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ORIGINAL ARTICLE

Recent colonization and expansion through the Lesser Sundas by seven amphibian and reptile species

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Abstract

The Lesser Sundas Archipelago is comprised of two parallel chains of islands that extend between the Asian continental shelf (Sundaland) and Australo-Papuan continental shelf (Sahul). These islands have served as stepping stones for taxa dispersing between the Asian and Australo-Papuan biogeographical realms. While the oceanic barriers have prevented many species from colonizing the archipelago, a number of terrestrial vertebrate species have colonized the islands either by rafting/swimming or by human introduction. Here, we examine phylogeographic structure within the Lesser Sundas for three snake, two lizard and two frog species that each has a Sunda Shelf origin. These species are suspected to have recently colonized the archipelago, though all have inhabited the Lesser Sundas for over 100 years. We sequenced mtDNA from 231 samples to test whether there is sufficiently deep genetic structure within any of these taxa to reject human-mediated introduction. Additionally, we tested for genetic signatures of population expansion consistent with recent introduction and estimated the ages of Lesser Sundas clades, if any exist. Our results show little to no genetic structure between populations on different islands in five species and moderate structure in two species. Nucleotide diversity is low for all species, and the ages of the most recent common ancestor for species with monophyletic Lesser Sundas lineages date to the Holocene or late Pleistocene. These results support the hypothesis that these species entered the archipelago relatively recently and either naturally colonized or were introduced by humans to most of the larger islands in the archipelago within a short time span.

KEYWORDS

biogeography, frogs, Indonesia, lizards, phylogeography, snakes

1 | INTRODUCTION

The oceanic islands of Wallacea are united by their historical isolation from the land masses of the Sunda Shelf to the west and the Sahul Shelf to the east and south (Figure 1). While oceanic islands tend to have lower biodiversity than adjacent continental regions, they also tend to have a higher proportion of endemic species in part because terrestrial fauna must cross oceanic barriers in order to reach them, making successful colonization rare and limiting subsequent gene flow between mainland and insular populations (Whittaker & Fernández-Palacios, 2007). Many other factors also influence the species diversity on oceanic islands such as their distance from continental sources, the presence or absence of stepping-stone islands, island size, habitat heterogeneity, elevation, latitude, age and intervening ocean currents. (MacArthur & Wilson, 2001; Whittaker & Fernández-Palacios, 2007). However, since humans began maritime travel, the accumulation of species by human-mediated introduction has had an immense impact on the species diversity of islands worldwide (see Austin, 1999; Capinha, Essl, Seebens, Moser, & Pereira, 2015). While identifying which species have been introduced to an island or archipelago can help clarify the islands' biogeographical history, it is perhaps most critical from a management perspective so that effective conservation measures can be taken to protect sensitive native and endemic species.

The Lesser Sundas Archipelago, comprising the southern portion of Wallacea, is composed of two parallel, linearly arranged chains of oceanic islands, the oldest of which have been continuously emergent for 10–12 Ma (Hall, 2009). This long period of isolation provided ample time for natural colonization of the islands, as well as for in situ diversification, and the Lesser Sundas are consequently home to many endemic species (Orme et al., 2005). Recent studies of Lesser Sundas amphibians and reptiles (including *Limnonectes* fanged frogs, *Sphenomorphus* forest skinks, *Draco* flying lizards and *Cryptoblepharus* snake-eyed skinks) have shown that many of these endemics exhibit deep inter- and intra-island divergences dating to ~2–10 million years before present that reflect the complex tectonic history of the archipelago (Blom et al., 2019; Reilly, 2016; Reilly et al., 2019). However, although much of the diversity of the Lesser Sundas originated via natural processes, the archipelago has been inhabited by sea-faring humans for over 40,000 years, and these humans maintained a long tradition of pelagic fishing and trade between islands (O'Connor, Ono, & Clarkson, 2011). Indonesia is currently the fourth most populous country on earth, and travelling and movement of goods between islands are still commonly undertaken by boat (Monk, Fretes, & Reksodiharjo-Lilley, 1997). The long period of habitation by sea-faring humans has resulted in substantial human-mediated dispersal of plants and animals, including many reptile and amphibian species, throughout the archipelago (Heinsohn, 2003; Monk et al., 1997; Reilly et al., 2017).

Many species of reptiles and amphibians have become highly invasive due to human-mediated dispersal with major impacts on native fauna (Kraus, 2015). In the Lesser Sundas, this phenomenon is easily observable by simply boarding one of the many ferries that traverse the archipelago, upon which multiple species of house geckos can be seen crawling

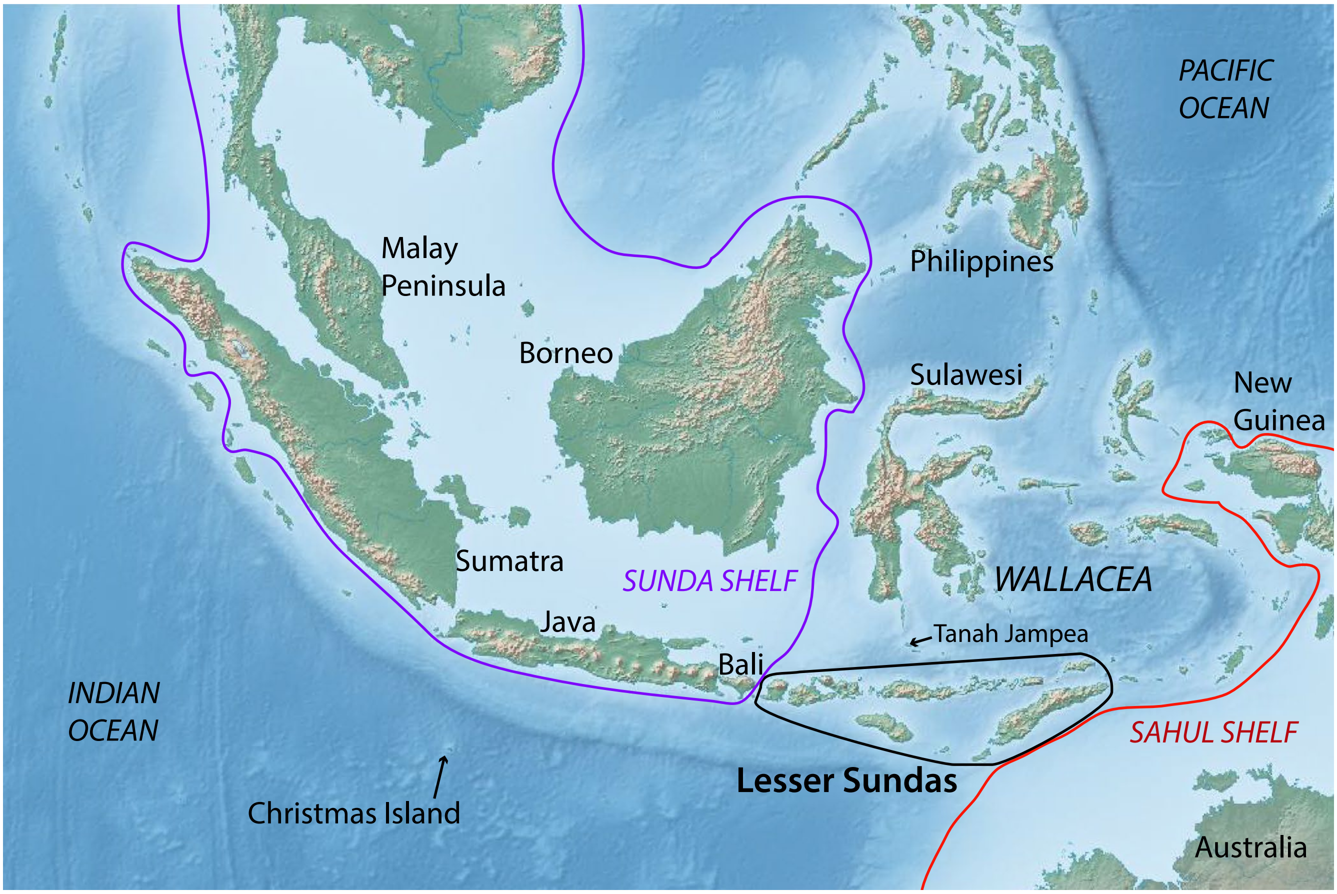

FIGURE 1 Map of the Sunda Shelf and Wallacea regions with the border of the Sunda Shelf shown as a purple line and the border of the Sahul Shelf shown as a red line. Wallacea consists of the oceanic islands between those two shelves [Colour figure can be viewed at wileyonlinelibrary.com]

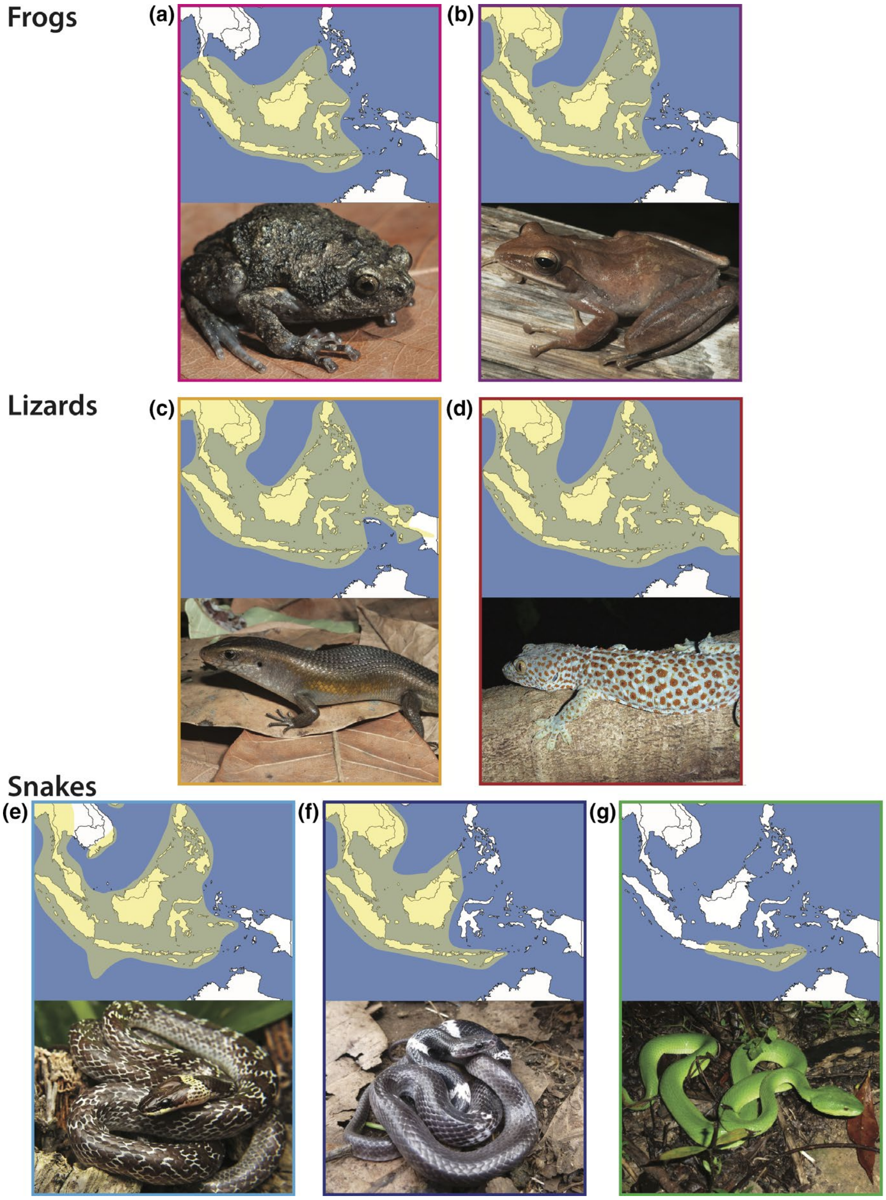

FIGURE 2 (a–g) Photographs of focal taxa and their geographic range within South-East Asia. (a) *Kaloula baleata,* (b) *Polypedates leucomystax*, (c) *Eutropis multifasciata*, (d) *Gekko gecko*, (e) *Lycodon capucinus*, (f) *Lycodon subcinctus* and (g) *Trimeresurus insularis* [Colour figure can be viewed at wileyonlinelibrary.com]

on the walls of the ship. However, there are a number of other species of reptiles and amphibians in the Lesser Sundas with colonization histories that remain unclear. Here, we examine seven species of reptiles and amphibians, each occurring on most of the major islands (Figure 2), that have either been shown to be recently introduced into other regions or are suspected of being moved between islands in the Lesser Sundas (Heinsohn, 2003). Nevertheless, these seven species have occupied islands within the archipelago for at least 100 years (Table S1), indicating either that they arrived via human-mediated introduction before the early 1900s (see van Lidth de Jeude, 1895; Boulenger, 1897; Barbour, 1912; De Rooij, 1917a, 1917b; van Kampen, 1923; Mertens, 1930) or that they arrived via natural colonization.

To distinguish human-mediated introductions that were not directly observed and recorded at the time of arrival from naturally occurring populations, it is typically necessary to employ some form of genetic analysis. Mitochondrial (mt) DNA is well suited for this purpose because of its rapid mutation rate relative to that of nuclear gene sequence data. If analysis of mtDNA finds well-supported monophyletic groupings consistent with geographically distinct local populations, such as samples from an island or set of islands, this would support the hypothesis that the species naturally colonized the archipelago long ago and have been isolated ever since. However, if there is a lack of phylogeographic structure (such as shared haplotypes between islands) or an absence of genetic diversity within the archipelago, this would suggest either a recent colonization (natural or anthropogenic) or prolific dispersal between islands. This pattern could be created by different scenarios, four of which are considered here: (a) natural introduction with prolific recent natural dispersals between islands; (b) natural introduction with human-mediated dispersal between islands; (c) a single human-mediated introduction and human-mediated dispersal between islands; or (d) multiple human-mediated introductions and human-mediated dispersal between islands.

Here, we utilize mtDNA sequence data from 231 newly sequenced samples, along with previously published data, to examine the phylogeographic structure in seven species of reptiles and amphibians with the goal of determining if there is sufficiently deep genetic structure within the Lesser Sundas to reject the hypothesis that they were introduced by humans. We pursue this goal by estimating summary statistics useful for the detection of recent population expansions consistent with recent introductions, estimating the age of monophyletic Lesser Sundas lineages and generating phylogenies that can shed light on the biogeographical history and any possible phylogeographic structure within the archipelago.

2 | MATERIALS AND METHODS

2.1 | Focal taxa

Lycodon capucinus (Family: Colubridae). The common wolf snake has been shown to be invasive in many regions of South-East Asia (Fritts, 1993; O'Shea, Kusuma, & Kaiser, 2018). Because sampling of this species within Indonesia is sparse, it remains unclear whether *L. capucinus* occurs naturally in the Lesser Sunda Islands.

Lycodon subcinctus (Family: Colubridae). The white-banded wolf snake also occurs throughout the Lesser Sundas and is similar in size and ecology to *L. capucinus*, suggesting it may have been transported between islands by human activity.

Trimeresurus insularis (Family: Viperidae). The venomous white-lipped island pit viper is widespread throughout the archipelago and exhibits regional colour morphs that are consistent with long-term isolation (see de Lang, 2011; personal observation). However, limited genetic analyses have found low divergence among island populations (David, Vogel, & Vidal, 2003; How, Schmitt, & Suyanto, 1996; Malhotra & Thorpe, 2004) suggesting the possibility of recent colonization or recent movement between islands. This hypothesis is also supported by a recent study (utilizing some of the same specimens examined in this study) which found that venom components are highly conserved between island populations of *T. insularis*, with the exception of Lombok, which has minor differences (Jones et al., 2019).

Gekko gecko (Family: Gekkonidae). Tokay geckos occur throughout the Indo-Australian Archipelago, and a study based on limited sampling found minimal genetic divergence between Timor and multiple Sunda Shelf localities including Sumatra, China, Malaysia and Cambodia (Roesler et al., 2011). This species is commonly found in human settlements, and its prey, insects and smaller geckos are abundant on boats travelling between islands.

Eutropis multifasciata (Family: Scincidae). Sun skinks were shown to be recently introduced to other regions of eastern Indonesia, and even to Australia and the USA (Ingram, 1987; Meshaka, Butterfield, & Hauge, 2004; O'Shea et al., 2018). A study based on allozyme data found limited divergence between islands within the Lesser Sundas, suggesting that inter-island movement may be prevalent (Schmitt, How, Hisheh, Goldberg, & Maryanto, 2000). *Eutropis multifasciata* from the nearest Sunda Shelf island, Bali, was once considered a distinct subspecies (*Eutropis m. balinensis*) from Lesser Sundas populations (*E. m. multifasciata*) based on scale morphology and coloration, though these subspecies have since been synonymized (Amarasinghe et al., 2018; Auffenberg, 1980; Mertens, 1927, 1930). A recent genomics study utilizing samples from mainland South-East Asia, the Philippines and one locality in Indonesia (Sulawesi) found high levels of connectivity across great distances including across some deep-water channels (e.g., Sulawesi and Luzon islands), but also found that the greatest levels of genetic divergence were associated with populations separated by deep-water channels (Barley, Monnahan, Thomson, Grismer, & Brown, 2015).

Polypedates leucomystax (Family: Rhacophoridae). The common tree frog has been shown to be introduced to various parts of Indonesia and the Philippines (Brown et al., 2010). However, relatively high genetic diversity on neighbouring Java combined with limited sampling from the Lesser Sundas has left their colonization history in the Lesser Sunda Islands an open question (Kuraishi et al., 2013).

Kaloula baleata (Family: Microhylidae). The smooth-fingered narrow-mouthed frog is common throughout the Sunda Shelf and the Lesser Sundas. The close relative *Kaloula pulchra* is suspected to have been introduced to multiple islands in Wallacea including Flores, suggesting that *K. baleata* may also have been introduced via human activity (Heinsohn, 2003; Whitten, Mustafa, & Henderson, 1987).

2.2 | Sampling

Herpetological surveys were conducted over the course of five expeditions between 2010 and 2014 on the islands of Bali, Nusa Penida, Lombok, Sumbawa, Flores, Lembata, Pantar, Alor, Wetar, Sumba, Savu, Rote and Timor. Sampling information for all newly sequenced samples can be found in Table S2. Voucher specimens and tissue samples are housed at either the UC Berkeley Museum of Vertebrate Zoology or the Museum Zoologicum Bogoriense in Cibinong, Indonesia.

2.3 | Data collection

Genomic DNA was extracted from liver or tail tip tissues using the Qiagen DNeasy Blood and Tissue Kit (Qiagen). For *T. insularis*, two mitochondrial genes, *ND4* (including tRNAs' serine, histidine and leucine) and cytochrome *b* (*cytb*), were PCR-amplified following standard procedures using the primers ND4 and LEU for the *ND4* gene (Arévalo, Davis, & Sites, 1994) and the primers THRSN2 and H14910 for the *cytb* gene (Burbrink, Lawson, & Slowinski, 2000). The *cytb* gene was sequenced for both species of *Lycodon* using the primers THRSN2 and H14910. The *ND4* gene (including tRNAs serine, histidine and leucine) was sequenced for both *E. multifasciata* and *G. gecko* using the primers ND4 and LEU. The *16S* gene was sequenced for *P. leucomystax* and *K. baleata* using the primers 16sc-L and 16sd-H (Evans et al., 2003). PCRs were carried out using standard Sanger sequencing methods and ethanol precipitation. DNA sequence visualization was performed on an ABI 3730 automated sequencer (Applied Biosystems). Forward and reverse sequence reads were combined in Geneious v11.1.5 (https://www.geneious.com). For each species, relevant DNA sequence data (i.e., for samples from the Lesser Sundas or other Sunda Shelf localities) were downloaded from GenBank. Sequence alignments were generated using MUSCLE (Edgar, 2004) with manual corrections where necessary.

2.4 | Data analysis

The software jModelTest 2 (Darriba, Taboada, Doallo, & Posada, 2012) was used to infer the appropriate models of sequence evolution for phylogenetic analyses. Maximum likelihood phylogenetic analysis was carried out using the program RAxML v8 (Stamatakis, 2014) with node support assessed with 1,000 non-parametric bootstrap replicates. For monophyletic Lesser Sundas clades with phylogenetic structure, the timing of entry into the archipelago was roughly estimated using divergence dating analyses implemented in the software BEAST v2.4.8 (Bouckaert et al., 2014). A strict molecular clock was applied with a rate of 2% divergence/million years for all taxa, as a median rate of 1.99% divergence per million years was estimated from a number of vertebrates (Allio, Donega, Galtier, & Nabholz, 2017). Additionally, rates more specific to each taxonomic group and mitochondrial gene were also applied including 1.3% for *K. baleata* (Macey et al., 2001), 1.3% for both *E. multifasciata* and *G. gecko* (Ceccarelli et al., 2014), 1.8% for *L. subcinctus* (Nabholz, Glémin, & Galtier, 2009) and 1.7% for *T. insularis* (Rodríguez-Robles, Jezkova, Fujita, Tolson, & García, 2015). For BEAST analyses, a data matrix composed of one sequence per unique haplotype was subjected to two separate runs of 10 million generations or more. Log files were viewed in Tracer v1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018) to ensure all parameter ESS values were >200. To obtain divergence estimates, the results of each pair of analyses were combined after discarding 10% of the samples as burn-in. Trees were viewed in FigTree.

Summary statistics including number of haplotypes, parsimony-informative sites, haplotype diversity, nucleotide divergence, Tajima's *D* (Tajima, 1989) and Fu's *Fs* were calculated with the software DnaSP v5 (Librado & Rozas, 2009). Tajima's *D* (when significantly negative) and Fu's *Fs* statistics can detect a rapid population expansion after a genetic bottleneck. All sequences are deposited in GenBank (see Table S2 for GenBank numbers).

3 | RESULTS

3.1 | Genetic structure

Kaloula baleata exhibits substantial genetic structure (Figure 3a). Samples from Timor are nested within samples from Java and Bali. This "Java/Bali/Timor" clade is sister to a Lesser Sundas clade composed of samples from Sumbawa, Sumba and Flores. The haplotype of the Bali sample is identical to that of two samples from Timor. Frogs from Sumbawa form a monophyletic assemblage with high support (bootstrap proportion [bp] = 94).

For *P. leucomystax*, the Lesser Sundas samples are most closely related to samples from Java, Bali and Nusa Penida (Figure 3b). A single common haplotype is shared across every island sampled within the Lesser Sundas as well as Java and Bali.

For *E. multifasciata*, our single sample from Timor is sister to a Bali clade, though still highly divergent. This Bali/Timor clade is sister to all remaining samples forming a well-supported Lesser Sundas clade (bp = 100; Figure 3c). These two haplotype groups are differentiated by ~4% sequence divergence at the *ND4* gene. Within the Lesser Sundas, lizards from Wetar and Pantar each form monophyletic groups, though only supported by one informative mutation for each clade. A single common haplotype is found on Lombok, Sumbawa, Flores and Alor.

Samples of *G. gecko* from the Lesser Sundas are found in three separate clades, each comprised of a collection of samples representing multiple overlapping localities. For example, samples from Sumbawa are present in each of the three clades. One of the two samples from China is nested in among the three Lesser Sundas clades, and a second sample from China is sister to all other samples (Figure 3d). The only monophyletic island is Bali, united by a single shared mutation. One common haplotype is found on Nusa Penida, Sumbawa, Sumba, Sabu and Timor, while a second common haplotype is found on Sumbawa, Flores, Pantar, Alor, Wetar, Timor and Rote.

Lycodon capucinus samples from the Lesser Sundas include a total of three haplotypes distinguished by two

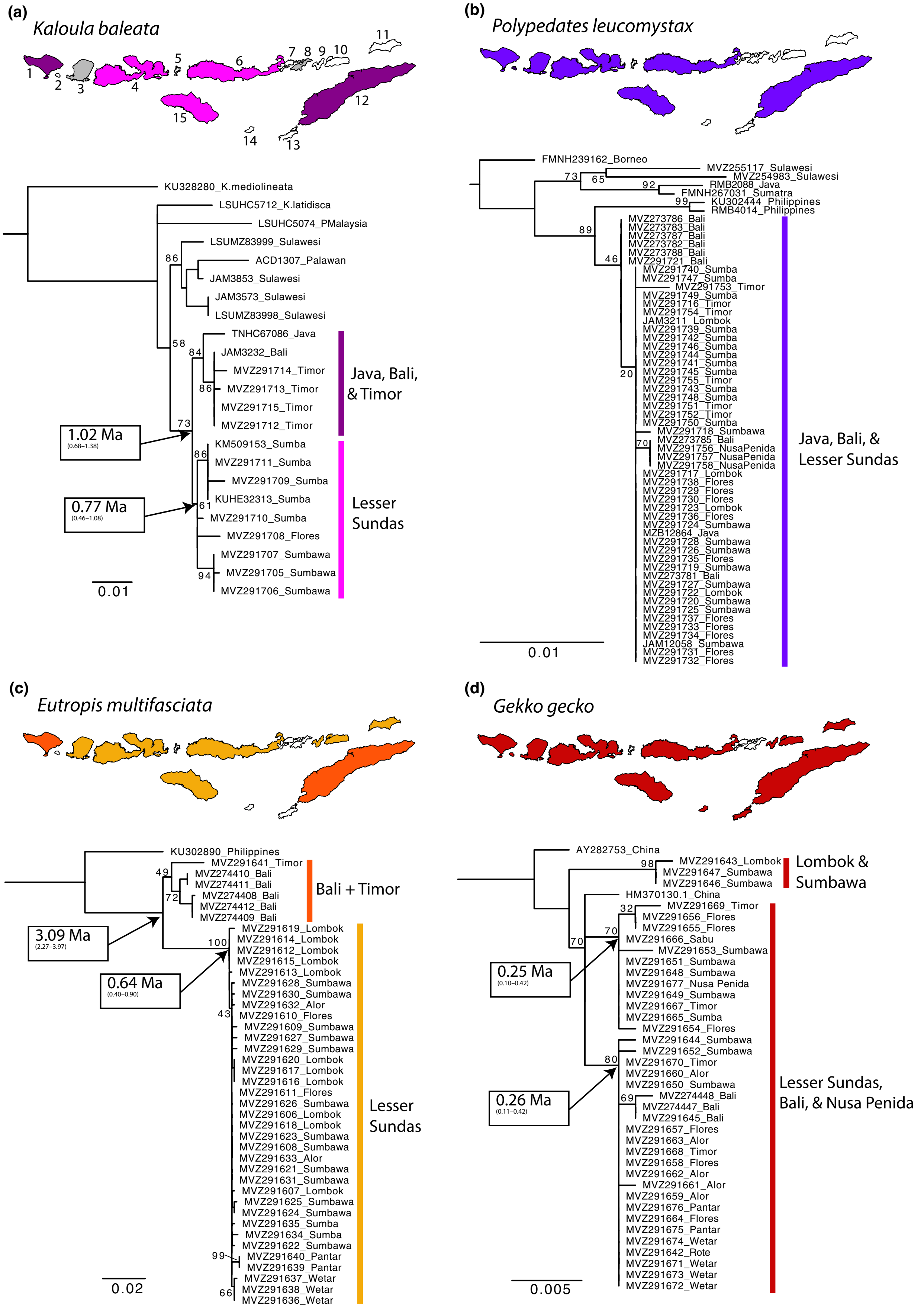
(a)
Kaloula baleata
1
2
3
4
5
6
7 8 9 10
11
12
13
14
15
KU328280_K.mediolineata
LSUHC5712_K.latidisca
LSUHC5074_PMalaysia
LSUMZ83999_Sulawesi
ACD1307_Palawan
JAM3853_Sulawesi
JAM3573_Sulawesi
LSUMZ83998_Sulawesi
TNHC67086_Java
JAM3232_Bali
MVZ291714_Timor
MVZ291713_Timor
MVZ291715_Timor
MVZ291712_Timor
KM509153_Sumba
MVZ291711_Sumba
MVZ291709_Sumba
KUHE32313_Sumba
MVZ291710_Sumba
MVZ291708_Flores
MVZ291707_Sumbawa
MVZ291705_Sumbawa
MVZ291706_Sumbawa
86
58
84
86
73
86
61
94
Java, Bali, & Timor
Lesser Sundas
1.02 Ma (0.68–1.38)
0.77 Ma (0.46–1.08)
0.01
(b)
Polypedates leucomystax
FMNH239162_Borneo
MVZ255117_Sulawesi
MVZ254983_Sulawesi
RMB2088_Java
FMNH267031_Sumatra
KU302444_Philippines
RMB4014_Philippines
73
65
92
99
89
46
20
70
MVZ273786_Bali
MVZ273783_Bali
MVZ273787_Bali
MVZ273782_Bali
MVZ273788_Bali
MVZ291721_Bali
MVZ291740_Sumba
MVZ291747_Sumba
MVZ291753_Timor
MVZ291749_Sumba
MVZ291716_Timor
MVZ291754_Timor
JAM3211_Lombok
MVZ291739_Sumba
MVZ291742_Sumba
MVZ291746_Sumba
MVZ291744_Sumba
MVZ291741_Sumba
MVZ291745_Sumba
MVZ291755_Timor
MVZ291743_Sumba
MVZ291748_Sumba
MVZ291751_Timor
MVZ291752_Timor
MVZ291750_Sumba
MVZ291718_Sumbawa
MVZ273785_Bali
MVZ291756_NusaPenida
MVZ291757_NusaPenida
MVZ291758_NusaPenida
MVZ291717_Lombok
MVZ291738_Flores
MVZ291729_Flores
MVZ291730_Flores
MVZ291723_Lombok
MVZ291736_Flores
MVZ291724_Sumbawa
MZB12864_Java
MVZ291728_Sumbawa
MVZ291726_Sumbawa
MVZ291735_Flores
MVZ291719_Sumbawa
MVZ273781_Bali
MVZ291727_Sumbawa
MVZ291722_Lombok
MVZ291720_Sumbawa
MVZ291725_Sumbawa
MVZ291737_Flores
MVZ291733_Flores
MVZ291734_Flores
JAM12058_Sumbawa
MVZ291731_Flores
MVZ291732_Flores
Java, Bali, & Lesser Sundas
0.01
(c)
Eutropis multifasciata
KU302890_Philippines
MVZ291641_Timor
MVZ274410_Bali
MVZ274411_Bali
MVZ274408_Bali
MVZ274412_Bali
MVZ274409_Bali
49
72
100
43
99
66
Bali + Timor
3.09 Ma (2.27–3.97)
0.64 Ma (0.40–0.90)
MVZ291619_Lombok
MVZ291614_Lombok
MVZ291612_Lombok
MVZ291615_Lombok
MVZ291613_Lombok
MVZ291628_Sumbawa
MVZ291630_Sumbawa
MVZ291632_Alor
MVZ291610_Flores
MVZ291609_Sumbawa
MVZ291627_Sumbawa
MVZ291629_Sumbawa
MVZ291620_Lombok
MVZ291617_Lombok
MVZ291616_Lombok
MVZ291611_Flores
MVZ291626_Sumbawa
MVZ291606_Lombok
MVZ291618_Lombok
MVZ291623_Sumbawa
MVZ291608_Sumbawa
MVZ291633_Alor
MVZ291621_Sumbawa
MVZ291631_Sumbawa
MVZ291607_Lombok
MVZ291625_Sumbawa
MVZ291624_Sumbawa
MVZ291635_Sumba
MVZ291634_Sumba
MVZ291622_Sumbawa
MVZ291640_Pantar
MVZ291639_Pantar
MVZ291637_Wetar
MVZ291638_Wetar
MVZ291636_Wetar
Lesser Sundas
0.02
(d)
Gekko gecko
AY282753_China
MVZ291643_Lombok
MVZ291647_Sumbawa
MVZ291646_Sumbawa
98
Lombok & Sumbawa
HM370130.1_China
32
70
70
80
69
MVZ291669_Timor
MVZ291656_Flores
MVZ291655_Flores
MVZ291666_Sabu
MVZ291653_Sumbawa
MVZ291651_Sumbawa
MVZ291648_Sumbawa
MVZ291677_Nusa Penida
MVZ291649_Sumbawa
MVZ291667_Timor
MVZ291665_Sumba
MVZ291654_Flores
MVZ291644_Sumbawa
MVZ291652_Sumbawa
MVZ291670_Timor
MVZ291660_Alor
MVZ291650_Sumbawa
MVZ274448_Bali
MVZ274447_Bali
MVZ291645_Bali
MVZ291657_Flores
MVZ291663_Alor
MVZ291668_Timor
MVZ291658_Flores
MVZ291662_Alor
MVZ291661_Alor
MVZ291659_Alor
MVZ291676_Pantar
MVZ291664_Flores
MVZ291675_Pantar
MVZ291674_Wetar
MVZ291642_Rote
MVZ291671_Wetar
MVZ291673_Wetar
MVZ291672_Wetar
0.25 Ma (0.10–0.42)
0.26 Ma (0.11–0.42)
Lesser Sundas, Bali, & Nusa Penida
0.005

FIGURE 3

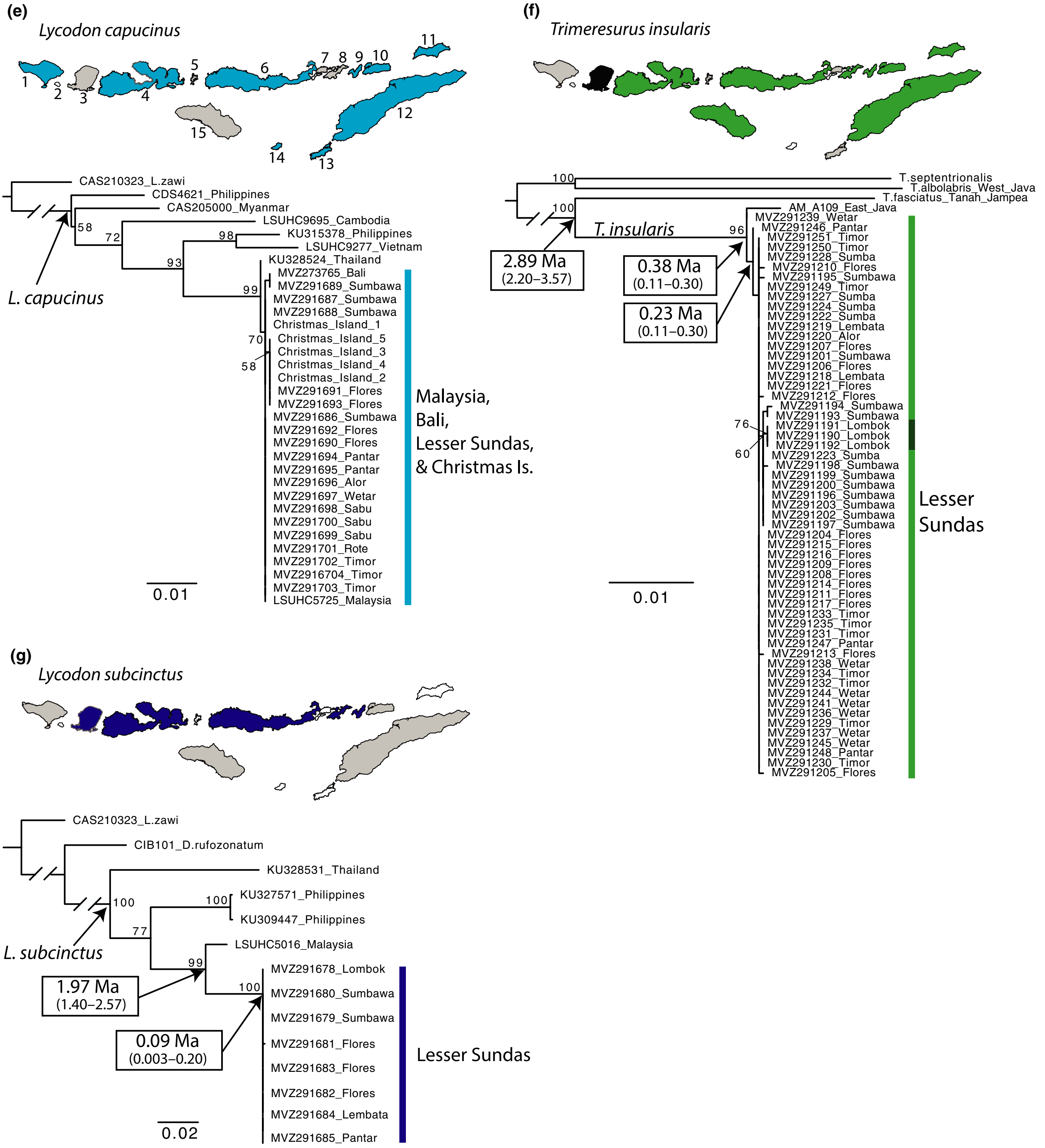

FIGURE 3 (a–g) Maximum likelihood phylogenies and represented islands. Each box contains a map of the Lesser Sundas region (including Bali and Nusa Penida) with sampled islands colour filled. Unsampled islands where the species is known to occur are shaded light grey. Numbers at nodes represent bootstrap support. Text in boxes represents node age estimates for relevant splits, with 95% posterior density distribution age ranges in parentheses. The numbers alongside islands in box (a) and (e) correspond to relevant islands: 1—Bali, 2—Nusa Penida, 3—Lombok, 4—Sumbawa, 5—Komodo, 6—Flores, 7—Adonara, 8—Lembata, 9—Pantar, 10—Alor, 11—Wetar, 12—Timor, 13—Rote, 14—Sabu and 15—Sumba [Colour figure can be viewed at wileyonlinelibrary.com]

parsimony-informative substitutions. The most common haplotype was found in samples from Malaysia, Sumbawa, Flores, Pantar, Alor, Wetar, Timor, Rote, Sabu and Christmas Island (Figure 3e). A second haplotype occurs on Christmas Island and Flores, and a third haplotype occurs on Bali and Sumbawa.

For *L. subcinctus*, a total of two haplotypes were recovered from the eight newly sequenced samples, with only one singleton mutation found in a Flores sample (Figure 3g). The common haplotype was found in snakes from Lombok, Sumbawa, Flores, Lembata and Pantar. The Lesser Sundas samples form a strongly supported clade relative to samples from Malaysia, Thailand and the Philippines, but we lack samples from adjacent Java or Bali.

A total of 11 haplotypes were recovered from the 57 *T. insularis* samples, with only three parsimony-informative sites present. The most common haplotype, belonging to 40/57 Lesser Sunda samples, is found on every island except Lombok. Lombok is the only monophyletic island population and is differentiated by a single unique mutation (Figure 3f). A *T. insularis* sample from eastern Java is sister to all Lesser Sundas samples. *Trimeresurus fasciatus* from Tanah Jampea Island (123 km north of Flores) was found to be the sister taxon of *T. insularis*.

3.2 | Summary statistics

Within the Lesser Sundas samples, genetic variability ranged from a low of one variable site and no parsimony-informative sites (*L. subcinctus*) to a high of 54 variable sites with 10 parsimony-informative sites (*E. multifasciata* “Lesser Sundas” clade + Timor sample; Table S3). However, when *E. multifasciata* samples from the Lesser Sundas without Timor are considered, there are only 23 variable sites, eight of which are parsimony-informative. Haplotype diversity within the Lesser Sundas samples ranged from 0.10 (*P. leucomystax*) to 0.91 (*E. multifasciata* “Lesser Sundas” clade; Table 1). Nucleotide diversities within Lesser Sundas samples were all low and ranged from a low of 0.0002 (*P. leucomystax* and *L. subcinctus*) to a high of 0.0044 (*G. gecko*; Table 1). Tajima's *D* statistics calculated from Lesser Sundas samples were negative for all species, ranging from −0.58 (*K. baleata*) to −2.20 (statistically significant value for *E. multifasciata* “Lesser Sundas” clade). Fu's *Fs* statistics were negative for all Lesser Sundas samples, ranging from −0.18 (*L. subcinctus*) to −16.52 (*E. multifasciata* “Lesser Sundas” clade), whereas the clades of *E. multifasciata* and *K. baleata* composed of samples from Timor and Bali had positive values (2.55 and 1.02, respectively).

3.3 | Ages of Lesser Sundas clades

The ages of the most recent common ancestors (MRCA) of each Lesser Sundas clade fell within the late Pleistocene (even considering 95% confidence intervals) with a range of 90,000–770,000 years (Table 1; Figure 3). Time-calibrated phylogenies using the taxon-/gene-specific rates can be found in Figure S2.

The MRCA age was estimated for the Lesser Sundas clade (not including Timor) of *K. baleata* using rates of 1.3% (0.77 Ma; 95% CI: 0.46–1.08 Ma) and 2% (0.49 Ma; 95% CI: 0.31–0.69 Ma). No estimate is given for *P. leucomystax* due to the nested Sundaland samples from Java, Bali and Nusa Penida. The MRCA ages were estimated for the Lesser Sundas lineage (not including Timor) of *E. multifasciata* using rates of 1.3% (0.64 Ma; 95% CI: 0.40–0.90 Ma) and 2% (0.42 Ma; 95% CI: 0.26–0.60 Ma). MRCA ages were estimated for two lineages of *G. gecko*: (a) a lineage containing samples from Sumbawa, Flores, Sumba, Sabu, Timor and Nusa Penida using a rate of 1.3% (0.25 Ma; 95% CI: 0.10–0.42) and 2% (0.16 Ma; 95% CI: 0.06–0.27 Ma); and (b) a lineage containing samples from Sumbawa, Flores, Pantar, Alor, Wetar, Timor, Rote and Bali using a rate of 1.3% (0.26 Ma; 95% CI: 0.11–0.42 Ma) and 2% (0.17 Ma; 95% CI: 0.07–0.27 Ma). MRCA ages were estimated for

TABLE 1 Mitochondrial marker summary statistics and divergence times within Lesser Sundas clades

Species	*h*	*π*	Tajima's *D*	Fu's *Fs*	MRCA (specific rate)	MRCA (2% rate)
Kaloula baleata						
“Java/Bali/Timor” clade	0.60	0.0031	−1.37 ($p > 0.10$)	1.02	0.65 Ma (0.34–0.97)	0.42 Ma (0.22–0.62)
“Lesser Sundas” clade	0.83	0.0037	−0.58 ($p > 0.10$)	−0.85	0.77 Ma (0.46–1.08)	0.49 Ma (0.31–0.69)
Polypedates leucomystax	0.10	0.0002	−1.49 ($p > 0.10$)	−2.66	N/A	N/A
Eutropis multifasciata						
“Bali/Timor” clade	0.87	0.0117	−0.39 ($p > 0.10$)	2.55	1.73 Ma (1.08–2.36)	1.12 Ma (0.71–1.54)
“Lesser Sundas” clade	0.91	0.0024	−2.20 ($p < 0.01$)[a]	−16.52	0.64 Ma (0.40–0.90)	0.42 Ma (0.26–0.60)
Gekko gecko	0.72	0.0044	−0.77 ($p > 0.10$)	−0.81	0.25 Ma (0.10–0.42)	0.16 Ma (0.06–0.27)
Lycodon capucinus	0.29	0.0003	−1.12 ($p > 0.10$)	−1.15	N/A	N/A
Lycodon subcinctus	0.25	0.0002	−1.05 ($p > 0.10$)	−0.18	0.09 Ma (0.002–0.20)	0.08 Ma (0.003–0.18)
Trimeresurus insularis	0.47	0.0006	−1.36 ($p > 0.10$)	−5.19	0.23 Ma (0.12–0.35)	0.19 Ma (0.11–0.30)

Note: Age ranges in parentheses represent the 95% posterior density distribution.

Abbreviations: *h*, haplotype diversity; Ma, millions of years ago; MRCA, most recent common ancestor; *π*, nucleotide diversity.

*Statistically significant.

Lesser Sundas lineages of *L. subcinctus* using rates of 1.8% (0.09 Ma; 95% CI: 0.002–0.20 Ma) and 2% (0.08 Ma; 95% CI: 0.003–0.18 Ma), and for *T. insularis* using rates of 1.7% (0.23 Ma; 95% CI: 0.12–0.35 Ma) and 2% (0.19 Ma; 95% CI: 0.11–0.30 Ma). No estimate is given for *L. capucinus* because no monophyletic Lesser Sunda lineage was recovered.

4 | DISCUSSION

4.1 | General patterns

The mitochondrial markers we have screened are well suited for rejecting human-mediated introductions since deep genetic structure would indicate that their occurrence in the region predated the arrival of humans. However, lack of structure does not necessarily mean that these species did not arrive via natural dispersal. We set out to determine whether there was sufficiently deep genetic structure within these taxa to reject the hypothesis that the Lesser Sundas populations are the result of human introductions. However, one potential complication is that independent introductions from structured populations outside of the Lesser Sundas could be misinterpreted as structure within the Lesser Sundas. Adding to this challenge is the possibility that some taxa may be composed of both native and non-native populations. To elucidate these alternative scenarios, more comprehensive geographic sampling of each species outside of the Lesser Sundas would be needed to pinpoint the source (or sources) and more accurately estimate the timings of colonization. Additionally, increased sampling of nuclear variation (e.g., RADSeq data) would produce a more robust phylogeny and allow for demographic and biogeographical model-testing analyses to better estimate the timing and sequence of island colonization. Even if a phylogenetic signal is obtained that is predicted under a human-mediated dispersal scenario, differentiating between natural and human-mediated dispersal is not always possible (see Hamilton, Zug, & Austin, 2010).

If any of these species had been long-established in the Lesser Sundas, we would expect each island population, or at least sets of islands, to have unique mutations resulting in phylogeographic structure. The results of our mitochondrial phylogenetic estimates and summary statistics have shown that each of the seven focal species has little to moderate genetic differentiation and little to no phylogeographic structure across the oceanic islands of the Lesser Sundas. It should be noted that the methods used in this study to estimate divergence dates tend to be biased towards older dates, suggesting that colonization and expansion through the archipelago occurred more recently (see Herman & Searle, 2011). Our MRCA ages, though only rough estimates, do suggest that some species may have occurred within the Lesser Sundas on the order of hundreds of thousands of years, and the negative Tajima's *D* values estimated for Lesser Sundas samples within each species are consistent with recent population expansions. Given that each of these species is known to have occurred throughout the majority of their currently known Lesser Sundas range for over 100 years (Table S1), any human-mediated introductions into the archipelago or specific islands would have occurred before the 20th century. While a primary goal of this study was to search for evidence of a natural invasion for these taxa, we can reject human-mediated introduction to the archipelago for only *K. baleata, E. multifasciata* and *T. insularis*. Even for these taxa, the general absence of phylogeographic structure within the Lesser Sundas indicates that we cannot reject human-mediated inter-island dispersal following natural colonization.

4.2 | Candidates for natural introduction with possible human-mediated dispersal

The white-lipped island pitviper has very low genetic diversity with a common haplotype found on every island except Lombok. However, *T. insularis* does contain some phylogeographic structure with Lombok island samples forming a monophyletic group (though only supported by a single mutation), which is in agreement with a recent venom study that showed venom differences between Lombok and the rest of the archipelago (Jones et al., 2019). Although the topology of our tree places samples from Wetar plus Pantar as basal within the Lesser Sundas, support for this branching pattern is very weak and it is more appropriate to interpret the tree as a large polytomy or star phylogeny. *Trimeresurus insularis* diverged from its sister species, the Tanah Jampea endemic *T. fasciatus*, ~2.9 Ma. It is unclear whether the ancestor of these two species dispersed from Tanah Jampea to the Lesser Sundas, or from the Lesser Sundas to Tanah Jampea, but the prevailing north-to-south oceanic currents in the Flores Sea suggest that a dispersal from Tanah Jampea to the Lesser Sundas is more likely (Gordon, 2005). If this is the case, then East Java may have been colonized by *T. insularis* from the Lesser Sundas. While it appears that this species has naturally occurred in the archipelago for some time, we note that both the high densities of *T. insularis* and the movement of large quantities of agricultural goods by boat could have transported these pit vipers between islands.

Both *K. baleata* and *E. multifasciata* have moderate phylogeographic structure and genetic diversity within the Lesser Sundas, suggesting that they have inhabited the archipelago for some time. Interestingly, for both species, our data indicate that samples from Timor are more closely related to samples from Bali (and Java in the case of *Kaloula*) than to samples representing the remainder of the Lesser Sundas. An important difference between these species is that the *K. baleata* sample from Bali had an identical haplotype to some Timor samples, whereas the *E. multifasciata* sample from Timor is clearly divergent and quite genetically distinct from samples from Bali. This suggests that *K. baleata* likely arrived on Timor via human-mediated introduction from Bali, whereas *E. multifasciata* on Timor may very well represent a separate natural introduction from Bali or elsewhere. Regarding the non-Timor populations of *Kaloula* in the Lesser Sundas, our limited data set exhibits phylogeographic structure, suggesting that these frogs are naturally occurring on the islands of Sumbawa, Sumba, Flores and likely others as well.

Eutropis multifasciata populations on Bali, Pantar and Wetar are each monophyletic, suggesting isolation on those islands, whereas Lombok, Sumbawa, Flores, Sumba and Alor share either a common haplotype or very closely related haplotypes, suggesting either recent or continued movement between those islands. *Eutropis multifasciata* has likely occurred in the Lesser Sundas for a significant amount of time, but it is unclear whether they have been moved between islands by people or whether they are such prolific natural dispersers that they remain genetically unstructured. Future sampling of both genes and localities throughout their range will certainly shed light on this unresolved question.

4.3 | Candidates for human-mediated introduction and dispersal

There is low phylogeographic structure within *P. leucomystax,* with one common haplotype found throughout the Lesser Sundas, as well as on Java and Bali. The extremely low nucleotide diversity (0.0002) suggests a recent and rapid spread through the Lesser Sundas. Though not yet proven, it has long been suspected that *P. leucomystax* has been moved around much of South-East Asia by people, a finding supported by Brown et al. (2010) and Kuraishi et al. (2013). Our findings support the hypotheses that *P. leucomystax* has either been recently introduced into and throughout the Lesser Sundas Archipelago or that it has naturally invaded the archipelago very recently followed by rapid colonization of nearly every major island.

Analyses of the snake fauna within the Lesser Sundas suggest that the archipelago's long-standing isolation from other islands in the region have influenced both their species composition and levels of variation between island populations (How & Kitchener, 1997). However, we find that both species of *Lycodon* wolf snakes have very little genetic diversity, no phylogeographic structure and negative Tajima's *D* values, suggesting that they have recently expanded their ranges and population sizes as they colonized the islands. *Lycodon capucinus* from the Lesser Sundas are nearly genetically identical to those from the Malay Peninsula, suggesting they were recently introduced by humans, and likely continue to be moved between islands. *Lycodon subcinctus* also exhibits minimal genetic diversity suggesting a recent and rapid spread through the archipelago and/or continued movement between islands. However, the lack of *L. subcinctus* sampling from the nearby Sunda Shelf islands of Java and Bali, as well as the monophyly of Lesser Sundas samples, prevents us from ruling out a natural introduction.

4.4 | Candidate for multiple human-mediated introductions and dispersals

Movement of reptiles between islands in the Lesser Sundas is certainly known for some species such as the geckos *Hemidactylus frenatus, Hemidactylus platyurus* and *Gehyra mutilata*, which are commonly seen on ferry boats travelling between islands (pers. obs. S. Reilly). This may be the case for *G. gecko* as well, which has more genetic diversity than the three snakes examined but no discernible phylogeographic structure. *ND4* sequence data for *G. gecko* outside the Lesser Sundas are sparse which prevents the inference of the source populations for the Lesser Sundas. *Gekko gecko* samples within the Lesser Sundas are rendered paraphyletic and nested within samples from China suggesting multiple human-mediated introductions. The introduction of the Chinese turtle, *Mauremys reevesii*, to Timor is evidence of the long history of travel and movement of animals between China and Indonesia (Kaiser, Carvalho, Freed, & O'Shea, 2010; Yuwono, 1998). *Gekko gecko* are common in dense human settlements in the region and are known to prey on smaller geckos (such as *Hemidactylus, Lepidodactylus* and *Gehyra*), which are common on boats travelling between islands (Aowphol, Thirakhupt, Nabhitabhata, & Voris, 2006). Thus, it would not be surprising if *G. gecko* occasionally or routinely stows away on these boats and is thereby moved between islands.

4.5 | Comparisons with confirmed native herpetofauna

While we have shed light on the recent arrival and low inter-island divergences within these seven focal species, there are many other species of herpetofauna whose history within the archipelago is much older with little to no connectivity between islands. For example, *Limnonectes* fanged frogs entered the archipelago at least 7 Ma with movement between islands between 2 and 7 Ma (Reilly et al., 2019), *Draco* flying lizards entered ~7–11 Ma with movements between islands ranging from 0.5 to 7 Ma (Reilly, 2016), and *Sphenomorphus* forest skinks entered before 6 Ma with early movements between islands ranging from recently (into Maluku) to 5.5 Ma (Reilly, 2016). Unlike the seven focal taxa for this study, the fanged frogs, flying lizards and forest skinks exhibit deep splits between monophyletic island populations (or populations from non-overlapping regions of larger islands), and estimates of their arrival into the archipelago are only slightly younger than the ages of the oldest continuously emergent islands.

Why have fanged frogs, flying lizards and forest skinks not been recently moved between the Lesser Sunda Islands while many of the seven focal taxa have? All seven species from this study, as well as *Draco* and *Sphenomorphus*, have close associations with human settlements or agriculture, whereas only *Limnonectes* primarily occur away from human-disturbed areas. Perhaps this pattern could be partially explained by each species propensity to be moved within agricultural products (e.g., fruits and potted plants) either as adults or as eggs. For frogs, the movement of eggs could account for *Polypedates* (which deposit foam nests out of the water (Yorke, 1983)), but not for *Kaloula* or *Limnonectes* which deposit eggs in water. *Gekko* and both *Lycodon* species could deposit eggs in the soil of potted plants, whereas *Eutropis* and *Trimeresurus* are ovoviviparous and would be moved as juveniles or adults. Egg deposition sites of *Draco* and *Sphenomorphus* may occur in soils or vegetation not readily moved by humans, whereas *Gekko* and *Lycodon* may deposit eggs in soils or vegetation more likely to be transported between islands. In summary, we are not aware of any clear association of the natural history/breeding biology with levels of divergence within Lesser Sundas herpetofauna.

4.6 | Conservation implications

Plants and animals have been moved between islands and introduced to new islands, both purposefully and unintentionally, within the Indo-Australian Archipelago for tens of thousands of years (Heinsohn, 2003). Certainly, the introduction of some species results in greater ecological impact than others, and special caution should be given to prevent the human-mediated spread of those species that could cause major damage such as toxic species (e.g., toads) or certain predators that can devastate naive native species. For example, the nearby Christmas Island has been heavily impacted by the human-mediated introduction of *L. capucinus*, which has likely caused the extinction of four species of lizards endemic to the island (Oliver et al., 2018; Smith et al., 2012). The detection of human-mediated introductions of ecologically harmful species will depend on accurate surveys of each island's fauna, both in the past (to determine a baseline) and in the present (to detect introductions), and as such, we recommend that comprehensive faunal surveys of the Lesser Sundas continue. When a confirmed or suspected introduction is detected, dietary analyses (e.g., stomach contents) of the intruding species could be particularly useful to understand their impacts on native fauna.

5 | CONCLUSIONS

The results from this study show that none of these seven taxa exhibit deep genetic structure as seen in old, naturally occurring taxa such as *Limnonectes* fanged frogs, *Draco* flying lizards and *Sphenomorphus* forest skinks (Reilly, 2016; Reilly et al., 2019). In the light of these results, we cannot definitively reject the hypothesis of human-mediated introductions and/or movements throughout the archipelago for these seven species. For some of these species (*K. baleata, E. multifasciata*, *T. insularis* and *L. subcinctus*), longer term natural introductions remain a viable possibility, whereas, for others (*P. leucomystax*, *G. gecko* and *L. capucinus*), natural introductions seem unlikely in the light of these new results, what we know about well-documented introductions elsewhere (such as for *L. capucinus*) and the biology of the species (rampant overwater dispersal over a short temporal extent would be quite unexpected for amphibians). While we have not definitively answered the question of the mode of introduction for most of these taxa, our data clearly indicate that each of these taxa is a relatively recent arrival to the Lesser Sundas Archipelago with little to no genetic divergence between islands. We have shed light on the biogeography of each of these taxa and set the stage for others to follow-up with more comprehensive sampling of localities and genes.

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REFERENCES

Allio, R., Donega, S., Galtier, N., & Nabholz, B. (2017). Large variation in the ratio of mitochondrial to nuclear mutation rate across animals: Implications for genetic diversity and the use of mitochondrial DNA as a molecular marker. *Molecular Biology and Evolution*, *34*, 2762–2772. https://doi.org/10.1093/molbev/msx197

Amarasinghe, A. A. T., Thammachoti, P., Campbell, P. D., Hallermann, J., Henkanaththegedara, S. M., Karunarathna, D. S., … Ineich, I. (2018). Systematic composition of the *Eutropis multifasciata* (Kuhl 1820) species complex (Squamata: Scincidae) and designation of a neotype. *Herpetologica*, *74*, 342–354. https://doi.org/10.1655/HERPETOLOGICA-D-15-00073.1

Aowphol, A., Thirakhupt, K., Nabhitabhata, J., & Voris, H. K. (2006). Foraging ecology of the Tokay gecko, *Gekko gecko*, in a residential area in Thailand. *Amphibia-Reptilia*, *27*, 491–503. https://doi.org/10.1163/156853806778877121

Arevalo, E., Davis, S. K., & Sites, J. W. Jr (1994). Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology*, *43*, 387–418. https://doi.org/10.1093/sysbio/43.3.387

Auffenberg, W. (1980). The herpetofauna of Komodo, with notes on adjacent areas. *Bulletin of the Florida State Museum, Biological Sciences*, *25*, 39–156.

Austin, C. C. (1999). Lizards took express train to Polynesia. *Nature*, *397*, 113–114. https://doi.org/10.1038/16365

Barbour, T. (1912). A contribution to the zoogeography of the East Indian Islands. *Memoirs of the Museum of Comparative Zoology*, *44*, 1–168.

Barley, A. J., Monnahan, P. J., Thomson, R. C., Grismer, L. L., & Brown, R. M. (2015). Sun skink landscape genomics: Assessing the roles of micro-evolutionary processes in shaping genetic and phenotypic diversity across a heterogeneous and fragmented landscape. *Molecular Ecology*, *24*, 1696–1712. https://doi.org/10.1111/mec.13151

Blom, M. P. K., Matzke, N. J., Bragg, J. G., Arida, E., Austin, C. C., Backlin, A. R., … Moritz, C. (2019). Habitat preference modulates trans-oceanic dispersal in a terrestrial vertebrate. *Proceedings of the Royal Society B: Biological Sciences*, *286*, 20182575. https://doi.org/10.1098/rspb.2018.2575

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., … Drummond, A. J. (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *10*(4), e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Boulenger, G. A. (1897). LII.—A list of the reptiles and batrachians collected by Mr. Alfred Everett in Lombok, Flores, Sumba, and Savu, with descriptions of new species. *Journal of Natural History*, *19*, 503–509.

Brown, R. M., Linkem, C. W., Siler, C. D., Sukumaran, J., Esselstyn, J. A., Diesmos, A. C., … Andayani, N. (2010). Phylogeography and historical demography of *Polypedates leucomystax* in the islands of Indonesia and the Philippines: Evidence for recent human-mediated range expansion? *Molecular Phylogenetics and Evolution*, *57*, 598–619. https://doi.org/10.1016/j.ympev.2010.06.015

Burbrink, F. T., Lawson, R., & Slowinski, J. B. (2000). Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): A critique of the subspecies concept. *Evolution*, *54*, 2107–2118. https://doi.org/10.1111/j.0014-3820.2000.tb01253.x

Capinha, C., Essl, F., Seebens, H., Moser, D., & Pereira, H. M. (2015). The dispersal of alien species redefines biogeography in the Anthropocene. *Science*, *348*, 1248–1251. https://doi.org/10.1126/science.aaa8913

Ceccarelli, F. S., Menegon, M., Tolley, K. A., Tilbury, C. R., Gower, D. J., Laserna, M. H., … Loader, S. P. (2014). Evolutionary relationships, species delimitation and biogeography of Eastern Afromontane horned chameleons (Chamaeleonidae: *Trioceros*). *Molecular Phylogenetics and Evolution*, *80*, 125–136. https://doi.org/10.1016/j.ympev.2014.07.023

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772. https://doi.org/10.1038/nmeth.2109

David, P., Vogel, G., & Vidal, N. (2003). On *Trimeresurus fasciatus* (Boulenger, 1896) (Serpentes: Crotalidae), with a discussion on its relationships based on morphological and molecular data. *Raffles Bulletin of Zoology*, *51*, 149–158.

de Lang, R. (2011). *The snakes of the Lesser Sunda Islands (Nusa Tenggara), Indonesia: A field guide to the terrestrial and semi-aquatic snakes with identification key*. Frankfurt am Main, Germany: Chimaira.

de Rooij, N. (1917a). *The reptiles of the Indo-Australian Archipelago: Lacertilia, chelonia, emydosauria*, Vol. 1. Leiden, the Netherlands: EJ Brill.

de Rooij, N. (1917b). *The reptiles of the Indo-Australian Archipelago: Ophidia*, Vol. 2. Leiden, the Netherlands: EJ Brill.

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797. https://doi.org/10.1093/nar/gkh340

Evans, B. J., Brown, R. M., McGuire, J. A., Supriatna, J., Andayani, N., Diesmos, A., … Cannatella, D. C. (2003). Phylogenetics of fanged frogs: Testing biogeographical hypotheses at the interface of the Asian and Australian faunal zones. *Systematic Biology*, *52*, 794–819. https://doi.org/10.1093/sysbio/52.6.794

Fritts, T. H. (1993). The common wolf snake, *Lycodon aulicus capucinus*, a recent colonist of Christmas Island in the Indian Ocean. *Wildlife Research*, *20*, 261–265. https://doi.org/10.1071/WR9930261

Gordon, A. L. (2005). The Indonesian Seas. *Oceanography*, *18*, 14–27.

Hall, R. (2009). Southeast Asia's changing paleogeography. *Blumea*, *54*, 148–161. https://doi.org/10.3767/000651909X475941

Hamilton, A. L., Zug, G. R., & Austin, C. C. (2010). Biogeographic anomaly or human introduction: A cryptogenic population of tree skink (Reptilia: Squamata) from the Cook Islands, Oceania. *Biological Journal of the Linnean Society*, *100*, 318–328. https://doi.org/10.1111/j.1095-8312.2010.01437.x

Heinsohn, T. (2003). Animal translocation: Long-term human influences on the vertebrate zoogeography of Australasia (natural dispersal versus ethnophoresy). *Australian Zoologist*, *32*, 351–376. https://doi.org/10.7882/AZ.2002.014

Herman, J. S., & Searle, J. B. (2011). Post-glacial partitioning of mitochondrial genetic variation in the field vole. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1724), 3601–3607. https://doi.org/10.1098/rspb.2011.0321

How, R. A., & Kitchener, D. J. (1997). Biogeography of Indonesian snakes. *Journal of Biogeography*, *24*, 725–735. https://doi.org/10.1046/j.1365-2699.1997.00150.x

How, R. A., Schmitt, L. H., & Suyanto, A. (1996). Geographical variation in the morphology of four snake species from the Lesser Sunda Islands, eastern Indonesia. *Biological Journal of the Linnean Society*, *59*, 439–456. https://doi.org/10.1111/j.1095-8312.1996.tb01476.x

Ingram, G. (1987). Does the skink, *Mabuya multifasciata*, occur in Australia? *Northern Territory Naturalist*, *10*, 11–12.

Jones, B. K., Saviola, A., Reilly, S. B., Stubbs, A. L., Arida, E., Iskandar, D. T., … Mackessy, S. P. (2019). Venom composition in a phenotypically variable pit viper (*Trimeresurus insularis*) across the Lesser Sunda Archipelago. *Journal of Proteome Research*, *18*, 2206–2220. https://doi.org/10.1021/acs.jproteome.9b00077

Kaiser, H., Carvalho, V. L., Freed, P., & O'Shea, M. (2010). A widely traveled turtle: *Mauremys reevesii* (Testudines: Geoemydidae) in Timor-Leste. *Herpetology Notes*, *3*, 93–96.

Kraus, F. (2015). Impacts from invasive reptiles and amphibians. *Annual Review of Ecology, Evolution, and Systematics*, *46*, 75–97. https://doi.org/10.1146/annurev-ecolsys-112414-054450

Kuraishi, N., Matsui, M., Hamidy, A., Belabut, D. M., Ahmad, N., Panha, S., … Thong, H. T. (2013). Phylogenetic and taxonomic relationships of the *Polypedates leucomystax* complex (Amphibia). *Zoologica Scripta*, *42*, 54–70. https://doi.org/10.1111/j.1463-6409.2012.00562.x

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

MacArthur, R. H., & Wilson, E. O. (2001). *The theory of island biogeography*, Vol. 1. Princeton, NJ: Princeton University Press.

Macey, J. R., Strasburg, J. L., Brisson, J. A., Vredenburg, V. T., Jennings, M., & Larson, A. (2001). Molecular phylogenetics of western North American frogs of the *Rana boylii* species group. *Molecular Phylogenetics and Evolution*, *19*, 131–143. https://doi.org/10.1006/mpev.2000.0908

Malhotra, A., & Thorpe, R. S. (2004). A phylogeny of four mitochondrial gene regions suggests a revised taxonomy for Asian pitvipers (*Trimeresurus* and *Ovophis*). *Molecular Phylogenetics and Evolution*, *32*, 83–100. https://doi.org/10.1016/j.ympev.2004.02.008

Mertens, R. (1927). Herpetologische Mitteilungen XVII, *Mabuya multifasciata*, Kuhl auf Bali. *Senckenbergiana*, *9*, 178–181.

Mertens, R. (1930). Die amphibien and reptilien der Inseln Bali, Lombok, Sumbawa and Flores. *Abhandlungen Senckenbergischen Naturforschenden Gesellschaft*, *42*, 115–344.

Meshaka, W. E., Butterfield, B. P., & Hauge, J. B. (2004). *The exotic amphibians and reptiles of Florida*. Malabar, FL: Krieger Pub. Co.

Monk, K. A., De Fretes, Y., & Reksodiharjo-Lilley, G. (1997). *The ecology of Nusa Tenggara and Maluku*. Singapore, Singapore: Periplus Editions.

Nabholz, B., Glémin, S., & Galtier, N. (2009). The erratic mitochondrial clock: Variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evolutionary Biology*, *9*, 54. https://doi.org/10.1186/1471-2148-9-54

O'Connor, S., Ono, R., & Clarkson, C. (2011). Pelagic fishing at 42,000 years before the present and the maritime skills of modern humans. *Science*, *334*, 1117–1121. https://doi.org/10.1126/science.1207703

Oliver, P. M., Blom, M. P.K., Cogger, H. G., Fisher, R. N., Richmond, J. Q., & Woinarski, J. C. (2018). Insular biogeographic origins and high phylogenetic distinctiveness for a recently depleted lizard fauna from Christmas Island, Australia. *Biology Letters*, *14*, 20170696. https://doi.org/10.1098/rsbl.2017.0696

Orme, C. D. L., Davies, R. G., Burgess, M., Eigenbrod, F., Pickup, N., Olson, V. A., … Owens, I. P. F. (2005). Global hotspots of species richness are not congruent with endemism or threat. *Nature*, *436*, 1016. https://doi.org/10.1038/nature03850

O'Shea, M., Kusuma, K. I., & Kaiser, H. (2018). First record of the Island Wolfsnake, *Lycodon capucinus*, from New Guinea, with comments on its widespread distribution and confused taxonomy, and a new record for the Common Sun Skink, *Eutropis multifasciata*. *IRCF Reptiles and Amphibians*, *25*, 70–84.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*, 901–904. https://doi.org/10.1093/sysbio/syy032

Reilly, S. B. (2016). *Historical biogeography of reptiles and amphibians from the Lesser Sunda Islands of Indonesia*. Doctoral Dissertation, University of California, Berkeley, CA.

Reilly, S. B., Stubbs, A. L., Karin, B. R., Bi, K., Arida, E., Iskandar, D. T., & McGuire, J. A. (2019). Leap-frog dispersal and mitochondrial introgression: Phylogenomics and biogeography of *Limnonectes* fanged frogs in the Lesser Sundas archipelago of Wallacea. *Journal of Biogeography*, *46*, 757–769. https://doi.org/10.1111/jbi.13526

Reilly, S. B., Wogan, G. O., Stubbs, A. L., Arida, E., Iskandar, D. T., & McGuire, J. A. (2017). Toxic toad invasion of Wallacea: A biodiversity hotspot characterized by extraordinary endemism. *Global Change Biology*, *23*, 5029–5031. https://doi.org/10.1111/gcb.13877

Rodríguez-Robles, J. A., Jezkova, T., Fujita, M. K., Tolson, P. J., & García, M. A. (2015). Genetic divergence and diversity in the Mona and Virgin Islands Boas, *Chilabothrus monensis* (*Epicrates monensis*) (Serpentes: Boidae), West Indian snakes of special conservation concern. *Molecular Phylogenetics and Evolution*, *88*, 144–153. https://doi.org/10.1016/j.ympev.2015.03.019

Roesler, H., Bauer, A. M., Heinicke, M. P., Greenbaum, E., Jackman, T., Nguyen, T. Q., & Ziegler, T. (2011). Phylogeny, taxonomy, and zoogeography of the genus *Gekko* Laurenti, 1768 with the revalidation of *G. reevesii* Gray, 1831 (Sauria: Gekkonidae). *Zootaxa*, *2989*, 1–50. https://doi.org/10.11646/zootaxa.2989.1.1

Schmitt, L. H., How, R. A., Hisheh, S., Goldberg, J., & Maryanto, I. (2000). Geographic patterns in genetic and morphological variation in two skink species along the Banda Arcs, southeastern Indonesia. *Journal of Herpetology*, *34*, 240–258. https://doi.org/10.2307/1565421

Smith, M. J., Cogger, H., Tiernan, B., Maple, D., Boland, C., Napier, F., … Smith, P. (2012). An oceanic island reptile community under threat: The decline of reptiles on Christmas Island, Indian Ocean. *Herpetological Conservation and Biology*, *7*, 206–218.

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*, 585–595.

van Kampen, P. N. (1923). *The amphibia of the Indo-Australian Archipelago* (p. 304). Leiden, the Netherlands: E. J. Brill Ltd.

van Lidth de Jeude, T. W. (1895). Reptiles from Timor and the neighbouring islands. *Notes from the Leyden Museum*, *16*, 119–127.

Whittaker, R. J., & Fernández-Palacios, J. M. (2007). *Island biogeography: Ecology, evolution, and conservation*. Oxford, UK: Oxford University Press.

Whitten, A. J., Mustafa, M., & Henderson, G. S. (1987). *The ecology of Sulawesi*. Yogyakarta, Indonesia: Gadjah Mada University Press.

Yorke, C. (1983). Survival of embryos and larvae of the frog *Polypedates leucomystax* in Malaysia. *Journal of Herpetology*, *17*, 235–241. https://doi.org/10.2307/1563825

Yuwono, F. B. (1998). The trade of live reptiles in Indonesia. *Mertensiella*, *9*, 9–15.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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