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Evolutionary biology

Rates of population differentiation and speciation are decoupled in sea snakes

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Comparative phylogeography can inform many macroevolutionary questions, such as whether species diversification is limited by rates of geographical population differentiation. We examined the link between population genetic structure and species diversification in the fully aquatic sea snakes (Hydrophiinae) by comparing mitochondrial phylogeography across northern Australia in 16 species from two closely related clades that show contrasting diversification dynamics. Contrary to expectations from theory and several empirical studies, our results show that, at the geographical scale studied here, rates of population differentiation and speciation are not positively linked in sea snakes. The eight species sampled from the rapidly speciating *Hydrophis* clade have weak population differentiation that lacks geographical structure. By contrast, all eight sampled *Aipysurus–Emydocephalus* species show clear geographical patterns and many deep intraspecific splits, but have threefold slower speciation rates. Alternative factors, such as ecological specialization, species duration and geographical range size, may underlie rapid speciation in sea snakes.

1. Background

Speciation biology predicts that if population differentiation and species diversification are limited by similar causal factors, their rates will be linked over macroevolutionary timescales [1,2]. However, the few studies that have examined relationships between rates of intraspecific differentiation and speciation show inconsistent patterns. For example, studies of birds [3] and fish [4] have found positive associations between genetic estimates of population geographical structure and speciation, supporting theory that the generation of differentiated populations contributes to broad-scale species diversity. However, work on orchids has revealed decoupled differentiation and diversification rates [5], indicating that speciation in this group is limited by other factors, such as ecological opportunity or population persistence. Better understanding of the links between population differentiation and species diversification requires phylogeographic comparisons of recently diverged groups that show contrasting diversification dynamics, ideally across a shared landscape. Such examples may be atypical but have the potential to provide important insights into the speciation mechanisms that explain diversity patterns in focal taxa.

Here, we compare phylogeographic patterns in two clades of sea snakes (Hydrophiinae) that share a common ancestor only approximately 6–16 Ma but have undergone very different rates of species diversification. The *Hydrophis* clade is the most rapidly speciating group of reptiles known, with 47 species that are ecologically diverse and typically have wide geographical distributions in the Indo-West Pacific [6]. By contrast, the *Aipysurus–Emydocephalus* clade has only nine species, most of which are less ecologically specialized and have narrower geographical ranges restricted to the Australasian region. Estimates of speciation rates based on Bayesian analyses of speciation and extinction (using molecular timetrees and correction for differential sampling across lineages) are more than three times higher for *Hydrophis* compared to *Aipysurus–Emydocephalus:* 0.333 versus 0.090 species per million years, respectively [7]. Many species in the two clades have overlapping distributions in various shallow-water habitats across northern Australia. These habitats experienced recurrent cycles of contraction and expansion in response to sea-level fluctuations from the Late Miocene to the Late Pleistocene [8]. Phases of habitat contraction during glacial maxima are thought to explain geographically concordant patterns of population differentiation in many marine taxa, including Australian sea snakes [9], and have been linked to speciation in some groups (e.g. [10]).

In this paper, we generated mitochondrial cytochrome b sequences to analyse phylogeographical histories of 16 sea snake species in the *Hydrophis* and *Aipysurus–Emydocephalus* clades. If rates of population geographical differentiation and species diversification are positively linked at the geographical scale studied here, we would expect to find stronger intraspecific differentiation in the *Hydrophis* taxa because these have threefold higher speciation rates compared with *Aipysurus–Emydocephalus*.

2. Methods

We analysed 373 individual samples from 16 species collected from across their ranges in northern Australia (electronic supplementary material, table S1). These species were initially recognized and described using morphology, but represent monophyletic groupings based on mitochondrial and nuclear genetic markers [11,12]. We sampled eight species from each of the *Aipysurus–Emydocephalus* and *Hydrophis* clades. Thirteen species (including one complex of two nominal species) were densely sampled, with 15–63 (mean 29) individuals sampled per species or species complex (table 1). Three *Hydrophis* species that were less densely sampled (six to eight individuals per species) were included only in the phylogenetic analysis (see below). Sampling localities were grouped into three major regions (figure 1): the Western Australian coast (WAC), Timor Sea Reefs (TS), and northern and eastern Australia (N&E Aus) (figures 1 and 2).

DNA was extracted and the mitochondrial cytochrome b gene was amplified and sequenced using standard protocols. A time-calibrated phylogeny was reconstructed using BEAST v.2.4.7 [13], haplotype networks were created using TCS network methods in PopART [14], and genetic diversity statistics and estimates of pairwise population genetic differentiation were calculated in ARELEQUIN v.3.5.2.2 [15] and DnaSP v.5 [16] (see the electronic supplementary material). An important caveat of our analyses of population differentiation is that they are based on a single mitochondrial gene. However, several previous studies have shown congruent patterns of population structure based on mitochondrial and fast evolving nuclear markers in sea snakes [9,17]. This supports the utility of mitochondrial data in providing meaningful estimates of phylogeographic histories in these species.

3. Results

The final alignment comprised 373 cytochrome b sequences of 1099 base pairs. Divergence time estimates are broadly consistent with previous studies [9,11] and most intraspecific splits are dated within the last approximately 2 Myr (figure 1). Species sampled from the two clades show contrasting phylogeographic patterns. All *Aipysurus–Emydocephalus* species have strong population structure that is broadly congruent with geographical regions. The BEAST tree (figure 1) recovered well-supported clades corresponding to the WAC versus TS and N&E Aus in *A. laevis*; WAC versus TS in *A. foliosquama*, *A. fuscus–A. tenuis*, *A. apraefrontalis*, and *E. annulatus;* and WAC versus N&E Aus in *A. mosaicus*. *A. foliosquama* also contained monophyletic groupings within the WAC (Shark Bay versus more northern WAC localities). Haplotype networks for *Aipysurus–Emydocephalus* species show clear geographical segregation with no haplotypes shared among regions (figure 2), and pairwise comparisons of Nei's genetic distance were significant for 7 of the 11 comparisons among geographical regions (table 1). The only significant Tajima's *D*-value was for the *A. laevis* WAC population (−1.66226; *p*-value: 0.034).

Table 1. Nei's pairwise population genetic distances between major regions; values in bold are significant ($p < 0.05$) and are italicized to show monophyletic clades in the BEAST tree. Superscripts denote numbers of haplotypes shared between regions. Shading delimits the *Aipysurus–Emydocephalus* versus *Hydrophis* clades.

	WAC versus TS	**WAC versus N&E AUS**	**TS versus N&E AUS**
E. annulatus	***0.461***	—	—
A. mosaicus	—	***0.305***	—
A. duboisii	0.103	0.023	**0.545**
A. foliosquama	*0.163*	—	—
A. apraefrontalis	***0.581***	—	—
A. fuscus/tenuis complex	***0.175***	—	—
A. laevis	***1.754***	*0.148*	**0.243**^{2}
H. elegans	—	**0.336**^{3}	—
H. ocellatus	—	−0.048^1	
H. stokesii	—	0.033	0.846
H. peronii	−0.015^1	0.083	0.365
H. major	—	0.147^2	—

None of the eight *Hydrophis* species showed clear phylogeographical structure. Two (*H. major, H. ocellatus*) were recovered in the BEAST tree as shallow clades with no discernable geographical structure (figure 1), and yielded star-shaped haplotype networks with haplotypes shared across distant localities (figure 2). Tajima's *D*-values were significantly negative for WAC populations of these species, at −2.00107 (*p*-value: 0.006) and −1.54236 (*p*-value: 0.02), respectively. The three other densely sampled *Hydrophis* species (*H. peronii*, *H. elegans*, *H. stokesii*) contained weakly supported clades in the BEAST tree but these did not correspond to geographical regions, and haplotypes were shared among regions in *H. peronii* and *H. elegans*. Of the eight pairwise comparisons of Nei's genetic distance in *Hydrophis*, only one was significant (table 1). Nucleotide and haplotype diversities were high within regions for most species (electronic supplementary material, table S2).

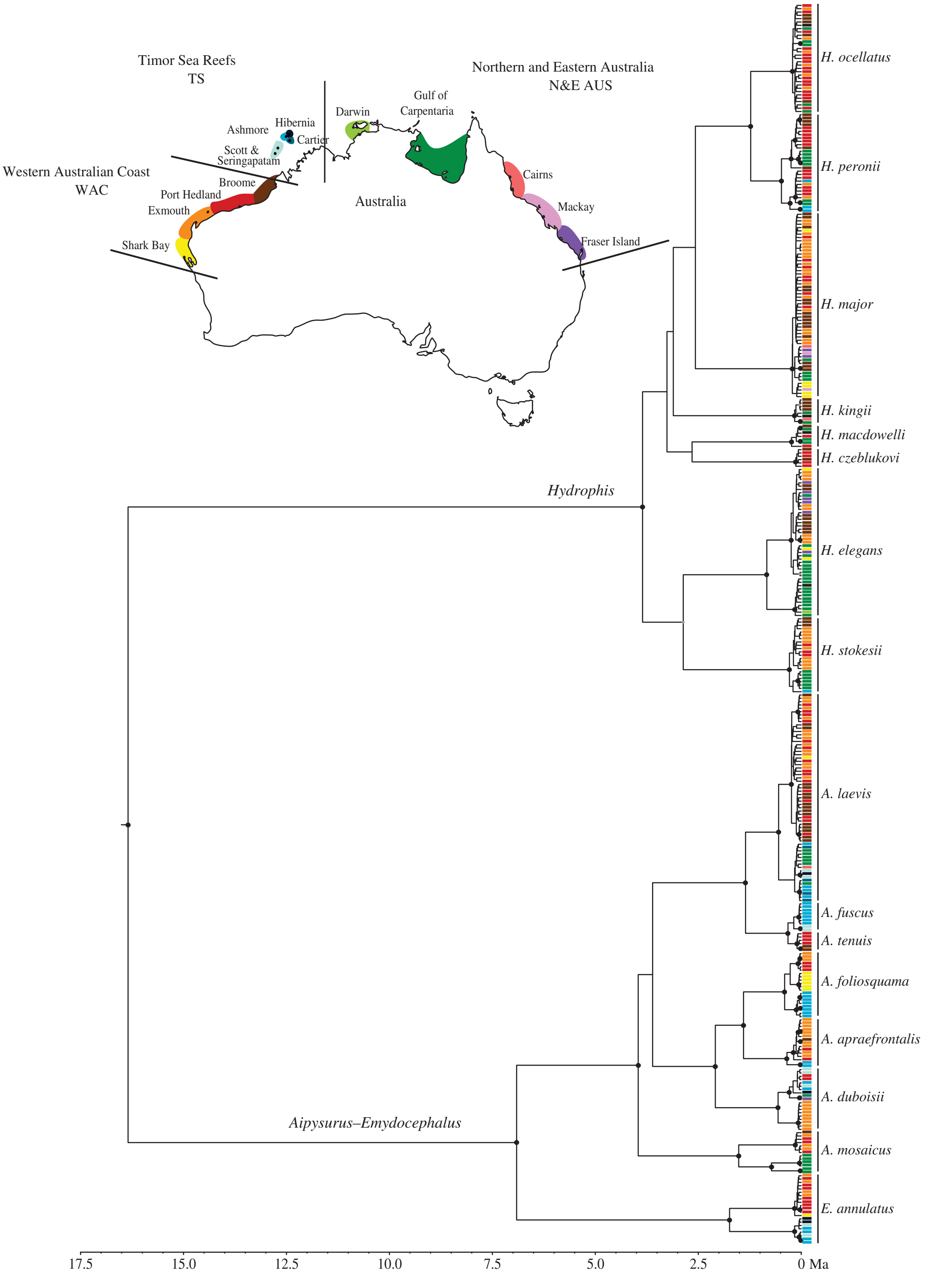

Figure 1. Mitochondrial maximum clade credibility tree for all 16 sampled species. Sampling localities are shown as colours and correspond to the map. Timescale is in millions of years ago (Ma). Posterior probability support values greater than 0.95 are shown as black dots. (Online version in colour.)

4. Discussion

Contrary to expectations from theory and several empirical studies, our results show that rates of fine-scale population differentiation are not positively linked to speciation in sea snakes. The species sampled from the rapidly speciating *Hydrophis* clade have weak population differentiation that lacks geographical structure. By contrast, all sampled *Aipysurus–Emydocephalus* species show clear geographical patterns and many deep intraspecific splits, but have threefold slower speciation rates (figures 1 and 2) [7]. Species in the two groups have diversified across very similar habitats and regions over the past approximately 2 Myr (figure 1). Hence, these lineages' contrasting phylogeographic patterns indicate heritable differences in their responses to historical landscape conditions.

All shallow marine species in northern Australia must have been impacted by the recurrent contractions of their habitats during the Miocene and Pleistocene [8]. However, the persistence of geographical population structure (and

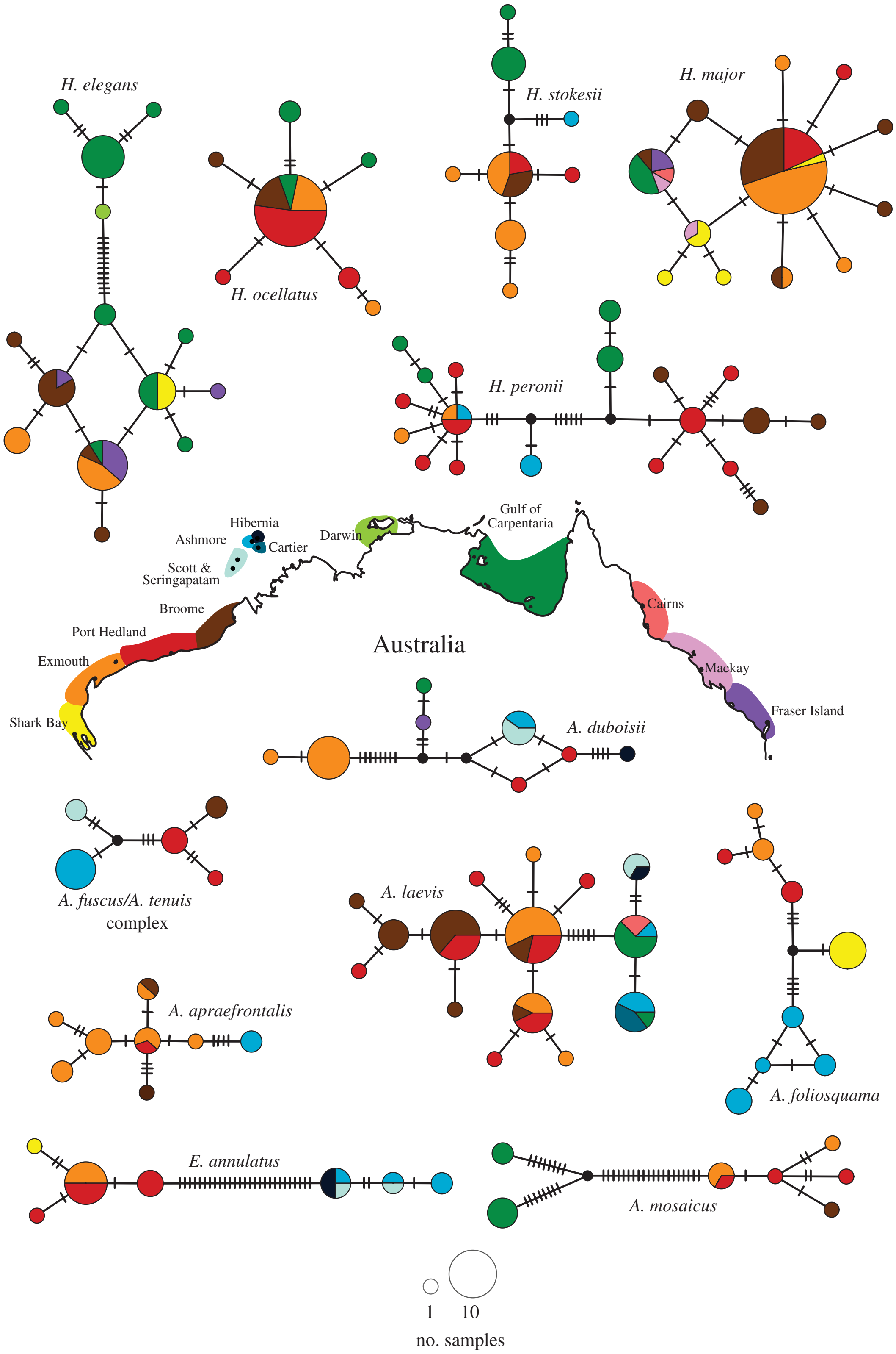

Figure 2. Mitochondrial haplotype networks for 12 densely sampled species or species complexes. Circles represent haplotypes, with sizes of nodes and pie segments proportional to haplotype frequency. Sampling localities are shown as colours based on the corresponding map. (Online version in colour.)

therefore the extent that it contributes to species diversity) will depend on the propensity of previously allopatric populations to introgress during expansion phases. Various demographic factors must influence the rate of gene flow in expanding populations that are incompletely reproductively isolated, particularly dispersal-related traits such as population size, intraspecific competition, habitat preference and dispersal ability. Unfortunately, most of these traits are poorly known for sea snakes. However, *Hydrophis* species typically have large geographical ranges in the Indo-West Pacific, whereas all but two *Aipysurus–Emydocephalus* species are restricted to Australasian waters. Species' range sizes are often indicative of their dispersal capacity [18]. If *Hydrophis* species underwent rapid post-glacial colonization, exporting haplotype diversity over large geographical distances, this may have eroded phylogeographic signal in genetically structured species *H. peronii* and *H. elegans*, and could explain the star-shaped haplotype networks and significantly negative Tajima's *D*-values (indicating recent population expansion) in *H. major* and *H. ocellatus*. It is also possible that range expansion of *Hydrophis* species is less constrained by interspecific competition, given that they are more ecologically specialized than most *Aipysurus* and often co-occur in diverse assemblages [19]. Future studies are needed to examine dispersal dynamics in sea snakes, and identify whether any clade-specific differences are owing to life-history traits and/or interspecific interactions. It will also be important to identify the locations of refugia (such as the remote Timor Sea reefs) used by the two clades during peak habitat contractions.

Regardless of their causative factors, the phylogeographic patterns reported in this paper have several important implications. It is clear that the anomalously high rates of speciation in *Hydrophis* are not limited by rates of population genetic differentiation at the geographical scale studied here. Instead, speciation rates may be promoted by greater range sizes in *Hydrophis* that enhance species' persistence and provide opportunities for divergence across major biogeographic and ecological boundaries. Our previous studies of *Hydrophis* have shown strong vicariance at inter-regional scales [12], and rapid morphological evolution driven by ecological specialization [20]. However, work is needed to identify links among geographical, ecological and life-history traits in sea snake species formation and diversity limits. Our findings also provide a valuable evolutionary context for sea snake conservation planning. In particular, the contrasting phylogeographic histories of *Hydrophis* and *Aipysurus–Emydocephalus* species suggest that they may respond differently to shared threats and require different spatial strategies to preserve genetic diversity and population processes.

Ethics. Work was carried out under the Department of Conservation, Biodivesity and Attactions, Western Australia, Regulation 17 licence no. SF010920, and The University of Adelaide Animal Ethics Committee permit no. S-2014-033.

Data accessibility. Data for all analyses reported in this paper are publicly accessible in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.h1t4h1m [21].

Authors' contributions. K.L.S. conceived the study; all authors contributed to sample collection; C.R.N. carried out laboratory work; C.R.N. and K.L.S. analysed the data and wrote the paper with contributions from M.H. and V.U. All authors approved the final version of the manuscript and agree to be held accountable for its content.

Competing interests. We declare we have no competing interests.

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