DO GEOLOGICAL OR CLIMATIC PROCESSES DRIVE SPECIATION IN DYNAMIC ARCHIPELAGOS? THE TEMPO AND MODE OF DIVERSIFICATION IN SOUTHEAST ASIAN SHREWS

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Geological and climatic processes potentially alter speciation rates by generating and modifying barriers to dispersal. In Southeast Asia, two processes have substantially altered the distribution of land. Volcanic uplift produced many new islands during the Miocene–Pliocene and repeated sea level fluctuations during the Pleistocene resulted in intermittent land connections among islands. Each process represents a potential driver of diversification. We use a phylogenetic analysis of a group of Southeast Asian shrews (*Crocidura*) to examine geographic and temporal processes of diversification. In general, diversification has taken place in allopatry following the colonization of new areas. Sulawesi provides an exception, where we cannot reject within-island speciation for a clade of eight sympatric and syntopic species. We find only weak support for temporally declining diversification rates, implying that neither volcanic uplift nor sea level fluctuations had a strong effect on diversification rates. We suggest that dynamic archipelagos continually offer new opportunities for allopatric diversification, thereby sustaining high speciation rates over long periods of time, or Southeast Asian shrews represent an immature radiation on a density-dependent trajectory that has yet to fill geographic and ecological space.

KEY WORDS: Biogeography, birth–death model, *Crocidura*, diversification rate, island, phylogeny.

A decline in the net rate of diversification through time is commonly inferred from molecular phylogenies (Kozak et al. 2006; McPeek 2008; Price 2008; Rabosky and Lovette 2008). This pattern is frequently characterized as evidence for density-dependent diversification (DDD), which supports the concept of a correlation between speciation rates and ecological opportunity (Seehausen 2007). Hence, DDD is a central tenet of the "ecological theory" of adaptive radiation and may apply broadly to nonadaptive radiations as well (Schluter 2000; McPeek 2008; Seehausen 2007). However, Phillimore and Price (2008) argued that the commonness of declining rates of diversification is partially due to the stochastic nature of birth–death processes. They demonstrated that clades that speciate rapidly early in their history tend to have many extant species, and thus are subject to phylogenetic study. Whatever the cause, most studies investigating the tempo of diversification examine continental radiations and many have inferred the putative density-dependent pattern (McPeek 2008; Phillimore and Price 2008; Price 2008). Although island faunas have been the focus of intensive study by evolutionary biologists, it remains an open question whether declining rates of diversification is the
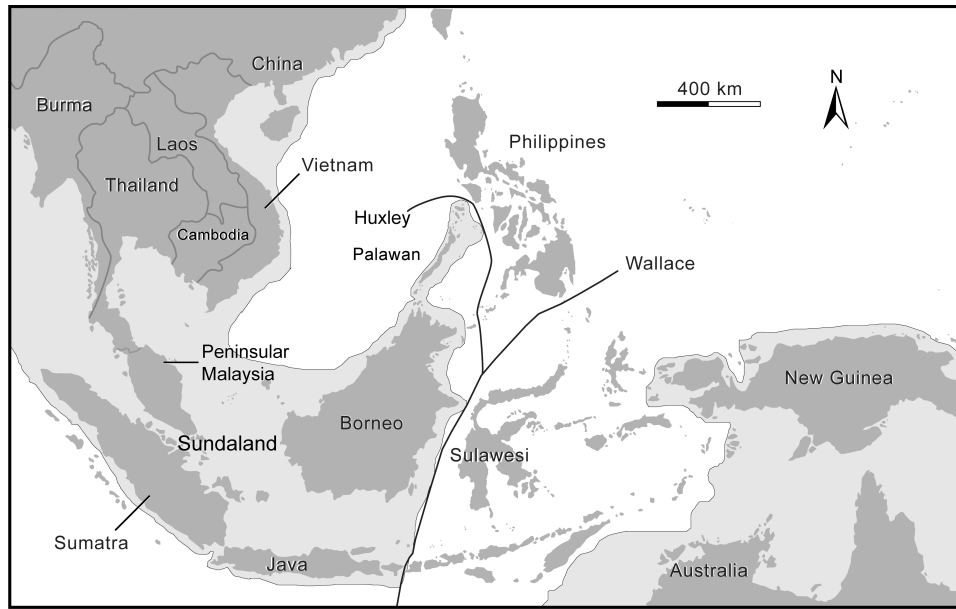
Figure 1. Map of Southeast Asia showing the extent of modern islands (medium gray) and continental shelves (light gray). Sundaland included the islands of Sumatra, Java, Borneo, and Palawan during Pleistocene glacial maxima. Wallace's Line and Huxley's modification of it are illustrated. Shrews (*Crocidura*) occur widely across the Sunda Shelf and cross Huxley's modification of Wallace's line into the Philippines and Sulawesi.

norm in island archipelagos, where there are enormous opportunities for allopatric diversification (Arbogast et al. 2006; Brown and Guttman 2002; Evans et al. 2003a; Filardi and Moyle 2005; Grant et al. 2000; Steppan et al. 2003).

The archipelagos of Southeast Asia represent the largest complex of islands in the world (Fig. 1), and they house a substantial proportion of global biodiversity (Mittermeier et al. 2004). The region is an aggregate of three globally significant hot spots divided by sharp, yet porous biogeographic boundaries (Evans et al. 2003a; Schmitt et al. 1995; Wallace 1860). Dynamic geological and climatic histories have combined to generate a matrix of islands in which the spatial distribution of terrestrial habitats has been altered extensively through time (Heaney 1985; Hall 1998; Voris 2000; Bird et al. 2005). The processes of volcanic uplift and repeated sea level fluctuations represent potential mechanisms promoting evolutionary diversification by providing opportunities for allopatric speciation (Heaney 2000; Steppan et al. 2003; Jansa et al. 2006; Outlaw and Voelker 2008). The two processes are temporally sea level fluctuations began (Haq et al. 1987; Hall 1998; Rohling et al. 1998; Zachos et al. 2001; Hall 2002). This scenario allows one to test for an impact of each process on diversification by examining temporal variation in the speciation and extinction rates of clades.

Southeast Asian shrews (Soricidae: *Crocidura* [hereafter, "shrews"]) provide an excellent model for testing the impacts of geological and climatic history on phylogenetic diversification. Shrews are broadly distributed across Southeast Asia and probably represent a recent arrival to the region. As species-level diversity in *Crocidura* is highest in Africa, and fossil dates of shrews from the continent are older than those in Eurasia, the group may have originated in Africa (or perhaps western Eurasia) and colonized east Asia relatively recently (Butler 1998; Storch et al. 1998; Hutterer 2005; Dubey et al. 2007, 2008). Dubey et al. (2007) estimated the divergence of African from Eurasian *Crocidura* at 5.4–10.7 million years ago (mya), thus the entire history of shrew evolution in Southeast Asia likely took place during the last 10 million years or so, a period over which we have a good understanding of geological history (Hall 1998, 2002). Shrews are found on all major islands of the Sunda Shelf, and cross Huxley's modification of Wallace's Line into the Philippines and Sulawesi (Fig. 1). They are widespread in the Philippines, with nine species currently recognized (Heaney and Ruedi 1994; Hutterer 2007); six species are known from Sulawesi (Ruedi 1995; Ruedi et al. 1998).

We use a multilocus phylogenetic analysis of Southeast Asian shrews to test competing hypotheses of the underlying causes of diversification. Specifically, we test for the monophyly of shrews in the Philippines and on Sulawesi (i.e., single founding colonization event per major landmass or archipelago), for sister relationships between sympatric/syntopic species in the Philippines and Sulawesi (within-island speciation), and for the biogeographical affinities of individual land masses adjacent to the Sunda Shelf. We further use maximum likelihood to fit a series of rate-constant and rate-variable birth–death models to the temporal distribution of speciation events in the phylogeny; we then consider whether
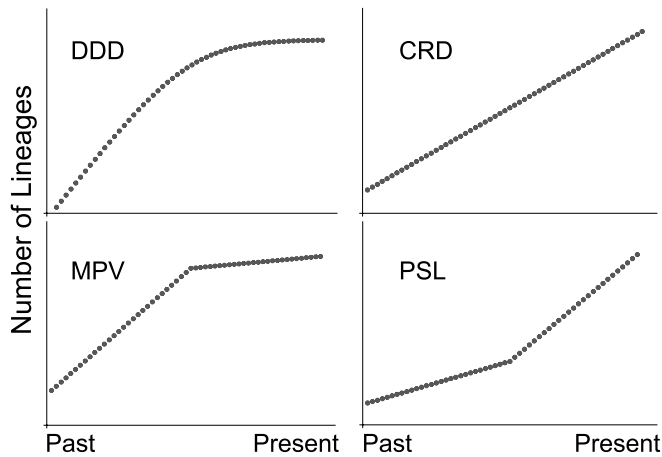
Figure 2. Idealized log lineage-through-time plots showing the expected patterns of speciation under hypotheses of density-dependent diversification (DDD), a constant rate of diversification (CRD), speciation promoted by Miocene–Pliocene volcanic (MPV) uplift, and speciation promoted by Pleistocene sea level (PSL) fluctuations.

the best-fitting models are consistent with the hypotheses of DDD, increased rates of diversification associated with volcanic uplift during the Miocene and Pliocene (MPV), increased rates of diversification associated with Pleistocene sea level (PSL) fluctuations, or a null hypothesis of a constant rate of diversification (CRD; Fig. 2).

Materials and Methods
GEOLOGICAL HISTORY OF SOUTHEAST ASIA

Southeast Asia has a long, complex geological history. The islands of the region are divided into the biogeographic zones of Sundaland (= Sunda Shelf), the oceanic Philippines, and Wallacea (dominated by Sulawesi Island). Sundaland (Malay Peninsula, Borneo, Java, Sumatra, and Palawan) is a complex of large islands currently separated by shallow water, lying south and east of the coasts of Thailand and Cambodia. The area was exposed as dry land repeatedly during Pleistocene glacial maxima (Rohling et al. 1998), thus opportunities for colonization by terrestrial organisms have been frequent, at least throughout the Pleistocene (Bird et al. 2005; Gorog et al. 2004; Heaney 1984; Meijaard and van der Zon 2003; Voris 2000). Sundaland is an important source from which the floras and faunas of the Philippines and Wallacea originated (Corbet and Hill 1992; Dickerson 1928).

Northeast of Sundaland, the Philippines includes >7000 modern islands (Fig. 3) that have been converging toward their present location over the last ca. 35 million years (Hall 1998, 2002). Most are volcanic in origin, but others are continental fragments that were submerged for long periods of time before emerging as islands (Hall 1998, 2002). The archipelago's fauna is thus derived from over-water colonization (Heaney 1985; Hall
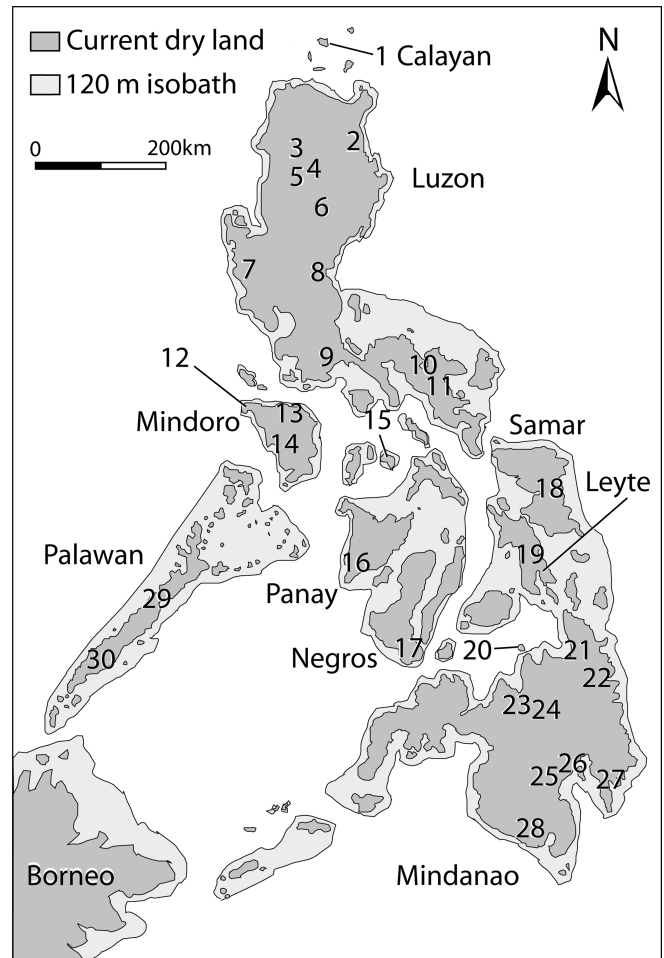

Figure 3. Map of the Philippine Islands showing the present distribution of dry land and the extent of dry land during Pleistocene glacial maxima (after Heaney 1985). Numbers show the approximate locations of Philippine sample sites.

1998; Evans et al. 1999; Brown and Guttman 2002; Evans et al. 2003a). One exception to this pattern is the Palawan group, which was isolated early in its history, but may have had a dry-land connection to Borneo as recently as 165,000 years ago (Hall 1998; Heaney 1984; Voris 2000). The mammalian and avian faunas of Palawan are most similar to those of Borneo (Dickerson 1928; Esselstyn et al. 2004), but the affinities of the herpetofauna are more complex (Brown and Diesmos in press; Inger 1954). A few studies have examined phylogenetic relationships within clades that span the Borneo–Palawan–Philippines region and several have shown Palawan to have biogeographic relationships with the oceanic Philippines and Sulawesi, often to the exclusion of Borneo (Brown and Guttman 2002; Evans et al. 2003a; McGuire and Kiew 2001).

Lying south of the Philippines and east of Sundaland, the island of Sulawesi probably represents a number of once distinct geological elements that recently coalesced (Evans et al. 2003b; Hall 1998). These former islands correspond today to

areas of endemism; each remains a distinctive biogeographic region within Sulawesi (Evans et al. 2003b, 2008). Sulawesi is surrounded by deep water and its individual components probably remained isolated from continental sources throughout their history (Hall 1998, 2002; Voris 2000); thus, the island's biodiversity is also most likely derived from over-water colonization.

TAXON SAMPLING

We gathered tissue samples from 227 shrews representing >30 species from populations throughout Southeast Asia. Our sampling is densest in the Philippines, where we obtained tissues from seven of nine named species; the two unsampled taxa are *Crocidura grandis*, which is known only from the holotype (Miller 1910), and *C. attenuata* from Batan (a small island lying midway between Taiwan and Luzon), which represents an outlying population of a mainland species (Heaney and Ruedi 1994). We include samples of *C. attenuata* from the Asian mainland. All other Philippine taxa are represented, most by multiple specimens from multiple localities; our sampling across geographic space is thorough, with all major Pleistocene island complexes represented (Fig. 3). Outside the Philippines, our sampling includes representatives of five species from Sulawesi and five from the Sunda Shelf, including taxa from Sumatra, Java, Borneo, and Peninsular Malaysia. Additional samples representing seven species from China, Vietnam, Taiwan, and India are included in the analyses.

When analyses were restricted to Cytochrome b (CytB), we further improved our taxonomic sampling with the addition of sequences from GenBank; these provided otherwise unsampled species from Sulawesi (4), the Sunda Shelf (4), Japan and the Ryukyu Islands (2), and the Asian mainland (4; see Appendix for details). Thus, with the addition of sequences from GenBank, our sampling includes 25 of 31 species known from the region encompassing the Sunda Shelf (including the Malay Peninsula), the Philippines, and Sulawesi (Ruedi 1995); and 34 of 46 species known from the region east of the Thai–Burmese border and south of the Ryukyu Islands (Hutterer 2005; Lunde et al. 2004; Ruedi 1995).

MOLECULAR GENETICS

We sequenced the mitochondrial genes CytB and NADH Dehydrogenase Subunit 2 (ND2) along with parts of four flanking tRNAs. We also sequenced three independent nuclear loci. These include the Y-linked Dead Box Intron 14 (DBY14), the autosomal Mast Cell Growth Factor Introns 5–6 (MCGF), and the autosomal exon Apolipoprotein B (ApoB).

We extracted DNA using a noncommercial guanidine thiocyanate method following Esselstyn et al. (2008). The polymerase chain reaction (PCR) was used to amplify target regions of mitochondrial and nuclear DNA. Thermal cycles for PCR followed the general protocol of initial denaturing at 94°C for 60 s, fol-

lowed by 30–40 cycles of denaturing (94°C for 30–60 s), annealing (35–60°C for 30–60 s), and extension (72°C for 30–120 s). Each PCR reaction ended with a final extension at 72°C for 5–7 min. We used several published primers and an array of newly developed, group-specific primers (Table 1). Methods of purification and sequencing follow Esselstyn et al. (2008). All sequences were deposited in GenBank under accession numbers FJ813604–FJ814618.

PHYLOGENETIC ANALYSES

We aligned sequences manually using Se-Al 2.0a11 (Rambaut 1996). The final alignment of the concatenated dataset was deposited in TreeBase. No indels were observed in the coding genes (CytB, ND2, ApoB); those found in the introns were short (<10 nucleotides) and alignments were unambiguous. Our phylogenetic inferences relied on parsimony, likelihood, and Bayesian approaches. We used *Suncus murinus* to root all trees because of its position relative to *Crocidura* in recent phylogenetic studies (Dubey et al. 2007; Ohdachi et al. 2006). A parsimony analysis was conducted in PAUP* 4.0b10 (Swofford 1999) on the concatenated dataset. All characters were weighted equally and gaps were treated as missing data. We completed heuristic searches with TBR branch swapping and 500 random addition sequences. One hundred nonparametric bootstraps were completed as measures of clade support.

Bayesian analyses were implemented in MrBayes 3.1 (Ronquist and Huelsenbeck 2003). Sequences were partitioned by codon position for each mitochondrial gene, the four flanking tRNAs were analyzed as a single partition, and each nuclear locus was modeled separately. Appropriate models of sequence evolution for each of the 10 partitions were identified using Akaike's Information Criterion (AIC), as implemented in Modeltest 3.7 (Posada and Buckley 2004; Posada and Crandall 1998). When AIC identified a submodel of the general class of GTR models, the GTR model was used (Table 2). Markov Chain Monte Carlo (MCMC) searches of tree space included four runs with four chains each and were run for 10^7 generations. Trees were sampled every 2000 generations and the first 2001 samples were discarded as burn-in, leaving 3000 postburnin trees from each run. We sought evidence of convergence among MCMC chains by examining log-likelihood plots in Tracer v1.4 (Rambaut and Drummond 2007). We also examined correlations of split frequencies between runs and cumulative split frequencies in AWTY (Nylander et al. 2008). Separate Bayesian analyses were conducted on CytB, the concatenated nuclear genes, and the entire matrix.

A maximum-likelihood analysis was conducted on the expanded CytB dataset in RAxMLHPC v7.0 (Stamatakis 2006). We completed 100 iterations of this analysis and selected the best tree among these searches. As our purpose for this inference was to test hypotheses related to rates of net diversification, we wanted

Table 1. Summary of primers and annealing temperatures used in this study. Annealing temperatures represent the full range used in successful reactions; TD indicates that a "touchdown" protocol was used. Primer names that begin with "Smr" and "Lyt" were designed specifically to amplify mtDNA from populations from the islands of Samar and Leyte, respectively.

Locus	Primer name	5′	3′	Primers paired with	Annealing temperatures	Primer source
ApoB	ApoBf	GCAATCATTTGACTTAAGTG		ApoBr	47–50°	Dubey et al. 2007
	ApoBr	GAGCAACAATATCTGATTGG		ApoBf	47–50°	Dubey et al. 2007
MCGF	MCGF56F	GTTCTCCTCAACATCAAGTCC		MCGF56R	40–55° (TD)	This study
	MCGF56R	GCAATTGCAGAGTTAGGTTCC		MCGF56F	40–55° (TD)	This study
	MCGF56NstF	TGAGAATGGTGYYTGTGTTGAG		MCGF56NstR	43–56° (TD)	This study
	MCGF56NstR	GCCRCCTTCTATTCACCACAG		MCGF56NstF	43–56° (TD)	This study
DBY14	DBY14F	GGGTAGTAAGTTATGTCCC		DBY14R	47–58° (TD)	This study
	DBY14R	GGTTACTCCTGGCTCTATGC		DBY14F	47–58° (TD)	This study
	DBY14NstF1	GTCCCAARATTAACTACTGYTGTTACT		DBY14NstR1	40–60° (TD)	This study
	DBY14NstR1	TATGCTCAGAAATCRCTYCTGGCAA		DBY14NstF1	40–60° (TD)	This study
ND2	Met-1	CTAATAAAGCTTTCGGGCCCATAC		ND2IntR1, LytND2IntR2, Trp-2	49–58° (TD)	Olson et al. 2004
	ND2IntF1	CAGGTTTAATTCTCTTCATGAC		Trp-2	49–56° (TD)	This study
	ND2IntF2	CTATCATAATTGGTGGCTGAGG		Trp-2	44–56° (TD)	This study
	LytND2IntF2	GACATCTATTATAATTGGTGGCTGAGG		Trp-2	35–55° (TD)	This study
	Trp-2	TTCTACTTAAGGCTTTGAAGGC		Met-1, ND2IntF1, ND2IntF2, LytND2IntF2	35–58° (TD)	Olson et al. 2004
	ND2IntR1	AAGTAAGTTTAGGAGGGAGAGG		Met-1	49–56° (TD)	This study
	LytND2IntR1	AGGGAGAGGTTAGGGTTATAG		Met-1	47–55° (TD)	This study
	LytND2IntR2	GACAAGGTAGAGGTAGTTGAAGTA		Met-1	47–55° (TD)	This study
CytB	L14724	CGAAGCTTGATATGAAAAACCATCGTTG		CroCBR, 597R, H15915	40–60° (TD)	Irwin et al. 1991
	29F	ATYCGAAARACYCACCCACT		CroCBF, 597R	45–60° (TD)	This study
	425F	GAGGCCAAATATCATTCTGAGG		1167R, H15915	55–60° (TD)	This study
	CroCBF	TACTTTCAGCTATCCCCTATATCGG		H15915	42–60° (TD)	This study
	SmrCytBNstF2	TCCCAGCACCCTCAAATATCTC		SmrCytBIntR1	50–60° (TD)	This study
	SmrCytBIntF1	ATCGTAGCAGCACTCGCAGGA		SmrCytBExtR	51–60° (TD)	This study
	CroCBR	AATAAGAGATGWACTCCTGCGAG		L14724, 29F	40–60° (TD)	This study
	597R	TTAGAGCCGGTTTCATGTAAG		L14724, 29F	45–60° (TD)	This study
	1167R	CTCCGGTTTACAAGACCAGTR		425F, CroCBF	45–60° (TD)	This study
	H15915	AACTGCAGTCATCTCCGGTTTACAAGAC		L14724, 425F, CroCBF	42–60° (TD)	Irwin et al. 1991
	SmrCytBIntR1	TGTCCGTGTCTGAGTTTAGTCCGGAT		SmrCytBNstF2	50–60° (TD)	This study
	SmrCytBExtR	GACCAGTGTATTARCTATACTACTAAGGC		SmrCytBIntF1	51–60° (TD)	This study

as complete taxon sampling as possible with each species represented by a single sequence. We therefore included all available GenBank sequences from east Asian *Crocidura*, but reduced the number of taxa to 50 by limiting each "species" to one sample. For most taxa, this meant a single sequence per named species. However, for several highly variable lineages, we included one representative from each island or each mountain range where populations were inferred to be monophyletic in the Bayesian analysis of the concatenated data. Thus, from the *C. beatus* and *C. grayi* complexes, we included six and five representatives, re-

spectively. We also included one representative of *C. mindorus* from each of the islands it occurs on (Mindoro and Sibuyan) and two highly divergent representatives from each of the mainland taxa, *C. fuliginosa* and *C. wuchihensis*.

THE ROLE OF INTERISLAND COLONIZATION

We test several hypotheses related to the origins of Southeast Asian shrew diversity and address the following questions: (1) Are Philippine and Sulawesian shrews each the result of a single founding colonization event? (2) Has within-island speciation

Table 2. Summary of models of evolution selected by AIC and used in model-based phylogenetic analyses.

Partition	AIC model	Model applied	Number of characters
Apolipoprotein B	HKY+G	HKY+G	577
Mast cell growth factor introns 5–6	TVM+G	GTR+G	635
Dead Box Y intron 14	K81uf+G	GTR+G	485
Cytochrome b, first codon position	SYM+I+G	GTR+I+G	380
Cytochrome b, second codon position	HKY+I	HKY+I	380
Cytochrome b, third codon position	GTR+I+G	GTR+I+G	380
NADH 2, first codon position	GTR+I+G	GTR+I+G	348
NADH 2, second codon position	TVM+I+G	GTR+I+G	348
NADH 2, third codon position	GTR+I+G	GTR+I+G	348
tRNAs Glu, Thr, Met, Trp	TrN+I+G	GTR+I+G	174

occurred in the Philippines or Sulawesi? (3) Do Palawan species (*C. batakorum* and *C. palawanensis*) show a close relationship to Bornean species and/or other taxa from the Sunda Shelf (Esselstyn et al. 2004; Everett 1889; Heaney and Ruedi 1994)? We evaluated each question using Bayesian methods and the approximately unbiased (AU) test (Shimodaira 2002). For these questions, the topological constraints consisted of monophyletic lineages including all Philippine species, all oceanic Philippine species, and all Sulawesian species (Hypothesis 1); sister relationships between *C. grayi halconus* and *C. mindorus* from Mindoro Island, between *C. palawanensis* and *C. batakorum* from Palawan Island, and among the several Sulawesian species (Hypothesis 2); *C. palawanensis* and/or *C. batakorum* sister to *C. foetida* or other Sunda Shelf taxa (*C. brunnea*, *C. fuliginosa*, *C. lepidura*, *C. malayana*, *C. maxi*, *C. orientalis*, and *C. paradoxura*; Hypothesis 3). For Hypothesis 3, we considered *C. palawanensis* and *C. batakorum* separately. In these calculations, we used the concatenated and CytB matrices separately. For the Bayesian approach, we took the percentage of 12,000 post-burnin trees consistent with each hypothesis to represent the posterior probability that the hypothesis is true. The AU test comparing the maximum-likelihood tree to the maximum-likelihood inference under 11 different constraints was implemented using CONSEL v0.1i (Shimodaira and Hasegawa 2001), with per-site likelihood scores generated by RAxMLHPC v7.0 (Stamatakis 2006).

TEMPORAL PATTERNS OF DIVERSIFICATION

We first tested the CytB alignment for the viability of a standard molecular clock. We optimized likelihood scores in PAUP* 4.0b10 with a molecular clock enforced and not enforced on the maximum-likelihood CytB topology. We then tested for significantly improved fit to the data with a likelihood ratio test ([LRT] Arbogast et al. 2002; Felsenstein 2004). As the LRT failed to reject a molecular clock, we implemented a strict clock assumption. We calculated two substitution rates derived from Figure 2 of Pesole et al. (1999) to place very approximate divergence date estimates

on the ultrametric phylogeny. The rates are one standard deviation greater than and one standard deviation less than the mean mammalian rates for CytB for synonymous and nonsynonymous substitutions (Pesole et al. 1999). We then calculated average rates weighted by the ratio of synonymous to nonsynonymous substitutions in the *Crocidura* CytB matrix. The resulting substitution rates (one fast and one slow) were then used to place time scales on the ultrametric tree. We then computed the accumulation of lineages through time (LTT) in GENIE v3.0 (Pybus and Rambaut 2002).

We used a maximum likelihood, model-fitting approach to test for variation in diversification rates (Rabosky 2006b). We chose this method over others because it is the only available technique capable of detecting increases in diversification rates through time, it has the potential to distinguish gradual from instantaneous changes in rates, and it outperforms other methods when extinction is present (Rabosky 2006b). We fit a variety of rate-constant and rate-variable versions of pure birth and birth–death models to the distribution of splitting events in the phylogeny using the R package, LASER 2.0 (Rabosky 2006a). The likelihood of each model was maximized over parameter space and model fit was measured using AIC; we compared the fit of the best rate-constant model to the fit of the best rate-variable model using the statistic, ΔAIC, as:

$$\Delta\text{AIC} = \text{AIC}_{\text{rc}} - \text{AIC}_{\text{rv}},$$

where AIC_{rc} is the AIC score of the best fitting rate-constant model and AIC_{rv} is the AIC score of the best fitting rate-variable model (Rabosky 2006b). ΔAIC is positive when a rate-variable model provides better fit than the rate-constant models and negative when a rate-constant model provides the best fit. Null distributions of ΔAIC scores were generated by fitting the same candidate models to 5000 trees simulated under the hypothesis of a constant-rate, pure-birth process. We accounted for uncertainty associated with incomplete taxon sampling by pruning randomly selected taxa from the simulated phylogenies before fitting the

birth–death models. Simulated trees held the same diversity (total number of taxa and number of missing taxa) as the empirical phylogenies. ΔAIC scores from the observed phylogeny were then compared to these null distributions to determine significance. Type I error rates can be high in model-fitting exercises when a lower AIC score is the sole criterion used to evaluate fit; generation of null distributions is therefore necessary to maintain Type I error rates near 0.05 (Rabosky 2006b).

We considered whether the results of these model-fitting analyses were consistent with the null hypothesis (CRD) or its alternatives (DDD, MPV, and PSL; Fig. 2). These hypotheses incorporate the following predictions: If shrews have diversified in a manner consistent with the null hypothesis (CRD), then either the rate-constant Yule model or the rate-constant birth–death model should provide the best fit. If Pleistocene sea level fluctuations resulted in elevated speciation rates (PSL), we expect to observe an instantaneous shift from a slow rate to a fast rate of diversification, with either the Yule-2-rate or rate-variable birth–death (RVBD) model providing the best fit. If either the MPV or DDD hypothesis is operating, we should see a decline in rates through time. MPV predicts an instantaneous shift (Yule-2-Rate or RVBD), whereas DDD predicts a gradual decline (logistic or exponential density-dependent models). In principle, MPV and DDD are distinguishable; in practice, differentiating between them will be difficult. Testing the MPV hypothesis requires the assumption that shrews arrived in Southeast Asia well before the Pliocene–Pleistocene boundary (1.8 mya). This assumption is reasonable, considering that Dubey et al. (2008) estimated the age of the earliest ingroup node in our *Crocidura* phylogeny at 6 mya and the origin of the primary clade that invaded Sundaland and the Philippines at 4.4 mya, suggesting that *Crocidura* colonized the islands of Southeast Asia at least 2 my before the beginning of the Pleistocene (1.8 mya). Because we calibrate the phylogeny to two potential time scales, either or both of which could be grossly incorrect, we allow shift times to vary in the models, and it is the relative position of fast and slow rates that will allow us to distinguish among hypotheses.

To evaluate statistical power, we simulated 1000 trees using a pure birth model with two rates of speciation, one fast and one slow (Python code provided by Mark T. Holder). These simulations were intended to mimic a shift in diversification rates at or near the Pliocene–Pleistocene boundary. We simulated data where diversification rates shifted to faster and slower rates by 1.5-, 2-, and 4-fold at three evenly spaced points in time. Rates shifted when the number of taxa in the growing tree was 0.25, 0.5, and 0.75 of the final number. Simulated trees contained the same diversity as the empirical phylogenies, with randomly selected taxa removed to accommodate uncertainty associated with incomplete taxon sampling. We fit the same candidate models to these simulated data, and used the distribution of ΔAIC scores to infer the

probability of rejecting the null hypothesis (CRD). The proportion of ΔAIC scores with higher values than the critical value in the null simulation was taken as the power to reject CRD under these scenarios. Because we were concerned that patterns of diversification might differ among individual clades within the entire dataset, all of these analyses were conducted separately for the entire phylogeny (49 species sampled and 12 missing) and a well-sampled, monophyletic group distributed across the Philippines, Sulawesi, and the Sunda Shelf (23 species sampled and six missing).

Results
PHYLOGENY ESTIMATION

The concatenated dataset consists of 4055 characters, 1143 of which are parsimony informative. Topological inferences among optimality criteria and individual loci versus concatenated datasets are largely congruent, though some differences exist. Most discrepancies are in areas of the tree that receive low support and/or have short internal branches. The partitioned Bayesian analysis of the concatenated matrix yields a largely resolved topology with most nodes receiving strong support (Fig. 4). The analysis restricted to nuclear loci was consistent with the concatenated topology, but relationships within the main Philippine clade (excluding *C. batakorum*) are unresolved (not shown). The ultrametric tree based on our likelihood analysis of CytB (Fig. 5) yields a similar topology to that from the partitioned Bayesian analysis. However, the relative positions of the three clades that make up the oceanic Philippine group are shuffled, the position of *C. palawanensis* has changed, and the clade that includes *C. foetida*, *C. nigripes*, and others is not inferred. All of these relationships received low support in the likelihood analysis restricted to CytB. In the Bayesian analysis of CytB (not shown), these relationships are inferred as in the combined analysis (Fig. 4).

Our topological inferences show three well-supported clades that include a basal group from Sulawesi and Palawan, a clade with a mixture of mainland Indochinese and Sunda Shelf taxa, and a clade that includes species from the Philippines, Sulawesi, and the Sunda Shelf (Clade Z; Fig. 4). A few species reside on long branches rooted in the basal portions of the tree.

Our analyses repeatedly recover three mostly allopatric clades that are distributed across the northern (*C. grayi* complex), central (*C. mindorus* + *C. negrina* + *C. panayensis*), and southern portions of the oceanic Philippines (*C. beatus* complex). The geographic distribution of these clades is congruent with earlier biogeographical delineations (e.g., Dickerson 1928; Heaney 1986). These clades are usually arranged with *C. beatus* and *C. grayi* sister to each other, with the central clade sister to the two, though support values for these relationships are always low and internode branches short.
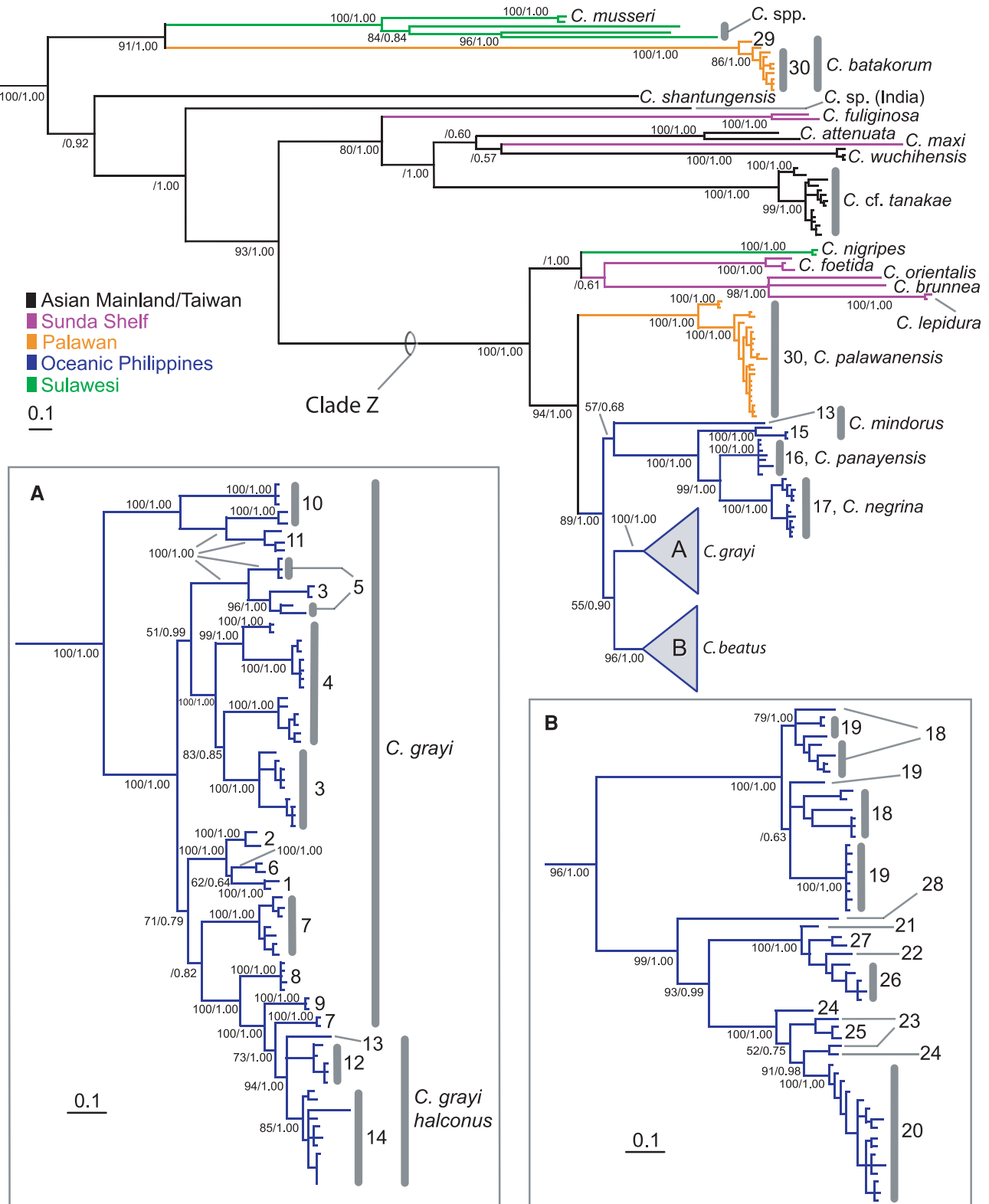
Figure 4. Bayesian estimate of phylogenetic relationships among species and populations of Southeast Asian shrews (genus *Crocidura*) as inferred from a partitioned analysis of two mitochondrial and three nuclear genes. Numbers at the nodes indicate bootstrap values from a maximum parsimony analysis, followed by Bayesian posterior probabilities. The outgroup (*Suncus murinus*) and node support values from within populations were removed for clarity of presentation. Numbers at the terminal branches refer to Philippine collection localities denoted in Figure 3.
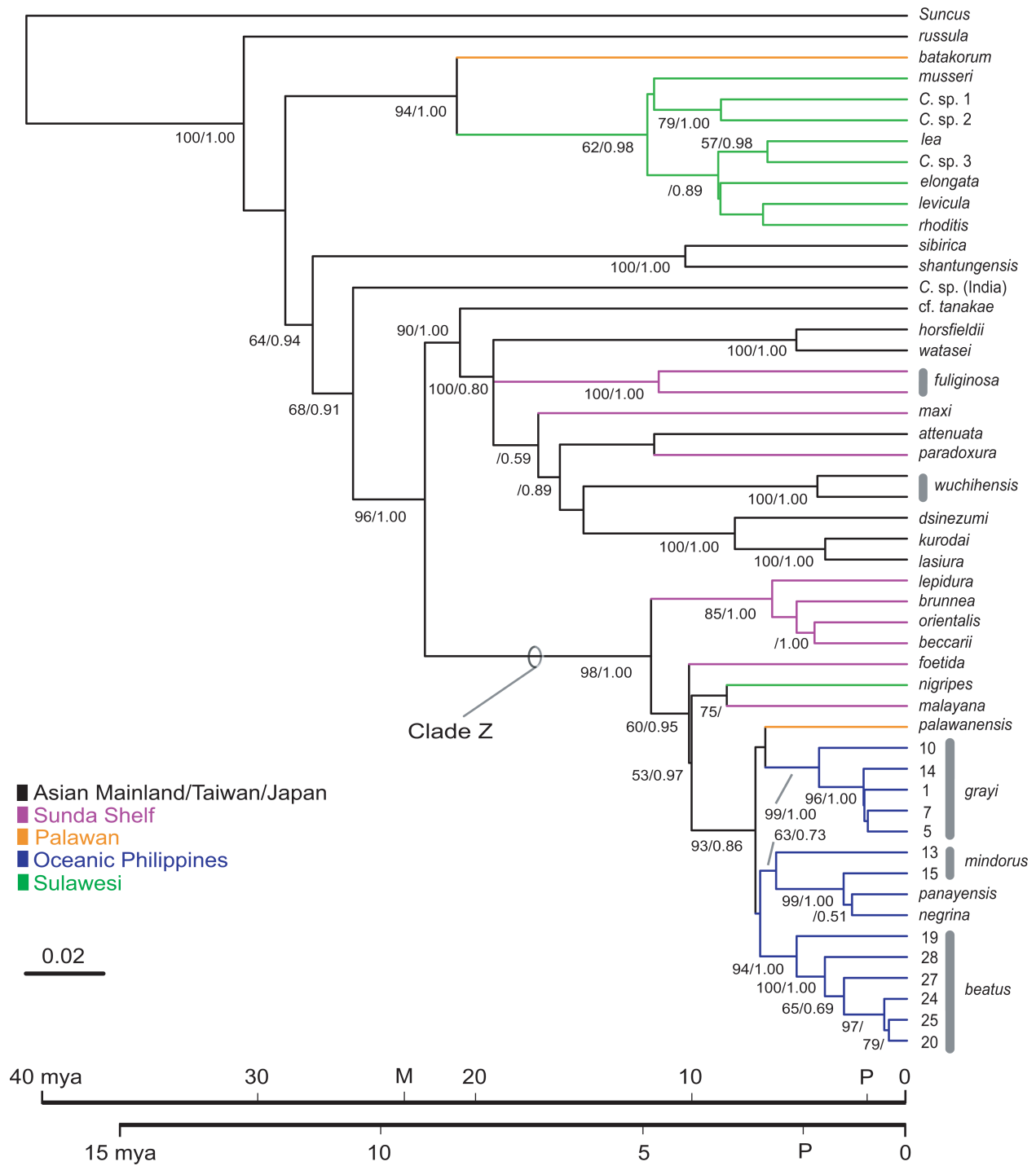
Figure 5. An ultrametric, maximum-likelihood phylogeny of Southeast Asian shrews inferred from cytochrome-b sequences and calibrated using two plausible substitution rates (see Materials and Methods). "P" and "M" on the time scales denote the beginning of the Pleistocene and Miocene, respectively. Redundant, within-population sampling has been eliminated. Numbers at the nodes represent bootstrap support (when >50%) followed by Bayesian posterior probabilities. Numbers at the terminal branches refer to Philippine collection localities denoted in Figure 3.

THE ROLE OF INTERISLAND COLONIZATION

Our evaluations of topological hypotheses provide several insights into the evolution of shrew diversity in Southeast Asia (Table 4). First, we soundly reject a single colonization event for the Philip-pines (including Palawan), but not for the oceanic portion of the archipelago (excluding Palawan).

Second, the biogeographical position of Palawan in our phy-logenetic analyses is not that of a simple extension of the Sunda

Table 3. Results of Bayesian and Approximately Unbiased (AU) evaluation of topological hypotheses. Posterior probabilities (PP) and *P*-values are presented for the complete concatenated (Concat) and cytochrome-b matrices (CytB). Evaluation of the last hypothesis (Palawan part of Sunda Shelf) involved multiple independent constraints on the relationships of *C. batakorum* and *C. palawanensis*; only the highest *P*-value among six distinct constraints is presented. *P*-values significant at $\alpha \leq 0.05$ are given in bold text.

Hypothesis	Constraint	PP Concat/CytB	AU Concat/CytB
Single colonization of Philippines	Monophyletic Philippine clade	0/0	**<0.001/<0.001**
Single colonization of oceanic Philippines	Monophyletic oceanic Philippine clade	1/0.71	0.971/0.500
Single colonization of Sulawesi	Monophyletic Sulawesian clade	0/0	**<0.001/<0.001**
Within-island speciation on Mindoro	*halconus & mindorus* sister taxa	0/0	**<0.001/0.001**
Within-island speciation on Palawan	*batakorum & palawanensis* sister species	0/0	**<0.001/0.037**
Palawan part of Sunda Shelf	*batakorum* or *palawanensis* sister to any species from the Sunda Shelf	0/0	**0.037**/0.077

Shelf. The clade that includes *C. batakorum* and *C. musseri* is shared between Palawan and Sulawesi, although this relationship could be altered with the addition of currently unavailable Sunda Shelf taxa. The other Palawan species (*C. palawanensis*) is part of a clade that includes all species from the oceanic Philippines, although it is sister to these. This relationship is well supported in the analyses of the concatenated matrix (Fig. 4) and by the Bayesian CytB analysis (not shown), but not recovered in the likelihood analysis of CytB (Fig. 5). *P*-values associated with the various *C. palawanensis*–Sunda Shelf sister relationship constraints are marginal (Table 3). However, with one relatively old Palawan species (*C. batakorum*) grouping with Sulawesi and one relatively young species (*C. palawanensis*) grouping with the oceanic Philippines, the characterization of the island group as an extension of Borneo is an over-simplification, a conclusion also reached by Brown and Diesmos (in press).

Neither of the syntopic Philippine species pairs (Palawan Island: *batakorum* and *palawanensis*; Mindoro Island: *grayi halconus* and *mindorus*) shows a sister relationship in any of our analyses and these hypotheses are rejected by our statistical tests (Table 3). It therefore appears that all speciation among currently named Philippine taxa has resulted from over-water colonization followed by divergence in allopatry. However, we note that some species, especially *C. beatus*, are genetically variable and represent several independently evolving lineages. It is evident (Fig. 4B) that extensive within-Pleistocene-island diversification has occurred, but current taxonomy does not reflect this variation.

In contrast to the allopatric distribution of Philippine shrew diversity, Sulawesi supports an exceptionally high level of sympatric diversity; Ruedi (1995) reported capturing five species in a small area near the center of the island. Our study is consistent with the conclusion of Ruedi et al. (1998) that shrews colonized Sulawesi at least twice. Two distantly related lineages occur on the island. One is represented by a single species, *C. nigripes*. The other clade consists of a monophyletic assemblage of eight species, three of which are undescribed (Fig. 5). This is a remarkable level of shrew diversity, especially considering that the nine species were sampled from only two of seven areas of endemism identified by Evans et al. (2003b). Given this result, within-island speciation, and perhaps sympatric speciation, may have played a prominent role in the diversification of Sulawesian shrews.

TEMPORAL PATTERNS OF DIVERSIFICATION

Log-likelihood scores with the molecular clock enforced and not enforced were $-13,953$ and $-13,827$, respectively. The LRT gave a nonsignificant result (χ^2_{252}, $P = 0.49$) and we proceeded to use a standard molecular clock (Fig. 5). The two substitution rates (0.00562 and 0.01385/site/my) used to estimate divergence dates provide a wide range of possible ages, but both indicate that our assumptions regarding the arrival of shrews in Southeast Asia are probably valid. The LTT plots of the entire dataset and Clade Z are each suggestive of either a constant rate of diversification or a subtle decline in rates through time (Fig. 6). For both LTTs, rate-variable models received lower AIC scores (i.e., better fit; Table 4) than the best rate-constant model (pure birth). However, ΔAIC scores were not significant in either case (All taxa, ΔAIC = 3.1, $P = 0.14$; Clade Z, ΔAIC = 3.8, $P = 0.07$). Power analyses indicate that we would have a moderate probability of rejecting CRD if rates declined twofold and a high probability of rejecting the null under a fourfold decline in rates (Fig. 7). Statistical power for detecting temporal increases in diversification rates was weaker, but a visual inspection of the LTTs indicates that temporal increases (PSL) are unlikely to represent a viable explanation of the data. We interpret these results as evidence that there is not a strong signal of diversification under the MPV or DDD hypotheses.
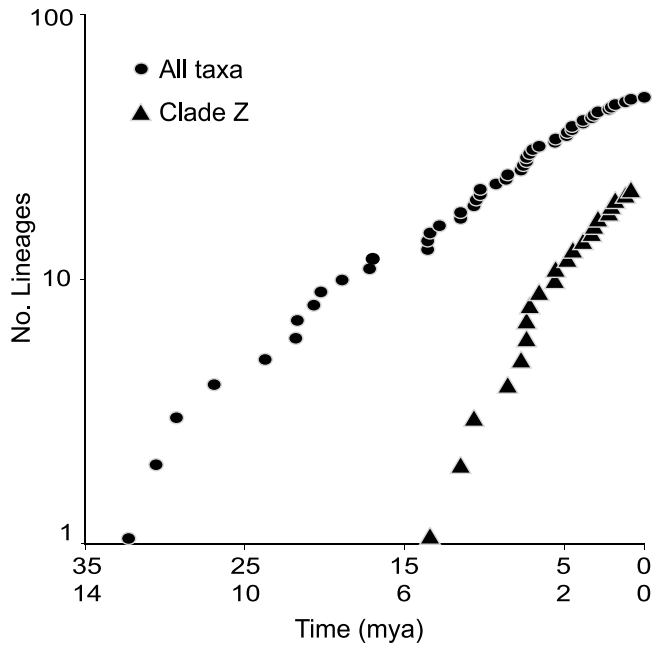
Figure 6. Lineage-through-time plots of Southeast Asian shrews derived from the phylogeny in Figure 5. Noted are the diversification rates for the entire phylogeny (circles) and Clade Z (triangles). The time scales on the *x*-axis are generated from two plausible substitution rates (see Materials and Methods).

Discussion

THE ROLE OF INTERISLAND COLONIZATION

Our topological inferences reveal a consistent pattern indicative of multiple invasions of most biogeographic regions. The Sunda Shelf holds multiple independent lineages of shrews. Our analyses using multiple loci and greater taxon sampling further support Ruedi et al.'s (1998) hypothesis that shrews colonized Sulawesi at least twice. The oceanic Philippines (i.e., excluding Palawan) apparently has been invaded only once, although extensive movements by shrews across water barriers within the Philippines

are necessary to explain current distributions and phylogenetic relationships.

The Palawan group of islands, which has generally been considered a peripheral portion of the Sunda Shelf, shows some surprising biogeographic affinities. With respect to the phylogenetic relationships among shrews, Palawan clearly has ties to both Sulawesi and the oceanic Philippines, but not a close relationship to the Sunda Shelf. This is contra to the hypothesis of Heaney and Ruedi (1994) that *C. palawanensis* is a close relative of *C. fuliginosa* and not part of the oceanic Philippine radiation. The Palawan group is probably most appropriately viewed as having a complex of faunal affinities, with various lineages having close phylogenetic relationships to forms on Borneo, the oceanic Philippines, and Sulawesi (Brown and Diesmos in press). The island chain may have played an important role as a colonization route into the oceanic Philippines for shrews and other taxa (Brown and Guttman 2002; Diamond and Gilpin 1983; Jones and Kennedy 2008).

All evidence from the Philippines indicates that currently recognized species are the result of over-water colonization events and subsequent divergence in allopatry. However, if current taxonomy reflected phylogenetic diversity, then *C. beatus* and perhaps *C. grayi*, would be split into multiple taxonomic entities (species) distributed allopatrically across the Mindanao and Luzon faunal regions. We further note that sympatry among Philippine shrews is achieved only among older lineages and all sympatric species differ substantially in body size and perhaps ecologically (elevational segregation and tolerance of habitat disturbance), suggestive of the idea of a "sympatry threshold" (e.g., Marshall et al. 2008).

In contrast, eight species from Sulawesi form a well-supported clade, indicating that within-island speciation, and perhaps sympatric speciation, may have played a significant role in the diversification process. However, modern Sulawesi is an aggregate of several once distinct islands (Hall 2002). A

Table 4. Rate-constant and rate-variable models of diversification fit to the ultrametric phylogeny of shrews (Fig.5). Model names as in LASER 2.0 (Rabosky 2006a). AIC scores are given for each of the empirical LTTs. AIC scores from the rate-constant and rate-variable models providing the best fit are noted with bold text, as are values for ∆AIC and *P*-values.

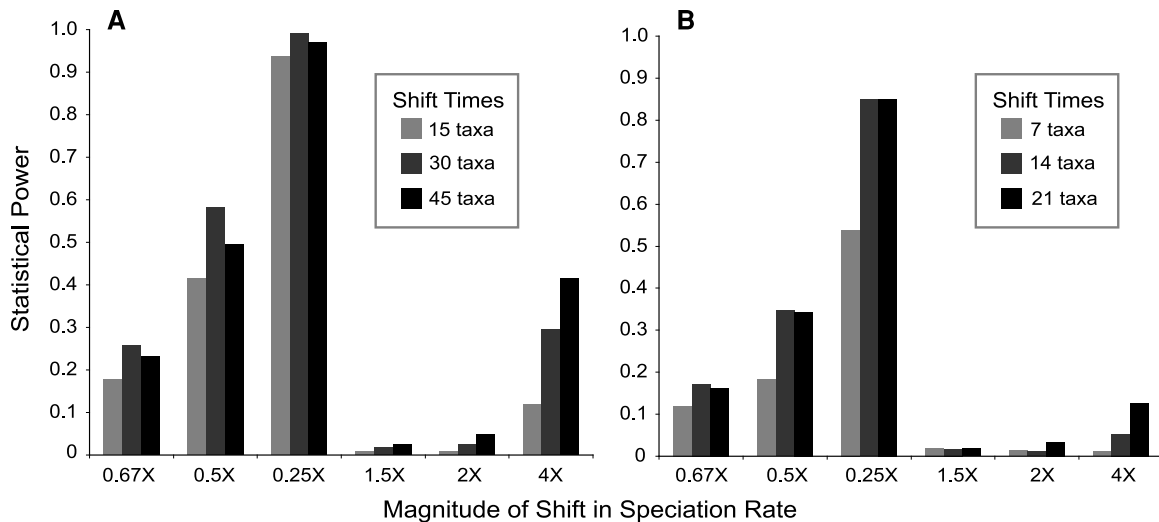| Model name	Rate category	Free parameters	Model type	AIC all taxa	AIC clade Z
Pure birth	Constant	1	Yule	**−446.4**	**−196.4**
bd	Constant	2	Birth-death	−444.4	−194.4
Yule-2-rate	Variable	3	Yule	**−449.5**	−198.6
RVBD	Variable	4	Birth-death	−447.5	−196.6
DDL	Variable	2	Density-dependent logistic	−448.0	**−200.2**
DDX	Variable	2	Density-dependent exponential	−446.7	−198.0
			∆AIC	**3.1**	**3.8**
			P-value	**0.14**	**0.07**

Figure 7. Probability of rejecting the null hypothesis of a constant rate of diversification (CRD) when birth rates decline and increase 1.5-, 2-, and 4-fold at three points in time in an expanding phylogeny. Statistical power is shown in simulated phylogenies with 49 taxa sampled from a clade of 61 species (A) and 23 taxa sampled from a clade of 29 species (B). Speciation rates shifted when the growing trees had 15, 30, and 45 terminals (A) and 7, 14, and 21 terminals (B).

phylogenetic estimate calibrated with multiple sources of data (e.g., fossils, group-specific substitution rates, and geological events) might provide important information on the timing of the arrival of shrews relative to the timing of the coalescence of the once independent islands and to their rifting from earlier landmasses.

TEMPORAL PATTERNS OF DIVERSIFICATION

Our birth–death analyses suggest that the net diversification rate has been relatively constant through time. Although models with declining rates provided the best fit, we are unable to reject the null, constant rate hypothesis. In contrast, most studies of diversification rates identify statistically significant temporal declines (Kozak et al. 2006; McPeek 2008; Phillimore and Price 2008; Price 2008).

The distribution of terrestrial habitats in Southeast Asia has been extremely dynamic through geological history (Heaney 1985, 1986; Defant et al. 1990; Hall 1998; Heaney 1991; Voris 2000; Bird et al. 2005) and two periods of time (Miocene–Pliocene and Pleistocene) are characterized by extensive changes in the distribution of land. The earlier period was a time of intensive volcanic uplift and numerous new islands were formed (Defant et al. 1990; Hall 1998; Ozawa et al. 2004). Afterward, during the Pleistocene, sea levels fluctuated extensively, repeatedly connecting and isolating many islands (Haq et al. 1987; Rohling et al. 1998; Voris 2000). Either process could have led to increased rates of diversification through the generation of new terrestrial habitats or intermittent connection of previously inaccessible lands. Our model-fitting analyses reject the notion that one of these processes had a strong effect on diversification rates. It is unlikely that biased

or incomplete taxon sampling drives our conclusions because our separate tests of the entire phylogeny and Clade Z lead to the same interpretation. Incomplete, random taxon sampling makes the inference of declining rates of diversification more likely, whereas biased taxon sampling can affect results in a variety of ways (Nee 2001). We doubt that a sampling bias has caused our failure to reject the null hypothesis because we sampled 81% (25 of 31) of the species known from the area occupied by Clade Z (Sunda Shelf, Sulawesi, and Philippines). Some species from the Sunda Shelf do not belong to Clade Z, thus we suspect that some of the six missing species also are not members of Clade Z. Therefore, the total number of known species missing from this clade is likely fewer than six. For this small number of species to affect our results, there would need to be a very strong bias in their ages (e.g., all old lineages). Nevertheless, it remains a possibility that either there are many yet undiscovered species of *Crocidura* in Southeast Asia or that this clade has experienced a decline in speciation rates through time, but a high rate of extinction has eroded the signal (Rabosky and Lovette 2008).

We note that the LTTs (Fig. 6) suggest the net rate of diversification has been faster in Clade Z than in the entire phylogeny. Clade Z is entirely insular and this may reflect a difference in the rate of diversification between the islands and the continent. However, our limited sampling from the mainland prevents an explicit test of this hypothesis. Nevertheless, our inference of a relatively constant diversification rate through time in analyses of both the entire phylogeny and Clade Z, in the presence of apparent rate variation across geography, is intriguing.

If shrews have indeed diversified at a constant rate, two potential explanations are conceivable. First, the extreme heterogeneity

(spatial and temporal) that characterizes large archipelagos may provide new opportunities for allopatric speciation over long periods of time. Second, an apparent constant rate of diversification could result from this group of shrews being an immature evolutionary radiation that has not existed long enough for the net diversification rate to plateau, as would be expected under a density-dependent model. These two hypotheses are not mutually exclusive, and the dynamic nature of large, old archipelagos may simply prolong the period of early, rapid speciation commonly noted in continental clades. Kozak et al. (2006) suggested that niche conservatism plays a role in promoting the diversification of lineages, especially where extensive opportunities for diversification in allopatry exist. Species of *Crocidura* have undergone limited morphological and ecological diversification in most of Southeast Asia. We note that the region has an unusually high diversity of shrew-like rodents (especially on Luzon; e.g., *Rhynchomys*, *Archboldomys*) and this may constrain ecological diversification in shrews.

TAXONOMIC HYPOTHESES AND MACROEVOLUTIONARY INFERENCES

Macroevolutionary studies implicitly rely on a foundation of taxonomic hypotheses, which contain their own biases and limitations. Taxonomic decisions usually are based on exclusivity criteria, such as complete fixation of morphological differences and monophyly of gene trees (de Queiroz 1998). Fixation of characters and gene tree monophyly generally take long periods of time to form after cessation of gene flow (Knowles and Carstens 2007), indicating that we probably are unable to recognize the most recently formed species. Studies of the temporal pattern of diversification would therefore be expected to show a decline in diversification rates near the present because of their reliance on a taxonomy incapable of recognizing young species.

In this study, we use information from taxonomy, supplemented with information on genetic diversity, and find that a model with a constant rate of diversification provides good fit to the data. In contrast, most such studies find a strong pattern of temporally declining rates of diversification (McPeek 2008; Phillimore and Price 2008). Clearly, more clades would show constant rates, lesser declines, or even increasing rates of diversification through time if phylogeographic diversity were commonly considered in concert with taxonomic information. It should be recognized that the limitations of taxonomic hypotheses (i.e., our inability to recognize young species), combined with the nature of stochastic birth–death processes (i.e., lineages that experience rapid, early diversification tend to be extant, diverse, and thus subject to phylogenetic estimation) may provide a viable explanation when temporally declining rates of diversification are inferred.

Conclusions

Southeast Asian shrews have diversified primarily through a process of repeated colonization of oceanic islands followed by divergence in allopatry, although the possibility remains that shrews speciated in sympatry on Sulawesi. The Sunda Shelf, Philippines (including Palawan), and Sulawesi all appear to have been colonized multiple times. Within the Philippines, shrews have colonized all major islands and substantial, within-island diversification has occurred on the large islands of Mindanao and Luzon (Fig. 4A,B). Closely related, unnamed lineages that inhabit these islands remain allopatric, but more distant relatives (species recognized by taxonomy) are sympatric or syntopic. In contrast, Sulawesi shrews may have diversified on a single paleoisland and of the nine species reported here, five are known to occur in sympatry (Ruedi 1995; Ruedi et al. 1998). Overall, diversification in this group has occurred in a manner consistent with a constant-rate, pure-birth process and with models that incorporate subtle declines in rates of diversification through time. There is not strong evidence that volcanic uplift during the Miocene and Pliocene (MPV hypothesis) resulted in an elevated diversification rate; the idea that Pleistocene sea level fluctuations resulted in an increased diversification rate (PSL hypothesis) is probably not viable for this group. The observation of a relatively constant rate of diversification is uncommon among studies that have explored the subject (McPeek 2008; Price 2008) and may reveal something unique about either the archipelago or the lineage under consideration. We suggest that (1) the dynamic history of Southeast Asia has generated a continuous supply of new opportunities for allopatric speciation, that (2) this group represents an immature radiation that has yet to fill geographical and ecological space, and/or (3) constant rates of diversification are in fact common, but rarely documented due to biases in taxonomic hypotheses and the nature of stochastic birth–death processes. Comparisons with other widespread Southeast Asian lineages should provide insights into which explanation(s) best accounts for the spectacular biodiversity of modern Southeast Asian archipelagos.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Samples used in this study.

Supporting Information may be found in the online version of this article.
(This link will take you to the article abstract).