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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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running head: Speciation and dispersal of sea snakes

ABSTRACT

Aim

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

Location

Indian Ocean and the West Pacific

Methods

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

Results

Phylogenetic analyses and novel Bayesian biogeographic reconstructions indicate that viviparous sea snakes underwent rapid speciation after colonizing SE Asia *c.* 3 million years ago. Most of the SE Asian sea snake diversity is the result of *in-situ* speciation, most consistent with the "centre of origin" and "centre of refuge" models for biodiversity hotspots. There is also speciation at the periphery, or entirely outside of, SE Asia; however, contrary to predictions of the "accumulation" and "overlap" models, these new outlying taxa do not preferentially disperse back into SE Asia. Instead, lineages are equally likely to disperse either into or away from SE Asia.

Main conclusion

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

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

INTRODUCTION

The Indo-Australian Archipelago (IAA), situated between the Indian and Pacific Oceans (Fig. S2), supports an exceptionally rich concentration of marine biodiversity (Hughes *et al.*, 2002), with more fish and coral species reported than for any other region (Hoeksema, 2007; Allen, 2008). A pattern of declining diversity with latitudinal and longitudinal distance from the central IAA in many taxa (Veron, 1995; Briggs, 1999; Mora *et al.*, 2003) suggests that a common process underlies this biodiversity hotspot. Theories proposed to explain the exceptional IAA marine diversity typically view the region as either: (1) a centre of origin/speciation, where new species form rapidly and subsequently disperse to peripheral areas (Ekman, 1953); (2) a centre of accumulation of diversity, with speciation in isolated locations at the periphery of the IAA and subsequent movement of newly-formed taxa into the region (Ladd, 1960); or alternatively (3) a region of overlap for marine biodiversity that originated in the Pacific and Indian Oceans, i.e. completely outside the IAA (Woodland, 1983). Finally (4), the centre of refuge model suggests that the temporally stable habitat-rich IAA has enabled prolonged speciation and survival for marine species (Jackson *et al.*, 1993; Bellwood & Hughes, 2001; Pellissier *et al.*, 2014), with the diversity gradient due to elevated extinction outside the IAA.

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

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

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

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

In this study we aimed to resolve the biogeographic history of viviparous sea snakes using a multi-locus time-calibrated phylogeny for *c.* 70% of described species, many sampled from multiple localities. We then compared rates and temporal concordance of inferred vicariance and dispersal events between marine basins in Australasia, SE Asia and the Indian Ocean. Specifically, our objective was to test whether viviparous sea snake diversity in the IAA is best explained by *in-situ* speciation, peripheral speciation and accumulation, or external speciation and subsequent overlap. We use several approaches including new Bayesian analyses that allow for clade-specific and event-specific dispersal rates.

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

METHODS

Sampling

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

We amplified and sequenced a total of 5792 base pairs (bp) from three mitochondrial markers (Cytochrome b [*Cyt-b*]: 1095bp, NADH dehydrogenase subunit 4 [*ND4*] and adjacent *tRNA* region: 838bp, 16S small subunit of ribosomal RNA [*16SrRNA*]: 531bp), two nuclear coding genes (Oocyte maturation factor [*c-mos*]: 918bp, recombination activation gene [*RAG-1*]: 1066bp) and three nuclear anonymous markers (*G1888*: 428bp, *G1894*: 422bp, *G1914*: 494bp) to reconstruct sea snake phylogeny. Details of DNA extraction, PCR amplification and sequencing are available in Appendix S1. The sequences generated in this study are deposited in the Genbank sequence database (see Appendix S2).

Phylogeny and divergence time estimates

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

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

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

Dispersal Dynamics and Ancestral Area Reconstruction (AAR)

Ancestral areas were reconstructed to examine the biogeographic history of sea snakes. Three oceanic regions/ancestral areas were recognised based on other studies (VLIZ, 2009) which considered dispersal barriers (e.g. deep-sea trenches) and patterns of endemism and species ranges replicated across separate taxa. The three regions (Fig. 1 inset map) are the (1) Indian Ocean, (2) SE Asia (comprising *c.* 70% of the IAA) and (3) Australasia (which includes the eastern end of the IAA). Ancestral area reconstructions were performed using the dated consensus tree (from the MrBayes analysis) using Bayesian inference in BEAST 1.8 (Drummond & Rambaut, 2007), parsimony as implemented in Mesquite 2.75 (Maddison & Maddison, 2009), and maximum-likelihood as implemented in Lagrange (Ree & Smith, 2008). For all analyses, each sample (tip) was assigned to one of the three oceanic regions based on the collection locality (See Appendix S1 for details of all analyses).

The BEAST analyses implemented novel methods to test whether rates of dispersal varied across lineages (clades) and/or events: the most appropriate model, selected using Bayes Factors, was adopted for Ancestral Area Reconstruction (see above) (See Appendix S3 for BEAST XML file). To test the importance of lineage-specific dispersal rates, we compared a model where different lineages (clades) were permitted different rates (using a "random local clock") (Drummond & Suchard, 2010) to a simpler model, which assumed a uniform dispersal rate across all lineages (a "strict clock"). To test whether certain dispersal events were more likely, we tested four dispersal models of decreasing complexity: (1) a "time-irreversible" model which assumed that all six dispersal events occurred at six different rates (Australasia → SE Asia; Indian Ocean → SE Asia; Australasia → Indian Ocean and the reverse), (2) a "time-reversible" model which assumed three such rates (Australasia ↔ SE Asia; Indian Ocean ↔ SE Asia; Australasia ↔ Indian Ocean), and (3) a single rate "unordered" model which assumed a single common rate for all six events. We further evaluated (4) a single-rate "ordered" model, which permitted only dispersals between adjacent regions (Australasia ↔ SE Asia; Indian Ocean ↔ SE Asia). There is no direct continental shelf connection between Australasia and the Northern/Western Indian Ocean, hence the "ordered" model evaluates the hypothesis that sea snakes (with the possible exception of the pelagic, planktonic *H. (Pelamis) platurus*) moving between these regions must generally pass through SE Asia. In all models, a posterior probability of > 0.7 for a region for a node was considered as strong support. These analyses used Markov-Chain Monte Carlo to sample reconstructions in proportion to their probability and recorded the exact number of each of the six dispersal events in each sampled reconstruction (inferring event numbers using consensus node reconstructions will underestimate events if there are often multiple events along single long branches). In addition to using BEAST to comparing these four event-specific models under a Random Local clock, we also tested the fit of these four models in BAYESTRAITS (Pagel *et al.*, 2004), assuming a uniform dispersal rate across lineages (BAYESTRAITS does not implement a RLC to accommodate lineage-specific dispersal rates).

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

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

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

RESULTS

Phylogeny and divergence time estimates

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

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

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

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

Dispersal Dynamics and Ancestral Area Reconstruction

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

All three AAR methods (Bayesian, parsimony, DEC: Figs. 1, 2, S3 in Appendix S1) recover an Australasian origin (MRCA) for viviparous sea snakes, approximately 6.9 Ma. Similarly, all three analyses indicate that the *Aipysurus* group also originated in Australasia, and subsequently diverged mostly within this region. Parsimony, Bayesian and DEC analyses support an Australasian origin for the two semi-aquatic lineages. BEAST analyses indicated (probability = 0.73) a SE Asian origin for the MRCA of the core *Hydrophis* group, which accounts for *c.* 75% of extant species richness. Parsimony and DEC analyses are consistent with either an Australasian or SE Asian origin for this group. DEC analysis estimated an overall dispersal rate of 0.156 events per lineage per Mya (and an extinction probability of 0.016 per Myr) whereas BEAST analyses suggest dispersal rates ranging from 0.31-0.34 per lineage per my in most lineages, up to 2.38 in *H. platurus*.

DISCUSSION

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

Divergence times, sea snake speciation and sea level changes

Our findings are consistent with previous studies that showed an accelerated rate of speciation in the core *Hydrophis* radiation, with other viviparous sea snakes and their terrestrial sister groups having a slower background rate (Sanders *et al.*, 2010). The recency of many speciation events is consistent with Pleistocene vicariance. The dated tree (Fig. S2 in Appendix S1) suggests that the majority of speciation events in both the *Aipysurus* lineage and the core *Hydrophis* group have occurred since *c.* 3 Ma; this is also broadly consistent with corrected pairwise genetic (*Cyt-b*) distances between sister lineages, which are typically < 6%, even for sister lineages spanning different oceans (see Table 1). Cyclic sea level changes that generated and dissolved barriers to dispersal between marine basins during the last 2.5 million years in the Indo-Australian Archipelago (IAA) (Voris, 2000; Lambeck *et al.*, 2002) are believed to have facilitated speciation of marine fauna via vicariance in isolated marine basins (e.g. De Bruyn & Mather, 2007; Crandall *et al.*, 2008). This may have been especially effective in sea snakes given that they are viviparous and thus lack the highly dispersive, planktonic larval stage that is expected to facilitate gene flow and population connectivity in many marine taxa.

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

Historical Biogeography of Indo-Pacific sea snakes

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

The best-fitting model evaluated in BEAST AAR favoured lineage-specific dispersal rates, and permitted dispersal only between adjacent regions (Table 2). This best-fitting model implies no significant bias in direction of dispersal: thus, contrary to predictions of the overlap or accumulation models, taxa are not more likely to disperse into, rather than out of, SE Asia and thus the IAA. Viviparous sea snakes therefore provide little support for the ‘region of accumulation hypothesis': there are few instances of peripheral speciation followed by subsequent recolonisation of SE Asia. Peripheral speciation is here identified as cladogenesis where one of the two resultant lineages is inferred to have (primitively) a SE Asian distribution and the other lineage to have (primitively) an external (Australasian or Indian Ocean) distribution. Across the entire tree, nodal reconstructions from BEAST, Parsimony, and DEC analyses indicated two such speciation events between Australasia and SE Asia (*A. mosaicus*-*A. eydouxii* and within *H. curtus*) and six such events between the Indian Ocean and SE Asia (*H. ornatus*-*H. lamberti* and within *M. gracilis, H. caerulescens, H. curtus, H. schistosus,* and *H. viperinus*) (Fig. 1, 2, 3 & S3 in Appendix S1). These findings support a role of geographic/historical isolation at the periphery of the IAA in generating overall species/genetic diversity (Ladd, 1960). However, these events do not increase diversity in SE Asia (i.e. the IAA): the ancestral lineage of each species pair is inferred to be from SE Asia, the peripheral speciation event thus adds a new species to the diversity in the adjacent area (Australasia or Indian Ocean), but there is no evidence of secondary range expansion of these extralimital species back into SE Asia.

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

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

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

According to the center of refuge model, the proximity to stable habitats during Quaternary glacioeustatic sea-level changes (Voris, 2000; Woodruffe, 2003) was a major determinant of species survival, enabling recolonisation of unstable shallow water habitats through exportation from the source (Pellissier *et al.*, 2014). Distance to stable habitats (source populations) might be especially important for the maintenance of sea snake diversity in peripheral marine habitats due to their limited dispersal capabilities and reliance on shallow water habitats. Indeed, the most severe known local extinctions of sea snakes have occurred in the very remote Timor Sea reefs (Lukoschek *et al.*, 2013). This scenario is harder to evaluate with molecular trees, as the prime driver (elevated extinction outside biodiversity hotspots) is difficult to estimate using living species alone (Rabosky, 2010). However, some of our patterns discussed above as being consistent with the centre of origin model would also fit the centre of refuge model.

An evolutionary history where taxa which leave the IAA are rapidly "pruned" by extinction would generate a phylogeny where most (inferred) speciation events are in the IAA, all the oldest clades are in the IAA, with subsequent and recent colonisation of the Indian Ocean and Australasia. As discussed above, most (inferred) speciation events are in the IAA, and the core *Hydrophis* lineage has its ancestral distribution in the IAA, and with multiple subsequent colonisations of the Indian and Australasian regions (Fig. 1). The broadly similar phylogenetic patterns expected by the "centre of origin" and "centre of refuge" models make them difficult to distinguish. However, the latter model would predict similar speciation rates and high diversity for all old clades (regardless of refuge region). The sea snake phylogeny here suggests long-term persistence (by itself) is not sufficient to generate high diversity, as the three most basal clades of sea snakes each have even longer inferred histories than the core *Hydrophis* group (though in the Australasian region: Fig. 1), yet have each attained only low to moderate diversity. However, huge phylogenies (several hundred taxa: (Goldberg *et al.*, 2011; Davis *et al.*, 2013) are required to properly tease apart the effects of elevated in-situ speciation versus higher extralimital extinction; this is in excess of the available species diversity of many relevant clades (including sea snakes, < 70 species).

Caveats

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

Conclusions

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

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

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

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SUPPORTING INFORMATION

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

Appendix S2: Details of the specimens, voucher numbers and the respective Genbank accession numbers used in the molecular phylogenetic analysis

Appendix S1: Supplementary Materials and Methods and supplementary Figures

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

BIOSKETCHES

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

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

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

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

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

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

A: BEAST (variable rates across lineages, ordered)

From \ To	**Australasia**	**SE Asia**	**Indian Ocean**
Australasia	-	17.1 (9)	*
SE Asia	18.2 (5)	-	17.7 (4)
Indian Ocean	*	11.6 (13)	-

B: Parsimony (ordered)

From \ To	**Australasia**	**SE Asia**	**Indian Ocean**
Australasia	-	5	*
SE Asia	4	-	7
Indian Ocean	*	1	-

C: Lagrange (ordered, interspecific events only)

From \ To	**Australasia**	**SE Asia**	**Indian Ocean**
Australasia	-	4	*
SE Asia	3	-	2
Indian Ocean	*	0	-

D: Fit of alternative dispersal models in BEAST

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

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

[+]The preferred model (number 4 in main text) assumes variable dispersal rates across lineages (RLC), and a common rate for all dispersal types, and also that dispersals are only possible between adjacent regions (i.e. ordered). See Appendix S1 for full description.

Figure legends

Fig. 1 Time-calibrated tree of viviparous sea snakes, with Bayesian (BEAST) ancestral area reconstructions. Time scale is in million years before present. Colours of the branches indicate the ancestral area reconstructions and correspond to the biogeographic/ancestral regions shown in map (Red: Indian Ocean (IO), Green: SE Asia (SEA), Blue: Australasia). Pie charts depict the relative posterior probability of the alternative ancestral areas for each node (WP - West Pacific, includes both SE Asia and Australia). See Fig. S1 for clade support values.

Fig. 2 Time-calibrated tree of viviparous sea snakes, with parsimony ancestral area reconstructions. Time scale is in million of years before present. Colours of the branches indicate the most parsimonious ancestral area reconstructions for the node at the younger end, and correspond to the biogeographic/ancestral regions shown in map (Red: Indian Ocean, Green: SE Asia, Blue: Australasia). Two colours (e.g. in the core *Hydrophis* branch) indicate 2 equally-parsimonious reconstructions. See Fig. S1 for clade support values.

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

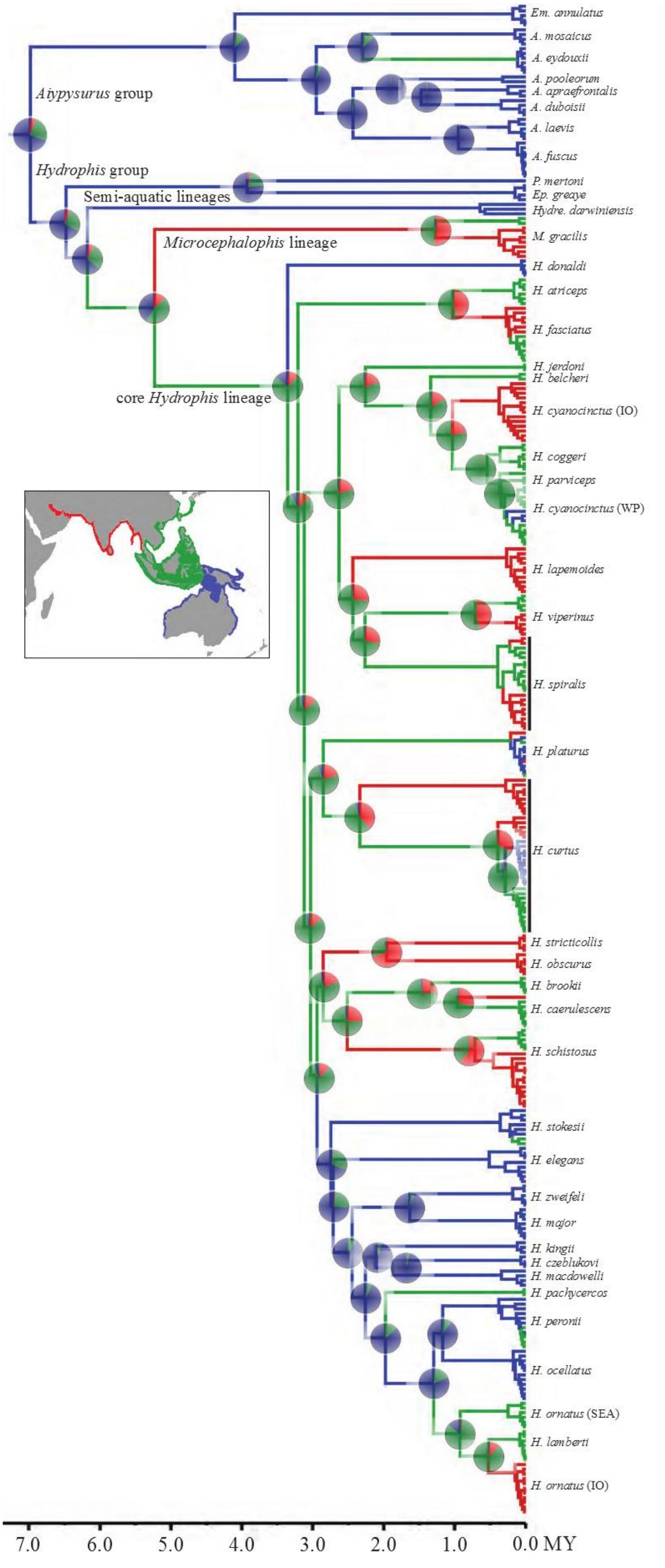
Aiypysurus group
Hydrophis group
Semi-aquatic lineages
Microcephalophis lineage
core Hydrophis lineage
Em. annulatus
A. mosaicus
A. eydouxii
A. pooleorum
A. apraefrontalis
A. duboisii
A. laevis
A. fuscus
P. mertoni
Ep. greaye
Hydre. darwiniensis
M. gracilis
H. donaldi
H. atriceps
H. fasciatus
H. jerdoni
H. belcheri
H. cyanocinctus (IO)
H. coggeri
H. parviceps
H. cyanocinctus (WP)
H. lapemoides
H. viperinus
H. spiralis
H. platurus
H. curtus
H. stricticollis
H. obscurus
H. brookii
H. caerulescens
H. schistosus
H. stokesii
H. elegans
H. zweifeli
H. major
H. kingii
H. czeblukovi
H. macdowelli
H. pachycercos
H. peronii
H. ocellatus
H. ornatus (SEA)
H. lamberti
H. ornatus (IO)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0 MY

Figure 1

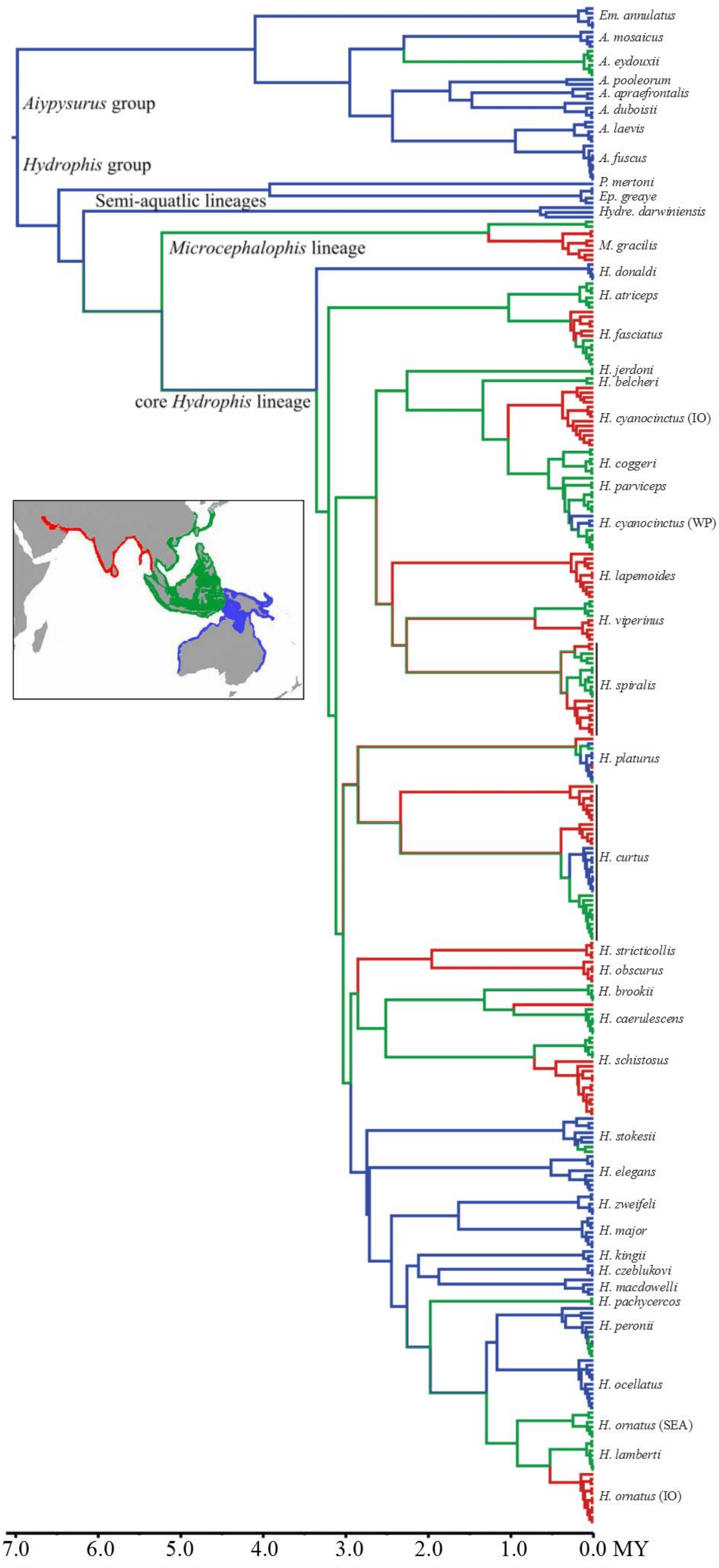
Em. annulatus
A. mosaicus
A. eydouxii
A. pooleorum
A. apraefrontalis
A. duboisii
A. laevis
A. fuscus
Aiypysurus group
Hydrophis group
Semi-aquatlic lineages
P. mertoni
Ep. greaye
Hydre. darwiniensis
Microcephalophis lineage
M. gracilis
H. donaldi
H. atriceps
H. fasciatus
core *Hydrophis* lineage
H. jerdoni
H. belcheri
H. cyanocinctus (IO)
H. coggeri
H. parviceps
H. cyanocinctus (WP)
H. lapemoides
H. viperinus
H. spiralis
H. platurus
H. curtus
H. stricticollis
H. obscurus
H. brookii
H. caerulescens
H. schistosus
H. stokesii
H. elegans
H. zweifeli
H. major
H. kingii
H. czeblukovi
H. macdowelli
H. pachycercos
H. peronii
H. ocellatus
H. ornatus (SEA)
H. lamberti
H. ornatus (IO)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0 MY

Figure 2

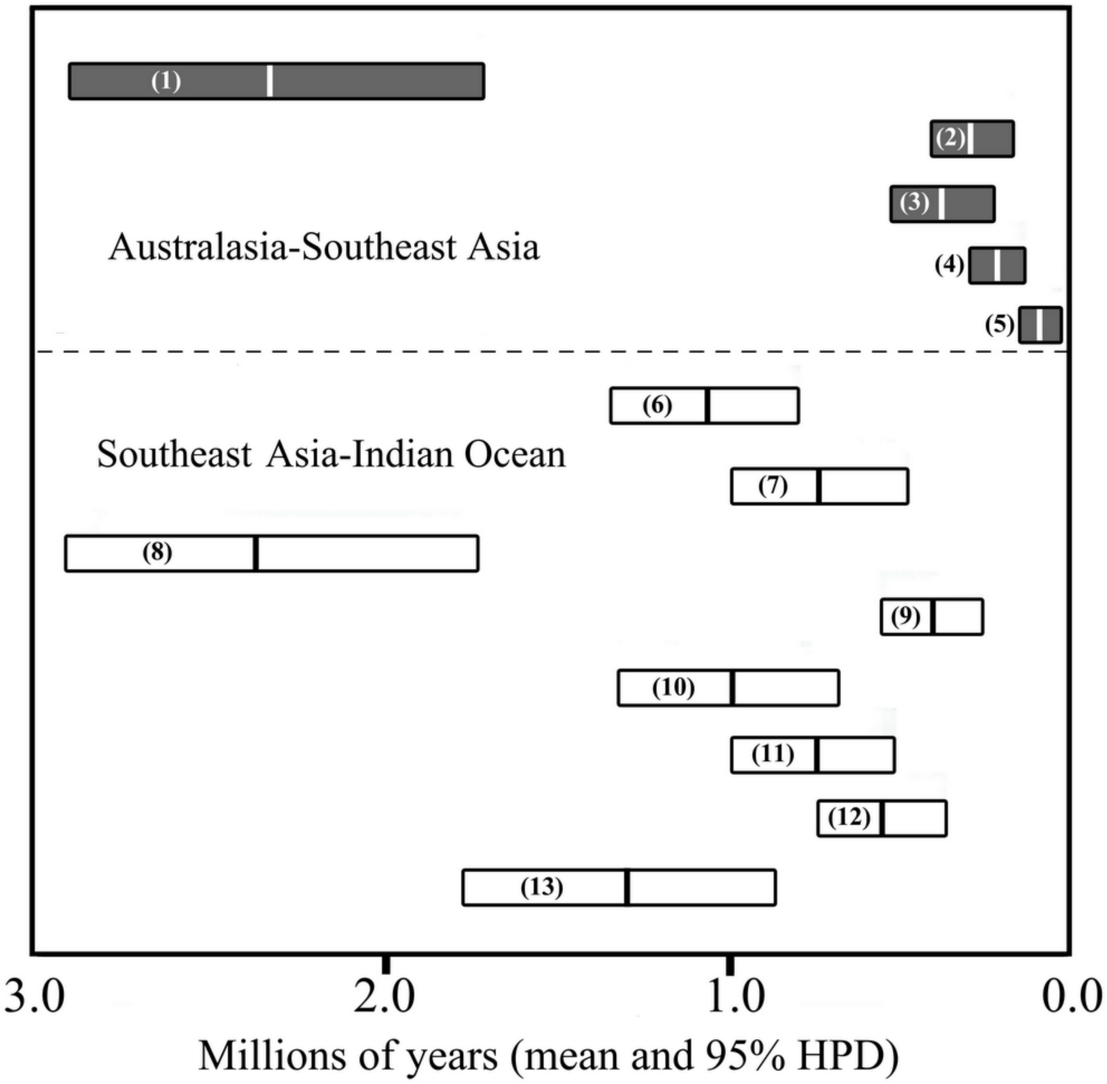
(1)
(2)
(3)
(4)
(5)
Australasia-Southeast Asia
(6)
Southeast Asia-Indian Ocean
(7)
(8)
(9)
(10)
(11)
(12)
(13)
3.0
2.0
1.0
0.0
Millions of years (mean and 95% HPD)

Figure 3