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Research Article

Diversity and endemism of Murinae rodents in Thai limestone karsts

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This study aims to investigate the species diversity of rodents living in karst ecosystems of Thailand. A survey has been conducted throughout Thailand, 122 karsts sampled and 477 Murinae rodents live-trapped. Phylogenetic reconstructions were carried out using two mitochondrial markers (cytb, COI). A sequence-based species delimitation method completed by the analysis of the level of genetic divergence was then applied to define species boundaries within our dataset. The phylogenetic position of *Niviventer hinpoon* was also investigated and sequences obtained from the holotype specimen of this species were used to reliably identify samples of *N. hinpoon*. A total of 12 described Murinae species, corresponding to 17 deeply divergent genetic lineages, were encountered in limestone karsts of Thailand. Our study revealed an important genetic diversity within the traditionally recognized species *Maxomys surifer* (four highly divergent genetic lineages), *Leopoldamys neilli* (two highly divergent genetic lineages) and *Berylmys bowersi* (two highly divergent genetic lineages). These species could be considered as species complex and require further taxonomic work. This study also provides valuable information on the distribution of the two rodent species endemic to limestone karsts of Thailand, *L. neilli* and *N. hinpoon*. *Leopoldamys neilli* was the most abundant species encountered in Thai karsts during our survey. However, *L. neilli* specimens from western Thailand are genetically highly divergent from the remaining populations of *L. neilli* and could represent a separate species. *Niviventer hinpoon*, phylogenetically closely related to *N. fulvescens*, is much rarer and its distribution limited to central Thailand. Most of the other captured species are typically associated with forest ecosystems. This study suggests that limestone karsts play a key role in the preservation of the rodent species endemic to such habitat, but they would also provide refuges for the forest-dwelling Murinae rodents in deforested regions.

Key words: GMYC lineages, limestone karsts, Murinae rodents, Rattini, Southeast Asia, species delimitation, Thailand

Introduction

Southeast Asia represents only 3% of the total land area of the world but this region includes four major biodiversity hotspots that host around 18% of the total plant and animal species of the earth (Myers *et al.*, 2000; ASEAN CFB, 2010). This region is a centre of diversity and endemism of Murinae rodents, a sub-family of the Muridae family. This highly speciose group includes 126 genera and 561 species largely distributed in the Old World (Musser & Carleton, 2005). Lecompte *et al.* (2008) subdivided the Murinae in several tribes, among which the Rattini tribe includes 37 genera and 170 rat species, most of them distributed in Southeast Asia. This exceptionally high diversification of Murinae in Southeast Asia is the result of several spectacular radiations during the last 10 million years (Chaimanee & Jaeger, 2001).

Southeast Asian limestone karsts were the site of several major rodent discoveries in the last few years, leading to the description of the mysterious *Laonastes aenigmamus* in Lao PDR (Jenkins *et al.*, 2005), the sole remaining representative of the Diatomyidae family that occurred in Asia from 33.9 to 11.6 million years ago (Mya) (Huchon *et al.*, 2007), and two new Murinae genera, *Saxatilomys*

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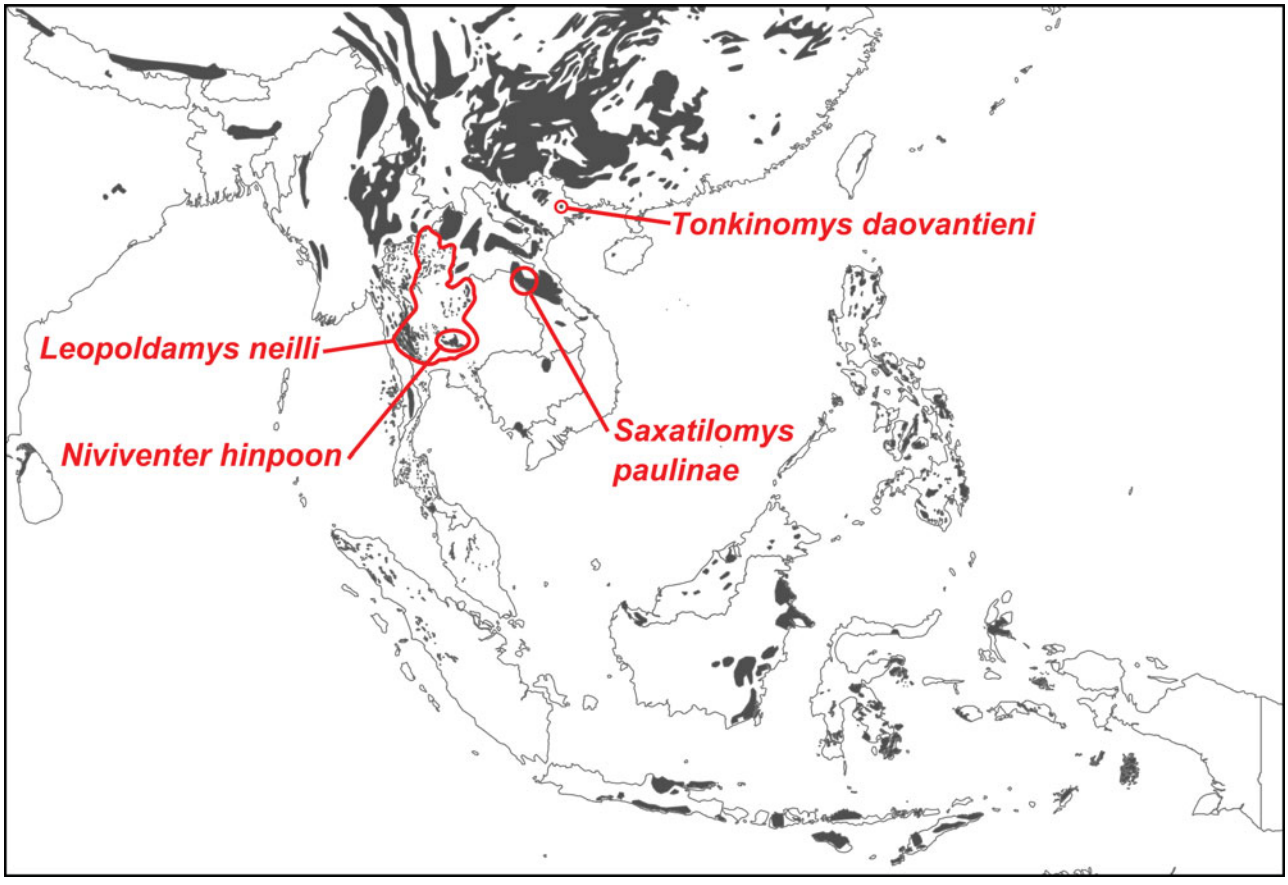


Fig. 1. Limestone karsts in Southeast Asia (dark grey) and distribution of Southeast Asian Murinae rodents endemic to limestone karsts (red): *Leopoldamys neilli* (distribution according to Latinne *et al.*, 2011, 2012), *Niviventer hinpoon* (Lekagul & McNeely 1988), *Saxatilomys paulinae* (Musser *et al.*, 2005), *Tonkinomys daovantieni* (Musser *et al*., 2006).

and *Tonkinomys* (Musser *et al.*, 2005, 2006). Limestone karsts are sedimentary rock outcrops consisting of calcium carbonate characterized by underground drainage systems and distinctive landforms resulting from solutional erosion and made of cone-shaped or sheer-sided hills riddled with caves and sinkholes (Gillieson, 2005; Simms, 2005). Some of the largest limestone karst regions of the world are located in Southeast Asia where they cover an area of 460 000 km^2 (Day & Urich, 2000) (Fig. 1). In Thailand, limestone karsts are widespread throughout most parts of the country, except for large areas on the northeastern Khorat Plateau. Karst biodiversity is characterized by an important species richness and high levels of endemic species of plants, vertebrates and invertebrates adapted to this particular environment (Vermeulen & Whitten, 1999). These high species diversity and endemism in karsts probably arise from the diverse and extreme environmental conditions of these ecosystems such as alkaline soils, rough topography, frequent drought, thin soil layers and darkness in caves (Vermeulen & Whitten, 1999; Clements *et al.*, 2006). Limestone karsts have also a significant economic value as limestone is an essential raw material used in major industries and karsts are currently over-exploited through quarrying of cement, lime and hard core (Vermeulen & Whitten, 1999). As a result of these activities, karst ecosystems are highly threatened and some endemic species became dramatically endangered. This situation is particularly alarming as karst biodiversity suffers from a considerable lack of scientific data and remains widely unknown throughout Southeast Asia despite its high biological importance (Clements *et al.*, 2006). Consequently, many undiscovered species could already be extinct or may become extinct within the next few years. In order to define conservation guidelines and achieve long-term preservation of karst ecosystems in Southeast Asia, it is therefore urgently necessary to increase our knowledge of this biodiversity.

Four species of Murinae rodents belonging to the Rattini tribe and endemic to limestone karsts are currently recognized in Southeast Asia, two in Thailand, *Leopoldamys neilli* (Marshall, 1976) and *Niviventer hinpoon* (Marshall, 1976), one in Lao PDR, *Saxatilomys paulinae* Musser, Smith, Robinson & Lunde, 2005, and one in Vietnam,

Tonkinomys daovantieni Musser, Lunde & Son, 2006 (Fig. 1). However little is currently known about these rare rodents. *Leopoldamys neilli* and *N. hinpoon* were discovered in 1973 in Saraburi and Lopburi provinces (central Thailand) (Lekagul & McNeely, 1988) and very little information about their distribution and ecological requirements were collected during more than 30 years. Due to this lack of data, the two species are currently listed as Data Deficient on the IUCN Red List (Lunde & Aplin, 2008*a*, 2008*b*). A few years ago, a field survey of Waengsothorn *et al.* (2007) in central Thailand confirmed the presence of these two species in sparse locations of this region. More recently, two studies revealed a complex phylogeographical structure for *L. neilli* in Thailand and a wider distribution range for this species than indicated by previous records (Latinne *et al.*, 2011, 2012) (Fig. 1).

However, the Murinae rodent diversity of Thai limestone karsts remains poorly understood and the aims of the present study were to investigate exhaustively the species composition of the rodent fauna in karst ecosystems of Thailand using DNA-based methods. As Thailand occupies a key biogeographical position at the intersection of the Indochinese and Sundaic biogeographical subregions (Hughes *et al.*, 2003; Woodruff & Turner, 2009), we could expect to observe a faunal transition of rodent diversity from northern to southern surveyed locations.

Most Rattini genera display several external and skull diagnostic characters and can be easily identified using morphological criteria. At the intrageneric level, however, species identification may be blurred by limited interspecific external morphological divergence and/or by large intraspecific morphological variations. This phenomenon combined with a lack of accurate morphological study for some species may lead to a confused taxonomy and an overabundance of species description and synonyms or, conversely, undiscovered cryptic species (two or more distinct species classified as a single species because they are at least superficially morphologically indistinguishable (Hebert *et al.*, 2004; Bickford *et al.*, 2007)). The genus *Rattus* and in particular the *Rattus rattus* complex (Aplin *et al.*, 2011) represents one of the best examples of this minimal external morphological divergence among Rattini species that evolved rapidly and recently and that are differentiated only by subtle morphological variations (Rowe *et al.*, 2011). DNA-based approaches have recently proved to be useful tools to clarify the complex taxonomy of Rattini and especially of the *Rattus* genus (Robins *et al.*, 2007; Pages *et al.*, 2010; Aplin *et al.*, 2011; Bastos *et al.*, 2011) and helped to elucidate the specific status of several *Rattus* cryptic species such as *Rattus losea* and *Rattus sakeratensis* (formerly treated as a synonym of *R. losea*) (Aplin *et al.*, 2011) or *Rattus rattus* and *Rattus satarae* (formerly treated as a subspecies of *R. rattus*) (Pages *et al.*, 2011). Discovery and description of cryptic species is particularly important for conservation and natural resources management as design of conservation strategies mainly relies on estimates of species richness and endemism (Bickford *et al.*, 2007). Moreover each species within cryptic complexes will require adapted conservation plans and their recognition is therefore essential.

In this study, we studied a large number of Murinae samples from Thai limestone karsts using DNA-based methods. Phylogenetic reconstructions were carried out using two mitochondrial markers. A sequence-based species delimitation method completed by the analysis of the level of genetic divergence was then applied to define species boundaries within our dataset. Finally, in order to name the delimited species, we used DNA sequences identified in previous studies and DNA sequences obtained from the holotype specimen of *N. hinpoon*.

Materials and methods

Sample collection

In order to study the Murinae rodent biodiversity of Thai limestone karsts, a survey was conducted and 122 karst localities were sampled in 25 provinces including all karst regions of Thailand (Appendix 1 and 2, see supplemental material, which is available on the Supplementary tab of the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2013.818587) using locally made live-capture traps (single-capture cage-traps made of wire mesh). Trapping sessions were organized so as to maximize the number and diversity of captures. At each site, traps baited with ripe banana were set in three habitat types: karst slopes, cave entrance and cave interior (provided that a cave was accessible on the site) for at least three consecutive nights. The number of traps set on karst slopes and within caves was distributed as equally as possible. All traps were checked every day in the early morning. During this survey, a total of 477 Murinae rodents were live-trapped. Captive rodents were handled using a cloth bag as described in Aplin *et al.* (2003). Field identifications of captured rodents were made based on geographical and morphological criteria according to Lekagul & McNeely (1988), Corbet & Hill (1992), Aplin *et al.* (2003) and Francis (2008) (these criteria are described in Appendix 3, see supplemental material online). Each rodent was also weighed, measured and its age/sex category determined. Finally, a small piece of ear tissue was collected and stored in 96% ethanol before the animal was released.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from skin samples using the DNeasy Tissue Kit (Qiagen Inc.) following the manufacturer's protocol. Two mitochondrial markers, the cytochrome b gene (cytb) and the cytochrome c oxidase sub-

unit I gene (COI), were amplified for all the samples. Universal primers L14723/H15915 (Irwin *et al.*, 1991) were used to amplify the cytb gene. However, due to amplification difficulties with some samples, several genus- and species-specific primers were designed to amplify a 900 base pairs (bp) fragment of the cytb gene: *Maxomys* (MaxoFw: 5′-TCCTTCATTGACCTACCYAC-3′/MaxoRv: 5′-ATGA TTGAGAAGTAGCTGATG-3’), *Niviventer* (NiviFw: 5′-TCCGAAAAACTCACCCCTTAC-3’/ NiviRv: 5′-GAAG TATGGAGGAATGGTAGG-3′), *Rattus* (RatFw: 5′-CATT CGRAAATCACACCC-3′/RatRv: 5′-GTTCTACTGGYT GKCCYCC-3′) and *Leopoldamys neilli* (LneilliFw: 5′-TCCATCCAACATCTCATCATG-3′/LneilliRv: 5′-GGAG GCTAGTTGGCCAATG-3′). Primers BatL5310 (5′-CCTA CTCRGCCATTTTACCTATG-3′) and R6036R (5′-ACT TCTGGGTGTCCAAAGAATCA-3′) were used to amplify COI (713 bp) (Robins *et al.*, 2007).

PCRs were carried out in 50 μL volume containing 12.5 μL of each 2 μM primers, 1 μL of 10 mM dNTP, 10 μL of 5× reaction buffer (Promega), 0.2 μL of 5 U/μL Promega Taq DNA Polymerase and approximately 30 ng of DNA extract. Amplifications were performed in a thermal cycler VWR Unocycler using one activation step (94°C for 4 min) followed by 40 cycles (94°C for 30 s, 48°C (COI) or 50°C (cytb) for 60 s, and finally 72°C for 90 s) with a final extension step at 72°C for 10 min. Sequencing reactions were performed on an ABI 3730 automatic sequencer.

Phylogenetic analyses

Sequences were aligned in BIOEDIT 7.0.9.0 (Hall, 1999) using ClustalW algorithm. Haplotypes were identified using ARLEQUIN 3.11 (Excoffier *et al.*, 2005). Phylogenetic reconstructions were performed on the combined dataset using the maximum likelihood (ML) and Bayesian inference (BI) approaches. Two sequences of the Eurasian harvest mouse *Micromys* belonging to the Rattini tribe (Lecompte *et al.*, 2008) and available on GenBank were used as outgroups of our phylogenetic analyses. The most suitable model of DNA substitution for each locus and dataset was determined using MODELTEST 3.0 (Posada & Crandall, 1998) according to the Akaike Information Criterion (AIC). PhyML 3.0 (Guindon *et al.*, 2010) was used to perform ML analyses. The transition/transversion ratio, the proportion of invariable sites and the gamma distribution parameter were estimated. The starting tree was determined by BioNJ analysis of the datasets. Robustness of the tree was assessed by 1000 bootstrap replicates. Bayesian analyses were performed with MRBAYES 3.1.1. (Ronquist & Huelsenbeck, 2003). Metropolis-coupled Markov chain Monte Carlo (MCMC) sampling was performed with 5 chains run for 6.10^6 generations with one tree sampled every 1000 generations, using default parameters as starting values. All trees obtained before the Markov chain reached stationary distribution (empirically determined by checking of likelihood values) were discarded as burn-in values. A 50% majority-rule consensus tree was then generated in PAUP 4.0b10 (Swofford, 1998).

Species delimitation and identification

To estimate putative species boundaries, we used the DNA-based approach developed by Pons *et al.* (2006). This method detects the transition between micro- and macroevolutionary patterns using an ultrametric tree. The null model assumes a single coalescent process for the entire tree. The alternative model, a General Mixed Yule-Coalescent (GMYC) model, combines equations that separately describe branching within populations (coalescent process) and branching between species (a Yule model that includes speciation and extinction rates). The point of highest likelihood of this mixed model estimates the switch from speciation to coalescent branching and can be interpreted as the species boundary. A more recent version of the GMYC model (multiple-threshold model) allows for multiple lineages to each have their own transition threshold within a single phylogenetic tree (Monaghan *et al.*, 2009). GMYC analyses were performed using the SPLITS package for R (http://r-forge.r-project.org/projects/splits/). An ultrametric tree was obtained using BEAST 1.6.2 (Drummond & Rambaut, 2007) using a GTR+G model with an uncorrelated lognormal relaxed molecular clock model (Drummond *et al.*, 2006) and a coalescent tree prior. Two runs of 25.10^6 generations, sampling every 10000th generation, were performed. Convergence of the chains to the stationary distribution was checked using TRACER 1.5 (Rambaut & Drummond, 2007). All Beast computations were performed on the freely available computational resource Bioportal at the University of Oslo (http://www.bioportal.uio.no).

To allow accurate species designation and confirm our field identification based on morphological and geographical criteria, sequences of voucher specimens available from a recent phylogenetic study of the Rattini tribe in the Indochinese region (Pages *et al.*, 2010) were added to our dataset. Cytb and COI sequences of a single representative of each of the 24 species delimited in this study were retrieved from GenBank. Therefore the final dataset includes 229 haplotypes from karst samples and 24 sequences from Pages *et al.* (2010).

According to the genetic species concept, the level of cytb net genetic distance between clusters could also be used to delimit species boundaries (Baker & Bradley, 2006). Matrices of pairwise net K2P distances for the cytb gene between GMYC lineages within the genera *Maxomys*, *Niviventer*, *Leopoldamys*, *Berylmys*, *Sundamys* and *Rattus* were therefore calculated in MEGA 4 (Tamura *et al.*, 2007).

Divergence time estimates

Divergence times among Rattini and our GMYC clusters were estimated using Bayesian inference, as implemented in BEAST 1.6.2 on the cytb dataset. Three fossil-based

calibration points were used: (i) the split between the tribe Phloemyini and the other tribes of Murinae at 12.3 million years (Myr) (Jacobs & Flynn, 2005) (ii) the divergence between *Apodemus mystacinus* and all the species of the subgenus *sylvaemus* (*A. flavicollis* and *A. sylvaticus*) at 7 Myr (Aguilar & Michaux, 1996; Michaux *et al.*, 1997); and (iii) the divergence of *Otomys*/*Parotomys* (Otomyinae) from the *Arvicanthis* clade at 5.7 Myr (Winkler, 2002). To constrain the age of these nodes in the tree, sequences of *Deomys ferrugineus* (GenBank accession number EU349745), *Gerbillus nigeriae* (AJ430555), *Batomys granti* (AY324458), *Phloeomys cumingi* (DQ191484), *Apodemus mystacinus* (AF159394), *Apodemus sylvaticus* (AB033695), *Apodemus flavicollis* (AB032853), *Arvicanthis somalicus* (EU349737), *Arvicanthis niloticus* (AF004569), *Oenomys hypoxanthus* (EU349769), *Grammomys macmillani* (AM408345), *Otomys angoniensis* (AM408343), *Parotomys* sp. (EU349773) were added to our dataset. Two analyses with different priors and dataset were performed independently following the strategy of Aplin *et al.* (2011). First, to deal with the combination of inter- and intra-specific diversity in the complete dataset, we used a coalescent tree prior, a relaxed molecular clock (uncorrelated lognormal) and a GTR+G substitution model (previously estimated by ModelTest). Secondly, we used a dataset restricted to one haplotype of each GMYC clusters combined to a Yule process speciation model as tree prior, a relaxed molecular clock (uncorrelated lognormal) and a GTR+G substitution model (previously estimated by ModelTest). Both analyses were run with MCMC chain length of 8×10^7, sampling every 10^4 generations.

Focus on *Niviventer* and *Maxomys* genera

To confirm our field identification of *Niviventer hinpoon* and assign unequivocally a species name to this cluster, we obtained DNA sequences from a piece of skin of the holotype specimen of *N. hinpoon*. The type specimen, an adult male, is located at the Centre for Thai National Reference Collections in Bangkok (reference number: CTNRC 54-3988) and was collected by W.A. Neill at Wat Tham Phrapothisat, Kaengkhoi District, Saraburi Province in 1973 (Waengsothorn *et al.*, 2007, 2009). As museum samples contained only small quantities of degraded DNA, we amplified a 85 bp fragment of the cytb gene that proved to be suitable for amplification of ancient DNA and to allow to discriminate among most vertebrate species (Teletchea *et al.*, 2008) and more specifically Rattini species (Pages *et al.*, 2010). Primers from Teletchea *et al.* (2008) were specifically modified for *N. hinpoon* (LNhinp: 5′-GACAAAATTCCATTTCACCC-3′/ HNhinp: 5′-TAGTTATCTGGGTCTCC-3′). DNA was extracted using the QIAamp DNA Micro Kit (Qiagen) following the protocol for isolation of genomic DNA from tissues. Amplifications were carried out in 25 μL volume containing 6.25 μL of each 2 μM primers, 0.625 μL of 10 mM dNTP, 5 μL of 5× reaction buffer (Promega), 2 μL of 25 mM $MgCl_2$, 2.5 μL of 10 mg/mL BSA, 0.5 μL of 5 U/μL GoTaq Hot Start Polymerase (Promega) and 2 μL of DNA extract. PCRs were performed using one activation step (95°C for 5 min) followed by 50 cycles (94°C for 30 s, 48°C for 30 s, and finally 72°C for 45 s). The precautions and criteria pertinent for ancient DNA studies (Gilbert *et al.*, 2005) were observed during all laboratory procedures: (i) All pre-PCR work was conducted in a physically remote area in ancient DNA laboratory where Rattini samples were never processed before this study; (ii) negative extraction and PCR controls were included; (iii) several independent PCRs were performed; and (iv) nucleotide substitutions were assessed for consistency with the molecular evolutionary pattern of ancient DNA sequences. Six PCR products from three independent PCRs were sequenced and resulted in the same nucleotide sequence. Extraction and PCRs controls remained negative. For all these reasons, we are fully confident that the sequence obtained is derived from the skin sample of the holotype specimen of *N. hinpoon*.

To confirm that the fragment obtained allowed us to discriminate among *Niviventer* species, cytb sequences of *Niviventer* available in this study and on GenBank were aligned, sorted by species and reduced to the 85 bp fragment. As *Niviventer* species misidentifications are numerous on GenBank (Pages *et al.*, 2010), sequences that seem obviously to be misidentified were removed from the dataset. The 85 bp cytb fragment obtained for *N. hinpoon* was then compared with other *Niviventer* species to identify diagnostically informative attributes. We used the definitions of Sarkar *et al.* (2008) to characterize these characteristic attributes (CAs): pure attributes exist across all members of a single clade, and never in any other clade while private attributes are present across some of the members of a clade, but never in any other clade.

To compare the level of genetic diversity within the two karst endemic species, *N. hinpoon* and *L. neilli*, we calculated the haplotype diversity (h) and the nucleotide diversity (Pi) (globally as well as at synonymous and non-synonymous sites) for both species using DnaSP 5.10 (Librado & Rozas, 2009).

In order to examine the phylogenetic position of *N. hinpoon* within the *Niviventer* genus, cytb and COI sequences of several *Niviventer* species available on GenBank were added to our dataset. As cytb and COI sequences deriving from the same specimen were not available, the two genes were analysed separately and two analyses performed. ML and BI analyses were performed as indicated above. Sequences of *Leopoldamys* were used as outgroups.

Finally, some doubts remain on species name assignment to clusters within the *Maxomys* genus because some *Maxomys* species reported in Thailand and in the Indochinese region (i.e. *M. whiteheadi*, *M. rajah* and *M. moi*) were

absent from the dataset of Pages *et al.* (2010). We therefore added cytb and COI sequences of *Maxomys* species available on GenBank to our own *Maxomys* sequences. ML and BI analyses were performed as indicated above on cytb and COI datasets separately. Sequences of *Leopoldamys* were used as outgroups.

Results

Phylogenetic analyses

Cytb and COI sequences were obtained, respectively, for 467 and 470 Murinae rodent samples, all of them belonging to the Rattini tribe. The final alignments included 199 cytb haplotypes, 161 COI haplotypes and 229 haplotypes for the combined dataset. All haplotype sequences have been deposited in GenBank database (GenBank Accession Numbers are listed in Appendix 4, see supplemental material online).

The ML and BI trees of the combined dataset gave congruent results at terminal nodes but present an inconsistency at a deeper node (Fig. 2). Three of the six Rattini divisions suggested by Musser & Carleton (2005) are represented in our sampling and correspond to three monophyletic groups in the Bayesian tree. The *Maxomys* division (Bootstrap support (BS) = 100% – Bayesian posterior probabilities (BP) = 1.0) is the first lineage to diverge, then the poorly supported *Dacnomys* division (BS = node not recovered – BP = 0.55) consisting of *Leopoldamys* and *Niviventer* and finally the *Rattus* division (BS = 81% – BP = 0.99) comprising *Berylmys*, *Sundamys* and *Rattus*. However, *Leopoldamys* and *Niviventer* do not form a monophyletic group in the ML tree. All terminal nodes are very well-supported in both ML and BI trees (Fig. 2).

Following our field identifications, we are able to suggest species names to the lineages belonging to *Maxomys* (*M. surifer*), *Niviventer* (*N. hinpoon*, *N. fulvescens*), *Leopoldamys* (*L. sabanus*, *L. edwardsi*, *L. neilli*), *Berylmys* (*B. berdmorei*, *B. bowersi*) and *Sundamys* (*S. muelleri*) (Fig. 2). At least four well-differentiated lineages are also observed within the *Rattus* genus but the field identifications of our *Rattus* specimens are confused and conflicting as most of these samples were identified as *Rattus tanezumi* or *Rattus* sp. in the field and therefore do not allow us to assign a species names to these lineages at this point of the analyses.

Our phylogenetic tree also indicates that some species (e.g. *Maxomys surifer* and *Leopoldamys neilli*) comprise several highly differentiated and well-supported phylogenetic lineages that could possibly belong to several distinct species.

Species delimitation and identification

In order to investigate the specific status of some lineages and estimate putative species boundaries, branch length pattern of the ultrametric tree including karsts samples and a single representative of each of the 24 Rattini species delimited in Pages *et al.* (2010) was analysed.

The likelihood of both single- and multiple-threshold GMYC model was significantly higher than that of the null model of uniform (coalescent) branching rates (LR = 56.82, $P < 0.0001$ and LR = 64.80, $P < 0.0001$, respectively). However, the multiple-threshold model did not fit the data better than the single-threshold model ($\chi^2 = 7.98$, d.f. = 6, $P = 0.24$), indicating the age of the switch from speciation to coalescent branching did not vary significantly among lineages.

The single-threshold GMYC model estimates 40 putative species within our dataset excluding outgroups (with a confidence interval from 36 to 48) composed of 27 distinct clusters and 13 singletons (Fig. 3). Karsts samples are distributed within 28 GMYC lineages belonging to six genera that have been labelled from Rat1 to Max6 (Fig. 3). Of the six putative species of *Rattus*, three could be named without ambiguity using sequences from Pages *et al.* (2010): Rat1 corresponds to *R. tanezumi* (*Rattus rattus* Lineage II *sensu* Aplin *et al.*, 2011), Rat5 to *R. tiomanicus* and Rat6 to *R. andamanensis*. The lineage Rat4 coincides with the lineages 'R3' in Pages *et al.* (2010) and '*Rattus rattus* Lineage IV' in Aplin *et al.* (2011) to which a species name could not be assigned. Lineages Rat2 and Rat3 could not be named either but are closely associated with the lineage Rat4. The percentage of match between field and molecular identifications of our *Rattus* specimens is very low for *R. tiomanicus* and *R. andamanensis* (Table 1).

The clustering of remaining Pages' sequences with some of our samples in the phylogenetic tree confirm our species designation for *M. surifer*, *N. fulvescens*, *L. sabanus*, *L. edwardsi*, *L. neilli*, *B. berdmorei* and *B. bowersi* (*N. hinpoon* and *S. muelleri* were not sampled in Pages *et al.* (2010)). The percentage of match between field and molecular identifications is very high (> 90%) for all these lineages, except for *L. edwardsi* (68%) and *N. fulvescens* (57%) (Table 1). However, *L. neilli*, *M. surifer*, *B. bowersi* and *S. muelleri* seem to consist of several putative species according to the method of Pons *et al.* (2006) as they correspond to eight, six, two and two GMYC lineages, respectively.

The amount of genetic differentiation (cytb net K2P distance) among GMYC lineages within the genera *Maxomys*, *Niviventer*, *Leopoldamys*, *Berylmys*, *Sundamys* and *Rattus* varied greatly and ranged from 0.3 to 16%. Pairwise distances among GMYC lineages have been categorized in five main groups (0–2.5%, 2.6–5%, 5.1–7.5%, 7.6–9.9%, > 10%) for more clarity (Table 2).

Divergence time estimates

Divergence time estimates among Rattini and our GMYC clusters obtained using the complete dataset and a coalescent tree prior are given in Fig. 4. Very similar divergence time estimates were obtained with the reduced dataset and

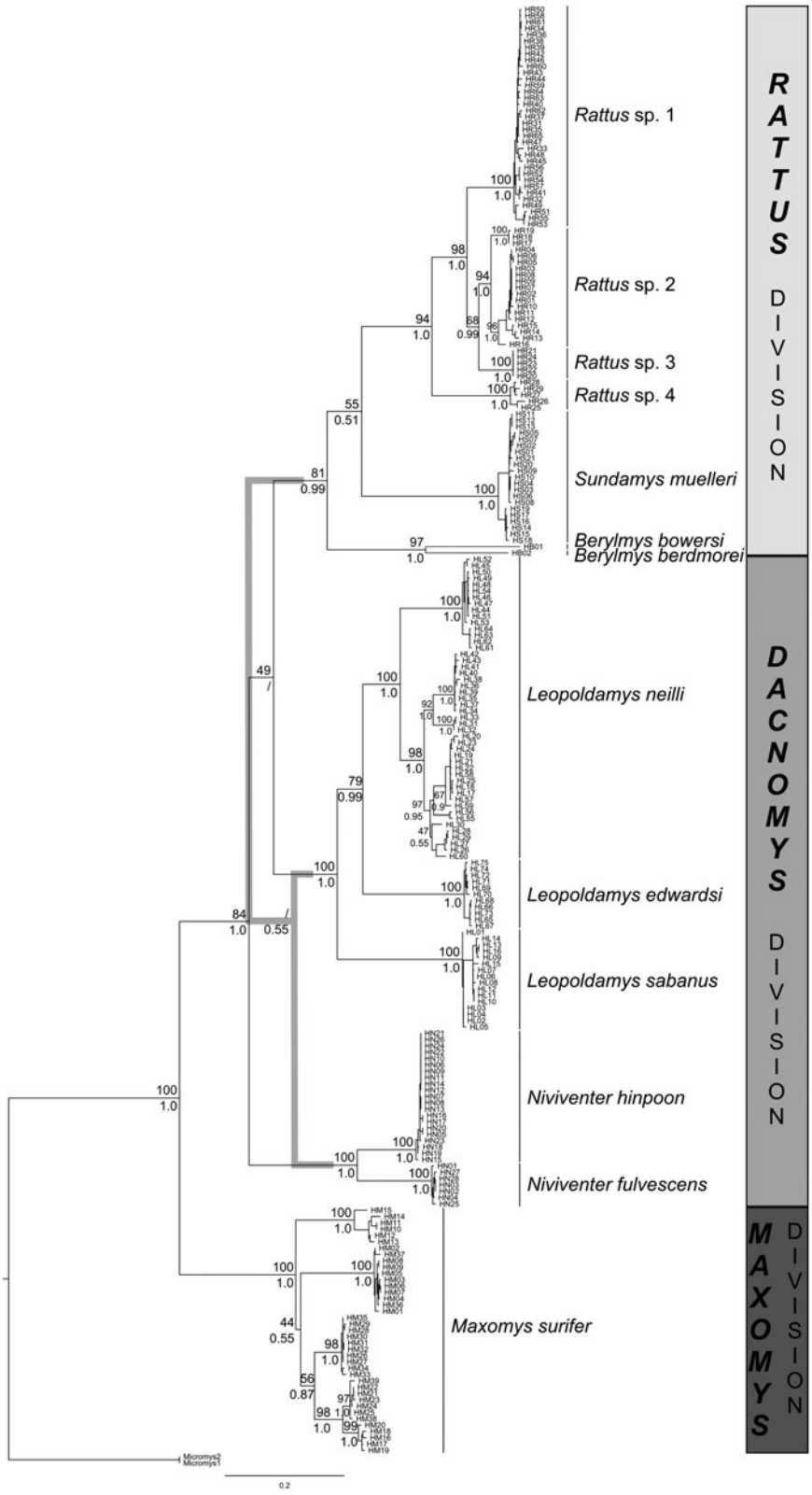


Fig. 2. Maximum likelihood (ML) tree (GTR + G) of the two combined mitochondrial genes (cytb, COI) of rodents collected in limestone karsts. Tree topologies of ML and Bayesian trees are different and the shaded lines indicate the internodes relationships recovered by Bayesian analyses. Bootstrap support (%, 1000 replicates) and posterior probabilities of nodes are indicated above and below the branches, respectively. Node supports from within lineages were removed for clarity of presentation.

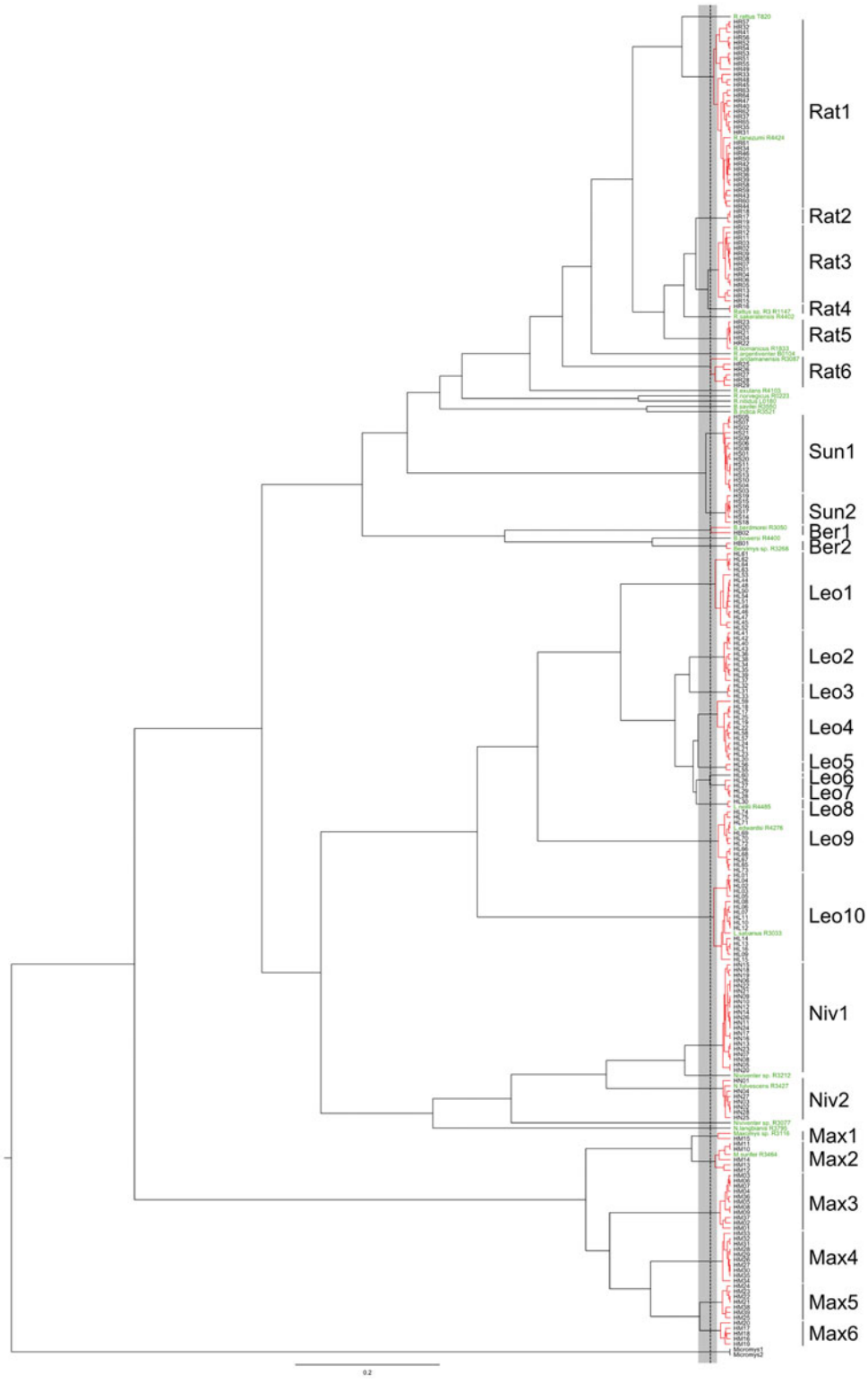


Fig. 3. Ultrametric tree obtained with BEAST including samples collected in limestone karsts (HXXX in black) and sequences from Pages *et al.* (2010) (green). The dashed vertical line indicates the maximum likelihood point of switch from speciation to coalescent branching estimated by a single-threshold GMYC model. The shaded zone indicated the confidence interval for this estimate (within two log-likelihood units of the maximum). Clusters corresponding to putative species are indicated in red. The 28 clusters including karst samples have been labelled from Rat1 to Max6.

Table 1. Distribution ranges and species names suggested for GMYC lineages encountered in Thai limestone karsts as well as percentages of match between field and molecular species identification.

GMYC lineage	Species name	Distribution range in this study (provinces) (see Figs 8–13)	N (% of total captures)	Percentage of match between field and molecular species identification
Rat1	***Rattus tanezumi*** Temminck, 1844 = *Rattus rattus* Lineage II *sensu* Aplin *et al.*, 2011	Chaiyaphum Chumphon Kanchanaburi Krabi Lampang Nakhon Ratchasima Nakhon Sawan Nakhon Si Thammarat Nan Petchabun Petchaburi Phang Nga Prachuap Khiri Khan Saraburi Surat Thani	59 (12.4%)	91% (54/59)
Rat2	***Rattus* sp.** = lineages 'R3' *sensu* Pages *et al*. (2010) and '*Rattus rattus* Lineage IV' *sensu* Aplin *et al.* (2011)	Ratchaburi	3 (0.6%)	
Rat3	***Rattus* sp.** = lineages 'R3' *sensu* Pages *et al*. (2010) and '*Rattus rattus* Lineage IV' *sensu* Aplin *et al.* (2011)	Chaiyaphum Kanchanaburi Loei Lopburi Nakhon Ratchasima Petchaburi Ratchaburi Saraburi	16 (3.6%)	90% (18/20) (samples identified as *R. tanezumi* were considered as correctly identified as *Rattus rattus* Lineages II and IV have similar external morphology according to Aplin *et al.* (2011) and could represent a single species (Lack *et al.*, 2012; Pages *et al.*, 2012)
Rat4	***Rattus* sp.** = lineages 'R3' *sensu* Pages *et al*. (2010) and '*Rattus rattus* Lineage IV' *sensu* Aplin *et al.*, 2011)	Nakhon Ratchasima	1 (0.2%)	
Rat5	***Rattus tiomanicus*** (Miller, 1900)	Krabi	6 (1.2%)	33% (2/6)
Rat6	***Rattus andamanensis*** (Blyth, 1860)	Chiang Rai Lampang Nan	5 (1.0%)	40% (2/5)
Sun1	***Sundamys muelleri*** (Jentink, 1879)	Chumphon Krabi Nakhon Si Thammarat	21 (4.4%)	100% (36/36)
Sun2	***Sundamys muelleri*** (Jentink, 1879)	Krabi Nakhon Si Thammarat Phang Nga	15 (3.1%)	
Ber1	***Berylmys berdmorei*** (Blyth, 1851)	Phrae	1 (0.2%)	100% (1/1)
Ber2	***Berylmys bowersi*** (Anderson, 1879) complex = lineage 'Be2b' in Pages *et al.* (2010)	Nakhon Si Thammarat	1 (0.2%)	100% (1/1)
Leo1	***Leopoldamys neilli*** (Marshall, 1976) complex sp. 1	Kanchanaburi Uthai Thani	70 (14.7%)	100% (70/70)
Leo2	***Leopoldamys neilli*** (Marshall, 1976) complex sp. 2	Lopburi Saraburi	22 (4.6%)	93% (144/155)
Leo3	***Leopoldamys neilli*** (Marshall, 1976) complex sp. 2	Nakhon Ratchasima Saraburi	29 (6.1%)	
Leo4	***Leopoldamys neilli*** (Marshall, 1976) complex sp. 2	Khon Kaen Loei Petchabun	68 (14.2%)	

(Continued on next page)

Table 1. (*Continued*)

GMYC lineage	Species name	Distribution range in this study (provinces) (see Figs 8–13)	N (% of total captures)	Percentage of match between field and molecular species identification
Leo5	***Leopoldamys neilli*** (Marshall, 1976) complex sp. 2	Chaiyaphum	7 (1.5%)	
Leo6	***Leopoldamys neilli*** (Marshall, 1976) complex sp. 2	Chiang Rai	2 (0.4%)	
Leo7	***Leopoldamys neilli*** (Marshall, 1976) complex sp. 2	Nan	16 (3.6%)	
Leo8	***Leopoldamys neilli*** (Marshall, 1976) complex sp. 2	Phrae	11 (2.3%)	
Leo9	***Leopoldamys edwardsi*** (Thomas, 1882)	Lampang Loei Nan Petchabun Phrae	22 (4.6%)	68% (15/22)
Leo10	***Leopoldamys sabanus*** (Thomas, 1887)	Chumphon Krabi Nakhon Si Thammarat Prachuap Khiri Khan Surat Thani	23 (4.8%)	100% (23/23)
Niv1	***Niviventer hinpoon*** (Marshall, 1976)	Lopburi Nakhon Sawan Saraburi	28 (5.9%)	100% (28/28)
Niv2	***Niviventer fulvescens*** (Gray, 1847)	Loei Chaiyaphum	7 (1.5%)	57% (4/7)
Max1	***Maxomys surifer*** (Miller, 1900) complex sp. 1	Phrae	1 (0.2%)	100% (8/8)
Max2	***Maxomys surifer*** (Miller, 1900) complex sp. 1	Lampang Nan Phrae	7 (1.5%)	
Max3	***Maxomys surifer*** (Miller, 1900) complex sp. 2	Chaiyaphum Loei Saraburi Petchabun	12 (2.5%)	100% (12/12)
Max4	***Maxomys surifer*** (Miller, 1900) complex sp. 3	Nakhon Si Thammarat Surat Thani	10 (2.1%)	90% (9/10)
Max5	***Maxomys surifer*** (Miller, 1900) complex sp. 4	Chumphon	9 (1.9%)	100% (14/14)
Max6	***Maxomys surifer*** (Miller, 1900) complex sp. 4	Krabi Nakhon Si Thammarat Surat Thani	5 (1.0%)	

a Yule process speciation model as tree prior (data not shown). Most Rattini species included in our dataset diverged during Middle/Late Pliocene with the exception of the species within the *Rattus rattus* complex that diverged more recently, during Early/Middle Pleistocene.

Niviventer genus

The selected 85 bp fragment of the cytb gene was well amplified from the holotype specimen of *Niviventer hinpoon*. An unambiguous consensus sequence was obtained from several PCR products (GenBank Accession no. KC010174) and compared with the sequences of our own *N. hinpoon* samples collected in limestone karsts. The holotype sequence corresponds exactly to the most abundant of three distinct variants of this fragment (H1) observed among our *N. hinpoon* dataset (Fig. 5). At least four CAs were identified between *N. hinpoon* and the other *Niviventer* species distributed in Thailand (*N. confucianus*, *N. cremoriventer*, *N. fulvescens*, *N. langbianis*, *N. tenaster*) (Fig. 5) as well as the remaining *Niviventer* species for which cytb sequences were available on GenBank (data not shown). This confirms that the selected 85 bp cytb fragment is highly suitable to discriminate among *Niviventer* species without ambiguity. Therefore the lineage Niv1 could be named unequivocally as *Niviventer hinpoon*.

Table 2. Pairwise cytb net K2P distance between GMYC lineages recovered by the single-threshold GMYC model. For clarity, species names of GMYC lineages with unambiguous specific status and that could be named using field identification and voucher specimens from Pages *et al.* (2010) were used.

cytb net K2P distance	Genus	Pairwise comparisons
0–2.5%	***Leopoldamys***	- All pairwise comparisons of Leo4, Leo5, Leo6, Leo7, Leo8
	Maxomys	- Max1 vs. Max2
		- Max5 vs. Max6
	Sundamys	- Sun1 vs. Sun2
	Rattus	- All pairwise comparisons of Rat2, Rat3, Rat4
	Niviventer	/
	Berylmys	/
2.6–5%	***Leopoldamys***	- Leo2 vs. Leo3, Leo4, Leo5, Leo6, Leo7, Leo8
		- Leo3 vs. Leo4, Leo5, Leo6, Leo7, Leo8
	Maxomys	/
	Sundamys	/
	Rattus	- ***R. tiomanicus*** vs. Rat2, Rat3, Rat4, ***R. rattus***, ***R. sakeratensis***
		- ***R. sakeratensis*** vs. Rat2, Rat3, Rat4
		- ***R. tanezumi*** vs. Rat3, Rat4, ***R. rattus***
		- ***R. rattus*** vs. Rat2, Rat3, Rat4
	Niviventer	- ***N. hinpoon*** vs. N2 *sensu* Pages (Pages *et al.* 2010)
	Berylmys	/
5.1–7.5%	***Leopoldamys***	/
	Maxomys	- Max4 vs. Max5, Max6
	Sundamys	/
	Rattus	- ***R. tanezumi*** vs. Rat2, ***R. tiomanicus***, ***R. sakeratensis***
		- ***R. rattus*** vs. ***R. sakeratensis***
		- ***R. norvegicus*** vs. ***R. nitidus***
	Niviventer	/
	Berylmys	- ***B. bowersi*** vs Ber2
7.6–9.9%	***Leopoldamys***	- Leo1 vs. Leo 2, Leo3, Leo4, Leo5, Leo6, Leo7, Leo8
		- ***L. edwardsi*** vs. Leo2, Leo4, Leo5, Leo6, Leo7, Leo8, ***L. sabanus***
	Maxomys	- Max1 vs. Max3, Max4, Max5, Max6
		- Max2 vs. Max3, Max4, Max5, Max6
		- Max3 vs. Max4, Max5, Max6
	Sundamys	/
	Rattus	- ***R. andamanensis*** vs. ***R. tanezumi***, Rat2, Rat3, Rat4, ***R. tiomanicus***, ***R. rattus, R. sakeratensis***, ***R. argentiventer***, ***R. exulans***
		- ***R. argentiventer*** vs. ***R. tanezumi***, Rat2, Rat3, Rat4, ***R. tiomanicus***, ***R. rattus, R. sakeratensis***, ***R. exulans***
	Niviventer	- ***N. fulvescens*** vs. ***N. hinpoon***, N2 *sensu* Pages (Pages *et al.,* 2010)
	Berylmys	/
>10%	***Leopoldamys***	- ***L. edwardsi*** vs. Leo1, Leo3
		- ***L. sabanus*** vs. Leo1, Leo 2, Leo3, Leo4, Leo5, Leo6, Leo7, Leo8
	Maxomys	/
	Sundamys	/
	Rattus	- ***R. exulans*** vs. ***R. tanezumi***, Rat2, Rat3, Rat4, ***R. tiomanicus***, ***R. rattus, R. sakeratensis***, ***R. norvegicus***, ***R. nitidus***
		- ***R. norvegicus*** vs. ***R. tanezumi***, Rat2, Rat3, Rat4, ***R. tiomanicus***, ***R. andamanensis***, ***R. rattus, R. sakeratensis***, ***R. argentiventer***
		- ***R. nitidus*** vs. ***R. tanezumi***, Rat2, Rat3, Rat4, ***R. tiomanicus***, ***R. andamanensis***, ***R. rattus, R. sakeratensis***, ***R. argentiventer***
	Niviventer	- ***N. langbianis*** vs. ***N. fulvescens***, ***N. hinpoon***, N2 *sensu* Pages, N3 *sensu* Pages
		- N3 *sensu* Pages vs. ***N. fulvescens***, ***N. hinpoon***, N2 *sensu* Pages
	Berylmys	- ***B. berdmorei*** vs. ***B. bowersi***, Ber2

The level of genetic diversity within *N. hinpoon* is much lower than within *L. neilli* (globally or limited to central populations), especially the nucleotide diversity which is 10 times lower within *N. hinpoon* than within *L. neilli* central lineages (Table 3).

Phylogenetic analyses indicated that the *N. hinpoon* lineage clustered with *N. fulvescens* lineages and formed a well-supported group in both COI (BS = 100% – BP = 1.0) (Fig. 6) and cytb (BS = 98% – BP = 0.99) (data not shown) trees. However, the two main COI

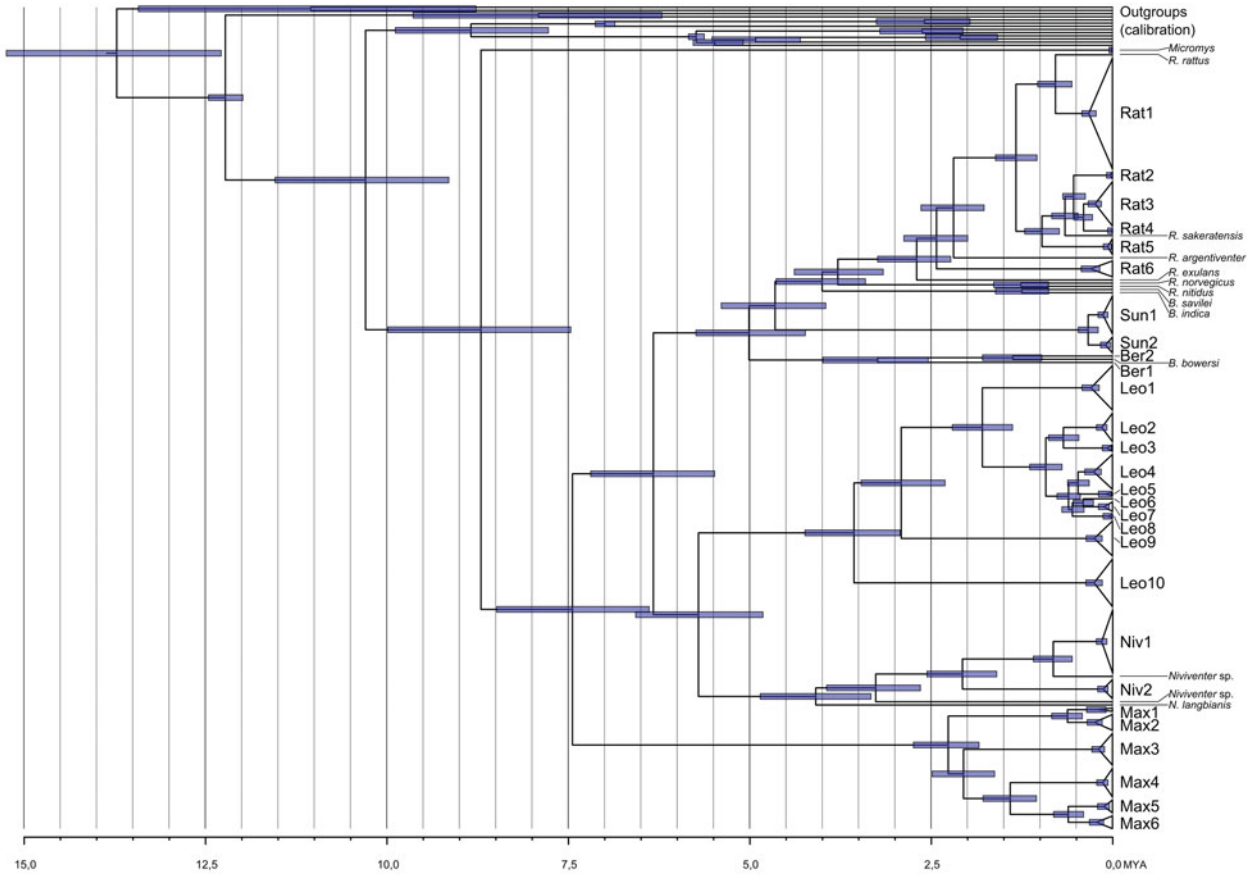


Fig. 4. Divergence time estimates and their 95% HPD (blue node bars) among our GMYC clusters computed with BEAST using the complete dataset and a coalescent tree prior.

lineages of *N. fulvescens* (Thailand + Vietnam + Guangxi (China)/Hainan + Guizhou (China)) are paraphyletic within this group. Paraphyletic lineages of *N. fulvescens* are also observed in the cytb tree. The group *N. hinpoon*/*N. fulvescens* is the sister clade to *N. cremoriventer* lineage in both trees and this relationship is very well supported.

Maxomys genus

Both COI (Fig. 7) and cytb (data not shown) phylogenetic trees indicated unambiguously that all our *Maxomys* samples belong to the well-supported *M. surifer* complex (COI: BS = 99% – BP = 1.0, cytb: BS = 100% – BP = 1.0). None of our six *Maxomys* GMYC lineages could be assigned to any other *Maxomys* species. Four well-supported groups that correspond to distinct regions of Southeast Asia are observed within the *M. surifer* complex in the COI tree: northern + western Thailand (lineages Max1 and Max2), Thai-Malay Peninsula (southern Thailand (lineages Max4, Max5 and Max6) and Malay Peninsula), eastern part of the Indochinese region (central and northeastern Thailand (lineage Max3), Laos, Vietnam and Cambodia) and finally Indonesia (Kalimantan Timur).

Discussion

Species delimitation and specific status of GMYC lineages

The accurate delimitation of species is of primary importance in conservation biology as this discipline mainly uses species as fundamental entities to define conservation guidelines. However this task is quite complicated and there is no firm consensus in the literature on how to define species boundaries (De Queiroz, 2005, 2007; Hausdorf, 2011). A lot of species concept and species delimitation methods have been suggested and could lead to very different estimates of species number, which have critical consequences for conservation (Agapow *et al.*, 2004). Methods for species delimitation using genetic data recommend

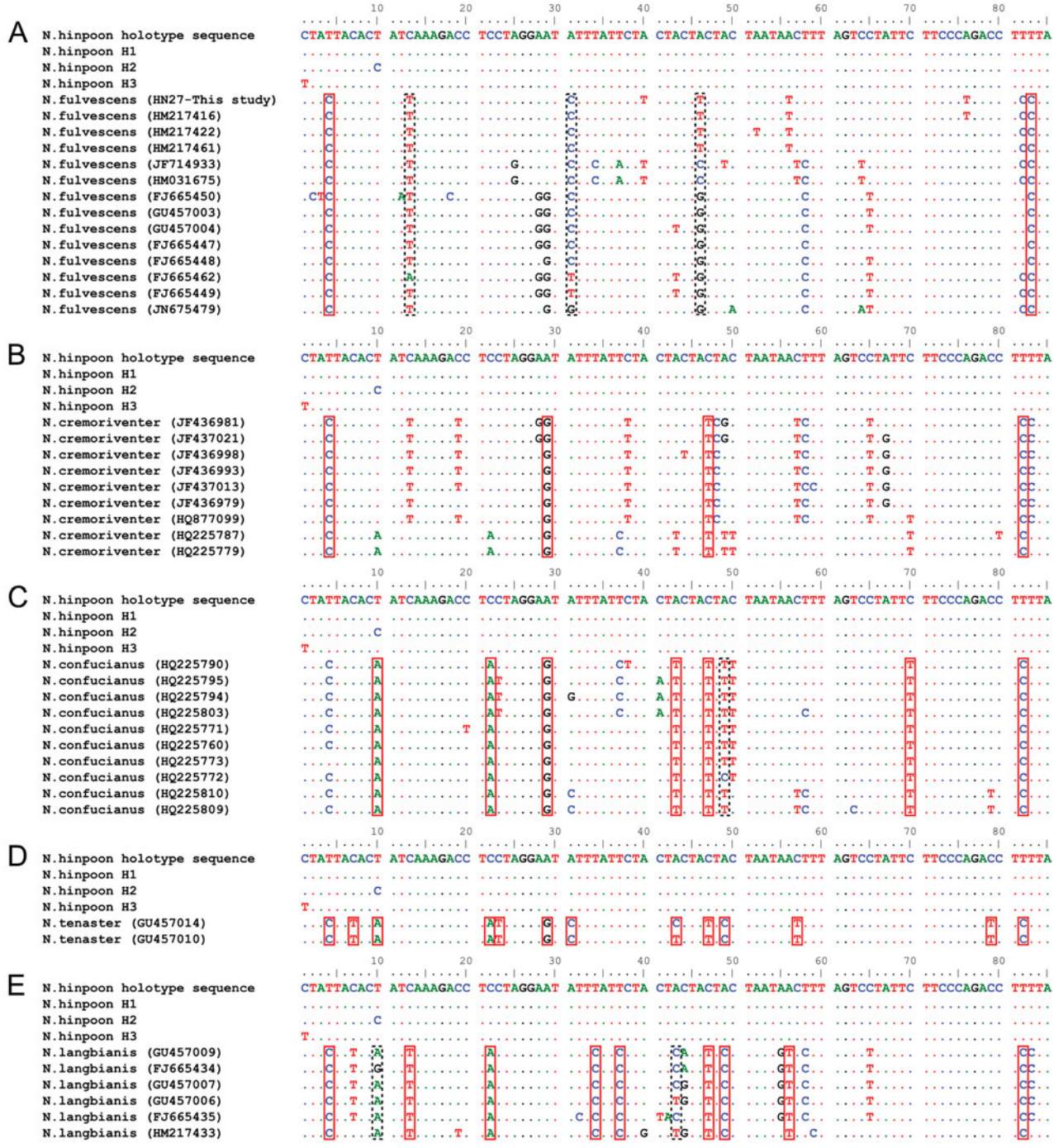


Fig. 5. Discrimination and identification of *N. hinpoon* among other *Niviventer* species distributed in Thailand using characteristic attributes (CAs) of a 85bp cytb fragment: *N. fulvescens* (A), *N. cremoriventer* (B), *N. confucianus* (C), *N. tenaster* (D), *N. langbianis* (E). Accession numbers of sequences retrieved from GenBank are indicated between brackets. Identical sequences are represented by just a single sequence. Identical positions as those of the reference sequence of *N. hinpoon* holotype are indicated by dots. Pure CAs and private CAs are outlined in red (solid line) and black (dashed line), respectively.

identifying clusters of organisms with similar genotypes (Bradley & Baker, 2006; Hausdorf, 2011). However, irrefutable criteria to recognize clusters representing species from others are still lacking (Sauer & Hausdorf, 2012).

The DNA-based approach of species delimitation developed by Pons *et al.* (2006) estimates 40 putative species belonging to seven genera within our dataset composed of karsts samples and a single representative of each of the 24

Table 3. Genetic diversity observed within *N. hinpoon* and *L. neilli*: haplotype diversity (h), nucleotide diversity (Pi), nucleotide diversity at synonymous site (Pi(s)) and nucleotide diversity at non-synonymous site (Pi(a)).

	cytb				COI			
	h	Pi	Pi(s)	Pi(a)	h	Pi	Pi(s)	Pi(a)
N. hinpoon	0.44	0.00141	0.00633	0	0.71	0.00542	0.02261	0
L. neilli (all lineages)	0.92	0.04605	0.14987	0.01001	0.87	0.03021	0.12662	0.00098
L. neilli (central lineages)	0.79	0.01709	0.03696	0.00601	0.59	0.01294	0.05232	0

Rattini species delimited in Pages *et al.* (2010). This number is much larger than the number of species described in Thailand for these genera (25: three *Maxomys*, six *Niviventer*, three *Leopoldamys*, two *Berylmys*, one *Sundamys*, two *Bandicota* and eight *Rattus*). It seems that *L. neilli*, *M. surifer*, *B. bowersi* and *S. muelleri* consist of several putative species according to this method as they correspond to eight, six, two and two GMYC lineages, respectively. However, these numerous lineages could represent real species but they could also simply represent geographically isolated populations evolving neutrally. Indeed, a limitation of this method is that a single species with a strong spatial population structure could wrongfully be split in several separate GMYC lineages (Pons *et al.*, 2006; Fontaneto *et al.*, 2007). Therefore the GMYC model provides an initial hypothesis of the number of species-level groups within our dataset that should be confirmed using other information (Papadopoulou *et al.*, 2009).

The cytb net genetic distance between clusters could also be useful to delimit species boundaries. According to Bradley & Baker (2001), within rodent, cytb genetic distance values smaller than 2% indicate intraspecific variation whereas values higher than 11% denote species delimitation in great majority of cases. Intermediate values could represent either intraspecific variation or species boundaries. Our results for the Rattini tribe corroborate these findings (Table 2). The range of distances from 0 to 2.5% includes only pairwise comparisons between closely related clusters that do not represent distinct recognized species. Distances comprised between 2.6 and 7.5% correspond mostly to pairwise comparisons among the Black rat complex (the *R. rattus* complex *sensu* Aplin *et al.*, 2011) including *R. rattus* (*R. rattus* Lineage I *sensu* Aplin *et al.*, 2011), *R. tanezumi* (*R. rattus* Lineage II *sensu* Aplin *et al.*, 2011), *Rattus* sp. R3 (*R. rattus* Lineage IV *sensu* Aplin *et al.*, 2011), *R. sakeratensis* and *R. tiomanicus*. The range of distances between 7.6 and 9.9% contains several pairwise comparisons between species traditionally recognized as valid such as *N. fulvescens*, *N. hinpoon*, *R. argentiventer*, *R. andamanensis*, *L. edwardsi* and *L. sabanus*. Distances higher than 10% comprise pairwise comparisons between well-differentiated described species. Therefore, based on these results, a minimum threshold genetic distance of 7.5% could be indicative of species delimitation within the Rattini tribe and could be lower for the closely related species of the *Rattus rattus* complex.

These findings allow us to discuss below the specific status of some GMYC lineages that remains unclear within our dataset (summarized in Table 1, see Figs 8–13 for their distribution).

Leopoldamys neilli

Recent phylogeographical studies based on a combination of mitochondrial and nuclear markers indicate a strong geographical structure of the *L. neilli* genetic diversity (Latinne *et al.*, 2011, 2012). The present study identified eight allopatric GMYC lineages (Leo1 to Leo8) within the species (with a confidence interval from seven to nine). Five of these clusters (Leo4, Leo5, Leo6, Leo7, Leo8) which diverged during Middle Pleistocene are separated by cytb genetic distances of less than 2.5% and the clustering may be interpreted as strong spatial population structure within a single species. The clusters Leo2 and Leo3 are differentiated from these five clusters and from each other by slightly higher genetic distance (between 2.5 and 5%). This level of differentiation is of the same order as those observed among the closely related species of the *Rattus rattus* complex whose taxonomic status is still confused. Therefore these values alone do not allow us to conclude that these clusters represent two separate species. Further information is required to determine the status of these lineages.

The Leo1 cluster is dated from Late Tertiary/Early Quaternary and is highly divergent from the other ones (cytb genetic distance between 7.5 and 9.9%). This level of genetic divergence is similar to the one observed among recognized *Leopoldamys* species in Thailand (*L. edwardsi*, *L. sabanus* and other clusters of *L. neilli*). Our previous phylogeographical studies also showed that this cluster formed a unique, highly supported and monophyletic clade in a phylogenetic tree based on two nuclear introns while all other *L. neilli* populations belong to undifferentiated clades (Latinne *et al.*, 2012). Therefore, following these genetic arguments, the cluster Leo1 including samples from western Thailand (Kanchanaburi and Uthai Thani provinces) could represent a separate species from the seven remaining clusters of *L. neilli*. A careful morphological study of this highly divergent lineage is now required to determine definitively its specific status.

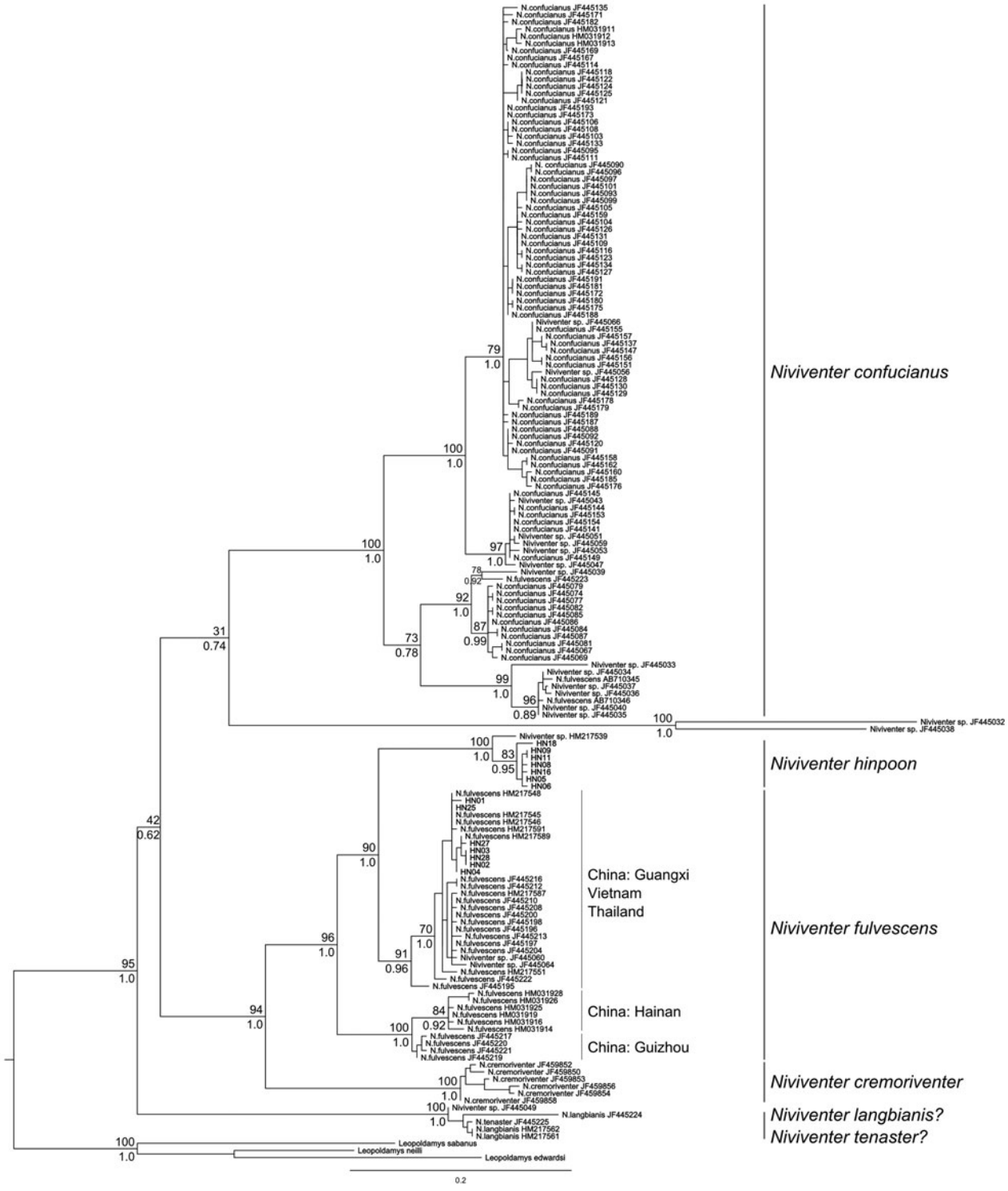


Fig. 6. ML tree (HKY + G) representing phylogenetic relationships within the *Niviventer* genus using COI dataset. Bootstrap support (%, 1000 replicates) and posterior probabilities of nodes are indicated above and below the branches, respectively. The dataset includes our karst samples (HNXX) and sequences retrieved from GenBank. GenBank Accession numbers are indicated.

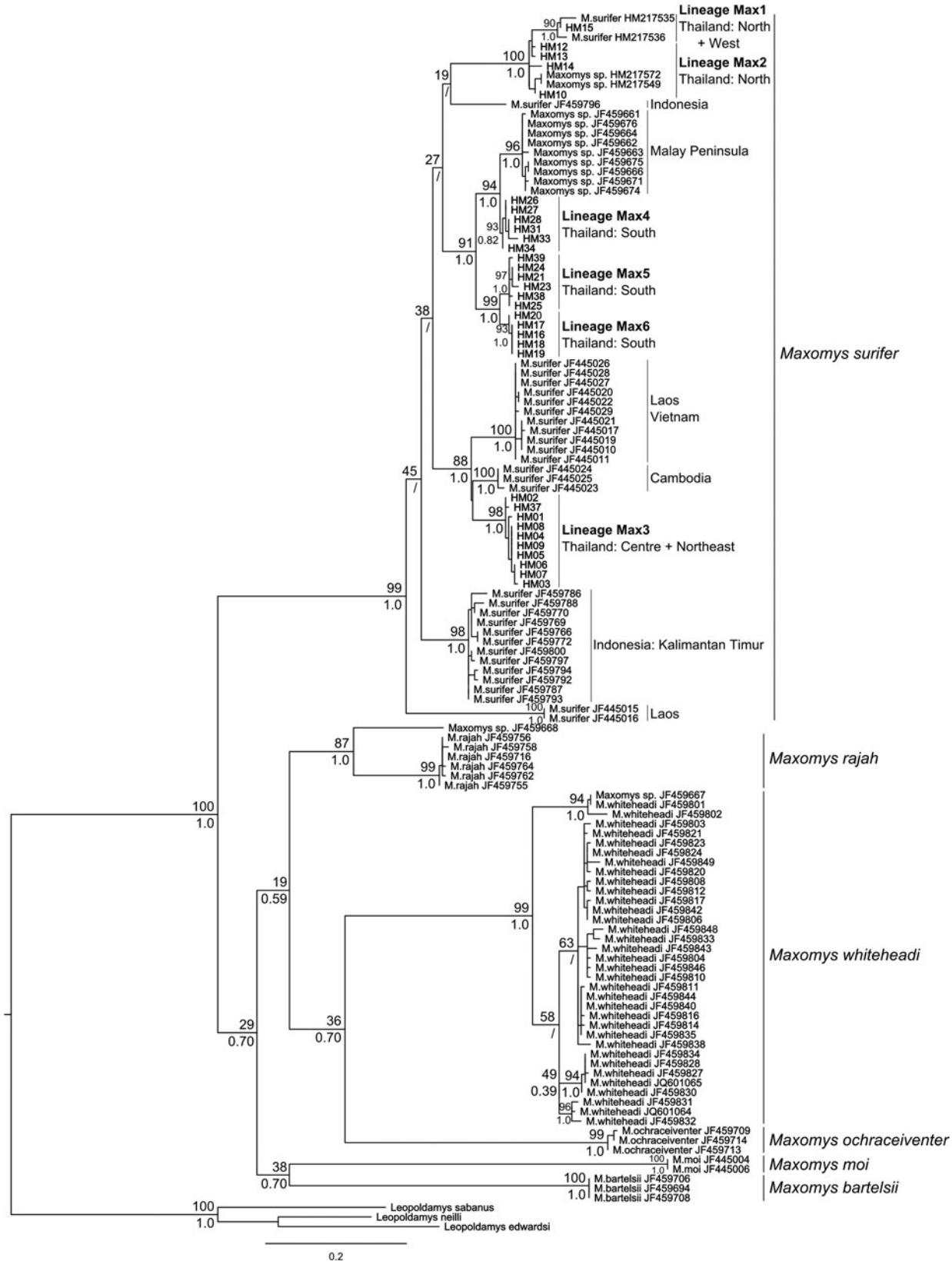
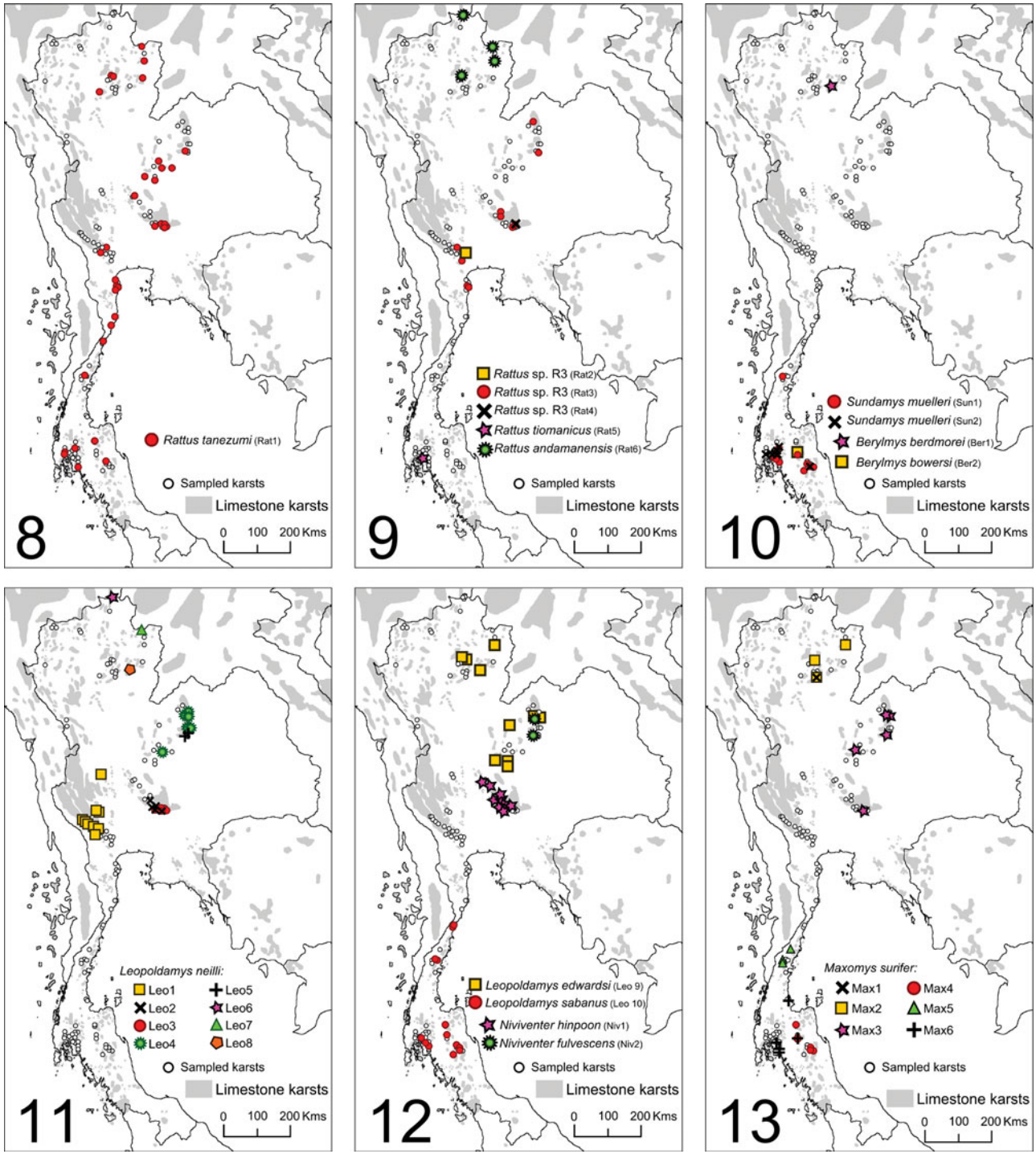


Fig. 7. ML tree (GTR + G) representing phylogenetic relationships within the *Maxomys* genus using COI dataset. Bootstrap support (%, 1000 replicates) and posterior probabilities of nodes are indicated above and below the branches, respectively. The dataset includes our karst samples (HMXX) and sequences retrieved from GenBank. GenBank Accession numbers are indicated.



Figs 8–13. Maps of surveyed Thai limestone karsts and presence/absence data of the species identified within our dataset and their corresponding GMYC lineages.

Maxomys surifer

Six GMYC lineages (Max1 to Max6) have been identified in Thailand within the species *M. surifer* (with a confidence interval from five to seven). Max1 and Max2 as well as Max5 and Max6 are separated by cytb genetic distances of less than 2.5%, values characteristic of intraspecific variation between geographically isolated populations rather than species delimitation. In contrast, four highly divergent lineages (Max1+Max2, Max3, Max4, Max5+Max6), separated by more than 7.5% of genetic distance (except

between Max4 and Max5+Max6: between 5.1 and 7.5%), could be delimited within our dataset. Our phylogenetic analysis clearly indicates that all these clusters could not be assigned to any other *Maxomys* species currently recognized in Thailand. These findings confirm that *M. surifer* may represent a complex of several species as already suggested by Musser & Carleton (2005). All the *Maxomys* samples belonging to these four highly divergent lineages were consistent with the external description of *M. surifer* (upperparts bright orange with black spines along the back and orange collar, underparts creamy-white, bicoloured tail) and may correspond to several cryptic species. However, future morphological studies as well as the sequencing of nuclear genes are now needed to justify the species status of these lineages based on mitochondrial DNA and to determine if these species are really cryptic. A possible ecological adaptation of one of these lineages to life in limestone karsts similar to the one observed for *L. neilli* and *N. hinpoon* should also be verified.

Sundamys muelleri

The two GMYC lineages (Sun1 and Sun2) detected within *S. muelleri* are separated by low cytb genetic distance (less than 2.5%) and diverged recently, around 400 000 years ago. Moreover, these two clusters are combined in a single lineage if the lower limit of the confidence interval of the GMYC analysis is taken into account. Therefore we consider that these two lineages do not represent separate species.

Berylmys bowersi

This study confirms the presence of two highly divergent genetic lineages within *B. bowersi* in Thailand as pointed out by Pages *et al.* (2010). The first lineage that includes northern samples has been identified as *B. bowersi* by Pages *et al.* (2010) and contains none of our karst samples. The second lineage (the lineage Ber2 corresponding to the lineage 'Be2b' in Pages *et al.* (2010)), that could not be named, includes southern samples from Kanchanaburi and Surat Thani provinces. However, the level of genetic divergence between these two lineages is moderate (between 5.1 and 7.5%) and lower than the distance between these two lineages and *B. berdmorei* (>10%) and is not indicative of a clear species delimitation. Several authors have already mentioned that southern populations of *B. bowersi* may represent a separate species or subspecies based on morphological criteria (Lekagul & McNeely, 1988; Francis, 2008). Further taxonomic work within *B. bowersi* is therefore required.

Rattus sp. R3

The clusters Rat2, Rat3, Rat4 are separated by less than 2.5% of cytb genetic distance. Moreover, Rat3 and Rat4 are combined in a single lineage if the lower limit of the confidence interval of the GMYC analysis is taken into account. Therefore, these results strongly suggest that these three lineages belong to a single species to which a name could not be assigned and identified as '*Rattus* sp. R3' in Pages *et al.* (2010), ' *R. rattus* Lineage IV' in Aplin *et al.* (2011) and Lack *et al.* (2012). Recent studies revealed that *Rattus* sp. R3 and *R. tanezumi* could not be distinguished using nuclear markers and morphological characters and suggest possible hybridization with introgression between *Rattus* sp. R3 and *R. tanezumi* (Lack *et al.*, 2012; Pages *et al.*, 2012). Therefore, the R3 species could even be invalidated and considered as *R. tanezumi*.

Identification of Rattini species

Most of the Rattini species included in our dataset were identified in the field with good success using external morphological characters and geographical criteria even if misidentifications were numerous (Table 1). However, the very low percentages of match between field and molecular identifications for some *Rattus* species reflect the difficulty of identifying *Rattus* specimens using only external characters as already pointed out by Robins *et al.* (2007) and Pages *et al.* (2010).

Our study also revealed frequent misidentifications of *Leopoldamys edwardsi* as *L. sabanus* in the field. During our field survey, the *Leopoldamys* specimens from northern Thailand that clearly did not belong to *L. neilli* (because of their very large body size and tail length) were assigned either to *L. edwardsi* or *L. sabanus* following the morphological criteria based on fur colour available in the literature (Appendix 3, see supplemental material online) as both species are assumed to occur in this region. However our phylogenetic analyses revealed that all these samples, regardless of their fur colour, clearly belong to a single phylogenetic lineage (Leo9) and were genetically highly differentiated from the *L. sabanus* samples trapped in peninsular Thailand (Leo10). Based on these results and the data of Pages *et al.* (2010), it seems that *L. sabanus* is restricted to the southern part of Thailand with a northern range limit in the Kanchanaburi province and that this species does not occur in sympatry with *L. edwardsi* in Thailand (Fig. 12). These findings are in opposition to data available in the literature (Lekagul & McNeely, 1988; Musser & Carleton, 2005). We suspect that this contradiction is probably due to frequent misidentifications between *L. sabanus* and *L. edwardsi* in the literature as these two species are morphologically very similar and easily confused. Therefore we recommend using DNA sequences to identify unambiguously the two species in future studies.

Rodent diversity of Thai limestone karsts

A total of 12 described Rattini species (*R. tanezumi*, *R. tiomanicus*, *R. andamanensis*, *S. muelleri*, *B. berdmorei*,

B. bowersi, *L. neilli*, *L. edwardsi*, *L. sabanus*, *N. hinpoon*, *N. fulvescens*, *M. surifer*), corresponding to 17 highly divergent genetic lineages (see above), were encountered in limestone karsts of Thailand (Table 1). Out of these 12 species, the two karst endemic species (*L. neilli* and *N. hinpoon*) represent 53.1% of the total number of captures, *L. neilli* being the most abundant species and representing almost half of the total captures (47.2%). On the contrary, *B. berdmorei* and *B. bowersi* were excessively rare (less than 1% of the captures).

Karsts as forest refuges?

Except karst endemic species, the majority of rodent species recorded in this study are associated with forest ecosystems (*R. tiomanicus*, *R. andamanensis*, *S. muelleri*, *L. edwardsi*, *L. sabanus*, *N. fulvescens*, *M. surifer*). Thailand has lost about 30% of its forest cover between 1950 and 2000 and estimates of current forest loss in Thailand range from 0.7% to 1% per year (World Bank, 2004; Dupuy *et al.*, 2012). The remaining forests that represent between 25 and 33% of the total land area of the country, depending on the calculation method (Charuppat, 1998; World Bank, 2004), are often fragmented and degraded (Dupuy *et al.*, 2012). As karsts are mainly unsuitable for agriculture due to their topography, deforestation is generally less extensive on karst hills than in valley floors. In many regions of Thailand, limestone karsts thus appear as forested islands surrounded by agricultural fields and urban areas. This study suggests that besides hosting karst endemic species, limestone karsts could also play a key role in the preservation of the global rodent biodiversity by providing important refuges for the forest-dwelling species in deforested and highly developed regions. Moreover, karst hillside forests could help to maintain the habitat connectivity and allow movements of species among isolated forest fragments. In order to confirm these hypotheses, a precise comparison of forest-dwelling rodent occurrence between karsts and adjacent habitats using an accurate methodology will be needed in the future. The important role of forested karsts to support forest-adapted species has also been mentioned by Furey *et al.* (2010) in their study of bat diversity in Vietnam. These observations strengthen the necessity of protecting karst ecosystems in Southeast Asia to preserve not only the karst endemic fauna but also the forest-dwelling species.

Rattini species commonly observed in agricultural and cultivated areas of Thailand (e.g. *Bandicota indica*, *Bandicota savilei*, *Rattus argentiventer*, *Rattus sakeratensis*, *Rattus nitidus*) were totally absent from our inventory. Highly commensal species such as *R. exulans* and *R. norvegicus* were also lacking in Thai limestone karsts. Only one very adaptable and generalist species, *R. tanezumi* (or two if we consider *Rattus* sp. R3 as a distinct species) inhabits commonly Thai limestone karsts.

The Indochinese–Sundaic biogeographical transition

The transition between the Indochinese and Sundaic biogeographical subregions has long been considered to occur around the Isthmus of Kra (e.g. Kloss, 1915, 1929; Wells, 1976). However, Woodruff & Turner (2009) showed that the transition in mammal species does not coincide with a narrow zone of the Thai–Malay peninsula but instead occurs within two areas situated around 5°N (northern part of Malay peninsula) and 14°N (where the Thai peninsula meets the mainland). These authors explained this pattern of faunistic transition by numerous significant faunal compressions in the Thai–Malay peninsula due to the repeated sea-level changes that occurred during the last 5 Myr.

Our divergence time analysis allows us to date the split among some Indochinese and Sundaic *Maxomys* and *Leopoldamys* lineages. The first divergence that occurred among our *Maxomys* clusters did not isolate the Indochinese from Sundaic lineages but separates the northern lineages (Max1+Max2) from the northeastern (Max3) and southern ones (Max4+Max5+Max6) around 2.3 Mya. However this node is not well-supported in our phylogenetic tree. Then, the southern lineages diverged from the northeastern one around 2 Mya, within the time frame of 1 to 4 Mya predicted by Woodruff & Turner (2009) following their hypothesis. The divergence of the Sundaic *L. sabanus* from the Indochinese *Leopoldamys* species (*L. edwardsi* and *L. neilli*) was also estimated to occur within this time frame, around 3.5 Mya, corroborating the hypothesis of Woodruff & Turner (2009).

Finally, our study evidenced a high number of genetic lineages in peninsular Thailand within *Maxomys surifer* (3 GMYC clusters) (Fig. 13), *Sundamys muelleri* (2 GMYC clusters) (Fig. 10) and *Leopoldamys sabanus* (1 GMYC cluster subdivided in 2 lineages) that diverged during Pleistocene. The strong phylogeographical pattern of these three species in this region may be due to the numerous repetitive marine transgressions that occurred during the last 4 Myr and strongly reduced the land area (around 50%) between Krabi, Surat Thani and Nakhon Si Thammarat (Woodruff & Turner, 2009). These marine transgressions may have influenced the genetic structure of these Murinae species in the central Thai–Malay peninsula by causing repeated demographic bottlenecks.

Karst endemic rodents of Thailand and their conservation status

More than 20% of limestone karsts in Thailand have already been quarried for cement, lime and hard core, and many have completely disappeared from the landscape (World Bank, 2004). The cement production of Thailand has increased by about 10% every year in the early 2000s but this rate has been slightly decreasing since 2007 (ESCAP,

2001; Shi, 2010). As a result of these activities, some endemic species are highly threatened.

Due to the lack of data about their distribution range and ecology, the two karst endemic rodent species of Thailand, *L. neilli* and *N. hinpoon*, are currently listed as Data Deficient on the IUCN Red List (Lunde & Aplin, 2008*a*, 2008*b*). However, the distribution range of *L. neilli* is much more extensive than indicated by previous records. This species is present in most karstic regions of central, northern and western Thailand (Fig. 11). Accordingly, Latinne *et al.* (2012) recommend classifying this species as 'Near Threatened' on the IUCN Red List. However, if we consider that the western populations of *L. neilli* represent a separate species, this species should be listed in a higher extinction risk category and qualified for the Vulnerable category (B1ab(iii)) due to its small extent of occurrence (less than 20 000 km^2), the high fragmentation of its habitat and the projected decline of the quality of this habitat in the future (threats of habitat destruction by limestone quarrying).

Contrary to *L. neilli*, our survey clearly indicated that the distribution range of *N. hinpoon* is limited to the limestone karsts of central Thailand (Saraburi, Lopburi, Nakhon Sawan provinces) (Fig. 12) where it seems to be quite common and abundant. Due to its small distribution range and the high threats that its habitat is facing in this region, *N. hinpoon* meets the same criteria as the western populations of *L. neilli* and would be qualified as Vulnerable (B1ab(iii)) on the IUCN Red List.

Our phylogenetic analyses indicate that *N. hinpoon* is closely related to *N. fulvescens*, as also suggested by morphological characters (Musser & Carleton, 2005). However, according to our phylogenetic tree combining GenBank sequences, *N. fulvescens* consists of several paraphyletic lineages, which confirms the need of a taxonomic revision of this species. At the intraspecific level, *N. hinpoon* is genetically homogeneous and, in contrast to *L. neilli* in this region, is characterized by a single mitochondrial lineage and a lower genetic diversity. *Leopoldamys neilli* and *N. hinpoon* frequently live in sympatry in the same caves of Saraburi and Lopburi provinces. Therefore competitive exclusion does not seem to occur between these two species adapted to limestone karsts which share presumably similar habitat requirements.

Conclusions

Our study revealed an important genetic diversity within traditionally recognized species *Maxomys surifer* (four highly divergent lineages), *Leopoldamys neilli* (two highly divergent lineages) and *Berylmys bowersi* (two highly divergent lineages) which could be considered as species complex. Further taxonomic work combining independent data (mitochondrial and nuclear markers, morphology) and an increased sampling is now required to confirm the specific status of these lineages. It will also be very important to determine if some of these putative cryptic species are endemic to limestone karsts as it will have very important implications for their conservation.

This study has also shown that the Murinae rodent diversity of limestone karsts in Thailand is not restricted to endemic species as karsts also host many forest-dwelling species to which they could provide forest refuge in deforested regions. Therefore limestone karsts should be protected to preserve not only endemic species, but also forest-dwelling species threatened by deforestation.

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References

Agapow, P.M., Bininda-Emonds, O.R.P., Crandall, K.A., Gittleman, J.L., Mace, G.M., Marshall, J.C. & Purvis, A. 2004. The impact of species concept on biodiversity studies. *Quarterly Review of Biology* **79**, 161–179.

Aguilar, J.P. & Michaux, J. 1996. The beginning of the age of Murinae (Mammalia: Rodentia) in southern France. *Acta Zoologica Cracoviensia* **39**, 35–45.

Aplin, K.P., Brown, P.R., Jacobs, J., Krebs, C.J. & Singleton, G.R. 2003. *Field Methods for Rodent Studies in Asia and the Indo-Pacific*. ACIAR, Canberra.

Aplin, K.P., Suzuki, H., Chinen, A.A., Chesser, R.T., Ten Have, J., Donnellan, S.C., Austin, J., Frost, A., Gonzalez, J.P., Herbreteau, V., Catzeflis, F., Soubrier, J., Fang, Y.P., Robins, J., Matisoo-Smith, E., Bastos, A.D.S., Maryanto, I., Sinaga, M.H., Denys, C., Van Den Bussche, R.A., Conroy, C., Rowe, K. & Cooper, A. 2011. Multiple geographic origins of commensalism and complex dispersal history of black rats. *Public Library of Science ONE* **6**, e26357.

ASEAN CFB. 2010. *ASEAN Biodiversity Outlook*, ASEAN Centre for Biodiversity, Philippines.

Baker, R.J. & Bradley, R.D. 2006. Speciation in mammals and the genetic species concept. *Journal of Mammalogy* **87**, 643–662.

Bastos, A., Nair, D., Taylor, P., Brettschneider, H., Kirsten, F., Mostert, E., Von Maltitz, E., Lamb, J., Van Hooft, P., Belmain, S., Contrafatto, G., Downs, S. & Chimimba, C. 2011. Genetic monitoring detects an overlooked cryptic species and reveals the diversity and distribution of three invasive *Rattus* congeners in South Africa. *BioMed Central Genetics* **12**, 26.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. & Das, I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22**, 148–155.

Bradley, R.D. & Baker, R.J. 2001. A test of the genetic species concept: cytochrome-b sequences and mammals. *Journal of Mammalogy* **82**, 960–973.

Chaimanee, Y. & Jaeger, J.-J. 2001. Evolution of *Rattus* (mammalia, Rodentia) during the plio-pleistocene in Thailand. *Historical Biology* **15**, 181–191.

Charuppat, T. 1998. *Forest Situation of Thailand in the Past 37 Years (1961–1998)*. Forest Resources Assessment Div. Forest Research Office. Royal Forest Department, Thailand.

Clements, R., Sodhi, N.S., Schilthuizen, M. & Ng, P.K.L. 2006. Limestone karsts of southeast Asia: imperiled arks of biodiversity. *Bioscience* **56**, 733–742.

Corbet, G. & Hill, J. 1992. *The Mammals of the Indomalayan Region: A Systematic Review*. Oxford University Press, Oxford, UK.

Day, M. & Urich, P. 2000. An assessment of protected karst landscapes in Southeast Asia. *Cave and Karst Science* **27**, 61–70.

De Queiroz, K. 2005. Different species problems and their resolution. *Bioessays* **27**, 1263–1269.

De Queiroz, K. 2007. Species concepts and species delimitation. *Systematic Biology* **56**, 879–886.

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

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. *Public Library of Science Biology* **4**, 699–710.

Dupuy, S., Herbreteau, V., Feyfant, T., Morand, S. & Tran, A. 2012. Land-cover dynamics in Southeast Asia: contribution of object-oriented techniques for change detection. In: *4th GEOBIA*. Rio de Janeiro, Brazil, pp. 217–222.

ESCAP. 2001. *Atlas of Mineral resources of the ESCAP region, Volume 16, Mineral Resources of Thailand*. United Nations, New York.

Excoffier, L., Laval, G. & Schneider, S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* **1**, 47–50.

Fontaneto, D., Herniou, E. A., Boschetti, C., Caprioli, M., Melone, G., Ricci, C. & Barraclough, T. G. 2007. Independently evolving species in asexual bdelloid rotifers. *Public Library of Science Biology* **5**, 914–921.

Francis, C.M. 2008. *A Field Guide to the Mammals of South-East Asia*. New Holland, London, UK.

Furey, N.M., Mackie, I.J. & Racey, P.A. 2010. Bat diversity in Vietnamese limestone karst areas and the implications of forest degradation. *Biodiversity and Conservation* **19**, 1821–1838.

Gilbert, M.T.P., Bandelt, H.J., Hofreiter, M. & Barnes, I. 2005. Assessing ancient DNA studies. *Trends in Ecology and Evolution* **20**, 541–544.

Gillieson, D. 2005. Karsts of Southeast Asia. In: Gupta, A., Ed., *The Physical Geography of Southeast Asia*. Oxford University Press, Oxford, UK, pp. 157–176.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**, 307–321.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Hausdorf, B. 2011. Progress toward a general species concept. *Evolution* **65**, 923–931.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proceedings of the National Academy of Sciences USA* **101**, 14812–14817.

Huchon, D., Chevret, P., Jordan, U., Kilpatrick, C.W., Ranwez, V., Jenkins, P.D., Brosius, J. & Schmitz, J. 2007. Multiple molecular evidences for a living mammalian fossil. *Proceedings of the National Academy of Sciences USA* **104**, 7495–7499.

Hughes, J.B., Round, P.D. & Woodruff, D.S. 2003. The Indochinese-Sundaic faunal transition at the Isthmus of Kra: an analysis of resident forest bird species distributions. *Journal of Biogeography* **30**, 569–580.

Irwin, D.M., Kocher, T.D. & Wilson, A.C. 1991. Evolution of the cytochrome b gene of mammals. *Journal of Molecular Evolution* **32**, 128–144.

Jacobs, L.L. & Flynn, L.J. 2005. Of mice ... again: the Siwalik rodent record, murine distribution, and molecular clocks. In: Lieberman, D., Smith, R. & Kelley, J., Eds., *Interpreting the Past: Essays on Human, Primate and Mammal Evolution*. Brill Academic, Leiden, The Netherlands, pp. 63–80.

Jenkins, P.D., Kilpatrick, C.W., Robinson, M.F. & Timmins, R.J. 2005. Morphological and molecular investigations of a new family, genus and species of rodent (Mammalia: Rodentia: Hystricognatha) from Lao PDR. *Systematics and Biodiversity* **2**, 419–454.

Kloss, C. B. 1915. Zoogeographical divisions for Siam. *Journal of the Natural History Society of Siam* **1**, 250–251.

Kloss, C. B. 1929. The zoo-geographical boundaries between Asia and Australia and some Oriental sub-regions. *Bulletin of the Raffles Museum* **2**, 1–10.

Lack, J.B., Greene, D.U., Conroy, C.J., Hamilton, M.J., Braun, J.K., Mares, M.A. & Van Den Bussche, R.A. 2012. Invasion facilitates hybridization with introgression in the *Rattus rattus* species complex. *Molecular Ecology* **21**, 3545–3561.

Latinne, A., Waengsothorn, S., Herbreteau, V. & Michaux, J.R. 2011. Evidence of complex phylogeographic structure for the threatened rodent *Leopoldamys neilli*, in Southeast Asia. *Conservation Genetics* **12**, 1495–1511.

Latinne, A., Waengsothorn, S., Rojanadilok, P., Eiamampai, K., Sribuarod, K. & Michaux, J.R. 2012. Combined mitochondrial and nuclear markers revealed a deep vicariant history for *Leopoldamys neilli*, a cave-dwelling rodent of Thailand. *Public Library of Science ONE* **7**, e47670.

Lecompte, E., Aplin, K., Denys, C., Catzeflis, F., Chades, M. & Chevret, P. 2008. Phylogeny and biogeography of African Murinae based on mitochondrial and nuclear gene sequences, with a new tribal classification of the subfamily. *BioMed Central Evolutionary Biology* **8**, 199.

Lekagul, B. & Mcneely, J.A. 1988. *Mammals of Thailand*. White Lotus Press, Bangkok.

Librado, P. & Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452.

Lunde, D.P. & Aplin, K. 2008*a*. *Leopoldamys neilli*. In: *IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1.* IUCN, Cambridge, UK.

Lunde, D.P. & Aplin, K. 2008*b*. *Niviventer hinpoon*. In: *IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1.* IUCN, Cambridge, UK.

Michaux, J., Aguilar, J.P., Montuire, S., Wolff, A. & Legendre, S. 1997. Les Murinae (Rodentia, Mammalia) neogenes du Sud de la France: Evolution et paleoenvironnements. *Geobios* **30**, 379–385.

Monaghan, M.T., Wild, R., Elliot, M., Fujisawa, T., Balke, M., Inward, D.J.G., Lees, D.C., Ranaivosolo, R., Eggleton, P., Barraclough, T.G. & Vogler, A.P. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Systematic Biology* **58**, 298–311.

Musser, G.G. & Carleton, M. 2005. Superfamily Muroidea. In: Wilson, D.E. & Reeder, D.M., Eds., *Mammal Species of the World: A Taxonomic and Geographic Reference*. Johns Hopkins University Press, Baltimore, USA, pp. 894–1531.

Musser, G.G., Smith, A.L., Robinson, M.F. & Lunde, D.P. 2005. Description of a new genus and species rodent (Murinae, Muridae, Rodentia) from Khammouan Limestone National Biodiversity Conservation Area in Lao PDR. *American Museum Novitates* **3497**, 1–31.

Musser, G.G., Lunde, D.P. & Son, N.T. 2006. Description of a new genus and species of rodent (Murinae, Muridae, Rodentia) from the tower karst region of northeastern Vietnam. *American Museum Novitates* **3517**, 1–41.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A.B. & Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**, 853–858.

Pages, M., Chaval, Y., Herbreteau, V., Waengsothorn, S., Cosson, J. F., Hugot, J.P., Morand, S. & Michaux, J. 2010. Revisiting the taxonomy of the Rattini tribe: a phylogeny-based delimitation of species boundaries. *BioMed Central Evolutionary Biology* **10**, 184.

Pages, M., Corbet, G., Orth, A., Volobouev, V., Michaux, J. & Catzeflis, F. 2011. Morphological, chromosomal, and genic differences between sympatric *Rattus rattus* and *Rattus satarae* in South India. *Journal of Mammalogy* **92**, 659–670.

Pages, M., Bazin, E., Galan, M., Chaval, Y., Claude, J., Herbreteau, V., Michaux, J.R., Piry, S., Morand, S. & Cosson, J.F. 2012. Cytonuclear discordance among Southeast Asian black rats (*Rattus rattus* complex). *Molecular Ecology* **22**, 1019–1034.

Papadopoulou, A., Monaghan, M.T., Barraclough, T.G. & Vogler, A.P. 2009. Sampling error does not invalidate the Yule-Coalescent model for species delimitation. A response to Lohse (2009). *Systematic Biology* **58**, 442–444.

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W.D. & Vogler, A.P. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* **55**, 595–609.

Posada, D. & Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Rambaut, A. & Drummond, A.J. 2007. *Tracer v1.4* (http://beast.bio.ed.ac.uk/Tracer, accessed 19 June 2013).

Robins, J.H., Hingston, M., Matisoo-Smith, E. & Ross, H.A. 2007. Identifying *Rattus* species using mitochondrial DNA. *Molecular Ecology Notes* **7**, 717–729.

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

Rowe, K.C., Aplin, K.P., Baverstock, P.R. & Moritz, C. 2011. Recent and rapid speciation with limited morphological disparity in the genus *Rattus*. *Systematic Biology* **60**, 188–203.

Sarkar, I.N., Planet, P.J. & Desalle, R. 2008. CAOS software for use in character-based DNA barcoding. *Molecular Ecology Resources* **8**, 1256–1259.

Sauer, J. & Hausdorf, B. 2012. A comparison of DNA-based methods for delimiting species in a Cretan land snail radiation reveals shortcomings of exclusively molecular taxonomy. *Cladistics* **28**, 300–316.

Shi, L. 2010. The mineral industry of Thailand. In: *2008 Minerals Yearbook, Thailand*. U.S. Geological Survey, USA.

Simms, M.J. 2005. Sedimentary processes, karsts and palaeokarst. In: Selley, R.C., Cocks, L.R.M. & Plimer, I.R., Eds., *Encyclopedia of Geology*. Elsevier, Oxford, UK, pp. 678–687.

Swofford, D.L. 1998. *PAUP*. Phylogenetic Analysis using Parsimony, (*and other Methods), version 4*. Sinauer Associates, Sunderland, MA, USA.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.

Teletchea, F., Bernillon, J., Duffraisse, M., Laudet, V. & Hanni, C. 2008. Molecular identification of vertebrate species by oligonucleotide microarray in food and forensic samples. *Journal of Applied Ecology* **45**, 967–975.

Vermeulen, J.J. & Whitten, T. 1999. *Biodiversity and Cultural Property in the Management of Limestone Resources – Lessons from East Asia*. World Bank, Washington, DC, USA.

Waengsothorn, S., Nabhitabhata, J. & Moochan, T. 2007. The ecological distribution of Thai endemic rodents with a new distributional range of *Niviventer hinpoon*. *Thailand Natural History Museum Journal* **2**, 31–42.

Waengsothorn, S., Kenthao, A., Latinne, A. & Hugot, J.P. 2009. Rodents within the Centre for Thai National Reference Collections (CTNRC), past, present and future. *Kasetsart Journal Natural Sciences* **43**, 118–124.

Wells, D.R. 1976. Resident birds. In: Medway, L. & Wells, D.R., Eds., *Vol. V: The Birds of the Malay Peninsula*. Whiterby, London, UK, pp. 1–33.

Winkler, A.J. 2002. Neogene paleobiogeography and East African paleoenvironments: contributions from the Tugen Hills rodents and lagomorphs. *Journal of Human Evolution* **42**, 237–256.

Woodruff, D.S. & Turner, L.M. 2009. The Indochinese-Sundaic zoogeographic transition: a description and analysis of terrestrial mammal species distributions. *Journal of Biogeography* **36**, 803–821.

WORLD BANK. 2004. *Thailand Environment Monitor 2004, Biodiversity Conservation*. World Bank, Washington, DC, USA.

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