Biological Journal of the Linnean Society, 2004, **81**, 91–109. With 8 figures

Vicariance or dispersal? Historical biogeography of three Sunda shelf murine rodents (*Maxomys surifer*, *Leopoldamys sabanus* and *Maxomys whiteheadi*)

ANTONIA J. GOROG[1]*, MARTUA H. SINAGA[2] and MARK D. ENGSTROM[3]

[1]*University of Michigan, Museum of Zoology and Department of Ecology and Evolutionary Biology, 1109 Geddes Ave., Ann Arbor, MI 48109, USA*
[2]*Indonesian Institute of Sciences (LIPI) & Museum Bogoriense, Jl. Raya Bogor-Jakarta KM 46, Cibinong, West Java, Indonesia 16911*
[3]*Royal Ontario Museum, Centre for Biodiversity and Conservation Biology, 100 Queens Park, Toronto, Ontario M5S 2C6, Canada*

Received 26 November 2002; accepted for publication 28 July 2003

The Sunda region of south-east Asia comprises the Malay Peninsula and the islands of Java, Sumatra and Borneo, all of which lie on a shallow continental shelf projecting from Indochina. Pleistocene glacial cycles caused sea levels to drop repeatedly, exposing vast areas of the Sunda shelf and creating land bridges among the islands and mainland. These land bridges, the latest of which connected all three of the major Sunda islands to the Malay Peninsula as recently as 9500 years ago, may have enabled mammalian migrations across the Sunda shelf. Pleistocene land bridges on the Sunda shelf have been invoked to explain the current distributions of mammalian taxa occupying ranges corresponding with the Pleistocene limits of land and the appearance of new mammal species in the Pleistocene fossil record. The ability of mammals to move throughout the exposed shelf during periods of low sea level would, however, have been influenced by topographic and ecological features, which have been variously described as savanna-like or as moist tropical rain forest. Using a phylogeographical approach, we test the hypothesis that Pleistocene land bridges enabled widespread movements in three rain-forest-restricted murine rodents of the Sunda shelf: *Maxomys surifer*, *Leopoldamys sabanus* and *Maxomys whiteheadi*. Our results do not support the hypothesis of broad Pleistocene migrations in these taxa, but instead suggest a deep history of vicariant evolution that may correspond with the Pliocene fragmentation of the Sunda block.

ADDITIONAL KEYWORDS: climate – control region – cytochrome *b* – land bridges – phylogeography – Pleistocene – Pliocene – refugia.

INTRODUCTION

Island archipelagos provide unique systems for the study of biological diversification and have been the subject of many foundational works in the fields of evolution and biogeography (e.g. Darwin, 1859; MacArthur & Wilson, 1967). The Indonesian archipelago, in particular, has fascinated biogeographers since Alfred Russell Wallace's pioneering studies in the 1850s documented extremely high levels of diversity in the region and the sharp biogeographical divide between the Sunda islands (the western part of the archipelago) and Sulawesi (Wallace, 1869). Because they lie on a shallow continental shelf that has been exposed repeatedly to create land bridges between islands in relatively recent geological history (Heaney, 1991; Voris, 2000), the Sunda islands are distinct from other well-studied island systems such as the Galapagos (Grant, 1999) and are especially interesting for biogeographical studies. The importance of these periodic connections to the evolution of the Sunda shelf fauna has been studied in detail for some groups (e.g. Heaney, 1986 for non-volant mammals; Ruedi & Fumagalli, 1996 for gymnures; Ruedi, 1996 for shrews; Schmitt, Kitchener & How, 1995 for fruit bats;

*Corresponding author. E-mail: ajgorog@umich.edu

Karns *et al*., 2000 for the Asian water snake; Inger & Voris, 2001 for frogs and snakes), but remains to be examined in a phylogenetic framework for the most diverse group of mammals on the Sunda shelf – the rodents. Here, we use a phylogeographical approach based on mitochondrial DNA to test the hypothesis that three species of rodents, widespread on the Sunda shelf, reached their current distributions via Pleistocene land bridges.

BACKGROUND

Geological evolution of the Sunda shelf

The Malay Peninsula and the major islands of Borneo, Java and Sumatra lie on the shallow continental Sunda shelf. These regions existed as a continental block with Indochina from the early Eocene (~50 Mya) to the late Oligocene (~25 Mya), after which Java and Sumatra were submerged until the middle Miocene (~15 Mya). By the early Pliocene (~5 Mya) the Sunda islands had obtained an essentially modern formation. Connections among the islands and peninsula/mainland had disappeared or had been reduced to narrow corridors, although the exact margins of the shallow sea and low-lying adjacent areas are unknown (Hall, 1998).

In the Pleistocene, drastic climate changes transformed the Sunda landscape when cyclical glaciations caused the sea level to drop and exposed land bridges among formerly isolated regions of the shelf. At least twice during this period, most recently about 11 000 years ago (Biswas, 1973), sea levels dropped 120 m, enough to reconnect Borneo, Sumatra and Java to the Malay Peninsula and Indochina in a single giant block. All three of the major Sunda islands remained connected to one another and, through the Malay Peninsula, to mainland Indochina until about 9500 years ago (Voris, 2000; Inger & Voris, 2001; Fig. 1). The Karimata Straits separating Borneo and Sumatra were formed as recently as 7000 years ago (Moss & Wilson, 1998), shortly before seas reached their present levels 6000 years ago (Voris, 2000).

Pleistocene movements of Sunda mammals

Pleistocene glacial maxima have long been discussed in the literature as times of widespread faunal migrations across the Sunda shelf (e.g. Banks, 1949). Some authors have argued that the current distributions of

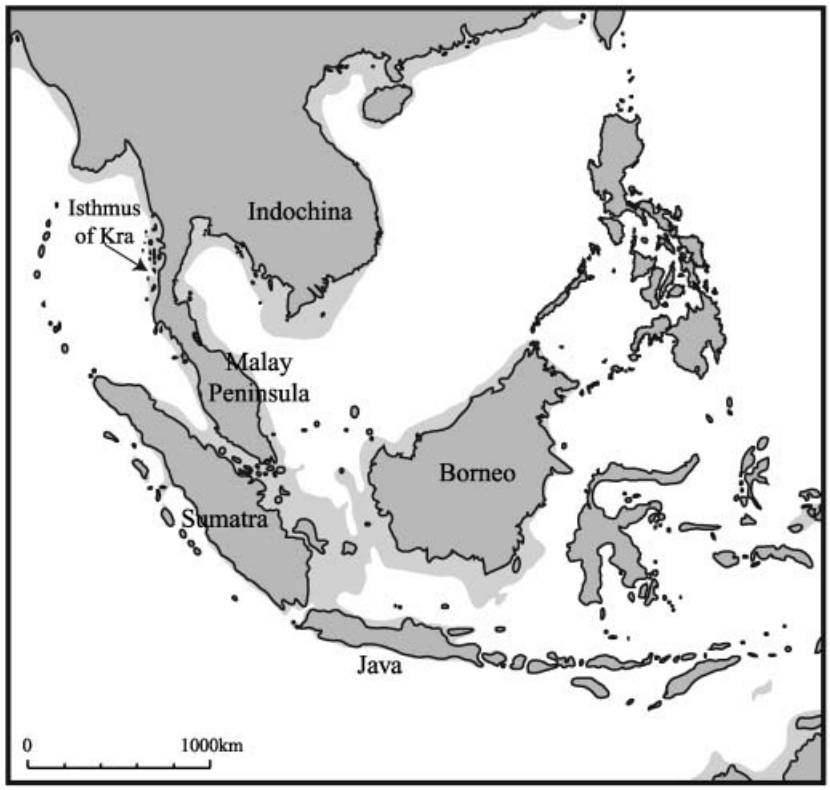

Figure 1. The Sunda region of south-east Asia showing the extent of land area at sea levels 40 m below present, conditions that characterized the Sunda landscape as recently as 9500 years ago. Adapted from the maps of C. R. Simpson and H. K. Voris in Voris (2000).

mammal species in south-east Asia correspond with the boundaries of land areas defined by Pleistocene sea levels (Heaney, 1986; Koopman, 1989), reflecting dispersals over land bridges. Others have similarly interpreted the mid-Pleistocene appearance in the fossil record of taxa such as the gibbon (*Hylobates*), orangutan (*Pongo*), barking deer (*Muntiacus*), ferret badger (*Melogale*) and gymnure (*Hylomys*), all lowland or montane forest-dwelling species, to be evidence of migrations over forested Pleistocene land bridges (Medway, 1963, 1972). Although the biogeographical patterns described in these studies may be consistent with the notion of widespread Pleistocene migrations, alternative scenarios involving the pre-Pleistocene connections among the Sunda islands and mainland have not been examined.

Factors influencing the Pleistocene movements of forest-dwelling mammals

The ability of rain forest animals to move across the exposed shelf in the Pleistocene would have been influenced by topographical and ecological features of the emergent palaeolandscape. The extent of rain forest in the Sunda region during this period is debated: some studies demonstrate a predominance of grassland plants characteristic of savanna or steppe vegetation (Verstappen, 1992, 1997; Morley, 1998, 2000; van der Kaars *et al*., 2001), whereas others indicate that tropical rain forest and mangrove swamp dominated at least the core southern region of the shelf (Sun *et al*., 2000; Kershaw *et al*., 2001).

The movements of rain forest taxa may have been facilitated by the giant rivers that dissected the Sunda region during this period. Deep troughs that today lie beneath the South China and Javan seas formerly connected extant Bornean rivers to extant rivers on Java and Sumatra. Other rivers flowed between Sumatra and the Malay Peninsula (Rainboth, 1996; Voris, 2000). These rivers enabled freshwater fish to move among islands (Dodson, Colombani & Ng, 1995), and, if the riverine systems supported gallery forests, may likewise have provided dispersal corridors for rain forest mammals.

The phylogeographical approach

How did Pleistocene sea-level changes and land bridges affect the evolution of forest-dwelling small mammals of the Sunda shelf? Phenetic tests of faunal similarity and fossil data support the hypothesis of large-scale faunal movements and provide a latest date for a number of colonization events, but neither represents an explicit test of this question. By contrast, a phylogeographical approach in which multiple groups are examined for common patterns of relationships across a landscape (Avise *et al*., 1987) can enable us to reconstruct the evolutionary histories of Sunda shelf rain forest taxa, and thus the history of isolation and fragmentation among forested regions of the shelf. The phylogeographical patterns that emerge from such a reconstruction can provide a test of the hypothesis that Pleistocene land bridges explain current distributional patterns in the Sunda region. The patterns found may also suggest alternative hypotheses.

Mitochondrial DNA is a useful tool in phylogeographical studies, as it is maternally inherited and therefore not subject to the effects of recombination (Avise, 1989). The mitochondrial control region (or d-loop) evolves at a rate sufficient for detecting intraspecific variation (Upholt & Dawid, 1977; Aquadro & Greenberg, 1983) and has been used effectively to address phylogeographical hypotheses in many mammal taxa, including bovids (Birungi & Arctander, 2000), felids (Eizirik *et al*., 1998), dasyurids (Firestone, 2000) and murine rodents (Matisoo-Smith *et al*., 1998). Likewise, the mitochondrial cytochrome *b* gene is an effective marker for phylogeographical studies and has been used to examine mammalian patterns of evolution at both intraspecific (e.g. Mustrangi & Patton, 1997; Harris, Rogers & Sullivan, 2001) and interspecific levels (e.g. Smith & Patton, 1993).

Taxa studied

Maxomys surifer Miller 1900, *Leopoldamys sabanus* Thomas 1887 and *Maxomys whiteheadi* Thomas 1889 are three common rain forest murines that represent a range of body sizes and life histories among small mammals of the Sunda region, and thus are good candidates for a phylogeographical study of the influence of Pleistocene sea-level changes on small mammals of the Sunda shelf. The red spiny rat *M. surifer* is a terrestrial medium-sized murine with a body mass up to 284 g, the long-tailed giant rat *L. sabanus* is a semi-arboreal murine of maximum body mass 532 g, and the spiny mouse *M. whiteheadi* is a small-bodied terrestrial murine with body mass as great as 83 g (Medway, 1969, 1977; Payne & Francis, 1998). All three of these taxa prefer forested habitat and appear not to exist commensally with humans, and therefore are unlikely to display phylogeographical patterns that are a result of human-influenced dispersal. Both *M. surifer* and *L. sabanus* are found on Borneo, Sumatra, Java, the Malay Peninsula and Indochina. *Maxomys whiteheadi* has a smaller, Sunda-restricted range: it is found on Borneo, Sumatra and the Malay Peninsula south of the Isthmus of Kra, but is absent from Java (Musser & Carleton, 1993). The possible multispecies nature of the two taxa with widespread continental and Sunda distributions (*M. surifer* and

L. sabanus) has been mentioned (Musser, Marshall & Boeadi, 1979; Musser, 1981), but none of these three species has been critically revised.

No comprehensive systematic analysis has resolved phylogenetic relationships among either all of the 19 *Maxomys* species or the four *Leopoldamys* species, nor have the relationships of these taxa to other south-east Asian genera been firmly established. Evidence for the evolutionary positions of *Maxomys* and *Leopoldamys* is reviewed in Musser & Carleton (1993). Data from dental and external morphology (Musser & Newcomb, 1983), karyotypes (Gadi & Sharma, 1983), albumin immunology (Watts & Baverstock, 1994) and spermatazoal morphology (Breed & Yong, 1986; Breed & Musser, 1991) are equivocal, as they indicate various possible relationships between the genera of interest here and others but examine different sets of taxa. Phylogenetic analysis of LINE-1 elements places *Maxomys* in a clade that is sister to a *Leopoldamys*/*Niviventer* group and another comprising *Rattus*, *Berylmys*, *Bandicota* and *Sundamys* (Verneau, Catzeflis & Furano, 1998). Both the distinctiveness of *Maxomys* and the close relationship of *Niviventer* and *Leopoldamys* are consistent with the results of a DNA–DNA hybridization study (Ruedas & Kirsch, 1997), but again it is important to stress that the sister groups to *Maxomys* and *Leopoldamys* will remain ambiguous until comprehensive analyses encompassing the broad diversity of murine genera are performed.

Hypotheses and predictions

We tested the hypothesis that Pleistocene land bridges enabled widespread movements of *M. surifer*, *L. sabanus* and *M. whiteheadi* among the islands and peninsular regions of the Sunda shelf. Borneo, Sumatra, Java and the Malay Peninsula were isolated from one another between 7000 and 9500 years ago. If large-scale migrations occurred periodically until this time, the subsequent period of isolation (7000–9500 years) is unlikely to have resulted in complete lineage sorting and pronounced genetic differentiation among populations in these regions. Hence, the observation of distinct, monophyletic lineages associated with each of these regions in the three taxa studied would reject the hypothesis of migrations up to and through the late Pleistocene. This pattern would instead be consistent with a vicariance scenario, in which *M. surifer*, *L. sabanus* and *M. whiteheadi* share a history of diversification resulting from barriers arising within their formerly continuous ranges.

Borneo is the focus of sampling for each group, and thus our strongest test is of the invasion of Borneo from other regions of the Sunda shelf. Borneo occupies a central position on the Sunda shelf with long coastlines facing all other major regions (Indochina, the Malay Peninsula, Sumatra and Java) and a history of broad Pleistocene connections to these regions. Despite the smaller samples from Sumatra and Java, the sampling of south-western Borneo, southern Sumatra and western Java for *M. surifer* provides a good test of the monophyly of populations in these regions. The former two may have been connected by riverine gallery forests in the Pleistocene and the latter two are separated today only by the narrow Sunda Straits. The sampling of the other Sunda regions and mainland is not extensive but provides initial evidence concerning our hypotheses; the patterns we find can be tested with additional data in the future.

METHODS

Sampling

Multiple samples are included in each study from regions representing most of the known range of the group (Table 1 and Fig. 2). For *Maxomys surifer*, 77 individuals were sequenced for d-loop from localities in Vietnam, the Malay Peninsula, Sumatra, Java, and north and south Borneo. For *Leopoldamys sabanus*, 52 samples from Vietnam, the Malay Peninsula, Sumatra, and north and south Borneo were included. It was not possible to obtain samples from Java, part of the known range of the group in recent history. For *Maxomys whiteheadi*, 52 individuals were included from the Malay Peninsula, Sumatra and a broad sampling of Bornean localities. This species is not known to occur on mainland Indochina (above the Isthmus of Kra) or Java. For all species, a subset of individuals was sequenced for cyt *b*. We also collected sequence data from closely related taxa and individuals representing appropriate outgroups for each analysis: for the studies of *M. surifer* and *M. whiteheadi*, we sequenced DNA from the congeners *Maxomys rajah* Thomas 1894 and *Maxomys moi* Robinson & Kloss 1922. For the study of *L. sabanus*, the south-east Asian murine species *Leopoldamys edwardsi* Thomas 1882, *Sundamys muelleri* Jentink 1879, *Niviventer rapit* Bonhote 1903 and *Niviventer cremoriventer* Miller 1900 were sequenced, and the *Rattus norvegicus* Berkenhout 1769 sequence was obtained from GenBank (NC001665). Specimen and locality information are given in the Appendix.

Molecular protocol

DNA was extracted from ethanol-preserved liver, kidney and/or heart samples using a Qiagen DNeasy Tissue Kit and protocols therein. Standard double-stranded 50-μL PCR amplifications were performed using the Perkin Elmer GeneAmp PCR System 2400.

Initially, approximately 1055 bp of the d-loop region were sequenced from several *M. surifer*, *L. sabanus*

Table 1. Control region haplotype information for *M. surifer*, *L. sabanus* and *M. whiteheadi*. Locality acronyms refer to sites indicated in Fig. 2. *N*, number of individuals sequenced per locality; N_h, number of haplotypes per locality; N_u, number of unique haplotypes per locality; S_R, number of haplotypes shared with other localities in the same region (e.g. within Borneo, Sumatra or north-east Vietnam); Max. Div., maximum pairwise divergence ('p' distance) within a locality

Locality	*N*	N_h	N_u	S_R	Max. Div.
M. surifer					
HT	4	3	3	0	0.5%
YD	3	2	2	0	2.8%
CT	1	1	1	0	–
CL	4	3	3	0	0.5%
PA	6	2	2	0	0.7%
LA	6	5	5	0	2.1%
KM	13	9	9	0	3.1%
GP	19	6	6	0	2.8%
BB	6	6	6	0	2.6%
BS	1	1	1	0	–
PK	14	6	6	0	0.7%
L. sabanus					
VP	1	1	1	0	–
HT	4	3	3	0	0.7%
CL	1	1	1	0	–
ER	4	4	4	0	1.1%
GL	1	1	1	0	–
LA	2	2	2	0	0.9%
KM	11	7	4	3	1.1%
GP	17	8	5	3	1.3%
BB	10	3	1	2	0.2%
LE	1	1	0	1	–
M. whiteheadi					
ER	2	2	2	0	2.3%
GL	4	3	3	0	3.8%
KM	16	10	10	0	1.9%
GP	13	8	6	2	5.4%
BB	7	5	3	2	1.7%
BS	5	5	3	2	3.8%
TL	2	2	2	0	1.2%
TW	3	3	3	0	2.1%

and *M. whiteheadi* using the conserved primers L15926 and H00651 (Kocher *et al*., 1989) and internal primers (L5′-CTCGATGGTA(A/C/T)(C/A)GGGTCTAA ACA-3′; H5′-TTCATGCCTTGACGGCTATGTTGA-3′) that were designed from conserved sites in published *Rattus* and *Mus* control region sequences (Brown *et al*., 1986). The reaction conditions for these PCR amplifications were as follows: initial denaturation at 94°C for 2 min; denature at 94°C for 20 s; anneal at 45–52°C for 15 s; and extension at 72°C for 30 s for 35 cycles. Each 50-μL reaction contained 0.125 μL Gibco Platinum *Taq*, 5 μL PCR buffer, 5 μL $MgCl_2$, 5 μL 10 mM dNTP, 2.5 μL of each 10 mM primer, and 1 μL DNA.

An alignment of these complete control region sequences generated using Clustal X (Thompson *et al*., 1997) enabled us to identify conserved regions in *M. surifer*, *L. sabanus* and *M. whiteheadi.* A specific primer pair was then designed from sites conserved in all three taxa (L5′-AGGCATCTGGTTCTTACTTC-3′; H5′-CATCTAAGCATTTTCAGTGC-3′) encompassing the ~530-bp region (beginning in the central domain and ending in the adjacent tRNA-phe) used in our phylogeographical analyses. The reaction conditions for PCR amplifications using these primers were as follows: initial denaturation at 94°C for 2 min; denature at 94°C for 15 s; anneal at 48–51°C for 20 s; and extension at 72°C for 1 min for 25–30 cycles. Each 50-μL reaction contained the same volumes of reactants as above except that 0.25 μL of Gibco *Taq* was used in place of 0.125 μL Platinum *Taq*.

To test the support for the phylogenetic hypotheses derived from the control region data, a segment of the cytochrome *b* gene (~830 bp) was PCR amplified for a subset of the animals sequenced for d-loop (19 *M. surifer*, 14 *L. sabanus* and 14 *M. whiteheadi*) using the primers MVZ05 and MVZ16 (Smith & Patton, 1993). The following reaction conditions were used: initial denaturation at 94°C for 2 min; denature at 94 °C for 15 s; anneal at 45–49°C for 20 s; and extension at 72°C for 80 s for 25–30 cycles. Each 50-μL reaction contained 0.25 μL Gibco *Taq*, 5 μL PCR buffer, 2.5 μL $MgCl_2$, 5 μL 10 mM dNTP, 2.5 μL of each 10 mM primer, and 1 μL DNA.

The target PCR products were purified by gel electrophoresis using 1.5% low-melting-point agarose (Gibco BRL) and cleaned of agarose using the Qiagen QIAquick Gel Extraction Kit and protocols therein. Sequencing reactions were performed using the Applied Biosystems Big Dye Terminal Cycle Sequencing Ready Reaction Kit and cleaned using Centrisep Spin Columns (Princeton Separations) packed with Sephadex G-50 (Sigma). The sequencing primers used for the control region were the same as the pair used for amplification of the ~530-bp segment. For the cyt *b* sequencing, we designed a set of internal primers that were used in combination with the PCR primers: the primer H5′-CCTCAGAAGGAAATTTGTCC-3′ was used with MVZ05 and L5′-GGACAAATATCCTTCTGA GG-3′ was used with MVZ16. Sequences were run on an ABI 377 according to the manufacturer's protocol.

Phylogenetic analyses

The control region sequences were aligned in Clustal X (Thompson *et al*., 1997) for each of the three data sets (*M. surifer*, *L. sabanus* and *M. whiteheadi*) using

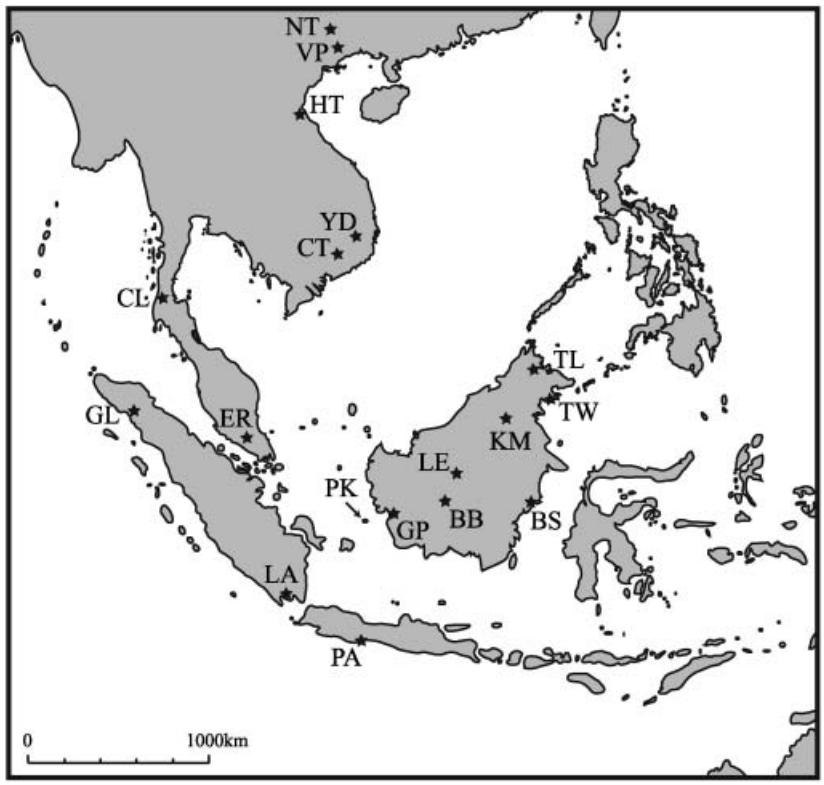

Figure 2. Sampling localities. See Appendix for specific locality information. Base map adapted from the maps of C. R. Simpson and H. K. Voris in Voris (2000).

the following default alignment parameters: gap opening cost = 15; gap extension cost = 6.66; delay divergent sequences setting = 30%; and DNA transition weight = 0.50. Because there is no standard approach to the complex issue of alignment, these parameters are reported here to enable repeatability.

The control region alignment was examined by eye in MacClade v.4.0 (Maddison & Maddison, 2000). Sequence length variation in each of the three control region data sets introduces problems of potential phylogenetic importance concerning the placement of gaps in the alignments. In each data set, several regions of high gap frequency were identified in which we could not justify one alignment over another. These regions were cut from the alignments. Subsequent to the excision of these 'gappy' regions, the lengths of the sequences in the d-loop alignments to be analysed were as follows: *M. surifer* 437 characters; *L. sabanus* 461; and *M. whiteheadi* 502. Duplicated sequences were pruned from the data sets leaving 44 haplotypes of *M. surifer*, 26 *L. sabanus* and 34 *M. whiteheadi*.

Remaining individual gaps in the control region data were treated as a fifth state in our analyses, a logically consistent cost regime in that: (1) gaps are phylogenetic events and (2) all character columns in the data matrix are equally weighted and treated as independent events (Giribet & Wheeler, 1999). Our conclusions are not altered by treating remaining gaps as missing data in analyses of these control region data sets.

The cyt *b* data were aligned in Clustal X separately for *M. surifer*, *L. sabanus* and *M. whiteheadi*. Because this protein-coding gene lacks insertions and deletions, alignment is unambiguous. The cyt *b* sequences were combined with the control region alignments for the same subset of individuals. For *M. surifer*, 836 base pairs of cyt *b* were combined with the d-loop data for a total of 1273 characters. The *L. sabanus* cyt *b* sequences were 841 base pairs, and the combined alignment was 1302 characters. The cyt *b* segment sequenced for *M. whiteheadi* was 819 base pairs in length and was combined with the control region data for a total sequence length of 1320 characters.

Phylogenetic analyses of both the control region data sets and the cyt *b* + control region data sets were performed with heuristic searches using parsimony. Analyses were conducted in NONA (Goloboff, 1993) spawned from Winclada (Nixon, 1999a) with 100 random addition replicates using the MultTBR+TBR option. Further analyses were conducted in NONA and our initial results rigorously tested using the Parsimony Ratchet (Nixon, 1999b) spawned from Win-

clada. By iteratively reweighting a subset of characters, the Ratchet improves the efficiency and speed of tree searching by enabling the search to move from an island of equally most parsimonious trees, where it can become 'mired' in branch-swapping on almost identical trees, to a new island that may possess more parsimonious trees. The trees collected during the iterations of the Ratchet represent a broader sample of tree space than does the sample collected from a single island (Nixon, 1999b). Each data set was subjected to 10 000 iterations of the Parsimony Ratchet with one starting tree and 15% of the characters reweighted per iteration. Because the Ratchet itself can become stranded on islands of similar trees (Nixon, 1999b), we also performed a set of analyses in which the data sets were subjected to 1000 iterations of the Ratchet ten times. For each of the taxa studied, heuristic analysis, 10 000 iterations of the parsimony Ratchet and ten repeated Ratchet analyses of 1000 iterations produced the same consensus topologies.

In the analysis of *M. surifer*, one *M. rajah* individual was designated as the outgroup (because in NONA only one terminal can be made outgroup). In preliminary analyses *L. sabanus* had been designated as outgroup to confirm the monophyly of *M. surifer* with respect to the other *Maxomys* species analysed. The monophyly of *M. surifer* was strongly supported, but, because of the difficulty of aligning *Maxomys* sequences against *L. sabanus* sequence, here we prefer to present analyses in which a congener was assigned outgroup. Other congeners were included but not specified as outgroups. The same argument follows for the *M. whiteheadi* analysis, for which *M. moi* was designated as the outgroup based on the results of analyses including *L. sabanus*.

In the analysis of *L. sabanus*, it was not possible to use the congener *L. edwardsi* as outgroup because the two species are paraphyletic in Indochina (see Results). Sympatric *L. sabanus* and *L. edwardsi* from the Malay Peninsula are distinct serologically, immunologically, karyotypically, behaviourally and morphologically (Yong, 1970). The paraphyly of Indochinese *L. sabanus* with respect to *L. edwardsi* may represent a case of mitochondrial introgression (Ferris *et al*., 1983; Ruedi, Smith & Patton, 1997) or it may be indicative of a greater evolutionary and taxonomic complexity among *Leopoldamys* species than is currently understood. Although we recognize that more data are required to address this question, this study provides initial insight into the relationships between Sunda shelf *L. sabanus* and Indochinese populations. A more distant relative, *R. norvegicus*, was designated as the outgroup and *S. muelleri*, *N. cremoriventer* and *N. rapit* were also included in the analysis.

Support for nodes was estimated in NONA using 1000 Jackknife replicates of 20 random addition replicates each. Bremer support (Bremer, 1994), which represents the difference in tree length between a cladogram having the group in question and one in which that group is collapsed, was estimated in PAUP 4.0b10 (Swofford, 2002) using a command file created in TreeRot.v.2 (Sorenson, 1999). 'p' distances (uncorrected percentage sequence divergence) within localities and within and among lineages were calculated using MEGA v.2.1 (Kumar *et al*., 2001).

RESULTS

Haplotype data

Control region haplotype data, including the number of unique and shared haplotypes and the maximum pairwise divergence within each general sampling locality, are presented in Table 1. No haplotypes are shared between sites on different landmasses (e.g. between Borneo and Sumatra) in any of the three murine species studied. In *M. surifer*, we found no haplotypes that are shared among sampling localities. In *L. sabanus* and *M. whiteheadi*, the only haplotypes shared among different sampling localities were found on Borneo in the GP, BB, BS and KM sites. Levels of sequence divergence within sampling localities are generally highest in *M. whiteheadi* and lowest in *L. sabanus*, but, because of the small sample sizes from some regions, we refrain here from presenting statistical comparisons of sequence diversity and divergence. No cyt *b* haplotypes were shared among sampling localities.

Phylogenetic analyses

Maxomys surifer

The phylogenetic analysis of control region from Indochinese and Sunda shelf *Maxomys surifer* included 152 parsimony-informative characters and resulted in 128 most parsimonious trees with a length of 449 steps, a CI of 0.60, and an RI of 0.81. The strict consensus is shown in Figure 3 with jackknife (JK) values above the branches and Bremer support values below. The number of individuals bearing each haplotype represented in the tree is presented in parentheses following the acronym for the sampling locality in which the haplotype was found; our *M. surifer* data set contained 33 redundant sequences.

Distinct groups associated with the following regions were identified in the control region tree: Borneo (including all populations sampled); Java; Sumatra; the Malay Peninsula; southern Vietnam; and north-eastern Vietnam. Support for these lineages is moderate (JK = 70%; Bremer = 3 for the Borneo group) to high (JK > 98%; Bremer = 4–14 for the groups associated with Java, Sumatra, the Malay Peninsula, southern Vietnam and north-eastern Vietnam).

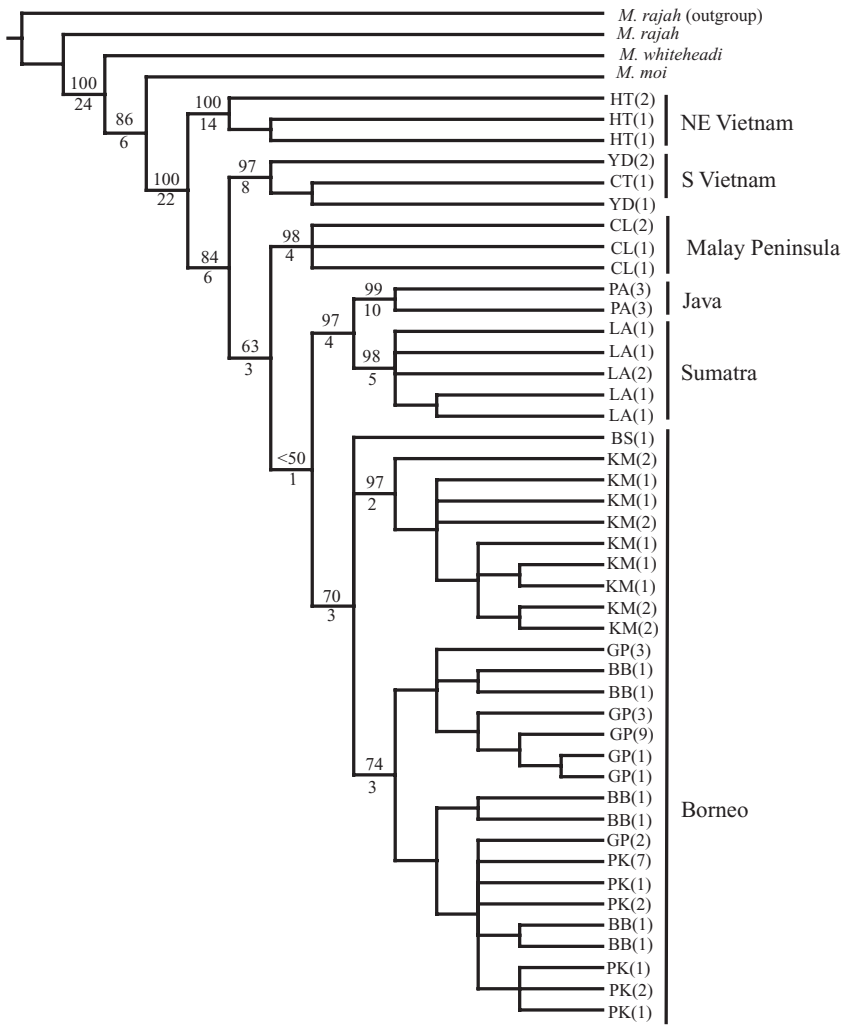

Figure 3. Strict consensus of most parsimonious trees obtained from the phylogeographical analysis of *M. surifer*. Jackknife support is shown above branches and Bremer support below. Numbers in parentheses refer to numbers of individuals bearing the haplotype of each terminal. Locality abbreviations are defined in the Appendix and mapped in Fig. 2.

Four sets of relationships among these distinct groups are apparent in the control region data: first, the Javan and Sumatran lineages form a clade (JK = 97%; Bremer = 4). Second, the Java/Sumatra group is sister to the Bornean group (JK < 50%; Bremer = 1). Third, the Malay Peninsula group is sister to the island group (JK = 63%; Bremer = 3). Fourth, the two Vietnamese lineages are basal to all other groups with the southern group as sister to the peninsular/insular clade (JK = 84%; Bremer = 6).

Sampling on Borneo was sufficiently widespread to enable several distinct control region lineages on the island to be distinguished: populations from three localities in south-west Borneo (GP, BB, and PK) including the small offshore island Karimata represent one group (JK = 74% and Bremer = 2), populations from a collecting site in north-east Borneo (KM) represent a second group (JK = 97%; Bremer = 2) and a single sample from the south-east (BS) falls into neither of the other groups. Identical haplotypes on Borneo are shared within general collecting localities, but not between them.

Average divergence (uncorrected 'p' distance) among the peninsular/insular control region lineages ranges from 5.5 to 8.8% and between the southern Vietnam and peninsular/insular lineages from 6.5 to 10.9%. Divergence between the north-eastern Vietnam group and all other groups ranges from 10.9 to 13.6%. Divergences among the Bornean lineages range from 5.8 to 6.7%, and divergences within the major Bornean groups are <1.9%.

Phylogenetic analysis of a combined cyt *b* + control region data set containing 311 parsimony-informative characters for 19 *M. surifer* individuals yielded one

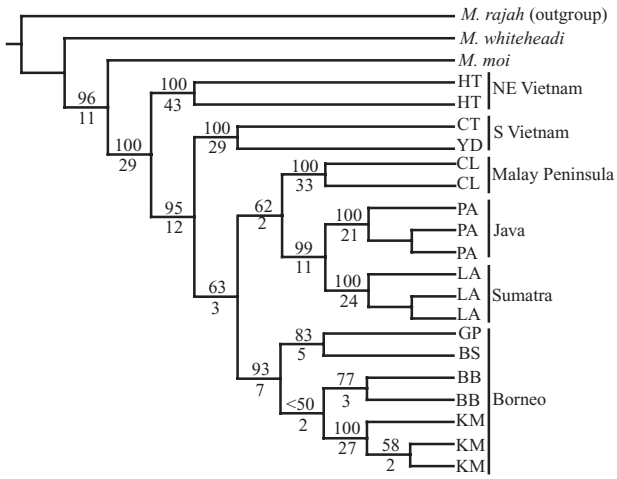

Figure 4. Single most parsimonious tree obtained by analysis of control region and cyt *b* data of *M. surifer*. Jackknife support is shown above branches and Bremer support below. Locality abbreviations are defined in the Appendix and mapped in Fig. 2.

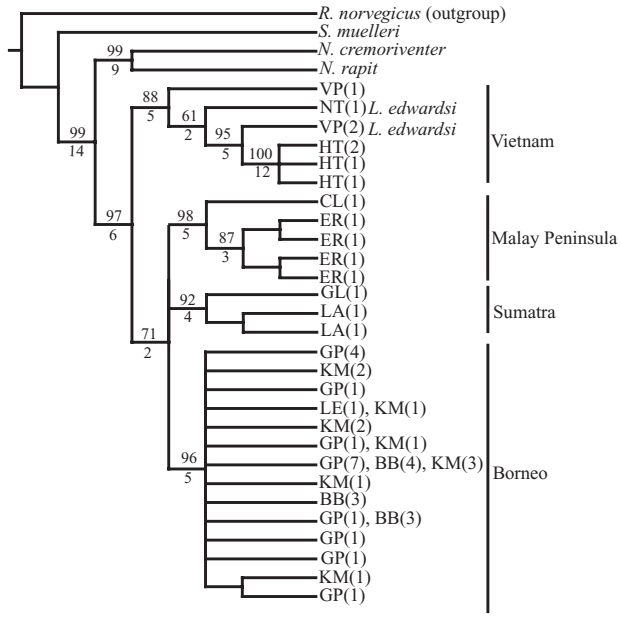

Figure 5. Strict consensus of most parsimonious trees obtained from the phylogeographical analysis of *L. sabanus*. Jackknife support is shown above branches and Bremer support below. Numbers in parentheses refer to numbers of individuals bearing the haplotype of each terminal. Locality abbreviations are defined in the Appendix and mapped in Fig. 2.

most parsimonious tree of 901 steps, CI = 0.57, RI = 0.69. This tree also demonstrated distinct lineages in each region sampled, including north-eastern and southern Vietnam groups (Fig. 4). Support for these groups is high (JK = 93–100%; Bremer = 7–43). Two topological differences between this tree and the control region tree (Fig. 3) are apparent: first, the Malay Peninsula lineage is sister to the Java/Sumatra lineage (JK = 62%), rather than basal to all Sunda island groups. Second, distinct northern and southern Bornean lineages are not apparent. Uncorrected 'p' distances in this combined data set show average divergences among peninsular/insular lineages of 6.1–9.3% (or 6.1–8.5% in the cyt *b* data alone) and of 8.7–9.0% (7.8–9.3% in the cyt *b* data alone) between southern Vietnam and the Sunda regions. Divergences between the north-eastern Vietnam group and all other lineages are 10.8–12.9% in the combined data (9.6–11.5% in the cyt *b* data alone).

Leopoldamys sabanus

The *L. sabanus* control region data set contained 138 parsimony-informative characters, and the phylogenetic analysis of *L. sabanus* from Indochina and the Sunda shelf resulted in 100 most parsimonious trees of length 449, CI = 0.64, RI = 0.76. The strict consensus is shown in Figure 5 (jackknife values above the branches and Bremer support below). The *L. sabanus* data set included 27 redundant haplotypes; the number of individuals bearing each haplotype is given in parentheses on the tree following the acronym representing the locality in which the haplotype was found.

The following well-supported groups were distinguished in the control region analysis: a Borneo group including all populations sampled (JK = 96%; Bremer = 5); a Malay Peninsula group including representatives from both the north and the south (JK = 98%; Bremer = 5); a Sumatra group including individuals from both the north and the south (JK = 92%; Bremer = 4); and an Indochinese group comprising a paraphyletic arrangement of all Vietnamese *L. sabanus* and *L. edwardsi* sampled (JK = 88%; Bremer = 5).

Two sets of relationships among these control region lineages are discernable: first, the Borneo, Sumatra and Malay Peninsula groups form an unresolved trichotomy (JK = 71%; Bremer = 2); second, this clade is sister to the Indochinese *L. sabanus*/*L. edwardsi* group.

No structure is apparent among the Bornean samples, which included individuals from the north-east (KM), centre (LE) and south-west (GP and BB) of the island. Identical haplotypes are shared between distant localities on Borneo, i.e. between a central locality (LE) and the north-east (KM), and between the south-west (GP and BB) and the north-east (KM).

Average distance among control region lineages in the island/peninsular trichotomy ranges from 5.9 to

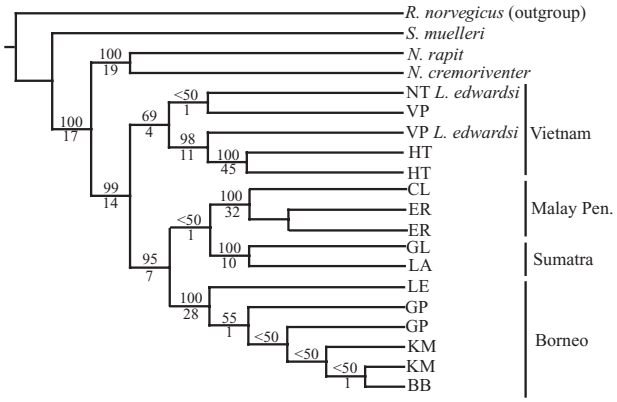

Figure 6. Single most parsimonious tree obtained by analysis of control region and cyt *b* data of *L. sabanus.* Jackknife support is shown above branches and Bremer support below. Locality abbreviations are defined in the Appendix and mapped in Fig. 2.

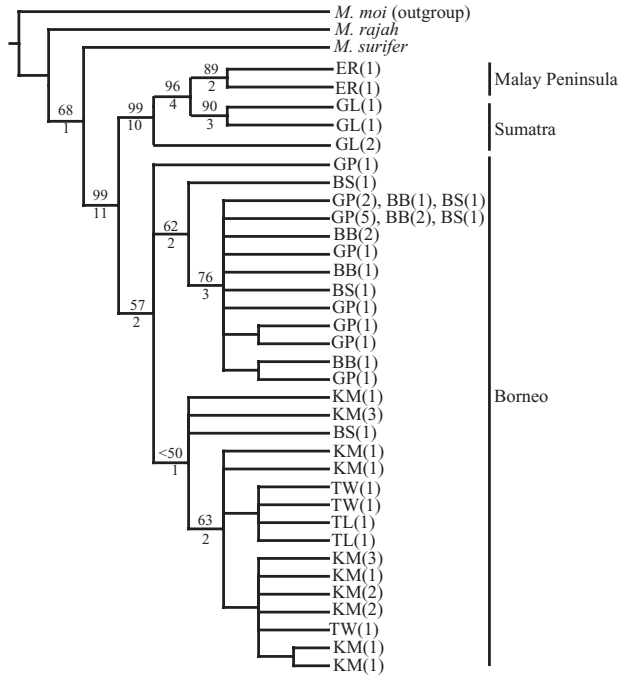

Figure 7. Strict consensus of most parsimonious trees obtained from the phylogeographical analysis of *M. whiteheadi.* Jackknife support is shown above branches and Bremer support below. Numbers in parentheses refer to numbers of individuals bearing the haplotype of each terminal. Locality abbreviations are defined in the Appendix and mapped in Fig. 2.

7.3%, and divergences between the Indochinese *L. sabanus*/*L. edwardsi* group and other lineages from 12.2 to 13.1%. Average within-group distance is <2.7%, except within the Indochinese group (8.1%).

The combined data set of control region and cyt *b* sequence for 14 *L. sabanus* contained 342 parsimony-informative characters and produced one most parsimonious tree of 1081 steps, CI = 0.58, RI = 0.66 (Fig. 6). This tree also demonstrates the presence of distinct lineages in each region sampled. Support for the insular and peninsular groups is high (JK = 98–100%; Bremer = 10–32). The Indochinese group, which includes a mixture of *L. edwardsi* and *L. sabanus* haplotypes as above (Fig. 5), is less well supported (JK = 69%; Bremer = 4). The tree is topologically consistent with that yielded in the analysis of the full control region data set (Fig. 5). Average divergences ('p' distances) among insular lineages and the peninsular group are 8.5–8.9% (8.9–9.1% in the cyt *b* data alone). The Vietnam group differs from the Sunda lineages by 12.9–13.3% in the combined data set (11.1–11.9% in the cyt *b* alone).

Maxomys whiteheadi

The phylogenetic analysis of *M. whiteheadi* control region sequence data included 122 parsimony-informative characters and resulted in 276 most parsimonious trees of length 420, CI = 0.70, RI = 0.77. The strict consensus of these is shown in Figure 7 with jackknife support values above the branches and Bremer support below. Eighteen duplicate haplotypes have been pared from the data set; numbers of individuals represented by each haplotype are given in parentheses on the tree.

Control region sequences representing the major regions covered by the range of *M. whiteheadi* comprise two lineages: a Borneo group (JK = 57%; Bremer = 2) and a group that includes all Malay Peninsula and Sumatra samples (JK = 99%; Bremer = 10).

Several control region lineages are discernable within the Borneo group: a primarily south-western group (GP and BB) that includes some individuals from the south-east (BS) (JK < 50; Bremer = 1); a primarily northern group (KM, TW and TL) that also includes some individuals from the south-east (BS) (JK = 62; Bremer = 1); and a single divergent individual from GP. Relationships among the three are unresolved. Haplotypes are shared between two southern localities (GP and BS) and between the two south-western localities (GP and BB).

The average divergence between control region sequences in the Malay Peninsula/Sumatra group and the Borneo group is 7.8% and among the three Borneo lineages is 2.9–4.3%. Variation within the Malay Peninsula/Sumatra group is 3.0% and within both Borneo groups is <1.8%.

Seven most parsimonious trees of 715 steps (CI = 0.70; RI = 0.65; 238 parsimony informative characters) were produced in the combined phylogenetic

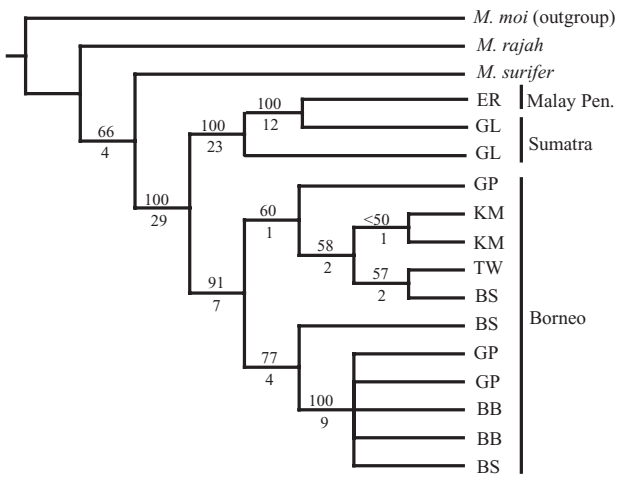

Figure 8. Strict concensus of seven most parsimonious trees obtained by analysis of control region and cyt *b* data of *M. whiteheadi.* Jackknife support is shown above branches and Bremer support below. Locality abbreviations are defined in the Appendix and mapped in Fig. 2.

analysis of control region and cyt *b* sequences for 14 *M. whiteheadi* (consensus shown in Fig. 8). The presence of two distinct lineages on the Sunda shelf, one on Borneo and one on the Malay Peninsula and Sumatra, is demonstrated as above (Fig. 7). Support for the Borneo lineage is considerably increased by the inclusion of the cyt *b* data (JK = 91%; Bremer = 7). The Sumatra/Peninsula group is similarly well supported (JK = 100%; Bremer = 23). The two lineages differ by an average pairwise distance of 7.7% in both the combined data set and in the cyt *b* data alone.

DISCUSSION

Pleistocene migrations or Pleistocene isolation?

Despite broad physical connections between Borneo and the other Sunda islands and Malay Peninsula less than 10 000 years ago, in each of the three taxa studied populations from Borneo represent a well-differentiated monophyletic lineage (Figs 3–8), suggesting a much longer period of isolation. A similar pattern of distinct lineages is observed for almost all of the other regions sampled, although more intensive sampling from regions other than Borneo will be needed to provide a rigorous test of, for example, Pleistocene movements between Java and Sumatra in *M. surifer* and *L. sabanus*. The analyses of all available data presented here, however, show no evidence of migrations over these land bridges, but rather provide strong support for a deeper history of vicariant diversification.

The depths of divergence among regional groups suggest that populations on Borneo, Java and Sumatra were isolated from one another and from the mainland well before the most recent glacial maximum (11 000 years ago) and quite possibly before the Pleistocene. Although we do not attempt a precise dating of the diversification of lineages in *M. surifer*, *L. sabanus* and *M. whiteheadi*, the cyt *b* data may be useful in obtaining a rough estimate of divergence times among the lineages identified (whereas the control region data, from which multiple hypervariable regions have been pared, are more difficult to consider). A standard molecular clock of 2% Myr^{-1} for mammalian mitochondrial DNA (Brown *et al.*, 1979), a rate that is consistent with that estimated from raw differences in *Rattus* and *Mus* cyt *b* sequence calibrated with the fossil split of these two genera (Jacobs & Downs, 1994), would place the divergences among islands at 3.1–4.7 Mya and between the islands and mainland at 4.8–6.0 Mya. A faster clock, such as the highest average rate for the whole mitochondrial genome (10% Myr^{-1}) estimated for species of *Mus* (She *et al*., 1990), would date the island divergences at 0.61–0.93 Mya and the island/mainland divergence at 0.96–1.19 Mya. If regional monophyly were to reflect postglacial vicariance, the rate of change required to account for the levels of divergence observed in our data (up to ~12% in the cyt *b* data) following isolation only 10 000 years ago is two orders of magnitude greater than would be predicted by either the standard 2% Myr^{-1} rate calculated for primates (Brown *et al*., 1979) or the 10% Myr^{-1} rate calculated for some species of *Mus* (She *et al*. (1990). Such a rapid rate has not been recorded previously.

An accurate estimate of divergence times in *M. surifer*, *L. sabanus* and *M. whiteheadi* would require a separate calibration of the molecular clock for each of these taxa. The only information that could be used for this purpose is the geological timing of the fragmentation and reconnection of the Sunda shelf and mainland, events that are not independent of the evolutionary events that we seek to date. Thus, it was not possible to estimate independently the rough ages of the lineages identified without recourse to published rates of divergence in other mammal lineages. Moreover, estimates based on the rough clocks above (ranging from 2 to 10% Myr^{-1}) cannot be reconciled with the known date for the last breakup of the shelf and the fragmentation of populations on different islands. A greater understanding of palaeoecological conditions on the Sunda shelf and the timing of changes in these conditions, in addition to the chronology of geological events, is needed to understand the precise timing of the evolution of independent lineages in the taxa studied.

Some authors have attempted to predict the relationships among animals inhabiting the Sunda region that would be expected from postglacial vicariance

caused by rising sea levels (Ruedi & Fumagalli, 1996). According to their bathymetry-based model, a postglacial fragmentation scenario would result in a sister group relationship between Java (Ruedi & Fumagalli, 1996; or Borneo, as in Voris, 2000) and all other island and mainland lineages, reflecting the early isolation of this island following the most recent glacial maximum. Peninsular and mainland populations would represent a clade in this scenario (Ruedi & Fumagalli, 1996) as a result of the continuity of these regions throughout the Pleistocene and Holocene (Haile, 1971).

The relationships among lineages in both *M. surifer* and *L. sabanus* (Figs 3–6) are inconsistent with the patterns that would theoretically result from postglacial vicariance caused by rising sea levels. Although relationships among regional lineages of *M. surifer* and *L. sabanus* are not fully resolved, neither displays this pattern. Rather, both show a closer relationship between peninsular and island lineages than between peninsular and Indochinese groups. This pattern was also observed in gymnures (Ruedi & Fumagalli, 1996) and is consistent with the sequential dispersal model proposed by these authors, wherein the mainland source area is represented by the most pleisiomorphic lineage and more recently colonized islands are represented by more derived lineages. However, the observation of this pattern in multiple, unrelated taxa is better explained by the occurrence of an older vicariance event affecting the northern Malay Peninsula followed by the vicariant isolation of island populations. We caution that alternative hypotheses involving postglacial vicariance among the Sunda islands layered onto an earlier vicariant event in the northern Malay Peninsula would also result in a sister group relationship between the Indochina group and the island/peninsula group. Again, to accept this scenario we would necessarily have to accept a rate of mitochondrial evolution in our data that is several orders of magnitude greater than those previously recorded.

The results of this study have two broad implications for understanding the Sunda shelf palaeoenvironment. First, they indicate that, at least for these rodent taxa, barriers between Borneo and the other islands and peninsula existed during some periods when Pleistocene land bridges connected these landmasses. This finding is in accordance with accumulating palynological evidence suggesting that the Pleistocene Sunda shelf was cool, arid and covered in large part by savanna-like vegetation (Heaney, 1991; Morley, 1998, 2000; van der Kaars *et al*., 2001) rather than by contiguous humid tropical forest. Second, the results indicate that, despite apparently inhospitable conditions on much of the exposed shelf, suitable rain forest habitat must have persisted throughout the Pleistocene in each region represented by a distinct lineage in the phylogenetic analyses. Previous studies of animals reached similar conclusions concerning the occurrence of refugia: first, studies of disjunct populations of leaf monkey (*Presbytis* and *Pygathrix*), proboscis monkey (*Nasalis*), loris (*Loris* and *Nycticebus*) and gibbon (*Hylobates*) species concluded that current populations represent relict groups that were confined to rain forest refugia during Pleistocene glacial periods (Brandon-Jones, 1996, 1998); the refugia suggested by the distributions of these primates are in north-eastern Indochina, northern Sumatra, western Java and northern Borneo. Second, a study of termite community composition found evidence of former refuges in northern Sumatra and northern and eastern Borneo based on information about rain-forest- and savanna-type termite faunas (Gathorne-Hardy *et al*., 2002). The data presented here suggest the presence of an additional refuge in the Malay Peninsula.

The presence of two clearly defined sister lineages in Bornean *M. surifer* (Fig. 3), and the suggestion of a similar pattern (though poorly supported) in *M. whiteheadi* (Fig. 7), could also be the result of Pleistocene restriction to multiple rain forest refugia. In *M. surifer*, haplotypes from the south-western localities (BB, GP and PK) form a clade that is sister to one composed of haplotypes from the north-east (KM). Neither of these clades is represented on any other island or mainland Indochina. In *M. whiteheadi*, a lineage composed of haplotypes from BB and GP is sister to a lineage composed of those from the north and north-east (TL, TW and KM), and haplotypes from the south-east (BS) appear in both groups. This may be indicative of glacial rain forest fragmentation on Borneo and the restriction of these animals to suitable habitat in the north and south-west of the island. Evidence from some mountainous regions of the Sunda shelf (Newsome & Flenley, 1988; Stuijts, 1993) suggests that these areas were less subject to Pleistocene aridification than lowland areas; the Schwaner Range of south-western Borneo and the topographically complex north may similarly have maintained pockets of moist forest that allowed rain forest mammal populations to persist through glacial maxima. In such a scenario, the mixing of *M. whiteheadi* northern and south-western lineages in the south-east (at BS) could be indicative of a re-invasion of the south-east following the last glacial maximum. Alternatively, this pattern could result from the persistence of ancestral polymorphisms in the BS animals. The large-bodied arboreal *L. sabanus* shows no such pattern on Borneo (Fig. 5); this taxon instead demonstrates a complete lack of phylogeographical structure on the island, with identical haplotypes found at distant localities in the north and south-west. The differences in the degree of phylogeographical structure on Borneo among the

three taxa studied may derive from a greater ability to disperse in forested environments in *L. sabanus* than in *M. surifer* and *M. whiteheadi*. Alternatively, the lack of structure in *L. sabanus* could indicate that it survived arid periods in a single refugium and subsequently dispersed throughout the island. The dispersal abilities of *M. surifer*, *L. sabanus* and *M. whiteheadi* have not been studied under comparable conditions, nor have the possible influences of Pleistocene habitat fragmentation and refugia on Borneo been examined in populations of small mammals.

One exception to the pattern of regional monophyly was revealed in the analyses: *M. whiteheadi* from the Malay Peninsula and Sumatra are not monophyletic relative to one another, although no haplotypes are shared between the two regions (Figs 7, 8). The average levels of control region divergence within this peninsula/Sumatra group (3.0%) are similar to levels within the Borneo *M. whiteheadi* group (3.5%), and the control region divergence between the peninsula/Sumatra group and Borneo (7.8%) is typical of the levels observed between islands in *M. surifer* and *L. sabanus*. This pattern might indicate limited dispersal between the two regions during glacial maxima. Given the lack of haplotypes shared between Sumatra and the Malay Peninsula and the levels of divergence among individual haplotypes (Table 1), it is also possible that the paraphyly of the Sumatran samples results from the retention of an ancestral haplotype in the northern Sumatra population.

An alternative scenario

The results presented clearly indicate that island and peninsular populations within *M. surifer*, *L. sabanus* and *M. whiteheadi* were isolated prior to the breakup of the last Pleistocene land bridges, an event that took place 7000–9500 years ago. Although we do not attempt to provide a precise estimate of dates for the origin of distinct regional lineages, we argue that the depths of divergence may be consistent with preglacial events and, thus, that an alternative scenario explaining the colonization of the Sunda shelf and subsequent diversification of these taxa must be considered. Given accumulating evidence that Pleistocene Sunda conditions were inhospitable for rain forest species, we discuss the possibility of an earlier invasion of the Sunda shelf and fragmentation of Sunda island populations. When might the Sunda region have been characterized by (1) broad connections among the islands, peninsula, and mainland and (2) widespread rain forest habitat that would enable the dispersal of the ancestors of these groups throughout their current ranges? What conditions changed that would then isolate the island groups long before the last glacial maximum?

Throughout the Miocene, Borneo was connected via the Malay Peninsula to Indochina, and, following their emergence from the seas about 10–15 Mya, to Java and Sumatra as well. These connections persisted for several million years until the beginning of the Pliocene (Hall, 1998). South-east Asia experienced a perhumid, or extremely moist, climate during the Miocene, with palynological records showing a predominance of rain forest species punctuated by only short maxima of Poaceae pollen representing brief dry intervals. These maxima increased in frequency in the Pliocene (Morley, 2000). Sediment cores from a 12-Myr record in Lake Baikal indicate that global climatic conditions deteriorated suddenly in the early to mid Pliocene (approximately 3 Mya), when the amplitudes of regular 1-Myr, 0.6-Myr and 0.4-Myr climatic oscillations increased dramatically (Kashiwaya *et al*., 2001). Quartz flux records from the Sea of Japan corroborate these findings, with evidence of humid climates at the Miocene–Pliocene boundary and a marked increase in aridification about 2.5 Mya (Dersch & Stein, 1994). This history of a relatively stable tropical environment through the Miocene followed by the early mid Pliocene deterioration of conditions in south-east Asia may provide an explanation for the vicariant patterns documented above.

The phylogenetic results suggest a mainland Indochina origin for *M. surifer* and *L. sabanus* (Figs 3–6). (A hypothesis concerning the geographical origin of *M. whiteheadi*, however, must await a robust phylogeny for *Maxomys*.) Moreover, in the analysis of *M. surifer* two lineages were identified in Vietnam, one of which is more closely related to the peninsular and Sunda island lineages than to the other Vietnamese group, a pattern indicative of a deep divergence within mainland *M. surifer* prior to its invasion of the Sunda shelf. One *M. surifer* lineage is composed of haplotypes from the coastal side of the northern Annamitique Range, which extends from Laos to southern Vietnam. This area represents a unique biogeographical region from which several new large mammal species have been described in the last decade (e.g. Schaller & Vrba, 1996). The second lineage is composed of haplotypes from the western side of the range at its southernmost limits. A major biogeographical disjunction corresponding with the Annamitique Range has been recorded in several lineages of Indochinese cave Collembola as well (L. Deharveng, pers. comm.).

We propose that *M. surifer*, *L. sabanus* and *M. whiteheadi* dispersed throughout their current ranges in the early Pliocene before the long-standing connections between Indochina, Java, Sumatra, Borneo and the Malay Peninsula were severed. At this time, a relatively stable perhumid climate had characterized the region for several million years and trop-

ical rain forest was widespread, presumably on the exposed regions of the shelf as well as on the islands, peninsula and mainland. Fossil evidence supports the early presence of *Maxomys* and *Leopoldamys* in the region: the putative sister taxon of *Maxomys*, the extinct *Ratchaburamys ruchae*, is known from the late Pliocene to early Pleistocene northern Malay Peninsula (Chaimanee, 1998). *Maxomys* is known from early Pleistocene deposits on eastern Java on the basis of a single molar (van der Meulen & Musser, 1999). Furthermore, *M. surifer* has been recorded from early to mid Pleistocene deposits south of the Isthmus of Kra (Chaimanee, 1998). *Leopoldamys minutus*, an extinct relative of *L. sabanus*, appears in late Pliocene to early Pleistocene deposits in the northern Malay Peninsula, and *L. sabanus* is known from the mid Pleistocene Kra region (Chaimanee, 1998). These fossil data corroborate the hypothesis of an early, preglacial presence of *M. surifer* and *L. sabanus* and their close relatives in the peninsular Sunda region.

Following the colonization of the Sunda shelf, the insular and peninsular populations were isolated from those in Indochina, giving rise to a distinct Sunda group. Although the timing of sea-level changes in the Pliocene and earlier is poorly understood for this region, there is geological evidence that the narrow Isthmus of Kra in the northern Malay Peninsula was periodically inundated during this period (Rangin *et al*., 1990; Ridder-Numan, 1998), an event that could explain the deep pattern of divergence between the Sunda region and the mainland in both *M. surifer* and *L. sabanus.* Differences in the Sunda and Indochinese faunas on either side of the isthmus have been noted at both taxonomic and intraspecific levels in many groups: in lowland birds (Wells, 1971; Medway & Wells, 1976) and amphibians (Inger, 1966) the isthmus represents a peak in the number of species limits of taxa associated with Indochina and those associated with the Sunda shelf. In murid rodents, 11 of the 17 species found in peninsular Malaysia have their northernmost limits at the Isthmus of Kra (Musser & Newcomb, 1983), and at least one of these (*N. cremoriventer*) is the sister taxon of an Indochinese species (Musser, 1973). Of the six that are found in Indochina as well, populations of *Berylmys bowersi*, *Chiropodomys gliroides*, *M. surifer*, *L. sabanus* and *L. edwardsi* north and south of the isthmus are morphologically distinct (Musser *et al*., 1979; Musser, 1979, 1981).

Following the initial vicariant event at the Isthmus of Kra, island populations of these rain forest taxa were isolated from one another by increasingly unstable climates in the mid-Pliocene that may have affected the distribution of humid forest habitat and by rising sea levels; the relative order of these events is difficult to determine. Some of the phylogenetic results (Fig. 4 in *M. surifer* and Fig. 8 in *M. whiteheadi*) suggest that Borneo was isolated first, and that connections between the Malay Peninsula and Sumatra (and Java, in the former case) were maintained for an additional period, resulting in a deep split between the Bornean and other Sunda lineages.

When falling sea levels in the Pleistocene again exposed land bridges among the Sunda islands and peninsula, these changes were accompanied by a cool and arid climate, drastically different from the perhumid and relatively stable climate of the Miocene and early Pliocene. Isolation among island populations was maintained by the unsuitable habitat characterizing much of the exposed shelf. Thus, in this scenario, the phylogenetic patterns we observed and the high levels of genetic differentiation are the result of several million years of isolation.

CONCLUSIONS

This study is the first to compare phylogeographical patterns among multiple widely distributed Sunda shelf rodents. Greater sampling and additional data, including nuclear DNA sequence and morphology, are required to conduct additional tests of regional monophyly and to assess the depth of divergences – indeed, to test if the lineages observed here may represent distinct species. However, the results clearly refute the hypothesis of widespread migration across the Late Pleistocene Sunda shelf and suggest a deeper history of preglacial vicariance in south-east Asia for *M. surifer*, *L. sabanus* and *M. whiteheadi*. These results challenge a long-standing idea that Pleistocene glacial cycles commonly erased pre-existing evolutionary and distributional patterns, and that the patterns we observe today are the results of these relatively recent events. The vicariance patterns observed in this study more likely have their roots in the Pliocene fragmentation of the Sunda block.

ACKNOWLEDGEMENTS

We thank the Indonesian Institute of Sciences (LIPI), the Museum Zoologicum Bogoriense (MZB) and the Indonesian Department of Forest Protection and Nature Conservation (PHKA) for their support. Special thanks to Farizal, Hon, Tang, Bastian, Rosichon Ubiadillah and Irvan Sadik for their help in the field. Agustinus Suyanto and R. Ubiadillah provided support at the MZB, and Tim Jessup and Nassir Foead of the Worldwide Fund for Nature helped to co-ordinate fieldwork in East Kalimantan (the Kayan Mentarang Project). Jennifer Ast, Lisa Curran, Tanya Dewey and Phil Myers gave insightful comments on earlier versions of this manuscript. Gary Paoli provided great

support in the field and throughout the writing of this manuscript. Jim Patton provided tissues from northern Sumatra and Vietnam, Guy Musser from Vietnam, François Catzeflis from Thailand and Sabah, and Kwai-Kin Han from Sabah and Sarawak. Funding for field and lab work was provided by grants from the University of Michigan, the Chicago Zoological Society, the American Society of Mammalogists, Sigma Xi the New York Explorer's Club, the Worldwide Fund for Nature, and the Foundation of the Royal Ontario Museum. Manuel Ruedi and Guy Musser reviewed this manuscript and improved it with their comments and suggestions.

REFERENCES

Aquadro CF, Greenberg BD. 1983. Human mitochondrial DNA variation and evolution: analysis of nucleotide sequences from seven individuals. *Genetics* **103:** 287–312.

Avise JC. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* **43:** 1192–1208.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. 1987. Intraspecific phylogeography: the mitochondrial bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18:** 489–522.

Banks E. 1949. *Bornean mammals*. Kuching: Kuching Press.

Birungi J, Arctander P. 2000. Large sequence divergence of mitochondrial DNA genotypes of the control region within populations of the African antelope, kob (*Kobus kob*). *Molecular Ecology* **9:** 1997–2008.

Biswas B. 1973. Quaternary changes in sea-level in the South China Sea. *Bulletin of the Geological Society of Malaysia* **6:** 229–256.

Brandon-Jones D. 1996. The Asian Colobinae (Mammalia: Cercopithecidae) as indicators of Quaternary climatic change. *Biological Journal of the Linnean Society* **59:** 327–350.

Brandon-Jones D. 1998. Pre-glacial Bornean primate impoverishment and Wallace's line. In: Holloway JD, Hall R, eds. *Biogeography and geological evolution of SE Asia*. Leiden: Backhuys, 393–404.

Breed WG, Musser GG. 1991. Sulawesi and Philippine rodents (Muridae): a survey of spermatozoal morphology and its significance for phylogenetic inference. *American Museum Novitates* **3003:** 1–15.

Breed G, Yong HS. 1986. Sperm morphology of murid rodents from Malaysia and its possible phylogenetic significance. *American Museum Novitates* **2855:** 1–12.

Bremer K. 1994. Branch support and tree stability. *Cladistics* **10:** 295–304.

Brown G, Gadaleta G, Pepe G, Saccone C, Sbisa E. 1986. Structural conservation and variation in the D-loop-containing region of vertebrate mitochondrial DNA. *Journal of Molecular Biology* **192:** 503–511.

Brown WB, George M Jr, Wilson AC. 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences, USA* **76:** 1967–1971.

Chaimanee Y. 1998. Plio-Pleistocene rodents of Thailand. *Thai Studies in Biodiversity* **3:** 1–303.

Darwin C. 1859. *Journal of researches into the geology and natural history of the various countries visited by H.M.S. Beagle, under the command of Captain Fitzroy, R.N. From 1832 to 1836.* London: Henry Colburn.

Dersch M, Stein R. 1994. Late Cenozoic records of eolian quartz flux in the Sea of Japan (ODP Leg 128, Sites 798 and 799) and paleoclimate in Asia. *Palaeogeography, Palaeoclimatology, Palaeoecology* **108:** 523–535.

Dodson J, Colombani F, Ng PKL. 1995. Phylogeographic structure in mitochondrial DNA of a South-east Asian freshwater fish, *Hemibagrus nemurus* (Siluroidei; Bagridae) and Pleistocene sea-level changes on the Sunda shelf. *Molecular Ecology* **4:** 331–346.

Eizirik E, Bonatto SL, Johnson WE, Crawshaw PG Jr, Vie JC, Brousset DM, O'Brien SJ, Salzano FM. 1998. Phylogenetic patterns and evolution of the mitochondrial DNA control region in two neotropical cats (Mammalia, Felidae). *Journal of Molecular Evolution* **47:** 613–624.

Ferris SD, Sage RD, Huang CM, Nielsen JT, Ritte U, Wilson A. 1983. Flow of mitochondrial DNA across a species boundary. *Proceedings of the National Academy of Sciences, USA* **80:** 2290–2294.

Firestone KB. 2000. Phylogenetic relationships among quolls revisited: the mtDNA control region as a useful tool. *Journal of Mammalian Evolution* **7:** 1–22.

Gadi IK, Sharma T. 1983. Cytogenetic rolationahsips in *Rattus*, *Cremnomys*, *Millardia*, *Nesokia* and *Bandicota* (Rodentia: Muridae). *Genetica* **61:** 21–40.

Gathorne-Hardy FJ, Syaukani Davies RG, Eggleton F, Jones DT. 2002. Quaternary rainforest refugia in south-east Asia: using termites (Isoptera) as indicators. *Biological Journal of the Linnean Society* **75:** 453–466.

Giribet G, Wheeler WC. 1999. On gaps. *Molecular Phylogenetics and Evolution* **13:** 132–143.

Goloboff PA. 1993. *NONA*, Version 2. Program and documentation.

Grant PR. 1999. *Ecology and evolution of Darwin's finches*. Princeton: Princeton University Press.

Haile NS. 1971. Quaternary shorelines in West Malaysia and adjacent parts of the Sunda shelf. *Quaternaria* **15:** 333–343.

Hall R. 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Holloway JD, Hall R, eds. *Biogeography and geological evolution of SE Asia*. Leiden: Backhuys, 99–131.

Harris D, Rogers DS, Sullivan J. 2001. Phylogeography of *Peromyscus furvus* (Rodentia; Muridae) based on cytochrome b sequence data. *Molecular Ecology* **9:** 2129–2135.

Heaney LR. 1986. Biogeography of mammals in SE Asia: estimates of rates of colonization, extinction and speciation. *Biological Journal of the Linnean Society* **28:** 127–165.

Heaney LR. 1991. A synopsis of climatic and vegetational change in Southeast Asia. *Climatic Change* **19:** 53–61.

Inger RF. 1966. The systematics and zoogeography of the Amphibia of Borneo. *Fieldiana Zoology* **52:** 1–402.

Inger RF, Voris HK. 2001. The biogeographical relations of the frogs and snakes of Sundaland. *Journal of Biogeography* **28:** 863–891.

Jacobs LL, Downs WR. 1994. The evolution of murine rodents in Asia. In: Tomida Y, Li Y, Setoguchi T, eds. *Rodent and lagomorph families of Asian origins and diversification.* Tokyo: National Science Museum, Monograph **8**: 149–156.

van der Kaars S, Penny D, Tibby J, Fluin J, Dam RAC, Suparan P. 2001. Late Quaternary palaeoecology, palynology and palaeolimnology of a tropical lowland swamp: Rawa Danau, West-Java, Indonesia. *Palaeogeography, Palaeoclimatology, Palaeoecology* **171:** 185–212.

Karns DR, O'Bannon A, Voris HK, Weigt LA. 2000. Biogeographical implications of mitochondrial DNA variation in the Bockadam Snake (*Cerberus rynchops*, Serpentes: Homalopsinae) in Southeast Asia. *Journal of Biogeography* **27:** 391–402.

Kashiwaya K, Ochiai S, Sakai H, Kawai T. 2001. Orbit-related long-term climate cycles revealed in a 12-myr continental record from Lake Baikal. *Nature* **410:** 71–74.

Kershaw AP, Penny D, van der Kaars S, Anshari G, Thamotherampillai A. 2001. Vegetation and climate in lowland Southeast Asia at the Last Glacial Maximum. In: Davidson I, Metcalf I, Morwood M, Smith JMB, eds. *Faunal and floral migrations and evolution in SE Asia–Australasia*. Lisse: A.A. Balkema, 227–236.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences* **86:** 6196–6200.

Koopman KF. 1989. Distributional patterns of Indo-Malayan bats (Mammalia: Chiroptera). *American Museum Novitates* **2942:** 1–19.

Kumar S, Tamura K, Jakobsen IB, Nei M. 2001. *MEGA2.1: Molecular Evolutionary Genetics Software.* Tempe: Arizona State University.

MacArthur RH, Wilson EO. 1967. *The theory of island biogeography*. Princeton: Princeton University Press.

Maddison DR, Maddison WP. 2000. *Macclade4: analysis of phylogeny and character evolution*, Version 4.0. Sunderland: Sinauer Associates.

Matisoo-Smith E, Roberts RM, Irwin GJ, Allen JS, Penny D, Lambert DM. 1998. Patterns of prehistoric human mobility in Polynesia indicated by mtDNA from the Pacific rat. *Proceedings of the National Academy of Sciences, USA* **95:** 15145–15150.

Medway G. 1963. Niah cave animal bone VI—new records. *Sarawak Museum Journal* **11:** 188–191.

Medway G. 1969. *The wild mammals of Malaya*. London: Oxford University Press.

Medway G. 1972. The Quaternary mammals of Malesia: a review. In: Ashton P, Ashton M, eds. *The Quaternary era in Malesia, Transactions of the second Aberdeen–Hull symposium on Malesian ecology*. Aberdeen: University of Hull, 63–83.

Medway G. 1977. *Mammals of Borneo. Monographs of the Malaysian branch of the Royal Asiatic Society no. 7*. Kuala Lumpur: Perchetakan Mas Sdn. Bhd.

Medway G, Wells DR. 1976. *The birds of the Malay Peninsula*. London: H.F. & G. Witherby Ltd.

Morley RJ. 1998. Palynological evidence for Tertiary plant dispersals in the SE Asian region in relation to plate tectonics and climate. In: Holloway JD, Hall R, eds. *Biogeography and geological evolution of SE Asia*. Leiden: Backhuys, 211–234.

Morley RJ. 2000. *Origin and evolution of tropical rain forests*. Chichester: John Wiley and Sons, Ltd.

Moss SJ, Wilson MEJ. 1998. Biogeographic implications from the Tertiary palaeogeographic evolution of Sulawesi and Borneo. In: Holloway JD, Hall R, eds. *Biogeography and geological evolution of SE Asia*. Leiden: Backhuys, 133–164.

van der Meulen AJ, Musser GG. 1999. New paleontological data from the continental Plio-Pleistocene in Thailand. *Historical Biology* **15:** 181–191.

Musser GG. 1973. Species-limits of *Rattus cremoriventer* and *Rattus langibianis*, murid rodents of Southeast Asia and the greater Sunda islands. *American Museum Novitates* **2525:** 1–65.

Musser GG. 1979. Results of the Archbold expeditions, 102. The species of *Chiropodomys*, arboreal mice of Indochina and the Malay Peninsula. *Bulletin of the American Museum of Natural History* **162:** 379–445.

Musser GG. 1981. Results of the Archbold expeditions, 105. Notes on systematics of Indo-Malayan murid rodents, and desciptions of new genera and species from Ceylon, Sulawesi, and the Philippines. *Bulletin of the American Museum of Natural History* **168:** 229–334.

Musser GG, Carleton MD. 1993. Family Muridae. In: Wilson DE, Reeder DM. *Mammal species of the world: a taxonomic and geographic reference.* Washington: Smithsonian University Press, 501–755.

Musser GG, Marshall JT, **Boeadi. 1979.** Definition and contents of the Sundaic genus *Maxomys* (Rodentia, Muridae). *Journal of Mammalogy* **60:** 592–606.

Musser GG, Newcomb C. 1983. Malaysian murids and the giant rat of Sumatra. *Bulletin of the American Museum of Natural History* **174:** 329–598.

Mustrangi M, Patton JL. 1997. Phylogoegraphy and systmatics of the slender ouse opossum *Marmosops* (Marsupialia, Didelphidae). *University of California Publications in Zoology* **130:** 1–86.

Newsome J, Flenley JR. 1988. Late Quaternary vegetational history of the Central Highlands of Sumatra II. Palaeopalynology and vegetational history. *Journal of Biogeography* **15:** 555–578.

Nixon KC. 1999a. *WinClada.* Program distributed by author, http://cladistics.com.

Nixon KC. 1999b. The Parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics* **15:** 407–414.

Payne J, Francis CM. 1998. *A field guide to the mammals of Borneo*. Kota Kinabalu: The Sabah Society.

Rainboth WJ. 1996. *Fishes of the Cambodian Mekong. FAO species identification field guide for fishery purposes*. Rome: Food and Agriculture Association of the United Nations.

Rangin C, Pubellier M, Azema J, Briais A, Chotin P, Fontaine J, Huchon P, Jolivet L, Maury R, Muller C, Rampnoux JP, Stephan JF, Tournon J, Cottereau N, Dercourt J, Ricou LE. 1990. The quest for Tethys in the western Pacific. 8 paleogeodynamic maps for Cenozoic time. *Bulletin do la Société Géologique de France* **8:** 909–913 + 8 colour maps.

Ridder-Numan J. 1998. Historical biogeography of *Spatholobus* (Leguminonsae-Papilionoideae) and allies in SE Asia. In: Holloway JD, Hall R, eds. *Biogeography and geological evolution of SE Asia*. Leiden: Backhuys, 259–277.

Ruedas LA, Kirsch JAW. 1997. Systematics of *Maxomys* Sody, 1936 (Rodentia: Muridae: Muridae): DNA/DNA hybridization studies of some Borneo-Javan species and allied Sundaic and Australo-Papuan genera. *Biological Journal of the Linnean Society* **61:** 385–408.

Ruedi M. 1996. Phylogenetic evolution and biogeography of Southeast Asian shrews (genus *Crocidura*: Soricidae). *Biological Journal of the Linnean Society* **58:** 197–219.

Ruedi M, Fumagalli L. 1996. Genetic structure of Gymnures (genus *Hylomys*; Erinaceidae) on continental islands of Southeast Asia: historical effects of fragmentation. *Journal of Zoological Systematics and Evolutionary Research* **34:** 153–162.

Ruedi M, Smith MF, Patton JL. 1997. Phylogenetic evidence of mitochondrial DNA introgression among pocket gophers in New Mexico (family Geomyidae). *Molecular Ecology* **6:** 453–462.

Schaller GB, Vrba ES. 1996. Description of the giant muntjac (*Megamuntiacus vuquangensis*) in Laos. *Journal of Mammalogy* **77:** 675–683.

Schmitt LH, Kitchener DJ, How RA. 1995. A genetic perspective of mammalian variation and evolution in the Indonesian archipelago: biogeographic correlates in the fruit bat genus *Cynopterus*. *Evolution* **49:** 399–412.

She JX, Bonhomme F, Boursot P, Thaler L, Catzeflis F. 1990. Molecular phylogenies in the genus *Mus*: comparative analysis of electrophoretic, scnDNA hybridization, and mtDNA RFLP data. *Biological Journal of the Linnean Society* **41:** 83–103.

Smith MF, Patton JL. 1993. The diversification of South Americn murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society* **50:** 149–177.

Sorenson MD. 1999. *Treerot*, Version 2. Boston: Boston University Department of Biology.

Stuijts IM. 1993. Late Pleistocene and Holocene vegetation of West Java, Indonesia. *Modern Quaternary Research in Southeast Asia* **12:** 1–173.

Sun X, Li X, Luo Y, Chen X. 2000. The vegetation and climate at the last glaciation on the emerged continental shelf of the South China Sea. *Palaeogeography, Palaeoclimatology, Palaeoecology* **160:** 301–316.

Swofford DL. 2002. *PAUP* 4.0b10: phylogenetic analysis using parsimony (*and other methods)*, Version 4.0b10. Sunderland: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignments aided by quality analysis tools. *Nucleic Acids Research* **24:** 4876–4882.

Upholt WB, Dawid IB. 1977. Mapping of mitochondrial DNA of individual sheep and goats: rapid evolution in the D loop region. *Cell* **11:** 571–583.

Verneau O, Catzeflis F, Furano AV. 1998. Determining and dating recent rodent speciation events by using L1 (LINE-1) retrotransposons. *Proceedings of the National Academy of Sciences, USA* **95:** 11284–11289.

Verstappen HT. 1992. Climatic change and geomorphology in south and south-east Asia. *Geo-Eco-Trop* **16:** 101–147.

Verstappen HT. 1997. The effect of climatic change on southeast Asian geomorphology. *Journal of Quaternary Science* **12:** 413–418.

Voris HK. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems, and time durations. *Journal of Biogeography* **27:** 1153–1167.

Wallace AR. 1869. *The Malay Archipelago, the land of the orang-utan and the bird of paradise: a narrative of travel with studies of man and nature*. London: Macmillan.

Watts CHS, Baverstock PR. 1994. Evolution in some South-east Asian Murinae (Rodentia) as assessed by microcomplement fixation of albumin, and their relationship to Australian murines. *Australian Journal of Zoology* **42:** 711–722.

Wells DR. 1971. Survival of the Malaysian bird fauna. *Malayan Nature Journal* **24:** 248–256.

Yong HS. 1970. A Malayan view of *Rattus edwardsi* and *Rattus sabanus* (Rodentia: Muridae). *Zoological Journal of the Linnean Society* **49:** 359–370.

APPENDIX

Specimens included in this study. Bold acronyms refer to collecting localities shown in Figure 2. AMNH, American Museum of Natural History; MVZ, Museum of Vertebrate Zoology; MZB, Museum Zoologicum Bogoriense; ROM, Royal Ontario Museum.

Maxomys surifer

Indochina

HT: Vietnam, Ha Tinh, Huong Son Camp: AMNH 272392; AMNH 272624; AMNH 272625; AMNH 272642.

YD: Vietnam, Dak Lak, Yok Don National Park: ROM 107723; ROM 107731; ROM 107778.

CT: Vietnam, Dong Nai, Cat Tien National Park: ROM 110900.

Malay Peninsula

CL: Thailand, Surat Thani, Chiew Larn Dam Reservoir: Montpellier University T 1433; T 1436; T 1437; T 1438.

Java

PA: Indonesia, West Java, Pangandaran Nature Reserve: Pakuan University HMB 30; HMB 33; HMB 34; HMB 35; HMB 36; HMB 37.

Sumatra

LA: Indonesia, Lampung, Talang Padang: Pakuan University HMB 01; HMB 16; HMB 22; HMB 23; HMB 25; HMB 28.

Borneo

KM: Indonesia, East Kalimantan, Kayan Mentarang Nature Reserve: ROM 102030; ROM 102072. Long Sungan: ROM 102206; ROM 102208; ROM 102209; ROM 102210; Puak: ROM 102256; ROM 102266; ROM 102267; ROM 102268; ROM 102280. Gunung Lunjut: ROM 102281.

GP: Indonesia, West Kalimantan, Gunung Palung National Park: UMMZ 174516; UMMZ 174517; UMMZ 174520; UMMZ 174521; UMMZ 174522; UMMZ 174523; UMMZ 174524; UMMZ 174537; UMMZ 174539; UMMZ 174540; UMMZ 174659 174660; UMMZ 174661; UMMZ 174662 & MZB 24241; MZB 24242. Kampung Sedahan: UMMZ 174531.

BB: Indonesia, West Kalimantan, Bukit Baka-Bukit Raya National Park: UMMZ 174525; UMMZ 174526; UMMZ 174527; UMMZ 174528; UMMZ 174529; UMMZ 174530.

BS: Indonesia, East Kalimantan, Bukit Soeharto Experimental Forest Reserve: ROM 101964.

PK: Indonesia, West Kalimantan, Pulau Karimata Nature Reserve: UMMZ 174541; UMMZ 174542; UMMZ 174543; UMMZ 174544; UMMZ 174548; UMMZ 174550; UMMZ 174551 & MZB 24249; MZB 24246; MZB 24243; MZB 24244; MZB 24245; MZB 24247; MZB 24248.

L. sabanus & L. edwardsi

Indochina

NT: Vietnam, Tuyen Quang, Nam Trang, Na Hang Nature Reserve: ROM 107692.

VP: Vietnam, Vinh Phu, Tam Dao: MVZ 186495; MVZ 186496; MVZ 186497.

HT: Vietnam, Ha Tinh, Huong Son Camp: AMNH 272636; AMNH 272402; AMNH 272461; AMNH 272620.

Malay Peninsula

CL: Thailand, Surat Thani, Chiew Larn Dam Reservoir: Montpellier University T 1428.

ER: Malaysia, Johor, Endau Rompin National Park: ROM 113037; ROM 113060; ROM 113066; ROM 113128.

Sumatra

GL: Indonesia, Aceh, Gunung Leuser National Park: MVZ 192201.

LA: Indonesia, Lampung, Talang Padang: Pakuan University HMB 12; HMB 13.

Borneo

KM: Indonesia, East Kalimantan, Kayan Mentarang Nature Reserve: ROM 102097; ROM 102121; ROM 102138. Long Sungan: ROM 102225; ROM 102226; ROM 102227; ROM 102228; ROM 102229. Puak: ROM 102266. Gunung Lunjut: ROM 102274; ROM 102283.

GP: Indonesia, West Kalimantan, Gunung Palung National Park: UMMZ 174497; UMMZ 174498, UMMZ 174499; UMMZ 174500; UMMZ 174501; UMMZ 174515; UMMZ 174654 & MZB 20605; MZB 20604; MZB 20603; AJG 55 (MZB uncat.); AJG 125 (MZB uncat.); AJG 544 (MZB uncat.). Kampung Sedahan: UMMZ 174510; UMMZ 174511; UMMZ 174512; UMMZ 174514.

BB: Indonesia, West Kalimantan, Bukit Baka-Bukit Raya National Park: UMMZ 174502; UMMZ 174505; UMMZ 174506; UMMZ 174509 & MZB 20596; MZB 20595; MZB 20597; MZB 20599; MZB 20594; MZB 20598.

LE: Malaysia, Sarawak, Lanjak-Entimau Wildlife Sanctuary: Louisiana State University KHH 127.

M. whiteheadi

Malay Peninsula

ER: Malaysia, Johor, Endau Rompin National Park: ROM 113074; ROM 113123.

Sumatra

GL: Indonesia, Aceh, Gunung Leuser National Park: MVZ 192215; MVZ 192216; MVZ 192217, MVZ 192218.

Borneo

TL: Malaysia, Sabah, Telupid: Montpellier University T 0739; T 0741.

TW: Malaysia, Sabah, Tawau: Universiti Malaysia Sarawak UMS 01706; UMS 01749; UMS 01819.

KM: Indonesia, East Kalimantan, Kayan Mentarang Nature Reserve: ROM 102034; ROM 102040; ROM 102041; ROM 102042; ROM 102882. Long Sungan: ROM 102211; ROM 102239; ROM 102240; ROM 102251. Puak: ROM 102257; ROM 102258; ROM 102264. Gunung Lunjut: ROM 102275; ROM 102276; ROM 102277; ROM 102278.

APPENDIX *Continued*

GP: Indonesia, West Kalimantan, Gunung Palung National Park: UMMZ 174466; UMMZ 174467; UMMZ 174470; UMMZ 174471; UMMZ 174473; UMMZ 174477; UMMZ 174478; UMMZ 174481; UMMZ 174482; UMMZ 174658 & MZB 22052; AJG 521 (MZB uncat.); AJG 543 (MZB uncat.).

BB: Indonesia, West Kalimantan, Bukit Baka-Bukit Raya National Park: UMMZ 174489; UMMZ 174491; UMMZ 174492 & MZB 20582; MZB 20577; MZB 20578; MZB 20579.

BS: Indonesia, East Kalimantan, Bukit Soeharto Experimental Forest Reserve: ROM 101959; ROM 101960; ROM 101961; ROM 101962; ROM 101963.

Additional taxa and outgroups

Maxomys moi
Vietnam, Quang Nam, Ngoc Linh Base Camp: ROM 111289.

Maxomys rajah
Indonesia, West Kalimantan, Bukit Baka-Bukit Raya National Park: UMMZ 174555; UMMZ 174556.

Maxomys whiteheadi
Indonesia, West Kalimantan, Gunung Palung National Park: UMMZ 174658.

Niviventer cremoriventer
Indonesia, West Kalimantan, Gunung Palung National Park: UMMZ 174430.

Niviventer rapit
Indonesia, West Kalimantan, Bukit Baka-Bukit Raya National Park: UMMZ 174435.

Rattus norvegicus
GenBank Accession No. NC001665. No locality data.

Sundamys muelleri
Indonesia, West Kalimantan, Bukit Baka-Bukit Raya National Park: UMMZ 174436.