

The impact of Pleistocene glaciations on population structure and systematics in five snake species in the Banda Arc islands of southern Wallacea: the views from genes, morphology and species assemblages

IBNU MARYANTO¹, SUSAN HISHEH^{2,3}, MAHARADATUNKAMSI^{1,2}, RICHARD A. HOW^{2,4} & LINCOLN H. SCHMITT^{2*}

¹ Museum Zoologicum Bogoriense, Research Centre in Biology, Indonesian Institute of Sciences (LIPI), Jl. Raya Cibinong KM 47, Cibinong, Indonesia

² School of Human Sciences, The University of Western Australia, Perth, WA 6907, Australia

³ Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Vic 3010, Australia

⁴ Research & Collections, Western Australia Museum, Welshpool, WA 6986, Australia

* Corresponding Author: ✉ linc.schmitt@uwa.edu.au

Abstract

Wallacea is a region of geographic overlap in biotas derived from both Asian and Australasian continental plates. Species have also evolved rapidly over the last few (ca. 5) million years, aided by isolation and dynamic changes in island extents during the Pleistocene, to produce high levels of endemism. Vertebrate surveys on 26 islands some 30 years ago confirmed the high diversity of reptiles in the Inner and Outer Banda Arcs of Wallacea and numerous species endemic to the region. Here we assess the genetic relationships of island populations of five snake taxa (*Dendrelaphis inornatus*, *Coelognathus subradiatus*, *Lycodon capucinus*, *Psammodynastes pulverulentus*, *Trimeresurus insularis*) that we had previously examined using only morphological variables. Island heterozygosity is within the range generally reported for reptiles, although on the low side, consistent with their island location. One of the five species we examined, *Trimeresurus insularis*, shows a statistically significant decline in island mean heterozygosity from west to east, a phenomenon we have observed in some mammalian species in the Banda Arcs. Genetic data confirm speciation and endemism in the Banda Arcs within the genus *Dendrelaphis*. Populations on Kai and Yamdena islands on the Outer Banda Arc are genetically distinct from those on the islands to their west and are closely allied to the recently described *D. grimeri* from the Maluku islands. *Dendrelaphis pictus* occurs on Lombok and Nusa Penida, whereas *D. inornatus* spans the intervening islands of the Lesser Sunda Islands. Within all taxa, aggregations of island populations that are genetically alike are almost always geographically separated from genetically different groups by straits that persisted through the last Pleistocene glaciation. However, genetic differentiation is not invariably associated with these Pleistocene-persistent straits. Several snake species on Alor, on the Inner Banda Arc, are more closely allied to those on Timor and other Outer Banda Arc islands, and some on Sumba, within the Outer Banda Arc, are genetically closest to populations on the nearby Inner Banda Arc islands. The major separation of snake assemblages across Lesser Sunda Islands coincides with a Pleistocene-persistent sea barrier.

Keywords: biogeography, genetic diversity, islands, morphology, population structure, snakes, species assemblages, systematics, Wallacea

Manuscript received 1 April 2021; accepted 9 November 2021

INTRODUCTION

The loss of biodiversity, particularly among endemic species, is focal to international conservation efforts as we enter the sixth major global biodiversity extinction pulse in the world's evolutionary history (Ceballos *et al.* 2020). Conversely, understanding the mechanisms that foster the development of biodiversity and endemism, particularly among islands, is imperative to maintaining diversity and is essential to enhancing conservation efforts and management. Indonesia is amongst the most biodiverse countries in the world having the greatest

number of mammal species, fourth highest number of bird species and third highest reptile diversity (McNeely *et al.* 1990). This enhanced diversity results from the country spanning two of the world's major biogeographic regions, the Australasian and the Asian, as well as covering two major tropical rainforest blocks, Borneo-Sumatra on the Asian tectonic plate and New Guinea on the Australian tectonic plate. Lying between these major blocks is Wallacea, a complex mosaic of islands that form a 'stepping-stone' corridor for species colonisation from the two main biogeographic regions and provides important opportunities for speciation amongst colonising taxa.

Wallacea, first proposed as a dynamic region in its own right by George Gaylord Simpson over 40 years ago (Simpson 1977; Ali & Heaney 2021), is now acknowledged for its high levels of vertebrate biodiversity and endemism as well as being one of the world's 35 major biodiversity hotspots (Myers *et al.* 2000). The southern islands of Wallacea (Fig. 1) are arranged in two adjacent arcs, the Inner and Outer Banda Arcs (IBA and OBA, respectively), arising from the collision of the Asian, Australian and Pacific continental plates (Veevers 1991). The western islands in the Banda Arcs comprise the Lesser Sunda Islands, so named for their close proximity to the major Sunda Islands of Sumatra, Borneo and Java on the Sunda Shelf. The high biodiversity and endemism across the islands of the Banda Arcs is a consequence of its tropical location, pronounced environmental gradients and the fragmentation of species populations on the myriad of islands in the region. The arrangement of islands in the Banda Arcs has also undergone change during the last Pleistocene glaciation as a consequence of fluctuating sea levels. These environmental circumstances, coupled with Wallacea's position between two of the major global biogeographic realms, highlights the potential for speciation, evolution, and adaptation within species populations. However,

despite Wallacea having been a focus of zoogeographic research for over 160 years, Kitchener and colleagues described 28 new taxa across the Banda Arcs during 1987–1994 (Kitchener & Suyanto 1996).

The fauna of the Banda Arc islands has strong morphological and genetic patterning (Schmitt *et al.* 1995; Schmitt *et al.* 2009) and high endemism (Vane-Wright 1991; Kitchener & Suyanto 1996; How & Kitchener 1997; Zug 2010) with most island populations exhibiting strong links to those on adjacent islands. Nonetheless, speciation has occurred between populations that occupy adjacent islands in both Banda Arcs, especially those that remained permanently separated throughout the Pleistocene when sea levels fluctuated between glacial and interglacial periods. This differentiation between species populations is particularly pronounced in the molecular patterning amongst populations of both the fruit and insectivorous bat families. At the peak of the last Pleistocene glacial maxima around 18000 years bp, sea levels were up to 120 m below present levels (Lewis *et al.* 2013) and many of the Banda Arc islands, presently separated by shallow seas, were joined by land-bridges to form considerably larger composite islands (Heaney 1991).

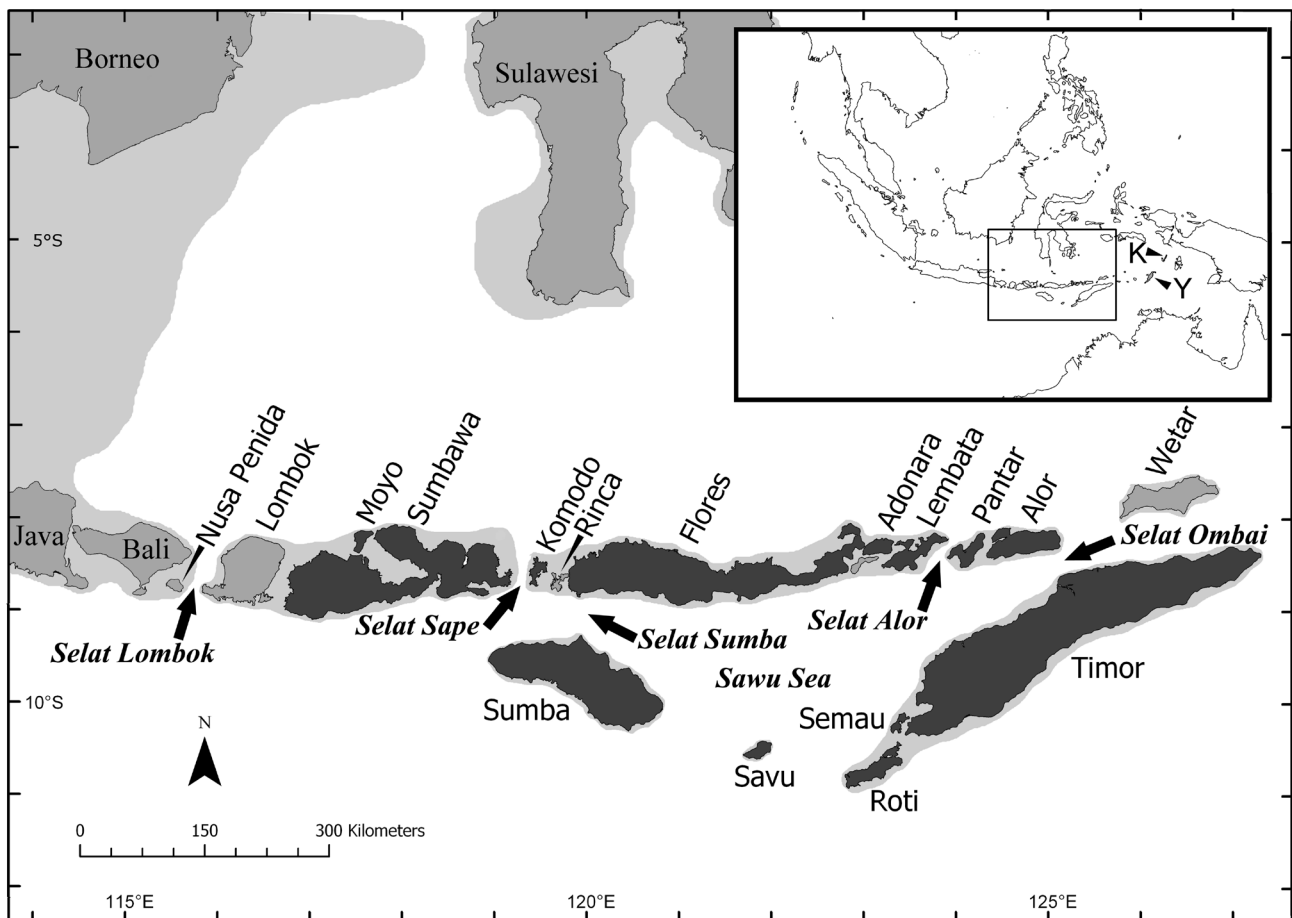


Figure 1. Regional island geography. Light shading indicates the maximum extent of islands and the Asian continent at the last Pleistocene glacial maximum. Arrows with italicized labels indicate the straits (selats) between sampled islands that persisted throughout the last Pleistocene glaciation. The 13 islands sampled for genetic analysis of the five taxa in this study have dark shading, with horizontal labels for Outer Banda Arc islands and angled labels for Inner Banda Arc islands. *Dendrelaphis* spp. on Nusa Penida, Lombok, Yamdena (Y in inset map) and Kai Besar (K) also were genetically assessed, and specimens from Rinca and Wetar were included in the species assemblage analysis.

The biogeographic relationship of snake faunas through the Indonesian islands indicates two principal groupings at both the generic and species level (How & Kitchener 1997). Those on Greater Sunda Islands on the Sunda shelf, Sulawesi and the Lesser Sunda Islands all are derived from Asia, whereas genera and species on islands of the southern and northern Maluku, West Irian and adjacent islands are more closely related to those in the Australo-Papuan region. Our previous work on morphological differentiation in five snake species across the Inner and Outer Banda Arcs (How *et al.* 1996a; How *et al.* 1996b) showed that populations on islands that remained separated throughout the Pleistocene had the highest morphological differentiation. However, in these snake and some mammalian taxa, this morphological differentiation was absent, or much reduced, in species that had good dispersal capabilities or were commensal with humans and easily transported (Kitchener & Suyanto 1996).

Important systematic research on the snake fauna of the Indonesian region in the last 20 years has highlighted the diversity of cryptic species in many of the genera through the region. Of particular relevance to the Lesser Sunda Islands are the reviews of *Trimeresurus* (David *et al.* 2003) and *Dendrelaphis* (van Rooijen & Vogel 2008; Vogel & van Rooijen 2008). In Indonesia both genera contain several taxa that show major geographic patterning. The molecular study of *Trimeresurus* confirmed the earlier findings of Giannasi *et al.* (2001) that the Lesser Sunda island form, *T. albolabris insularis*, warranted distinct specific status. The above revisions of the *Dendrelaphis pictus* complex from southeast Asia described new taxa for the Mollucca region and evaluated the systematic status of many species and subspecies across the complex's range. Additionally, a more recent evaluation of snake biogeography across the Lesser Sunda Islands (de Lang 2011) highlighted the high endemism (28%) within snakes of those islands and endorsed the biogeographic relationships described by How & Kitchener (1997). De Lang (2011, p. 50) also stated for the Lesser Sunda Islands that 'The taxonomy of several genera is uncertain and needs to be reviewed'. This state of flux in the taxonomy of many snakes can be extended to other reptilian genera across the whole Wallacean region (Schmitt *et al.* 2000; Zug 2010).

This study emanates from an extensive geographic assessment of variation in the distribution, morphology and genetics of terrestrial vertebrates from across the southern Wallacean region (Kitchener & Suyanto 1996; How & Kitchener 1997; Schmitt *et al.* 2009). Here we assess genetic variation in five snake taxa (Fig. 2) on the islands of the Lesser Sundas, a viperid (*Trimeresurus insularis*) and four colubrid (*Coelognathus subradiatus*, *Dendrelaphis inornatus*, *Lycodon capucinus* and *Psammodynastes pulverulentus*); nomenclature follows the Reptile Database (Uetz *et al.* 2021). The geographic patterns in variation are interpreted in the context of the biogeographic setting, and especially related to the longevity of sea barriers during the Pleistocene glacial fluctuations. They are also compared with morphometric assessments of the same taxa. We also assess the snake assemblages on the Lesser Sunda Islands in light of new distributional information and recent changes in systematic determinations and interpret these within

the context of their relationship to those in the broader Wallacean region. For all analyses, we assume the taxonomic status of *Dendrelaphis inornatus* (Boulenger 1897) and provide genetic evidence in support of that assessment.

MATERIALS AND METHODS

As part of a large survey between 1987 and 1993 (Kitchener & Suyanto 1996; How & Kitchener 1997), we sampled reptiles on 26 Wallacean islands in the Banda Arcs and the Sunda and Sahul Shelves. All specimen material and tissue reside in the collections of the Museum Zoologicum Bogoriense or the Western Australian Museum. Unless otherwise noted, GENSTAT (VSN International 2020) was used for all statistical analyses.

Genetics

Genetic variation for the five species compared here—*Trimeresurus insularis*, *Coelognathus subradiatus*, *Dendrelaphis inornatus*, *Lycodon capucinus* and *Psammodynastes pulverulentus*—was assessed by allozyme electrophoresis on a cellulose acetate support medium according to Richardson *et al.* (1986) using liver tissue from 13 islands (Table 1; Appendix 1). Initially, we also assessed genetic variation in two geographically-adjacent cognates of *D. inornatus*; i.e. *D. pictus* from Nusa Penida (N = 2) and Lombok (N = 4), and *D. calligaster* from Yamdena (N = 2) and Kai Besar (N = 2). All four populations were excluded from further analysis because of their genetic differentiation from *D. inornatus* (see **Results**).

The extent of genetic variation within islands was assessed using mean unbiased expected heterozygosity (Nei 1978). Longitudinal trends in mean island heterozygosity were investigated using regression. Hierarchical *F*-statistics (Weir & Cockerham 1984) were used to estimate genetic variation between islands (F_{ST}), between localities within islands (F_{DS}), and within localities (F_{ID}) and tested for statistical significance using permutation (Excoffier 2003). To ascertain the interrelationships between populations as indicated by genetic data, genetic distances between island populations were estimated by the arc measure of Cavalli-Sforza & Edwards (1967) and subjected to principal coordinates analysis (PCO; Gower 1966).

Morphology

We undertook stepwise discriminant function analyses of our previously published morphological data (Appendix 2; How *et al.* 1996a; How *et al.* 1996b), limiting each taxon to those islands where genetic data were available. First, forward selection was used to obtain the four 'best' discriminating variables (based on minimizing Wilks' lambda) and then repeated using backward selection. From all variables selected by these two methods we used backward selection to obtain the best four variables to compute the final discriminant functions. The plots of island centroids on the first two discriminant functions were then examined and compared with their relative positions on the first two PCO coordinates. We also computed the correlations between the Mahalanobis



Figure 2. Images of the snake species evaluated with their location, island, and month and year of capture: **a)** *Coelognathus subradiata*, Waelonda Sumba, June 1989, [N Cooper]; **b)** *Dendrelaphis pictus*, Kuta Lombok, June 1988 [R Johnstone]; **c)** *Dendrelaphis inornatus*, Batu Tering Sumbawa, May 1988, [R Johnstone];. **d)** *Dendrelaphis inornatus*, Camplong Timor, October 1990 [R Johnstone]; **e)** *Lycodon capucinus*, Meraran Sumbawa, May 1988, [R Johnstone]; **f)** *Psammodynastes pulverulentus*, Robo Flores, May 1990, [R Johnstone]; **g)** *Trimeresurus insularis* (blue morph), Gn. Api Komodo, May 1990, [R Johnstone]; and **h)** *Trimeresurus insularis* (yellow and green morphs), Ipokol Wetar, September 1993 [R How]. Square brackets indicate photographic credits.

and genetic distance matrices, and tested statistical significance using a modification of Mantel's test (Manly 2005).

Snake Assemblages

We assessed the biogeographic relationships of snake assemblages on 13 islands within southern Wallacea that had more than five snake taxa. This included 11 of the islands in the genetic study described above, and the nearby islands of Rinca and Wetar. We first constructed a species-by-island matrix of presence/absence of species and then used PRIMER E (Clarke & Gorley 2006) to cluster the matrix of Sorensen's similarity index using the unweighted pair group method with arithmetic mean (UPGMA) algorithm. The similarity profiling routine SIMPROF (Clarke *et al.* 2008) was used to determine statistically significant clusters and these relationships, along with relative similarity between islands—these are illustrated using non-metric multidimensional scaling (NMDS).

RESULTS

Dendrelaphis Systematics

Our initial analysis of the genetic relationship between island populations of *D. inornatus*, *D. pictus* and *D. calligaster*, confirmed that the latter was distinct on the first principal coordinate (Fig. 3a). After excluding *D. calligaster* and repeating the PCO analysis, *D. pictus*

also appeared distinct from *D. inornatus* (Fig. 3b). Consequently, we only considered samples from the latter taxon in all further genetic and morphological analyses.

Genetic Diversity

Across all species, within-island mean heterozygosity was 3.4%, and 76% of islands had a mean less than 5% (Table 1). One species, *T. insularis*, showed a statistically significant relationship between mean heterozygosity and longitude (Fig. 4).

There were strong genetic differences between islands for three species, with F_{ST} much greater than zero (Table 2). This statistic was not significant in *L. capucinus* or *P. pulverulentus*, but the latter show statistically significant genetic differentiation between localities within islands (F_{DS}) and evidence of heterogeneity within localities (F_{ID}).

Principal coordinates analyses revealed the nature of the genetic relationships between island populations (Fig. 5, left frames). For all five species, the first two axes combined account for the majority of the variation, ranging from 59% to 93%, and generally provide a good reflection of the information in the genetic distance matrices.

Of the three species where there is statistically significant inter-island variation, *T. insularis* populations aggregate into three regional groups along the Banda Arcs (west, central and east; Fig. 5a). There are also three groups in *C. subradiatus*, one being the OBA islands, another of three IBA islands then Alor, which is distinct

Table 1. Island tissue sample size (N) and mean unbiased percent heterozygosity (\bar{H}). Islands are ordered from west to east within the Inner Banda Arc above the internal horizontal line, and the Outer Banda Arc below.

Island	Code	<i>Trimeresurus insularis</i>		<i>Coelognathus subradiatus</i>		<i>Dendrelaphis inornatus</i>		<i>Lycodon capucinus</i>		<i>Psammodynastes pulverulentus</i>	
		N	\bar{H}	N	\bar{H}	N	\bar{H}	N	\bar{H}	N	\bar{H}
Sumbawa	SW	13	7.0			10	7.7	1	0		
Moyo	MO					1	3.7	1	0		
Komodo	KO	1	8.0	1	0					1	3.4
Flores	FL	4	1.7	4	3.9	2	3.7	1	0	13	6.3
Adonara	AD							1	0		
Lembata	LE	9	2.1	3	2.9	7	5.3	1	3.8	4	5.9
Pantar	PA	2	0			2	3.7	3	4.4		
Alor	AL	12	0	3	2.9	14	2.6	11	3.9	6	4.6
Sumba	SB	6	1.2	4	1.7	3	3.2	6	3.6	2	7.5
Savu	SV					1	3.7				
Roti	RO					1	3.7	3	5.9		
Semau	SE	1	0	2	2.2			1	7.7		
Timor	TI	12	0.3	5	1.1	7	8.7				
Mean			2.3		2.1		4.6		2.9		5.5

Table 2. F -statistics for five snake species of the Lesser Sunda islands.

Species	F_{ST}	F_{DS}	F_{ID}
<i>Trimeresurus insularis</i>	0.59 ($p < 0.001$)	0.24 ($p = 0.052$)	0.15 ($p = 0.047$)
<i>Coelognathus subradiatus</i>	0.69 ($p = 0.003$)	0.11 ($p = 0.49$)	-0.22 ($p = 1$)
<i>Dendrelaphis inornatus</i>	0.62 ($p < 0.001$)	0.04 ($p = 0.67$)	-0.01 ($p = 0.58$)
<i>Lycodon capucinus</i>	0.10 ($p = 0.35$)	0.13 ($p = 0.30$)	-0.16 ($p = 0.96$)
<i>Psammodynastes pulverulentus</i>	0.26 ($p = 0.10$)	0.33 ($p = 0.002$)	0.27 ($p = 0.006$)

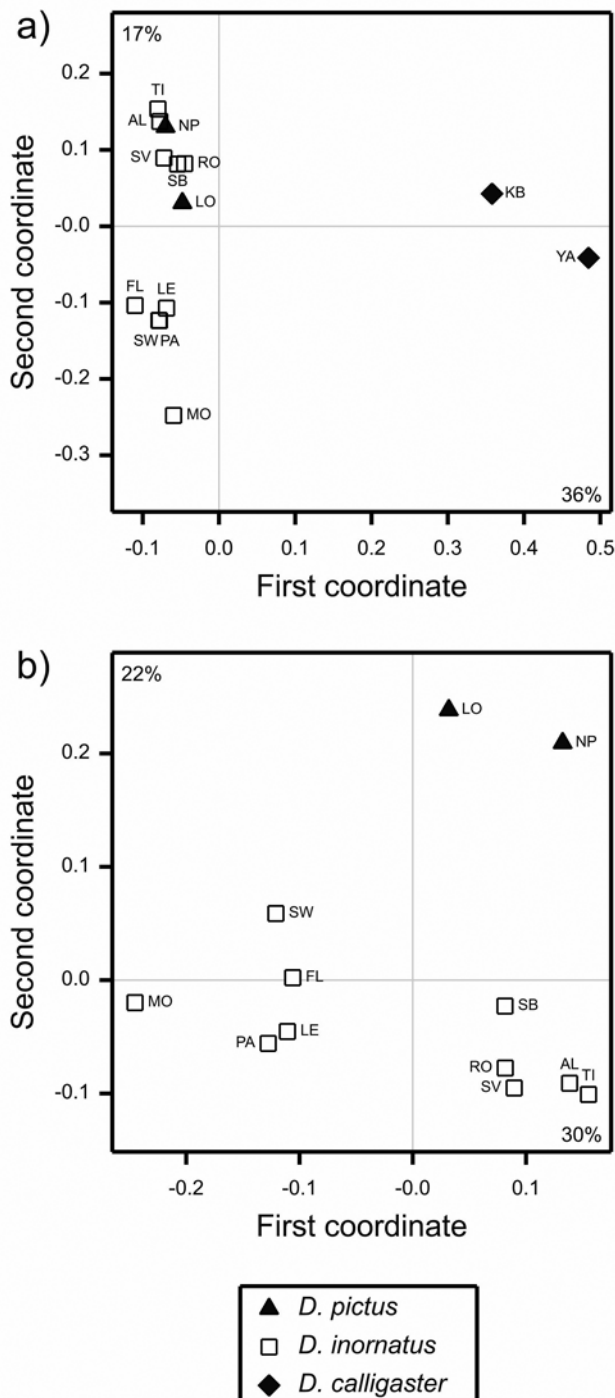


Figure 3. Plots of the first two axes from principal coordinates analysis of genetic distance between Wallacean island populations of *Dendrelaphis* spp.: **a)** island populations ascribed to *D. pictus*, *D. inornatus* and *D. calligaster*; and **b)** island populations ascribed to *D. pictus* and *D. inornatus*. Island codes in Table 1; KB = Kai Besar, YA = Yamdena.

from the other two (Fig. 5b). There is a marked separation into east and west groups of *D. inornatus* islands on the first axis. The eastern group, comprising all the OBA islands and Alor, show marked diversity on the second axis whereas the western group of IBA islands remains tightly aggregated on that axis (Fig. 5c). It is notable that the Alor population of these three species shows a greater affinity to the adjacent OBA islands than to those on the IBA. Considering these three species as a whole, the groupings that they exhibit, with one exception—the Roti population of *D. inornatus*—are separated by perennial sea barriers.

For the two species that do not have statistically significant F_{ST} values, *L. capucinus* and *P. pulverulentus*, the estimate is relatively large (especially so for the latter) and both hint at some genetic structure. The OBA islands of *L. capucinus* aggregate in one corner of the plot, with Alor and Pantar adjacent to that group and Sumbawa, the most western island, is marginal on the second axis (Fig. 5d). In *P. pulverulentus*, the one OBA island sampled, Sumba, is associated with its nearest IBA neighbour (Flores) whereas Alor is distinct from the other four islands sampled (Fig. 5e).

Genetics vs Morphology

The first two discriminant functions derived from morphological variables accounted for at least 80% of the inter-island variation in each species (Table 3). No species shows a significant correlation between genetic distance and morphological distance, although the estimates are higher and approach statistical significance for *Coelognathus* and *Dendrelaphis* compared to the other three species. A visual comparison of the morphology and genetic plots of the first two axes for *Coelognathus* (Fig. 5b) indicates a separation of the IBA and OBA islands in both. There are less obvious similarities in island relationships for genetic and morphological variation in *Dendrelaphis*, although Timor, Roti and Alor are marginal on the first axis for both plots (Fig. 5c). In general the two biological measures do not show many similarities in their geographic patterning.

Snake Assemblage Relationships

Two significantly distinct island groupings are currently identified in southern Wallacea on the basis of their snake assemblages (Fig. 6). A significantly divergent group is present on the islands of the western Inner Banda Arc (excluding Alor and Pantar, but including Sumba), while the other is on the islands on the Outer Banda Arc (excluding Sumba, but including Alor and Pantar).

DISCUSSION

Systematics

Bronzeback colubrid tree snakes (*Dendrelaphis* spp. complex) represent the only genera of the five examined that has a distribution extending from the Asian Sunda shelf to the Australasian Sahul shelf (Mertens 1934; How *et al.* 1996a; Vogel & van Rooijen 2008). *Dendrelaphis* populations on the Maluku islands of Yamdena, Kai and Ambon contain morphologically distinct taxa (How *et al.* 1996a) and could be assigned to the *D. calligaster* complex,

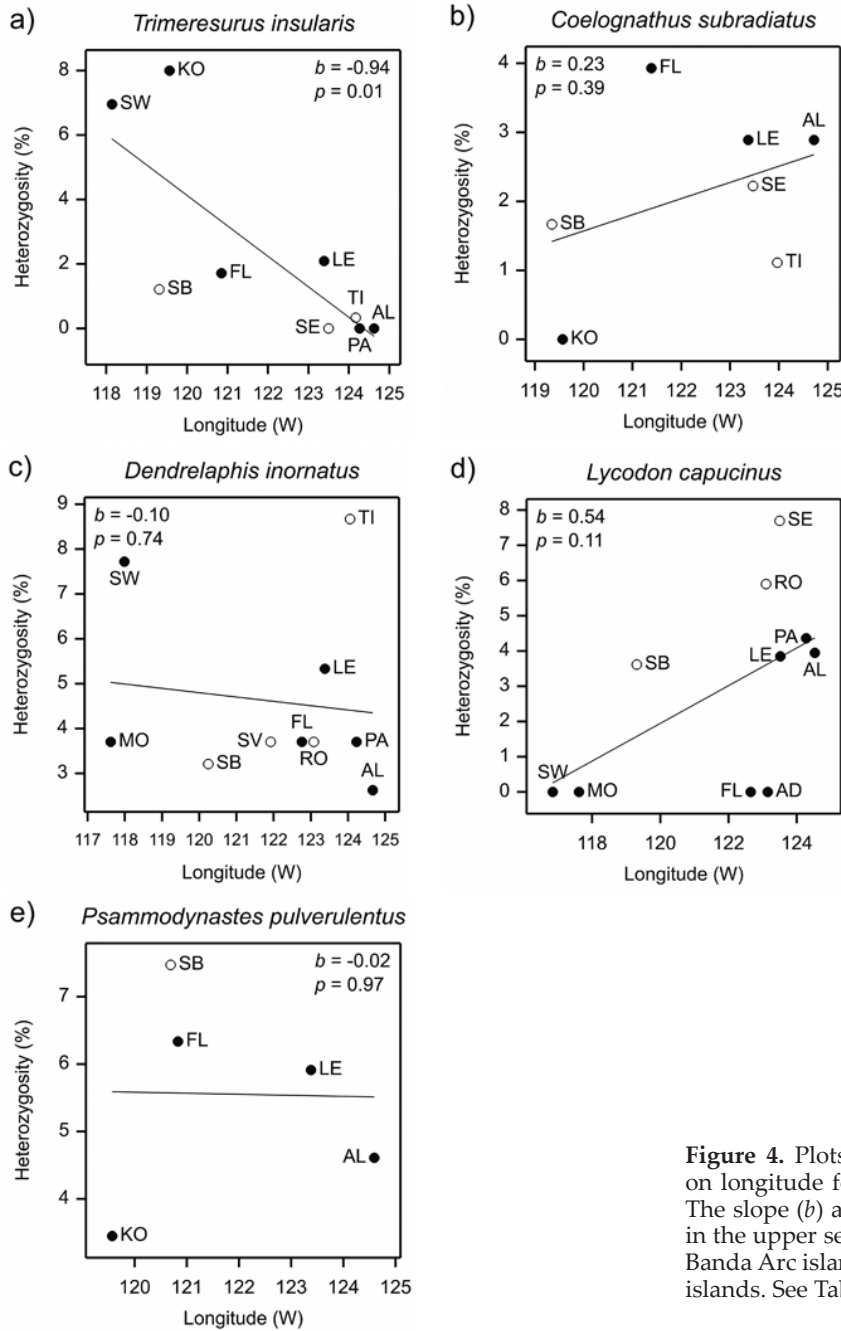


Figure 4. Plots of unbiased mean island heterozygosity on longitude for five taxa with the fitted regression line. The slope (b) and its statistical significance (p) are shown in the upper section of each plot. Filled symbols for Inner Banda Arc islands and open symbols for Outer Banda Arc islands. See Table 1 for the island codes.

Table 3. The four morphological variables selected by stepwise discriminant function analyses, the variance accounted for (%) by the first two latent roots, and the correlation between the Mahalanobis distance matrix and the genetic distance matrix (r), with its statistical significance (p) for five snake species. See Appendix 2 for variable codes.

Species	Selected variables	Function 1	Function 2	r
<i>Trimeresurus insularis</i>	EYNAL, INNAW, NASL, PREOLO	47%	39%	0.06 ($p = 0.41$)
<i>Coelognathus subradiatus</i>	FROW, INNAL, LORV, PARL	45%	37%	0.39 ($p = 0.056$)
<i>Dendrelaphis inornatus</i>	INNAL, PARL, PREFW, ROSW	74%	15%	0.29 ($p = 0.069$)
<i>Lycodon capucinus</i>	EYED, FROL, PREFW, SUOCL	46%	34%	0.03 ($p = 0.42$)
<i>Psammodynastes pulverulentus</i>	FROW, INNAW, LORV, ROSW	60%	36%	-0.03 ($p = 0.29$)

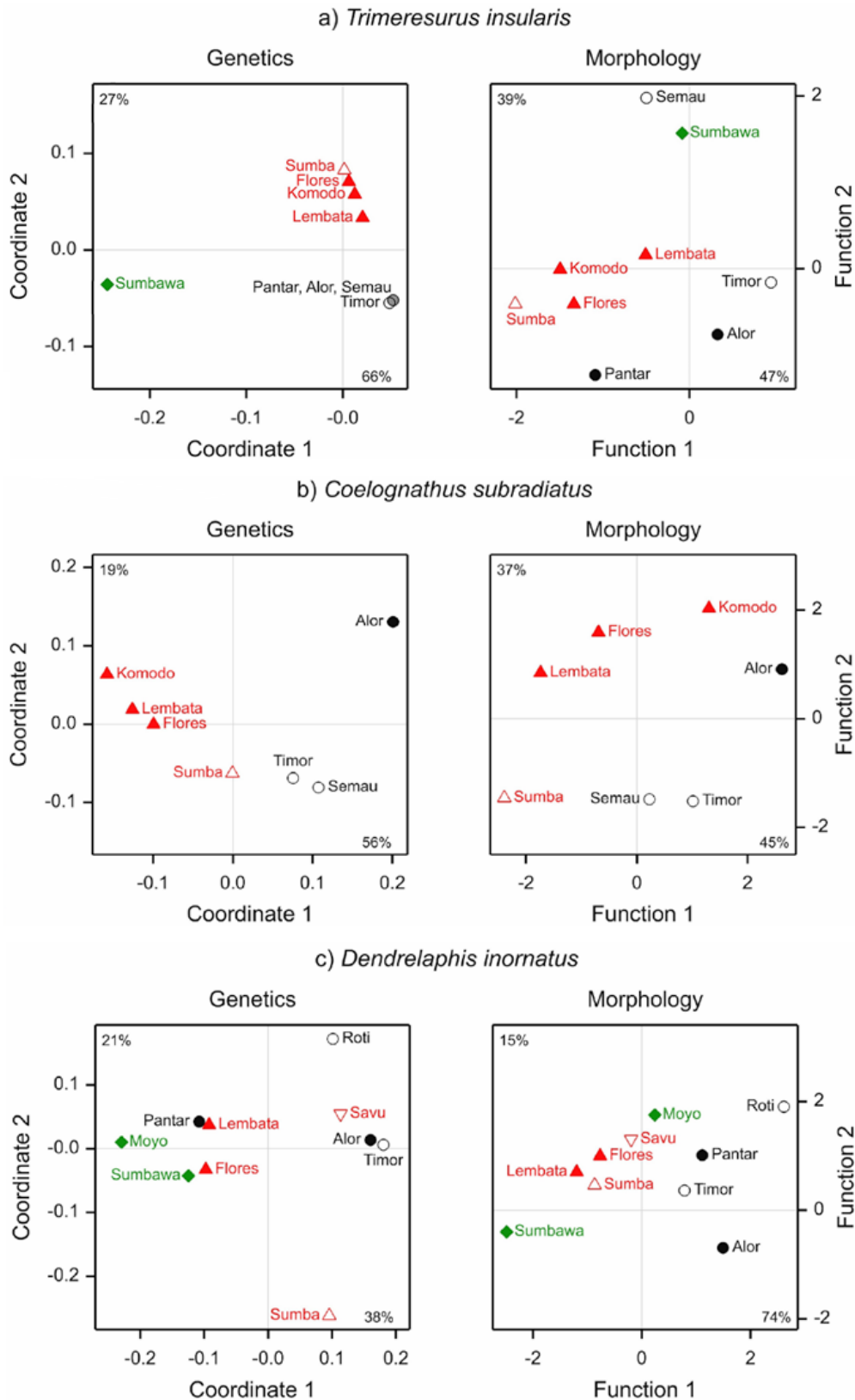


Figure 5. Plots of the genetic (left) and morphological (right) relationship of islands for five species. The genetic relationships represented are the first two coordinates from a principal coordinate analysis, and the morphological relationships are represented by the first two discriminant functions. The contribution to the total variation of each coordinate or function is shown at the upper end of each axis. Filled symbols for Inner Banda Arc islands and open symbols for Outer Banda Arc islands. Islands that were part of the same meta-island during the last Pleistocene glacial maximum share the same symbol and colour. Islands in the Outer Banda Arc have the same symbol and colour as the nearest Inner Banda Arc island (with the exception of ∇ for Savu).

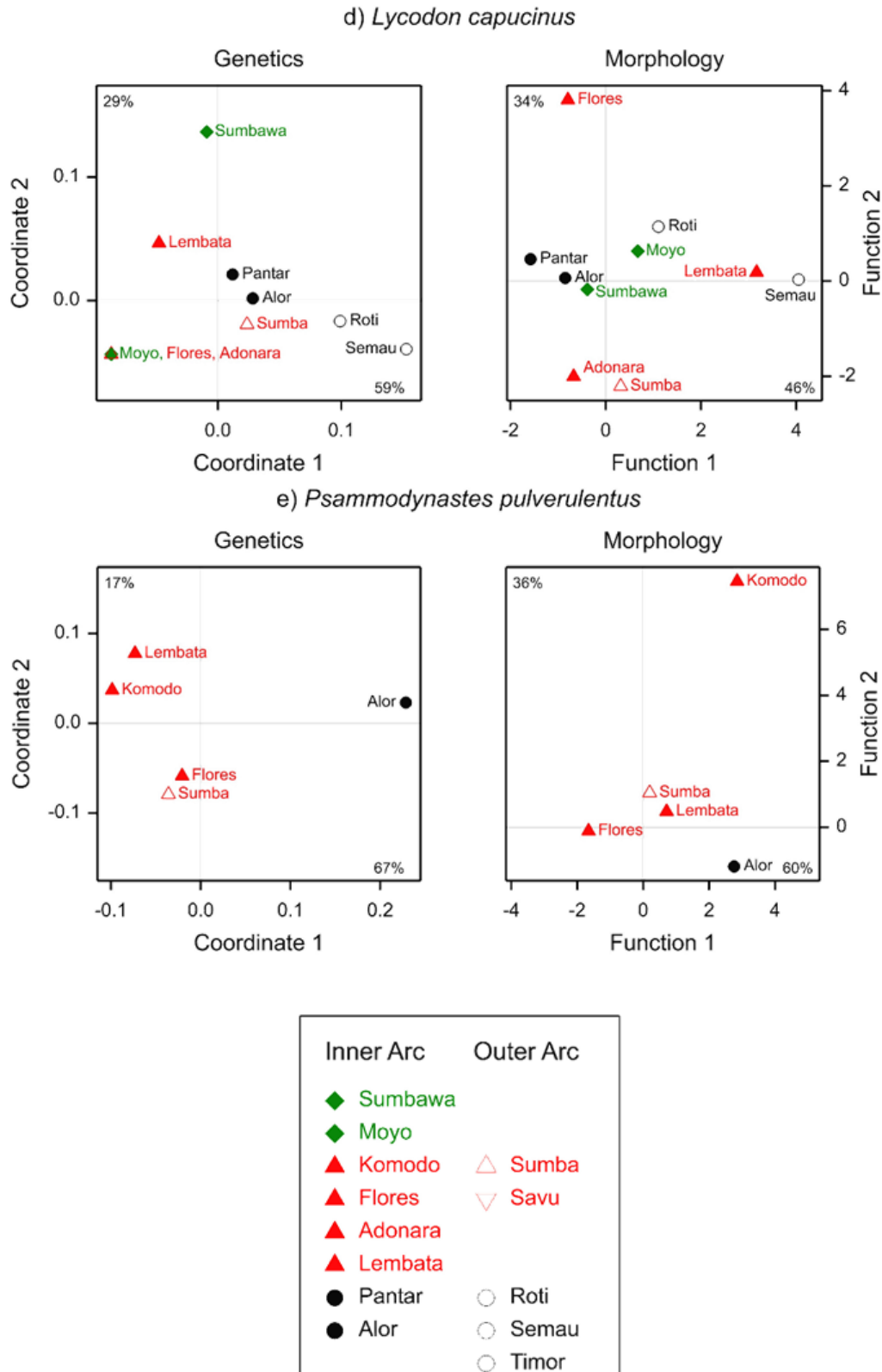


Figure 5. (Cont.)

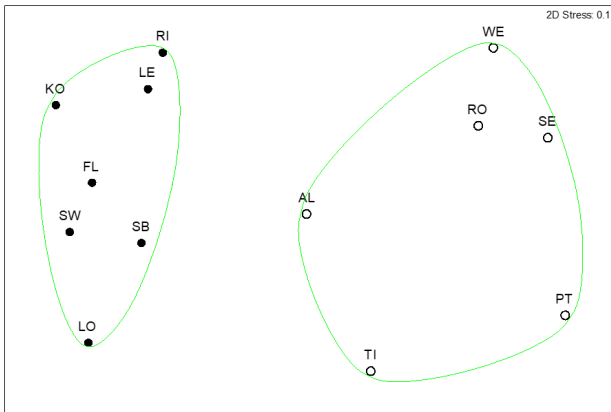


Figure 6. Non-metric multidimensional scale plot (NMDS) of snake assemblages from southern Wallacea, specifically islands across the Lesser Sundas that contain five or more snake taxa. The different symbols indicate significantly different groups of islands and the ellipses mark the 50% similarity groups. Island codes in Table 1; RI = Rinca, WE = Wetar. The two island groups are separated by the Sawu Sea on the Outer Banda Arc, and Selat Alor on the Inner Banda Arc; both straits persisted throughout the Pleistocene sea-level fluctuations.

later described as *D. grismeri* (Vogel & van Rooijen 2008), as all have 13 mid-body scale rows whereas all *Dendrelaphis* individuals on the Lesser Sunda Islands have 15 mid-body scale rows. How *et al.* (1996a) also indicated that the population on Kai Besar should be assigned to the described taxon *D. kaiensis*, but the population on Yamdena, in the Tanimbar Archipelago, differed significantly and probably represents a new taxon.

Our genetic study confirms that the *Dendrelaphis* spp. populations on the eastern OBA islands of Yamdena and Kai Besar are discrete taxa, probably with close affinities to *D. grismeri*, the taxon described from other Maluku islands (Vogel & van Rooijen 2008). Genetics also confirms two species of *Dendrelaphis* spp. (*sensu pictus*) are present along the Lesser Sunda Islands of southern Indonesia. The populations on Nusa Penida and Lombok islands represent the more widespread taxon of *D. pictus* (Fig. 4b) but are discrete from populations of *D. inornatus* along the remaining Lesser Sunda Islands.

Dendrelaphis pictus (*sensu stricto*) from the islands of south-eastern Indonesia was examined in morphological detail by Mertens (1934): he recognised *Dendrelaphis pictus pictus* on Bali and Lombok, but also extending onto Java and Sumatra; *D. p. inornatus* on Sumba, Timor and Alor; *D. p. intermedius* on Sumbawa and Flores; and considered *D. p. timorensis* on Timor was synonymous with *D. p. inornatus*. Our reappraisal of the morphology of *Dendrelaphis* populations from Bali and the Lesser Sunda Islands (How *et al.* 1996a) determined those on Bali, Nusa Penida and Lombok should be assigned to the widely distributed taxon, *D. pictus*, whereas those on the remaining islands of the Lesser Sundas should be referred to *D. inornatus*. This genetic study confirmed the specific status of *D. pictus* and *D. inornatus*, but also added molecular support for the morphological subspecies defined by Mertens (1934), with his *D. p.*

inornatus on Sumba Timor and Alor, and *D. p. intermedius* on Sumbawa, Moyo, Flores, Lembata and Pantar.

The Rat Snakes, *Coelognathus* spp, are a wide-ranging and variable genus having the highest species richness for any colubrid genus and an extensive distribution (Schulz & Entzeroth 1996). Morphological differences in *Coelognathus* are such that the population on the Lesser Sunda Islands (*C. subradiatus*) warranted full specific status (in den Bosch 1985). Thus the Lesser Sunda Rat Snake, *C. subradiatus*, is endemic to the islands of the Lesser Sundas and consists of three discrete groups. The population on Alor is genetically distinct from those on other IBA islands of Komodo, Flores and Lembata, as well as from those on the OBA islands of Timor, Semaun and Sumba, which are discrete from the IBA on the second axis of Figure 4b. This pattern of genetic separation resembles earlier morphology evidence (How *et al.* 1996b) that showed populations on Alor and Wetar were morphologically distinct from those on Timor+Semaun+Sumba and are, in turn, distinct from those on Komodo+Flores+Lembata. However, these genetic data do not support the differentiation of the Sumba population, as implied by morphology (How *et al.* 1996b), but do show major genetic differentiation across several of the Pleistocene-persistent sea barriers. Recent genetic examination of *C. subradiatus* populations from the Lesser Sunda Islands using the ND4 gene (Reilly *et al.* 2021) defined three distinct clades (Alor, Wetar+Timor, Sumbawa+Flores). They postulate that the clades represent a species complex, supporting our earlier morphological study, but suggest more extensive examination is needed to confirm this speciation across the Lesser Sunda Islands.

The Wolf Snake, *Lycodon capucinus*, is widely distributed throughout southeast Asia, south-eastern China, the Philippines and Indonesia (except Borneo), and in this study showed limited genetic variation between island populations across the Lesser Sunda Islands—a finding supported by the limited mtDNA structure in the species throughout its range (Reilly *et al.* 2019). This lack of genetic patterning is consistent with the morphology (How *et al.* 1996b), and its known distribution and ecological parameters. *Lycodon capucinus* is recognised as a rapid coloniser (Smith 1998) of new environments and easily transported from one island to another by human activity (Leviton 1965; Reilly *et al.* 2019). These ecological characteristics are reflected in its relatively undifferentiated island populations in both morphological and genetic space.

The Mock Viper, *Psammodynastes pulverulentus*, has the widest distribution of all species examined in this study, extending from north-eastern India, throughout southeast Asia, southern China, Taiwan, the Philippines and in Indonesia as far east on the IBA as Alor (de Lang 2011). However, on the OBA islands it only occurs on Sumba. Genetic data indicates a distinct separation of the population on Alor, in accordance with our earlier morphological study (How *et al.* 1996b).

The White-lipped Island Pit Viper, *Trimeresurus insularis*, extends from eastern Java onto the Lesser Sunda Islands, with its easternmost record from the island of Kisar (David *et al.* 2003) and it showed limited morphological variation along the island chains of eastern Indonesia (How *et al.* 1996b). Malhotra & Thorpe (1997)

concluded that a complex phylogenetic pattern existed within *Trimeresurus* in Indonesia such that species and their relationships were difficult to determine using morphological and molecular techniques. Within *T. insularis*, the mtDNA patterns of island populations did not correspond with morphological patterns (Malhotra and Thorpe *op. cit.*), making the differentiation of taxa impossible using morphological techniques alone. The later study by David *et al.* (2003) indicated that there was limited mtDNA separation of populations between Java and Wetar despite reporting a Java clade, a Flores clade and a Komodo to Alor clade. Reilly *et al.* (2019) explored mtDNA sequences in the species across the Lesser Sunda Islands and showed low genetic diversity with Lombok forming a monophyletic group, but supported by just one mutation. The present study indicates a genetic difference only in the Sumbawa population.

Important revisions of reptile taxa across the Lesser Sunda Islands, indicate that many have 'species' level differences based solely on morphological criteria. The widely distributed colubrid tree snake, *Boiga cynodon*, has been revised using both morphology and genetics (Ramadhan *et al.* 2010; Weinell *et al.* 2021), and determined to have a Lesser Sunda endemic species, *Boiga hoeseli*. Wüster & Thorpe (1989) revised the Asiatic Cobra, *Naja sputatrix*, a species that extends along much of the IBA (de Lang 2011), and detected three morphologically distinct groups: the populations on Komodo and Flores differ from those to the west on Sumbawa through to east and central Java, and in turn these differed from the western Java population. The morphological documentation of subspecific separation in the volant agamid, *Draco volans*, across the islands of Nusa Tenggara (Musters 1983) is remarkably similar to the morphologic differentiation of subspecies determined for *Dendrelaphis pictus* (Mertens 1934). Separation in *Draco* occurred somewhere between Adonara and Alor on the IBA, and Sumba and Roti on the OBA. Similarly, How *et al.* (1998) determined that the skink *Emoia* on Sumba was morphologically distinct from *Emoia similis* on Flores and Komodo and represented an endemic taxon.

Genetic diversity

At 3.4%, mean heterozygosity within the five taxa we examined is generally a little lower than the 5–7% typically reported in reviews of reptilian studies (Nei 1975). However, most studies are of continental populations and it is well known that island populations generally have lower levels of genetic diversity than their mainland counterparts, so these five taxa seem to have typical levels of genetic diversity given their insular locations.

We previously reported that some Lesser Sunda mammalian species of Asian origin show a decline in heterozygosity from west to east (Schmitt *et al.* 2009). In the present study we observed this in *Trimeresurus insularis* but not in the other four species. Determining the cause of such trends is problematic. An obvious explanation is that population sizes may decline from west to east but that leaves the question of what may cause this trend. There are known environmental gradients from west to east, such as decreasing rainfall and increasing woodland habitats, that may be driving heterozygosity directly or via population size, but we

have virtually no information that could lead to a credible explanation.

Our observation of strong genetic differences between islands for three species is most likely, in our view, to be the consequence of restricted gene flow between islands and the consequent impact of random genetic drift. Nonetheless, we cannot exclude differential natural selection, although variation in the loci we have utilized is, typically, ascribed to a balance between gene flow and genetic drift.

A balance between gene flow and genetic drift as a major cause of genetic diversity among islands is supported by an association between the degree of inter-island genetic differentiation and the type of sea barrier between the islands—in general, genetically-determined island groups are separated by sea barriers that persisted throughout the Pleistocene. Genetic differentiation across Selat Alor seems to be particularly strong and suggests this strait has constrained gene flow in all five species with Alor populations being distinct from their IBA cognates. We have constructed a somewhat subjective summary of the impact of the Pleistocene-persistent straits on genetic differentiation for each species to illustrate the extent of this generality (Table 4).

Such barriers, however, do not inevitably lead to strong genetic differentiation. The Sumba populations of *Trimeresurus*, *Lycodon* and *Psammodynastes* all have strong affinity with Sumbawa, despite separation by the large Selat Sumba. The wide Selat Ombai also appears to be a relatively weak barrier to gene flow for *Dendrelaphis*, *Trimeresurus* and *Lycodon*, with their Alor populations showing close affinity with Timor and the other OBA islands.

Islands separated by ephemeral sea barriers—those that disappeared during Pleistocene glacial maxima—almost always contain genetically similar faunas. A notable exception is the Roti population of *Dendrelaphis*, which is distinct from Timor's (although the sample size of the former was only one) and these two populations occupy similar positions on the first PCO coordinate. Similar observations and conclusions can be made for the differentiation of the Flores population of *Psammodynastes* compared to that from Komodo and Lembata.

Morphological island relationships

This study provides modest evidence for a correlation between genetic and morphological pattern in *Dendrelaphis* and *Coelognathus* populations across the Lesser Sunda Islands. The lack of a significant correlation between the genetic and morphological patterns for the other three genera illustrates the importance of combining both measures of variation in order to be confident of population level differences that may translate to taxonomic separation.

The genetic and morphological appraisal of two skink species along the Banda Arcs of eastern Indonesia (Schmitt *et al.* 2000) showed that genetic variation was not matched by similar separation in morphological characters in either taxa. They suggested that sibling taxa may be evolving in the Lesser Sunda Islands, which were not detectable morphologically. The wide-ranging skink *Mabuya multifasciata*, showed little morphological or genetic variation along the Banda Arcs; however, a

Table 4. Summary of the effects of Pleistocene sea barriers on the population structure of five snake species.

Species	Selat Sape	Selat Alor	Selat Sumba	Sawu Sea	Selat Ombai
<i>Trimeresurus insularis</i>	++	++	0	++	0
<i>Coelognathus subradiatus</i>		++	+	+	++
<i>Dendrelaphis inornatus</i>	0	++	++	?	?
<i>Lycodon capucinus</i>	+	+	0	?	0
<i>Psammodynastes pulverulentus</i>		++	0		

Note: + and ++ indicate weak and strong differentiation across a barrier; 0 indicates no differentiation; ? indicates variable or uncertain differentiation. Empty cells indicate no assessment was possible.

similarly wide-ranging skink, *Lamprolepis elberti*, showed a genetic separation between populations on the Lesser Sunda Islands that was not matched by morphological characters such that cryptic species probably existed. Morphologically conservative but genetically distinct taxa have also been recorded in the skink genera *Emoia* (Bruna *et al.* 1996) and *Lipinia* (Austin 1995) on islands in the Pacific Ocean.

Biogeography of snake assemblages

Biogeographic relationships of 36 islands in the Indonesian archipelago, using land and freshwater snakes (How & Kitchener 1997), documented two main groups incorporating: (1) the Greater Sunda Islands, the Lesser Sunda Islands, Sulawesi and the Banggai and Sula archipelagos; and (2) the islands of northern and southern Maluku, New Guinea and adjacent islands and Australia. The former group contains principally Asian snake genera and species, whereas Australo-Papuan genera and species dominate in the latter. This major division in snake faunas is evident between the islands of Maluku in the east and Sulawesi and the Lesser Sunda Islands in the west, indicating the major boundary in the snake fauna of Indonesia is Weber's Line, rather than Wallace's Line. Holloway (2003) reanalysed How and Kitchener's data, and their results agreed, for the greater part, with those published earlier.

The present reappraisal of biogeographic relationships of islands of southern Wallacea is based on their snake assemblages using currently determined distributions of species and their taxonomy. Within the Lesser Sunda Islands, the IBA islands of Alor and Wetar have snake faunas more similar to Savu, Roti, Semau and Timor on the OBA, than to the more westerly Lesser Sunda Islands of Sumbawa, Moyo, Komodo, Flores, Lembata and Pantar that are also IBA islands. However, the Sumba fauna on the OBA is more closely allied to that on proximal IBA islands than to any other OBA island. This paper provides strong support to Alor linking with OBA islands, except Sumba, as described by How & Kitchener (1997), although it does not support their separation of Lembata, Flores and Komodo from either Pantar, Sumba or Sumbawa.

CONCLUSIONS

By the nature of its geographic location and extent, Wallacea's biota is subjected to an island environment

and high insularity. The region is geologically young and has experienced variations in sea-level of up to 120 m associated with recent Pleistocene glaciations that influenced island size and connectivity. The glacial cycles led to the coalescence of some islands as sea-levels declined during cooler periods and, conversely, the fragmentation of larger composite islands during warmer periods. These fluctuations in island arrangements facilitated evolution of many endemic species throughout the region. Isolation by straits that persisted throughout the Pleistocene seems, at least for less mobile species, to be a primary cause of their population structure, whereas the genetic affinities of island populations separated by ephemeral straits is generally much greater. Consequently, significant differentiation occurs in most Wallacean vertebrates separated by persistent sea barriers over the last two million years, giving rise to populations with high local endemism that are important for active conservation management.

ACKNOWLEDGEMENTS

The former directors of the Puslitbang Biologi Bogor, Dr Kardasan, Dr Amir, Dr Soetikno and Dr Hainald and of the Western Australian Museum, John Bannister, provided continuous support and encouragement of the survey of the vertebrate fauna of Wallacean islands. The survey was conceived, planned and orchestrated by the drive and persistence of our colleague, Darrell Kitchener. The collecting would not have been possible without his field assistance and that of many other colleagues, including Ken Aplin, Boedi, Norah Cooper, John Dell, Ron Johnstone, Dennis King, Najamuddin, Laurie Smith, Augustine Suyanto, Kirstin Tullis, and Chris Watts, as well as many volunteers. We greatly appreciate the generosity of Ron Johnstone and Norah Copper for allowing us access to their photographic images of the snakes considered in the paper, while improvements to the manuscript were suggested by Paul Doughty, John Dell and an anonymous referee. Aspects of this work received financial support from the Western Australian Museum, The University of Western Australia, the Australian Research Council, The Australian Government, the National Geographic Society (USA), and the Harry (WH) Butler Fund. This manuscript formed the basis of a presentation to the 2020 symposium *Wallacea: Connecting Asia to the Australian Continent* organized by David Haig under the auspices of The Royal Society of Western Australia.

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Appendix 1. Allele frequencies in five snake species of southern Wallacea.

Trimeresurus insularis

		Sumbawa	Komodo	Flores	Lembata	Pantar	Alor	Sumba	Semau	Timor
ACYC	<i>a</i>	0.88	1	1	1	1	1	1	1	0.96
	<i>b</i>	0.12								0.04
ME2	<i>a</i>	0.17								
	<i>b</i>	0.29	0.50	0.25	0.56	1	1	0.17	1	1
	<i>c</i>	0.54	0.50	0.75	0.44			0.83		
ME3	<i>a</i>		1	1	1	1	1	1	1	1
	<i>b</i>	1								
PEP C	<i>a</i>	0.54								
	<i>b</i>	0.46	1	1	1	1	1	1	1	1
6PGD	<i>a</i>	0.77								
	<i>b</i>	0.23	0.50	1	1	1	1	1	1	1
	<i>c</i>		0.50							
N		13	1	4	9	2	12	6	1	12

The following loci were invariant: *ACON1*, *ACON2*, *ACP1*, *ACP2*, *ADA*, *ENOL*, *FDP*, *GOT1*, *GOT2*, *aGPD*, *IDH1*, *IDH2*, *LDH1*, *LDH2*, *MDH1*, *MDH2*, *ME1*, *PEP A*, *PGM1*, *PGM2*.

Coelognathus subradiatus

		Komodo	Flores	Lembata	Alor	Sumba	Semau	Timor
GDA	<i>a</i>				0.33			
	<i>b</i>	1	1	1		1		0.17
	<i>c</i>				0.67		1	0.83
IDH2	<i>a</i>	1						
	<i>b</i>		1	1	1	1	0.50	1
	<i>c</i>						0.50	
ME1	<i>a</i>	1	0.88	1	1	1	1	1
	<i>b</i>		0.12					
ME2	<i>a</i>				0.83			
	<i>b</i>	1	1	1	0.17	1	1	1
PEP B	<i>a</i>					0.12		
	<i>b</i>	1	1	1		0.88	1	1
	<i>c</i>				1			
PEP C1	<i>a</i>	1	1	0.83	1	1	1	1
	<i>b</i>			0.17				
6PGD	<i>a</i>		0.12		1	1	1	1
	<i>b</i>	1	0.88	1				
PGM1	<i>a</i>					0.12		
	<i>b</i>		0.12					
	<i>c</i>	1	0.88	1	1	0.88	1	1
N		1	4	3	3	4	2	5

The following loci are invariant: *ACON1*, *ACON2*, *ACP*, *ACYC*, *ADA*, *ENOL*, *GOT1*, *GOT2*, *aGPD1*, *aGPD2*, *IDH1*, *LDH1*, *LDH2*, *MDH1*, *MDH2*, *ME3*, *NP*, *PEP A*, *PEP C2*, *PEP C3*, *PEP D*, *PGM2*.

Dendrelaphis spp.

		Nusa Penida	Lombok	Sumbawa	Moyo	Flores	Lembata	Pantar	Alor	Sumba	Savu	Roti	Timor	Yamdena	Kai Besar	
ACON1	a	1	1	0.2												
	b			0.8	1	1	1	1	1	1	1	1	1	1	1	1
ACON2	a							0.25								
	b									0.33			0.07			
	c	0.25							1		1	1	0.93			
	d	0.75	1	1	1	1	1	0.75		0.67						
	e													1		
	f															1
ENOL	a													1	1	
	b	1	1	1		1	1	1	0.96	1	1	1	0.93			
	c				1				0.04				0.07			
FDP	a	1	1	1	1	1	1	1	1	1	1	1	1		1	
	b													1		
GDA	a													1	1	
	b		0.125	0.1												
	c	0.5	0.875	0.8	1	1	1	1	1	1	1	1	1			
	d			0.1												
	e	0.5														
GOT2	a	1	1	1	1	1	1	1	1		1	1	1			
	b									1						
	c													1		
	d														1	
GPI	a	1	1	1	1	1	1	1	1		1	1	1	1	1	
	b									1						
GPT	a						0.57		0.04							
	b		0.25	0.95	1	1										
	c						0.36	1								
	d	0.75	0.75	0.05			0.07		0.96	1	1	1	1	1	1	
	e	0.25														
IDH2	a													1	1	
	b	1	1	1	1	1	1	1	1	1	1	1	1			
MDH1	a						0.36	0.25						1	1	
	b	1	1	1	1	1	0.64	0.75	1	1	1	1	0.93			
	c												0.07			

Dendrelaphis spp. (Cont.)

		Nusa Penida	Lombok	Sumbawa	Moyo	Flores	Lembata	Pantar	Alor	Sumba	Savu	Roti	Timor	Yamdena	Kai Besar
NP	a	1	1	1	1	1	1	1	1	1	1	1	0.86	1	1
	b												0.14		
PEP A	a													0.75	
	b		0.12												
	c			0.8	0.5									0.25	
	d	1	0.88	0.2		1	1	1	1	1	1	1	1		1
	e				0.5										
PEP B	a											1			
	b			0.9	1	1	1	1	1	1	1		1		
	c	1	0.38												
	d		0.62	0.1										1	1
PEP C	a												0.21		
	b	1	1	1	1	1	1	1	1	1	1	0.5	0.71	1	1
	c											0.5			
	d												0.07		
PEP D1	a			0.05											
	b		0.12	0.45	1	0.5	1	1			0.5	1		0.5	0.5
	c	1	0.38	0.5		0.5			1	1	0.5		0.93	0.5	0.5
	d		0.5										0.07		
PEP D2	a	1	1	1	1	1	1	1	0.61	0.83	1	1	0.36	1	1
	b								0.39				0.64		
	c									0.17					
6PGD	a			0.1											
	b														0.25
	c												0.29		
	d	1	1	0.9	1	1	1	1	0.96	1	1	1	0.64	1	0.75
	e								0.04						
	f												0.07		
SORDH	a		0.25				0.21								
	b	1	0.75	1	1	1	0.79	1	1	1	1	1	1		1
	c													1	
N		2	4	10	1	2	7	2	14	3	1	1	7	2	2

The following loci were invariant: *ACP, GAPD, GOT1, LDH1, LDH2, MDH2, PGAM, PGM, SOD*.

Lycodon capucinus

		Sumbawa	Moyo	Flores	Adonara	Lembata	Pantar	Alor	Sumba	Roti	Semau
ADA	a	1	1	1	1	1	0.67	0.46	0.42	0.5	
	b						0.33	0.54	0.58	0.5	1
IDH1	a	1				0.5	0.5	0.41	0.25	0.5	0.5
	b		1	1	1	0.5	0.5	0.59	0.75	0.5	0.5
IDH2	a									0.83	0.5
	b	1	1	1	1	1	1	1	1	0.17	0.5
N		1	1	1	1	1	3	11	6	3	1

The following loci were invariant: *ACON1*, *ACON2*, *ACP*, *ENOL*, *GDA*, *GOT*, *GPI*, *αGPD*, *LDH1*, *LDH2*, *MDH1*, *MDH2*, *ME*, *MPI*, *NP*, *PEP A*, *PEP B*, *PEP C*, *PEP D*, *6PGD*, *PGM1*, *PGM2*, *SORDH*.

Psammodynastes pulverulentus

		Komodo	Flores	Lembata	Alor	Sumba
ACYC	a		0.04			
	b	1	0.96	1	1	1
GDA	a	1	1	0.88	1	1
	b			0.12		
GPT	a	1	0.73	0.62	0.17	0.5
	b		0.27	0.38	0.83	0.5
IDH2	a	1	0.96	0.88	0.83	1
	b		0.04	0.12	0.17	
LDH2	a	1	1	1		1
	b				1	
NP	a	1	0.81	1	0.9	1
	b		0.19		0.1	
PEP C2	a	1	0.96	1	1	1
	b		0.04			
PEP C3	a	1	0.81	0.75	0.42	0.75
	b		0.19	0.25	0.58	0.25
PEP D	a					0.25
	b	0.5	0.81	0.12	1	0.75
	c	0.5		0.88		
	d		0.19			
6PGD	a		0.04			
	b	1	0.92	1	1	1
	c		0.04			
PGM1	a		0.04		1	
	b	1	0.96	1		1
SORDH	a					0.25
	b	1	1	1	1	0.75
N		1	13	4	6	2

The following loci were invariant: *ACON1*, *ACON2*, *ACP*, *ADA*, *ENOL*, *GLDH*, *GOT1*, *GOT2*, *αGPD*, *IDH1*, *LDH1*, *MDH1*, *MDH2*, *PEP A*, *PEP B*, *PEP C1*, *PGM2*.

Gene codes

Code	Enzyme
<i>ACON</i>	Aconitase
<i>ACP</i>	Acid phosphatase
<i>ACYC</i>	Acyclase
<i>ADA</i>	Adenosine deaminase
<i>ENOL</i>	Enolase
<i>FDP</i>	Fructose-1,6-diphosphatase
<i>GAPD</i>	Glyceraldehyde-3-phosphate dehydrogenase
<i>GDA</i>	Guanine deaminase
<i>GLDH</i>	Glutamate dehydrogenase
<i>GOT</i>	Aspartate aminotransferase
<i>αGPD</i>	Glycerol-3-phosphate dehydrogenase
<i>GPI</i>	Glucose-phosphate isomerase
<i>GPT</i>	Alanine aminotransferase
<i>IDH</i>	Isocitrate dehydrogenase
<i>LDH</i>	Lactate dehydrogenase
<i>MDH</i>	Malate dehydrogenase
<i>ME</i>	Malic enzyme
<i>MPI</i>	Mannose-phosphate isomerase
<i>NP</i>	Nucleoside phosphorylase
<i>PEP A</i>	Peptidase A
<i>PEP B</i>	Peptidase B
<i>PEP C</i>	Peptidase C
<i>PEP C1</i>	Peptidase C1
<i>PEP C2</i>	Peptidase C2
<i>PEP C3</i>	Peptidase C3
<i>PEP D</i>	Peptidase D
<i>PEP D1</i>	Peptidase D1
<i>PEP D2</i>	Peptidase D2
<i>6PGD</i>	6-phosphogluconate dehydrogenase
<i>PGAM</i>	Phosphoglycerate mutase
<i>PGM</i>	Phosphoglucomutase
<i>SOD</i>	Superoxide dismutase
<i>SORDH</i>	L-Iditol dehydrogenase

Appendix 2. Morphometric features measured for five snake species (How *et al.* 1996a; How *et al.* 1996b).

Morphometric	Code	<i>Trimeresurus insularis</i>	<i>Coelognathus subradiatus</i>	<i>Dendrelaphis inornatus</i>	<i>Lycodon capucinus</i>	<i>Psammodynastes pulverulentus</i>
Snout to vent length	SVL	✓	✓	✓	✓	✓
Tail length	TAIL	✓	✓		✓	✓
Ventral scale count	VENT	✓	✓	✓	✓	✓
Sub-caudal scale count	CAUD	✓	✓		✓	✓
Eye diameter	EYED	✓	✓	✓	✓	✓
Eye-nostril length	EYEN		✓	✓	✓	✓
Eye-nasal scale length	EYNAL	✓				
Frontal medial suture length	FROL		✓	✓	✓	✓
Frontal width	FROW		✓	✓	✓	✓
Internasal medial suture length	INNAL	✓	✓	✓	✓	✓
Internasal width	INNAW	✓	✓	✓	✓	✓
Length of upper labial one	LAU1	✓				
Length of upper labial two	LAU2	✓				
Length of upper labial three	LAU3	✓				
Loreal dorsal margin length	LORD		✓	✓	✓	✓
Loreal ventral margin length	LORV		✓	✓	✓	✓
Loreal diagonal length	LORL				✓	
Nasal length	NASL	✓				
Parietal length	PARL		✓	✓	✓	✓
Prefrontal medial suture length	PREFL		✓	✓	✓	✓
Prefrontal width	PREFW		✓	✓	✓	✓
Lower preoculars length	PREOLO	✓				
Upper preoculars length	PREOUP	✓				
Dorsal rostral width	ROSD	✓				
Ventral rostral width	ROSV	✓				
Rostral height	ROSH	✓				
Rostral width	ROSW		✓	✓	✓	✓
Subocular length	SUBOL	✓				
Superoocular length	SUOCL	✓	✓	✓	✓	
Maximum superocular width	SUOCW	✓	✓		✓	
Scale count between superoculars	SUPSUP	✓				
Width of the head between the superoculars	SUPSUPW	✓				
Supralabial six length at mid-height	UL6			✓		
Supralabial seven length at mid-height	UL7			✓		