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Rates of population differentiation and speciation are decoupled in sea snakes

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Rates of population differentiation and speciation are decoupled in sea snakes

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2 Title: Rates of population differentiation and speciation are decoupled in sea snakes

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34 Abstract

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

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52 Keywords: Phylogeography, speciation, sea snake, Australia, marine

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68 Background

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70 Speciation biology predicts that if population differentiation and species
71 diversification are limited by similar causal factors, their rates will be linked over
72 macroevolutionary timescales (TEMPLETON 1986; TURELLI *et al.* 2001). However, the
73 few studies that have examined relationships between rates of intraspecific
74 differentiation and speciation show inconsistent patterns. For example, studies of
75 birds (HARVEY *et al.* 2017a) and fish (RIGINOS *et al.* 2014) have found positive
76 associations between genetic estimates of population geographic structure and
77 speciation, supporting theory that the generation of differentiated populations
78 contributes to broad-scale species diversity. However, work on orchids has revealed
79 decoupled differentiation and diversification rates (KISEL *et al.* 2012), indicating that
80 speciation in this group is limited by other factors, such as ecological opportunity or
81 population persistence. Better understanding of the links between population
82 differentiation and species diversification requires phylogeographic comparisons of
83 recently diverged groups that show contrasting diversification dynamics, ideally
84 across a shared landscape. Such examples may be atypical but have the potential to
85 provide important insights into the speciation mechanisms that explain diversity
86 patterns in focal taxa.

87 Here, we compare phylogeographic patterns in two clades of sea snakes
88 (Hydrophiinae) that share a common ancestor only ~6-16 million years ago but have
89 undergone very different rates of species diversification. The *Hydrophis* clade is the
90 most rapidly speciating group of reptiles known, with 47 species that are ecologically
91 diverse and typically have wide geographic distributions in the Indo-West Pacific
92 (RASMUSSEN *et al.* 2011). In contrast, the *Aipysurus-Emydocephalus* clade has only
93 nine species, most of which are less ecologically specialised and have narrower
94 geographic ranges restricted to the Australasian region. Estimates of speciation rates
95 based on dated molecular trees are more than three times higher for *Hydrophis*
96 compared to *Aipysurus-Emydocephalus*: 0.333 versus 0.090 species per million years,
97 respectively (LEE *et al.* 2016). Many species in the two clades have overlapping
98 distributions in various shallow-water habitats across northern Australia. These
99 habitats experienced recurrent cycles of contraction and expansion in response to sea
100 level fluctuations from the late Miocene to the late Pleistocene (BOWEN *et al.* 2016).
101 Phases of habitat contraction during glacial maxima are thought to explain

102 geographically concordant patterns of population differentiation in many marine taxa,
103 including Australian sea snakes (LUKOSCHEK 2018), and have been linked to
104 speciation in some groups (e.g. SHEN *et al.* 2011).

105 In this paper, we generated mitochondrial cytochrome b sequences to analyse
106 phylogeographical histories of 16 sea snake species in the *Hydrophis* and *Aipysurus-*
107 *Emydocephalus* clades. If rates of population geographic differentiation and species
108 diversification are positively linked at the geographic scale studied here, we would
109 expect to find stronger intraspecific differentiation in the *Hydrophis* taxa because
110 these have three-fold higher speciation rates compared to *Aipysurus-Emydocephalus*.

111

112 Methods

113

114 We analysed 375 individual samples from sixteen species collected from across their
115 ranges in northern Australia (electronic supplementary table S1). Eight species were
116 sampled from each of the *Aipysurus-Emydocephalus* and *Hydrophis* clades. Thirteen
117 species (including one complex of two nominal species) were densely sampled, with
118 15-63 (mean 29) individuals sampled per species or species complex (Table 1). Three
119 *Hydrophis* species that were less densely sampled (6-8 individuals per species) were
120 included only in the phylogenetic analysis (see below). Sampling localities were
121 grouped into three major regions (Figure 1): the Western Australia coast (WAC),
122 Timor Sea Reefs (TS), and northern and eastern Australia (N&E Aus) (Figures 1 and
123 2).

124 DNA was extracted and mitochondrial cytochrome b gene was amplified and
125 sequenced using standard protocol. A time-calibrated phylogeny was reconstructed
126 using BEAST v2.4.7 (BOUCKAERT *et al.* 2014), haplotype networks were created
127 using TCS network methods in PopART (LEIGH *et al.* 2015), and genetic diversity
128 statistics and estimates of pairwise population genetic differentiation were calculated
129 in Arelequin v3.5.2.2 (Excoffier and Lischer, 2010) and DnaSP v5 (LIBRADO AND
130 ROZAS 2009) (see electronic supplementary material).

131

132 Results

133

134 The final alignment comprised 375 cytochrome b sequences of 1099 base pairs.
135 Divergence time estimates are broadly consistent with previous studies (Sanders *et al.*

136 2013; Lukoschek 2018) and most intraspecific splits are dated within the last ~2
137 million years (Figure 1). Species sampled from the two clades show contrasting
138 phylogeographic patterns. All *Aipysurus-Emydocephalus* species have strong
139 population structure that is broadly congruent with geographic regions. The BEAST
140 tree (Figure 1) recovered well-supported clades corresponding to the WAC versus TS
141 and N&E Aus in *A. laevis*; WAC versus TS in *A. foliosquama*, *A. fuscus-A. tenuis*, *A.*
142 *apraefrontalis*, and *E. annulatus*; and WAC versus N&E Aus in *A. mosaicus*. *A.*
143 *foliosquama* also contained monophyletic groupings within the WAC (Shark Bay
144 versus more northern WAC localities). Haplotype networks for *Aipysurus-*
145 *Emydocephalus* species show clear geographic segregation with no haplotypes shared
146 among regions (Figure 2), and pairwise comparisons of Nei's genetic distance were
147 significant for 7 of the 11 comparisons among geographic regions (Table 1). The only
148 significant Tajima's D value was for the *A. laevis* WAC population (-1.66226; p-
149 value: 0.034).

150 None of the eight *Hydrophis* species showed clear phylogeographical
151 structure. Two (*H. major*, *H. ocellatus*) were recovered in the BEAST tree as shallow
152 clades with no discernable geographic structure (Figure 1), and yielded star-shaped
153 haplotype networks with haplotypes shared across distant localities (Figure
154 2). Tajima's D values were significantly negative for WAC populations of these
155 species, at -2.00107 (p-value: 0.006) and -1.54236 (p-value: 0.02), respectively. The
156 three other densely-sampled *Hydrophis* species (*H. peronii*, *H. elegans*, *H. stokesii*)
157 contained weakly supported clades in the BEAST tree but these did not correspond to
158 geographic regions, and haplotypes were shared among regions in *H. peronii* and *H.*
159 *elegans*. Of the 8 pairwise comparisons of Nei's genetic distance in *Hydrophis*, only
160 one was significant (Table 1). Nucleotide and haplotype diversities were high within
161 regions for most species (electronic supplementary table S2).

162

163 Discussion

164

165 Contrary to expectations from theory and several empirical studies, our results show
166 that rates of fine-scale population differentiation are not positively linked to
167 speciation in sea snakes. The species sampled from the rapidly speciating *Hydrophis*
168 clade have weak population differentiation that lacks geographic structure. In
169 contrast, all sampled *Aipysurus-Emydocephalus* species show clear geographical

170 patterns and many deep intraspecific splits, but have three-fold slower speciation rates
171 (Figures 1 and 2) (Lee et al. 2016). Species in the two groups have diversified across
172 very similar habitats and regions over the last ~2 million years (Figure 1). Hence,
173 these lineages' contrasting phylogeographic patterns indicate heritable differences in
174 their responses to historical landscape conditions.

175 All shallow marine species in northern Australia must have been impacted by
176 the recurrent contractions of their habitats during the Miocene and Pleistocene
177 (BOWEN *et al.* 2016). However, the persistence of geographic population structure
178 (and therefore the extent that it contributes to species diversity) will depend on the
179 propensity of previously allopatric populations to introgress during expansion phases.
180 Various demographic factors must influence the rate of gene flow in expanding
181 populations that are incompletely reproductively isolated, particularly dispersal-
182 related traits such as population size, intra-specific competition, habitat preference,
183 and dispersal ability. Unfortunately, most of these traits are poorly known for sea
184 snakes. However, *Hydrophis* species typically have large geographic ranges in the
185 Indo-West Pacific, whereas all but two *Aipysurus-Emydocephalus* species are
186 restricted to Australasian waters. Species' range sizes are often indicative of their
187 dispersal capacity (e.g. JABLONSKI 2008). If *Hydrophis* species underwent rapid post-
188 glacial colonisation, exporting haplotype diversity over large geographic distances,
189 this may have eroded phylogeographic signal in genetically structured species *H.*
190 *peronii* and *H. elegans*, and could explain the star-shaped haplotype networks and
191 significant, negative Tajima's D values (indicating recent population expansion) in *H.*
192 *major* and *H. ocellatus*. It is also possible that range expansion of *Hydrophis* species
193 is less constrained by interspecific competition, given that they are more ecologically
194 specialised than most *Aipysurus* and often co-occur in diverse assemblages
195 (HEATWOLE AND COGGER 1994). Future studies are needed to examine dispersal
196 dynamics in sea snakes, and identify whether any clade-specific differences are due to
197 life-history traits and/or interspecific interactions. It will also be important to identify
198 the locations of refugia (such as the remote Timor Sea reefs) used by the two clades
199 during peak habitat contractions.

200 Regardless of their causative factors, the phylogeographic patterns reported in
201 this paper have several important implications. It is clear that the anomalously high
202 rates of speciation in *Hydrophis* are not limited by rates of population genetic
203 differentiation at the geographic scale studied here. Instead, speciation rates may be

204 promoted by greater range sizes in *Hydrophis* that enhance species persistence and
205 provide opportunities for divergence across major biogeographic and ecological
206 boundaries. Our previous studies of *Hydrophis* have shown strong vicariance at inter-
207 regional scales (E.G. UKUWELA *et al.* 2016), and rapid morphological evolution driven
208 by ecological specialization (e.g. SHERRATT *et al.* 2018). However, work is needed to
209 identify links among geographic, ecological and life-history traits in sea snake species
210 formation and diversity limits. Our findings also provide a valuable evolutionary
211 context for sea snake conservation planning. In particular, the contrasting
212 phylogeographic histories of *Hydrophis* and *Aipysurus-Emydocephalus* species
213 suggest that they may respond differently to shared threats and require different
214 spatial strategies to preserve genetic diversity and population processes.

215

216 Ethics.

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218 Attractions, Western Australia, Regulation 17 licence number: SF010920, and The
219 University of Adelaide Animal Ethics Committee permit number: S-2014-033.

220

221 Data accessibility.

222 Data are available in the electronic supporting information and newly generated
223 sequences have been deposited in GenBank (Table S1).

224

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228

229 Authors' contributions.

230 KLS conceived the study; all authors contributed to sample collection; CN carried out
231 laboratory work; KLS and CN analysed the data and wrote the paper with edits from
232 MH and VU; all authors read and approved the final manuscript.

233

234 Competing interests.

235 The authors have no competing interests.

236

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245 References

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247 Bouckaert, R., J. Heled, D. Kuhnert, T. Vaughan, C. H. Wu *et al.*, 2014 BEAST 2: a
248 software platform for Bayesian evolutionary analysis. PLoS Comput Biol 10:
249 e1003537.

250 Bowen, B. W., M. R. Gaither, J. D. DiBattista, M. Iacchei, K. R. Andrews *et al.*, 2016
251 Comparative phylogeography of the ocean planet. Proc Natl Acad Sci U S A
252 113: 7962-7969.

253 Excoffier, L., and Lischer, H.E.L. (2010) Arlequin Suite ver 3.5, a New Series of
254 Programs to Perform Population Genetics Analyses under Linux and
255 Windows. Molecular Ecology Resources, 10, 564-567.
256 <https://doi.org/10.1111/j.1755-0998.2010.02847.x>

257 Harvey, M. G., G. F. Seeholzer, B. T. Smith, D. L. Rabosky, A. M. Cuervo *et al.*,
258 2017 Positive association between population genetic differentiation and
259 speciation rates in New World birds. Proceedings of the National Academy of
260 Sciences of the United States of America 114: 6328-6333.

261 Heatwole, H., and H. Cogger, 1994 Sea snakes of Australia. Sea snake toxinology,
262 edited by P. Gopalakrishnakone. Singapore University Press, pp. 167-205.

263 Jablonski, D., 2008 Species Selection: Theory and Data. Annual Review of Ecology
264 Evolution and Systematics 39: 501-524.

265 Kisel, Y., A. C. Moreno-Letelier, D. Bogarin, M. P. Powell, M. W. Chase *et al.*, 2012
266 Testing the link between population genetic differentiation and clade
267 diversification in Costa Rican orchids. Evolution 66: 3035-3052.

268 Lee, M. S., K. L. Sanders, B. King and A. Palci, 2016 Diversification rates and
269 phenotypic evolution in venomous snakes (Elapidae). Royal Society Open
270 Science 3: 150277.

- 271 Librado, P., and J. Rozas, 2009 DnaSP v5: a software for comprehensive analysis of
272 DNA polymorphism data. *Bioinformatics* 25: 1451-1452.
- 273 Lukoschek, V., 2018 Congruent phylogeographic patterns in a young radiation of
274 live-bearing marine snakes: Pleistocene vicariance and the conservation
275 implications of cryptic genetic diversity. *Diversity and Distributions* 24: 325-
276 340.
- 277 Rasmussen, A. R., J. C. Murphy, M. Ompi, J. W. Gibbons and P. Uetz, 2011 Marine
278 Reptiles. *PLoS ONE* 6: 1-12.
- 279 Riginos, C., Y. M. Buckley, S. P. Blomberg and E. A. Treml, 2014 Dispersal capacity
280 predicts both population genetic structure and species richness in reef fishes.
281 *Am Nat* 184: 52-64.
- 282 Sanders K.L., Lee MSY, Mumpuni, Bertozzi T, Rasmussen AR. 2013. Multilocus
283 phylogeny and recent rapid radiation of the viviparous sea snakes (Elapidae:
284 Hydrophiinae). *Mol Phylogenet Evol.* 66(3): 575-91. doi:
285 10.1016/j.ympev.2012.09.021.
- 286 Shen, K.-N., Jamandre, B.W., Hsu, C.-C., Tzeng, W.-N., & Durand, J.-D. 2011. Plio-
287 Pleistocene sea level and temperature fluctuations in the northwestern Pacific
288 promoted speciation in the globally- distributed flathead mullet *Mugil*
289 *cephalus*. *BMC Evolutionary Biology*: 11, 83.
- 290 Sherratt, E., A. R. Rasmussen and K. L. Sanders, 2018 Trophic specialization drives
291 morphological evolution in sea snakes. *R Soc Open Sci* 5: 172141.
- 292 Templeton, A., 1986 The Relation Between Speciation Mechanisms And
293 Macroevolutionary Patterns. 497-512.
- 294 Turelli, M., N. H. Barton and J. A. Coyne, 2001 Theory and speciation. *Trends in*
295 *Ecology & Evolution* 16: 330-343.
- 296 Ukuwela, K. D., M. S. Lee, A. R. Rasmussen, A. Silva, B. G. Fry *et al.*, 2016
297 Evaluating the drivers of Indo-Pacific biodiversity: speciation and dispersal of
298 sea snakes (Elapidae: Hydrophiinae). *Journal of Biogeography* 43: 243-255.
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303 Table 1. Nei's pairwise population genetic distances between major regions; values in
304 bold are significant ($p < 0.05$) and are underlined to show monophyletic clades in the
305 BEAST tree. Superscripts denote numbers of haplotypes shared between regions.

306

307 Figure 1. Mitochondrial maximum clade credibility tree for all 16 sampled species.
308 Sampling localities are shown as colours and correspond to the map. Timescale is in
309 millions of years ago (MYA). Posterior probability support values > 0.95 are shown as
310 black dots.

311

312 Figure 2. Mitochondrial haplotype networks for twelve densely sampled species or
313 species complexes. Circles represent haplotypes with sizes of nodes and pie
314 segments proportional to haplotype frequency. Sampling localities are shown as
315 colours based on the corresponding map.

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337 Table 1.

	WAC v TS	WAC v N&E AUS	TS v N&E AUS
<i>E. annulatus</i>	<u>0.461</u>	-	-
<i>A. mosaicus</i>	-	<u>0.305</u>	-
<i>A. duboisii</i>	0.103	0.023	0.545
<i>A. foliosquama</i>	<u>0.163</u>	-	-
<i>A. apraefrontalis</i>	<u>0.581</u>	-	-
<i>A. fuscus/tenuis</i> complex	<u>0.175</u>	-	-
<i>A. laevis</i>	<u>1.754</u>	<u>0.148</u>	0.243²
<i>H. elegans</i>	-	0.336³	-
<i>H. ocellatus</i>	-	-0.048 ¹	
<i>H. stokesii</i>	-	0.033	0.846
<i>H. peronii</i>	-0.015 ¹	0.083	0.365
<i>H. major</i>	-	0.147 ²	-

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