Journal of Biogeography (J. Biogeogr.) (2015) 42, 51–64

ORIGINAL ARTICLE

Testing diversification models of endemic Philippine forest mice (*Apomys*) with nuclear phylogenies across elevational gradients reveals repeated colonization of isolated mountain ranges

Rebecca Justiniano[1†], John J. Schenk[1‡], Danilo S. Balete[2], Eric A. Rickart[3], Jacob A. Esselstyn[4], Lawrence R. Heaney[2] and Scott J. Steppan[1*]

[1]*Department of Biological Science, Florida State University, Tallahassee, FL 32306-4295, USA,* [2]*Field Museum of Natural History, Chicago, IL 60605-2496, USA,* [3]*Natural History Museum of Utah, Salt Lake City, UT 84108, USA,* [4]*Department of Biological Sciences and Museum of Natural Science, Louisiana State University, Baton Rouge, LA 70803, USA*

*Correspondence: Scott Steppan, Department of Biological Science, Florida State University, Tallahassee, FL 32306-4295, USA.
E-mail: steppan@bio.fsu.edu

[†]Current address: College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA.

[‡]Current address: Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA 70118-5698, USA

ABSTRACT

Aim Our aims were to document the extent of diversification in an endemic clade of small mammals (*Apomys*, subgenus *Megapomys*) on a large oceanic island and to test whether speciation occurred primarily (1) along isolated elevational gradients or (2) among montane areas, as would be expected if diversification were driven by glacial cycles.

Location The Philippine archipelago, particularly Luzon Island and two smaller adjacent islands (Mindoro and Lubang).

Methods We analysed newly generated nuclear DNA sequences from five unlinked genes and mitochondrial cytochrome *b* using concatenation (likelihood and Bayesian) and coalescent-based methods to estimate the species tree for all 13 species. We tested *a priori* models of speciation using both topological constraints and reconstructed elevational ranges of ancestors.

Results All individual gene trees recovered at least four of the morphologically defined *Megapomys* species as monophyletic, while the concatenated approaches recovered all 13 species as monophyletic. Each species was confined to a single mountain range or off-shore island. Most mountain ranges had two species, but these species pairs usually were not sisters. *Megapomys* originated at medium to high elevation followed by three transitions into lower elevations and possibly one to high elevation. Both *a priori* models of speciation (elevational gradient and glacial cycle) were rejected by topology tests. The speciation rate was approximately constant through time.

Main conclusions Nuclear sequence data strongly corroborated the species status of recently described taxa. A well-supported phylogeny showed that *Megapomys* diversified by splitting into a predominantly high-elevation clade and an entirely low-elevation clade. Neither adaptation along elevational gradients on single mountain ranges nor vicariance of high-elevation species following glacial cycle-induced dispersals fitted the data. Rather, the most likely process explaining species distributions is repeated colonization of isolated mountain ranges by distantly related species.

Keywords

Coalescent methods, elevational gradient, mammals, *Megapomys*, multi-locus, nuclear DNA, oceanic island biogeography, speciation, species tree, The Philippines.

Journal of Biogeography

INTRODUCTION

The evolution and ecology of biological diversity on islands has been a topic of interest since the initial development of biogeography as a field. Recent efforts to develop a comprehensive model of the dynamics of species richness on islands have emphasized the role of *in situ* diversification, along with colonization and extinction, especially on oceanic islands (e.g. Heaney, 2000; Whittaker *et al.*, 2007). However, the extent of *in situ* diversification, and the factors that promote and/or limit diversification, are not sufficiently well known, particularly in the context of the varied geological histories of oceanic islands and archipelagos (e.g. Borges & Hortal, 2009; Gillespie & Baldwin, 2010; Steinbauer *et al.*, 2012).

The Philippine archipelago, most of which is oceanic in origin, is an ideal place for such studies; it has a complex biogeography shaped by dramatic tectonic events (Hall, 2002) and Pleistocene climatic and sea-level fluctuations (Heaney, 1991; Steppan *et al.*, 2003). More than 7000 islands of varying sizes make up the archipelago, and multiple mountain ranges of varying ages and extents occur on the larger islands. The archipelago's exceptional species diversity has led to its description as a 'megadiverse' nation (Mittermeier *et al.*, 1997), as well as a conservation 'hotspot' (Mittermeier *et al.*, 1999). Recent studies have explored the roles of geological and climate change and geographical complexity in shaping Philippine biodiversity (e.g. Heaney *et al.*, 2005; Jansa *et al.*, 2006; Esselstyn *et al.*, 2009; Miranda *et al.*, 2011). For example, Steppan *et al.* (2003) rejected a 'species pump' model of Pleistocene glacial cycles for small-bodied, southern Philippine 'forest mice' (*Apomys* subgenus *Apomys*), in which repeated coalescence and vicariance of islands on shallow-water platforms drove speciation on the southern and eastern islands. Rather, colonization among nearby oceanic islands and some speciation within large islands were the predominant modes of diversification. Subsequent studies of the species-rich, endemic genera of Philippine murids have detected similar patterns (e.g. Balete *et al.*, 2007, 2012), but the mode of within-island diversification has not been analysed, including within *Apomys*.

The genus *Apomys* is a member of the endemic vermivore clade of Philippine murids that also includes *Archboldomys*, *Chrotomys*, *Rhynchomys* and *Soricomys* (Jansa *et al.*, 2006; Balete *et al.*, 2012). *Apomys* includes 19 described and at least three undescribed species, most of which are endemic to Luzon (Steppan *et al.*, 2003; Musser & Carleton, 2005; Heaney *et al.*, 2011). The genus is partitioned into two well-supported monophyletic subgenera: *Megapomys* and *Apomys*. Members of the subgenus *Apomys* are predominantly small and arboreal (18–41 g; Heaney *et al.*, 2010), while *Megapomys* species are large and ground-dwelling (70–110 g; Heaney *et al.*, 2011, 2014). Recent biodiversity surveys have increased our awareness of the species-rich mammalian fauna on Luzon, the largest and most topographically complex island in the Philippines (e.g. Heaney *et al.*, 2010; Heaney, 2013), including the discovery of eight new *Megapomys* species on Luzon, plus one on an adjacent small island (Heaney *et al.*, 2011, 2014), for a total of 13 species in the subgenus. Most species of *Megapomys* are limited to one mountain range on Luzon Island (Fig. 1; Heaney *et al.*, 2011). Others are endemic to Mindoro (*A. gracilirostrus*), Lubang (*A. lubangensis*), or to two adjacent mountain ranges on Luzon (*A. sierrae*). Most mountain ranges support two (Central Cordillera, Banahaw and Mingan) or three (Zambales) species. However, despite intensive sampling on southern Luzon (the Bicol Peninsula), no representatives of *Megapomys* are known to be present (Rickart *et al.*, 1991; Balete *et al.*, 2013).

Morphologically defined species were monophyletic in a mitochondrial gene tree (Heaney *et al.*, 2011), with the exceptions of the morphologically distinguishable, but reciprocally paraphyletic *A. abrae* and *A. datae*. Their distributions overlap both elevationally and spatially in the Central Cordillera of northern Luzon (Fig. 1). The interdigitation of mitochondrial linages of *A. abrae* and *A. datae* may be due to ancestral polymorphism, to conspecificity, or to mitochondrial introgression from hybridization, an issue we address further in this paper.

Mitochondrial cytochrome *b* (cyt *b*) sequence data indicated that when two *Apomys* species occur on the same mountain or range, they are usually not sister species (Heaney *et al.*, 2011). Species primarily segregate into high (> 1500 m) or low (predominantly < 1500 m) elevations (Fig. 1b). The segregation of species into different elevational zones may be indicative of several speciation models; however, little is known about how evolution into these elevation zones has influenced Philippine species diversity.

Two common models provide possible explanations for the geographical pattern of speciation among animals with limited dispersal ability, such as *Apomys*. The ecological gradient model posits that local adaptation drives divergence along an ecological gradient, such that elevationally segregated pairs of species from the same mountain will form sister species (Cadle & Patton, 1988; Patton & Smith, 1992; Schneider *et al.*, 1999; Guarnizo *et al.*, 2009; Caro *et al.*, 2013). Alternatively, past climate changes may have been a primary driver of speciation. In this model of climatic vicariance, cooler temperatures associated with Pleistocene glacial periods would have shifted high-elevation species to lower elevations where they could more easily disperse to other ranges. Species associated with high-elevation habitats might have then migrated back up the mountains when temperatures warmed, becoming isolated and speciating. If this were the case, multiple ranges could have been colonized around the same time, and we would predict that high-elevation species would form a clade derived from a low elevation, possibly paraphyletic, grade, or exhibit concordant times of origin for high-elevation species. Species in lowland areas are less likely to have been affected by such changes. Here we reconstructed the evolutionary history of *Megapomys* with a species-tree approach to test the mitochondrial DNA-based hypothesis of phylogeny and to determine which of the geographical hypotheses of speciation is most concordant with those phylogenetic patterns in *Apomys* in order to better

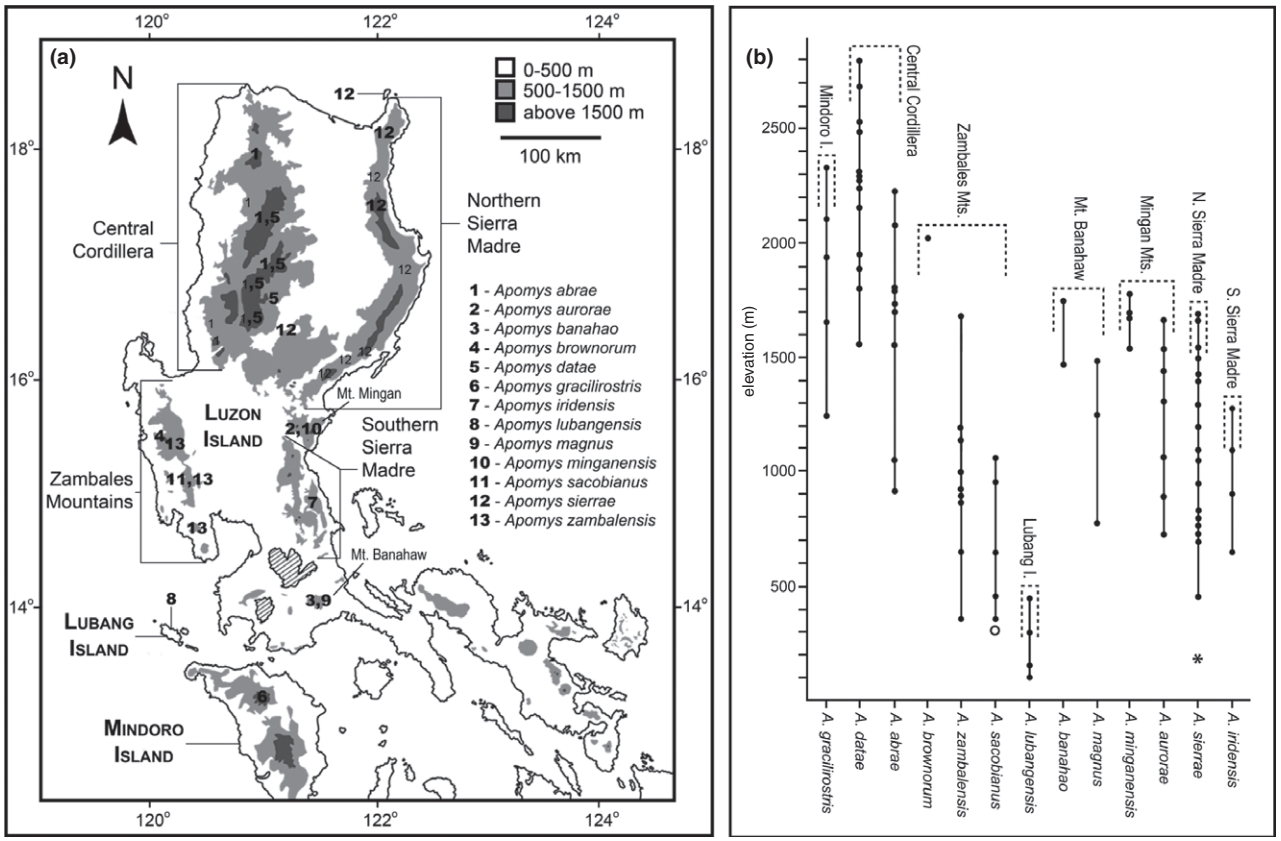

Figure 1 (a) Map of Luzon Island, Philippines, and approximate distribution of *Apomys* subgenus *Megapomys*. Locations of samples analysed in this study (Appendix 1) are shown in boldface. (b) Elevational distribution for all known samples of *Megapomys* species (data from Heaney *et al.*, 2011, 2014).

understand the processes generating the exceptional taxonomic diversity on Luzon.

MATERIALS AND METHODS

Species sampling

We collected *Megapomys* along elevational gradients throughout north and central Luzon (e.g. Balete *et al.*, 2009; Heaney, 2011, 2013), sequencing 45 specimens in 13 species (see Appendix 1 below and Appendix S1 in the Supporting Information). Six montane areas were sampled: Central Cordillera, Mingan Mts, Sierra Madre, Zambales Mts, Mt Banahaw and Mt Pinatubo (Fig. 1b). In addition, we included *A. gracilirostris* from Mindoro Island, and *A. lubangensis* from Lubang Island (Fig. 1a), a small island off the south-west coast of Luzon. Outside *Megapomys*, we included four species from the subgenus *Apomys* (*A. camiguinensis*, *A. insignis*, *A. musculus* and *A. microdon*), along with *Archboldomys*, *Rhynchomys* and *Chrotomys* as outgroups (Jansa *et al.*, 2006; Rowe *et al.*, 2008).

Laboratory methods

We extracted genomic DNA from liver or skeletal muscle following standard phenol/chloroform protocols (Sambrook *et al.*, 1989). We amplified four nuclear genes: the divergent exonic domain of recombination activating gene one (*RAG1*), intron two and parts of bounding exons of acid phosphatase type V (*Acp5*), intron three of benzodiazepine receptor (*BDR*), and exon one and part of exon two of acrosin. These genes were informative in previous studies at the species-genus level (e.g. Good *et al.*, 2008; Rowe *et al.*, 2008). Acrosin was chosen because it is a sperm-expressed protease that may be under species-specific selection (Good *et al.*, 2008; Raterman & Springer, 2008) and therefore might reflect biological species boundaries better than neutrally evolving genes. PCR reactions contained 21 μL of Platinum Blue PCR Supermix (Invitrogen, Carlsbad, CA, USA), 10 μM forward and reverse primers, 25 μM $MgCl_2$ and 25 ng template DNA. Some PCRs were conducted with 2.5 μL of 10× GoTaq buffer (Promega, Madison, WI, USA), 2 units of GoTaq polymerase, 1.5 μL of 25 mM dNTPs, 1.0 μL of 1.6% dimethyl sulfoxide (DMSO), and a total volume of 25 μL was reached with the addition of ddH_20.

We used the following amplification primers: cyt *b*, P484, P485, S128 and P1185 (Steppan *et al.*, 2003); 1100 bp of acrosin, acr11 and acr15 (Good *et al.*, 2008); 500 bp of *Acp5*, 120fwd and 564rev (DeBry & Seshadri, 2001); 950 bp of *BDR*, S151 and S153 (modified from Rowe *et al.*, 2008); 1200 bp of the first section of *RAG1* with primer pairs S70

and S142 (Steppan *et al.*, 2004) or S278 and S279 (Schenk *et al.*, 2013). Cycling conditions included an initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation (40 s at 94 °C), annealing (40 s at 56–62 °C), and extension (1:30 min at 72 °C), with a final extension (6 min at 72 °C).

Amplicons were purified with ExoSAP-IT (Affymetrix, Cleveland, OH, USA). PCR products were sequenced bi-directionally at the FSU sequencing core facility or the DNA Analysis Facility at Yale University. *RAG1* amplicons were sequenced with external primers and newly designed *Apomys*-specific internal primers S280 (5′-CCCTACCGAAT TCTGCCATA-3′) and S281 (5′-GGTGCTTACAACTGGTCT CCA-3′). We sequenced *Acp5* with internal primers 139fwd and 545rev (DeBry & Seshadri, 2001), acrosin with primers acr11 and acr15 (Good *et al.*, 2008), and *BDR* with external primers and newly designed internal primers S324 (5′-CCCTCTCGGATATGCTGTGT-3′), and S325 (5′-GGAGGTT GAAGTGGCACAAT-3′).

Length polymorphisms were identified in a few *BDR*, *Acp5* and acrosin sequences. Sequences that displayed length polymorphisms or multiple heterozygous sites were cloned at the FSU cloning facility, using the TA cloning vector, pDK101 (Invitrogen, Carlsbad, CA, USA), and sequenced with primers M13 forward and M13 reverse. Haplotypes were randomly designated A and B. For species-tree analyses, we selected a random haplotype to represent an individual. The sequences were edited and aligned using Sequencher 4.7 (GeneCodes Inc., Ann Arbor, MI, USA). Manual adjustments and concatenations were made to the alignment by eye in MacClade 4.08 (Maddison & Maddison, 2005). Sequences are available from GenBank under accession numbers KM099685–KM099879.

Phylogenetic estimation of gene trees and concatenated data

Recombination has the potential to misinform species tree estimates (Liu *et al.*, 2009). We tested nuclear genes for recombination using the Recombination Detection Program (RDP) 4.13 (Martin *et al.*, 2010), under the default settings for 100 permutations of each of the following methods: RDP (Martin & Rybicki, 2000), GeneConv (Padidam *et al.*, 1999), MaxChi (Maynard Smith, 1992) and Chimaera (Posada & Crandall, 2001).

Individual gene trees were estimated with maximum likelihood (ML), with data partitioned by codon position and intron (3–4 partitions) with RAxML 7.2.6 (Stamatakis, 2006) using the GTR+Γ substitution model. ML searches were initiated with 1000 most-parsimonious trees and were performed with 1000 replicates for each analysis. We estimated nonparametric bootstrap (BS; Felsenstein, 1985) values for 2000 pseudoreplicated datasets under the GTRCAT substitution model. We used Bayesian inference (BI) as implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), employing two independent Markov chain Monte Carlo (MCMC) runs, each with four chains. The analyses ran for 4×10^7 generations, sampling every 1000th generation, under the GTR+I+Γ substitution model. Parameter values among partitions were unlinked and the tree prior was set to a Dirichlet distribution. Convergence was determined when the standard deviation of split frequencies was less than 0.01. We examined stationarity diagnostics using Tracer 1.5 (Rambaut & Drummond, 2005) to determine burn-in. The first 10% of trees were discarded as burn-in and a 50% majority rule consensus tree was reconstructed in paup* 4.0a123 (Swofford, 2002).

The two concatenated matrices, nuclear DNA (nDNA) plus mtDNA genes and nDNA genes alone, were partitioned by codon positions across all exons (three partitions), with introns as a separate partition, and cyt *b* was partitioned by codon position separately, yielding seven partitions for the nDNA+mtDNA data set. These schemes were applied to ML, BS and BI analyses. We used beast 1.6.1 (Drummond & Rambaut, 2007) to estimate an ultrametric tree using the nDNA+mtDNA dataset with the uncorrelated lognormal relaxed molecular clock model for 10^8 generations, sampling every 1000 generations, and 10% burn-in. Because no internal fossil calibrations exist for this endemic Philippine clade, we set the root to a depth of 4.47 Ma, as estimated by Schenk *et al.* (2013) from a taxonomically broad, fossil-calibrated sampling of muroids.

Phylogenetic estimation of species tree

Each gene potentially has a different evolutionary history, and the differential sorting of haplotypes can yield gene trees that might not represent the species phylogeny (Degnan & Rosenberg, 2006). To infer the species tree from multi-locus data, we used two different methods: data concatenation (described above) and coalescent approaches. Mitochondrial genes may improve the efficiency by showing recent evolutionary history because they coalesce, on average, four times faster than nuclear genes (Moore, 1995). Only individuals sequenced for at least two genes were used in species-tree estimations.

The concatenation approach can reconstruct incorrect species trees because it does not account for gene tree variation due to incomplete lineage sorting (Kubatko & Degnan, 2007). To contend with potential discordance among the gene trees, we used *beast version 3.0 (Heled & Drummond, 2010; not to be confused with the concatenation beast method above), and stem 2.0 (Kubatko *et al.*, 2009). Two analyses were run for 2×10^8 generations, sampling every 1000, with the first 10% discarded as burn-in. The two runs were analysed for convergence diagnostics with the web-based system awty (Wilgenbusch *et al.*, 2004) and we summarized the data from one, randomly chosen analysis. To analyse a more taxonomically complete data set, but one with some missing character data, we used the ML approach implemented in stem 2.0, which is more tolerant of missing data. stem requires time-calibrated ultrametric gene trees to

reconstruct a species-tree, and, therefore, ML gene trees were ultrametricized with penalized likelihood in r8s (Sanderson, 2002). The smoothing parameter was first determined with the recommended cross-validation procedures. In STEM, we used the default settings and varied the effective population size (θ) from 0.1 to 0.0001. Changing θ had no effect on the inferred relationships, but minor effects on the branch lengths; we arbitrarily report results from $\theta = 0.001$.

Testing elevational-gradient and glacial-cycle models

We employed topological constraints to test our *a priori* hypotheses of elevational and glacial-cycle speciation (Fig. 2). For the elevational-gradient model, we created four constraints, one for each of the four mountain ranges (Central Cordillera, Mingan, Mt Banahaw and Zambales; Fig. 2a), wherein each species pair was constrained to form a monophyletic group. This model is consistent with an elevational-gradient model (e.g. Cadle & Patton, 1988; Caro *et al.*, 2013) where species diversify across an elevational gradient proximally on the same mountain. We also created a comprehensive constraint tree that simultaneously constrained all four mountain ranges. To test the glacial-cycle model, we constrained all high-elevation species to be monophyletic (Fig. 2b). This model posits a high-elevation species migrated to lower elevations, dispersed to other mountain ranges during glacial advances, and then speciated after the ranges became isolated. Constrained ML searches were conducted with RAxML and 1000 repetitions on the nDNA+mtDNA matrix. We used PAUP* to conduct an approximately unbiased (AU; Shimodaira, 2002) test to determine whether there is a significant difference in likelihood scores between unconstrained and constrained trees. Analyses were conducted with 10,000 BS replicates and were repeated with and without a distribution constructed from a subset of 500 sub-optimal RAxML trees.

Patterns of elevational diversification

We estimated ancestral elevations in MESQUITE 2.72 (Maddison & Maddison, 2009) using maximum parsimony (ordered character states) and maximum likelihood (Markov *k*-state model; Lewis, 2001) on the nDNA+mtDNA concatenated ML tree. Taxa were designated as high (predominantly > 1500 m), middle (median approximately 1500 m), or low (predominantly < 1500 m) elevation based on their distributions described in Heaney *et al.* (2011, 2014; Fig. 1b). This resulted in overlap among the three categories, but best described the clearly trimodal distributions of ranges.

We estimated the tempo of species diversification in *Megapomys* by plotting the log-lineages-through-time plot with the APE package (Paradis *et al.*, 2004) in the R statistical language, version 3.0 (R Development Core Team, 2005), as estimated on the BEAST nDNA+mtDNA chronogram. Outgroups and redundant representatives of species were pruned from the tree.

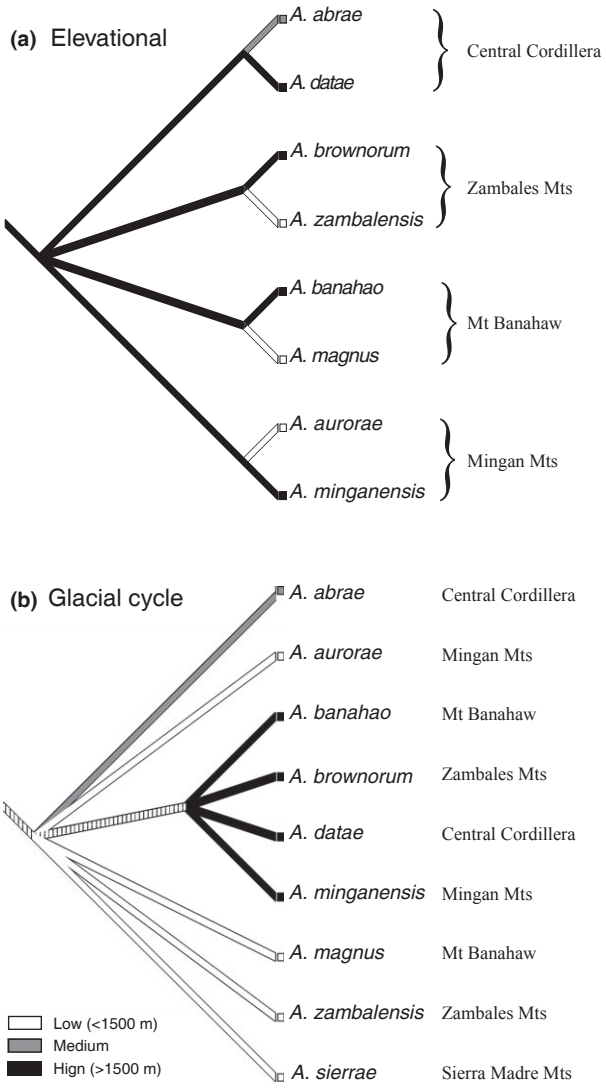

Figure 2 Backbone constraint trees used to test *a priori* speciation models in *Megapomys* on Luzon Island: (a) elevational and (b) glacial cycle. Geographical distributions are indicated in the right hand column. The elevational tree presented is the master constraint tree wherein all four mountain ranges are monophyletic for the species pairs present. Four additional constraints were applied, holding each range monophyletic separately.

RESULTS

Phylogenetic estimation of gene trees

No evidence of recombination was identified in *Megapomys* genes. In the cyt *b* phylogram (Fig. 3), we identified five lineages recognized previously with cyt *b* (Heaney *et al.*, 2011) and follow that notation for clades (A, B, C, D, with the modification of splitting D into E and F; Fig. 3). Clade B included *A. lubangensis*, *A. banahao*, *A. brownorum* and *A. sacobianus*. Clade C, which included the reciprocally paraphyletic *A. abrae* and *A. datae*, both of the Central Cordil-

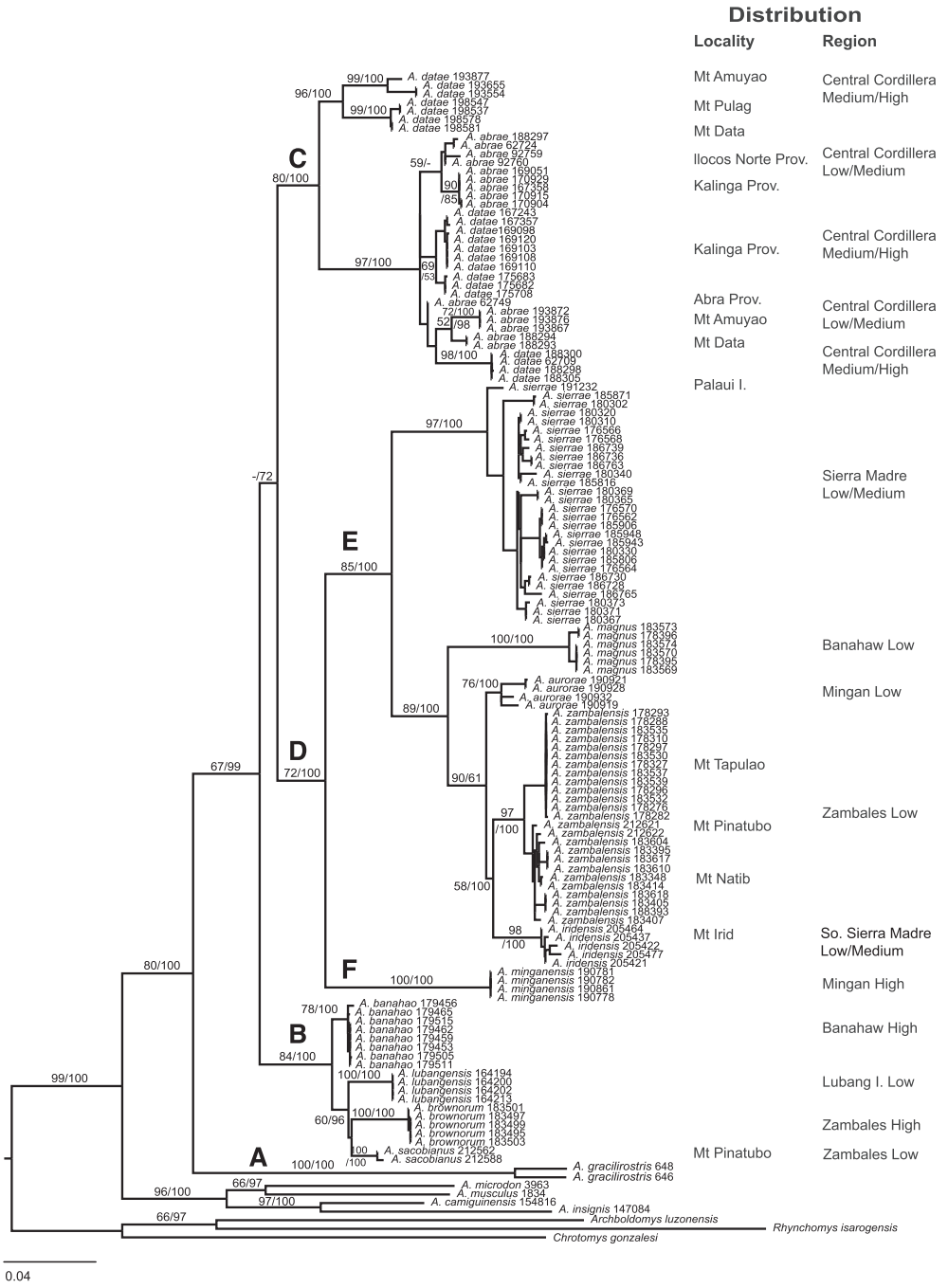

Figure 3 Phylogram of the Philippine endemic genus *Apomys* based on the mitochondrial gene cytochrome *b*, optimized with maximum likelihood for all codon partitions with RAxML. Numbers above the branches are maximum likelihood bootstrap proportions followed by posterior probabilities. Distribution information, including the locality and region, are indicated to the right of the specimen catalogue numbers. Scale bar represents probability of change.

lera, with the deepest split between *A. datae* from Mt Amuyao and Mt Pulag and the remaining samples, was sister to clade D, which consisted of low-elevation species (clade E), plus the high-elevation *A. minganensis* (clade F).

Cytochrome *b* recovered all putative species as monophyletic except *A. abrae* and *A. datae*. Individual nuclear genes did not recover all clades found in the cyt *b* tree (Figs S1–S4 in Appendix S2). On average, monophyly was supported by any given gene for 46% of the 13 operational taxonomic units (OTUs): 10 by *BDR*, six by *RAG1*, five by acrosin, and three by *Acp5* (Table 1). Although acrosin is involved in sperm recognition, it recovered only five monophyletic species. Among OTUs, only *A. gracilirostris* – sampled from a single population – was monophyletic for all loci, and *A. minganensis* for all but one. In contrast, *A. magnus* was paraphyletic for every nuclear locus. When all nuclear loci were concatenated, all OTUs

Table 1 Support for monophyly of species in the Philippine endemic genus *Apomys* based on individual gene trees and concatenated data. Bootstrap proportions are indicated before the forward slash, followed by Bayesian posterior probabilities. Polyphyletic is abbreviated as 'poly.' and paraphyletic is abbreviated as 'para.'.

Taxon	Cyt *b*	*Acp5*	Acrosin	*BDR*	*RAG1*	All nuclear	All genes
PIF	334	34	42	78	57	211	545
Total	1144	442	1081	957	1185	3665	4809
A. abrae	Poly. w/*A. datae*	96/100	99/100	Para. w/*A. datae*	Poly.	61/83	83/80
A. aurorae	76/100	Unresolved	Unresolved	100/100	Unresolved	69/94	100/100
A. banahao	78/100	Unresolved	Poly.	86/97	98/100	100/100	100/100
A. brownorum	100/100	Unresolved	Unresolved	86/100	93/100	100/100	100/100
A. datae	Poly. w/*A. abrae*	98/100	99/100	84/97	Unresolved	100/100	< 50/100
A. gracilirostris	100/100	96/100	100/100	100/100	99/100	100/100	100/100
A. iridensis	98/100	Unresolved	One sample	75/80	Poly.	< 50/< 50	96/100
A. lubangensis	100/100	Poly.	Poly.	85/97	Unresolved	Para.	89/100
A. magnus	100/100	Unresolved	Unresolved	Poly.	Unresolved	Poly.	100/100
A. minganensis	100/100	Unresolved	98/100	92/99	98/100	100/100	100/100
A. sacobianus	100/100	Unresolved	<50/58	99/100	Unresolved	62/86	100/100
A. sierrae	97/100	72/<50	Unresolved	Poly.	73/100	86/100	100/100
A. zambalensis	97/100	Unresolved	Unresolved	96/100	1000/100	98/100	100/100
No. monophyletic species:	11	4	5	10	6	11	13

PIF, number of parsimony informative characters within *Apomys*; Total, total number of nucleotides in each alignment.

except *A. lubangensis* and *A. magnus* were monophyletic (see Fig. S5). When species were not monophyletic, they were typically grouped with close relatives (based on cyt *b*; Fig. 3) and several more-inclusive clades were widely supported. Although some nuclear genes did not effectively differentiate *A. aurorae*, *A. magnus* and *A. zambalensis*, they were part of a low-elevation clade. Cytochrome *b*, *BDR* and *RAG1* showed a complex relationship between *A. abrae* and *A. datae*, with *A. abrae* being polyphyletic with respect to *A. datae*. *Apomys datae*, but not *A. abrae*, was monoplyletic in acrosin and *Acp5*, but they were not sister species.

Phylogenetic estimation of species tree

The nDNA+mtDNA concatenated ML tree (see Fig. S6 in Appendix S2) recovered all *Megapomys* species as monophyletic (Table 1). Bootstrap support exceeded 88% for all species except *A. datae* and *A. abrae*. This tree was largely congruent with both the nDNA concatenated tree (Fig. S5) and the cyt *b* tree (Fig. 3), and all three had similar relative branch lengths. The important differences between the cyt *b* and the nDNA or mt+nDNA trees were the switching in placement of clades B (*A. banahao/A. brownorum/A. sacobianus/A. lubangensis*) and E (the low-elevation *A. sierrae* clade) relative to clades C (*A. abrae/A. datae*) and F (*A. minganensis*). Relevant clades were moderately well supported [BS, 73% and 89%, posterior probability (PP) 100% and 100%, for C/B/F and B/F respectively]. All three trees differed in relationships within clade E. The addition of nuclear data made *A. abrae* and *A. datae* reciprocally monophyletic (BS, 100%; PP, 100%, Fig. S6; nDNA concatenated, Fig. S5); the only strong signal for paraphyly came from cyt *b*. In all ML multi-gene analyses, *A. datae* and *A. abrae* were sister species and together they were sister to the high (*A. banahao*, *A. brownorum* and *A. minganensis*) plus low (*A. lubangensis* and *A. sacobianus*) elevation clade. The position of *A. sierrae* as sister to the rest of the low-elevation taxa was recovered in all analyses with a BS value of 97–100%.

The nDNA-concatenated tree (Fig. S5) recovered all *Megapomys* species as monophyletic except for *A. magnus* and *A. lubangensis* (Table 1). The monophyly of seven species was strongly supported (≥ 85% BS; ≥ 95% PP), while the monophyly of *A. abrae*, *A. sacobianus*, *A. aurorae* and *A. iridensis* received limited support (BS < 85%).

The mt+nDNA concatenated BEAST tree (Fig. 4) was concordant with ML (Fig. S6) for all but two regions: (1) relationships within low-elevation clade E; and (2) the paraphyly of *A. datae* with respect to *A. abrae*, wherein both respects it matched cyt *b* (Fig. 3).

Species-tree analyses yielded slight topological variants with respect to less robustly resolved regions. *BEAST is the only analysis to yield the relationship (F(C,B)) (Fig. 5a), and relationships with clades B and E are a mixture found in other analyses. The primary difference between the STEM (Fig. 5b) and the concatenated tree (Fig. S6) was also within clade B and short-branch region of the low-elevation clade E. The nDNA STEM tree inferred the same interspecific relationships as the nDNA+mtDNA STEM (Fig. 5b) but with minor branch length differences.

Species from within a given mountain range (Mingan, Banahao and Zambales) were never closely related; instead, they fell into clades with species from similar elevations on different mountain ranges. For example, *A. zambalensis* from low-elevation Zambales Mountains was more closely related to low-elevation species from the Mingan range and Mt Banahaw than to the high-elevation species from the

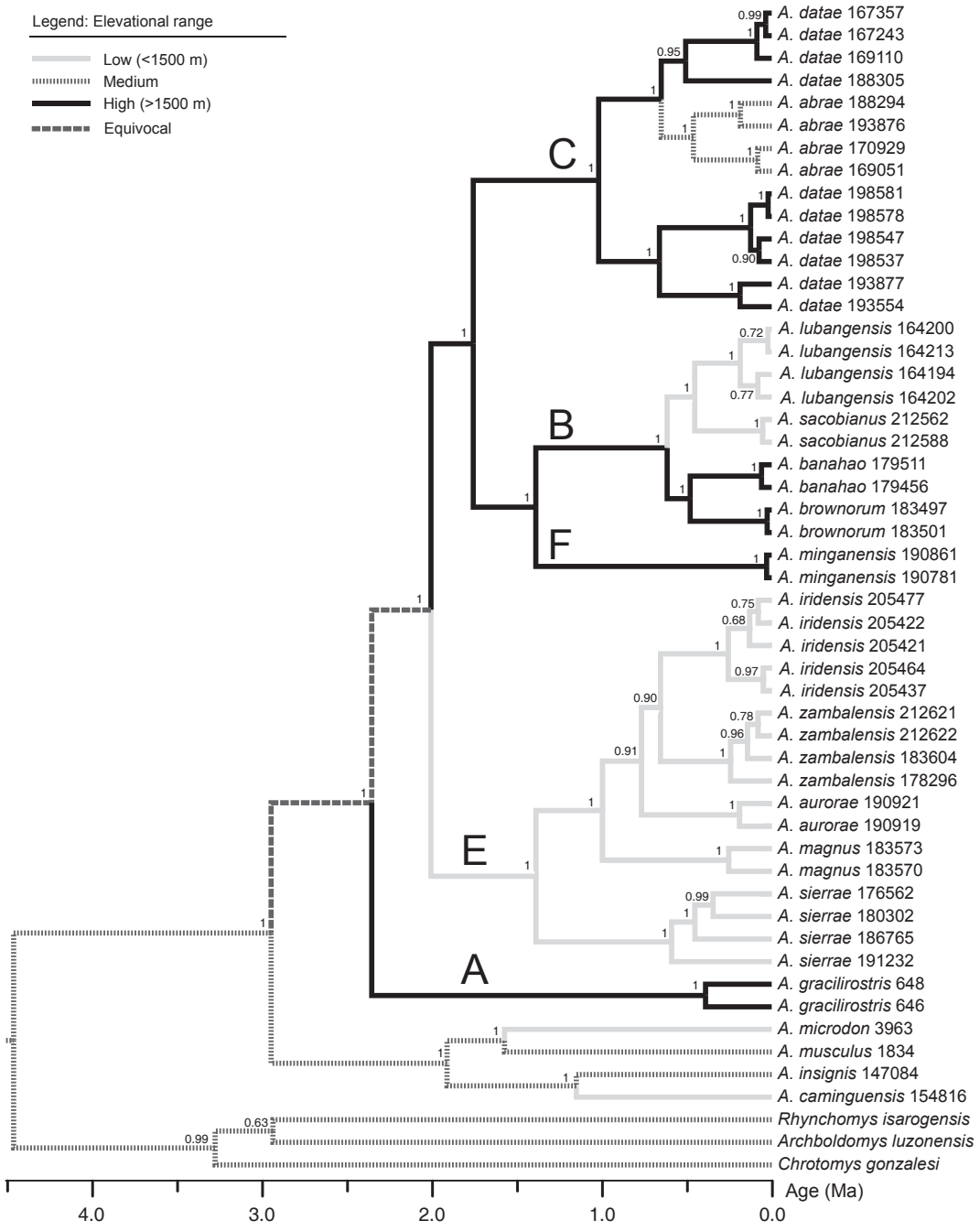

Figure 4 Maximum parsimony reconstruction of elevational range for species of *Apomys* (Philippine Islands), mapped onto the BEAST chronogram. Maximum likelihood reconstructions were nearly identical, and indicated that the parsimoniously equivocal reconstruction for the most recent common ancestor of all species of *Apomys* subgenus *Megapomys* excluding *A. gracilostris* was also uncertain, with marginal proportional likelihoods for each of the three states between 0.25 and 0.50.

Zambales Mountains, *A. brownorum*. The one exception to this pattern was observed in the *A. abrae* and *A. datae* clade that inhabit the Central Cordillera.

Testing speciation models

When the species from each range were constrained to be monophyletic, as expected under the elevational gradient model, the tree was significantly worse under the AU test than the unconstrained phylogeny ($P < 0.001$). When these constraints were tested for each mountain group individually, we rejected sister relationships for Mingan, Mt Banahaw, and Zambales mountain species pairs (all $P < 0.001$). In contrast, the monophyly of the *A. datae* and *A. abrae* clade from Central Cordillera was not rejected ($P = 0.137$). When high-elevation species were constrained to be monophyletic, as

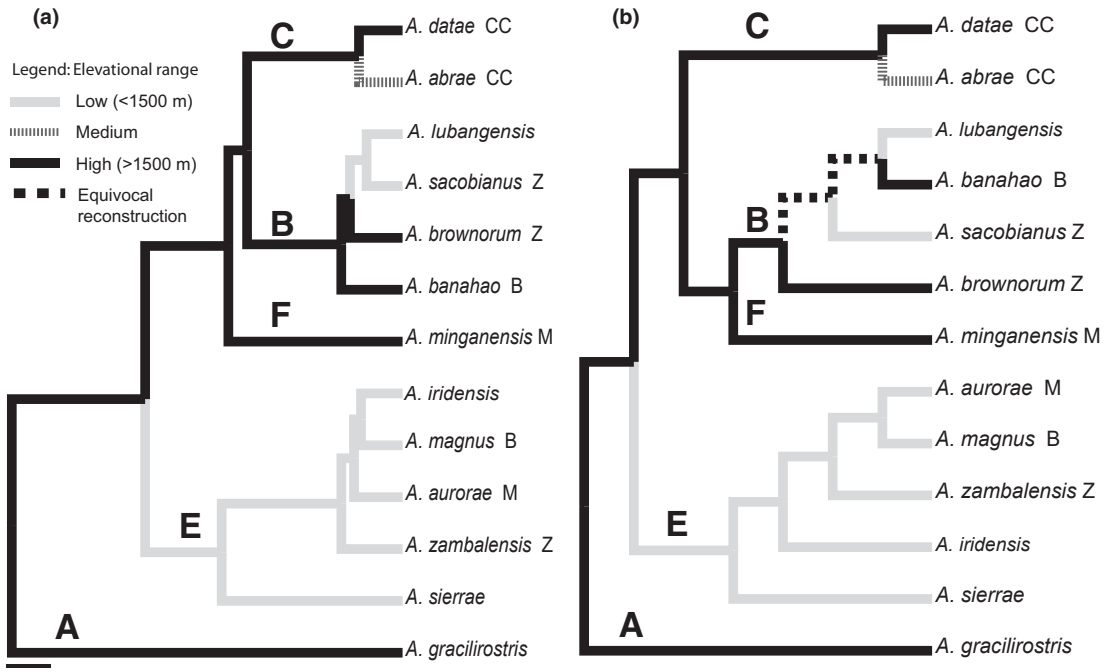

Figure 5 Multi-locus species trees for *Apomys* subgenus *Megapomys* (Philippine Islands) including four nuclear genes and cytochrome *b* from (a) *BEAST and (b) STEM. Letters above clades correspond to those in the text. Branch shading indicates ancestral reconstructions of elevation as determined with parsimony optimizations. Letters after species names indicate the mountain ranges for those ranges with more than one species: B – Mt Banahao, CC – Cordillera Central, M – Mingan Mountains, Z – Zambales Mountains. Scale bar represents probability of change.

predicted under a glacial-cycle driven range expansion followed by vicariance, that resolution was also rejected ($P < 0.001$), principally because of the presence of the lowland *A. sacobianus/A. lubangensis* clade and the *A. abrae* clade within the otherwise highland clade (Fig. 4, Fig. S6).

Patterns of elevational diversification

Both MP and ML (the latter not shown) ancestral state reconstruction of elevation indicated the root of *Megapomys* as either high or medium elevation followed by one shift to higher elevations (MP only) and three shifts into lower elevations (Fig. 4). One shift into low-elevation occurred at clade E. The second decrease in elevation (from high to medium) occurred in *A. abrae*, although this reconstruction is equivocal depending on whether *A. datae* is monophyletic. The range of *A. abrae* extends up to 2250 m, a 700-m overlap with *A. datae*, by far the largest extent of sympatry among *Megapomys* species pairs. The third shift to low elevation occurred in the *A. sacobianus/A. lubangensis* clade.

Relative timing of diversification

The rate of diversification within *Megapomys* does not appear to have occurred in pulses; instead, the rate is nearly constant (Appendix S3). Thus, we see no evidence for a scenario in which speciation occurred as a result of one or more specific periods of expansion followed by a period of isolation, as might be expected under the glacial-cycle expansion model.

DISCUSSION

Megapomys *phylogeny*

Megapomys is remarkably diverse for a mammalian taxon within a single island, with 11 species-level taxa endemic to Luzon (approximately 103,000 km^2) and another two on nearby small islands; most species occur within small areas (Heaney *et al.*, 2011, 2014). Here, we use multiple nuclear loci and additional cyt *b* sequences to corroborate the species status of previous assignments that were based on morphology and mitochondrial cyt *b*. We also tested the species status of two recently described species (*A. lubangensis* and *A. iridensis*; Heaney *et al.*, 2014) and our results supported their recognition of these as distinct species. Furthermore, concatenated phylogenetic and species-tree analyses refined our phylogenetic understanding of this clade. All multiple-gene analyses (concatenated and species-tree, except for *BEAST), with and without cyt *b*, yield the same topology among the major clades: (A(E(C(B,F)))), leading us to favour this common topology as the best estimate for the phylogenetic relationships in *Megapomys*. Only cyt *b*, analysed alone, differed significantly, yielding (A(B(C(E,F)))). Our preferred phylogeny

therefore differs from the cyt *b* tree published recently (Heaney *et al.*, 2011) with respect to the relative positioning of the low-and high-elevation clades.

It is notable that *A. datae* and *A. abrae* were the only sister species to occupy the same mountain range, had by far the greatest elevational overlap (700 m), and exhibited the only evidence for possible hybridization. The nuclear-only trees recovered these two species as reciprocally monophyletic with moderate to strong support (Appendix S2). In contrast, cyt *b* revealed two to three mitochondrial lineages for each species that are basally interdigitated (Fig. 3; Heaney *et al.*, 2011); these data suggest a relatively old mitochondrial introgression event because the depth of the paraphyly is not consistent with recent introgression.

Patterns of elevational diversification and biogeographical reconstructions

We note that the elevational ranges shown in Fig. 1b are likely to represent actual upper and lower limits of species distributions because, in all but two cases, we trapped intensively in suitable habitat above and below these limits. The two exceptions were Mt Irid, where we were unable to sample below 700 m, and the Central Cordillera, where we were unable to sample below 900 m. Thus, our data (Balete *et al.*, 2009; Heaney, 2011, 2013; Rickart *et al.*, 2011) indicate that *Megapomys* occur below 400 m elevation only on small islands offshore of Luzon, and rarely below 700 m. We conclude that even the 'lowland' *Megapomys* were truly isolated from each other by extensive areas of lowland plains, even before modern deforestation.

Maximum parsimony and ML reconstructed the most recent common ancestor of *Megapomys* as inhabiting middle or high elevations, with possibly an early shift into high elevations (> 1500 m, if ancestrally medium) and three shifts to lower elevations (Fig. 4). Both *a priori* models, whether driven by speciation within individual elevational gradients or facilitated by glacial cycles, are rejected by topological AU tests. Therefore, we do not find evidence for the elevational gradient model of speciation (Cadle & Patton, 1988; Caro *et al.*, 2013), wherein divergent selection along the gradient would drive speciation. There is also no evidence that high-elevation species colonized more distant ranges in parallel during a climatic period of lowered habitat zones associated with glacial maxima that allowed a highland species to spread widely, followed by a climatic period that caused populations to move up-slope and become isolated; rather, we found that speciation did not occur in temporal pulses, and instead occurred at a constant rate (Appendix S3). This 'species pump' model also lacked evidence among southern species of *Apomys* that diverged through colonization of continuously separated 'Pleistocene islands' (Steppan *et al.*, 2003).

In the absence of evidence for pulses of speciation events associated with major times of climate change during the past half-million years (Appendix S3), and given the evidence that sister species usually occur on different mountain ranges, and that pairs of species that do occur within a single mountain range are distantly related within *Megapomys*, our results are most consistent with a gradual process of independent colonization of isolated mountain ranges, followed by differentiation and speciation. This within-island pattern of colonization-based allopatric speciation is similar to between-island patterns of diversification described for subgenus *Apomys* (Steppan *et al.*, 2003), shrews (Esselstyn *et al.*, 2011), and fanged frogs (Evans *et al.*, 2003). Sympatry thus appears to usually require that substantial periods of time have passed before any two species are sufficiently different that they can occur in a single mountain range, with even partial elevational overlap. The degree of ecological and morphological overlap among sympatric species, and the potential for competitive exclusion, is thus highlighted as a worthy topic for future study.

Our results showed that high- and low-elevation species diverged at approximately 2.0 Ma, that all within-Luzon events occurred during the Pleistocene, and that the *A. datae/A. abrae* split occurred at approximately 1.0 Ma (Fig. 4). This was a time of rapid climatic and geological change on Luzon, with much volcanic activity that changed the topography of the island in dramatic but still imprecisely understood ways (Hall, 2002; Ku *et al.*, 2009). It is possible that the seemingly individualistic speciation events will fall into a pattern when placed into the context of specific geological history, rather than the context of general speciation models; this topic will be investigated in future studies.

CONCLUSIONS

We conclude that speciation has produced notable endemic diversity in *Megapomys* on a small geographical scale within Luzon Island, and note that *Apomys* and four other genera of murid rodents form a clade endemic to the archipelago that contains at least 47 species (Heaney *et al.*, 2011, 2014; Balete *et al.*, 2012). While colonization among oceanic islands is clearly a major factor in the generation of mammalian diversity within the archipelago, our study demonstrates that speciation within the larger islands of the archipelago can also produce substantial diversity through a similar process involving the colonization of isolated mountain ranges.

ACKNOWLEDGEMENTS

This project was supported by the Tri-Beta National Biology Honor Society Research Grant Award and the Bess H. Ward Honors Thesis Award (FSU) to R.J., Florida State University Small Grant Program grant and National Science Foundation (NSF) grant DEB-0841447 to S.J.S. We thank the Biological Science Core Facilities and High Performance Computing at FSU for use of their facilities. Fieldwork was supported by the American Society of Mammalogists and the University of Kansas E. R. Hall Fund (to J.A.E.); and by the Negaunee Foundation, Grainger Foundation, and the Field Museum's Ellen Thorne Smith and Marshall Field Funds and the Barbara Brown Fund for Mammal Research (to L.R.H.).

REFERENCES

Balete, D.S., Rickart, E.A., Rosell-Ambal, R.G.B., Jansa, S. & Heaney, L.R. (2007) Description of two new species of *Rhynchomys* Thomas (Rodentia: Muridae: Murinae) from Luzon Island, Philippines. *Journal of Mammalogy*, **88**, 287–301.

Balete, D.S., Heaney, L.R., Veluz, M.J. & Rickart, E.A. (2009) Diversity patterns of small mammals in the Zambales Mts., Luzon, Philippines. *Mammalian Biology*, **74**, 456–466.

Balete, D.S., Rickart, E.A., Heaney, L.R., Alviola, P.A., Duya, M.R.M., Duya, M.V., Sosa, T. & Jansa, S. (2012) *Archboldomys* (Muridae: Murinae) reconsidered: a new genus and three new species of shrew mice from Luzon Island, Philippines. *American Museum Novitates*, **3754**, 1–60.

Balete, D.S., Heaney, L.R. & Rickart, E.A. (2013) Diversity and distribution of small mammals in the Bicol Volcanic Belt of Southern Luzon Island, Philippines. *National Museum of the Philippines Journal of Natural History*, **1**, 61–86.

Borges, P.A.V. & Hortal, J. (2009) Time, area, and isolation: factors driving arthropod speciation at the Azorean Archipelago. *Journal of Biogeography*, **36**, 178–191.

Cadle, J.E. & Patton, J.L. (1988) Distribution patterns of some amphibians, reptiles, and mammals of the eastern Andean slope of southern Peru. *Proceedings of a workshop on Neotropical distributional patterns* (ed. by P.E. Vanzolini and W.R. Heyer) pp. 225–244. Academia Brasileira de Ciencias, Rio de Janeiro, Brazil.

Caro, L.M., Caycedo-Rosales, P.C., Bowie, R.C.K., Slabbekoorn, H. & Cadena, C.D. (2013) Ecological speciation along an elevational gradient in a tropical passerine bird? *Journal of Evolutionary Biology*, **26**, 357–374.

DeBry, R.W. & Seshadri, S. (2001) Nuclear intron sequences for phylogenetics of closely related mammals: an example using the phylogeny of *Mus*. *Journal of Mammalogy*, **82**, 280–288.

Degnan, J.H. & Rosenberg, N.A. (2006) Discordance of species trees with their most likely gene trees. *PLoS Genetics*, **2**, 762–768.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Esselstyn, J.A., Timm, R.M. & Brown, R.M. (2009) Do geological or climatic processes drive speciation in dynamic archipelagos? The tempo and mode of diversification in South East Asian Shrews. *Evolution*, **63**, 2595–2610.

Esselstyn, J.A., Maher, S.P. & Brown, R.M. (2011) Species interactions during diversification and community assembly in an island radiation of shrews. *PLoS ONE*, **6**, e21885.

Evans, B.J., Brown, R.M., McGuire, J.A., Supriatna, J., Andayani, N., Diesmos, A.C., Iskandar, D.T., Melnick, D.J. & Cannatella, D.C. (2003) Phylogenetics of fanged frogs: testing biogeographical hypotheses at the interface of the Asian and Australian faunal zones. *Systematic Biology*, **52**, 794–819.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Gillespie, R.G. & Baldwin, B.G. (2010) Island biogeography of remote archipelagoes: interplay between ecological and evolutionary processes. *The theory of island biogeography revisited* (ed. by J.B. Losos and R.E. Ricklefs), pp. 358–387. Princeton University Press, Princeton, NJ.

Good, J.M., Hird, S., Reid, N., Demboski, J., Steppan, S.J., Martin-Nims, T.R. & Sullivan, J. (2008) Ancient hybridization and mitochondrial capture between two species of chipmunks. *Molecular Ecology*, **17**, 1313–1327.

Guarnizo, C.E., Amézquita, A. & Bermingham, E. (2009) The relative roles of vicariance versus elevational gradients in the genetic differentiation of the high Andean tree frog, *Dendropsophus labialis*. *Molecular Phylogenetics and Evolution*, **50**, 84–92.

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 Science*, **20**, 353–434.

Heaney, L.R. (1991) A synopsis of climatic and vegetational change in Southeast Asia. *Climate Change*, **19**, 53–61.

Heaney, L.R. (2000) Dynamic disequilibrium: a long-term, large-scale perspective on the equilibrium model of island biogeography. *Global Ecology and Biogeography*, **9**, 59–74.

Heaney, L.R. (ed.) (2011) *Discovering diversity: studies of the mammals of Luzon Island, Philippines*. Fieldiana: Life and Earth Sciences, 2. The Field Museum of Natural History, Chicago.

Heaney, L.R. (ed.) (2013) Studies of mammalian biodiversity on Luzon Island, Philippines. *National Museum of the Philippines Journal of Natural History*, **1**, v + 1–86.

Heaney, L.R., Walsh, J.S., Jr & Peterson, A.T. (2005) The roles of geological history and colonization abilities in genetic differentiation between mammalian populations in the Philippine archipelago. *Journal of Biogeography*, **32**, 229–247.

Heaney, L.R., Dolar, M.L., Balete, D.S., Esselstyn, J.A., Rickart, E.A. & Sedlock, J.L. (2010) *Synopsis of Philippine mammals*. The Field Museum of Natural History in cooperation with the Philippine Department of Environment and Natural Resources, Protected Areas and Wildlife Bureau. Available at: http://www.fieldmuseum.org/philippine_mammals/.

Heaney, L.R., Balete, D.S., Rickart, E.A., Alviola, P.A., Duya, M.R.M., Duya, M.V., Veluz, M.J., VandeVrede, L. & Steppan, S.J. (2011) Seven new species and a new subgenus of forest mice (Rodentia: Muridae; *Apomys*) from Luzon Island. *Discovering diversity: studies of the mammals of Luzon Island, Philippines* (ed. by L.R. Heaney), pp. 1–60. Fieldiana: Life and Earth Sciences, 2. The Field Museum of Natural History, Chicago.

Heaney, L.R., Veluz, M.J., Balete, D.S., Steppan, S.J., Esselstyn, J.A., Pfieffer, A. & Rickart, E.A. (2014) Two new species of Philippine forest mice (*Apomys*, Muridae, Rodentia) from Lubang and Luzon Islands, with a redescription of *Apomys sacobianus* Johnson, 1962. *Proceedings of the Biological Society of Washington*, **126**, 395–413.

Heled, J. & Drummond, A.J. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570–580.

Jansa, S.A., Barker, F.K. & Heaney, L.R. (2006) The pattern and timing of diversification of Philippine endemic rodents: evidence from mitochondrial and nuclear gene sequences. *Systematic Biology*, **53**, 73–88.

Ku, Y., Chen, C., Song, S., Iizuka, Y. & Shen, J.J. (2009) A 2 Ma record of explosive volcanism in southwestern Luzon: implications for the timing of subducted slab steepening. *Geochemistry, Geophysics, Geosystems*, **10**, Q06017.

Kubatko, L.S. & Degnan, J.H. (2007) Inconsistency of phylogenetic estimates from concatenated data under coalescence. *Systematic Biology*, **56**, 17–24.

Kubatko, L.S., Carstens, B.C. & Knowles, L.L. (2009) STEM: species tree estimation using maximum likelihood for gene trees under coalescence. *Bioinformatics*, **25**, 971–973.

Lewis, P.O. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, **50**, 913–925.

Liu, L., Yu, L., Kubatko, L., Pearl, D.K. & Edwards, S.V. (2009) Coalescent methods for estimating phylogenetic trees. *Molecular Phylogenetics and Evolution*, **53**, 320–328.

Maddison, D.R. & Maddison, W.P. (2005) *MacClade 4.08 for OS X*. Sinauer Associates, Sunderland, MA.

Maddison, W.P. & Maddison, D.R. (2009) *Mesquite: a modular system for evolutionary analysis*. Version 2.71. Available at: http://mesquiteproject.org/.

Martin, D. & Rybicki, E. (2000) RDP: detection of recombination amongst aligned sequences. *Bioinformatics*, **16**, 562–563.

Martin, D.P., Lemey, P., Lott, M., Moulton, V., Posada, D. & Lefeuvre, P. (2010) RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics*, **26**, 2462–2463.

Maynard Smith, J. (1992) Analyzing the mosaic structure of genes. *Journal of Molecular Evolution*, **34**, 126–129.

Miranda, H.C., Jr, Brooks, D.M. & Kennedy, R.S. (2011) Phylogeny and taxonomic review of Philippine lowland scops owls (Strigiformes): parallel diversification of highland and lowland clades. *The Wilson Journal of Ornithology*, **123**, 441–452.

Mittermeier, R.A., Gil, P.R. & Mittermeier, C.G. (1997) *Mega-diversity Earth's biologically wealthiest nations*. CEMEX, Mexico City.

Mittermeier, R.A., Myers, N., Gil, P.R. & Mittermeier, C.G. (1999) *Hotspots: Earth's biologically richest and most endangered terrestrial ecoregions*. CEMEX, Mexico City.

Moore, W.S. (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution*, **49**, 718–726.

Musser, G.M. & Carleton, M.D. (2005) Superfamily Muroidea. *Mammal species of the world: a taxonomic and geographic reference* (ed. by D.E. Wilson and D.M. Reeder), pp. 894–1531. Smithsonian Institution, Washington, DC.

Padidam, M., Sawyer, S. & Fauquet, C.M. (1999) Possible emergence of new geminiviruses by frequent recombination. *Virology*, **265**, 218–225.

Paradis, E., Claude, J. & Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.

Patton, J.L. & Smith, M.F. (1992) MtDNA phylogeny of Andean mice: a test of diversification across ecological gradients. *Evolution*, **46**, 174–183.

Posada, D. & Crandall, K.A. (2001) Evaluation of methods for detecting recombination from DNA sequences: computer simulations. *Proceedings of the National Academy of Sciences USA*, **98**, 13757–13762.

R Development Core Team (2005) *R: a language and environment for statistical computing*. Available at: http://cran.r-project.org/.

Rambaut, A. & Drummond, A.J. (2005) *Tracer v. 1.5*. Available at: http://tree.bio.ed.ac.uk/software/tracer/.

Raterman, D. & Springer, M.S. (2008) The molecular evolution of acrosin in placental mammals. *Molecular Reproduction and Development*, **75**, 1196–1207.

Rickart, E.A., Heaney, L.R. & Utzurrum, R.B. (1991) Distribution and ecology of small mammals along an elevational transect in southeastern Luzon, Philippines. *Journal of Mammalogy*, **72**, 458–469.

Rickart, E.A., Heaney, L.R., Balete, D.S. & Tabaranza, B.R., Jr (2011) Small mammal diversity along an elevational gradient in northern Luzon, Philippines. *Mammalian Biology*, **76**, 12–21.

Ronquist, F. & Huelsenbeck, J.P. (2003) Mr Bayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Rowe, K., Reno, M.L., Richmond, D.M., Adkins, R.M. & Steppan, S.J. (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.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular cloning: a laboratory manual*. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York.

Sanderson, M.J. (2002) Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution*, **19**, 101–109.

Schenk, J.J., Rowe, K.C. & Steppan, S.J. (2013) Ecological opportunity and incumbency in the diversification of repeated continental colonizations by muroid rodents. *Systematic Biology*, **62**, 837–864.

Schneider, C.J., Smith, T.B., Larison, B. & Moritz, C. (1999) A test of alternative models of diversification in tropical rainforests: ecological gradients vs. rainforest refugia. *Proceedings of the National Academy of Sciences USA*, **96**, 13869–13873.

Shimodaira, H. (2002) An approximately unbiased test of phylogenetic tree selection. *Systematic Biology*, **51**, 492–508.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Steinbauer, M.J., Otto, R., Naranjo-Cigala, A., Beierkuhnlein, C. & Fernández-Palacios, J.-M. (2012) Increase of island endemism with altitude – speciation processes on oceanic islands. *Ecography*, **35**, 23–32.

Steppan, S.J., Zawadzki, C. & Heaney, L.R. (2003) Molecular phylogeny of the endemic Philippine rodent *Apomys* (Muridae) and the dynamics of diversification in a oceanic archipelago. *Biological Journal of the Linnean Society*, **80**, 699–715.

Steppan, S.J., Storz, B.L. & Hoffmann, R.S. (2004) Nuclear DNA phylogeny of the squirrels (Mammalia: Rodentia) and the evolution of arboreality from c-myc and RAG1. *Molecular Phylogenetics and Evolution*, **30**, 703–719.

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

Whittaker, R.J., Ladle, R.J., Araújo, M.B., Fernández-Palacios, J.M., Delgado, J. & Arévalo, J.R. (2007) The island immaturity-speciation pulse model of island evolution: an alternative to the "diversity begets diversity" model. *Ecography*, **30**, 321–327.

Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2004) *AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference*. Available at: http://king2.scs.fsu.edu/CEBProjects/awty/awty_start.php.

SUPPORTING INFORMATION

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

Appendix S1 GenBank accession numbers for *Apomys*.
Appendix S2 Maximum likelihood phylograms of individual gene trees and concatenated nuclear data for *Apomys*.
Appendix S3 Lineages-through-time plot.

BIOSKETCH

Rebecca Justiniano received a Bachelor of Science in Biological Science from Florida State University. She is currently at the University of Arizona pursuing a PhD in Pharmacology and Toxicology. The research team is interested in how lineages diversify across landscapes through time, with particular interests in Philippine biogeography and small mammals.

Author contributions: J.J.S., L.R.H., R.J. and S.J.S. conceived the ideas; D.S.B., E.A.R., J.A.E. and L.R.H. conducted fieldwork and species sampling; R.J. collected the molecular data; and J.J.S., R.J. and S.J.S. analysed the data and led the writing with important contributions from L.R.H., J.A.E. and all authors.

Editor: Brett Riddle

APPENDIX 1

Specimens of *Apomys* sequenced. All specimens were collected in the Philippines on Luzon Island, unless indicated otherwise. See Appendix S1 for cytochrome *b* sequences from Heaney *et al.* (2011). FMNH, Field Museum of Natural History; CMNH, Cincinnati Museum of Natural History & Science; KUNHM, University of Kansas Natural History Museum; USNM, National Museum of Natural History, Smithsonian Institution.

Apomys abrae: FMNH 169051, Kalinga Province, Balbalan Municipality; FMNH 170929, Kalinga Province, Balbalan Municipality, Bgy Balbalasang, Mapga; FMNH 188293, Mountain Province, Bauko Municipality, Mt Data: 1.0 km N, 1.25 km E south peak; FMNH 188294, Mountain Province, Bauko Municipality, Mt Data, 0.9 km N, 1.75 km E south peak; FMNH 193872, FMNH 193876, Mountain Province, Barlig Municipality, 0.6 km N Barlig Municipal Hall; FMNH 92759, Ilocos Norte Province, Mt Simminubular.

Apomys aurorae: FMNH 190919, FMNH 190921, Aurora Province, Dingalan Municipality, Mingan peak.

Apomys banahao: FMNH 179456, FMNH 179511, Quezon Province, Tayabas Municipality, Bgy Barangay, Mt Banahaw, Lalo.

Apomys brownorum: FMNH 183497, FMNH 183501, Zambales Province, Palauig Municipality, Bgy Salasa, Mt Tapulao.

Apomys camiguinensis: FMNH 154816, Camiguin I., Mt Tim- poong, 2 km N, 6.5 km W Mahinog.

Apomys datae: FMNH 167243, FMNH 167357, Kalinga Province, Balbalan Municipality, Bgy Balbalasang, Mapga; FMNH 169110, Kalinga Province, Balbalan Municipality, Bgy Balbalasang, Am-licao; FMNH 188305, Mountain Province, Bauko Municipality, Mt Data, 0.1 km E south peak; FMNH 193554, Mountain Province, Barlig Municipality, 0.5 km N W Mt Amuyao peak; FMNH 193877, Mountain Province, Barlig Municipality, 0.6 km N Barlig Municipal Hall; FMNH 198537, FMNH 198547, Benguet Province, Kabayan Municipality, Mt Pulag NP, 1.35 km S, 0.8 km E Mt Pulag peak; FMNH 198578, Benguet Province, Kabayan Municipality, Mt Pulag NP, 1.15 km S, 1.35 km E Mt Pulag peak; FMNH 198581, Benguet Province, Kabayan Municipality, Mt Pulag NP, 0.8 km S, 0.4 km W Mt Babadak peak.

Apomys gracilirostris: CMNH 646, CMNH 648, Mindoro Island, Mindoro Oriental Province, north ridge approach to Mt Halcon, Hanglo.

Apomys insignis: FMNH 147084, Mindanao Island, Bukidnon Province, Mt Kitanglad Range, 17 km S, 7 km E Baungon.

Apomys iridensis: FMNH 205421, FMNH 205422, Rizal Province, Rodriguez Municipality, 1.5 km S, 1 km W Mt Irid peak; FMNH 205437, Rizal Province, Rodriguez Municipality, 1.25 km S, 0.5 km W Mt Irid peak; FMNH 205464, Rizal Province, Rodriguez Municipality, 0.5 km S, 0.1 km W Mt Irid peak; FMNH 205477, FMNH 205480, Rizal Province, Rodriguez Municipality, 0.25 km S, 0.15 km W Mt Irid peak.

Apomys lubangensis: KUNHM 164194, KUNHM 164200, KUNHM 164202, KUNHM 164213, Lubang Island, Occidental Mindoro Province, Lubang Municipality.

Apomys magnus: FMNH 183570, Quezon Province, Tayabas Municipality, Idoro, Mt Banahaw; FMNH 183573, Quezon Province, Tayabas Municipality, Hasa-an, Mt Banahaw.

Apomys microdon: USNM 458907, Camarines Sur Province, Mt Isarog.

Apomys minganensis: FMNH 190781, FMNH 190861, Aurora Province, Dingalan Municipality, Mingan peak.

Apomys musculus: USNM 458925, Camarines Sur Prov., Mt Isarog.

Apomys sacobianus: FMNH 212562, Pampanga Province, Mabalacat Municipality, 7.4 km N, 13 km E Mt Pinatubo peak; FMNH 212588, Pampanga Province, Mabalacat Municiplality, 6 km N, 12.7 km E Mt Pinatubo peak.

Apomys sierrae: FMNH 176562, Cagayan Province, Gonzaga Municipality, Bgy Magrafil, Sitio Masok, Mt Cagua; FMNH 180302, Cagayan Province, Peñablanca Municipality, Bgy Lapi, Sitio Baua, Mt Cetaceo; FMNH 186765, Nueva Vizcaya Province, Quezon Municipality, Mt Palali; FMNH 191232, Palaui Island, Cagayan Province, Sta. Ana Municipality, Bgy San Vicente, 4.25 km S of Cape Engaño Lighthouse.

Apomys zambalensis: FMNH 178296, Zambales Province, Palauig Municipality, Bgy Salasa, Mt Tapulao; FMNH 183604, Bataan Province, Orani Municipality, 0.7 km N, 0.2 km W Mt Natib peak; FMNH 212621, FMNH 212621, Pampanga Province, Mabalacat Municipality, 3.2 km N, 11.5 km E Mt Pinatubo peak.

Archboldomys luzonensis: EAR 1826, Luzon I., Camarines Sur Prov., Mt Isarog.

Chrotomys gonzalezi: USNM 458952, Luzon I., Camarines Sur Prov., Mt Isarog.

Rhynchomys isarogensis: USNM 573905, Luzon I., Camarines Sur Prov., Mt Isarog.