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Phylogenetic relationships in genus *Niviventer* (Rodentia: Muridae) in China inferred from complete mitochondrial cytochrome *b* gene

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Abstract

Chinese species of the genus *Niviventer*, predominantly distributed in the southeastern Tibetan Plateau and in Taiwan, are a diverse group and have not yet received a thorough molecular phylogenetic analysis. Here, we reconstructed the phylogenetic relationships of 32 specimens representing nine Chinese species of *Niviventer*, based on sequences of the complete mitochondrial cytochrome *b* gene. Maximum parsimony, maximum likelihood and Bayesian analysis resulted in three consistent trees, each supported by high bootstrap values. The results showed that the *Niviventer* species included here are monophyletic. The nine species were classified into three distinct clades: clade A with *Niviventer brahma*, *N. confucianus*, *N. coxingi*, *N. culturatus*, *N. eha* and *N. fulvescens*; clade B with *N. andersoni* and *N. excelsior*; clade C with *N. cremoriventer*. Our results also suggested that *N. culturatus* should be a valid species rather than a subspecies of *N. confucianus*. Divergence times among species were calibrated according to the middle-late Pleistocene (1.2–0.13 Mya) fossil records of *N. confucianus*. The results demonstrated that the first radiation event of the genus *Niviventer* occurred in early Pleistocene (about 1.66 Mya), followed by the divergence of clades A and B at about 1.46 Mya. Most of the extant *Niviventer* species appeared during early to middle Pleistocene (about 1.29–0.67 Mya). These divergence times are coincidental with the last uplift events of the Tibetan Plateau, Kun-Huang movement, Pleistocene glaciations and the vicariant formation of Taiwan Strait. Consequently geographical events and Pleistocene glaciations have played a great role in the diversification of *Niviventer*.

Keywords: Biogeography; Chinese species of *Niviventer*; Cytochrome *b*; Molecular phylogeny; Vicariant

1. Introduction

The genus *Niviventer* (Rodentia: Muridae) contains 15 species, occurring from the Himalayas and China to the Greater Sunda Islands (Corbet and Hill, 1992; Musser, 1981; Musser and Carleton, 1993, Fig. 1A). All *Niviventer* species are well distinguished from other murid rodents by the long, slender, flat craniums and the tail-tip on tails (Musser, 1981). These medium-sized rats are cursorial, scansorial or arboreal, and they can live in various kinds of forest in both lowlands and mountains (Marshall, 1977; Musser, 1981; Musser and Chiu, 1979; Yu, 1994).

Niviventer are traditionally included in *Rattus* sensu lato (Allen, 1940; Ellerman and Morrison-Scott, 1966; Marshall, 1977; Osgood, 1932). Misonne (1969) placed it in the genus *Maxomys*, which was subsequently rejected by Musser et al. (1979). Marshall (1979) proposed *Niviventer* as a subgenus of *Rattus*. Finally, Musser (1981) elevated *Niviventer* to the generic rank. Combining morphological comparison, chromosomes and geographical distribution, Musser (1981) also investigated systematics and taxonomy of this genus from the Indo-Malayan region. He divided the genus into two divisions: the *Niviventer andersoni* division (*N. andersoni*, *N. excelsior*) and the *Niviventer nivivet-*

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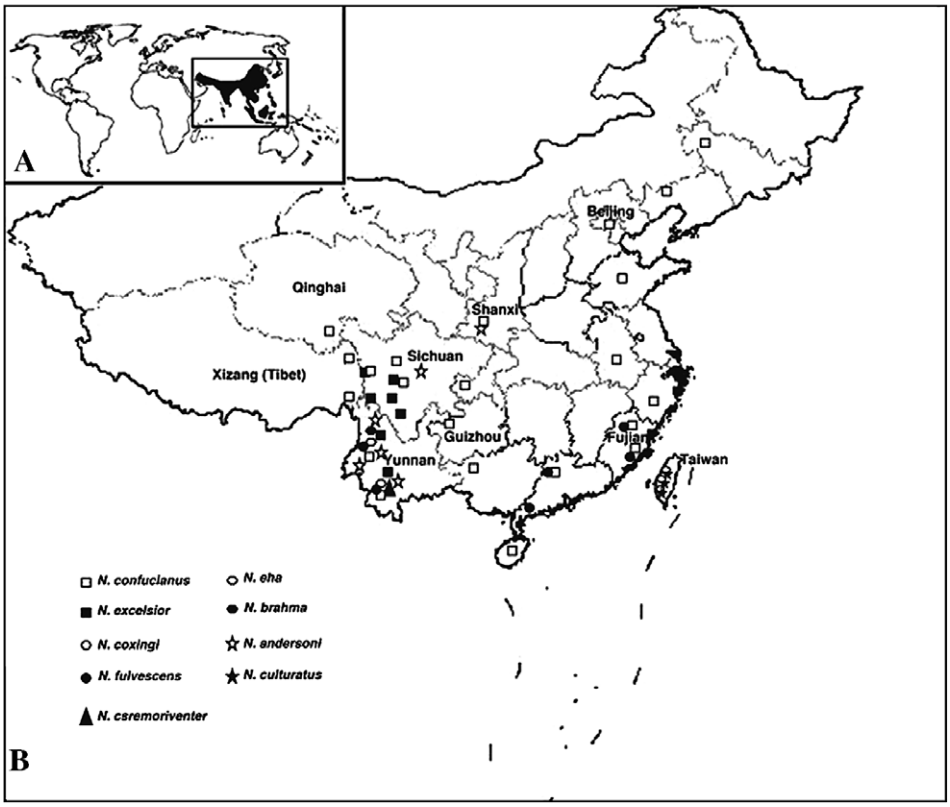

Fig. 1. (A) Map showing the distribution of *Niviventer* species in world. (B) The locations of sites where the specimens of nine species of *Niviventer* in China have been reported by Corbet and Hill (1992) and Wang (2003).

ner division (*N. niviventer*, *N. confucianus*, *N. tenaster*, *N. fulvescens*, *N. coxingi*, *N. rapit*, *N. lepturus*, *N. bukit*, *N. brahma*, *N. eha*, *N. langbianis*, *N. hinpoon* and *N. cremoriventer*). Later Musser and Carleton (1993) reviewed taxonomy of this genus based on morphology, and suggested that *N. bukit* was a synonym of *N. fulvencens* and that *N. culturatus* was not a subspecies of *N. confucianus* but a valid species. These views were followed by Nowak (1999), Wang (2003) and Yu (1994, 1995).

In China and Taiwan, according to Musser and Carleton (1993), there are nine *Niviventer* species (*N. andersoni*, *N. excelsior*, *N. confucianus*, *N. fulvescens*, *N. brahma*, *N. eha*, *N. cremoriventer*, *N. coxingi*, *N. culturatus*). Among these nine species, two (*N. coxingi* and *N. culturatus*) are island forms endemic to Taiwan, and the remaining seven species are mainly distributed in the southeastern shoulder of the Tibetan Plateau (Musser and Carleton, 1993, Fig. 1). Perhaps due to unavailability of specimens, all Chinese species of the *Niviventer* genus have never been included in any molecular systematic studies.

The Tibetan Plateau has undergone dramatic geological and climatic changes over the past 25 million years, including uplift of mountains, cutting of rivers and glaciation events, which produced complicated shifts of habitats (Harrison et al., 1992; Lehmkuhl and Haselein, 2000). This series of geographic events were hypothesized to have played important roles in evolutionary histories of many mammals in this region (Liu et al., 2004; Luo et al., 2004; Yu et al., 2000). For the *Niviventer* species, it is unclear that how these geographic events had facilitated their speciation and adaptation processes. Moreover, Taiwan became an island approximately 3–5 million years ago (Teng, 1987; Shaw, 1996) and was connected to the continent more than once since the Quaternary (Lin and Zhou, 1974). The relevancy between island vicariance of Taiwan and the geographic distribution of *N. coxingi* and *N. culturatus* is still not clear but see Yu (1995) for a discussion.

The mitochondrial cytochrome *b* (cyt *b*) gene is informative to reveal genetic divergence between sister species and their congeners. The cyt *b* gene is usually not affected by severe saturation effects involving multiple nucleotide substitutions (Avise et al., 1998; Meyer, 1993; Moritz et al., 1987). Hence, it has often been used to reconstruct phylogenetic relationships within and among numerous vertebrate groups (Andrews et al., 1998; Irwin et al., 1991; Avise et al., 1998), including rodents from western China (Liu et al., 2004; Luo et al., 2004). To explore the molecular phylogenetic relationships of *Niviventer* species, we sequenced the complete mitochondrial cyt *b* gene of Chinese *Niviventer* species. We aimed to address the following issues: (1) to elucidate the phylogeny of *Niviventer* from the southeastern shoulder of the Tibetan Plateau and Taiwan; (2) to clarify the taxonomic status of *N. culturatus* which was treated as a subspecies of *N. confucianus* (Musser, 1981) but a valid species (Musser and Carleton, 1993) and (3) to investigate the correlation between the diver-

gence events in the genus and recent geographic events of the Tibetan Plateau and the formation of Taiwan Strait.

2. Materials and methods

2.1. Specimens

Tissues of 32 specimens from 9 species were collected from the southeastern shoulder of the Tibetan Plateau and from Taiwan (Table 1). All specimens were identified based on external characteristics and skull morphology, following the system of Corbet and Hill (1992) and Musser (1981). The tissue samples were associated with voucher specimens at the Museum of Kunming Institute of Zoology, Chinese Academy of Sciences (CAS) and the Museum of Taiwan University (Table 1). *Rattus rattus* (AB033702), *Leopoldamys edwarsi* (AJ698881) and *Mus musculus* (J01420) were used as outgroups in all analysis.

2.2. DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from muscle tissues preserved in 95% ethanol using the DNeasy Tissue Kit (Qiagen). A 1.2 kb fragment of the cyt *b* gene was amplified for each individual by two universal cyt *b* primers: L14724 (5′-CGAAGCTTGATATGAAAAACC TCGTTG-3′) (Paäbo and Wilson, 1988) and H15915R (5′-GGAATT CATCT CTCCGGT TTACAAGAC-3′) (Irwin et al., 1991). In order to obtain complete sequences of the cyt *b* gene, a region of 800 bp was also amplified using primers: L15162 (5′-GCAAGCTTC TACCATGAGGACAAA TATC-3′) (Irwin et al., 1991) and H15915R.

Table 1
Species included in this study with common name, sample codes, sample localities and GenBank accession numbers for the cyt *b* sequences

Species	Common name	Sample codes	Sample localities	Accession No.
N. andersoni	Anserson's white-bellied rat	002	Qinglin, Meixian, SX	EF053003
		003	Mount YuLong, Lijiang, YN	EF053001
		04047	Mount Ailao, Jingdong, YN	EF053002
N. brahma	Brahma white-bellied rat	001	Mount Gaoligong, Lushui, YN	EF053010
		GLGS017	Mount Gaoligong, Lushui, YN	EF053011
N. confucianus	Chinese white-bellied rat	04093	Mount Qinglin, Ningqiang, SX	EF053020
		04094	Mount Qinglin, Ningqiang, Sx	EF053021
		05042	Mount Ailao, Jingdong, YN	EF053022
		05044	Mount Ailao, Jingdong, YN	EF053023
N. coxingi	Taiwan white-bellied rat	9605	Yunlin, TW	EF053024
		9608	Yunlin, TW	EF053025
		Yu675	Gaoxiong, TW	EF053026
N. cremoriventer	Dark-tailed tree rat	WLS011	Mount Wuliang, Jingdong ,YN	EF053030
		WLS022	Mount Wuliang, Jingdong, YN	EF053032
		WLS023	Mount Wuliang, Jingdong ,YN	EF053031
N. culturatus	Oldfield white-bellied rat	Yu1024	Nantou, TW	EF053027
		Yu926	Gaoxiong, TW	EF053029
		Yu1154	Nantou, TW	EF053028
N. eha	Smoke-bellied rat	GLGS103	Mount Goaligong, Gongshan, YN	EF053018
		GLGS104	Mount Goaligong, Gongshan, YN	EF053019
N. excelsior	Large white-bellied rat	05029	Mount Ailao, Jingdong, YN	EF053007
		05030	Mount Ailao, Jingdong, YN	EF053004
		05043	Mount Ailao, Jingdong, YN	EF053005
		05070	Mount Ailao, Jingdong, YN	EF053006
		GLGS065	Mount Gaoligong, Lushui, YN	EF053008
		GLGS066	Mount Gaoligong, Lushui, YN	EF053009
N. fulvescens	Chestnut white-bellied rat	WLS001	Mount Wuliang, Jingdong, YN	EF053012
		WLS002	Mount Wuliang, Jingdong, YN	EF053013
		WLS005	Mount Wuliang, Jingdong, YN	EF053014
		05019	Mount Ailao, Jingdong, YN	EF053015
		05069	Mount Ailao, Jingdong, YN	EF053016
		GLGS034	Mount Gaoligong, Lushui, YN	EF053017
Rattus rattus	Black rat		Miyazaki, Japan	**AB033702**
Leopoldamys edwarsi	Edwards rat		Vietnam	**AJ698881**
Mus musculus	House mouse			**J01420**

Note. YN, Yunnan; SX, Shanxi; TW, Taiwan.

All PCRs were carried out in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems) with 50 μl reaction volume, including 36.7 μl of sterile distilled water, 5 μl of $10\times{}^{EX}$Taq buffer (Mg^{2+} Free; Takara Biotech), 4 μl dNTPs mix (a 2.5 mM concentration of each dNTP), 3 μl $MgCl_2$ (25 mM), 1μl of each primer (10 μM) and 0.3 μl ^{EX}Taq polymerase (5 U/μl, Takara Biotech), and approximately 20–50 ng total genomic DNA. PCR reaction was composed of 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 90 s, plus a final extension at 72 °C for 10 min. Each round of PCR reaction also included one negative control to check for contamination. The PCR products were stored at 4 °C until purification and sequencing.

PCR products were purified with a gel extraction kit (Sangon BioMedical). Double-stranded PCR products were directly sequenced from both directions with an ABi 3100 automatic sequencer (Perkin–Elmer) using the ABi PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (with AmpliTaq DNA polymerase FS, Applied Biosystems). The inadvertent amplification and possible inclusion of nuclear pseudogene sequences was checked by observing if the obtained sequences translated properly, that is, whether they possessed conventionally positioned start and stop codons, and no false stop codons, insertions or deletions.

2.3. Data analyses

All sequences were aligned using the DNASTAR software package 5.0 (DNASTAR) and manually confirmed. Parameters (variable sites, parsimony informative sites and base composition biases) were obtained with Mega 3.1 (Kumar et al., 2004). We performed a wide array of phylogenetic analyses using different methods to gauge the robustness of the trees. These methods were maximum parsimony (MP) and maximum likelihood (ML) as implemented in PAUP* Version 4.0 b10 (Swofford, 2002), and a Bayesian (BI) approach as implemented in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). Likelihood ratio tests (Goldman, 1993a,b; Huelsenbeck and Crandall, 1997), as implemented in MODELTEST 3.7 (Posada and Crandall, 1998), were employed to choose models for model-based methods (ML analysis and Bayesian analysis). The TrN + I + G model was selected. The ML method was then performed with a heuristic search and random addition of sequences as implemented in PAUP* 4.0b10 (Swofford, 2002), with starting tree obtained via stepwise addition of taxa, and then swapped using the tree-bisection-reconnection (TBR) algorithm. The MP trees were constructed by using 100 repetitions of random sequence additions of taxa, starting trees obtained by step-wise addition, and branches swapped using the TBR option. Supports for branches in the MP trees were tested by bootstrap analysis with 1000 replicates. For the Bayesian procedure, four independent MCMC chains were simultaneously run for 4,000,000 replicates by sampling one tree per 1000 replicates. We discarded the first 1000 trees as part of a burn-in procedure, and used the remaining 3000 sampling trees (of which log likelihoods converged to stable values) to construct a 50% majority rule consensus tree.

2.4. Molecular dating

We compared log likelihood scores of trees with ($-\ln L = 6472.0603$) and without ($-\ln L = 5502.1724$) the molecular clock enforced from PAUP. A significant difference was observed between them, where p values were less than 0.05. The divergence time between species were estimated through Bayesian molecular dating using PAML/MULTIDIVTIME developed by Kishino et al. (2001), Thorne et al. (1998) and Yang and Yoder (2003).

The molecular dating was run in three steps. First, the program BASEML calculated parameters of transition/transversion rate ratio and rate heterogeneity among sites. Second, the program ESTBRANCHES recalculated the branch lengths of the constrained topology and the corresponding variance–covariance matrix from amino acid data sets. Third, the program MULTIDIVTIME used this variance–covariance matrix to run a Markov chain and calculate divergence times of nodes 95% confidence intervals (CI). After a ''burn-in'' stage of 100,000 cycles, the Markov chain was sampled 100,000 times every 100 cycles. We ran this step three times and did not observe significant difference, and so we chose result randomly.

A fossil based calibration point was used for estimating divergence time. Among the extant species, *N. confucianus* has reliable fossil records. The ancestor of *N. confucianus* was *N. preconfucianus* from the early Pleistocene site of Zhoukoudian (1.2 Mya), Beijing (Cheng et al., 1995). The latest fossil records of *N. confucianus* was from Panxian Dadong, Guizhou Province, a middle-late Pleistocene (0.30–0.13 Mya) cave in south China (Bekken et al., 2004; Schepartz et al., 2003). For this study, we took the middle-late Pleistocene (1.2–0.13 Mya) fossil records of *N. confucianus* as a calibration point to infer divergence time for the different lineages of *Niviventer*.

3. Results

3.1. Description of data

The 32 complete cyt *b* sequences (1143 bp) were aligned together with three outgroups: *Rattus rattus*, *Leopoldamys edwarsi* and *Mus musculus*. No insertion/deletion and stop codons were observed. As expected, all sequences began with the conserved initiating methionine codon ATG and there was no complete stop codon. These sequences were translated according to the vertebrate mitochondrial genetic code to the expected 380 amino acids. Additionally, PCR did not produce more than one band or bands of different sizes. Thus, no nuclear mitochondrial pseudogenes were included in our analysis (Zhang and Hewitt, 1996).

Of the 1143 characters, 394 were variable across all samples; 329 of these were phylogenetically informative. Exclusion of outgroup taxa (*Rattus rattus*, *Leopoldamys edwarsi* and *Mus musculus*) reduced number of variable and phylogenetically informative characters to 340 and 322, respectively. Nucleotide composition was similar to those reported for the majority of mammals (Irwin et al., 1991). In this study, guanines (12.5%) occurred less than adenine (29.6%), cytosine (28.7%) and thymine (29.2%). Levels of sequence variation based on uncorrected pairwise distance were summarized in Table 2, as calculated using Mega 3.1 (Kumar et al., 2004). Sequence divergence ranged from 4% to 16.7% between species. These values are within the range of variation observed between congener species for mammals (Avise et al., 1998; Bradley and Baker, 2001; Johns and Avise, 1998). In contrast, very low sequence divergences were observed within species, such as *N. coxingi* (0.4%) and *N. excelsior* (1%) (Table 2).

3.2. Phylogenetic analysis

Fig. 2 shows a 50% majority rule maximum likelihood tree constructed from a set of 35 cyt *b* sequences. Maximum parsimony and Bayesian analysis yielded the same topology (trees not shown). Based on hierarchical likelihood ratio tests, the best model selected was the TrN + I + G model with a proportion of invariable sites and α distribution corrected heterogeneity. Parameters of the TrN + I + G model contained estimated base frequencies (A = 0.3173; C = 0.3164; G = 0.0949; T = 0.2714) and the nucleotide substitution rate matrix (A–C = 5.3238; A–G = 15.3472; A–T = 5.6628; C–G = 1.0078; C–T = 55.9670; G–T = 1.000). The proportion of invariables sites was estimated to be 0.567 and the shape of the α parameter was 0.991. The heuristic search produced a single phylogenetic tree with a negative log likelihood score ($-\ln L$) of 6472.06.

All three trees support the monophyletic state of *Niviventer* with high bootstrap values (79 in ML, 0.85 in BI, 100 in MP). Three major clades within the *Niviventer* were identified (Fig. 2). Clade A contains six species: *N. confucianus*, *N. culturatus*, *N. coxingi*, *N. fulvescens*, *N. eha* and *N. brahma*. Clade B comprises two species endemic to mainland China: *N. andersoni*, *N. excelsior*. Clade A and B are sister-groups including eight of the nine species. Both clade A and clade B are monophyletic supported by high bootstrap values (Fig. 2). Clade C has only one species: *N. cremoriventer*.

3.3. Divergence time estimations

Divergence times were estimated using the early to middle-late Pleistocene (1.2–0.125 Mya) fossil records of *N. confucianus* as calibration point. Transversion rate was 0.0238 per Myr. The divergence time of each node were given in (Table 3). The fossil record of *N. confucianus* in mainland China occurs in a layer dated the early Pleistocene (Cheng et al., 1995). The first separation within *Niviventer* occurred in early Pleistocene (1.644 Mya), suggesting that the upper bound of the value obtained may represent an overestimation. Clade A and clade B were separated in late Pleistocene (1.462 Mya). Within clade A, *N. brahma* originated at late Pleistocene (1.285 Mya), while *N. coxingi*, *N. fulvenscens*, *N. eha*, *N. confucianus* and *N. culturatus* originated at late Pleistocene (1.104–0.667 Mya). Species in clade B (*N. excelsior* and *N. andersoni*) split from each other 1.169 million year ago.

4. Discussion

4.1. Systematic review

In this study, 32 specimens representing nine recognized *Niviventer* species were collected from the southeastern shoulder of the Tibetan Plateau of mainland China and Taiwan. The samples were subjected to molecular systematic analysis using the complete mitochondrial cyt *b*. Based on MP, ML and BI analyses, three major evolutionary lineages were recognized within the specimens. Clade A (*N. confucianus*, *N. culturatus*, *N. coxingi*, *N. fulvescens*, *N. eha* and *N. brahma*) and clade B (*N. andersoni* and *N. excelsior*) are sister groups, while clade C (*N. cremoriventer*) is

Table 2
Uncorrected pairwise distance (P-distance) among the nine Chinese *Niviventer* species based on the complete cyt *b* sequences

Taxa	1	2	3	4	5	6	7	8	9
N. brahma	**0.047**								
N. andersoni	0.146	**0.033**							
N. confucianus	0.119	0.126	**0.013**						
N. fulvescens	0.113	0.094	0.081	**0.011**					
N. excelsior	0.149	0.072	0.134	0.139	**0.010**				
N. coxingi	0.140	0.141	0.097	0.099	0.138	**0.004**			
N. eha	0.110	0.104	0.084	0.040	0.140	0.087	**0.019**		
N. cremoriventer	0.153	0.159	0.152	0.167	0.147	0.162	0.161	**0.005**	
N. culturatus	0.154	0.154	0.119	0.147	0.140	0.140	0.151	0.149	**0.002**

Note. The bold values on the diagonal indicated sequence diversity within species.

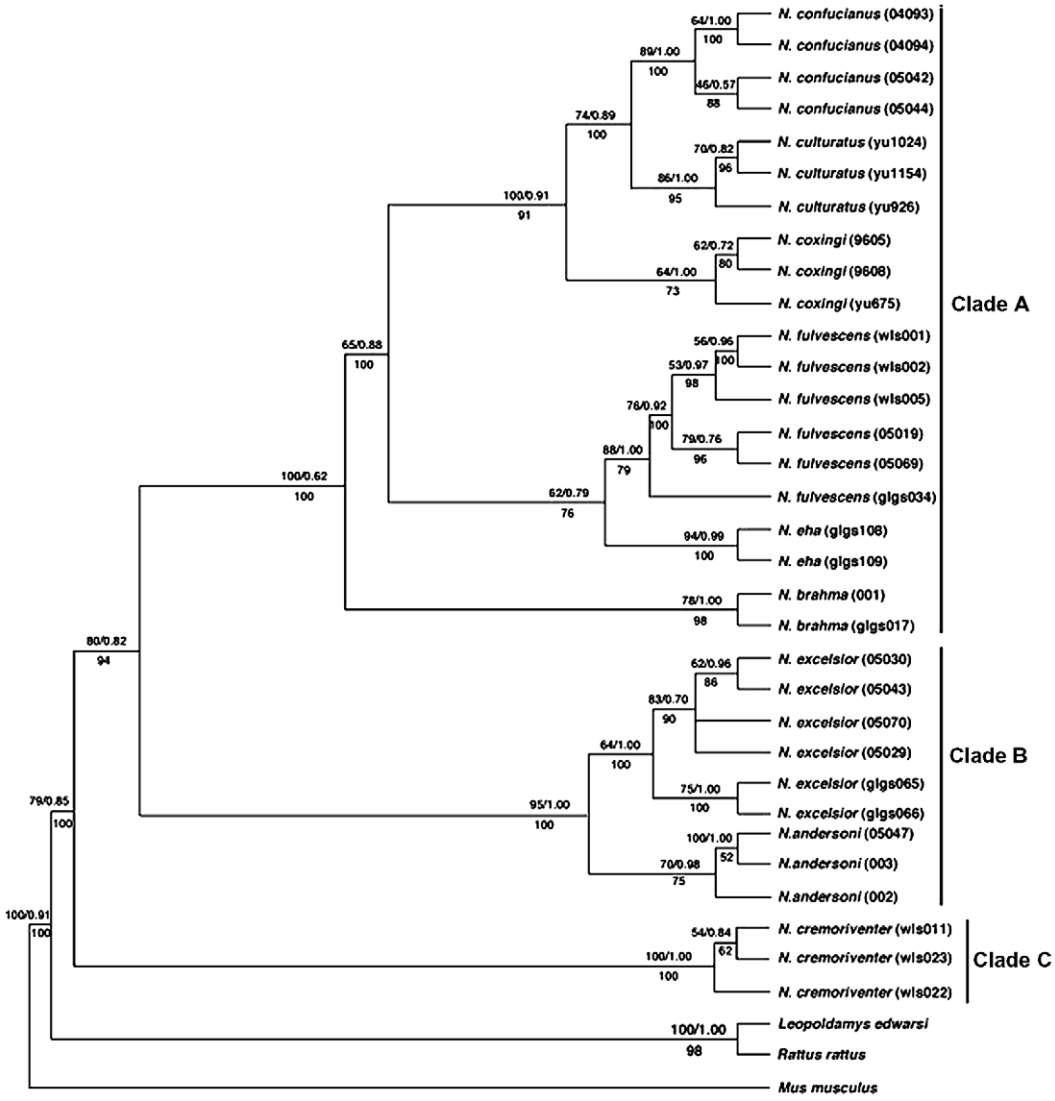

Fig. 2. Maximum likelihood tree ($-\ln L = 6472.0603$) using TrN + I + G model (see text). The numbers above the branches is the bootstrap values of Maximum likelihood (ML) and Bayesian inference (BI), respectively. Maximum parsimony (MP) bootstrap values are shown under the branches.

Table 3
Estimates of divergence times (expressed in million of years) and credibility intervals at 95% (CI)

Nodes	Posterior divergence time			
	Date	95% CI	Date	95% CI
Clade C/Clade A + B	1.644	0.050–5.804	3.821	1.435–8.153
Clade A/Clade B	1.462	0.044–5.266	3.531	1.312–7.550
brahma/confucianus + culturatus + coxingi + fulvescens + eha	1.285	0.039–4.700	3.226	1.194–6.976
confucianus + culturatus + coxingi/fulvescens + eha	1.104	0.032–4.130	2.900	1.043–6.280
confucianus + culturatus/conxingi	0.889	0.023–3.418	2.591	0.908–5.710
confucianus/culturatus	0.667	0.016–2.710	2.416	0.833–5.376
eha/fulvescens	0.926	0.025–3.574	2.641	0.928–5.819
excelsior/andersoni	1.169	0.034–4.346	2.635	0.899–5.832

the basal lineage (Fig. 2). The monophyletic states of both clades A and B were supported by high bootstrap values.

According to previous studies (Musser, 1981), the *Niviventer* was separated into two primary divisions based on morphological characters: the *N. andersoni* division (*N. andersoni* and *N. excelsior*) and the *N. niviventer* division (the remaining 13 species). Species in the *N. andersoni* division are endemic to the high mountains along the eastern edge of the Tibetan Plateau and the Himalayas (Musser and Chiu, 1979). The *N. niviventer* division has two complexes: the *niviventer* complex (*N. brahma*, *N. eha*, *N. langbianis*, *N. hinpoon* and *N. cremoriventer*) and other species

(*N. niviventer*, *N. confucianus*, *N. tenaster*, *N. fulvescens*, *N. coxingi*, *N. rapit*, *N. lepturus* and *N. bukit*). However, our results are different from Musser's results (1981). In our three trees, clade A and clade B are sister-groups, supported by high bootstrap values (95 of ML, 1.00 of BI and 100 of MP). Clade B (bootstrap values 95, 1.00 and 100, respectively) contains *N. excelsior* and *N. andersoni* which is endemic to the mountain regions of western China, consistent with the *Niviventer andersoni* division. Clade C only has one species: *N. cremoriventer*, which was classified in the *niviventer* complex previously (Musser, 1981). Clade A comprises the other six species belonging to the *N. niviventer* division.

4.2. Taxonomic status of *N. culturatus*

In previous studies, *N. culturatus* was identified as a subspecies of *N. confucianus* (Allen, 1940; Ellerman and Morrison-Scott, 1966; Musser, 1981). Musser and Carleton (1993) listed it as a distinct species based on morphological data. Though the morphological characters of *N. culturatus* resemble those of mainland China *N. confucianus*, the differences between them are sufficient enough to suggest a valid species status of *N. culturatus* (Musser and Carleton, 1993). They also pointed out that the relationships between the two species need to be assessed in a systematic revision of the genus. In our molecular phylogeny, *N. confucianus* and *N. culturatus* are sister species supported by high bootstraps values (74, 0.89, 100), and the P-distance between them is 0.119 (Table 2). According to the concept of DNA-based taxonomy (Blaxter, 2004; Tautz et al., 2002, 2003), the genetic distance and phylogenetic tree validate that *N. culturatus* is a distinct species, which is consistent with the view of Wang (2003) and Yu (1994, 1995).

4.3. Evolutionary history of genus Niviventer and its correlation with geological events

Five species (*N. andersoni*, *N. brahma*, *N. cremoriventer*, *N. eha* and *N. excelsior*) in *Niviventer* from mainland China occurred in the Trans-Himalayan region (Fig. 1). *N. confucianus* is a common and adaptive species distributed all over China in a wide spectrum of habitats from forests to farmlands. *N. fulvescens* is found in south China (Musser, 1981; Corbet and Hill, 1992, Fig. 1). *N. culturatus* and *N. coxingi* are endemic to Taiwan. *N. coxingi* lives in regions no higher than 2000 m and *N. culturatus* inhabits on forested slopes between 2000 and 3600 m (Yu, 1994). The relationships between mainland China species and Taiwan species have never been investigated. Among the remaining six species that do not occur in China, three species (*N. lepturus*, *N. langbianis* and *N. niviventer*) are distributed in Assam and Pakistan; two species (*N. bukit* and *N. hinpoon*) are distributed in Thailand; *N. rapit* is distributed in Malaya, Sumatra and Borneo (Corbet and Hill, 1992). So far little is known about the molecular systematics of these six species. Further phylogenetic analyses including these six species will improve our understanding of the relationships among species of the whole genus.

The present Trans-Himalayan Range includes various north–south extending ranges and adjacent mountainous areas on the eastern skirts of the Tibetan Plateau. The geological configuration of this area is complicated, and it is composed of several heterogeneous landform assemblages. Three main areas are generally defined: the western high mountain and gorge area, the northeastern piedmont plain-gorge area and the southeastern plateau-lake basin area. The first two areas belong to the Tibetan Plateau while the third one is a part of the Yunnan-Guizhou Plateau (Li and Wang, 1986; Luo et al., 2004). Geological studies indicated that the uplift events of the Tibetan plateau occurred most intensely and frequently from 3.6 to 1.6 Mya: the first uplift occurred at 3.6 Mya, the second at 2.6 Mya and the third at 1.6 Mya. After the uplift events, the environments of this area were complicated by two factors: the Kun-Huang movement took place (between 1.1 and 0.6 Mya) and the Plateau underwent the glaciation events (Paillard, 1998; Shi et al., 1998; Sun and Zheng, 1998).

Molecular clock indicated that the first diversification of *Niviventer* species happened at the early Pleistocene (1.64 Mya, Table 3), which was followed by the last uplift events of the Tibetan Plateau (Shi et al., 1998; Sun and Zheng, 1998). The large-scale uplifts caused strong orogenic movement, including the formation of the Trans-Himalayan Range. Most extant species of *Niviventer* from mainland China occurred in the Trans-Himalayan region (Fig. 1). Clade A and clade B diverged at about 1.46 Mya, which was consistent with the time of transition between the last uplift events of the Tibetan plateau and Kun-Huang movement. Interestingly, the clade B was endemic to the Trans-Himalayan Range (Musser and Chiu, 1979, Fig. 1). The distribution of extant species of *Niviventer* implied the close correlation between the last uplift event of Tibetan Plateau and the early diversification of this genus. Our data suggested that most extant Chinese species in *Niviventer* diverged during 1.285–0.667 Mya (Table 3), and there was a rapid species radiation in the middle-late Pleistocene. This period was characterized by Kun-Huang movement and large-scale of glaciation (Paillard, 1998; Shi et al., 1998; Sun and Zheng, 1998).

N. culturatus and *N. coxingi* diverged at about 0.667 and 0.889 Mya, respectively. These two species are endemic to Taiwan, which became an island approximately 3–5 million years ago (Teng, 1987; Shaw, 1996). Sea level alteration caused by glaciations affected the pattern of connections between mainland China and Taiwan, especially in the Pleistocene (Lin and Zhou, 1974). In some occasions, glaciations would have lowered the sea level and connected Taiwan with mainland China through the presence of a land bridge. When sea levels rose again, the land bridge would have been disrupted. In most cases, terrestrial animal species with poor oversea dispersal abilities could only disperse by dry lands, rafting or introduction by humans

(Lin et al., 2002; Matisoo-smith and Robins, 2004). The periodic formation of a land bridge during glaciations offered the opportunities to disperse from mainland China to Taiwan.

However, when the glaciations ended, the rising of sea level would result in vicariant isolation. The phylogenetic positions of *N. culturatus* and *N. coxingi* support a vicariant speciation model. We speculate that *N. culturatus* most likely arose due to allopatric speciation as populations of *N. confucianus* were isolated on Taiwan by rising sea levels. We also presume that *N. coxingi* could have diverged from an ancestral population of *N. fulvescens* due to similar vicariant processes. Modern *N. fulvescens* is mainly distributed in south China (Fig. 1), and fossils have been found in a Pleistocene layer (0.73 Mya) in Fujian province (You and Cai, 1996). In summary, vicariant speciation could be the major mechanism for the formation of these two island-endemic species.

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