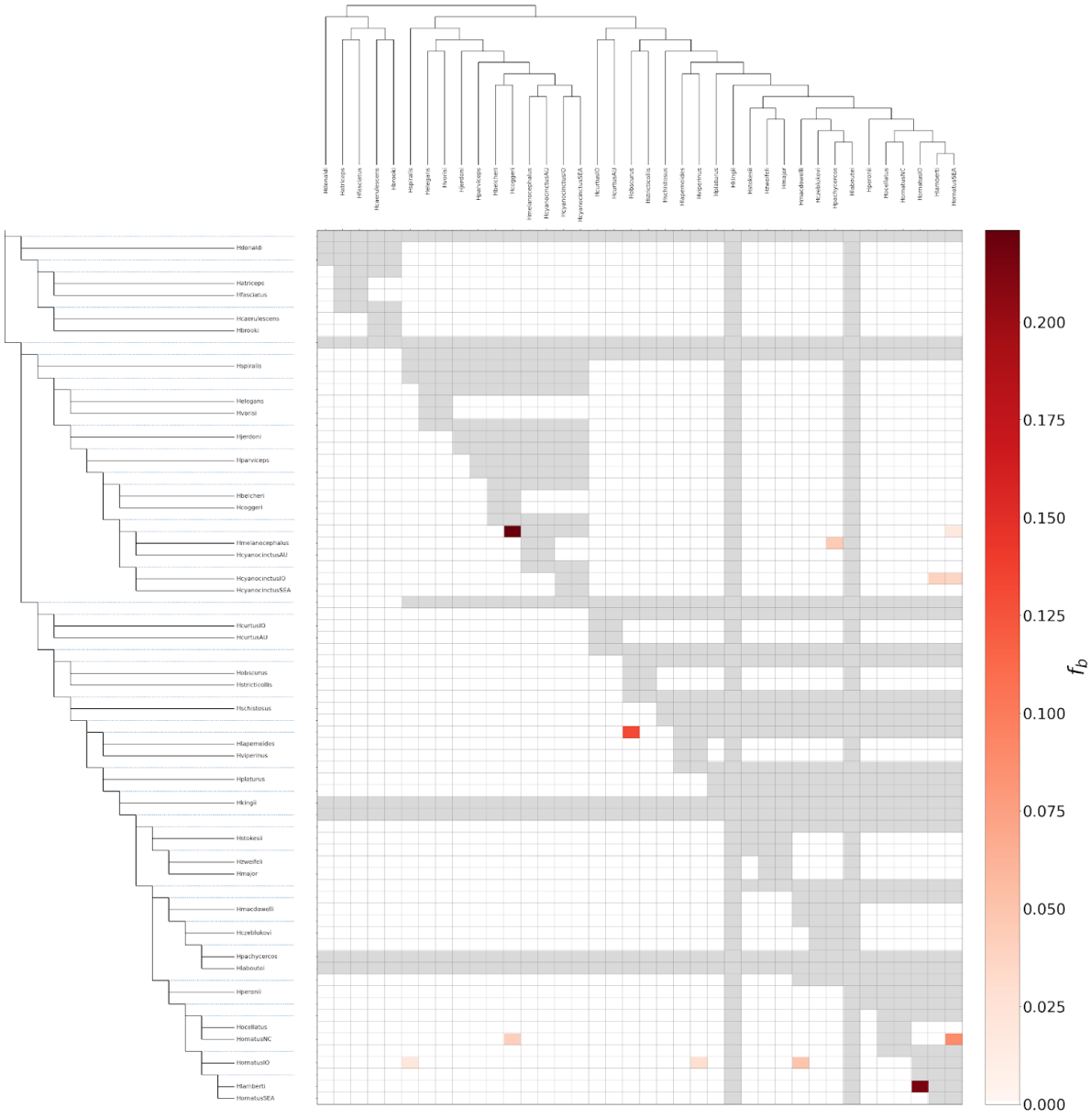

Figure 3. - Mirrored cladograms depicting fbranch values of introgression between non sister lineages reconstructed using Dsuite. Columns on the x-axis represent tips of the species tree used in the analysis; rows on the y-axis represent both terminal and internal branches. Darker red (higher fbranch scores) indicates putative elevated introgression. Grey shading indicates comparisons that can not be calculated. Only pairs with significant Z scores from ABBA-BABA tests are plotted.

Ecomorph identification and patterns of adaptive diversification

Based on non-overlapping ranges for three axes of body size and shape variation, plus congruent morphological and dietary differences, we recognized five broad ecomorphs. These were: 1) Four species of “crevice giants”, which are long bodied with high vertebral counts and similar fore-hind body girths, and feed largely on crevice eels such as morays and congers; 2) ten species of “microcephalics”, which are intermediate in length with high vertebral counts and relative girths (proportionally small forebodies), and feed almost exclusively on burrowing eels; 3) nine species of “shallow burrow" which are intermediate with respect to the other ecomorphs in body size, vertebral count and relative girth, and either specialise on gobies or feed on a combination of burrowing and crevice eels; 4) five species of “spinous-toxic” which are intermediate to large in body length with low to intermediate vertebral counts and low-moderate relative girths, and feed largely on a variety of spinous and toxic prey such as eeltail catfish (Plotosidae), dragonets (Callionymidae) and pufferfish (Tetradontidae); 5) four species of “generalists" which are relatively short in body length with low vertebral counts and low relative girths, and feed on a wide variety of fish, many of which are not elongated (i.e. are ‘mullet’ or ‘discoid’ in shape sensu Voris & Voris, 1983), thus differ from the prey of the first three ecomorphs (Table 1). We use the term “generalist” for these species in the context of diet and morphological characters used here, although many of the species contained within have unusual aspects of life history in the context of sea snakes. Spinous-toxic and generalist ecomorphs broadly overlap in the three morphological variables, but differ substantially in diet preferences. One species, *H. vorisi*, was not assigned to any ecomorph category due to a lack of dietary and morphological data. For species where we sampled multiple geographic lineages (*H. curtus*, *H. cyanocinctus* and *H. ornatus*), we treated them as the same ecomorph due to inconsistency in data availability.

Ecomorph category	diet	body length (cm)	body shape (relative girth)	vertebral count
Crevice giant	>40% crevice eels	>105	1.5-2	>220
Shallow burrow	>80% burrowing fishes	60-110	1.6 - 2.2	170-205
Microcephalic	>50% burrowing eels	70-95	>2.5	190-230
Spinous-toxic	>50% spinous-toxic fishes	60-105	1.2-1.65	150-180
Generalist	<50% any single prey category	55-85	1.2-1.6	135-170

Table 1 - Morphological and dietary characteristics used to define the five ecomorph categories.

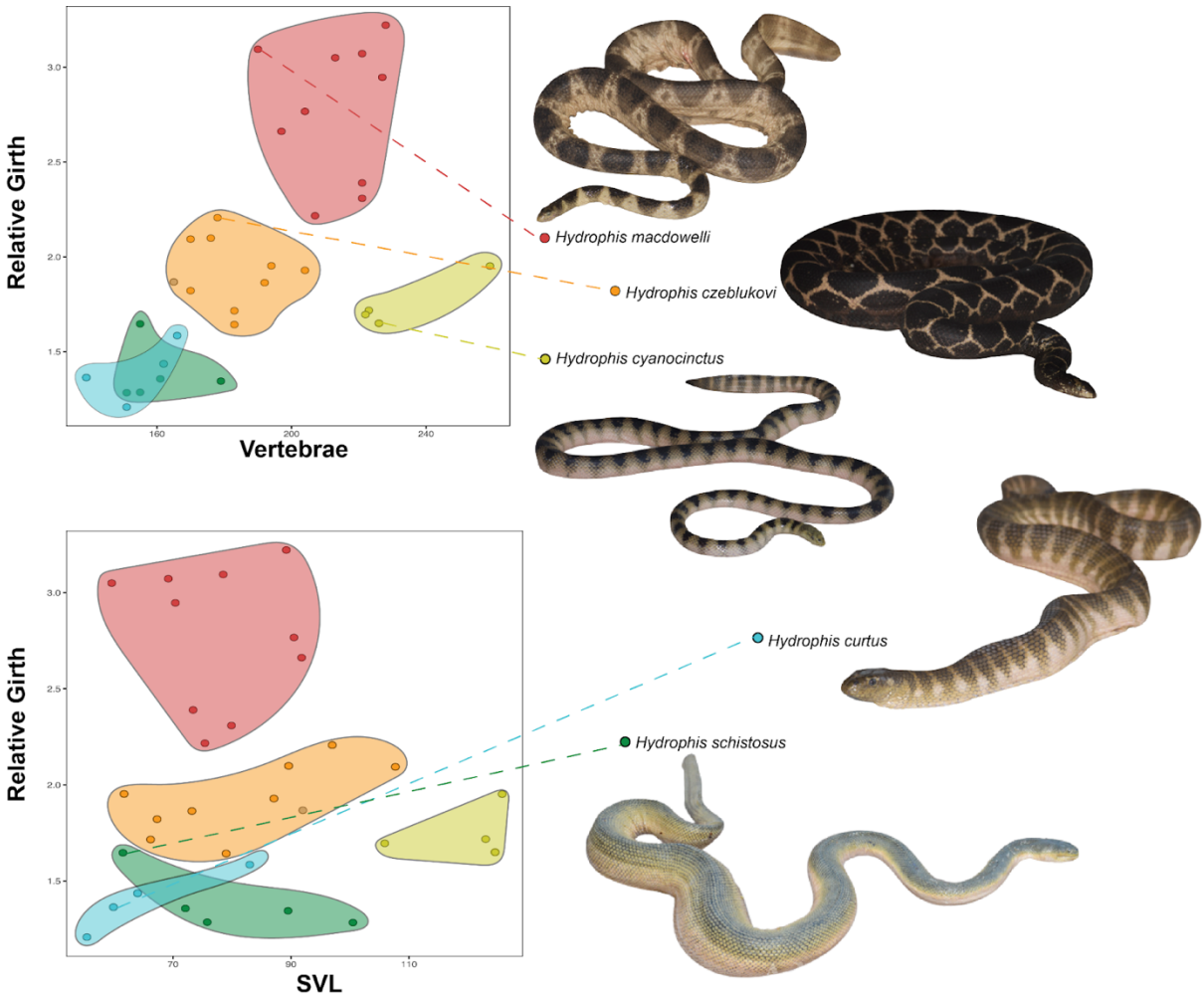

Figure 4 - Bivariate scatterplots illustrating the morphological variation used to define five ecomorphs (top relative girth vs. vertebrae; bottom relative girth vs SVL) and representatives of each ecomorph. Photos of *H. macdowelli*, *H. czeblukovi*, *H. cyanocinctus*, *H. curtus* (James Nankivell); *H. schistosus* (Luke Allen).

Ecomorph	C1	C2	C3	C4	C5
Crevice Giant	0.754759	46.8657	0.404078	0.0310730	6
Microcephalic	0.717611	42.1573	0.439010	0.0279512	7
Generalist	0.718519	40.2407	0.417457	0.0266805	7
Shallow Burrow	0.659192	34.9197	0.392313	0.0231526	5
Spinous Toxic	0.781434	52.4066	0.563799	0.0347468	8

Table 2 - C_1-C_5 convergence metrics derived from Convevol (ref). Higher C_1-C_4 values indicate stronger convergence while C_5 represents the number of times taxa have invaded the morphospace occupied by each of the five ecomorphs. Note the broad overlap between spinous-toxic and generalist ecomorphs inflates the C_5 values for these taxa.

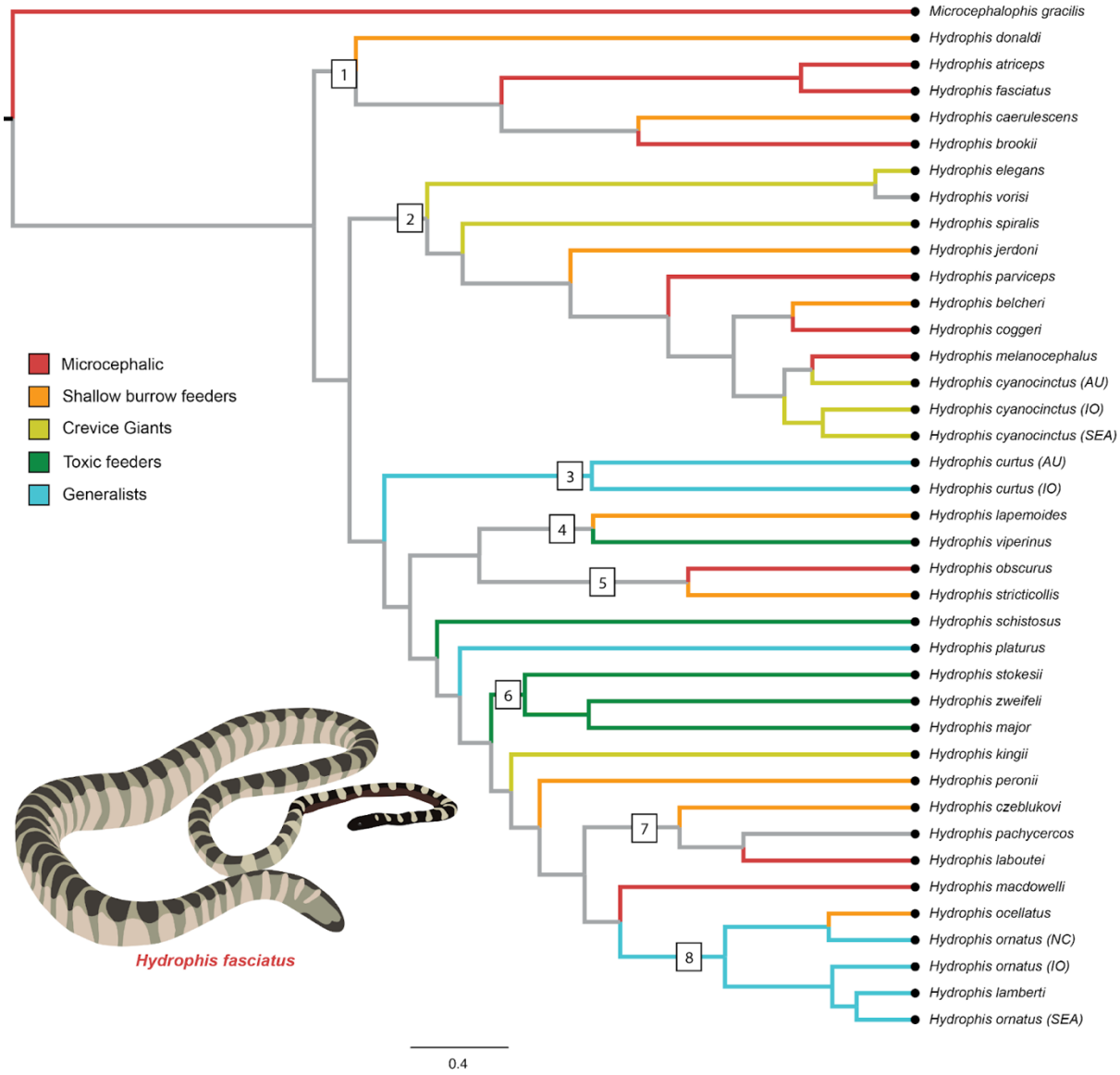

Figure 5. - Maximum likelihood phylogenetic tree (IQTREE) with ecomorphs mapped onto terminal branches. Numbered lineages supported by both ML and species trees (BS > 58) are discussed below. Scale bar is in Mya.

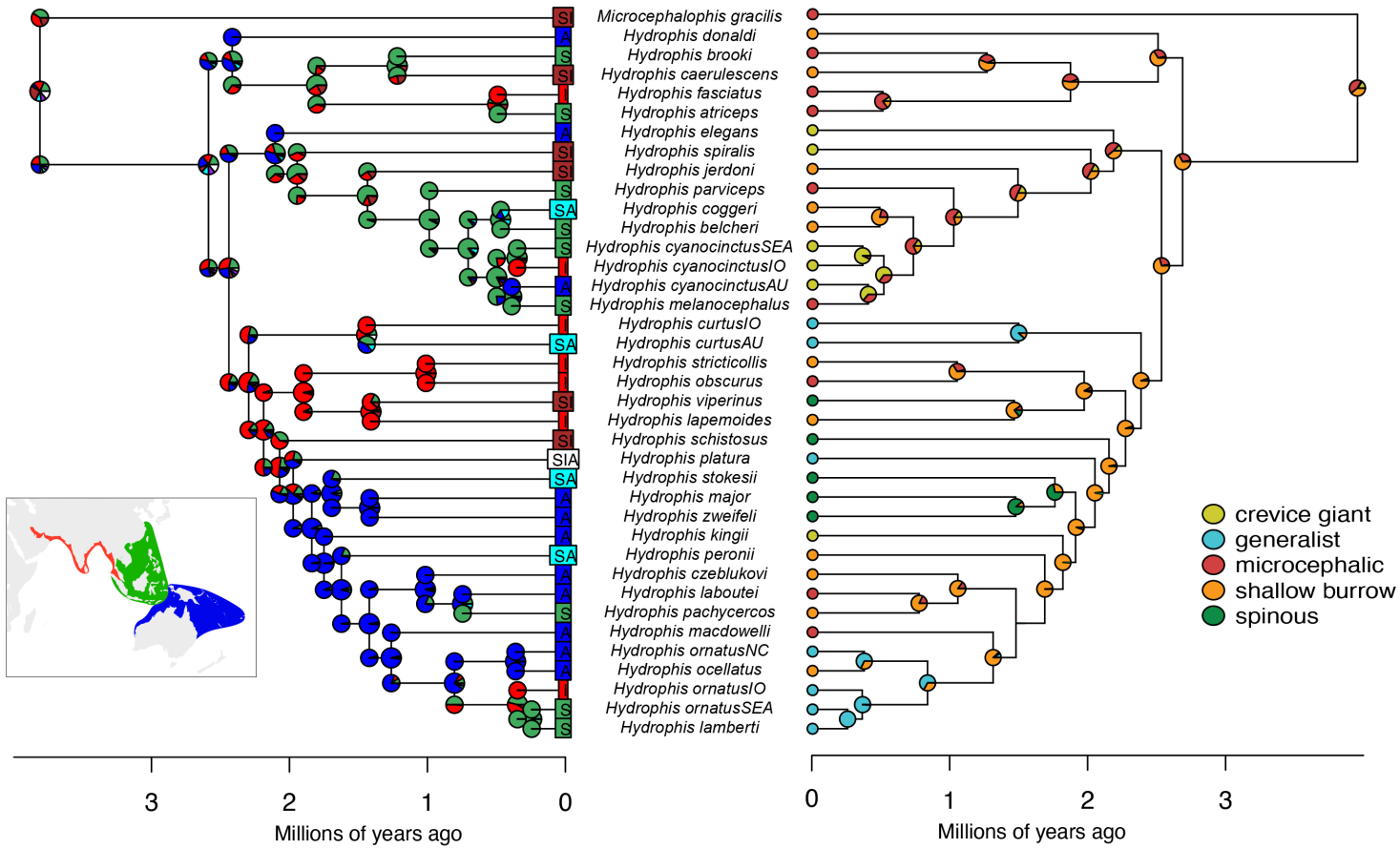

Figure 6 - Ancestral area reconstruction using BioGeoBEARs (left), and ancestral state reconstruction of ecomorphs defined in Fig. 4. using phytools (right) and the ML SNP tree. Terminal points on biogeographic reconstruction are coded by geographic regions (A = Australia, I = Indian Ocean, S =Sundaland), with species distributed in multiple regions coded with multiple letters. Scale bar is in Mya.

Mapping of the five ecomorph categories to the ML tree (Fig. 5 - 6) categories showed all species in the early diverging clade [1] as well as *M. gracilis* belonged to the microcephalic or shallow burrow ecomorphs. Clade [2] contains both of the burrowing prey ecomorphs as well as the majority of crevice giant species, both at the base and deeply nested within the clade. These three ecomorphs make up all of the moderately to highly microcephalic species as well as the ecomorphs with moderate to high vertebral counts. The remaining large clade contains all five ecomorphs occurring at varying frequency, indicating a shift to greater ecomorphological diversity. Two of these ecomorphs, generalists and spinous-toxic, have considerably reduced relative girth and vertebrae counts compared to other *Hydrophis*.

Ancestral state reconstruction supports one of the three eel feeding ecomorphs (Shallow-Burrow, Crevice Giant, Microcephalic) at the ancestral node of *Hydrophis* with a relatively even posterior probability for each. Numerous transitions between these ecomorphs are implied within clades 1 and 2 even on relatively short timescales. Within the remaining large nested clade, the morphologically intermediate shallow-burrow ecomorph is nearly universally recovered as the ancestral state with a high posterior probability. Many of the lineages within this clade are on relatively long branches, however transitions between the eel feeding ecomorphs and the remaining stockier (generalist and spinous-toxic) morphs also occur on short timescales between sister species pairs in some cases (e.g. *H. lapemoides* and *H. viperinus*). Analysis of convergence metrics using convevol showed strong signals of convergence among all ecomorphs, with the spinous-toxic ecomorph having the highest C_1 while the shallow-burrow group had the lowest (Table 2).

Ancestral area reconstructions

The DEC+J model for ancestral area reconstruction had a higher log likelihood than the DEC model (-59.99004 vs. -73.65814). Based on the DEC+J model, the ancestral region of occupancy of *Hydrophis* was ambiguous with similar probabilities for all three regions. Clearer patterns were present in the subclades. Both clades 1 and 2 showed similar patterns of historical biogeography with an Australian origin

followed by relatively concurrent dispersal and diversification within the Sunda region approximately 2 Mya. The remaining nested clade was reconstructed as originating in the Indian Ocean with the three earliest diverging lineages having Indian Ocean origins. At approximately 2 Mya there was another dispersal and subsequent diversification event centred on Australia with a small number of transitions back and forth later. The estimated timing of these major dispersal events coincides with the early Pleistocene and major sea level fluctuations in the Indo-West Pacific.

Discussion

Using SNP data generated from reduced representation genome sequencing, this study obtained substantially greater resolution of *Hydrophis* phylogeny compared to previous analyses of mitochondrial and (few) nuclear loci. Our likelihood tree of concatenated SNPs resolved 33 of 37 nodes with >90% bootstrap support, including several new ecomorphological and biogeographic groupings. These results are discussed below in relation to patterns of mito-nuclear discordance, past introgression, biogeographic history and adaptive radiation.

Clade 1: Shallow-habitat, burrowing prey feeders: *Hydrophis donaldi, H. caerulescens, H. brookii, H. fasciatus* and *H. atriceps*

This clade was recovered as sister to the rest of *Hydrophis* and is well supported in the maximum likelihood analysis, although the inclusion of *H. donaldi* is not well supported in the species tree phylogeny. This study is the first to recover these species as a monophyletic clade; however, previous phylogenies have robustly recovered sister relationships between *H.atriceps* - *H. fasciatus* and *H. brookii* - *H. caerulescens,* while *H. donaldi* has typically been recovered either on a long branch or as distant sister to *H. annandalei* (which was not sampled in our SNP analysis). All five species in this clade are relatively small bodied, are predominantly found in estuarine environments, and belong either to the microcephalic or shallow burrow ecomorphs. They are all simply banded with blackish heads and are typically shades of black, grey and white with prominently keeled scales as adults. All species have noticeably rounded snouts. Based on the ND4 mitochondrial gene tree and morphology it is likely *H. torquatus* and *H. sibauensis* belong to this clade (Lee et al., 2016).

Species within this group have been reported primarily from estuaries and shallow coastal environments. *Hydrophis donaldi* is known only from a single estuary in Northern Australia but may be more widespread given current gaps in sampling. The remaining species are widely distributed in South-east Asia and the Indian Ocean, with *H.atriceps, H. brookii* and *H. caerulescens* (plus *H. torquatus*) found in syntopy within the Straits of Malacca (Voris, 2017). *H. fasciatus* - *H. atriceps* are an allopatric species pair that likely diverged in isolation either side of the Sunda Shelf during glacial cycles. The small goby specialist *H. caerulescens* is currently recognised as a single widespread species, but similarly shows deep mitochondrial divergence between Southeast Asian and Indian Ocean populations. Records of *H. caerulescens* from Australia are tentative and require further investigation.

Clade 2: *Hydrophis cyanocinctus* group

Previous morphological and molecular studies have both recovered a *Hydrophis cyanocinctus* group of varying composition. The subgenus *Leioselasma* of McDowell (1972) incorporated numerous morphologically similar species (*H. cyanocinctus, H. spiralis, H. melanocephalus, H. coggeri* and *H. elegans*) while molecular work incorporated a more morphologically diverse group (*H. cyanocinctus*, *H. melanocephalus, H. coggeri, H. parviceps, H. belcheri, H. jerdoni*). Our SNP analyses robustly resolved a group incorporating all of these species, in addition to the previously unsampled taxon *H. vorisi,* which was once thought to be a regional variant of *H. obscurus* (McDowell, 1972; Kharin, 1984) and is resolved here as close sister to *H. elegans*. The species in this group all specialise on elongate burrowing or crevice-sheltering prey, and are morphologically separated along axes of relative girth and body length (Fig. 4). Five of the six lineages assigned to the crevice giant ecomorph are found within this clade, from the two most basal lineages (*H. elegans* and *H. spiralis*) to the nested *H. cyanocinctus* lineages. In addition to the crevice giants there are a number of ecomorphological transitions to (and likely from) the shallow-burrow and microcephalic ecomorphs (Figs. 5 - 6).

Within the broader *H. cyanocinctus* group is a taxonomically problematic group of four geographically overlapping species: ‘eastern’ *H. cyanocinctus*, *H. melanocephalus, H. coggeri* and *H. parviceps.* Mitochondrial analyses recovered these species as highly interdigitated lineages that formed a shallow sister clade to a

relatively distant ‘western’ (Indian ocean) *H. cyanocinctus* (Sanders et al 2013; this study, Fig. 1). However, our SNP phylogenies robustly recover the microcephalic *H. parviceps* as sister to a clade that also includes *H. belcheri* (recovered as sister to the morphologically similar *H. coggeri*), and a paraphyletic grouping of both ‘eastern’ and ‘western’ lineages of *H. cyanocinctus* plus *H. melanocephalus*. The strong signal of past introgression in this clade (Fig. 3) suggests that mitochondrial capture has contributed to this instance of mito-nuclear discordance. *H. cyanocinctus*-*H. melanocephalus* in particular contains several morphologically divergent subpopulations whose relationships might be represented best by reticulate networks. A remaining taxonomic issue in this group is the status of *H. pacificus*. Australian populations of *H. cyanocinctus* are frequently referred to as *H. pacificus*; however, morphological and molecular work shows that these are deeply nested within the *H. cyanocinctus* complex (Sanders et al., 2013a). Topotypic specimens of *H. pacificus* from New Guinea differ morphologically from *H. cyanocinctus* (Kharin, 2005). Additional sampling of topotypic *H. pacificus* will be needed to determine the validity of this species. Population level sequencing will also be needed to determine the relationship between *H. cyanocinctus* and *H. melanocephalus*.

Clade 3: *Hydrophis curtus*

H. curtus is recovered on a long branch nested within *Hydrophis* in both of our SNP based analyses. This result is similar to mitochondrial phylogenies (Fig. 1 this study; Sanders et al., 2013b; Ukuwela et al., 2016) which are characterised by poorly resolved basal branches and did not recover *H. curtus* as particularly close to any other species. *H. curtus* is morphologically distinct with an unusually low vertebral count. It is thought to hunt in the water column (Marcos & Lanyon, 2004) and has the broadest diet of any sea snake (Supplementary Table 1). Deep population structure between *H. curtus* populations in major ocean basins may warrant recognition of separate species, however more work is needed to define these accurately (Gritis & Voris, 1990; Ukuwela et al. 2014).

Clade 4: *Hydrophis obscurus and H. stricticollis* (Bay of Bengal group)

Hydrophis obscurus and *Hydrophis stricticollis* were robustly supported as sister species in our SNP phylogeny, which is consistent with previous molecular phylogenetic analyses (Sanders et al., 2013b). Simoes et al., (2020) also recovered the enigmatic species *Hydrophis hendersoni* within this clade using the ND4 mitochondrial gene, but we were unable to sample this species in our SNP tree. These three species are sympatric with relatively small distributions in the estuarine environments surrounding the Bay of Bengal. They are diverse in body shape, ranging from robust (*H. hendersoni*) through to extremely microcephalic (*H. obscurus*), although all species are united in possessing striking black and yellow bands. Given the clear morphological disparity in this closely related group of sympatric species this could represent a useful system to investigate ecological speciation-with-gene-flow (Via, 2001). In the concatenated SNP tree, this clade was recovered as sister to *H. lapemoides* and *H. viperinus*, another group distributed in the Indian Ocean, although this relationship was not supported by the SVDquartets analysis.

Clade 5: *Hydrophis lapemoides and H. viperinus*

Previous molecular analyses have indicated a close phylogenetic affinity between these species (Sanders et al., 2013b); however, this study is the first to robustly recover them as a monophyletic clade. Both species have wide distributions in the Indian Ocean and the range of *H. viperinus* extends to the flooded Sunda Shelf. These species differ considerably in skull and ventral scale morphology (Smith, 1926) although juveniles have similar colour patterns and juxtaposed hexagonal scales. Maximum likelihood analysis recovered a sister relationship with clade 4 suggesting this group may represent a small Indian Ocean radiation, however the other molecular analyses are equivocal as to whether they form a monophyletic group (Fig. 1).

Clade 6: Toxic-spinous clade: *Hydrophis stokesii, H. major* and *H. zweifeli*

Clade 6 from Fig. 5 is a well-supported clade of three species belonging to the toxic-spinous ecomorph. Previous phylogenies established a close relationship between *H. major* and *H. zweifeli* (Ukuwela et al 2013), but until now the placement of *H.*

stokesii was uncertain. All three species are broadly sympatric in Australia but have distinct prey preferences. *H. major* feeds almost exclusively on elongate but spinous and highly venomous plotosid catfish (Shine et al., 2019) while *H. stokesii* has a broader diet consisting of various rounder-bodied and spiny-toxic fishes (e.g. puffer- and toad-fish). *H. zweifeli* shares a slit-like mental (chin) scale with *H. schistosus;* this convergent adaptation is thought to increase gape to facilitate swallowing of spinous prey. The relationships resolved by our analyses will aid future studies of the mechanisms of toxin resistance in these species, including whether resistance evolved convergently or arose early in this group and facilitated dietary specialisation.

Clade 7: *Hydrophis czeblukovi, H. laboutei and H. pachyceros*

Lineage 7 consists of at least three poorly known species that are distributed in Australia and Southeast Asia. It is likely that *H. macdowelli* also belongs in this clade based on strong support in the SVDquartets analysis and clear morphological similarity with other species in this group, particularly *H. czeblukovi* (Nankivell, pers obs.). Previous coalescent analyses of a small number of mitochondrial and nuclear genes recovered close affinities between *H. czeblukovi* and *H. macdowelli* as well as *H. pachycercos* (Sanders et al., 2013b). Mitochondrial and morphological analyses also suggest the inclusion of *H. bituberculatus* in this group (Rasmussen et al., 2008). The placement of *H. laboutei* with *H. pachycercos* confirms the previous suggestion of a close affinity of these species plus *H. bituberculatus* based on morphological characters (Rasmussen and Ineich, 2000).

All species in this clade are strongly laterally compressed with broad tails, which likely contributes to swimming efficiency. The *H. laboutei* specimen used in the molecular analysis was collected in deep water (Crowe-Riddell et al., 2019) and *H. czeblukovi* is also known to primarily inhabit deeper water (Nankivell, Sanders and Rasmussen, pers obs.). Members of this clade group are primarily mid-sized and belong either to the microcephalic or shallow burrow ecomorphs, with substantial variation in forebody girth. All species from this clade are only known to feed on eels (including *H. bituberculatus)* although diet data are sparse for most of these poorly known species, and no diet data are available for *H. pachycercos.*

Clade 8: *Hydrophis ornatus* group

Lineage 8 comprises three nominal species (*H. ornatus, H. ocellatus, H. lamberti*), all of which were once part of a more widespread *H. ornatus*. Both SNP and mitochondrial analyses show that *H. ornatus* as presently defined is polyphyletic with a minimum of three divergent, non-monophyletic lineages within it. One clade contains the Australasian *H. ocellatus* and *H. ornatus* “New Caledonia” which are allopatrically distributed. *H. ocellatus* belongs to the shallow burrow ecomorph, and is morphologically distinct from other species in the group by virtue of its large body size and spotted colour pattern. In contrast, *H. ornatus* “New Caledonia” is small and banded much like topotypic and other lineages currently classified as *H. ornatus*. There is a significant signal of introgression between *H. ornatus* “New Caledonia” and *H. ornatus* “SEA”, which prompts an interesting question of whether colour pattern traits were shared across species during past hybridisation (Wellenreuther et al., 2014).

Among the remaining three species there is significant mito-nuclear discordance which is accompanied by signals of introgression. Both SNP trees show *H. ornatus* “Indian Ocean” as the sister to the very closely related sympatric species *H. ornatus* “SEA” and *H. lamberti*. In contrast the mitochondrial tree suggests a closer relationship between *H. ornatus* “Indian Ocean” and *H. lamberti* and these also show evidence of introgression in the ABBA-BABA tests. This suggests that ancient hybridisation and mitochondrial capture may have influenced phylogenetic patterns within this group. This pattern is unexpected as *H. ornatus* “IO” and H. *lamberti* are not known to occur in sympatry, yet show a signal of introgression, while the extremely closely related *H. ornatus* “SEA” has maintained a coherent species boundary with *H. lamberti*. This apparently unusual biogeographic pattern may be related to the temporally variable seascape of the Sunda Shelf but this needs further investigation (Sathiamurthy and Voris, 2006).

Other lineages

A further four species (*H. schistosus, H. platura platura, H. kingii, H. peronii*) were not recovered in any of the lineages listed above and instead are interdigitated between them. All four of these species differ in placement between the two SNP analyses, and bootstrap support values are poor in some cases. Poor resolution of

these species was also observed in the mitochondrial tree, except for *H. peronii* which was recovered inside the *H. ornatus* group. These species are from four of the five ecomorph categories and all were placed in separate genera to *Hydrophis* (three of which were monotypic) with no clear phylogenetic affinities.

Our study confirms that the striking similarity in morphology and diet of *H. schistosus* and *H. zweifeli* is due to convergence rather than recent shared ancestry or hybridisation (Ukuwela et al., 2013). *H. platura platura* is unique among marine snakes in being a pelagic specialist that feeds by ambush near the top of the water column. *H. platura platura* was formerly placed in the monotypic genus *Pelamis* largely because of the unusually elongated snout used to snatch small fish in the water column. McDowell (1972) retained this arrangement but noted some similarities to the *H. ornatus* group in skull shape, while our SNP analyses confirm that *H. platura platura* is relatively closely related to that group. *H. kingii* is the only crevice giant outside of Clade 2, although even among these species it has an unusually thin body with the highest vertebral count as well as a low count of maxillary teeth (1 - 2). *H. peronii* was placed in the monotypic genus *Acalyptophis* due to its unusual horned cephalic scales, however McDowell (1972) noted its similarity to *H. ornatus* in hemipenal and skull morphology. Subsequent molecular work also suggested a close relationship (Ukuwela et al., 2016) to the *H. ornatus* group and our SNP phylogeny confirms this, albeit with different branching order from the mitochondrial phylogeny.

Patterns of ecomorphological and biogeographic diversification

Previous work has shown that *Hydrophis* exhibits the classic hallmarks of adaptive radiation (Schluter, 2000; Rundell & Price, 2009). Speciation is three-folder faster in this clade compared to all other hydrophiines, including the contemporaneous and geographically overlapping *Aipysurus-Emydocephalus* sea snakes (Sanders et al. 2010; Lee et al 2016). *Hydrophis* species also show high sympatric ecomorphological diversity (Voris, 2017; Elfes et al., 2017), and pronounced ecomorphological convergence (Sanders et al. 2013; Ukuwela et al., 2013; Sherratt et al., 2018; 2019a; 2022). In this paper, we assessed the degree of ecomorph convergence between a broader set of ecomorphs and the partitioning of ecomorphs

among subclades and geographic regions. These results are discussed below in the context of generalities about the characteristics of adaptive radiations.

Our study identified five ecomorph categories within *Hydrophis* and showed that species in all of these categories have convergently evolved distinct trophic habits, body proportions and sizes. Most conspicuous is the microcephalic ecomorph; this has the highest species richness and has evolved repeatedly at all levels of the phylogeny (consistent with Sherratt et al., 2018). New resolution of the *Hydrophis cyanocinctus* group (clade 2) shows that transitions from 'crevice eel' and 'shallow burrow' to 'microcephalic' forms were the most frequent ecomorphological changes in this subclade. Relatively distantly related 'crevice giants' appear to have evolved between two and three times, and share elongate bodies with high vertebral counts. The 'generalist' category was assigned to three separate lineages. These have short-moderate body lengths, with low vertebral counts and similar fore-to-hindbody girths. However, they show divergent trophic behaviour; *H. platura platura* is pelagic and feeds at the sea surface, whereas *H. ornatus-lamberti* are benthic feeders and *H. curtus* feeds in both open water and benthic habitats. The 'spinous-toxic' ecomorph broadly overlaps the morphospace of the generalist ecomorph, however spinous-toxic species all have unique adaptations for consuming difficult to handle prey such as a slit-like mental scale to facilitate expanding the mouth. Many of these prey items are toxic and it is likely that there are molecular and physiological adaptations associated with using this resource. This ecomorph may have arisen as few as three times but includes one of the most striking examples of convergent morphological evolution (*H. schistosus* and *H. zweifeli:* Ukuwela et al., 2013).

All five major ecomorphs appeared within only 1.7 Ma of the crown age of *Hydrophis*. This might suggest that *Hydrophis* conforms to the tendency for adaptive radiations to reach high ecomorphological disparity early in their history (the 'early burst' model: Harmon et al., 2010). However, it is also notable that all five species in the 'generalist' and 'spinous-toxic' ecomorphs occur in a single nested clade. The earliest splits within the *Microcephalophis-Hydrophis* phylogeny involve clades composed of eel (and other burrowing) prey specialists. This suggests that the ancestral *Hydrophis* may have resembled the 'shallow burrow' or 'microcephalic' ecomorphs. If this were the case, the 'generalist' and 'spinous-toxic' ecomorphs would represent secondary reversals to the typical body proportions (low relative girths) seen in terrestrial and other marine elapids.

Regardless of whether the *Hydrophis* ancestor was microcephalic, it is clear that a capacity to exploit eel prey has been important in the expansion of the clade. 'Crevice giant', 'microcephalic', and 'shallow burrow' ecomorphs account for at least twice as much of the species richness in *Hydrophis* compared to the 'toxic-spinous' and 'generalist' species (25 versus 12 species, respectively, with 2 species undesignated). Being elongate, limbless predators with well-developed chemoreception, sea snakes are superbly adapted to exploit prey in burrows and crevices. Eels and eel-like fishes are usually unarmoured and rely on inaccessibility for predator avoidance, consequently there are few other known predators of eels in their hiding places.

Finally, our ancestral reconstructions of biogeographic range and ecomorph categories (Figure 6) allow more robust inferences of the geographic partitioning of ecomorph diversity within *Hydrophis* than was possible in previous studies. Some sister pairs have overlapping ranges and belong to different ecomorph categories (e.g *H. brookii* - *H. caerulescens* in Southeast Asia), whereas others belong to the same ecomorphs, show very similar morphology, and are allopatrically distributed (e.g *H. atriceps* - *H. fasciatus* either side of the Sunda Shelf). In general, however, the results show low levels of geographic isolation between close relatives of different ecomorphs. Larger groupings are mostly composed of species that share the same ancestral geographic region and represent at least three ecomorph categories. For example, clades 6, 7 and 8 form a larger clade in which most interspecific lineages are 'crevice giant', 'toxic-spinous', 'shallow-burrow' or 'microcephalic' species and have diversified within the Australia-Pacific region. Clades 4 and 5 together comprise a primarily Indian Ocean clade of only four species but these belong to three different ecomorphs. Nested within clade 2 is a shallow subclade (encompassing the most recent common ancestor of *H. cyanocinctus* and *H. parviceps*) of 'crevice giant', 'shallow burrow' and 'microcephalic' species with highly overlapping ranges in Southeast Asia. These observations at the macroevolutionary level provide promising candidates for model-based tests of speciation mechanisms. It will be especially important to quantify the level and timing of gene flow during species divergence (speciation-with-gene flow), which is an important focus in studies of adaptive radiation (Ford et al., 2015; Malinsky et al., 2018).

Caveats and conclusions

While we endeavoured to sample as many lineages as available, a significant number of sea snakes lineages have remained unsampled in our molecular phylogeny. Some of these do not have clear affinities to any other species (e.g. *Hydrophis anomalus*), while others such as *Microcephalophis cantoris* are likely important to understanding the early evolution of *Hydrophis* due to their likely close relationship to *M. gracilis*, the sister to the remaining *Hydrophis* group. Additionally, our study aimed to reconstruct ecomorphological groupings of sea snakes using morphology and diet. However, for most species diet is only understood at a rudimentary level with only a few, often non species specific, dietary items recorded. Geographical variation in morphology is found within closely related *Aipysurus* (Chapter #; this thesis), but has not been investigated yet in *Hydrophis*. Additional work on sea snake ecology will help clarify ecomorphs in sea snakes and how numerous apparently ecomorphologically similar guilds of species occur in the same assemblages. Despite these limitations, our study has robustly resolved much of the *Hydrophis* phylogeny and provided insights into this spectacular adaptive radiation. It has provided further evidence of the importance of key innovations related to feeding on burrowing and elongated fishes in providing ecological opportunity for radiation.

Acknowledgements

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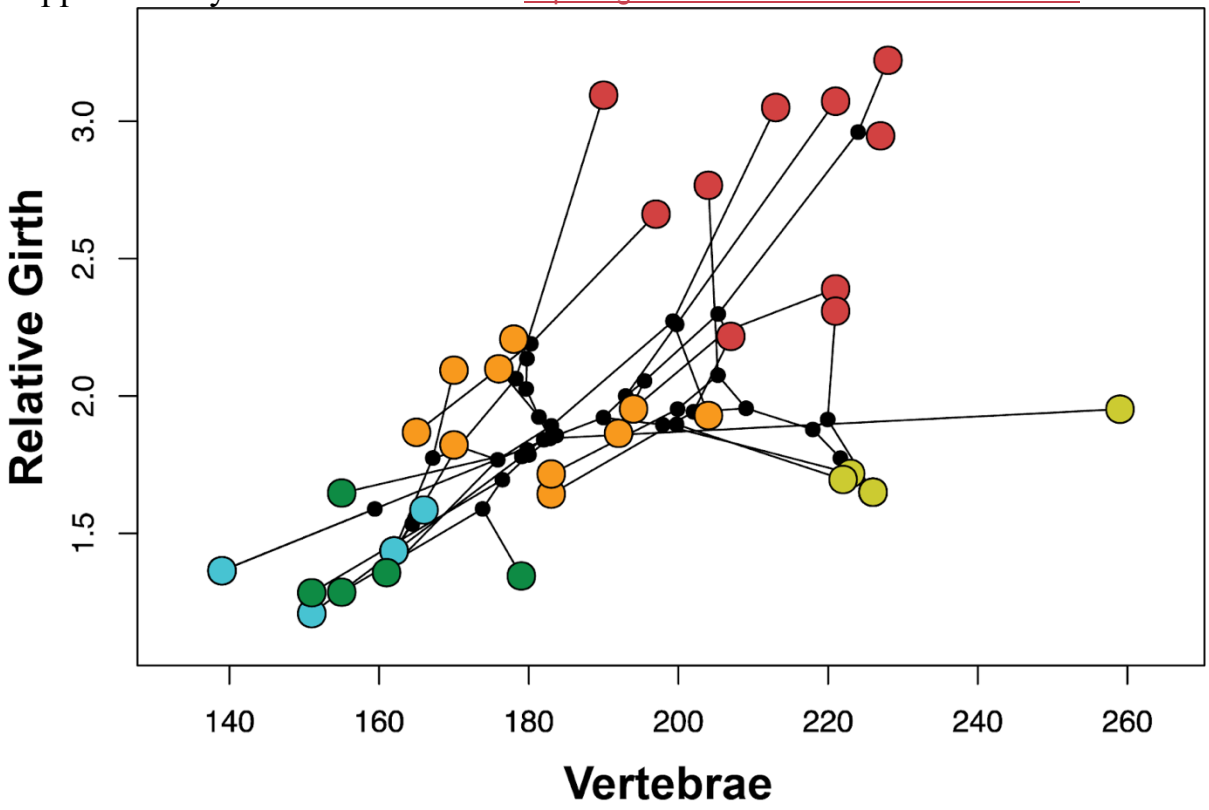
Supplementary Material

Supplementary data is available at https://figshare.com/s/64646eb0a098d6e7c8b9



Supplementary Figure 1 - Phylomorphospace of *Hydrophis* group sea snakes, on a bivariate plot comparing relative girth (y axis) and number of vertebrae (x axis). Tree projected into the morphospace is the maximum likelihood tree from Figure 1. Colours correspond to ecomorphs defined in figure 5.

Supplementary Table 1 - Table of specimen data sequenced using DArTseq

Genus	Species	Sample	Location	Country
Hydrelaps	*darwiniensis*	KLS0534	Broome	Australia
Microcephalophis	*gracilis*	KLS0100	Jaffna	Sri Lanka
Hydrophis	*atriceps*	atr1_VT	Vung Tau	Vietnam
Hydrophis	*belcheri*	mel1_VT	Vung Tau	Vietnam
Hydrophis	*brooki*	R3-178	Thale Luang	Thailand
Hydrophis	*caerulescens*	MW04470	Kalimantan East	Indonesia
Hydrophis	*coggeri*	NC_Jan2019_248	Noumea	New Caledonia

Hydrophis	*curtus*	CAS204975	Mwe Hauk	Myanmar
Hydrophis	*curtus*	MW04675	Weipa	Australia
Hydrophis	*cyanocinctus*	MW04635	Pelabuhanratu	Indonesia
Hydrophis	*cyanocinctus*	MAGNT R37129	Gulf of Carpentaria	Australia
Hydrophis	*cyanocinctus*	FMNH 249656	Phuket	Thailand
Hydrophis	*czeblukovi*	SS171018-06	Pilbara	Australia
Hydrophis	*donaldi*	MW04668	Weipa	Australia
Hydrophis	*elegans*	MW04683	Weipa	Australia
Hydrophis	*fasciatus*	KLS0074	Jaffna	Sri Lanka
Hydrophis	*jerdoni*	X01014	Gulf of Thailand	Vietnam
Hydrophis	*kingii*	KLS0589	Broome	Australia
Hydrophis	*lamberti*	MW04732	Makassar	Indonesia
Hydrophis	*lapemoides*	CAS216443	Kanythaya	Myanmar
Hydrophis	*macdowelli*	KLS00775	Broome	Australia
Hydrophis	*major*	WAMR R154980	Shark Bay	Australia
Hydrophis	*melanocephalus*	MW04738	Makassar	Indonesia
Hydrophis	*obscurus*	R3-328	Chittagong	Bangladesh
Hydrophis	*ocellatus*	KLS0885	Exmouth Gulf	Australia
Hydrophis	*ornatus*	GS136	Makassar	Indonesia
Hydrophis	*ornatus*	FMNH 249576	Phuket	Thailand
Hydrophis	*ornatus*	NC_Jan2019_264	Noumea	New Caledonia
Hydrophis	*pachycercos*	X01009	Phat Thien	Vietnam
Hydrophis	*parviceps*	X01010	Ham Tan	Vietnam
Hydrophis	*peronii*	KLS0555	Broome	Australia

Hydrophis	*platura platura*	WAMR R101240	Perth	Australia
Hydrophis	*schistosus*	KLS0028	Puttlam	Sri Lanka
Hydrophis	*cf. laboutei*	MAGNT R16697	Arafura Sea	Australia
Hydrophis	*spiralis*	KLS0071	Trincomallee	Sri Lanka
Hydrophis	*stokesii*	KLS0794	Exmouth Gulf	Australia
Hydrophis	*stricticollis*	R3-245	Chittagong	Bangladesh
Hydrophis	*viperinus*	KLS0079	Jaffna	Sri Lanka
Hydrophis	*vorisi*	MEL-475	Kikori	Papua New Guinea
Hydrophis	*zweifeli*	MW04666	Weipa	Australia

Supplementary Table 2 - Table of specimen data sequenced for cytochrome b

Genus	Species	Sample	Location	Country
Hydrelaps	*darwiniensis*	EU547084	Bing Bong Station	Australia
Hydrophis	*annandalei*	X01015	Gulf of Thailand	Vietnam
Hydrophis	*atriceps*	GS153	Pasurwan	Indonesia
Hydrophis	*belcheri*	Mel1_VT	Vung Tau	Vietnam
Hydrophis	*brooki*	ZMUC 661293	Thale Sap	Thailand
Hydrophis	*caerulescens*	MW04470	East Kalimantan	Indonesia
Hydrophis	*coggeri*	GS137	Makassar	Indonesia
Hydrophis	*curtus*	MW04675	Weipa	Australia
Hydrophis	*curtus*	CAS204975	Mwe Hauk	Myanmar
Hydrophis	*cyanocinctus*	FMNH 249656	Phuket	Thailand
Hydrophis	*cyanocinctus*	MW04632	Pelenbuhratu	Australia
Hydrophis	*cyanocinctus*	ABTC101328	Gulf of Carpentaria	Australia

Hydrophis	*czeblukovi*	SS171018-06	Pilbara	Australia
Hydrophis	*donaldi*	MW04492	Weipa	Australia
Hydrophis	*elegans*	MW04683	Weipa	Australia
Hydrophis	*fasciatus*	KLS0074	Jaffna	Sri Lanka
Hydrophis	*jerdoni*	X01014	Gulf of Thailand	Vietnam
Hydrophis	*kingii*	KLS0589	Broome	Australia
Hydrophis	*lamberti*	MW04732	Makassar	Indonesia
Hydrophis	*lapemoides*	AR6	Phuket	Thailand
Hydrophis	*macdowelli*	KLS0778	Broome	Australia
Hydrophis	*major*	WAM R154978	Shark Bay	Australia
Hydrophis	*melanocephalus*	MW04738	Makassar	Australia
Hydrophis	*obscurus*	R3-332	Chittagong	Bangladesh
Hydrophis	*ocellatus*	KLS0884	Exmouth Gulf	Australia
Hydrophis	*ornatus*	NC_Jan2019_264	Noumea	New Caledonia
Hydrophis	*ornatus*	GS136	Makassar	Indonesia
Hydrophis	*ornatus*	FMNH 249567	Phuket	Thailand
Hydrophis	*pachycercos*	X01009	Gulf of Thailand	Vietnam
Hydrophis	*parviceps*	X01011	Ham Tan	Vietnam
Hydrophis	*peronii*	KLS0544	Broome	Australia
Hydrophis	*platura platura*	WAM R101240	Perth	Australia
Hydrophis	*schistosus*	MZB12121	East Kalimantan	Indonesia
Hydrophis	*spiralis*	KLS0071	Trincomallee	Sri Lanka
Hydrophis	*stokesii*	KLS0794	Exmouth 0	Australia

Hydrophis	*stricticollis*	R3-245	Chittagong	Bangladesh
Hydrophis	*viperinus*	KLS0118		Sri Lanka
Hydrophis	*vorisi*	MEL-475	Kikori	Papua New Guinea
Hydrophis	*zweifeli*	MW04666	Weipa	Australia
Microcephalophis	*gracilis*	GS147	Makassar	Indonesia

Supplementary Table 3 - Table of available diet records for *Hydrophis* and *Microcephalophis* included in this study

Species	Prey type	Counts	Percentage of prey type
Hydrophis atriceps	Eel	5	5.70%
	Burrowing Eel	80	90.90%
	Crevice Eel	1	1.10%
	Burrowing Goby	2	2.30%
Hydrophis belcheri	Eel	1	50.00%
	Burrowing Eel	1	50.00%
Hydrophis bituberculatus	Eel	7	100.00%
Hydrophis brookii	Eel	9	31.03%
	Burrowing Eel	18	62.07%
	Goby	2	6.90%
Hydrophis caerulescens	Burrowing Eel	6	42.90%
	Burrowing Goby	8	57.10%
Hydrophis coggeri	Burrowing Eel	41	93.18%
	Crevice Eel	3	6.82%
Hydrophis curtus	Eel	79	28.80%
	Burrowing Eel	4	1.50%

	Burrowing Goby	12	4.40%
	Goby	20	7.30%
	Toxic/Spinous	19	6.91%
	All other fish/prey	141	51.30%
Hydrophis cyanocinctus	Eel	1	7.70%
	Burrowing Eel	3	23.10%
	Crevice Eel	6	46.20%
	Goby	2	15.40%
	Toxic/Spinous	1	7.70%
Hydrophis czeblukovi	Crevice Eel	2	100.00%
Hydrophis elegans	Burrowing Eel	6	7.70%
	Crevice Eel	39	50.00%
	Swimming Eel	7	9.00%
	Toxic/Spinous	3	3.85%
	All other fish/prey	23	29.50%
Hydrophis fasciatus	Burrowing Eel	3	100.00%
Hydrophis jerdoni	Burrowing Eel	2	50.00%
	Crevice Eel	2	50.00%
Hydrophis kingii	Eel	1	16.67%
	Burrowing Eel	1	16.67%
	Crevice Eel	2	33.30%
	Toxic/Spinous	2	33.00%
Hydrophis cf. laboutei	Eel	1	100.00%
Hydrophis lapemoides	Eel	1	14.29%

	Burrowing Eel	1	14.29%
	Goby	3	42.86%
	All other fish/prey	2	28.57%
Hydrophis macdowelli	Crevice Eel	1	50.00%
	Burrowing Eel	1	50.00%
Hydrophis major	Burrowing Eel	1	2.44%
	Swimming Eel	3	7.32%
	Goby	2	4.88%
	Toxic/Spinous	27	65.85%
	All other fish/prey	8	21.10%
Hydrophis obscurus	Burrowing Eel	1	100.00%
Hydrophis ornatus	Burrowing Eel	1	14.00%
	Goby	3	43.00%
	Toxic/Spinous	2	29.00%
	All other fish/prey	1	19.51%
Hydrophis parviceps	Burrowing Eel	2	100.00%
Hydrophis peronii	Crevice Eel	1	5.00%
	Burrowing Goby	15	75.00%
	Goby	1	5.00%
	All other fish/prey	3	15.00%
Hydrophis platura platura	Swimming Eel	2	0.90%
	Burrowing Goby	2	0.90%
	Toxic/Spinous	84	35.70%
	All other fish/prey	147	62.60%

Hydrophis schistosus	Toxic/Spinous	162	93.10%
	All other fish/prey	12	6.90%
Hydrophis spiralis	Burrowing Eel	8	57.10%
	Crevice Eel	6	42.90%
Hydrophis stokesii	Burrowing Goby	1	3.70%
	Goby	3	11.00%
	Toxic/Spinous	16	59.30%
	All other fish/prey	7	25.90%
Hydrophis viperinus	Eel	1	7.10%
	Goby	1	7.10%
	Toxic/Spinous	12	85.70%
Hydrophis zweifeli	Toxic/Spinous	1	50.00%
	All other fish/prey	1	50.00%
Microcephalophis gracilis	Burrowing Eel	24	100.00%

Chapter 6

General Discussion

Discussion

Thesis Overview

The Hydrophiinae are a species-rich and ecologically diverse group of snakes distributed primarily in Australia as well as the Indo-West Pacific (Keogh, 1998; Uetz et al., 1998). They are the dominant snake family in Australia and include the most successful group of marine reptiles in the viviparous sea snakes. In this thesis I explored the diversity of Hydrophiinae along the speciation continuum, from the incipient speciation in *Aipysurus* through to the classic adaptive radiation of *Hydrophis*. I uncovered undescribed diversity in both marine and terrestrial lineages and described two new species. To do this I integrated data from a broad array of sources including molecular, morphological and ecological data as well as data available from the fishing industry. In this final chapter I summarise the findings of my research and contextualise them within the broader literature.

Lineage formation and incipient species

In my Chapter 2 I defined Evolutionarily Significant Units (ESUs) in four (of five) codistributed *Aipysurus* species on the North-west Shelf of Australia. Three of these species are of conservation concern following local extinctions in the Timor Sea (Guinea, 2006; Lukoschek et al., 2013), however rediscoveries on the Western Australian Coast have complicated assessments of their status (Sanders et al., 2015). I used high throughput sequencing to generate thousands of nuclear SNPs and showed that for four species the genetic patterns of divergence mirror that of earlier mitochondrial based studies. I also measured a small number of ecologically relevant morphological characters. In all species there was parallel evolution in colour pattern, body size and in two species an increase in fineness. The Western Australian Coast ESUs were generally paler in colouration and longer bodied, which I suggest is a result of their relatively featureless and deep sandy habitat. In contrast the Timor Sea ESUs were darker and relatively shorter bodied, which I argue is an adaptation to their relatively shallow and complex coral reef environment. These patterns of parallel evolution suggest ecological factors have driven the formation of the ESUs in conjunction with biogeographic factors. Intriguingly in the largest and most generalist of these species, *A. laevis*, the nuclear data suggested

contemporary or recent gene flow between populations despite strong mitochondrial divergence.

Understanding population structure and connectivity is important for conservation, as individual populations may have different management requirements. In the short-term, isolated populations may be smaller and more vulnerable to stochastic events, which may well explain the near extinction of various sea snakes on Ashmore Reef. However, another aspect is that of preserving evolutionary potential for the long term (Milot et al., 2020). The interplay of local adaptation and population genetic diversity may well play a role in preserving species through climate fluctuations, which is increasingly relevant in an era of rapid climate change (Jump et al., 2009). Finally, the integration of molecular and morphological data allowed for an insight into the early stages of speciation. While allopatric speciation is considered the dominant mechanism in speciation biology (Phillimore et al., 2008), there is no obvious contemporary barrier separating the *Aipysurus* ESUs and so the ecological factors may also be important, particularly during periods of gene flow, such as during warm interglacials. This is particularly striking in *A. laevis* where the mitochondrial data suggests a historic barrier that has subsequently broken down with gene flow, providing an insight into the ebbs and flows of the early stages of speciation.

Uncovering undescribed diversity in Hydrophiinae

In my third and fourth chapters I described two species of hydrophiine snakes from vastly different environments. In Chapter three I described a new species of turtle-headed sea snake from Western Australia, which is strikingly divergent from its congeners molecularly but nearly cryptic morphologically except for much greater body size. In Chapter four, I described a new species of terrestrial whipsnake from the deserts of central Australia; the new species is distinct morphologically although it is within a genus characterised partly by a great degree of morphological conservatism.

The newly described turtle-headed Sea Snake, *Emydocephalus orarius*, showed strikingly similar biogeographic pattern to the codistributed *Aipysurus* lineages from Chapter 2. *E. orarius* was deeply divergent from the geographically proximate *E. annulatus*, reached a much greater size, differed in colour pattern and

was found on soft bottomed habitats as opposed to blue water coral reefs where *e. annulatus* is known from. The significantly larger size and colour pattern differences suggests that similar processes have driven the divergence in *Emydocephalus* as in the *Aipysurus* species. Curiously though, *E. orarius* was estimated to have diverged approximately four times earlier than the *Aipysurus* species. Various life history factors could cause this, such as more limited dispersal abilities which well documented in other *Emydocephalus* (Lukoschek and Shine, 2008) or faster generation times, which would bias towards deeper divergence estimates. However, while *Emydocephalus* is among the most studied sea snakes due to extensive ecological field surveys in New Caledonia (e.g. Shine et al., 2004; Shine et al., 2005), comparatively little is known about the life history of most *Aipysurus* species.

To describe the new species of *Demansia* in Chapter 4, I reconstructed the first comprehensive phylogeny of *Demansia*, the most speciose genus of terrestrial elapids in Australia. These snakes are highly conservative in morphology and are a classic non-adaptive radiation, instead it appears they have diversified along habitat lines. The new species I described inhabits the relatively cool deserts of Southern Australia, but among its close relatives are species that inhabit temperate, semi-arid and tropical monsoonal climates. Much of the species diversity of *Demansia* is concentrated in the monsoonal tropics, and this appears to be the ancestral geographic range of the genus, with descendent clades invading temperate and arid habitats down the east and west coasts of Australia respectively.

These taxonomy focused chapters were all investigated within an integrative framework (Dayrat, 2005). Use of single or few markers can lead to taxonomic inflation because of inherent bias in so few independent loci, however methods utilising high throughput sequencing can also lead to endless dividing of populations also causing inflation (Zachos et al., 2013). Instead, the current best practice is to use a combination of molecular and morphological data, with strong consideration of any available ecological and biogeographic information. An important source of data in both of these chapters came indirectly through industry partnerships which offered a useful source of samples in regions which remain difficult for researchers to access. For sea snakes, fishing trawlers were an important source of samples. An explosion of environmental surveys has occurred in Western Australia and South Australia associated with mining exploration, and these surveys frequently use pitfall and funnel trapping which is effective for catching elusive snakes. While mining and

fishing clearly pose a threat to biodiversity, it is unlikely much of the material used in this thesis would be available without cooperation between academic researchers and these industries.

Effective environmental monitoring requires good taxonomy and species descriptions, yet in Australia a significant number of squamates remain undescribed (Melville et al., 2021). While neither of the species I described in Chapters three and four is likely to be seriously imperilled at present, awareness of the diversity in a given assemblage is important baseline knowledge. For example, when the sea snake population in Ashmore collapsed unexpectedly, this was flagged only because of a number of baseline surveys and descriptions of species that were thought to be endemic. Had earlier collections and taxonomic work not occurred at Ashmore (Smith, 1926; Heatwole, 1999), the depauperate fauna of modern times would not have been noted as unusual. Finally, as complete as possible sampling of lineages is vital for macroevolutionary studies as just a few missing key lineages can affect outcomes in these studies (Wright et al., 2015). The ecological data included in species descriptions are also important for macroevolutionary studies, which often mine taxonomic papers for morphological, dietary and geographical information. It was my intention in my taxonomic chapters to present accurate information that can be used long into the future for macroevolutionary studies.

Hydrophis phylogeny and diversification dynamics

In my final chapter I reconstructed a phylogeny of *Hydrophis* using thousands of nuclear SNPs. Previous studies had struggled to robustly infer the *Hydrophis* phylogeny with a small number of markers, and this has impacted studies of biogeographical and ecomorphological evolution. I found at least two candidate introgression events using ABBA-BABA statistics, which is a relatively common feature of rapid adaptive radiations. I further built upon studies of shape evolution in *Hydrophis* by defining five ecomorphs using morphological and ecological data. Ancestral state reconstruction showed that ecomorphs that feed on burrowing prey arose early in the radiation, with an intermediate “shallow burrow” ecomorph being the ancestral state across much of the backbone of the tree. Biogeographic reconstruction suggested that three of the four major clades arose in Australia and

one in the Indian Ocean, but two of the Australian clades underwent extensive diversification within the flooded Sunda Shelf region.

Chapter four provides insights into one of the key observations of evolutionary biology; that life on earth is distributed unevenly both geographically and across phylogenies. Explanations for why some groups diversify prodigiously (and others do not) are centred around ideas of ecological opportunity and evolutionary potential (Stroud and Losos, 2016). Sea snakes provide a rare opportunity to investigate these ideas because of the presence of two likely separate but closely related marine invasions, where one of these (*Aipysurus*) has speciation rates similar to the ancestral background rates while the other (*Hydrophis)* has prodigious rates well above the background. With the obvious explanation of invasion of marine habitats alone being unlikely in explaining the *Hydrophis* adaptive radiation, the answer likely lies elsewhere. The leading candidate for explaining the increased speciation rates in *Hydrophis* is that of a convergent evolution in response to ecological opportunity, and in *Hydrophis* this appears to be related to a key innovation that facilitates transitions to microcephaly to take advantage of burrowing fishes as prey resource (Sherratt et al., 2018; 2022). However, *Hydrophis* has a broad diversity of non-microcephalic species that feed on other prey sources as well and so this is only part of the story. My fourth chapter showed that feeding on elongate and burrowing fish arose early in *Hydrophis* and was one of the major drivers of diversification, likely taking advantage of developments in axial skeleton evolution (Sherratt et al., 2022).

Another key insight from my fourth chapter was the timing and evolution of the *Hydrophis* ecomorphs that do not specialise on burrowing and elongate prey. Despite being most similar to ancestral elapids in morphology, these species likely arose secondarily from the more specialised lineages. Biogeographic analyses suggest that these species predominately arose outside of Australia in the Sunda Shelf and Indian Ocean, although later reinvaded the Australian region. Unlike Australia, the Sunda Shelf and Indian Ocean lack generalist *Aipysurus* therefore incumbency effects may partly explain the presence of generalists within only a single large nested clade. I suggest that the initial radiation of *Hydrophis* was driven by changes in axial development that allowed them to exploit new prey sources that are unavailable to most predators. Once freed from competition with *Aipysurus* in Asia further ecological opportunity may have arisen and caused another pulse of diversification. Like all macroevolutionary studies these analyses are contingent on

sampling and basic ecomorphological data, as further species are sampled and measured the story will continue to evolve.

Future Directions

Within the genus *Aipysurus* I have defined ESUs for conservation purposes; however, most conservation management and recovery plans are focussed at the species level. An obvious further step will be to determine whether these ESUs would be more accurately represented as species. To do this well, more comprehensive sampling is required, particularly north of the Dampier Peninsula where putative contact zones are located. The presence of a stable and narrow contact zone or a lack of interbreeding would suggest these ESUs are on separate evolutionary trajectories and should be recognised as species. It will also be important to test the generality of the observed patterns by examining reef versus non-reef populations in eastern Australia where *A. laevis* and *A. duboisii* occur in both habitats. Preliminary observations of duboisii in Queensland suggest that this reef versus non-reef dynamic may be more widespread and should be a priority for future studies. My work has identified a previously overlooked system for understanding the roles of trait divergence and reproductive isolation in generating biodiversity along the speciation continuum.

While my study has highlighted pathways for future research, sampling gaps currently block progress. Many of the factors that apply to lineage formation in *Aipysurus* on the North-western Shelf of Australia likely also do to the newly described *E. orarius* and its congeners, but its power is currently severely limited by the existence of only seven *E. orarius* specimens in museums. Sea snakes have an unusual pattern in collections, with a great number of specimens available from small geographic regions targeted by trawl fisheries. In between these collection sites are vast regions with little known of the sea snake fauna. Filling these gaps will help not just solve taxonomic quandaries, but provide better insights into population status and conservation. In contrast our new species of *Demansia* has been collected extensively throughout its range, particularly in South Australia. Our sampling of other *Demansia* species was notably poorer, as this was largely outside the scope of our study, but our phylogeny still flagged a number of future targets for revision. It remains surprising that Australia's terrestrial elapid fauna has been poorly

documented, and while this is partially because of the vast and sparsely populated nature of the continent, taxonomic vandalism has hit this group harder than most (Wüster et al., 2021). While a number of issues remain, limited data suggest taxonomic problems remain the norm in Australian elapids and will require greater targeted sampling and further taxonomic investment to solve.

Sampling is important for evolutionary studies at all scales of organisation, and the *Hydrophis* radiation is no exception. With only ~70% of described species included in this phylogeny, there is scope for some changes as a result of improved sampling. Improved sampling does not just apply to taxon, but also to morphological and ecological data. While comprehensive diet data is available for a subset of species that Voris and Voris (1983) studied intensively in the 20th century, for even relatively common species in Australia comparatively little is known. Collecting for dietary and life history data can be expensive, but prawn trawl bycatch offers a relatively reliable source of these data and can be utilised more into the future. Sampling is likely to remain a consideration in sea snakes for years to come, but in some regions, it is likely to remain difficult to obtain adequate sea snake sampling well into the future, as such leveraging existing samples and data to understand evolution is required. Some of the most rapid leaps in feasibility and affordability have come in the field of genomics and this is a promising avenue for further exploration. Utilising the developing field of museum genomics to improve sampling is a clear future direction to improve sampling (Card et al., 2021).

The new phylogenetic relationships and population patterns revealed by my work has led to hypotheses that can be addressed by whole genome sequencing. For example, the rapid axial evolution of *Hydrophis* must surely have a genomic basis, and research into genes underpinning regionalisation and segmentation should possible with high quality genomes that are rapidly becoming available. Another striking trait in *Hydrophis* is the propensity to feed on highly toxic prey in many cases, but it remains unknown whether these adaptations are species specific, or rather arose early in *Hydrophis* and provided another key innovation opening up evolutionary potential. Within *Aipysurus* whole genome sequencing can be used to understand the demographic context of the speciation continuum, for example determining whether bottlenecks during glacial cycles play a role in promoting divergence. With technology ever improving undoubtedly more avenues will open up to understand this fascinating group. Hydrophiine snakes are an excellent system to

test evolutionary mechanisms, they contain immense diversity in ecology with multiple major ecological transitions and at least one adaptive radiation, yet are not overwhelmingly diverse. However, there is still much to discover and many species remain only understood at the most rudimentary level in taxonomy and ecology.

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