



# Sun skink diversification across the Indian–Southeast Asian biogeographical interface

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## ABSTRACT

**Aim** Widespread, transcontinental vertebrate groups represent ideal systems for biogeographical studies, because they can shed light on a wide range of questions relating to species diversification across the geographical template. We combined extensive geographical and genetic sampling from across multiple biogeographical realms to examine the timing and location of diversification in Asian sun skinks, a clade characterized by problematic species boundaries and a particularly enigmatic evolutionary history.

**Location** Indian subcontinent, the Philippines, Southeast Asia and Sundaland.

**Methods** We sequenced one mitochondrial and nine nuclear genes for most species in the genus *Eutropis*, and estimated phylogenetic relationships and divergence times using coalescent methods. To investigate the location of diversification events, we also estimated ancestral geographical ranges using several methods. Finally, we explored patterns of genetic diversity within several poorly understood, but widely distributed species.

**Results** Divergence-time estimates indicate that *Eutropis* began to diversify during the Eocene. Biogeographical reconstructions show that species diversification was associated with dispersal into three biogeographical realms: India, Sundaland and the Philippines.

**Main conclusions** The results of this study clarify several questions related to the evolutionary history of *Eutropis*, and place them in the context of classic Southeast Asian biogeography. Our study represents one of the first to compile a heavily sampled multilocus dataset ranging across international boundaries in southern Asia that have historically prevented a unified understanding of biogeographical and evolutionary processes involving the Indian subcontinent, mainland southern Asia and the island archipelagos of Southeast Asia.

## Keywords

Biogeographical range evolution, divergence time, *Eutropis*, India, Philippines, phylogenetics, Sundaland.

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## INTRODUCTION

Given the complex geography (Hall, 1998; Yumul *et al.*, 2009), historical climatic fluctuations (Heaney, 1991; Woodruff, 2010) and remarkably high concentration of land vertebrate biodiversity (Myers *et al.*, 2000) in Southeast Asia, the region is an ideal setting for investigating species diversification and biogeographical patterns (Lohman *et al.*, 2011; Brown *et al.*, 2013). In many cases, researchers have found multiple, codistributed taxa that exhibit shared biogeographi-

cal patterns, the examination of which has yielded insight into the historical processes that may drive species diversification (e.g. Heaney, 1986; Woodruff, 2003; Brown & Diesmos, 2009). However, empirical studies include numerous exceptions to predictions of common mechanisms (e.g. Brown & Guttman, 2002; Esselstyn *et al.*, 2009; Lim *et al.*, 2011).

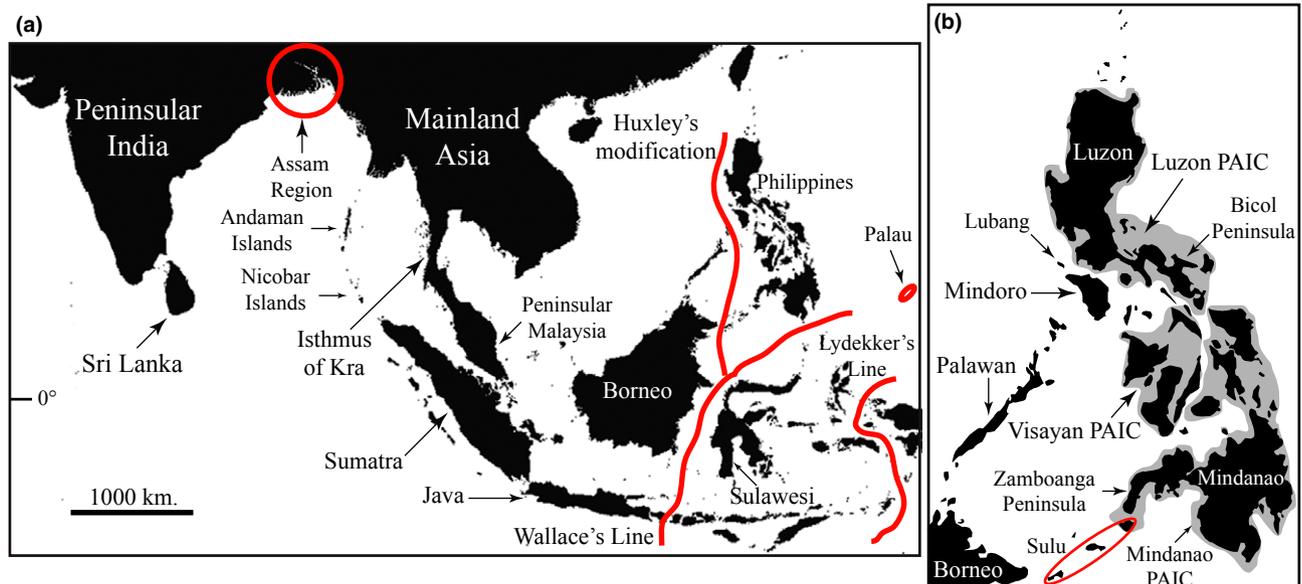
The study of Southeast Asian biogeography has often focused on identifying how regional differences in geological and environmental factors affect species composition across this large geographical area (Turner *et al.*, 2001; Corlett,

2009; Woodruff, 2010). Initially, a number of studies that were focused on species distributions led to the identification of celebrated biogeographical barriers that were hypothesized to explain species distribution patterns (e.g. Wallace, 1860; Huxley, 1868; Lydekker, 1903; van Steenis, 1950; Mani, 1974). Within Southeast Asia, the Philippines has long been identified as a unique biogeographical realm, in part because the majority of the archipelago has never been connected to mainland Asia as other island groups were in the recent past, and is therefore primarily considered a true ‘oceanic island archipelago’ (Yumul *et al.*, 2009). Studies of Philippine biogeography have often focused on routes of dispersal into the Philippines, patterns of colonization within the archipelago and how regional faunal composition patterns have been affected by changes in island connectivity due to sea level fluctuations (e.g. Inger, 1954; Diamond & Gilpin, 1983; Brown *et al.*, 2013). The Indonesian and Malaysian Archipelagos make up a large proportion of the Sundaland biogeographical region, and portions were connected to mainland Asia as part of the Sunda Shelf when sea levels were lower, most recently in the late Pleistocene. Many studies have focused on identifying regions of faunal turnover between Sundaland and Wallacea (which are separated by deep-water ocean channels), as these regions represent a zone of transition and mixing between the Oriental and the Australian faunas (e.g. Evans *et al.*, 2003; Stelbrink *et al.*, 2012). Another important region of faunal turnover that has been extensively studied is the transition between the Sundaic and the Indochinese biogeographical regions, often demarcated by the Isthmus of Kra (Fig. 1; Parnell, 2013). India has also consistently been identified as a unique Asian biogeographical region, so much so that it has often been left out of

studies of Asian biogeography (Briggs, 1989; Meijaard & Groves, 2006). Although many clades are shared across these different biogeographical regions, India is arguably the most distinct, harbouring high levels of endemism (e.g. Mani, 1974; Kamei *et al.*, 2012).

Widespread taxonomic groups are ideal systems for testing biogeographical hypotheses, because they offer the opportunity to examine how geographical barriers, as well as dispersal and colonization, affect diversification and determine biogeographical patterns of distribution (e.g. Irschick *et al.*, 1997; Setiadi *et al.*, 2011; Linkem *et al.*, 2013). Although studies of such groups are important for understanding the general biogeographical history of a region, few clades contain both geographically widespread species and narrowly distributed microendemic taxa (the cat-eyed snakes of the genus *Boiga* may represent one example in Southeast Asia; Greene, 1989). Additionally, logistical obstacles to research across international boundaries have historically made the study of widespread Southeast Asian vertebrate taxa problematic (e.g. Brown *et al.*, 2012; Datta-Roy *et al.*, 2013).

*Eutropis* is a species-rich genus of skinks that occur throughout tropical Asia (Taylor, 1922; Mausfeld & Schmitz, 2003; Das, 2004; Grismer, 2011). The group is an intriguing evolutionary radiation, both in terms of its large geographical distribution and the fact that it has not diversified substantially from an ecological perspective, as most species appear to occupy similar ecological niches. Although some previous studies have investigated coarse biogeographical patterns in *Eutropis* (Mausfeld & Schmitz, 2003; Whiting *et al.*, 2006; Das *et al.*, 2008; Skinner *et al.*, 2011; Datta-Roy *et al.*, 2012), poor taxonomic sampling, a paucity of data from nuclear markers and the presence of cryptic species diversity (Barley



**Figure 1** Map of (a) tropical Asia and (b) the Philippines encompassing the range of *Eutropis*. The map illustrates several important biogeographical features discussed in this study (red lines). Grey areas indicate exposed land within the 120-m isobath that formed the aggregate island complexes, when sea levels were lower during Pleistocene glacial periods.

*et al.*, 2013) have prevented a comprehensive understanding of the evolutionary history and patterns of diversification in this clade.

Many *Eutropis* lineages appear to be capable of long-distance dispersal across oceanic barriers, because they presently inhabit extensive geographical ranges that span well-known biogeographical barriers between major geographical and faunal realms (Kuhl, 1820; Hallowell, 1857; Grismer, 2011; Holt *et al.*, 2013). The pattern of large geographical ranges in selected *Eutropis* species may be indicative of this radiation being composed primarily of habitat-generalist species that may be capable of surviving in a wide range of ecological and climatic environments. In contrast, a number of *Eutropis* species are also restricted to very small geographical ranges (Smith, 1935; Brown & Alcalá, 1980; Bobrov, 1992; Barley *et al.*, 2013).

In this paper, we employ geographically extensive taxonomic sampling (including numerous individuals from across the ranges of the widespread taxa), and data from multiple genetic markers, to investigate the timing of diversification and biogeographical patterns within *Eutropis*. In doing so, we relate the biogeographical patterns to the complex geography of tropical Asia in an attempt to understand the processes leading to speciation in this group. We also employ our phylogenetic estimate to infer the routes by which sun skinks colonized the different biogeographical regions of tropical Asia, and consider these results in the context of previously articulated Southeast Asian biogeographical hypotheses.

## MATERIALS AND METHODS

### Taxonomic and genetic sampling

We obtained genetic samples for 20 of the 30 described species of *Eutropis*, including at least three individuals per species whenever possible. Because several previous studies have indicated that cryptic and undescribed species diversity is likely to be present in multiple species complexes (Mausfeld & Schmitz, 2003; Das *et al.*, 2008; Barley *et al.*, 2013), we included multiple individuals from diverse sampling localities in an effort to include as many suspected species as possible (see Appendix S1 in Supporting Information for details of sampling). As outgroups, we also included species from three genera that appear to be closely related to *Eutropis* (*Dasia grisea*, *Trachylepis perrotetii* and *Mabuya mabouya*), as well as a more distantly related species, *Emoia atrocostata* (Mausfeld & Schmitz, 2003; Datta-Roy *et al.*, 2012).

Genomic DNA was extracted, amplified and sequenced using the methods described in Barley *et al.* (2013). We sequenced one mitochondrial gene and seven nuclear genes that have been previously shown to be informative in studies of *Eutropis*: nicotinamide adenine dinucleotide dehydrogenase subunit 2 (*ND2*), ATP synthetase-B subunit (*ATPSB*), selenoprotein-T (*SELT*), N-acetyltransferase 15 (*NAT15*),

nitric oxide synthase 1 (*NOS1*), forkheadbox P2 (*FOXP2*) and L-lactate dehydrogenase M chain (*LDHA*) (see Barley *et al.*, 2013). We also sequenced the RNA fingerprint protein 35 (*R35*; Leaché, 2009), the melanocortin receptor 1 (*MC1R*) gene (Pinho *et al.*, 2010), and the ribosomal protein 40 (*RP40*) gene (Friesen *et al.*, 1999). The following redesigned primers were used for *RP40*: *RP40.F* 5'-ATGTGGTGGATG YTGCTCGTGAAGTC-3' and *RP40.R* 5'-GCTTCTCA GCWGCRCGCTGCTC-3'. Sequences were edited and subsequently aligned using MAFFT in GENEIOUS PRO 5.3 (Katoh *et al.*, 2005). All sequence data were deposited in GenBank (see Appendix S1 for details). The alignments were visually examined, translated along coding regions as a check for stop codons in protein-coding genes, and models of molecular evolution were selected using decision theory, as implemented in DT-MODEL (Minin *et al.*, 2003).

### Phylogenetic analyses

We estimated phylogenetic relationships using several model-based methods. Maximum-likelihood (ML) phylogenetic analysis was performed for each gene individually and a partitioned, concatenated analysis was run for the full 10-gene dataset using RAxML 7.03 (Stamatakis, 2006), with each partition being assigned a GTR+ $\Gamma$  model and with support assessed via 1000 bootstrap replicates. A partitioned, concatenated Bayesian phylogenetic analysis of the full dataset was performed using MRBAYES 3.2.1 (Ronquist *et al.*, 2012) utilizing two replicates with four chains each for 20 million generations, sampling every 2000 generations, and discarding samples from the first 5 million generations as burn-in. Convergence was assessed by confirming that all parameters had reached stationarity and had sufficient effective sample sizes (> 200) using TRACER 1.4 (Rambaut & Drummond, 2007), and topological convergence was assessed using AWTY (Wilgenbusch *et al.*, 2004; Nylander *et al.*, 2008). In the Bayesian phylogenetic analyses, we first attempted to partition the protein-coding genes by codon position. The analyses were, however, unable to appropriately sample the posterior for some of the associated parameters in some partitions because of the small number of sites included (poor mixing resulted in low effective sample sizes). The dataset was therefore partitioned by gene for all analyses.

Our goal was to obtain a temporal framework for examining biogeography and diversification in *Eutropis*, and to use a coalescent species-tree model to estimate the phylogeny in a simultaneous analysis. Unfortunately, no fossil calibration points exist for taxa within the genus or closely related species, and the ages of islands in Southeast Asia only provide maximum bounds for colonization times, with no minimum bounds. As a rough approximation of the general temporal framework for diversification, we therefore employed a mitochondrial DNA (mtDNA) substitution rate derived from closely related taxa to time-calibrate our tree. Although these limitations prevent precise divergence-dating, using a molecular rate for calibration can provide a rough estimate

of the divergence times of the major radiations within the genus (Caccone *et al.*, 1997; Calsbeek *et al.*, 2003; Rabosky *et al.*, 2007; Linkem *et al.*, 2013).

We performed divergence time estimation with an uncorrelated relaxed lognormal clock in BEAST 1.7.5 (Drummond & Rambaut, 2007), using the species tree ancestral reconstruction (\*BEAST; Heled & Drummond, 2010). Each gene was assigned a separate unlinked relaxed clock model in the analysis. For the *ND2* data, we used a 95% normal distribution prior (with a mean of 0.00895 and a standard deviation of 0.0025) as a substitution rate to calibrate our tree. This corresponded to a rate distribution of 0.48–1.31% Myr<sup>-1</sup>, which encompasses the mtDNA rates estimated for several different reptile groups, including skinks (Zamudio & Greene, 1997; Rabosky *et al.*, 2007; Linkem *et al.*, 2013). For the nuclear genes, the clock means were assigned a uniform distribution from 0 to 50 and the standard deviations were assigned exponential distributions with a mean of 0.05. These priors were chosen to allow for substantial rate variation among genes, and to facilitate efficient mixing of the MCMC chain. The species population-mean hyperprior and the species Yule-process birth prior were both assigned exponential distributions, with means of 0.01 and 1.0, respectively. We ran the analysis for 400 million generations, sampling every 32,000 generations, and discarding the first 100 million generations as burn-in. Convergence was assessed using TRACER and AWTY as described above. Sequence alignments and phylogenetic trees generated for this research were deposited in TreeBASE (accession S16117).

### Biogeographical analyses

In order to examine biogeographical range evolution and test hypothesized colonization routes, we used several different methods to infer ancestral states across our species-tree topology. We used a dispersal–extinction–cladogenesis (DEC) ML model implemented in the program LAGRANGE 20130526 (Ree & Smith, 2008), and we employed the ML and rjMCMC (reversible-jump Markov chain Monte Carlo) models of character evolution available in BAYESTRAITS 2.0 (Pagel, 1999; Pagel & Meade, 2006). We reconstructed ancestral states in order to examine both broad-scale biogeography across tropical Asia, and finer patterns within the Philippines. We coded species distributions into four biogeographical subregions: peninsular India and Sri Lanka, mainland Southeast Asia (starting from the Assam region of India and including Peninsular Malaysia), Sundaland (excluding the Malay Peninsula) and the Philippines. Although these regions share certain faunal elements and some level of connectivity exists among them, we chose to partition tropical Asia in this way because: (1) significant geographical barriers separate each of these four regions; (2) previous biogeographical studies have shown each of these regions to harbour endemic radiations of species (Wallace, 1876; Mani, 1974; Corlett, 2009; Woodruff, 2010; Datta-Roy *et al.*, 2012, 2014); (3) these regions separate the distributions of many of the species and major clades within

*Eutropis*; and (4) many biogeographical range evolution models perform best when a small number of areas are considered in the analysis (Ree & Smith, 2008). For analyses of the endemic Philippine radiation, we divided the islands into four regions based on geography and the Pleistocene aggregate island complex (PAIC) model (Inger, 1954; Heaney, 1985; Brown & Diesmos, 2009): (1) the Luzon PAIC + Mindoro, Lubang and Semirara; (2) the Mindanao PAIC; (3) the Visayan PAIC; and (4) Palawan and Borneo.

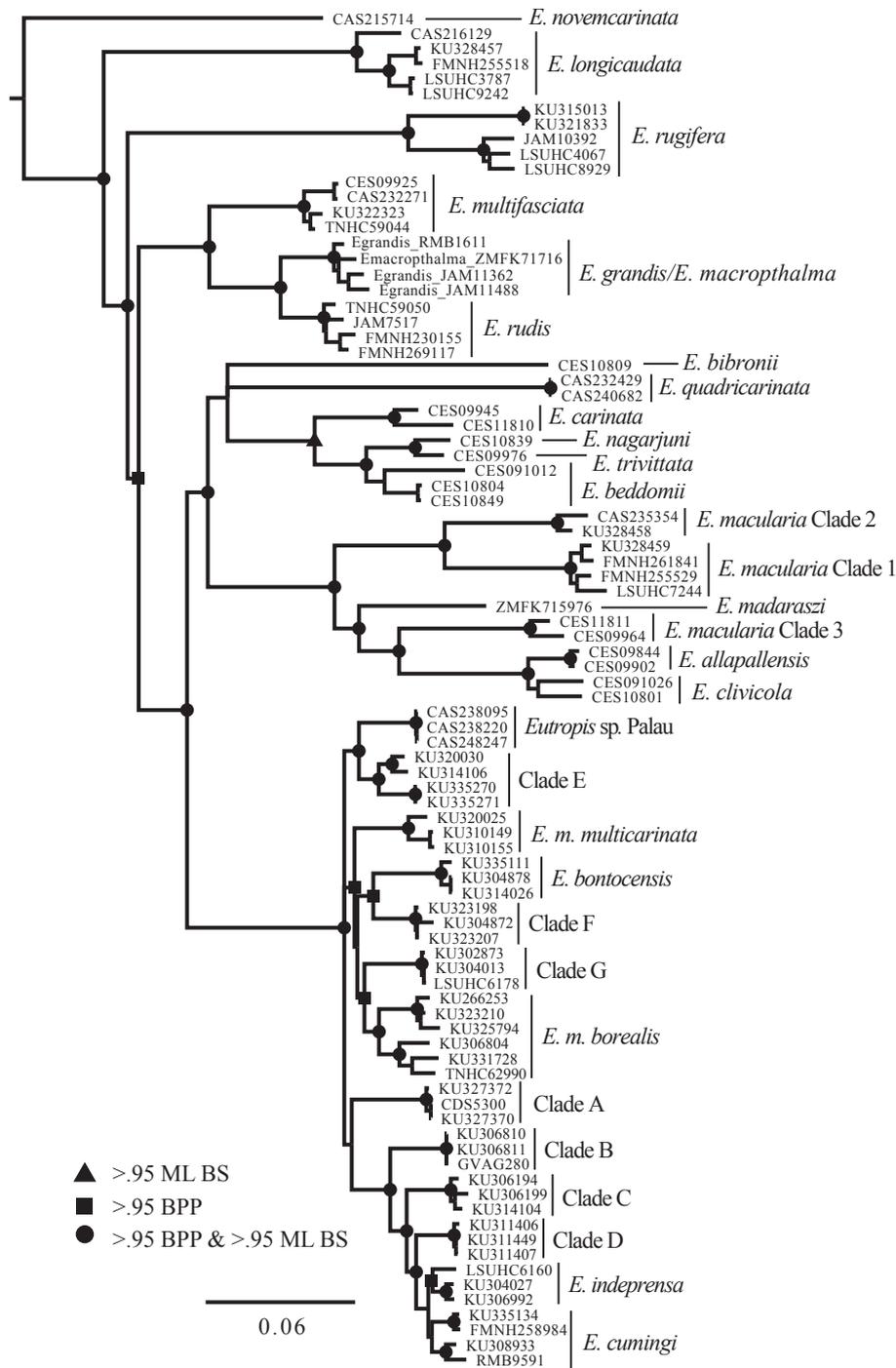
For the LAGRANGE analyses, we configured our analyses using the LAGRANGE CONFIGURATOR (beta) 20130526 web tool (available at: <http://www.reelab.net/lagrange/>), setting the dispersal rates between India and the Philippines to be half of that between the other areas, as those were the only two areas not adjacent to each other. We initially ran several unconstrained BAYESTRAITS analyses where free dispersal parameters were estimated between all areas and in both directions. In these analyses, estimates of the dispersal rate parameters between some areas were, however, unrealistically high (e.g. between India and the Philippines, where direct dispersal between regions is not possible). We subsequently adopted a parameterization that was a more biologically realistic reflection of biogeography and dispersal in this system. For analyses of the entire genus, we estimated two free dispersal parameters: one for dispersal rates between India and the Philippines, and one for dispersal rates between the remaining areas. For the BAYESTRAITS analyses of the Philippine lineages, we estimated six free dispersal parameters, representing bidirectional dispersal rates between each of the four geographical regions. The rjMCMC analyses were run for  $1 \times 10^8$  generations, sampling every 25,000 generations, and discarding samples from the first  $5.0 \times 10^7$  generations as burn-in. Stationarity of all model parameters was confirmed using TRACER.

### Genetic structure in widely distributed species

We also sought to make a preliminary characterization of the genetic diversity across the ranges of the biogeographically widespread *Eutropis* species. To do this, we sequenced the *ND2* gene from a larger sampling of individuals for *E. longicaudata*, *E. macularia*, *E. multifasciata*, *E. rudis* and *E. rugifera* from a diverse range of localities. We then used these data to calculate several population genetics summary statistics using DNASP 5.10.1 (Librado & Rozas, 2009) for each species.

## RESULTS

Our final dataset used for the phylogenetic analyses consisted of 1016 base pairs (bp) of mitochondrial data (*ND2*) and 8094 bp of nuclear data from nine different nuclear genes: *ATPSB* (1293 bp), *FOXP2* (671 bp), *LDHA* (589 bp), *MC1R* (661 bp), *NAT15* (779 bp), *NOS1* (1685 bp), *R35* (664 bp), *RP40* (380 bp) and *SELT* (1372 bp) for 94 individuals. Our data matrix was mostly complete, with only 5.4% missing

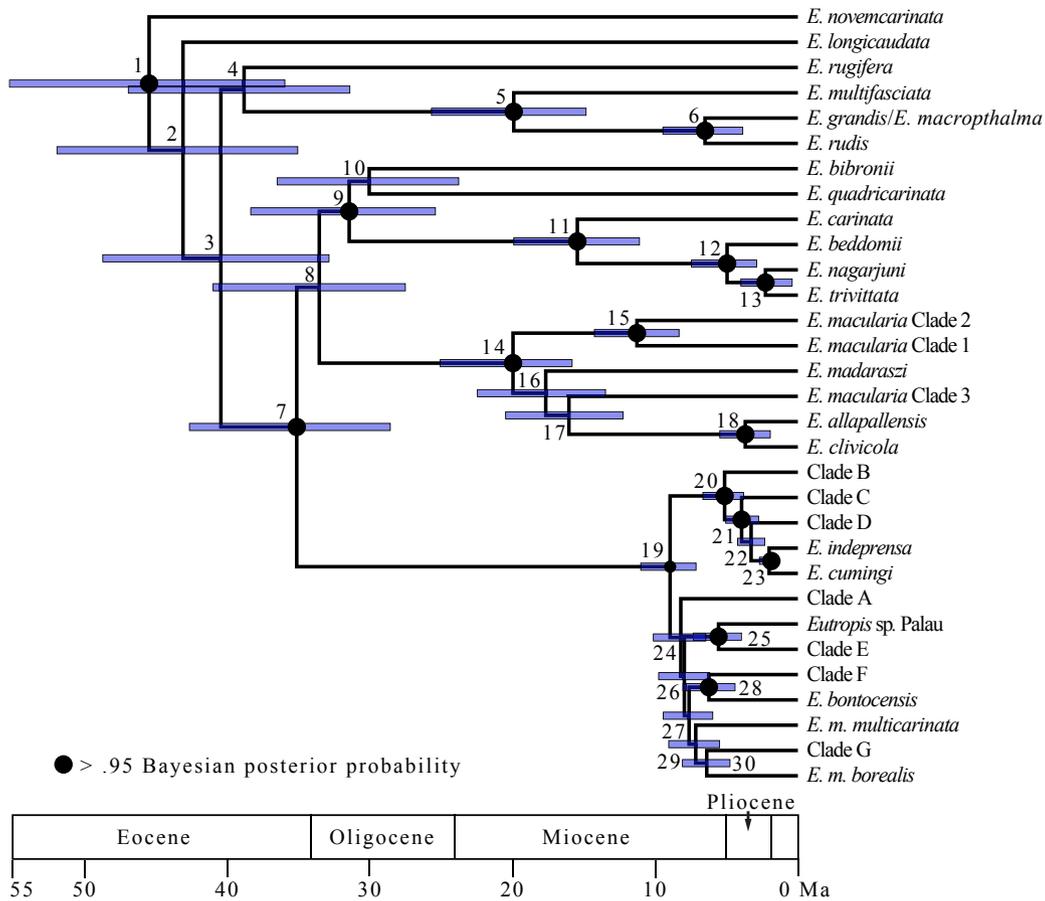


**Figure 2** Majority-rule consensus tree from concatenated, Bayesian phylogenetic analysis of Asian *Eutropis*. Specimen numbers are shown adjacent to species identities (see Appendix S1 for specimen information; outgroups have been pruned from figure to ease visualization). *Eutropis macularia* clades 1 and 2 consist of individuals from populations in mainland Southeast Asia; Clade 3 represents populations from peninsular India. ML BS, maximum likelihood bootstrap support; BPP, Bayesian posterior probabilities. Scale bar represents number of substitutions per site.

data (see Appendix S1 for details). We tested for saturation of the *ND2* data using *DAMBE* (Xia *et al.*, 2003), which indicated no substantial evidence for saturation in the data.

The concatenated phylogenetic analyses identified two cases in which populations of several species were strongly supported as non-monophyletic (*E. grandis* and

*E. macrophthalma*, as well as populations of *E. macularia* from India and mainland Southeast Asia; Fig. 2). In order to accommodate this problematic taxonomy, we designated species identities for these individuals in the coalescent-based species tree analysis based on their appropriate genetic clade identified in the concatenated-data analyses.



**Figure 3** Maximum clade credibility tree from the combined species tree and divergence-time-rooted phylogenetic analysis of Asian *Eutropis*. Confidence intervals show 95% highest posterior density for divergence times based on mtDNA substitution rate. Node numbers correspond to those in Table 1 showing divergence-time confidence intervals.

Our time-tree analysis (Fig. 3) reveals that the earliest-diverging clades consist of species with geographical ranges across mainland Southeast Asia. We also recovered a clade consisting primarily of taxa endemic to India, another Sundaic clade consisting of *E. rugifera*, *E. multifasciata*, *E. grandis* / *E. macrophthalma* and *E. rudis*, and a third group of species from the Philippines (+ Palau) (Fig. 3). Our species-tree analysis using \*BEAST is likely to be our best estimate of phylogenetic relationships within *Eutropis*, because (1) we noted evidence of gene-tree heterogeneity among loci (see Appendix S2), and (2) previous studies have shown that coalescent analyses are more appropriate and perform better than concatenation in many situations when analysing multilocus datasets (Kubatko & Degnan, 2007; Liu & Pearl, 2007).

We used the maximum clade credibility tree from our \*BEAST analysis (generated using TREEANNOTATOR 1.7.5) to estimate ancestral states in the biogeographical analyses. Each of the biogeographical range evolution methods produced similar results for both the full phylogeny and the Philippine-focused analyses in terms of general dispersal patterns. Differences were related to the timing of dispersal (i.e. the specific node at which dispersal occurred) into different

biogeographical realms (Table 1, Appendix S3). We focus primarily on the results from the LAGRANGE analyses, because the DEC model can allow for widespread biogeographical ranges encompassing more than one area, and for lineage divergence within a species to occur either between a single area and the remainder of its range, or within an area (Ree & Smith, 2008). Because of this, we feel this model more accurately characterizes biogeographical range evolution in this group (Fig. 4).

Our characterization of genetic diversity within the widespread *Eutropis* species identified several different patterns, with some species exhibiting high genetic divergence between some sampling localities, as well as some species showing little genetic differentiation across large geographical distances (Table 2). Of all the widespread species, *E. multifasciata* exhibits the least geographically based genetic differentiation across its range; we noted little sequence divergence between individuals from adjacent geographical regions and a maximum of c. 8.0% uncorrected pairwise mtDNA sequence divergence across the entire range of this widely distributed species (Table 2). *Eutropis longicaudata* (which is widespread across mainland Southeast Asia) was sampled from populations in Myanmar, Laos,

**Table 1** Divergence-time estimates and biogeographical reconstruction results for Asian *Eutropis*. Node numbers refer to those in Figs 3 & 4; ages are 95% highest posterior densities; DEC (dispersal–extinction–cladogenesis) model results show relative probability for the most likely range inheritance scenario, depicted in Fig. 4; most probable states are shown for BAYESTRAITS analyses with the corresponding maximum likelihood or posterior mean relative probability.

Node	Age (Ma)	DEC model	BAYESTRAITS ML model	BAYESTRAITS rjMCMC model
1	36–55	0.49	Mainland, 0.57	Mainland, 0.53
2	35–52	0.48	India, 0.47	India, 0.39
3	33–49	0.36	India, 0.61	India, 0.49
4	31–47	0.49	Sundaland, 0.60	Sundaland, 0.56
5	15–26	0.13	Sundaland, 0.76	Sundaland, 0.72
6	4–9	0.52	Sundaland, 0.95	Sundaland, 0.94
7	29–43	0.63	India, 0.92	India, 0.76
8	28–41	0.30	India, 0.96	India, 0.93
9	25–38	0.63	India, 0.90	India, 0.84
10	24–37	0.81	India, 0.47	India, 0.43
11	11–20	1.0	India, 0.99	India, 0.97
12	3–7	1.0	India, 1.0	India, 1.0
13	0.4–4	1.0	India, 1.0	India, 1.0
14	16–25	0.98	India, 0.78	India, 0.76
15	8–14	0.99	Mainland, 0.98	Mainland, 0.97
16	13–22	1.0	India, 1.0	India, 0.98
17	12–21	1.0	India, 0.98	India, 0.96
18	2–5	1.0	India, 1.0	India, 1.0
19	7–11	0.10	Luzon, 0.48	Luzon, 0.38
20	4–7	0.16	Visayas, 0.47	Luzon, 0.39
21	3–5	0.16	Luzon, 0.56	Luzon, 0.51
22	2–4	1.0	Luzon, 0.54	Luzon, 0.51
23	1–3	1.0	Luzon, 0.92	Luzon, 0.83
24	6–10	0.51	Mindanao, 0.72	Mindanao, 0.50
25	4–8	1.0	Mindanao, 0.92	Mindanao, 0.73
26	6–10	0.58	Mindanao, 0.52	Luzon, 0.39
27	6–9	0.84	Luzon, 0.74	Luzon, 0.58
28	4–8	1.0	Luzon, 0.63	Luzon, 0.57
29	5–9	0.84	Luzon, 0.55	Luzon, 0.39
30	5–8	0.77	Luzon, 0.39	Luzon, 0.42

ML, maximum likelihood; rjMCMC, reversible-jump Markov chain Monte Carlo.

Thailand and Peninsular Malaysia. Populations from Laos and Thailand exhibited limited genetic divergence, whereas the remaining populations exhibited some geographical structure across the landscape (with uncorrected  $p$ -distances for  $ND2$  of 13–18% among individuals). Other species exhibited greater potential for unrecognized species diversity (Table 2; see below).

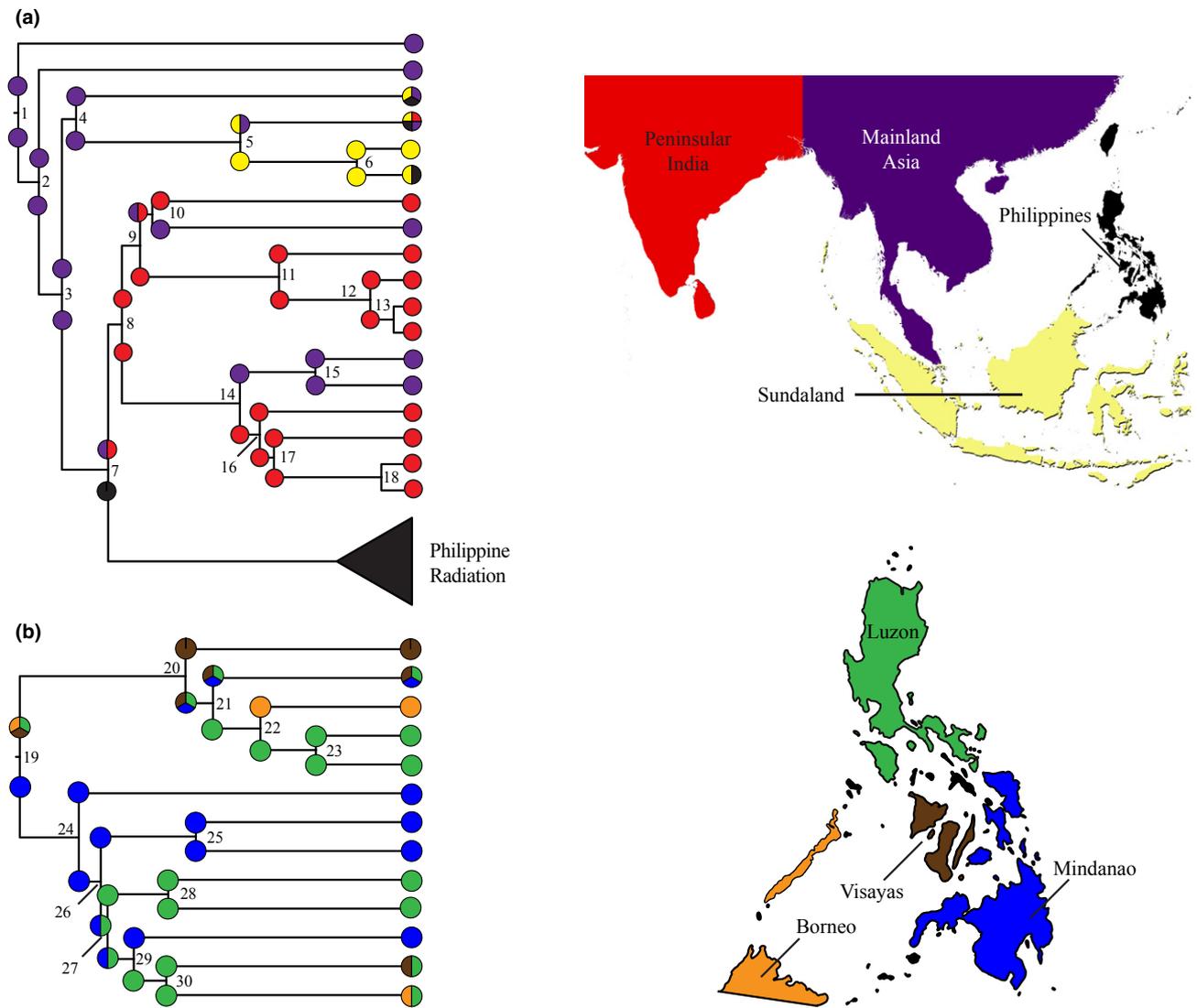
## DISCUSSION

### Biogeography and divergence times

This study provides a robust examination of biogeographical patterns and elucidates the nature of the timing and origin of diversification of *Eutropis* across some of tropical Asia's most celebrated biogeographical boundaries. Our analyses indicate that the initial lineages that gave rise to *Eutropis* began diversifying in mainland Southeast Asia during the Eocene (Fig. 3). Subsequently, the group's geographical distribution diffused outward, with three distinct lineages invading Sundaland, peninsular India and the Philippines

(Fig. 4). This geographical spread of lineages, followed by a subsequent period of diversification gave rise to endemic species in each biogeographical region, and diversification appears to have been most extensive in India and the Philippine archipelago (Fig. 4).

Interestingly, the complex biogeographical patterns exhibited by sun skinks indicate that there are likely to have been extensive back-dispersal events out of – and multiple invasions into – each of the different biogeographical subregions. Our results suggest a dispersal event from mainland Southeast Asia into India relatively early (*c.* 29–43 million years ago, Ma), conceivably around the time modern-day India was colliding with mainland Asia (Aitchison *et al.*, 2007). Two subsequent and independent back-dispersals into mainland Southeast Asia from India are inferred (Datta-Roy *et al.*, 2012), the first of which involved the lineage that gave rise to *E. quadricarinata* (Fig. 4). It also appears that the ancestor of the *E. macularia* species complex was initially distributed in India, and subsequently dispersed back into mainland Southeast Asia. The most likely route of these faunal exchanges would have been through the Assam region of



**Figure 4** Results of biogeographical character-state reconstructions using the DEC (dispersal–extinction–cladogenesis) model in LAGRANGE for (a) the full Asian *Eutropis* phylogeny and (b) the Philippine radiation. The biogeographical regions are coded on the associate maps. The maximum clade credibility tree from the species tree analysis was used in the LAGRANGE analyses (shown). The most probable range inheritance scenarios are shown for each node, with multiple colours indicating a distribution across multiple biogeographical realms. Numbers at nodes correspond to those in Table 1 showing relative probabilities. The tips indicate the present-day ranges for each species. Species diversity estimates for each biogeographical region based on current taxonomy, this study, and Barley *et al.* (2013) are as follows: India & Sri Lanka, 13 species; Mainland Asia, 9 species; Sundaland, 6 species; and Philippines + Palau, 17 species.

**Table 2** Summary of sampling localities for several widespread species of *Eutropis* included in study, as well as numbers of polymorphic sites ( $P_N$ ), nucleotide diversity ( $\pi$ ), and ranges of pairwise sequence divergence ( $S$ ) for *ND2* data.  $n$ , number of individuals included;  $D$ , largest geographical distance (km) between populations sampled. See Appendix S1 for full details of sampling.

Species	$n$	Sampling localities	$P_N$	$\pi$	$S$	$D$
<i>E. longicaudata</i>	6	Laos, Malaysia, Myanmar, Thailand, Vietnam	217	0.1136 ± 0.0169	0.4–16.0	2400
<i>E. macularia</i>	11	Cambodia, Laos, Malaysia, Myanmar, Thailand, India	232	0.1332 ± 0.0131	0.6–22.8	2900
<i>E. multifasciata</i>	23	India, Myanmar, Philippines, Sulawesi, Thailand	131	0.0438 ± 0.0049	0.0–7.7	3300
<i>E. rudis</i>	8	Sabah, Sarawak, Sulawesi	93	0.0379 ± 0.0055	0.3–5.9	1000
<i>E. rugifera</i>	7	Malaysia, Philippines, Sarawak, Sulawesi	203	0.1071 ± 0.0143	0.0–15.7	2105

north-eastern India (Mani, 1974; Myers *et al.*, 2000). The dispersal of *Eutropis* into Sundaland appears to have occurred slightly later, and this region appears to contain the lowest *Eutropis* species diversity within the range of the genus (Fig. 4).

Our analyses indicate that the Philippines has been invaded multiple times (with at least two, but perhaps as many as four independent colonizations), but only one of these invasions has led to significant *in situ* diversification (Fig. 4; Barley *et al.*, 2013). Several species from the Sundaland group have colonized the south-western portion of the archipelago more recently, with *E. rugifera* now known only from the Zamboanga Peninsula, and *E. rudis* only from the Sulu Archipelago (Brown & Alcalá, 1980). Lastly, *E. multifasciata* also has successfully colonized the entire archipelago (Brown & Alcalá, 1980). Interestingly, the Philippine radiation is not closely related to any species that currently occur in Sundaland, but is instead sister to a clade consisting of species distributed across India and mainland Southeast Asia (Fig. 3). The Philippine radiation is composed of two species complexes – the *E. multicolorata* species complex and the *E. indeprensa* species complex (Brown & Alcalá, 1980; Barley *et al.*, 2013) – with the split between these two groups appearing to have occurred during the Miocene.

Ancestral-state reconstructions indicate that the ancestor of the *E. indeprensa* species complex may have had a broad distribution across the archipelago (Fig. 4). In contrast, our results show that the ancestor of the *E. multicolorata* species complex was likely to have been distributed on islands that later formed the Mindanao PAIC, and which were then located further south than their present-day position (with the exception of the Zamboanga Peninsula; Yumul *et al.*, 2004, 2009). Lineages in this species complex subsequently dispersed northwards into the Luzon PAIC and diversified (which may have been facilitated by the northward movement of the Bicol Peninsula and its subsequent collision with the Luzon palaeoisland), whereas a long-distance dispersal event into the Palau archipelago also appears to have occurred from Mindanao. The species in this complex ultimately appear to have dispersed from Luzon into the western Visayan Islands (central Philippines), completing an anti-clockwise colonization pattern of the archipelago. The dispersal out of the Philippines into Borneo by several species in this radiation is inferred to have occurred more recently, in one instance by a species in the *E. multicolorata* complex (Clade G; also known from the islands of Lubang and Semirara; Fig. 4). A divergent lineage, most closely related to *E. indeprensa* (from Mindoro; Fig. 2), also occurs on Borneo.

### Phylogeographical patterns in 'widespread species'

Although there are multiple examples of range-restricted *Eutropis* species (Smith, 1935; Brown & Alcalá, 1980; Bobrov, 1992; Barley *et al.*, 2013), several species are considered to be widespread across multiple biogeographical regions. We carried out a preliminary survey of the genetic diversity across

these species' ranges, in order to assess the potential for any unrecognized cryptic species diversity. *Eutropis rugifera* is distributed throughout the Sundaic region of Southeast Asia, in Malaysia and Indonesia, and the Andaman and Nicobar Islands (Das, 2004). Here, we report the first discovery of *E. rugifera* in the Philippines, from the Zamboanga Peninsula of south-western Mindanao in the southernmost Philippines (Figs 1 & 2). Our phylogenetic analysis included specimens of *E. rugifera* from the Philippines, Sulawesi, Peninsular Malaysia and Sarawak, which form a monophyletic clade. The newly discovered Philippine population is, however, highly divergent from the other populations (*c.* 17–18% uncorrected *p*-distance for *ND2*).

One of the most problematic *Eutropis* species-groups taxonomically has been the *E. macularia* species complex. A number of subspecies have been proposed, and a number of populations have been described as distinct species (Blythe, 1853; Schmidt, 1926; Inger *et al.*, 1984; Das *et al.*, 2008). We amassed the largest molecular dataset to date for this species complex, sampling within and across several geographical regions. Our results indicate that the species complex began to diversify between 15 and 25 Ma from an ancestor that was probably distributed in India, and that there was a subsequent dispersal into mainland Asia (Figs 3 & 4). Not surprisingly, our results also indicate that there may be additional cryptic species diversity within the complex, and several described species render some populations of '*E. macularia*' paraphyletic (Fig. 3). Populations of *E. macularia* from mainland Southeast Asia also appear to consist of at least two distinct species, as we found two highly divergent clades (*c.* 23% *p*-distance for *ND2*): one clade consisting of populations from Myanmar and Thailand and another consisting of populations from Thailand, Peninsular Malaysia, Cambodia and Laos (Figs 2 & 3, Table 2). This result, suggesting that multiple species may masquerade within *E. macularia*, is consistent with a previous study that found variation in chromosome number in populations of *E. macularia* from Thailand (Ota *et al.*, 2001).

Our phylogenetic analyses indicate that populations of *E. macrophthalma* (originally described from Java; Mausfeld & Böhme, 2002) and *E. grandis* (originally described from Sulawesi; Howard *et al.*, 2007) exhibit virtually no genetic divergence between these biogeographically distinct islands. There is no mention of populations on Sulawesi in Mausfeld & Böhme (2002) and no comparison of the two species in Howard *et al.* (2007). Thus, it appears these species may represent a single taxon distributed more widely across the Sundaic region than was originally thought; reconsideration of the taxonomic validity of the latter name (*E. grandis*) may be advisable in the near future.

### CONCLUSIONS

Tropical Asia has been the geographical setting for the formulation of many seminal hypotheses in the field of biogeography (e.g. Wallace, 1860; Huxley, 1868; van Steenis, 1950;

Inger, 1954; Diamond & Gilpin, 1983; Briggs, 1989). Thus, consideration of how the biogeographical patterns observed in *Eutropis* fit within a more general context is warranted. Lydekker's Line (Lydekker, 1903), which demarcates the easternmost zone of mixing between the Oriental and Australian faunal regions also represents the eastern edge of the distribution of the genus. Additionally, the Assam region of north-eastern India (Fig. 1) appears to represent an important region of species turnover within *Eutropis*, in that it separates the Indian radiation from the Southeast Asian species, although the region may have served as a corridor for dispersal in several cases (see above). It also marks the distributional limit of eight *Eutropis* species (*E. beddomii*, *E. bibronii*, *E. carinata*, *E. longicaudata*, *E. macularia*, *E. novemcarinata*, *E. quadricarinata* and *E. trivittata*), with *E. multifasciata* being the only species with a distribution that currently spans both subcontinents.

In contrast, the frequently cited biogeographical barriers represented by Wallace's Line (Wallace, 1860) and Huxley's corresponding modification (Huxley, 1868) appear to have had little significance with regard to *Eutropis* diversification, and no taxa exhibit ranges that abut this barrier. The results of our biogeographical analyses indicate that Wallace and Huxley's lines have each been crossed at least once: by the Sundaland clade and the endemic Philippine radiation, respectively (Figs 1 & 4). The Sundaland clade consists of four taxa that have present-day distributions spanning the original position of Wallace's Line, and five *Eutropis* species have geographical ranges spanning Huxley's extension of Wallace's Line into the Philippines (Figs 1 & 4; *E. rudis*, *E. rugifera*, *E. multifasciata*, *E. indeprensa* and Clade G in the Philippines). Similarly, in mainland Southeast Asia, three *Eutropis* species exhibit distributions that span the Isthmus of Kra (*E. longicaudata*, *E. macularia* and *E. multifasciata*), whereas only one species shows a distributional break there (with *E. rugifera* only known to occur south of that point; Grismer, 2011). Within the Philippines, five taxa have distributions restricted to a single Pleistocene aggregate island complex (Inger, 1954; Heaney, 1985; Brown & Diesmos, 2009), whereas seven species are distributed across multiple PAICs (Fig. 4). Finally, back-dispersals out of the Philippines to Borneo (Figs 1 & 4) also appear to support the importance of Palawan as a route facilitating faunal exchange (Diamond & Gilpin, 1983; Esselstyn *et al.*, 2010).

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## SUPPORTING INFORMATION

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

**Appendix S1** GenBank accession numbers of specimens used and locality information.

**Appendix S2** Individual maximum-likelihood gene trees for each locus.

**Appendix S3** Results from BAYESTRAITS biogeographical character state reconstructions.

## BIOSKETCH

The authors are interested in evolution and biodiversity in reptile and amphibian systems, with the current research focus of A.J.B. and R.M.B. being in Southeast Asia, and that of A.D.R. and K.P.K. being in India.

Author contributions: All authors conceived the ideas, participated in fieldwork, and contributed to the composition of the manuscript. A.J.B. and A.D.R. collected and analysed the data.

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