

Phylogeny, diversity, and biogeography of Southeast Asian spiny rats (*Maxomys*)

ANANG S. ACHMADI, JACOB A. ESSELSTYN,* KEVIN C. ROWE, IBNU MARYANTO, AND M. T. ABDULLAH

Museum Zoologicum Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Jl. Raya Jakarta–Bogor, Cibinong Km. 46, Indonesia (ASA, IM)

Department of Biological Sciences and Museum of Natural Science, 119 Foster Hall, Louisiana State University, Baton Rouge, LA 70803, USA (JAE)

Museum Victoria, Sciences Department, G.P.O. Box 666, Melbourne, Victoria 3001, Australia (KCR)

Molecular Ecology Laboratory, Department of Zoology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia (MTA)

* Correspondent: esselstyn@lsu.edu

Knowledge of the diversity and relationships of species in many groups of plants and animals in Southeast Asia is severely limited, preventing an integrative understanding of evolutionary and ecological processes in island archipelagos. We generated a 3-locus DNA sequence data set to estimate phylogenetic relationships among species and populations of *Maxomys*, a genus of rodents endemic to Southeast Asia. Our inclusion of *Crunomys* as a potential outgroup supported the monophyly of *Crunomys*, but the genus was deeply nested within *Maxomys*. Because of the relatively ancient divergences (mean uncorrected p -distances up to 0.15 in cytochrome- b sequences) among species of *Maxomys* and short branch lengths among basal lineages of the phylogeny, we obtained little support for the oldest relationships in *Maxomys* + *Crunomys*. However, our analyses revealed unrecognized diversity in the form of divergent populations both between and within islands and the presence of 2 potentially undescribed species from Sulawesi. The *Maxomys* and *Crunomys* of Sulawesi belonged to 4 clades sister to extralimital species, suggesting that repeated overwater dispersal between Sundaland–Philippines and Sulawesi was an important isolating mechanism in the history of this group.

Key words: *Crunomys*, Indonesia, island colonization, Murinae, phylogeography, Sunda Shelf, Sulawesi

© 2013 American Society of Mammalogists

DOI: 10.1644/13-MAMM-A-092.1

Island archipelagos provide important systems for the study of biological diversification and have been the subject of many foundational works in the fields of evolution and ecology (e.g., Darwin 1859; Wallace 1869; MacArthur and Wilson 1963; Grant and Grant 1993). The Indomalayan archipelago, in particular, offers a potential model for island biogeographic study because of its exceptional endemism, its broad transition zone between the Sundaic and Sahulian biotas, and its geographic history, involving sea-level fluctuations and geological changes (Wallace 1869; Myers et al. 2000; Dirzo and Raven 2003; Corlett 2009; Lohman et al. 2011). Although the archipelago is known to contain a mix of Asian and Australian faunal and floral elements, the provenance of many Indonesian lineages remains uncertain, limiting the current value of the region as a model system for understanding ecological and evolutionary processes in a biogeographic context (Lohman et al. 2011).

One of the most diverse mammalian groups in the archipelago is the Old World rats and mice (Murinae), with at least 213 species occurring in the Indomalayan region (Corbet and Hill 1992). Despite the high diversity and abundance of murines in Southeast Asia, few studies have investigated molecular genetic diversity at the phylogeographic level in these animals (but see Steppan et al. [2003] and Gorog et al. [2004]). Among the Murinae, the spiny rats (*Maxomys*) represent a prime subject for biogeographic study because of their abundance, ubiquity, and widespread distribution across the region (Ruedas and Kirsch 1997). Eighteen species are currently recognized, with a generic distribution from mainland Southeast Asia east throughout the Sunda Shelf to some neighboring oceanic islands. Species of *Maxomys* are present



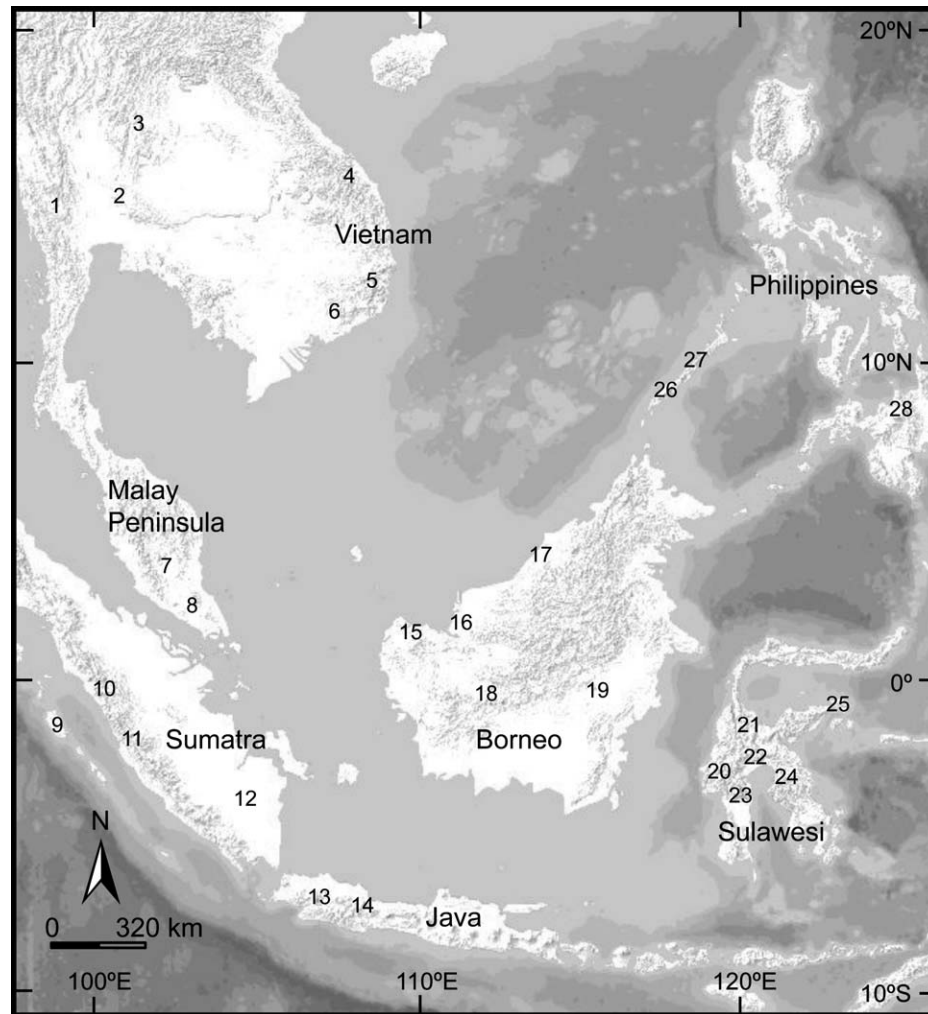


FIG. 1.—Map of Southeast Asia, showing localities of specimens of *Maxomys* and *Crunomys* used in this study. Locality numbers are referenced in the phylogeny of Figs. 2 and 3.

on Sumatra, Borneo, the Mentawai Islands, Palawan, many of the smaller islands of the Sunda Shelf, and Sulawesi (Musser and Carleton 2005). Specimens representing an undescribed species from Mindoro Island may extend the range of *Maxomys* to the oceanic Philippines (Musser and Carleton 2005).

The taxonomy of *Maxomys* has been unstable. Sody (1936) proposed the genus for *Rattus bartelsii* (originally named as *Mus bartelsii* [Jentink 1879]) and Musser et al. (1979) later refined this definition by adding 4 species previously associated with *Leopoldamys* (at that time in the genus *Rattus*). New species of *Maxomys* continue to be described (e.g., Achmadi et al. 2012), and undescribed species have been reported from the Philippines and Sulawesi (Corbet and Hill 1992; Musser and Carleton 2005). In addition, many authors have stated the need for improved information on species limits and phylogenetic relationships (Musser 1969; Musser et al. 1979; Ruedas and Kirsch 1997; Gorog et al. 2004; Musser and Carleton 2005; Achmadi et al. 2012).

The relationships of *Maxomys* to other genera in the Murinae also remain uncertain, and the genus is currently treated as a

distinct division (Musser and Carleton 2005). A DNA–DNA hybridization study inferred *Maxomys* to be sister to a clade composed of *Rattus* and *Dacnomys* division members, including the genera *Sundamys*, *Rattus*, *Niviventer*, and *Leopoldamys* (Ruedas and Kirsch 1997). Jansa et al. (2006) inferred a sister relationship between *Maxomys* and *Crunomys* (*Crunomys* division), but included only 1 species of *Maxomys* in their analyses. Buzan et al. (2011) found *Crunomys* was nested within *Maxomys*, but they included only 1 species of *Crunomys*. Within *Maxomys*, Ruedas and Kirsch (1997) inferred a sister relationship between *M. surifer* and *M. bartelsii*, whereas the phylogenetic positions of *M. ochraceiventer*, *M. rajah*, and *M. whiteheadi* varied among analyses. Otherwise, relationships among species of *Maxomys* have not been examined in an explicit phylogenetic context. Gorog et al. (2004) investigated phylogeographic patterns within *M. whiteheadi* and *M. surifer* and found relatively ancient divergences among populations that were connected by dry land during Pleistocene sea-level lowstands; they therefore rejected the hypothesis of widespread migration of these lineages across the exposed Sunda Shelf during the late Pleistocene.

TABLE 1.—Mean uncorrected mitochondrial genetic distances (*p*-distances) between species of *Maxomys* and *Crunomys* (below diagonal) and maximum intraspecific uncorrected genetic distances (bold font on diagonal). Intraspecific distances are only shown for those species sampled from multiple localities.

	<i>C. celebensis</i>	<i>C. melanius</i>	<i>C. suncooides</i>	<i>M. bartelsii</i>	<i>M. cf. hellwaldii</i>	<i>M. dollmani</i>	<i>M. hellwaldii</i>	<i>M. hylomyoides</i>	<i>M. moi</i>
<i>C. celebensis</i>	—								
<i>C. melanius</i>	0.07	—							
<i>C. suncooides</i>	0.07	0.04	—						
<i>M. bartelsii</i>	0.14	0.12	0.12	0.01					
<i>M. cf. hellwaldii</i>	0.12	0.12	0.13	0.12	—				
<i>M. dollmani</i>	0.13	0.12	0.13	0.13	0.13	0.02			
<i>M. hellwaldii</i>	0.12	0.11	0.11	0.13	0.12	0.09	—		
<i>M. hylomyoides</i>	0.13	0.13	0.13	0.13	0.13	0.12	0.13	0.07	
<i>M. moi</i>	0.12	0.11	0.12	0.12	0.13	0.14	0.13	0.12	—
<i>M. musschenbroekii</i>	0.14	0.13	0.12	0.13	0.13	0.14	0.14	0.14	0.14
<i>M. ochraceiventer</i>	0.12	0.10	0.11	0.12	0.11	0.13	0.13	0.11	0.13
<i>M. pagensis</i>	0.12	0.11	0.11	0.14	0.12	0.14	0.13	0.12	0.13
<i>M. panglima</i>	0.13	0.12	0.12	0.14	0.13	0.14	0.13	0.13	0.12
<i>M. rajah</i>	0.12	0.10	0.11	0.13	0.11	0.13	0.12	0.12	0.11
<i>M. sp. (Sulawesi)</i>	0.13	0.12	0.12	0.13	0.11	0.10	0.12	0.13	0.13
<i>M. surifer</i>	0.14	0.12	0.12	0.13	0.13	0.14	0.14	0.12	0.12
<i>M. tajuddinii</i>	0.11	0.12	0.12	0.13	0.13	0.13	0.13	0.08	0.12
<i>M. whiteheadi</i>	0.10	0.14	0.11	0.14	0.12	0.13	0.15	0.09	0.13

With the basic goal of improving knowledge of the diversity and biogeography of *Maxomys*, we investigated relationships in a broad sample of species and populations using new and published sequences of 1 mitochondrial and 2 nuclear loci. Specifically, we attempted to answer the following questions: Where does *Maxomys* fit phylogenetically among Indomalayan murines? Is *Maxomys* closely related to species of the *Rattus*, *Dacnomys*, or *Crunomys* divisions? Is *Maxomys* monophyletic? Are the *Maxomys* of Sulawesi the product of an in situ radiation or the result of repeated colonization? Do distinct phylogenetic units exist within species of *Maxomys* that may represent cryptic species? To address these questions we performed phylogenetic analyses of 13 of the 18 known species of *Maxomys*, plus a broad sample of outgroup species, including members of the *Chrotomys*, *Crunomys*, *Dacnomys*, *Dasymys*, *Phloeomys*, *Pseudomys*, and *Rattus* divisions.

MATERIALS AND METHODS

Taxon sampling.—There are 18 currently recognized species of *Maxomys*, many of which have broad distributions in Southeast Asia and occur on multiple islands (Musser et al. 1979; Musser and Carleton 2005; Achmadi et al. 2012). *Maxomys rajah*, *M. surifer*, and *M. whiteheadi* are known from the Malay Peninsula, Sumatra, Borneo, and Java; *M. alticola*, *M. baeodon*, *M. ochraceiventer*, and *M. tajuddinii* are endemic to Borneo; *M. hylomyoides* and *M. inflatus* are endemic to Sumatra; *M. bartelsii* is endemic to Java; *M. pagensis* is endemic to the Mentawai Islands; *M. dollmani*, *M. hellwaldii* (sometimes referred to as *M. hellwaldi* [e.g., Musser 1969]), *M. wattsi*, and *M. musschenbroekii* are endemic to Sulawesi; *M. panglima* is endemic to the Palawan group of islands in the Philippines; *M. inas* is endemic to the Malay Peninsula; and *M. moi* is found in Thailand, Laos, Vietnam, and China.

We sequenced the mitochondrial cytochrome-*b* (*Cytb*) gene in 130 specimens of *Maxomys* and 2 unlinked coding nuclear genes (interphotoreceptor retinoid-binding protein [IRBP] and growth hormone receptor [GHR]) in a subset of this diversity (24 IRBP and 23 GHR samples). The subset of specimens for which we obtained nuclear DNA sequences was chosen to represent divergent lineages on a preliminary estimate of the mitochondrial gene tree. Sequenced specimens cover the geographic range of the genus, with samples taken from sites in Vietnam, the Malay Peninsula, Borneo, Sumatra, Java, Sulawesi, Palawan, and the Mentawai Islands (Fig. 1; Supporting Information S1, DOI: 10.1644/13-MAMM-A-092.S1). Our sampling represents 13 currently recognized species, including *M. bartelsii*, *M. dollmani*, *M. hellwaldii*, *M. hylomyoides*, *M. moi*, *M. musschenbroekii*, *M. ochraceiventer*, *M. pagensis*, *M. panglima*, *M. rajah*, *M. surifer*, *M. tajuddinii*, *M. whiteheadi*, and 2 potentially undescribed species from Sulawesi. To these data, we added 26 previously published *Cytb* sequences from additional localities for *M. ochraceiventer*, *M. rajah*, *M. surifer*, and *M. whiteheadi* (Fig. 1; Supporting Information S1). We failed to obtain samples of *M. alticola*, *M. baeodon*, *M. inas*, *M. inflatus*, and *M. wattsi*. For use as outgroups, we sequenced and downloaded sequences from several other genera of murines (Supporting Information S1). All new sequences were deposited in GenBank (KC878024–KC878203, KC878206–KC878208, and KC878210–KC878238 [Supporting Information S1]).

Specimen identifications and taxonomic usage.—Most specimens of *Maxomys* used in this paper were identified by A. S. Achmadi or one of the other authors. However, we have not examined many of the specimens associated with published GenBank sequences. We therefore retain the taxonomic names published on GenBank in our figures and in Supporting Information S1, even when they conflict with our identifications of closely related specimens. We have not

TABLE 1.—Extended.

	<i>M. musschenbroekii</i>	<i>M. ochraceiventor</i>	<i>M. pagensis</i>	<i>M. panglima</i>	<i>M. rajah</i>	<i>M. sp.</i> (Sulawesi)	<i>M. surifer</i>	<i>M. tajuddinii</i>	<i>M. whiteheadi</i>
0.07									
0.11		—							
0.14		0.13	—						
0.14		0.12	0.12	0.04					
0.14		0.11	0.06	0.12	0.01				
0.15		0.12	0.13	0.13	0.13	—			
0.13		0.11	0.12	0.14	0.12	0.13	0.08		
0.09		0.12	0.14	0.13	0.13	0.12	0.15	—	
0.09		0.09	0.11	0.13	0.12	0.12	0.13	0.10	0.08

examined Museum of Texas Tech University (TK) 152985 from Borneo and we therefore label it as *Maxomys* sp., despite its close relationship to *Maxomys tajuddinii* in mitochondrial DNA (mtDNA; see below). We also refer to 2 potentially undescribed species from Sulawesi as *M. sp.* because we have not examined the specimens (Museum Zoologicum Bogoriense [MZB] 23104, 23105) and as *M. cf. hellwaldii* because the external morphology of this series (Field Museum of Natural History [FMNH] 213370–213372, 213451, 213452) qualitatively resembles that of *M. hellwaldii*.

Molecular genetics.—Genomic DNA was isolated from muscle or liver tissue using either the cetyltrimethylammonium bromide (Ducroz et al. 1998; Lecompte et al. 2005) or guanidine thiocyanate (Esselstyn et al. 2008) protocol. We amplified and sequenced *Cytb* using the standard conserved mammalian primers MVZ05 and MVZ16 following Smith and Patton (1993), with annealing temperatures of 52–54°C. We also amplified and sequenced fragments of 2 nuclear exons, GHR and IRBP, using primers GHREXON10, GHREND, 119A2, and 878F (Jansa and Voss 2000; Adkins et al. 2001). We used annealing temperatures of 47–50°C for these loci. Thermal cycling profiles for polymerase chain reaction and cycle sequencing followed Jansa and Weksler (2004), Steppan et al. (2005), and Rowe et al. (2008).

Phylogenetic analyses.—We estimated Bayesian and likelihood topologies, branch lengths, and nodal support independently for each gene and for a concatenated alignment. DNA sequences were edited using Geneious Pro 5.5.6 (Drummond et al. 2011). We aligned sequences using the native algorithm of the same software. Initial alignments were examined by eye and found to be unambiguous. We then removed identical haplotypes to minimize the computational burden during phylogenetic analyses. The concatenated alignment included all mitochondrial haplotypes, with individuals preferentially chosen as those with nuclear gene

sequences. This matrix was thus mostly complete at *Cytb*, but sparsely sampled for the 2 nuclear genes (see Supporting Information S1). We estimated an appropriate model of sequence evolution for each gene (GTR + Γ + I for *Cytb* and IRBP; HKY + Γ + I for GHR) using the Bayesian information criterion in MrModeltest 2.3 (Nylander 2004). We used the preferred model in all Bayesian and likelihood phylogenetic analyses, with the concatenated analysis partitioned by gene. This partitioning strategy was determined a priori. Phylogenies were rooted with *Phloeomys*, but also included species of *Anisomys*, *Apomys*, *Batomys*, *Berylmys*, *Bunomys*, *Chiromyscus*, *Conilurus*, *Crunomys*, *Dacnomys*, *Dasymys*, *Leopoldamys*, *Mastomys*, *Mus*, *Niviventer*, *Paruromys*, *Rattus*, *Rhynchomys*, and *Srilankamys* to test the monophyly of *Maxomys*. Our Bayesian analyses used MrBayes 3.2 (Ronquist et al. 2012). Markov chain Monte Carlo searches of tree space included 4 runs with 4 chains each. Each run consisted of 5×10^6 generations with parameters sampled every 1,000 generations. Initial analyses had very low swap rates among cold and hot chains, thus we lowered the temperature setting to 0.1. After examining the trends and distributions of parameters, including the likelihood, in Tracer version 1.4 (Rambaut and Drummond 2007), we discarded the first 501 samples of each run as burn-in, and pooled the 4 runs to give 1.8×10^4 trees in the posterior distribution. Effective sample sizes of all parameters were $> 2,000$. In addition to our Bayesian analyses, we used the Web-based RAxML Black Box software (Stamatakis 2006) to estimate maximum-likelihood topologies and provide maximum-likelihood bootstrap support values. Bootstrap support values were derived from 100 replicates.

RESULTS

Our initial *Cytb* alignment contained sequences from 188 individuals, including outgroups; after eliminating duplicate



FIG. 2.—A) Bayesian estimate of phylogeny of Southeast Asian *Maxomys* and *Crunomys* derived from analysis of concatenated DNA sequences. The phylogeny is separated arbitrarily into panels A and B for ease of presentation. Nodes that received strong support in both Bayesian and likelihood analyses are marked with a black diamond. Terminals are labeled with species names and either a museum voucher number or a GenBank accession number. The bracketed number on ingroup terminals refers to localities in Fig. 1.

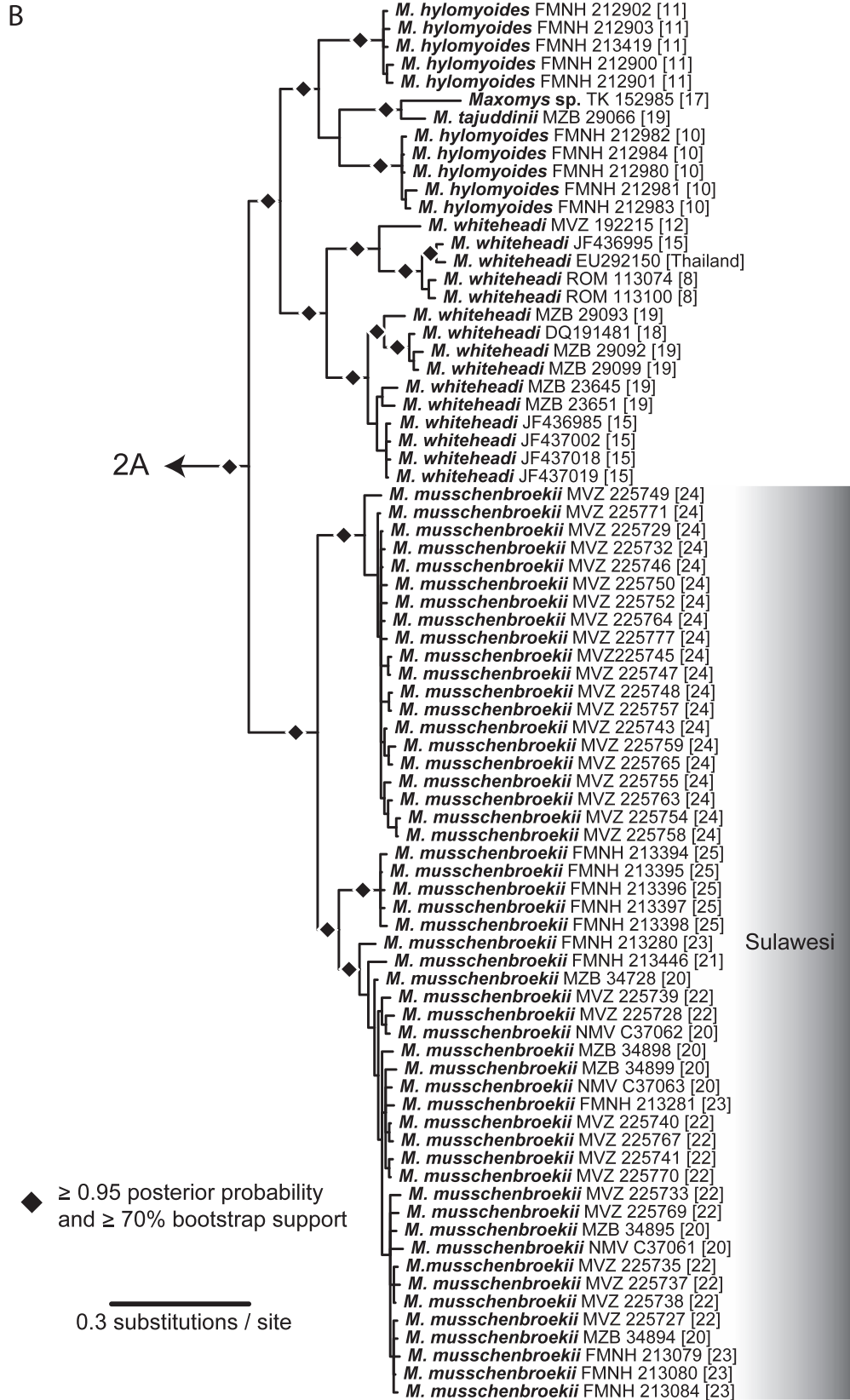


FIG. 2.—Continued. Part B.

haplotypes, the data set was 72% complete (28% of characters missing); it contained 1,140 nucleotide positions and 153 unique haplotypes. No insertions, deletions, or premature stop codons were observed. Mean, uncorrected, interspecific (*Crunomys* and *Maxomys*) sequence divergences in *Cytb* were 0.04–0.15, whereas maximum intraspecific divergences for species sampled from multiple localities were 0.01–0.08 (Table 1). The GHR alignment contained 50 taxa, 822 nucleotide positions, 3 insertions–deletions (indels), and 4% missing data. The IRBP alignment contained 52 taxa, 747 nucleotide positions, 1 indel, and 5% missing data.

Maxomys was not monophyletic in any of our analyses because *Crunomys* was nested within it. However, the monophyly of *Crunomys* + *Maxomys* to the exclusion of other sampled murines was well supported (posterior probability [PP] \geq 0.95, bootstrap support [BS] \geq 70) in analyses of all data sets except IRBP, where PP = 0.91 and BS = 84 (Figs. 2–4). Our phylogenetic estimates provided strong support for many recent relationships, but several deep nodes within the clade (*Maxomys* + *Crunomys*), especially those surrounded by short internal branches, received little support (Figs. 2–4). Although *Crunomys* is nested within *Maxomys*, species of *Crunomys* formed a monophyletic group in all trees except the GHR gene tree, in which the 2 sampled species were recovered as paraphyletic with respect to *M. dollmani* and *M. hellwaldii* (Fig. 3). *Maxomys* + *Crunomys* was recovered as a well-supported sister to a clade of *Dacnomys* and *Rattus* division members, in which the latter division was monophyletic, but the former was not (Figs. 2–4). As reported previously, the paraphyly of the *Dacnomys* division was caused by the position of *Srilankamys* as sister to the *Rattus* division (Buzan et al. 2011; Figs. 2–4).

Within *Crunomys* + *Maxomys* relationships among species of *Maxomys* were not well supported in any of our analyses, with a few notable exceptions. All 3 analyses (Figs. 2–4) recovered a clade uniting *M. panglima*, *M. pagensis*, and *M. rajah*. One *Cytb* sequence from GenBank (JF436990) was reported as *M. rajah* in an earlier study, but clusters with specimens we identified as *M. surifer*. We have not examined this specimen, but it appears to be misidentified. Setting this specimen aside, all of our samples of *M. rajah* form a clade. The combined-data analysis also recovered a well-supported clade that groups an unrecognized species of *Maxomys* from Sulawesi with a clade comprising the Sulawesi species *M. dollmani* and *M. hellwaldii* (Fig. 2A). The latter 2 species also were recovered as a clade in independent analyses of the 2 nuclear genes (Figs. 3 and 4). Finally, a clade comprising *M. hylomyoides*, *M. whiteheadi*, and *M. musschenbroekii* is recovered in all 3 analyses, although IRBP lends little support to this clade (Figs. 2–4). A clade comprising 1 or 2 additional species from Borneo (*M. tajuddinii* and an unidentified *Maxomys*) also join this clade in the combined-data analysis (Fig. 2B).

We found 4 independent clades of species from Sulawesi, including *M. dollmani* + *M. hellwaldii* + *Maxomys* sp., *M. musschenbroekii*, *M. cf. hellwaldii*, and *Crunomys celebensis*.

If each of these lineages represents a separate invasion of Sulawesi, then the island has been colonized at least 4 times by this group of rats. However, back-colonizations of Sundaland or the Philippines, or both, from Sulawesi provide alternative explanations that cannot be tested without comprehensive taxon sampling and well-supported species-level relationships. Only 1 of the clades from Sulawesi contains multiple currently recognized species, but substantial geographic variation is present in mtDNA of *M. musschenbroekii*.

Several currently recognized species contain divergent lineages worthy of closer taxonomic research. *M. whiteheadi*, one of the widespread species, contains divergent lineages from Sumatra, the Malay Peninsula, Thailand, and Borneo. Within *M. surifer*, a species with a similar distribution, we found 5 distinct clades represented by populations from Vietnam, Borneo, Sumatra, Thailand, and the Malay Peninsula. Several GenBank sequences identified by others as either *Maxomys* sp. or *M. rajah* are closely related to specimens we identified as *M. surifer* (Fig. 2A). Setting aside the specimens we have not examined, all remaining *M. surifer* formed a clade. Two populations of *M. hylomyoides*, a Sumatran endemic, were not recovered as a clade, because *M. tajuddinii* and an unidentified specimen from Borneo (TK 152985) cluster with the Mt. Singgalang (site 10; Fig. 1) population of *M. hylomyoides* (although with poor support [Fig. 2B]). Among the samples of *M. musschenbroekii*, a Sulawesi endemic, we recovered 3 distinct and geographically structured haplogroups, which correspond to previous designations of Areas of Endemism (Evans et al. 2003b). The distinct haplogroups of *M. musschenbroekii* include samples from Malili (SE Area of Endemism, site 24; Fig. 1); Mt. Tompotika at the terminus of the east peninsula (E Area of Endemism, site 25); and Mts. Balease (site 22), Gandangdewata (site 20), Rorekatimbo (site 21), and Latimojong (site 23), all in Sulawesi's Central Area of Endemism.

DISCUSSION

Our phylogenetic analyses of 13 recognized and 2 potentially undescribed species (*M. cf. hellwaldii* and *Maxomys* sp., both from Sulawesi) of *Maxomys* revealed several interesting findings that bear on the taxonomy and biogeography of *Maxomys*. First, regarding taxonomy, most currently named species that we sampled and 2 putative new species (both from Sulawesi) appear to be genetically distinct from one another and form monophyletic groups (Fig. 2). Potential exceptions include *M. pagensis*, which is closely related to *M. rajah*, and *M. tajuddinii* and TK 152985, which are nested within *M. hylomyoides* (Fig. 2B). Previous authors have noted the probable existence of undescribed species of *Maxomys* on Sulawesi, but have yet to formally describe these putative species (e.g., Musser 1969; Corbet and Hill 1992); the putative undescribed species we sampled here (*M. cf. hellwaldii* and *Maxomys* sp.) may correspond to those mentioned in earlier reports. One specimen we identified as *M. dollmani* (Museum of Vertebrate Zoology [MVZ] 225725) has mtDNA most

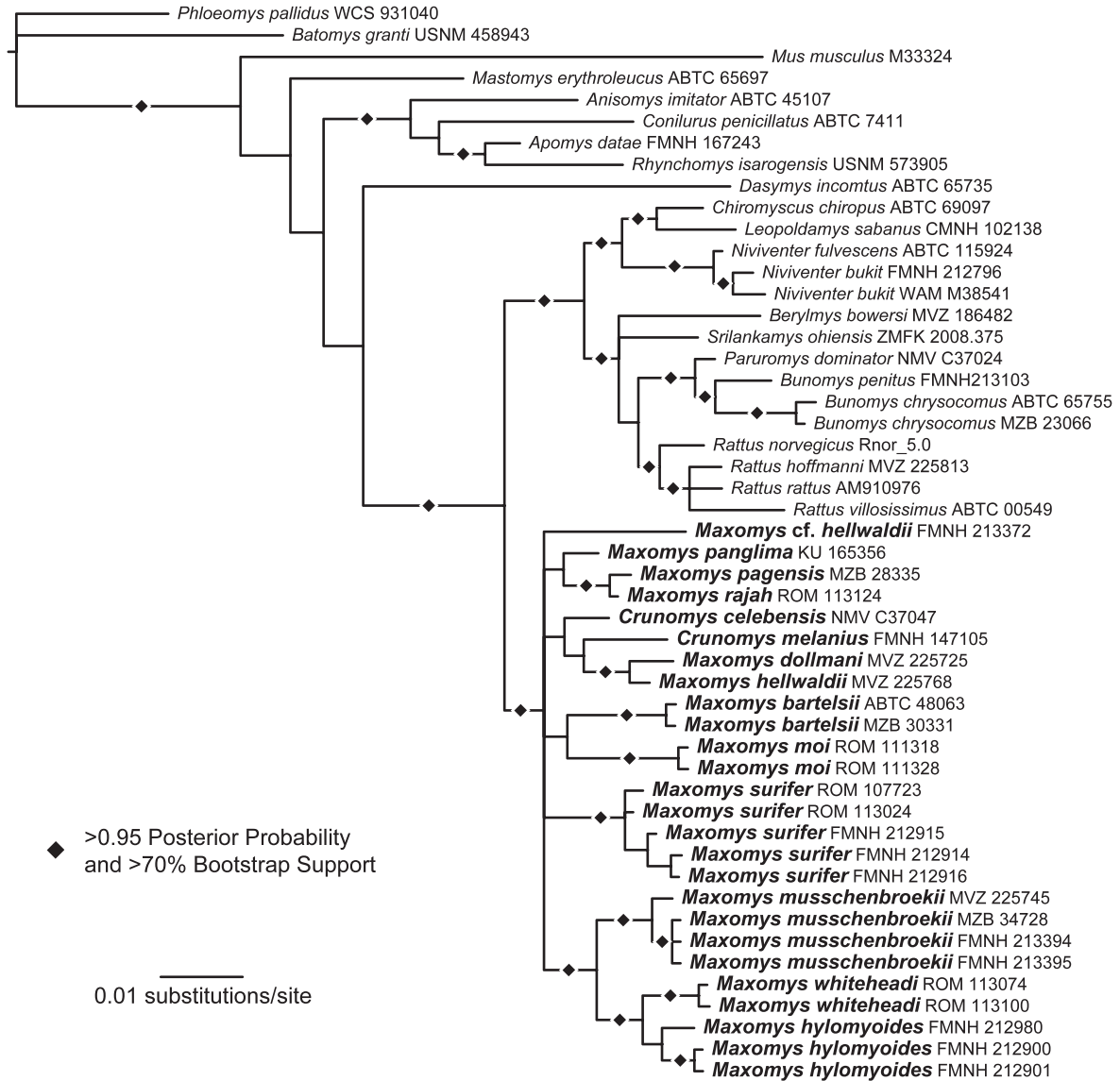


FIG. 3.—Bayesian estimate of phylogeny of Southeast Asian *Maxomys* and *Crunomys* derived from analysis of growth hormone receptor sequences. Nodes that received strong support in both Bayesian and likelihood analyses are marked with a black diamond. Terminals are labeled with species names and either a museum voucher number or a GenBank accession number.

closely related to specimens of *M. hellwaldii* (Fig. 2), suggesting that mitochondrial introgression or other forms of gene flow may occur between these morphologically distinguishable sister species. Additional genetic and population sampling will be necessary to understand the extent of interaction between these species.

Second, we identified several species with geographically structured genetic diversity that require closer taxonomic investigations, such as the populations of *M. surifer* and *M. whiteheadi* from the Sunda Shelf (Gorog et al. 2004) and *M. hylomyoides* from Sumatra (Table 1). Similar observations have been made in other Sunda Shelf taxa (Esselstyn et al. 2010; Oliveros and Moyle 2010; Wilting et al. 2012), which, given the very recent dry land connections between Java, Sumatra, Borneo, and the Malay Peninsula, begs explanation. Plausible isolating mechanisms include climatic and habitat

barriers (Heaney 1991; Bird et al. 2005) and idiosyncratic extinctions (Wilting et al. 2012). *M. musschenbroekii*, an endemic of Sulawesi, also contains substantial geographic variation in its mtDNA (Fig. 3; Table 1). Distinct haplogroups of this species correspond to previous delimitations of Areas of Endemism (Evans et al. 2003b). Areas of Endemism on Sulawesi have been defined based on genetic diversity of primates and amphibians, but may provide a consistent geographic framework for distinct lineages of many plants and animals on Sulawesi (Evans et al. 2003b). If boundaries of Areas of Endemism are indeed consistent among many lineages, these may have been caused by the island's history as an archipelago (Hall 2002), with current borders between Areas of Endemism corresponding to geological sutures between paleoislands. However, testing this hypothesis and plausible alternatives is difficult because of the lack of

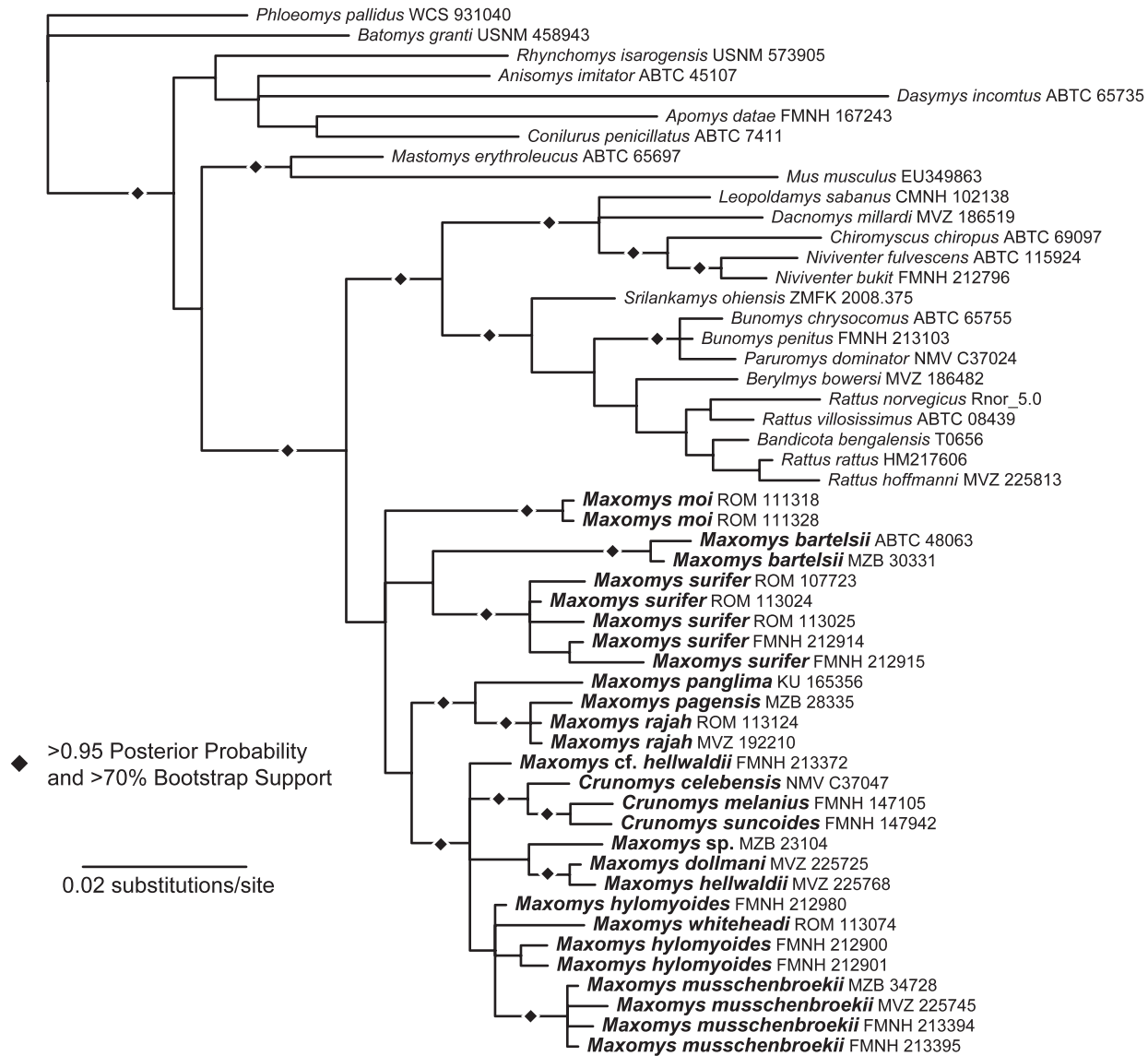


FIG. 4.—Bayesian estimate of phylogeny of Southeast Asian *Maxomys* and *Crunomys* derived from analysis of interphotoreceptor retinoid-binding protein sequences. Nodes that received strong support in both Bayesian and likelihood analyses are marked with a black diamond. Terminals are labeled with species names and either a museum voucher number or a GenBank accession number.

literature on paleoclimatic or paleohabitat differences that might have generated similar patterns.

Third, we find that *Crunomys* (with 3 of the 4 named species sampled) is monophyletic, but nested within *Maxomys* (Figs. 2–4). In nomenclature, *Crunomys* (Thomas, 1898) has priority over *Maxomys* (Sody, 1936). However, we refrain from synonymizing these 2 genera because of the lack of resolution in the deeper nodes of our phylogenetic estimates. Moreover, the type species of *Crunomys*, *C. fallax*, has yet to be included in phylogenetic analyses. Assuming *C. fallax* is closely related to the other species of *Crunomys*, some taxonomic changes will be necessary. However, lacking greater phylogenetic resolution, we cannot say whether all species of *Maxomys*, or a subset of them, should be placed in *Crunomys*.

Previously, both *Crunomys* and *Maxomys* have been difficult to place in a phylogenetic context. Species of *Maxomys* were often treated as members of *Rattus* until Misonne’s (1969) revision. Musser et al. (1979) confirmed the distinction of *Maxomys*, but changed the composition of the genus substantially by uniting several members of *Rattus* under *Maxomys*. Several previous studies using disparate approaches such as electrophoretic data (Chan et al. 1979), albumin immunology (Watts and Baverstock 1994), karyotypes (Gadi and Sharma 1983), and DNA–DNA hybridization (Ruedas and Kirsch 1997) have supported the distinction of *Maxomys*. Thomas (1898) initially placed *Crunomys* in the Hydromyinae, but later expressed doubt regarding this arrangement (Thomas 1907). Ellerman (1941) and Misonne (1969) put *Crunomys* in Murinae, and Musser (1982) suggested it as a possible member

of an endemic Philippine radiation of murines including shrew-rats (e.g., *Archboldomys* and *Chrotomys*) and terrestrial and arboreal rats (e.g., *Batomys*, *Carpomys*, and *Crateromys*). Musser and Heaney (1992) refined this hypothesis, suggesting a close relationship between *Archboldomys* and *Crunomys*, but Rickart et al. (1998) and Rickart and Heaney (2002) refuted this idea. More recently, Jansa et al. (2006) found a sister relationship between *Crunomys* and *M. whiteheadi*, the only *Maxomys* included in their phylogeny, and Buzan et al. (2011) inferred *Crunomys* to be nested in a clade of 3 species of *Maxomys*. Our results support the definition by Musser et al. (1979) of *Maxomys*, with the exceptions that *Crunomys* should be a member and *Crunomys* is the appropriate name for some or all of this clade. In addition, our results show *Maxomys* + *Crunomys* is sister to a clade of *Dacnomys* and *Rattus* division members, as previously inferred by Buzan et al. (2011) and Balakirev et al. (2012). Our results regarding membership in the *Rattus* and *Dacnomys* divisions are consistent with those of Buzan et al. (2011) and we therefore recommend *Srilankamys* be transferred to the *Rattus* division.

Biogeographically the relationships among species of *Maxomys* provide insights on the diversification of *Maxomys* across Wallace's Line, on both the Sunda Shelf and Sulawesi. Sulawesi is neither Asian nor Australian, but rather contains an exceptional number of endemic species derived from a mixture of Sundaic and Sahulian lineages (Whitten et al. 2002; Lohman et al. 2011). These species have assembled through a combination of colonization and in situ diversification (Stelbrink et al. 2012), but the role of each process remains obscure in many lineages. Our inferred phylogenetic topology, with 4 independent clades of *Maxomys* + *Crunomys* on Sulawesi, suggests that these rats have crossed between Sulawesi and the Sunda Shelf multiple times. Although the exact number and direction of colonizations remains ambiguous, it is clear from the polyphyly of the *Maxomys* of Sulawesi that multiple colonization events (in one direction or the other) took place. This indicates that for these rats, the deepwater barriers surrounding Sulawesi have been relatively permeable. In addition to the important role of interisland colonization in generating diversity, the presence of multiple species in 1 Sulawesi clade (*M. dollmani* + *M. hellwaldii* + *Maxomys* sp. [Figs. 2–4]) suggests in situ processes also have been important generators of diversity. Shrews, fanged frogs, and various lineages of arthropods also have colonized Sulawesi multiple times and experienced in situ diversification (Ruedi et al. 1998; Evans et al. 2003a; Esselstyn et al. 2009; Stelbrink et al. 2012). In the case of *Maxomys*, only the lineage containing *M. dollmani*, *M. hellwaldii*, and *Maxomys* sp. appears to have produced species in situ. However, denser sampling and finer-scale taxonomic research of species such as *M. musschenbroekii* or inclusion of currently unsampled species (*M. watsi*), or both, may bring forth additional cases of in situ diversification.

In summary, we find evidence suggesting that *Crunomys* of the Philippines and Sulawesi form a monophyletic group nested within *Maxomys*; taxonomic revision is necessary to

move all or some *Maxomys* to *Crunomys* so that genera represent natural groupings; potentially undescribed species of *Maxomys* are present on Sulawesi; multiple interisland colonization events are necessary to explain the current species distributions and relationships of *Maxomys*; and several species (*M. hylomyoides*, *M. musschenbroekii*, *M. surifer*, and *M. whiteheadi*) contain substantial geographic structure in their mtDNA.

ACKNOWLEDGMENTS

We gratefully acknowledge financial support provided by the National Science Foundation (OISE-0965856 and DEB-1145251), National Geographic Society (9025-11), Australia and Pacific Science Foundation (12-6), a Ministry of Higher Education-Fundamental Research Grant Scheme grant (06086602007), a Universiti Malaysia Sarawak (UNIMAS) Eco-Zoonosis Grant (ZRC/03/2007/03), and an Ontario Ministry of Research and Innovation fellowship. ASA thanks UNIMAS for granting the Zamalah Scholarship (UNIMAS 2008/10) and J. Patton for generously providing sequences and financial support. S. Stepan and J. Schenk (Florida State University) contributed unpublished sequences and G. Musser helped identify specimens. The MZB, UNIMAS, MVZ, FMNH, and University of Kansas Biodiversity Institute generously provided samples, support, and facilities. We thank L. Heaney and T. Horton for their warm hospitality during our visits to FMNH. Several MZB technicians (M. H. Sinaga and N. Supriatna), UNIMAS laboratory assistants (B. Ketol, W. Marni, I. Sait, and H. Irwan) and others (F. A. Anwarali Khan, M. Ridwan Rahman, S. Wiantoro, R. Chaya T. T. Eileen A. L., N. H. Hasan, M. Phuong, M. Fizi Sidiq, P. Rindang, R. Kurnia, and M. Sarkam) assisted with fieldwork and laboratory research. Finally, we thank L. Heaney and an anonymous reviewer for helpful feedback on earlier versions of this manuscript.

SUPPORTING INFORMATION

SUPPORTING INFORMATION S1.—List of specimens, localities, and GenBank accession numbers of samples used in this study. Found at DOI: 10.1644/13-MAMM-A-092.S1

LITERATURE CITED

- ACHMADI, A. S., I. MARYANTO, AND MAHARADATUNKAMSI. 2012. Systematic and descriptions of new species within genus *Maxomys* from East Kalimantan, Borneo Island. *Treubia* 39:1–28.
- ADKINS, R. M., E. L. GELKE, D. ROWE, AND R. L. HONEYCUTT. 2001. Molecular phylogeny and divergence time estimates for major rodent groups: evidence from multiple genes. *Molecular Biology and Evolution* 18:777–791.
- BALAKIREV, A. E., A. V. ABRAMOV, A. N. TIKHONOV, AND V. V. ROZHNOV. 2012. Molecular phylogeny of the *Dacnomys* division (Rodentia, Muridae): the taxonomic positions of *Saxatilomys* and *Leopoldamys*. *Doklady Biological Sciences* 445:251–254.
- BIRD, M. I., D. TAYLOR, AND C. HUNT. 2005. Palaeoenvironments of insular Southeast Asia during the Last Glacial Period: a savanna corridor in Sundaland? *Quaternary Science Reviews* 24:2228–2242.
- BUZAN, E. V., M. PAGÈS, J. MICHAUX, AND B. KRYSSTUFEK. 2011. Phylogenetic position of the Ohiya rat (*Srilankamys ohiensis*) based on mitochondrial and nuclear gene sequence analysis. *Zoologica Scripta* 40:545–553.

- CHAN, K. L., S. S. DHALIWAL, AND H. S. YONG. 1979. Protein variation and systematics of three subgenera of Malayan rats (Rodentia: Muridae, genus *Rattus* Fischer). *Comparative Biochemistry and Physiology, B. Comparative Biochemistry* 64:329–337.
- CORBET, G. B., AND J. E. HILL. 1992. The mammals of the Indomalayan region: a systematic review. Oxford University Press, Oxford, United Kingdom.
- CORLETT, R. T. 2009. The ecology of tropical East Asia. Oxford University Press, New York.
- DARWIN, C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London, United Kingdom.
- DIRZO, R., AND P. H. RAVEN. 2003. Global state of biodiversity and loss. *Annual Review of the Environment and Resources* 28:137–167.
- DRUMMOND, A. J., ET AL. 2011. Geneious v5.5. <http://www.geneious.com>. Accessed 1 April 2011.
- DUCROZ, J. F., V. VOLOBOUEV, AND L. GRANJON. 1998. A molecular perspective on the systematics and evolution of the genus *Arvicanthis* (Rodentia, Muridae): inferences from complete cytochrome *b* gene sequences. *Molecular Phylogenetics and Evolution* 10:104–117.
- ELLERMAN, J. R. 1941. The families and genera of living rodents. British Museum, London, United Kingdom.
- ESSELSTYN, J. A., H. J. D. GARCIA, M. G. SAULOG, AND L. R. HEANEY. 2008. A new species of *Desmalopex* (Pteropodidae) from the Philippines, with a phylogenetic analysis of the Pteropodini. *Journal of Mammalogy* 89:815–825.
- ESSELSTYN, J. A., C. H. OLIVEROS, R. G. MOYLE, A. T. PETERSON, J. A. MCGUIRE, AND R. M. BROWN. 2010. Integrating phylogenetic and taxonomic evidence illuminates complex biogeographic patterns along Huxley's modification of Wallace's Line. *Journal of Biogeography* 37:2054–2066.
- ESSELSTYN, J. A., R. M. TIMM, AND R. M. BROWN. 2009. Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in Southeast Asian shrews. *Evolution* 63:2595–2610.
- EVANS, B. J., ET AL. 2003a. Phylogenetics of fanged frogs: testing biogeographical hypotheses at the interface of the Asian and Australian faunal zones. *Systematic Biology* 52:794–819.
- EVANS, B. J., J. SUPRIATNA, N. ANDAYANI, M. I. SETIADI, D. C. CANNATELLA, AND D. J. MELNICK. 2003b. Monkeys and toads define areas of endemism on Sulawesi. *Evolution* 57:1436–1443.
- GADI, I. K., AND T. SHARMA. 1983. Cytogenetic relationships in *Rattus*, *Cremnomys*, *Millardia*, *Nesokia* and *Bandicota* (Rodentia: Muridae). *Genetica* 61:21–40.
- GOROG, A. J., M. H. SINAGA, AND M. D. ENGSTROM. 2004. Vicariance or dispersal? Historical biogeography of three Sunda shelf murine rodents (*Maxomys surifer*, *Leopoldamys sabanus* and *Maxomys whiteheadi*). *Biological Journal of the Linnean Society* 81:91–109.
- GRANT, B. R., AND P. R. GRANT. 1993. Evolution of Darwin's finches caused by a rare climatic event. *Proceedings of the Royal Society, B. Biological Sciences* 251:111–117.
- HALL, R. 2002. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions and animations. *Journal of Asian Earth Sciences* 20:353–431.
- HEANEY, L. R. 1991. A synopsis of climatic and vegetational change in Southeast Asia. *Climatic Change* 19:53–61.
- JANSA, S. A., F. K. BARKER, AND L. R. HEANEY. 2006. The pattern and timing of diversification of Philippine endemic rodents: evidence from mitochondrial and nuclear gene sequences. *Systematic Biology* 55:73–88.
- JANSA, S. A., AND R. S. VOSS. 2000. Phylogenetic studies on didelphid marsupials I. Introduction and preliminary results from nuclear IRBP gene sequences. *Journal of Mammalian Evolution* 7:43–77.
- JANSA, S. A., AND M. WEKSLER. 2004. Phylogeny of muroid rodents: relationships within and among major lineages as determined by IRBP gene sequences. *Molecular Phylogenetics and Evolution* 31:256–276.
- JENTINK, F. A. 1879. On various species of *Mus*, collected by S. C. I. W. Van Musschenbroek Esq. in Celebes. *Notes from the Leyden Museum* 1(1878):7–13.
- LECOMPTÉ, E., C. DENYS, AND L. GRANJON. 2005. Confrontation of morphological and molecular data: the *Praomys* group (Rodentia, Murinae) as a case of adaptive convergences and morphological stasis. *Molecular Phylogenetics and Evolution* 37:899–919.
- LOHMAN, D. J., ET AL. 2011. Biogeography of the Indo-Australian Archipelago. *Annual Review of Ecology, Evolution, and Systematics* 42:205–226.
- MACARTHUR, R. H., AND E. O. WILSON. 1963. An equilibrium theory of insular zoogeography. *Evolution* 17:373–387.
- MISONNE, X. 1969. African and Indo-Australian Muridae. Evolutionary trends. *Annales du Musée Royal d'Afrique Centrale, Tervuren* 172:1–219.
- MUSSER, G. G. 1969. Results of the Archbold Expeditions. No. 92. Taxonomic notes on *Rattus dollmani* and *Rattus hellwaldi* (Rodentia, Muridae) of Celebes. *American Museum Novitates* 2386:1–24.
- MUSSER, G. G. 1982. Results of the Archbold Expeditions. No. 110. *Crunomys* and the small-bodied shrew rats native to the Philippine Islands and Sulawesi (Celebes). *Bulletin of American Museum of Natural History* 174:1–95.
- MUSSER, G. G., AND M. D. CARLETON. 2005. Superfamily Muroidea. Pp. 894–1531 in *Mammal species of the world: a taxonomic and geographic reference* (D. E. Wilson and D. M. Reeder, eds.). 3rd ed. Johns Hopkins University Press, Baltimore, Maryland.
- MUSSER, G. G., AND L. R. HEANEY. 1992. Philippine rodents: definitions of *Tarsomys* and *Limnomys* plus a preliminary assessment of phylogenetic patterns among native Philippine murines (Murinae, Muridae). *Bulletin of the American Museum of Natural History* 211:1–138.
- MUSSER, G. G., J. T. MARSHALL, JR., AND BOEADI. 1979. Definition and contents of the Sundaic genus *Maxomys* (Rodentia, Muridae). *Journal of Mammalogy* 60:592–606.
- MYERS, N., R. A. MITTERMEIER, C. G. MITTERMEIER, G. A. B. DA FONSECA, AND J. KENT. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403:853–858.
- NYLANDER, J. A. A. 2004. MrModeltest v2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- OLIVEROS, C. H., AND R. G. MOYLE. 2010. Origin and diversification of Philippine bulbuls. *Molecular Phylogenetics and Evolution* 54:822–832.
- RAMBAUT, A., AND A. J. DRUMMOND. 2007. Tracer v1.4. <http://beast.bio.ed.ac.uk/Tracer>. Accessed 1 April 2011.
- RICKART, E. A., AND L. R. HEANEY. 2002. Further studies on the chromosomes of Philippine rodents (Muridae: Murinae). *Proceedings of the Biological Society of Washington* 115:473–487.
- RICKART, E. A., L. R. HEANEY, B. R. TABARANZA, JR., AND D. S. BALETE. 1998. A review of the genera *Crunomys* and *Archboldomys*

- (Rodentia: Muridae: Murinae), with descriptions of two new species from the Philippines. *Fieldiana: Zoology (New Series)* 89:1–24.
- RONQUIST, F., ET AL. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61:539–542.
- ROWE, K. C., M. L. RENO, D. M. RICHMOND, R. M. ADKINS, AND S. J. STEPPAN. 2008. Pliocene colonization and adaptive radiations in Australia and New Guinea (Sahul): multilocus systematics of the old endemic rodents (Muroidea: Murinae). *Molecular Phylogenetics and Evolution* 47:84–101.
- RUEDAS, L. A., AND J. A.W. KIRSCH. 1997. Systematics of *Maxomys* Sody, 1936 (Rodentia: Muridae: Murinae): 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.
- RUEDEI, M., M. AUBERSON, AND V. SAVOLAINEN. 1998. Biogeography of Sulawesi shrews: testing for their origin with a parametric bootstrap on molecular data. *Molecular Phylogenetics and Evolution* 9:567–571.
- SMITH, M. F., AND J. L. PATTON. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society* 50:149–177.
- SODY, H. J. V. 1936. Seventeen new generic, specific, and subspecific names for Dutch East Indian mammals. *Natuurkundig Tijdschrift voor Nederlandsch Indië* 96:42–55.
- STAMATAKIS A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690.
- STELBRINK, B., C. ALBRECHT, R. HALL, AND T. VON RINTELEN. 2012. The biogeography of Sulawesi revisited: is there evidence for a vicariant origin of taxa on Wallace's "anomalous island"? *Evolution* 66:2252–2271.
- STEPPAN, S. J., R. M. ADKINS, P. Q. SPINKS, AND C. HALE. 2005. Multigene phylogeny of the Old World mice, Murinae, reveals distinct geographic lineages and the declining utility of mitochondrial genes compared to nuclear genes. *Molecular Phylogenetics and Evolution* 37:370–388.
- STEPPAN, S. J., C. ZAWADZKI, AND L. R. HEANEY. 2003. Molecular phylogeny of the endemic Philippine rodent *Apomys* (Muridae) and the dynamics of diversification in an oceanic archipelago. *Biological Journal of Linnean Society* 80:699–715.
- THOMAS, O. 1898. On the mammals obtained by Mr. John Whitehead during his recent expedition to the Philippines. *Transactions of the Zoological Society of London* 14:377–414.
- THOMAS, O. 1907. The Duke of Bedford's zoological exploration in eastern Asia.—III. On mammals obtained by Mr. M. P. Anderson in the Philippine Islands. *Proceedings of the Zoological Society of London* 1907:140–142.
- WALLACE, A. R. 1869. *The Malay Archipelago*. MacMillan, London, United Kingdom.
- WATTS, C. H. S., AND P. R. BAVERSTOCK. 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.
- WHITTEN, A. J., M. MUSTAFA, AND G. S. HENDERSON. 2002. *The ecology of Sulawesi*. Periplus, Singapore, Singapore.
- WILTING, A., R. SOLLMANN, E. MEIJAARD, K. M. HELGEN, AND J. FICKEL. 2012. Mentawai's endemic, relictual fauna: is it evidence for Pleistocene extinctions on Sumatra? *Journal of Biogeography* 39:1608–1620.

Submitted 15 April 2013. Accepted 3 June 2013.

Associate Editor was Sharon A. Jansa.