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Original Article

Evaluating the drivers of Indo-Pacific biodiversity: speciation and dispersal of sea snakes (Elapidae: Hydrophiinae)

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26 running head: Speciation and dispersal of sea snakes

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49 **ABSTRACT**

50 Aim

51 There are several competing hypotheses to explain the high species richness of the Indo-
52 Australian Archipelago (IAA) marine biodiversity hotspot centered within Southeast Asia.
53 We use phylogenetic methods to provide a novel perspective on this problem using viviparous
54 sea snakes, a group with high species richness in the IAA that is highly distinct from other
55 taxa previously studied, both phylogenetically (Reptilia, Amniota) and biologically (e.g.
56 viviparity and direct development).

57

58 Location

59 Indian Ocean and the West Pacific

60

61 Methods

62 We used likelihood and Bayesian methods to reconstruct a multi-locus time-calibrated
63 phylogeny for *c.* 70% of viviparous sea snake species, many sampled from multiple localities
64 in Australasia, Southeast Asia and the Indian Ocean. We then compared rates and temporal
65 concordance of inferred vicariance and dispersal events between marine basins using several
66 approaches including new Bayesian analyses that allow for clade-specific and event-specific
67 dispersal rates.

68

69 Results

70 Phylogenetic analyses and novel Bayesian biogeographic reconstructions indicate that
71 viviparous sea snakes underwent rapid speciation after colonizing SE Asia *c.* 3 million years
72 ago. Most of the SE Asian sea snake diversity is the result of *in-situ* speciation, most

73 consistent with the "centre of origin" and "centre of refuge" models for biodiversity hotspots.
74 There is also speciation at the periphery, or entirely outside of, SE Asia; however, contrary to
75 predictions of the "accumulation" and "overlap" models, these new outlying taxa do not
76 preferentially disperse back into SE Asia. Instead, lineages are equally likely to disperse
77 either into or away from SE Asia.

78

79 Main conclusion

80 The high diversity of sea snakes in SE Asia (and hence the IAA) is mostly explained by *in-*
81 *situ* speciation rather than accumulation or overlap. Most speciation events are
82 contemporaneous with sea level changes that generated and dissolved barriers between
83 marine basins during the last 2.5 million years.

84

85 **KEYWORDS:** biodiversity hotspot, centre of origin, Coral triangle, evolutionary radiation,
86 Indo-Australian Archipelago, Pleistocene

87

88 INTRODUCTION

89 The Indo-Australian Archipelago (IAA), situated between the Indian and Pacific Oceans (Fig.
90 S2), supports an exceptionally rich concentration of marine biodiversity (Hughes *et al.*, 2002),
91 with more fish and coral species reported than for any other region (Hoeksema, 2007; Allen,
92 2008). A pattern of declining diversity with latitudinal and longitudinal distance from the
93 central IAA in many taxa (Veron, 1995; Briggs, 1999; Mora *et al.*, 2003) suggests that a
94 common process underlies this biodiversity hotspot. Theories proposed to explain the
95 exceptional IAA marine diversity typically view the region as either: (1) a centre of
96 origin/speciation, where new species form rapidly and subsequently disperse to peripheral

97 areas (Ekman, 1953); (2) a centre of accumulation of diversity, with speciation in isolated
98 locations at the periphery of the IAA and subsequent movement of newly-formed taxa into the
99 region (Ladd, 1960); or alternatively (3) a region of overlap for marine biodiversity that
100 originated in the Pacific and Indian Oceans, i.e. completely outside the IAA (Woodland,
101 1983). Finally (4), the centre of refuge model suggests that the temporally stable habitat-rich
102 IAA has enabled prolonged speciation and survival for marine species (Jackson *et al.*, 1993;
103 Bellwood & Hughes, 2001; Pellissier *et al.*, 2014), with the diversity gradient due to elevated
104 extinction outside the IAA.

105 Studies on various marine taxa from the region have provided support for the ‘centre of
106 origin’ (Veron, 1995; Carpenter & Springer, 2005; Barber *et al.*, 2006; Tornabene *et al.*, 2015),
107 ‘centre of accumulation’ (Drew & Barber, 2009; Eble *et al.*, 2011; Hodge *et al.*, 2012), ‘region
108 of overlap’(Santini & Winterbottom, 2002; Hubert *et al.*, 2012; Gaither & Rocha, 2013), and
109 the ‘centre of refuge’ (Pellissier *et al.*, 2014) models. Taken together, these studies suggest that
110 multiple processes could contribute towards higher IAA marine biodiversity in various taxa
111 (Randall, 1998; Bernardi *et al.*, 2004; Barber & Bellwood, 2005; Mironov, 2006), and have led
112 to a ‘biodiversity feedback model’ under which the IAA and other tropical marine biodiversity
113 hotspots act as centres of speciation, accumulation and/or overlap (Bowen *et al.*, 2013).

114 Determining the relative importance of the these potential processes generating IAA
115 marine biodiversity requires study groups that span the Indo-Pacific, are highly speciose, are
116 relatively young (thus preserving recent biogeographic events) and can be well-sampled for
117 phylogenetic analysis. The viviparous sea snakes (Elapidae: Hydrophiinae) offer high species
118 diversity, with 62 described species that share a terrestrial Australian ancestor only *c.* 10.6-6.5
119 million years ago (Ma) (Sanders & Lee, 2008; Sanders *et al.*, 2008; Lukoschek *et al.*, 2012).
120 They occupy shallow-marine habitats throughout the tropical and subtropical Indian and

121 Pacific Oceans, but like many other marine groups in the Indo-Pacific, reach peak species
122 diversity in the IAA hotspot (Elfes *et al.*, 2013). Moreover, at least 75% of sea snake species
123 are part of a single, explosively speciating ‘core *Hydrophis* clade’, less than *c.* 3 million years
124 old (Sanders *et al.*, 2010; Sanders *et al.*, 2013a) and widespread throughout the IAA.

125 The majority of sea snake diversification, including the rapid core *Hydrophis*
126 radiation, occurred during major climatic and geological events (Voris, 2000; Woodruffe,
127 2003; Sanders *et al.*, 2013a) that drove vicariant population and species divergence in many
128 of the region’s marine groups (reviewed in Carpenter *et al.*, 2011). Viviparous sea snakes
129 might be particularly influenced by ‘soft’ biogeographic barriers (such as incomplete and thus
130 permeable land bridges) because they undergo direct development (i.e. give birth to live
131 young) and thus lack the dispersing planktonic larval stage that is expected to promote
132 population connectivity in most other marine groups (many fish and invertebrates) (Hoskin,
133 1997). Several sea snake species accordingly show strong intraspecific genetic structure
134 corresponding to deep-water and historical land barriers (Lukoschek *et al.*, 2007; Sanders *et*
135 *al.*, 2013b; Ukuwela *et al.*, 2014). However, biogeographic patterns and the diversification
136 dynamics of the entire sea snake radiation have not previously been quantitatively
137 investigated.

138 In this study we aimed to resolve the biogeographic history of viviparous sea snakes
139 using a multi-locus time-calibrated phylogeny for *c.* 70% of described species, many sampled
140 from multiple localities. We then compared rates and temporal concordance of inferred
141 vicariance and dispersal events between marine basins in Australasia, SE Asia and the Indian
142 Ocean. Specifically, our objective was to test whether viviparous sea snake diversity in the
143 IAA is best explained by *in-situ* speciation, peripheral speciation and accumulation, or

144 external speciation and subsequent overlap. We use several approaches including new
145 Bayesian analyses that allow for clade-specific and event-specific dispersal rates.

146 Although numerous studies have investigated the biogeography of Indo-Pacific marine
147 taxa, most of these have involved a single (Williams & Benzie, 1998; Gaither *et al.*, 2011) or
148 a few species (Halas & Winterbottom, 2009; Gaither *et al.*, 2010), and many have been
149 restricted to sub-regions/single marine basins (Barber *et al.*, 2000; Lourie & Vincent, 2004;
150 Lukoschek *et al.*, 2007). The few broad scale biogeographic studies of species-rich, widely
151 distributed groups have focused primarily on reef fish (Barber & Bellwood, 2005; Gaither &
152 Rocha, 2013). Our study of sea snakes thus provides a novel insight towards understanding
153 the biogeographic processes that have shaped this important marine region.

154

155 **METHODS**

156

157 **Sampling**

158 We sampled a total of 320 individuals from 42 species of viviparous sea snakes from
159 Australia, Indonesia, Myanmar, Malaysia, Vietnam, Thailand, Bangladesh, Sri Lanka, India
160 and Iran (see Fig. S2 in Appendix S1 in Supporting Information; taxonomy and nomenclature
161 follows Sanders *et al.* 2013a). Liver/muscle tissue samples preserved in 90% Ethanol/Iso-
162 propanol were obtained from specimens collected primarily as fisheries by-catch (233
163 individuals, 36 species) and from specimens accessioned in museums (57 individuals, 22
164 species). Additional mitochondrial and nuclear sequences were also obtained from Genbank
165 (30 individuals, 16 species). Specimen collection localities and museum voucher numbers are
166 provided in Appendix S2.

167 We amplified and sequenced a total of 5792 base pairs (bp) from three mitochondrial markers
168 (Cytochrome b [*Cyt-b*]: 1095bp, NADH dehydrogenase subunit 4 [*ND4*] and adjacent *tRNA*
169 region: 838bp, 16S small subunit of ribosomal RNA [*16SrRNA*]: 531bp), two nuclear coding
170 genes (Oocyte maturation factor [*c-mos*]: 918bp, recombination activation gene [*RAG-I*]:
171 1066bp) and three nuclear anonymous markers (*G1888*: 428bp, *G1894*: 422bp, *G1914*:
172 494bp) to reconstruct sea snake phylogeny. Details of DNA extraction, PCR amplification
173 and sequencing are available in Appendix S1. The sequences generated in this study are
174 deposited in the Genbank sequence database (see Appendix S2).

175

176 Phylogeny and divergence time estimates

177 Time-calibrated sea snake phylogenies were inferred using maximum likelihood (ML)
178 and Bayesian analyses of the concatenated mitochondrial and nuclear alignment (See
179 Appendix S1 for details). The Australasian terrestrial elapid *Hemiaspis damielli* was used as
180 an outgroup because there is strong molecular and morphological evidence that *Hemiaspis* is
181 a close relative of the viviparous sea snakes (= Hydrophiini) (Rasmussen, 2002; Lukoschek &
182 Keogh, 2006; Sanders *et al.*, 2008). Maximum Likelihood analyses (undated, no clock) were
183 implemented in RAxML 7.2.8 (Stamatakis, 2006). For the (dated) Bayesian analyses, Bayes
184 Factors (ΔBF ; *sensu* Kass & Raftery, 1995) strongly supported the strict clock over the
185 uncorrelated gamma relaxed clock ($\Delta BF = 1938$); this was consistent with undated (clock-
186 free) trees being approximately ultrametric. The prior on overall rate was set to encompass a
187 broad range, with a lower bound of zero and an upper 95% bound of 20% per lineage per
188 million years, *c.* 20X the "typical" rate of mtDNA (normal distribution with mean 0.01
189 substitutions per million years and a standard deviation of 0.1, truncated at 0). Bayesian
190 analyses with estimation of the divergence times were performed in MRBAYES 3.2 (Ronquist

191 & Huelsenbeck, 2003) (see Appendix S3 for Nexus alignment with MrBayes command
192 block). Since there are no known Hydrophiini fossils that could be used to calibrate the tree,
193 secondary calibrations (uniform distributions 6.5-10.6 Ma and 4.5-7.9 Ma) were applied,
194 respectively, to the root divergence and the *Aipysurus-Hydrophis* divergence. These bounds
195 correspond to the 95% HPD distributions estimated for these two divergences in wider
196 squamate analyses using long nuclear sequences and several reliable squamate fossil
197 calibrations (Sanders *et al.*, 2008; Scanlon & Lee, 2011; Lukoschek *et al.*, 2012).
198 Convergence of the independent runs in topology was assessed by examining similar clade
199 (split) frequencies across runs (standard deviation < 0.05); convergence in numerical
200 parameters was assessed through essentially identical distributions with high effective sample
201 sizes (> 200) as shown by TRACER 1.5 (Rambaut & Drummond, 2007).

202 In addition to the phylogenetic analyses, we estimated the genetic distance between
203 sister lineages in different ocean basins to examine the amount of genetic divergence between
204 these distinct lineages: corrected (HKY) pairwise sequence divergence was calculated in
205 GENEIOUS PRO 5.4 software (Drummond *et al.*, 2009) for the mitochondrial *Cyt-b* gene, which
206 is widely used in phylogeographic studies.

207

208 Dispersal Dynamics and Ancestral Area Reconstruction (AAR)

209 Ancestral areas were reconstructed to examine the biogeographic history of sea snakes. Three
210 oceanic regions/ancestral areas were recognised based on other studies (VLIZ, 2009) which
211 considered dispersal barriers (e.g. deep-sea trenches) and patterns of endemism and species
212 ranges replicated across separate taxa. The three regions (Fig. 1 inset map) are the (1) Indian
213 Ocean, (2) SE Asia (comprising *c.* 70% of the IAA) and (3) Australasia (which includes the
214 eastern end of the IAA). Ancestral area reconstructions were performed using the dated

215 consensus tree (from the MRBAYES analysis) using Bayesian inference in BEAST 1.8
216 (Drummond & Rambaut, 2007), parsimony as implemented in MESQUITE 2.75 (Maddison &
217 Maddison, 2009), and maximum-likelihood as implemented in LAGRANGE (Ree & Smith,
218 2008). For all analyses, each sample (tip) was assigned to one of the three oceanic regions
219 based on the collection locality (See Appendix S1 for details of all analyses).

220 The BEAST analyses implemented novel methods to test whether rates of dispersal
221 varied across lineages (clades) and/or events: the most appropriate model, selected using Bayes
222 Factors, was adopted for Ancestral Area Reconstruction (see above) (See Appendix S3 for
223 BEAST XML file). To test the importance of lineage-specific dispersal rates, we compared a
224 model where different lineages (clades) were permitted different rates (using a "random local
225 clock") (Drummond & Suchard, 2010) to a simpler model, which assumed a uniform dispersal
226 rate across all lineages (a "strict clock"). To test whether certain dispersal events were more
227 likely, we tested four dispersal models of decreasing complexity: (1) a "time-irreversible"
228 model which assumed that all six dispersal events occurred at six different rates (Australasia →
229 SE Asia; Indian Ocean → SE Asia; Australasia → Indian Ocean and the reverse), (2) a "time-
230 reversible" model which assumed three such rates (Australasia ↔ SE Asia; Indian Ocean ↔
231 SE Asia; Australasia ↔ Indian Ocean), and (3) a single rate "unordered" model which assumed
232 a single common rate for all six events. We further evaluated (4) a single-rate "ordered" model,
233 which permitted only dispersals between adjacent regions (Australasia ↔ SE Asia; Indian
234 Ocean ↔ SE Asia). There is no direct continental shelf connection between Australasia and the
235 Northern/Western Indian Ocean, hence the "ordered" model evaluates the hypothesis that sea
236 snakes (with the possible exception of the pelagic, planktonic *H. (Pelamis) platurus*) moving
237 between these regions must generally pass through SE Asia. In all models, a posterior
238 probability of > 0.7 for a region for a node was considered as strong support. These analyses

239 used Markov-Chain Monte Carlo to sample reconstructions in proportion to their probability
240 and recorded the exact number of each of the six dispersal events in each sampled
241 reconstruction (inferring event numbers using consensus node reconstructions will
242 underestimate events if there are often multiple events along single long branches). In addition
243 to using BEAST to comparing these four event-specific models under a Random Local clock,
244 we also tested the fit of these four models in BAYESTRAITS (Pagel *et al.*, 2004), assuming a
245 uniform dispersal rate across lineages (BAYESTRAITS does not implement a RLC to
246 accommodate lineage-specific dispersal rates).

247 The parsimony analyses used MESQUITE 2.75 (Maddison & Maddison, 2009), and
248 optimised regions and dispersals on the tree using an "ordered model" (model number 4),
249 which was the best-supported model identified in model testing (see above).

250 Maximum-Likelihood was implemented in the Dispersal-Extinction-Cladogenesis
251 (DEC) model in LAGRANGE (Ree & Smith, 2008) with ordered and unordered dispersal
252 models. Likelihood ratio tests on Lagrange did not strongly favour either model, but both
253 models produced generally similar results. Thus we provide only the results of the ordered
254 model, which is favoured in the Bayesian analyses and is biologically most reasonable (see
255 above). At each node, range inheritance scenarios > 2 log-likelihood units better than all other
256 possible scenarios were considered as strong support.

257 Even though the *Hydrophis* sea snakes are among the most rapidly speciating
258 tetrapods known (Sanders *et al.*, 2010), this diversity was insufficient to permit statistical tests
259 of relationship between geographic areas and speciation rate (BiSSE, GeoSSE), with robust
260 results requiring "roughly one or two hundred tip species" (Goldberg *et al.*, 2011).

261

262 **RESULTS**

263
264 Phylogeny and divergence time estimates
265 ML (undated) and Bayesian (dated) analyses of the concatenated alignment recovered similar
266 topologies, relative branch lengths and levels of support (Fig. S1 in Appendix S1). Both our
267 ML and Bayesian analyses strongly recovered every sampled species except the *Hydrophis*
268 *ornatus* complex as monophyletic (posterior probabilities (PP) > 0.9 and bootstrap values
269 (BS) > 70%) (Fig. S1 in Appendix S1). Both analyses strongly recovered (PP > 0.9 and BS >
270 70%) reciprocally monophyletic clades within species that correspond to Indian Ocean versus
271 SE Asian/West Pacific populations for *Microcephalophis (Hydrophis) gracilis*, *Hydrophis*
272 *caerulescens*, *H. (Lapemis) curtus*, *H. (Enhydrina) schistosus* and *H. (Thalassophina)*
273 *viperinus* (Fig. S1 in Appendix S1). *Hydrophis curtus* showed further population divergence
274 with distinct clades in Phuket, Thailand (Indian Ocean), SE Asia and Australasia. The
275 analysis also recovered distantly related cryptic lineages of *H. cyanocinctus* and *H. ornatus*
276 with allopatric distributions in the Indian Ocean or West Pacific/SE Asia (Fig. S1 in
277 Appendix S1). However, the widely distributed species *H. (Astrotia) stokesii*, *H.*
278 *(Acalyptophis) peronii* and *H. (Pelamis) platurus* did not display clear geographic genetic
279 structure.

280 Divergence time estimates indicate that the speciation of the *Aipysurus* clade
281 (containing the species of the genera *Aipysurus* and *Emydocephalus*) and the core *Hydrophis*
282 clade (containing the species of the genus *Hydrophis* sensu Sanders *et al.*, 2013a) each
283 commenced *c.* 3.5 Ma (*Aipysurus*: 5.002-2.922 95% HPD; *Hydrophis*: 4.130-2.285 95%
284 HPD) (Fig. S1 in Appendix S1, Fig.1). However, the majority of the divergence time
285 estimates between sister species and sister lineages (within species) ranged from 2.34 to 0.53

286 Ma (2.878-0.343 95% HPD) indicating a rapid late Pliocene or Pleistocene diversification
287 (Table 1).

288 Corrected pairwise genetic (*Cyt-b*) distances between sister lineages in the Indian
289 Ocean and SE Asia ranged between 9.96-2.36%, and for sister lineages in Australasia and SE
290 Asia ranged between 0.72-0.78% (Table 1). This was again consistent with a late Pliocene-
291 Pleistocene speciation with respect to the estimated pairwise substitution rate of 3.3% per
292 million years for the *Cyt-b* gene in Hydrophiinae (Sanders *et al.*, 2013a).

293

294 Dispersal Dynamics and Ancestral Area Reconstruction

295 The best-fitting model, as evaluated in BEAST, allowed lineage-specific dispersal
296 rates, and permitted dispersal only between adjacent regions ("ordered" model), with a single
297 common rate for all four possible dispersal events (Australasia ↔ SE Asia; Indian Ocean ↔
298 SE Asia) (Table 2). Dispersal rates are relatively similar across most lineages, but planktonic
299 *H. platurus* exhibits great (*c.* eightfold = 2.38) increase in dispersal rate compared to other sea
300 snakes (Figs. 1, 2, S3 in Appendix S1; see below). BAYESTRAITS, which tested the four
301 alternative event-specific dispersal models but under the assumption of a common dispersal
302 rate across lineages, could not distinguish between the "ordered", 3-rate and 6-rate models (all
303 $\Delta\text{BF} < 5$ compared to best model) but rejected the unordered model ($\Delta\text{BF}=14.1$).

304 All three AAR methods (Bayesian, parsimony, DEC: Figs. 1, 2, S3 in Appendix S1)
305 recover an Australasian origin (MRCA) for viviparous sea snakes, approximately 6.9 Ma.
306 Similarly, all three analyses indicate that the *Aipysurus* group also originated in Australasia,
307 and subsequently diverged mostly within this region. Parsimony, Bayesian and DEC analyses
308 support an Australasian origin for the two semi-aquatic lineages. BEAST analyses indicated
309 (probability = 0.73) a SE Asian origin for the MRCA of the core *Hydrophis* group, which

310 accounts for *c.* 75% of extant species richness. Parsimony and DEC analyses are consistent
311 with either an Australasian or SE Asian origin for this group. DEC analysis estimated an
312 overall dispersal rate of 0.156 events per lineage per Mya (and an extinction probability of
313 0.016 per Myr) whereas BEAST analyses suggest dispersal rates ranging from 0.31-0.34 per
314 lineage per my in most lineages, up to 2.38 in *H. platurus*.

315

316 **DISCUSSION**

317 Our time-calibrated molecular phylogenetic analyses and ancestral area
318 reconstructions reveal that although viviparous sea snakes had their origins in Australasia,
319 they underwent rapid speciation after colonizing SE Asia during the last 3 million years.
320 Phylogenetic analyses further recover reciprocally monophyletic clades that correspond to
321 Indian Ocean versus SE Asian/West Pacific populations of five species of sea snakes
322 indicating cryptic lineage diversity. Ancestral area reconstructions suggest that most of the SE
323 Asian or the Indo-Australian Archipelago sea snake diversity is the result of *in-situ* speciation.
324 We discuss these findings here with reference to the geo-climatic history of the region,
325 dispersal dynamics and the origins of IAA marine biodiversity.

326

327 *Divergence times, sea snake speciation and sea level changes*

328 Our findings are consistent with previous studies that showed an accelerated rate of
329 speciation in the core *Hydrophis* radiation, with other viviparous sea snakes and their
330 terrestrial sister groups having a slower background rate (Sanders *et al.*, 2010). The recency
331 of many speciation events is consistent with Pleistocene vicariance. The dated tree (Fig. S2 in
332 Appendix S1) suggests that the majority of speciation events in both the *Aipysurus* lineage
333 and the core *Hydrophis* group have occurred since *c.* 3 Ma; this is also broadly consistent with

334 corrected pairwise genetic (*Cyt-b*) distances between sister lineages, which are typically <
335 6%, even for sister lineages spanning different oceans (see Table 1). Cyclic sea level changes
336 that generated and dissolved barriers to dispersal between marine basins during the last 2.5
337 million years in the Indo-Australian Archipelago (IAA) (Voris, 2000; Lambeck *et al.*, 2002)
338 are believed to have facilitated speciation of marine fauna via vicariance in isolated marine
339 basins (e.g. De Bruyn & Mather, 2007; Crandall *et al.*, 2008). This may have been especially
340 effective in sea snakes given that they are viviparous and thus lack the highly dispersive,
341 planktonic larval stage that is expected to facilitate gene flow and population connectivity in
342 many marine taxa.

343 The temporal diversification patterns uncovered for Indo-Pacific sea snakes are also
344 consistent with species of marine invertebrates (Lavery *et al.*, 1996; Williams & Benzie,
345 1998; Benzie, 1999; Duda & Palumbi, 1999) and fish (Timm *et al.*, 2008; Drew & Barber,
346 2009; Leray *et al.*, 2010; Gaither *et al.*, 2011; Tornabene *et al.*, 2015) studied in this region.
347 However, studies on other marine taxa from this region indicate that many taxa currently
348 recognized as species pre-date the Pleistocene and potentially represent complexes of cryptic
349 species (Barber & Bellwood, 2005; Renema *et al.*, 2008; Williams & Duda Jr, 2008; Cowman
350 & Bellwood, 2013). Thus, while Pleistocene vicariance has demonstrably played an important
351 role in generating species and genetic diversity in many Indo-Pacific marine taxa, its
352 contribution to total alpha diversity remains uncertain due to inadequate knowledge of species
353 boundaries and thus, total species numbers. The current work and previous work has
354 identified candidate new (cryptic) species in sea snakes (e.g. Ukuwela *et al.*, 2014), but this
355 unappreciated alpha diversity is likely to be most prevalent in less studied groups such as
356 many invertebrates. Comprehensive taxonomic revisions that incorporate dense molecular
357 sampling from populations up to higher-taxon clades are thus needed to clarify the

358 diversification history and conservation status of marine groups in the IAA biodiversity
359 hotspot.

360

361 *Historical Biogeography of Indo-Pacific sea snakes*

362 Ancestral Area Reconstruction methods recover an Australasian origin for viviparous
363 sea snakes, c. 6.9 million years ago. Similarly, AARs indicate that the *Aipysurus* group also
364 originated in Australasia, and speciated mainly within this region. Of the *Aipysurus* group
365 species, only the specialist fish egg-eaters *Emydocephalus ijimae*, *E. szczyrbaki* (not sampled
366 here) and *A. eydouxii* have colonized SE Asia and none have expanded into the Indian Ocean
367 beyond the coast of Western Australia. BEAST analyses recovered a SE Asian origin for the
368 core *Hydrophis* group, and all three AAR methods indicated that subsequent diversification in
369 this rapidly speciating clade occurred primarily in SE Asia, with subsequent dispersals into
370 the Indian Ocean and re-colonisation of Australasia. In the BEAST AAR (Fig. 1), for
371 instance, there are 34 divergences between lineages older than 0.5 Ma (candidate speciation
372 events); 22 of these have > 0.7 posterior probabilities of occurring in SE Asia, 10 in
373 Australasia, and 2 in the Indian Ocean (Figs 1, 2 & 3). This suggests that most of the sea
374 snake diversity in the SE Asia is derived from a period of rapid *in-situ* diversification. Thus,
375 although viviparous sea snakes originated in Australasia, SE Asia (which comprises most of
376 the IAA) appears to be their primary ‘centre of speciation’.

377 The best-fitting model evaluated in BEAST AAR favoured lineage-specific dispersal
378 rates, and permitted dispersal only between adjacent regions (Table 2). This best-fitting model
379 implies no significant bias in direction of dispersal: thus, contrary to predictions of the
380 overlap or accumulation models, taxa are not more likely to disperse into, rather than out of,
381 SE Asia and thus the IAA. Viviparous sea snakes therefore provide little support for the

382 'region of accumulation hypothesis': there are few instances of peripheral speciation followed
383 by subsequent recolonisation of SE Asia. Peripheral speciation is here identified as
384 cladogenesis where one of the two resultant lineages is inferred to have (primitively) a SE
385 Asian distribution and the other lineage to have (primitively) an external (Australasian or
386 Indian Ocean) distribution. Across the entire tree, nodal reconstructions from BEAST,
387 Parsimony, and DEC analyses indicated two such speciation events between Australasia and
388 SE Asia (*A. mosaicus*-*A. eydouxii* and within *H. curtus*) and six such events between the
389 Indian Ocean and SE Asia (*H. ornatus*-*H. lamberti* and within *M. gracilis*, *H. caerulescens*,
390 *H. curtus*, *H. schistosus*, and *H. viperinus*) (Fig. 1, 2, 3 & S3 in Appendix S1). These findings
391 support a role of geographic/historical isolation at the periphery of the IAA in generating
392 overall species/genetic diversity (Ladd, 1960). However, these events do not increase
393 diversity in SE Asia (i.e. the IAA): the ancestral lineage of each species pair is inferred to be
394 from SE Asia, the peripheral speciation event thus adds a new species to the diversity in the
395 adjacent area (Australasia or Indian Ocean), but there is no evidence of secondary range
396 expansion of these extralimital species back into SE Asia.

397 A small proportion of the sea snake diversity in SE Asia/IAA is consistent with the
398 "overlap" model: speciation entirely outside of SE Asia and subsequent recolonisation. When
399 nodal reconstructions are examined in all three AAR methods, the only major external
400 contribution appears to be from the *H. ornatus* clade (*H. stokesii*, *H. pachycercos*, *H. peroni*,
401 *H. ornatus*, *H. ocellatus*, *H. lamberti*: sensu Sanders *et al.*, 2013); a few lineages from this
402 predominantly Australasian clade have secondarily extended their ranges back into SE Asia
403 (*H. stokesii*, *H. pachycercos*, *H. peroni*, and the *H. ornatus*-*H. lamberti* clade). The Indian
404 Ocean fauna has made little or no contribution to the SE Asian sea snake diversity (the only
405 possible recolonisations involve *H. fasciatus* and *H. spiralis*). The majority of sampled Indian

406 Ocean species and lineages have a SE Asian origin and the regional sea snake fauna seems to
407 be mainly derived from direct dispersal from SE Asia, with few dispersals in the other
408 direction. These findings indicate that considerable speciation occurs outside of the IAA;
409 however, subsequent inward dispersal into the IAA is not a major driver of species richness
410 there.

411 Consistent with the inferences from nodal reconstructions above, all analyses
412 suggested overall dispersals between SE Asia and Australasia occurred at the same
413 frequencies in both directions (Table 2). The BEAST analyses suggested that dispersals
414 between SE Asia and the Indian Ocean also occurred at approximately the same frequency in
415 both directions; however, parsimony and DEC analyses indicated that dispersals from SE
416 Asia to the Indian Ocean were more frequent than the reverse. However, the DEC analysis
417 reconstructed very few events in total, by only considering events between rather than within
418 species. The comparatively slower overall dispersal rate inferred in the DEC analysis might
419 be due to the fact that it only evaluates rates in interspecific branches (the numerous recent
420 dispersals on intraspecific branches were not considered). Alternatively, the broad (flat) prior
421 in the BEAST analysis might have allowed fast rates (see Appendix S1). Dispersal rates were
422 very similar across most lineages (0.31-0.34) with the exception of *H. platurus* (2.38). The
423 relatively high dispersal rates seen in *H. platurus* likely reflect this species' unique ecology:
424 *H. platurus* is the only species of sea snake with pelagic, planktonic habits (drifting with
425 surface and subsurface currents) and consequently has the largest distribution of any squamate
426 reptile (Heatwole, 1999).

427 According to the center of refuge model, the proximity to stable habitats during
428 Quaternary glacioeustatic sea-level changes (Voris, 2000; Woodruffe, 2003) was a major
429 determinant of species survival, enabling recolonisation of unstable shallow water habitats

430 through exportation from the source (Pellissier *et al.*, 2014). Distance to stable habitats
431 (source populations) might be especially important for the maintenance of sea snake diversity
432 in peripheral marine habitats due to their limited dispersal capabilities and reliance on shallow
433 water habitats. Indeed, the most severe known local extinctions of sea snakes have occurred in
434 the very remote Timor Sea reefs (Lukoschek *et al.*, 2013). This scenario is harder to evaluate
435 with molecular trees, as the prime driver (elevated extinction outside biodiversity hotspots) is
436 difficult to estimate using living species alone (Rabosky, 2010). However, some of our
437 patterns discussed above as being consistent with the centre of origin model would also fit the
438 centre of refuge model.

439 An evolutionary history where taxa which leave the IAA are rapidly "pruned" by
440 extinction would generate a phylogeny where most (inferred) speciation events are in the
441 IAA, all the oldest clades are in the IAA, with subsequent and recent colonisation of the
442 Indian Ocean and Australasia. As discussed above, most (inferred) speciation events are in
443 the IAA, and the core *Hydrophis* lineage has its ancestral distribution in the IAA, and with
444 multiple subsequent colonisations of the Indian and Australasian regions (Fig. 1). The broadly
445 similar phylogenetic patterns expected by the "centre of origin" and "centre of refuge" models
446 make them difficult to distinguish. However, the latter model would predict similar speciation
447 rates and high diversity for all old clades (regardless of refuge region). The sea snake
448 phylogeny here suggests long-term persistence (by itself) is not sufficient to generate high
449 diversity, as the three most basal clades of sea snakes each have even longer inferred histories
450 than the core *Hydrophis* group (though in the Australasian region: Fig. 1), yet have each
451 attained only low to moderate diversity. However, huge phylogenies (several hundred taxa:
452 (Goldberg *et al.*, 2011; Davis *et al.*, 2013) are required to properly tease apart the effects of

453 elevated in-situ speciation versus higher extralimital extinction; this is in excess of the
454 available species diversity of many relevant clades (including sea snakes, < 70 species).

455

456 **Caveats**

457 Incomplete taxon sampling can affect biogeographic reconstructions and inferred
458 dispersal patterns (Turner *et al.*, 2009). In this study *c.* 70% of viviparous sea snake species
459 were sampled: sampling was more complete for Australasian and Indian Ocean taxa (both >
460 75%), but less complete for SE Asia (< 60%). This would tend to bias results against
461 reconstructing SE Asia for ancestral nodes. Despite this potential bias, our AARs nevertheless
462 recovered a SE Asian distribution for all basal, and most subsequent, speciation events in the
463 core *Hydrophis* group. Hence, the importance of SE Asia as a centre of speciation for
464 viviparous sea snakes is likely to remain and perhaps be amplified with additional species
465 sampling. Similarly, two species (*H. coggeri* and *H. caeruleus*) were not sampled in one of
466 the geographic areas they are known to occur (Australia). However both species are highly
467 nested in the *Hydrophis* clade so that their intraspecific relationships and distributions are
468 unlikely to significantly impact the AARs at deeper nodes, including the initial diversification
469 of the rapid *Hydrophis* radiation.

470

471 **Conclusions**

472 The drivers of the elevated diversification rate in the core *Hydrophis* group still need
473 to be identified. They could involve extrinsic (geographical) factors, such as the formation of
474 transient barriers (Palumbi, 1994) and proximity to habitat refugia (Pellissier *et al.*, 2014) in
475 the Plio-Pleistocene, or intense competition (Briggs, 2005; Bowen *et al.*, 2013), or divergent
476 selection in a highly heterogeneous and biodiverse environment (Rocha & Bowen, 2008).

477 Alternatively, they could be intrinsic: a recent study has suggested that plasticity of head size
478 evolution contributed to rapid speciation in one clade within this group (Sanders *et al.*,
479 2013b). Evaluation of whether the core *Hydrophis* group exhibits different diversification
480 rates in different regions would answer this question, but robust inferences would require far
481 more species than exist: at least 100-200 (Goldberg *et al.*, 2011) or > 300 (Davis *et al.*, 2013).
482 However, pooling phylogenies of sea snakes and other vertebrate groups (fish) spanning this
483 region might provide sufficient sample size (Goldberg *et al.*, 2011), though even with
484 sufficiently large taxon sets, current implementations of these methods are highly problematic
485 (Rabosky & Goldberg, 2015).

486 Distinguishing alternative diversification scenarios for the origins and maintenance of
487 extraordinary marine biodiversity in the IAA remains a central goal in marine biogeography.
488 Analyses of viviparous sea snakes suggest that SE Asia, which includes most of the IAA, has
489 functioned mainly as a ‘centre’ or a ‘cradle’ of speciation for viviparous sea snakes: the core
490 *Hydrophis* group underwent rapid and largely *in-situ* diversification during the last 3 Mya in
491 SE Asia. Speciation either at the periphery (or outside) of SE Asia, followed by biased
492 inwards range shifts, does not appear to be an important contributor of marine snake
493 biodiversity of SE Asia and the IAA.

494

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503

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721 **SUPPORTING INFORMATION**

722 Additional Supporting Information may be found in the online version of this article:

723 Appendix S2: Details of the specimens, voucher numbers and the respective Genbank

724 accession numbers used in the molecular phylogenetic analysis

725 Appendix S1: Supplementary Materials and Methods and supplementary Figures

726 Appendix S3: Aligned dataset in Nexus format with MrBayes commands and BEAST xml
 727 file for biogeographic reconstruction

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729 **BIOSKETCHES**

730 Kanishka D.B. Ukuwela is a recent PhD graduate from the University of Adelaide, Australia,
 731 now a Lecturer at Rajarata University of Sri Lanka. His current research is focused on the
 732 origins, evolution and systematics of the South Asian herpetofauna.

733 Author contributions: KDBU, MSYL, KLS conceived the study. KDBU, ARR, AdeS, Mu,
 734 BGF, PG, MR and KLS collected samples. KDBU and KLS generated data. KDBU, KLS and
 735 MSYL analysed data and KDBU, MSYL and KLS wrote the paper.

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737 **Table 1** Percentage pairwise corrected genetic divergences, and mean divergence times
 738 (millions of years) between sister species/lineages in different Ocean basins. Abbreviations:
 739 IO- Indian Ocean, SEA- SE Asia, AUS-Australasia, WP-West Pacific (includes both SEA and
 740 SEA)

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Species/Lineage	Genetic divergence (corrected; %)	Mean divergence time (Ma)	Divergence Time (95% HPD, Ma)
<i>A. eydouxii</i> - <i>A. mosaicus</i>	7.10-7.39	2.297	2.878-1.679
<i>H. atriceps</i> - <i>H. fasciatus</i>	2.02-2.92	1.027	1.366-0.654
<i>H. caeruleus</i> (IO-SEA)	2.36-2.91	0.965	1.292-0.651
<i>H. curtus</i> (IO-WP)	8.64-9.96	2.337	2.895-1.698
<i>H. curtus</i> (SEA-AUS)	0.72-0.78	0.289	0.411-0.174
<i>H. cyanocinctus</i> (IO-WP)*	4.01-4.96	-	-
<i>H. ornatus</i> (IO-SEA)*	3.33-4.12	-	-
<i>H. schistosus</i> (IO-SEA)	4.05-4.96	0.716	0.967-0.490
<i>H. lamberti</i> - <i>H. ornatus</i> (IO)	1.04-1.30	0.526	0.718-0.343
<i>H. viperina</i> (IO-SEA)	4.05-4.85	0.708	0.977-0.457
<i>M. gracilis</i> (IO-SEA)	4.53-5.44	1.270	1.756-0.841

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743 *These species are each currently considered single species. However, molecular analyses
 744 indicate that each consist of two cryptic lineages that do not show a sister species/lineage
 745 relationship (hence divergence time is not shown).

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751 **Table 2** Inferred dispersal events from the three ancestral area reconstruction methods (A-C)
 752 and the fit of alternative dispersal models (D), which assume uniform or variable dispersal
 753 rates across lineages (clades) and across events. In the BEAST table (A), the actual numbers
 754 of events in the individual MCMC samples are listed first; the events "inferred" by only
 755 examining nodal reconstructions in the Bayesian consensus tree are shown in parentheses

756

<i>A: BEAST (variable rates across lineages, ordered)</i>			
From \ To	Australasia	SE Asia	Indian Ocean
Australasia	-	17.1 (9)	*
SE Asia	18.2 (5)	-	17.7 (4)
Indian Ocean	*	11.6 (13)	-
<i>B: Parsimony (ordered)</i>			
From \ To	Australasia	SE Asia	Indian Ocean
Australasia	-	5	*
SE Asia	4	-	7
Indian Ocean	*	1	-
<i>C: Lagrange (ordered, interspecific events only)</i>			
From \ To	Australasia	SE Asia	Indian Ocean
Australasia	-	4	*
SE Asia	3	-	2
Indian Ocean	*	0	-
<i>D: Fit of alternative dispersal models in BEAST</i>			
Dispersal models	-LognL	BayesFactor	
Variable rates across lineages, 1 event rate (ordered) ⁺	113.506	0 (best)	

Variable rates across lineages, 1 event rate (unordered)	121.073	-15.134
Variable rates across lineages, 3 event rates (reversible)	117.398	-7.784
Variable rates across lineages, 6 event rates (irreversible)	118.378	-9.744
Uniform rates across lineages, 1 event rate (ordered)	122.758	-18.504

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758 * = fixed to zero (see model testing in Appendix S1).

759 +The preferred model (number 4 in main text) assumes variable dispersal rates across lineages

760 (RLC), and a common rate for all dispersal types, and also that dispersals are only possible

761 between adjacent regions (i.e. ordered). See Appendix S1 for full description.

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765 **Figure legends**

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767 **Fig. 1** Time-calibrated tree of viviparous sea snakes, with Bayesian (BEAST) ancestral area

768 reconstructions. Time scale is in million years before present. Colours of the branches

769 indicate the ancestral area reconstructions and correspond to the biogeographic/ancestral

770 regions shown in map (Red: Indian Ocean (IO), Green: SE Asia (SEA), Blue: Australasia).

771 Pie charts depict the relative posterior probability of the alternative ancestral areas for each

772 node (WP - West Pacific, includes both SE Asia and Australia). See Fig. S1 for clade support

773 values.

774

775 **Fig. 2** Time-calibrated tree of viviparous sea snakes, with parsimony ancestral area

776 reconstructions. Time scale is in million of years before present. Colours of the branches

777 indicate the most parsimonious ancestral area reconstructions for the node at the younger end,

778 and correspond to the biogeographic/ancestral regions shown in map (Red: Indian Ocean,

779 Green: SE Asia, Blue: Australasia). Two colours (e.g. in the core *Hydrophis* branch) indicate
780 2 equally-parsimonious reconstructions. See Fig. S1 for clade support values.

781

782 **Fig. 3** Divergence times (mean and 95% HPD intervals) between pairs of sister allopatric
783 lineages, spanning Australasia (AUS) and SE Asia (SEA) (5 pairs, grey bars), and spanning SE
784 Asia and the Indian Ocean (IO) (8 pairs, white bars). Most divergence events occurred in the
785 last million years in both cases. The numbers in parentheses refer to the following divergences:
786 (1) *Aipysurus eydouxii*-*Aipysurus mosaicus*, (2) *H. cyanocinctus* (AUS-SEA), (3) *H. curtus*
787 (AUS-SEA), (4) *H. stokesii* (AUS-SEA), (5) *H. peronii* (AUS-SEA), (6) *H. atriceps*-*H.*
788 *fasciatus*, (7) *H. viperina* (IO-SEA), (8) *H. curtus* (IO-Phuket+SEA+AUS), (9) *H. curtus*
789 (Phuket-SEA+AUS), (10) *H. caeruleus* (IO-SEA), (11) *H. schistosus* (IO-SEA), (12) *H.*
790 *lamberti*-*H. ornatus* (IO), (13) *M. gracilis* (SEA-IO).

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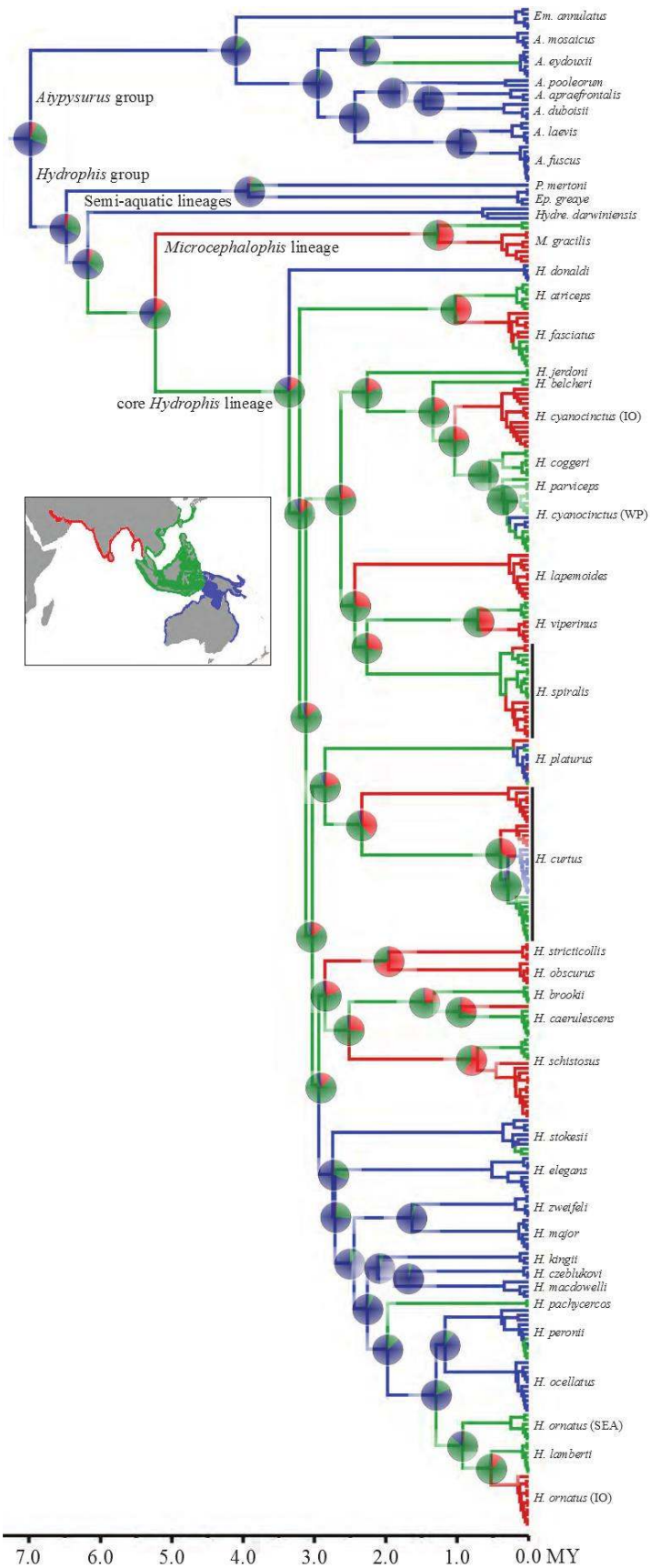
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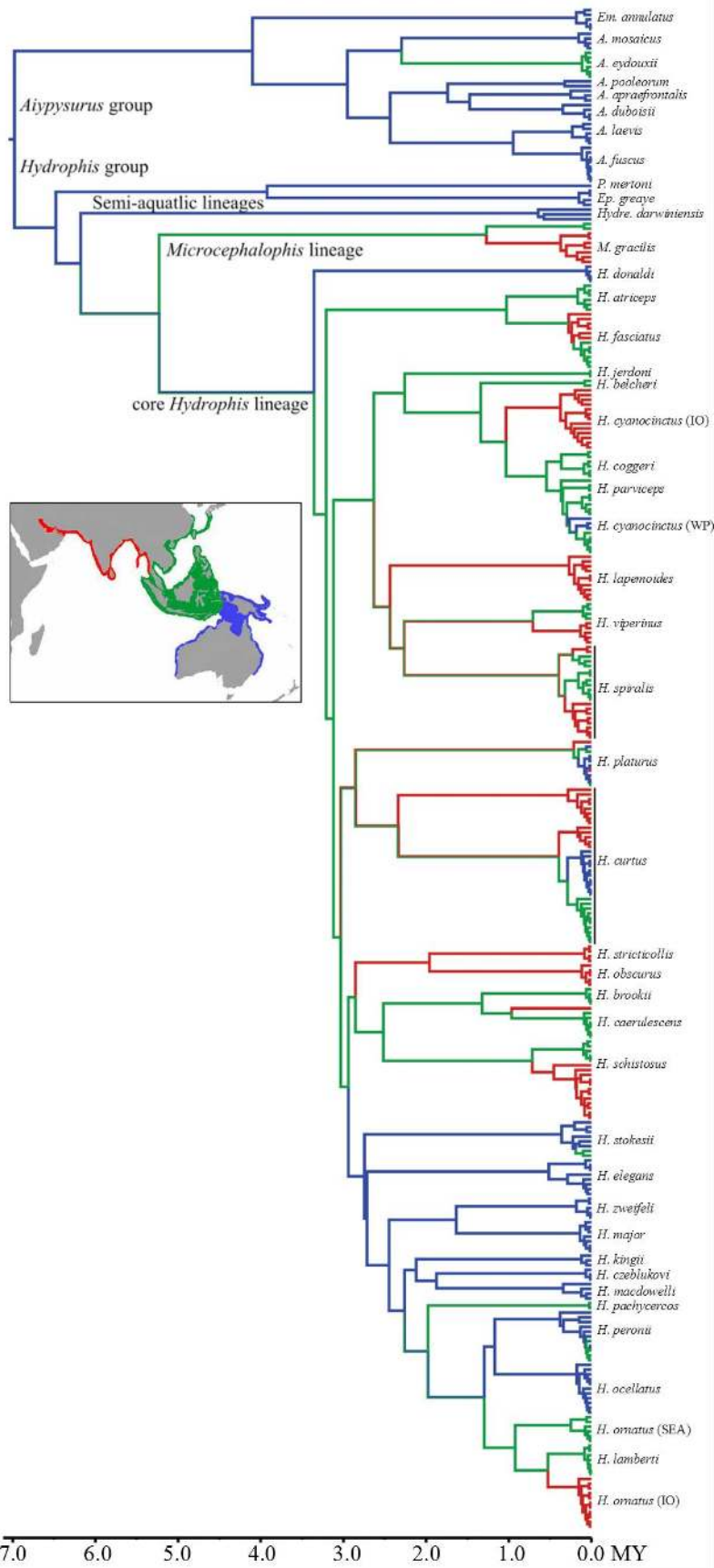
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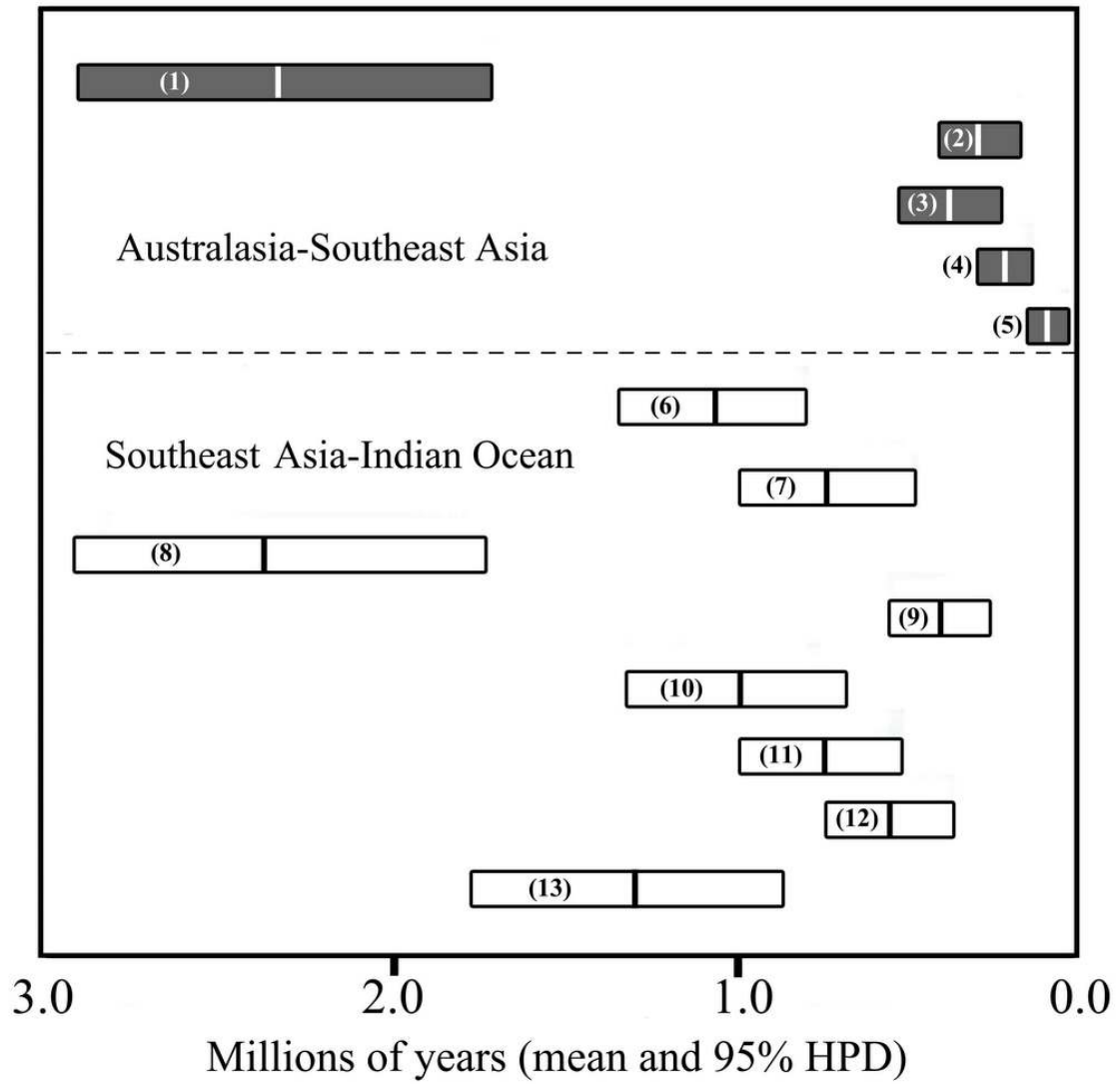
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Figure 1



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Figure 2



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 832 Figure 3
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