1 Original article

2 LRH: J. A. Leonard *et al.*

3 RRH: Phylogeography of the Sunda Shelf

4

5 **Phylogeography of vertebrates on the Sunda Shelf: a multi-species comparison**

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26 ABSTRACT

27 **Aim** Pleistocene environmental fluctuations had well-characterized impacts on the
28 patterns of within-species divergences and diversity in temperate habitats. Here we
29 examine the impact the Pleistocene had on widely distributed forest vertebrates in a
30 tropical system where the distribution of the habitat was affected by those
31 fluctuations.

32 **Location** Sundaland, tropical Southeast Asia.

33 **Methods** We conducted a comparative phylogeographical analysis of 28 non-
34 migratory, forest-dependent vertebrates for which we constructed rooted, intraspecific
35 phylogenies based on mitochondrial DNA sequences of individuals from at least the
36 three major landmasses in the area (Borneo, Sumatra and the Malay Peninsula) and
37 compared them to hypothetical phylogenies based on independent geological data and
38 climate models regarding connections and relationships between the major
39 landmasses of Sundaland. Java was included where possible. We dated the
40 phylogenies to determine whether patterns of differentiation were concordant across
41 species.

42 **Results** In most species, populations on the Malay Peninsula and Sumatra were most
43 closely related, and sister to those from Borneo. The dates of these divergences,
44 however, varied extensively between species. Borneo harbours multiple deeply
45 divergent lineages of many species compared to the diversity within those species.
46 Javan populations of several birds were most divergent relative to those from the rest
47 of the Sunda Shelf.

48 **Main conclusions** These results suggest a dynamic history, including recurrent
49 population extinctions and replacements and a strong priority effect for local
50 populations. The close relationship between populations in Sumatra and the Malay

51 Peninsula supports the existence of forest on the exposed shelf during the Pleistocene
52 at many different times, and suggests that proximity was more important than the
53 presence of palaeorivers for dispersal of forest taxa between landmasses.

54 **Keywords**

55 **Comparative phylogeography, extinction, molecular dating, Pleistocene**
56 **divergence, population divergence, priority effect, Sundaland.**

57

58 INTRODUCTION

59 Phylogeographical patterns have been used extensively to improve our understanding
60 of the evolutionary history of individual species or species complexes across many
61 taxa (e.g. González-Porter *et al.*, 2011; Koblmüller *et al.*, 2012; Den Tex & Leonard,
62 2014). Despite the large number of phylogeographical studies, relatively few
63 comparative phylogeographical studies have been undertaken (Bermingham &
64 Moritz, 1998; Arbogast & Kenagy, 2001; Riddle *et al.*, 2008). Comparative
65 phylogeographical studies have the potential to uncover the evolutionary history of
66 ecosystems by simultaneously admitting spatial and temporal elements in the
67 hypotheses (Arbogast & Kenagy, 2001), and uniquely allow for biological replication
68 in the form of the different species studied (Bermingham & Moritz, 1998). Some
69 examples from tropical systems include the Australian tropical rain forest (Schneider
70 *et al.*, 1998), the Guiana shield in the Amazon (Fouquet *et al.*, 2012), and most
71 relevant to our study, Southeast Asia (Lim *et al.*, 2011). Each of these studies
72 compared the phylogeography of six to 16 co-distributed species to test hypotheses
73 associated with Pleistocene environmental changes that could have caused
74 divergence. Widespread support for the importance of Pleistocene events in driving
75 within species divergences was found in Australia and the Amazon, but tropical
76 Southeast Asia yielded mixed results, with some Pleistocene and some earlier
77 divergences. Pleistocene age divergences thought to be associated with environmental
78 changes were also identified in comparative phylogenetic studies of five or six species
79 in temperate regions (Carstens *et al.*, 2005; Qu *et al.*, 2010).

80 Here we applied comparative phylogeography to Sundaland, a biodiversity
81 hotspot in tropical Southeast Asia that inspired Alfred Russel Wallace to conclude
82 that species originated through natural selection (Wallace, 1858). The forest across

83 this region is currently divided by sea. The three largest landmasses are the Malay
84 Peninsula, Sumatra and Borneo, but there are also many other islands of various sizes
85 including Java (Fig. 1). Pleistocene climatic fluctuations led to repeated changes in
86 sea level resulting in the periodic connection of the landmasses across the shelf (Fig.
87 1).

88 Although there are many locally endemic species in this biodiversity hotspot
89 (Myers *et al.*, 2000), there are also many species widespread across the region
90 (Heaney, 1986). We used data from all non-migratory, forest dependent vertebrates
91 for which mitochondrial DNA data are available from at least Sumatra, Borneo and
92 the Malay Peninsula to test the following hypotheses based on independent,
93 geological data. The periodic land connections suggest the hypothesis that there were
94 panmictic populations across the region during these times. This is further supported
95 by climatic reconstructions suggesting that both highland and lowland forests across
96 Sundaland were more extensive in periods of glacial maxima (Cannon *et al.*, 2009).
97 This predicts very recent or no divergence between populations (Fig. 2a). However, it
98 has been hypothesized that the Pleistocene land bridges were much drier than the
99 islands and were either completely or partially covered by grasslands or savanna
100 (Heaney, 1991; Meijaard, 2003; Bird *et al.*, 2005; Wurster *et al.*, 2010). In this case,
101 the land that connected what today is Borneo, Sumatra and the Malay Peninsula may
102 have been as efficient a barrier as the sea for inhibiting gene flow within forest-
103 dependent species. This would predict that genetic divergences between populations
104 would be ancient, and geographical distances would be the primary factor determining
105 relationships (Fig. 2b). Alternatively, it has been hypothesized that gallery forests
106 along palaeorivers running through the xeric exposed land during periods of low sea
107 level could have acted as corridors for forest-dependent taxa (Heaney, 1991; Voris,

2000), and thus would have led to a pattern of gene flow more associated with these routes than distance (Fig. 2c). The other possible way the populations of a species on the three landmasses could be related, with Borneo and the Malay Peninsula being most closely related, is illustrated in Fig. 2d.

MATERIALS AND METHODS

Materials

Data from all forest-dependent, non-migratory species for which mitochondrial DNA sequences were available from at least the Malay Peninsula, Sumatra and Borneo, were collected from the literature. Although Java is also a large island, it has been included in few phylogeographical studies of the region, probably because many widely distributed species are absent, perhaps due to its different climate (Wilting *et al.*, 2012). In total the data set comprised 31 taxa (Table 1), including 12 species of mammal [six rodents, two primates, one artiodactyl (*Sus barbatus*), one erinaceomorph (*Hylomys suillus*), one carnivoran (*Paradoxurus hermaphroditus*) and one proboscidean, the Asian elephant (*Elephas maximus*)], 17 species of bird [12 songbirds, four barbets and one kingfisher (*Ceyx erithaca*)], one flying lizard (*Draco sumatranus*) and one frog (*Rana chalconota* s.l.). Of these 31 taxa, three were excluded from further analysis because of taxonomic uncertainty due to lack of monophyly of the species: the erinaceomorph (*Hylomys suillus*), one primate (*Macaca nemestrina*) and a tree squirrel (*Sundasciurus tenuis*) (Ruedi & Fumagalli, 1996; Ziegler *et al.*, 2007; Den Tex *et al.*, 2010), leaving 28 species for analysis. Half of the species are endemic to Sundaland ($n = 15$), but some are also distributed north of the Isthmus of Kra (Table 1). It has been suggested in the literature that several of the species distributed beyond Sundaland may represent multiple species ($n = 5$; Table 1),

133 indicating that the Sundaland populations may be distinct species. Within-species
134 phylogenies included in the original publications were compared with the population
135 phylogenies illustrated in Fig. 2 (see Table 1). The exception was the barbets because
136 within-species phylogenies were not included in the original publication (Den Tex &
137 Leonard, 2013). For this reason, within-species phylogenies with an appropriate
138 outgroup for each species of barbet were constructed (see Appendix S1 in Supporting
139 Information).

140

141 **Dating**

142 When available, dates from the original publication were used. Eleven data sets for
143 which dates were not estimated in other publications (for four rodents *Leopoldamys*
144 *sabanus*, *Maxomys whiteheadi*, *M. rajah* and *M. surifer*, the frog *Rana chalconota*,
145 the lizard *Draco sumatranus*, the forktail *Enicurus leschenaulti*, and the four barbets
146 *Megalaima rafflesii*, *M. henricii*, *M. chrysopogon* and *M. australis*) were tested for
147 deviance from the molecular clock (Table 1). First, we used the Akaike information
148 criterion (AIC) as implemented in MODELTEST 3.7 (Posada & Crandall, 1998) to
149 obtain the most appropriate model of sequence evolution for the ingroup taxa. Then
150 MEGA (Tamura *et al.*, 2013) or PAUP* 4b10 (Swofford, 2002) was used to obtain
151 maximum likelihood (ML) scores for both the constrained and unconstrained trees.
152 Lastly, the maximum likelihood ratio test was applied to the scores to test whether the
153 molecular clock for each data set was violated.

154 Out of these 11 data sets, we were able to date nine: the four barbet
155 phylogenies (Den Tex & Leonard, 2013), the four rodent phylogenies (Gorog *et al.*,
156 2004; Achmadi *et al.*, 2013) and the forktail phylogeny (Moyle *et al.*, 2005). For all
157 rodents, the divergence at *cytb* third codon transversions used was the rate of 1.85%

per million years (Myr) (as estimated in Den Tex *et al.*, 2010), for the barbets (*Megalaima* sp.) we used a rate of 1.88% (SD 0.23%) per Myr (Nabholz *et al.*, 2009), and for the forktail a rate of 2.5% (SD 1.15%) per Myr (Nabholz *et al.*, 2009). The date estimated is for the divergence between the sequences in the different populations, which is always older than the actual divergence between the taxa, introducing a bias towards older dates. Because of uncertainty regarding the mutation rates of the markers used in the flying lizard (*Draco sumatranus*) and the frog (*Rana chalconota* s.l.), *ND2* and *ND3*, respectively, we refrained from dating these phylogenies.

RESULTS

Phylogenetic patterns

In 16 of the 28 taxa analysed, Borneo was basal to Sumatra and the Malay Peninsula (Fig. 2b). No geographical structure was found in five taxa, and the relationships among the three landmasses were unresolved in six taxa (Fig. 2a). The last taxon, the macaque (*Macaca fascicularis*), had structure that was inconsistent with the pattern found in the other taxa. The macaques on the islands (Borneo and Sumatra) formed a reciprocally monophyletic clade with respect to all of the continental populations with mitochondrial DNA (Tosi & Coke, 2007). In this case the lineages found on Borneo, Sumatra and the Malay Peninsula do not share a most recent common ancestor (the Malay Peninsula lineages share a most recent common ancestor with the other mainland lineages).

Java

Thirteen of the 27 species included in these analyses are also distributed on the large island of Java, which is adjacent to the south-east of Sumatra (see Fig. 1) and is also located on the Sunda Shelf. We were able to obtain data from Javan populations for eight taxa. In two cases the Javan population was most closely related to the neighbouring island of Sumatra (the rodent *Maxomys surifer* and the carnivoran *Paradoxurus hermaphroditus*), whereas the relationships among the Sumatran, Javan and Bornean populations of the macaque (Tosi & Coke, 2007) and the kingfisher (Lim *et al.*, 2010) were unresolved. In the other four cases (all birds), the Java populations were very divergent to Malay Peninsula, Sumatra and Borneo (*Megalaima australis*, *Enicurus leschenaulti*, *Arachnothera longirostra* and *Pteruthius flaviscapis*). The barbet *M. australis* distributed on Java and Bali were reciprocally monophyletic with respect to the Malay Peninsula, Sumatra and Borneo populations also with high support (see Fig. S1 in Appendix S1). The taxonomy of the shrike-babblers is uncertain (Rheindt & Eaton, 2009), but the population/ species on Java is divergent from the population/ species found on Borneo, Sumatra and the Malay Peninsula. In two other cases (*M. henricii* and *M. chrysopogon*), the sister taxon is endemic to Java or Java and Bali (*M. armillaris* and *M. corvine*, respectively; Den Tex & Leonard, 2013).

200

201 **Dating**

A molecular clock could not be rejected for any of the phylogenies of the nine taxa (four rodents and five birds) tested here. Dates from previously published papers were used for the other six taxa.

All but one intra-specific splitting event dated within the Pleistocene. Only the rodent *Leopoldamys sabanus* showed divergence of Sumatra and the Malay Peninsula

207 and those with Borneo before 2 Ma. The divergence between Borneo, Sumatra and
 208 the Malay Peninsula populations of the other four rodents (*Maxomys surifer*, *M.*
 209 *whiteheadi*, *M. rajah* and *Sundasciurus hippurus*) ranged from latest Pleistocene to
 210 1.51 Ma. Similarly, the divergence between Borneo, Sumatra and the Malay
 211 Peninsula populations of birds diverged between the latest Pleistocene and 1.8 Ma.
 212 Both thrushes (*Copsychus saularis* and *Enicurus leschenaulti*) also have earlier
 213 Pleistocene divergences on Borneo. This suggests no great difference in
 214 phylogeographical patterns in small terrestrial mammals and similarly sized birds.
 215 The populations of the large mammals (pigs and elephants) diverged most recently, in
 216 the latest Pleistocene. Polytomies or non-geographically structured clades in the
 217 barbet and the babbler (*Megalaima rafflesii* and *Pteruthius flaviscapis*) were inferred
 218 to be of late Pleistocene age.

219 Based on the data presented here, the most common biogeographical pattern
 220 identified was a close relationship between Sumatra and the Malay Peninsula, with
 221 Borneo more divergent. In those cases, the Sumatra and Malay Peninsula lineages
 222 diverged between the mid- and latest Pleistocene in 10 of 11 of the dated phylogenies
 223 except for the rodent *Leopoldamys sabanus*, which diverged much earlier. The times
 224 of divergence do not cluster around a particular glacial cycle or other geological
 225 event, such as the eruption of Toba 0.735 Ma (Fig. 3). In the subset of phylogenies
 226 where Java was also sampled, it often diverged earlier than the other landmasses ($n =$
 227 4).

228

229 DISCUSSION

230 Phylogenetic patterns

231 We found substantial genetic structure among most animal populations surveyed here
232 that are widely distributed across the Sunda Shelf. In the majority of cases where
233 structure was identified between populations (16 out of 17), the Sumatra populations
234 were more closely related to the Malay Peninsula populations, with the Bornean
235 populations more divergent from the other two (Fig. 2b). Published phylogenies on
236 other species distributed or sampled only on Borneo and Sumatra or the Malay
237 Peninsula (but not both) also found deep divergences, such as the clouded leopard
238 (*Neofelis nebulosa*), the orang-utan (*Pongo pygmaeus*) and the flying lemur
239 (*Galeopterus variegatus*) (Wilting *et al.*, 2007; Steiper, 2006; Janecka *et al.*, 2008).
240 The only exception to this pattern was the macaque (*Macaca fascicularis*), in which
241 the Borneo and Sumatra populations form a clade, and the Malay Peninsula
242 population is more closely related to the rest of mainland tropical East Asian
243 macaques (Tosi & Coke, 2007). The other cases ($n = 11$) were unresolved or no
244 geographical pattern was observed.

245 Borneo is not only most divergent from Sumatra and the Malay Peninsula. In
246 several of the cases where multiple regions of Borneo were sampled, a large amount
247 of divergence was identified between populations within Borneo (Moyle *et al.*, 2005;
248 Den Tex *et al.*, 2010; Lim *et al.*, 2011; Den Tex & Leonard, 2013). This pattern is
249 indicative of a long evolutionary history and warrants further investigation.

250

251 **Java**

252 Java contains fewer species than neighbouring (and larger) Sumatra, but relatively
253 more of the species are endemic (MacKinnon & Phillipps, 1993). This is probably a
254 result of both history and environment. Java has a climate and vegetation that is
255 different from the other islands on the Shelf (Wilting *et al.*, 2012). Many taxa that are

widespread across Sundaland are absent from Java, such as tree squirrels of the genus *Sundasciurus* (Thorington *et al.*, 2012). The climate of Java is drier than in many other parts of the Sunda Shelf, and there are grassy areas as well as tropical forests (Heaney, 1991). In a few of the cases where data were available from Java as well as Sumatra, Borneo and the Malay Peninsula, the geographically close Sumatra was genetically most similar ($n = 2$). However, in most cases, specimens from Java represented the most divergent lineage ($n = 4$) or the sister species. We find it to be noteworthy that these within-species patterns were anticipated by some of Alfred Russel Wallace's observations of faunal similarity among the Malay Peninsula, Sumatra, Borneo and Java based on the distribution patterns of species and genera (Wallace, 1880, pp. 348–362), and his recognition of the importance of the shallow seas over continental shelf that separate these land areas.

Pleistocene divergence

Most intra-specific divergences, many of which represent named sub-species, date to the Pleistocene. Some closely related species/ taxa, which have been referred to both as species and as subspecies, also diverged in the Pleistocene. This suggests that the Pleistocene had important environmental effects that impacted a wide variety of taxa. Our results indicate that the Pleistocene climatic/environmental fluctuations largely caused divergence and phylogeographical structure within species. This is similar to the pattern of Pleistocene age intra-specific divergence found in Europe (Hewitt, 2000) and North America (Zink *et al.*, 2004). Through the Pleistocene, as the glaciers expanded, temperate Holarctic habitats receded into refugia. As the climate changed again and the ice melted, additional habitat became available to these temperate species, and the populations expanded. The pattern in Sundaland is the opposite of

281 that for temperate forests. During glacial maximums sea level was lower, which
282 exposed large parts of the Sunda shelf and potentially allowed a large expansion of
283 both highland and lowland tropical forest in this region (Cannon *et al.*, 2009). These
284 forests have a minimum extent in the interglacial periods, such as now (Woodruff,
285 2010).

286 Although the within-species divergence times occurred in the Pleistocene, they
287 do not date back to the same time period (Fig. 3). This suggests that the common
288 pattern was not caused by a single, perhaps particularly dramatic, climatic fluctuation
289 or sea-level change, or another event such as a major volcanic eruption. Instead, this
290 suggests that populations diverged and persisted for different periods of time, up to
291 millions of years (Table 1). Eventually, some populations went extinct, in a somewhat
292 stochastic fashion. At some point, surviving populations recolonised the areas where
293 the species had gone locally extinct.

294 This scenario relies on the ability of forest-dependent taxa to expand out of
295 their refugia and reach the other areas, which requires forest connections between
296 Borneo, Sumatra and the Malay Peninsula on a relatively regular basis. Some forest-
297 dependent taxa may be able to disperse across other habitats (especially the larger
298 mammals), but we assume that this is less frequent than dispersal within forest
299 (Heaney, 1986). When these forest taxa greatly expanded their ranges as more forest
300 habitat became available across the Sunda Shelf, how could the observed common
301 pattern arise? The rate of expansion may be much faster in areas where there is not
302 already another population of the same species. This does not require that the
303 divergent lineages stay isolated, just that the spread of genes is slowed down by the
304 presence of other individuals already occupying the regions. This is similar to the
305 ecological concept of ‘priority effect’, which is generally used in reference to a

species' ability to survive and exclude other species, even when the other species may be better adapted to the environment, simply because they were there first (i.e. Mergeay *et al.*, 2011). In this case we refer to different populations of a single species. This is supported by the pattern of expansion out of refugia observed in many European temperate taxa (Hofreiter *et al.*, 2004).

311

312 CONCLUSIONS

Across a wide variety of forest dependent vertebrates in Sundaland, populations on the Malay Peninsula and Sumatra are generally more closely related to one another than they are to populations on Borneo. The few cases where data were available from multiple populations on Borneo suggest that this land-mass may harbour large amounts of genetic diversity and interesting patterns of divergence which require further study based on better sampling. The different habitat available on Java may explain why only about half of the species studied here are distributed on this island. The birds from Java for which data were available suggest that the populations of these species on Java are the most divergent of the four land-masses. Although a common pattern was observed across many species, the timing of the divergences between populations on different land-masses varied widely and can not be attributed to a single event, such as a particularly extreme glacial cycle or massive volcanic eruption. The wide variation in dates suggests a more stochastic driver, resulting in different rates of population survival over tens of thousands to a few million years.

327

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586

587 **SUPPORTING INFORMATION**

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

589

590 **Appendix S1** Phylogenetic reconstruction for the four species of barbet.

591

592 **BIOSKETCH**

593 This research group, headed by J.A.L., J.E.M. and R.T., is studying the ecology and

594 evolution of the biodiversity of the Sunda Shelf, with a focus on forest dependent

595 vertebrates. This system is particularly well suited to study alternative hypotheses

596 regarding differentiation and speciation in the tropics due to the geography and

597 history of isolation and connection associated with Pleistocene climatic fluctuations.

598 More information can be found at <http://www.consevol.org/>.

599

600 Author contributions: J.A.L. conceived the idea based on discussions with R.T. and

601 J.E.M., J.A.L. and R.-J.d.T. collected and analyzed the data, JAL wrote the

602 manuscript with support from all authors.

603

604 Editor: Lawrence Heaney

605

606 **Figure legends**

607

608 **Figure 1** Map of Southeast Asia illustrating the positions of Borneo, Sumatra and
609 Java relative to mainland Southeast Asia and the Malay Peninsula and the Isthmus of
610 Kra. Current land is shown in dark grey, and the maximum exposure of the Sunda
611 Shelf (the -116m contour line) is indicated with the light grey, and ocean in white.
612 Palaeorivers are shown as in Heaney (1991). Figure modified after Sathiamurthy &
613 Voris (2006).

614

615 **Figure 2** Four possible population phylogenies that illustrate the theoretical
616 relationships between populations of a species distributed across the Malay Peninsula,
617 Sumatra and Borneo.

618

619 **Figure 3** Age estimates of population splits within vertebrate species superimposed
620 on the sea-level change curve during the Quaternary (0 to 1.8 Ma). The squares at the
621 top represent the age estimates of the split of Borneo versus Sumatra and Malay
622 Peninsula populations and the circles at the bottom indicate the age estimates of the
623 split of the Sumatra versus Malay Peninsula populations, from Table 1. Proxy for sea-
624 level changes after and modified from Lisiecki & Raymo (2005).

Table 1 Species list of vertebrate taxa that show the distribution of at least the Malay Peninsula (MP), Sumatra and Borneo and for which genetic data are available. Species endemic to Sundaland (to Huxley's Line) are in bold. Also tabulated here are the mitochondrial sequences (mt gene) on which the respective taxon phylogenies and splitting events are based, and the estimated dates of divergences.

Taxon summary			Dating results		
Order	Taxon	Reference	Mt gene	Borneo (MP–Sumatra)	MP–Sumatra
Rodentia	<i>Leopoldamys sabanus</i> ^a Thomas, 1887	Gorog <i>et al.</i> , 2004	<i>cytb</i>	2.29	2.24
	<i>Maxomys whiteheadi</i> Thomas, 1894	Gorog <i>et al.</i> , 2004	<i>cytb</i>	0.75	0.29
	<i>Maxomys surifer</i> ^b Müller, 1900	Gorog <i>et al.</i> , 2004	<i>cytb</i>	1.51	0.94
	<i>Maxomys rajah</i> [†] Thomas 1894	Achmadi <i>et al.</i> , 2013	<i>cytb</i>	0.24	0.13
	<i>Sundasciurus hippurus</i> Geoffroy, 1831	Den Tex <i>et al.</i> , 2010	<i>cytb</i>	0.3	latest Pleistocene
Primates	<i>Sundasciurus tenuis</i> [*] Horsfield, 1824	Den Tex <i>et al.</i> , 2010	<i>cytb</i>		
	<i>Macaca fascicularis</i> ^c Linnaeus 1758	Tosi & Coke, 2007	<i>cytb</i>	mainland–islands split 1.2; island relationships unresolved ¹	
	<i>Macaca nemestrina</i> [*] Linnaeus 1766	Ziegler <i>et al.</i> , 2007	<i>cytb</i>		
Artiodactyla	<i>Sus barbatus</i> Müller, 1838	Larson <i>et al.</i> , 2007	D loop	no geographical structure	
Insectivora	<i>Hylomys suillus</i> ^{ad} Müller, 1941	Ruedi & Fumagalli, 1996	<i>cytb</i>		
	<i>Paradoxurus hermaphroditus</i> ^c Pallas, in Schreber, 1777	Patou <i>et al.</i> , 2010	<i>cytb</i>	3.4	1.95
Proboscidea	<i>Elaphas maximus</i> Linnaeus, 1758	Fernando <i>et al.</i> , 2003	D loop	unresolved relationships ²	
Coraciiformes	<i>Ceyx erithaca</i> Linnaeus, 1758	Lim <i>et al.</i> , 2010	ND2	no geographical structure	
Piciformes	<i>Megalaima rafflesii</i> Lesson, 1839	Den Tex & Leonard, 2013	<i>cytb</i>	no geographical structure ³	
	<i>Megalaima henricii</i> Temminck, 1831	Den Tex & Leonard, 2013	<i>cytb</i>	0.53	0.085
	<i>Megalaima chrysopogon</i> Temminck, 1824	Den Tex & Leonard, 2013	<i>cytb</i>	0.91	0.11
	<i>Megalaima australis</i> ^g Horsfield, 1821	Den Tex & Leonard, 2013	<i>cytb</i>	0.23	0.075
Passeriformes	<i>Enicurus leschenaulti</i> ^g Vieillot, 1818	Moyle <i>et al.</i> , 2005	ND2	0.27	no geographical structure
	<i>Pteruthius flaviscapit</i> ^g Temminck, 1836	Reddy, 2008	<i>cytb</i>	unresolved relationships ⁴	
	<i>Copsychus saularis</i> ^c Linnaeus, 1758	Lohman <i>et al.</i> , 2010	<i>cytb</i>	0.32	no geographical structure
	<i>Copsychus saularis</i> ^c Linnaeus, 1758	Sheldon <i>et al.</i> , 2009	ND2, <i>COI</i>	0.47	no geographical structure
	<i>Arachnothera longirostra</i> ^c Latham, 1790	Lohman <i>et al.</i> , 2010	<i>cytb</i>	unresolved relationships ⁵	
	<i>Pycnonotus goiavier</i> ^c Scopoli, 1786	Lohman <i>et al.</i> , 2010	<i>cytb</i>	1.37	no geographical structure
	<i>Rhipidura javanica</i> ^c Sparman, 1788	Lohman <i>et al.</i> , 2010	<i>cytb</i>	no geographical structure	

<i>Hypothymis azurea</i> ^c	Boddaert, 1783	Lim <i>et al.</i> , 2011	ND2	unresolved relationships
<i>Orthotomus sericeus</i>	Temminck, 1836	Lim <i>et al.</i> , 2011	ND2	no geographical structure
<i>Hypogramma hypogrammicum</i> ^e	Müller, 1843	Lim <i>et al.</i> , 2011	ND2	unresolved relationships
<i>Stachyris erythroptera</i>	Blyth, 1842	Lim <i>et al.</i> , 2011	ND2	no geographical structure
<i>Stachyris poliocephala</i>	Temminck, 1836	Lim <i>et al.</i> , 2011	ND2	3.9
<i>Malacocincla malaccensis</i>	Hartlaub, 1844	Lim <i>et al.</i> , 2011	ND2	1.03
<i>Draco sumatranus</i>	McGuire & Heang, 2001	McGuire & Heang, 2001	ND2	2.55
<i>Rana chalconota</i>	group Schlegel, 1837	Stuart <i>et al.</i> , 2006	ND3	unresolved relationship
				no dating performed
				no dating performed

^aExcluded: not a natural group.

^bOnly haplotypes that form a monophyletic group included (as in Achmadi *et al.*, 2013).

^cDistributed across Sundaland and north of Isthmus of Kra, probably multiple species (Carleton & Musser, 2005; Lunde *et al.*, 2008).

^dDistributed across Sundaland, Philippines and north of Isthmus of Kra, probably multiple species (Aplin *et al.*, 2008).

^eDistributed across Sundaland, Philippines and north of Isthmus of Kra.

^fDistributed across Sundaland and north of Isthmus of Kra, probably multiple species (Ruedi & Fumagalli 1996).

^gDistributed across Sundaland, Philippines and north of Isthmus of Kra, probably multiple species (Duckworth *et al.*, 2008).

^hDistributed across Sundaland and north of Isthmus of Kra, probably three species (Birdlife International, 2014).

ⁱDistributed across Sundaland and north of Isthmus of Kra.

¹From Tosi *et al.* (2007).

²Substructuring of 'Pleistocene origin' from Fernando *et al.* (2003).

³'Ancient' split 0.23 Ma.

⁴MP–Sumatra–Borneo split 0.4 Ma from Reddy (2008).

⁵Substructuring of late Pleistocene age.

Sea level

Time before present in millions of years

Journal of Biogeography

SUPPORTING INFORMATION**Phylogeography of vertebrates on the Sunda Shelf: a multi-species comparison**

Jennifer A. Leonard, Robert-Jan den Tex, Melissa T. R. Hawkins, Violeta Muñoz-Fuentes, Richard Thorington and Jesus E. Maldonado

Appendix S1 Phylogenetic reconstruction for the four species of barbets.

Within-species phylogenies of the barbets were not published as part of the original paper from which we took the data (Den Tex & Leonard, 2013). For this reason, within-species phylogenies with an appropriate outgroup for each barbet were constructed. Saturation plots constructed for each of the barbets showed no saturation and, therefore, maximum parsimony (MP) and maximum likelihood (ML) methods were applied to construct the phylogenetic trees using PAUP* 4b10 (Swofford, 2002). Branch support was tested by MP bootstrapping (1000 times) with 1000 additional replicates each time and a heuristic search algorithm with tree bisection–reconnection (TBR). For the ML analysis the most appropriate model of sequence evolution of each ingroup was determined based on the Akaike information criterion (AIC) as implemented in MODELTEST 3.7 (Posada & Crandall, 1998). ML bootstrapping (100 times and 10 additional replicates each) used also a heuristic search algorithm with TBR as implemented in PAUP* 4.10 (Swofford, 2002). Because these intraspecific phylogenies are not available elsewhere in the literature (as the other species are, see references in Table 1 in the main paper), they are included as supplementary files (see Figs S1–S4 below).

Figure S1 The maximum likelihood (ML) clock tree based on mitochondrial DNA sequences from *Maxomys australis* with first number referring to the maximum parsimony bootstrap value and the second number to the ML bootstrap value providing statistical support for that particular branch. The model of sequence evolution used was HKY+G with base frequencies A = 0.2681, C = 0.3508, G = 0.1168, the transition–transversion ratio = 23.2237 and the α shape = 0.0804. Scale bar in percent divergence.

Figure S2 The maximum likelihood (ML) clock tree based on mitochondrial DNA sequences from *Maxomys chrysopogon* with the first number referring to the maximum parsimony bootstrap value and the second number to the ML bootstrap value providing statistical support for that particular branch. The model of sequence evolution used was k81uf with base frequencies A = 0.2785, C = 0.3229, G = 0.1207 and rate matrix = (1, 14.0178, 0.014, 0.014, 14.0178). Scale bar in percent divergence.

Figure S3 The maximum likelihood (ML) clock tree based on mitochondrial DNA sequences from *Maxomys henricii* with the first number referring to the maximum parsimony bootstrap value and the second number to the ML bootstrap value providing statistical support for that particular branch. The model of sequence evolution used was HKY with base frequencies A = 0.2794, C = 0.343, G = 0.1195 and the transition–transversion ratio = 24.3457. Scale bar in percent divergence.

Figure S4 The maximum likelihood (ML) clock tree based on mitochondrial DNA sequences from *Maxomys rafflesii* with the first number indicating maximum parsimony bootstrap and the second ML bootstrap support for two clades within *M. rafflesii* that do not show geographical substructuring. The model of sequence evolution used was HKY with base frequencies A = 0.2764, C = 0.3344, G = 0.1159 and the transition–transversion ratio = 5.3622. Scale bar in percent divergence.