

RESEARCH ARTICLE

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Geographic variation of the large-eared field mouse (*Apodemus latronum* Thomas, 1911) (Rodentia: Muridae) with one new subspecies description verified via cranial morphometric variables and pelage characteristics

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Abstract

Background: The large-eared field mouse (*Apodemus latronum* Thomas, 1911), a common rodent, mainly inhabits southwestern China. Since its first description nearly a century ago, there have been numerous taxonomical and morphological arguments as to its validity, but relatively little work was done on mapping out the geographic variations observed in samples of the species. In this study, we used 142 specimens of *A. latronum* from Xizang, Sichuan, and Yunnan to conduct a multivariate analysis, coefficient of difference (CD) analysis of cranial measurements, and a comparison of some pelage characteristics.

Results: The results of the analysis on 15 measurable cranial characters indicated that the specimens from Lijiang, Weixi, and Binchuan areas of the Yunnan province are apparently different from all the other specimens of *A. latronum* described so far and are allopatrically distributed.

Conclusions: These samples form the core of our new description for *A. latronum lijiangensis* subsp. nov. as a new subspecies, and a detailed discussion on the relationships between the differentiation of *A. latronum* and its evolvement in southwestern China is provided.

Keywords: New subspecies; Geographic variation; Statistic analysis; *Apodemus latronum*; Morphometry

Background

The large-eared field mouse (*Apodemus latronum* Thomas, 1911) mainly inhabits southwestern China, including both Sichuan and Yunnan provinces as well as southeastern Tibet and northern Burma (Corbet and Hill 1992; Musser and Carleton 2005). The species was first named *Apodemus speciosus latronum* (Thomas 1911) based on an adult male specimen from Tatsienlu, Szechwan (modern-day Kangding, Sichuan province), and due to its large body size and big blackish ears, Osgood (1932) recognized

it as a species that might have been related to *A. flavicollis* (Melchior, 1834), and Allen (1940) also accepted the naming as *A. latronum* in subgenus *Sylvaemus*. Ellerman (1941), however, thought it should be *A. speciosus latronum* in the *speciosus* group of *Apodemus*, and later, Ellerman and Morrison-Scott (1950) regarded it as *A. flavicollis latronum*. Zimmermann (1962) then identified it as a valid species, and Corbet (1978) accepted this result. Nearly a decade later, Feng et al. (1986) rearranged it as *A. draco latronum*, followed by arguments from Corbet and Hill (1992) and Musser and Carleton (1993), all of whom insisted on the earlier result of Zimmermann (1962). This finding was later supported by Huang et al. (1995) and Musser et al. (1996) who went on to expatiate on the validity of *A. latronum* with morphological or published genetic evidence (Suzuki et al. 2003). Finally, both

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Wang (2003) and Musser and Carleton (2005) listed it as *A. latronum*.

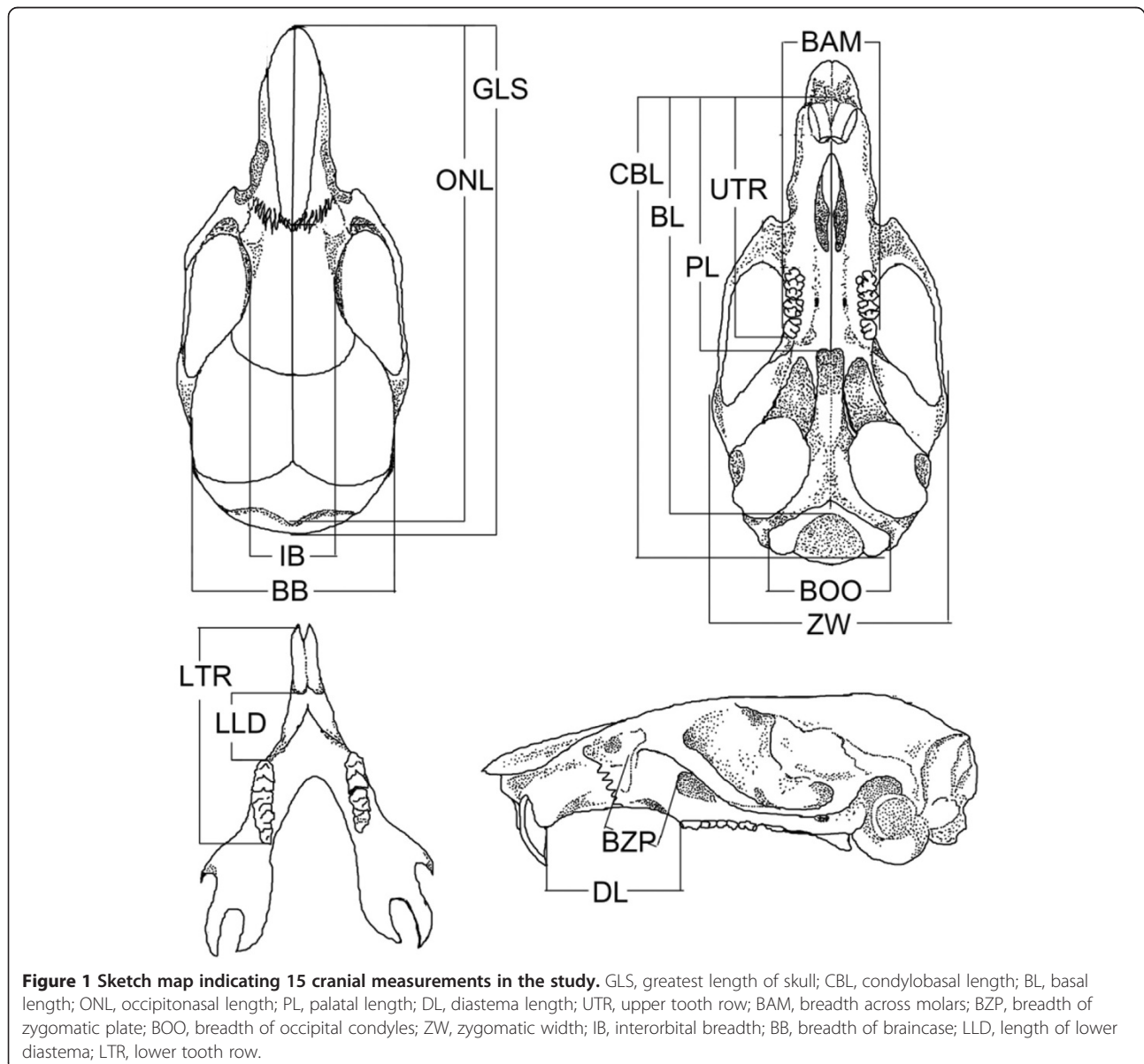
After so many arguments on its taxonomic status and the subsequent genetic studies to clarify any inconsistencies, there has been no recent disagreement as to the status of *A. latronum* as a valid species. Interestingly though, there have been no further follow-up morphological studies conducted to obtain a more well-rounded view of this species, especially in regard to measureable cranial variables or comparisons of pelage characteristics as it pertains to the geographic variation of *A. latronum*. In this study, we opted to conduct a typical morphometric analysis of both morphometrics and related statistical analyses on the skull of *A. latronum*, and comparison on their pelage characteristics is performed in order to study

their geographic variation. Additionally, we also discussed the relationships between the differentiation of *A. latronum* and its environment in southwestern China.

Methods

Data collection

The specimens used in the study were obtained from Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences (KIZ, CAS) (Kunming, China), Institute of Zoology, Chinese Academy of Sciences (IOZ, CAS) (Beijing, China), and Sichuan Academy of Forestry (SAF) (Chengdu, China). The numbers and collection localities of specimens examined in the study are listed in detail in the Appendix.



A total of 142 specimens were studied. Excluding four specimens that have no noted sexual record, the remaining 138 specimens include 64 male samples and 74 female samples. All specimens used in this study are considered to have been adults at the time of their collection, because it was evident from examination that molar eruption had finished, and the grinding surface of M² appears as a longitudinal worn dentine link (Lu et al. 1987). Totally, 15 cranial measurements were taken with a digital caliper at the greatest possible accuracy (0.01 mm) as follows: greatest length of skull (GLS), condylobasal length (CBL), basal length (BL), occipitonasal length (ONL), palatal length (PL), diastema length (DL), upper tooth row (UTR), breadth across molars (BAM), breadth of zygomatic plate (BZP), breadth of occipital condyles (BOO), zygomatic width (ZW), interorbital breadth (IB), breadth of braincase (BB), length of lower diastema (LLD), and lower tooth row (LTR) (Figure 1). In addition, four external measurements were taken from the initial accession labels attached to the skin: head and body length (HB), tail length (TL), hind foot length (HFL), and ear length (EL). Since these measurements were taken by a variety of different collectors who likely used different tools and methodologies, these four metrics were not included either in the multivariate or coefficient of difference (CD) analyses.

Data analysis

Following the aforementioned data collection, the 15 cranial measurements we took were log-transformed and analyzed for sexual dimorphism (for the 138 samples with known sex records). After which, principal component analyses (PCA) without assuming a prior group was used to identify the various groupings of the samples. The groups identified via PCA were then assigned with names; then, multiple comparisons between the groups were performed for all 15 cranial variables, and the coefficient of difference analysis (CD) (Mayr 1969) between the groups was calculated using the following equation: $CD = (Mb - Ma) / (SDa + SDb)$, where *Mb* is the mean of population b, *Ma* is the mean of population a, *SDa* is the standard deviation of population a, and *SDb* is the standard deviation of population b. The principal component analyses and multiple comparisons were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

Pelage comparisons

Pelage characteristics are well known to change during different seasons, and as such, it can be difficult to make comparisons between samples taken at different times of the year. In order to eliminate the influence of seasonal pelage characteristic variation, we selected samples with the same collection date of topotype of *A. latronum latronum* and the type of *A. latronum lijiangensis* to compare the pelage characteristics.

Table 1 Tests of equality of group means by gender

Variables	Wilks' Lambda	F	d.f. 1	d.f. 2	p
GLS	0.961	5.543	1	135	0.020*
CBL	0.959	5.825	1	135	0.017*
BL	0.963	5.168	1	135	0.025*
ONL	0.976	3.352	1	135	0.069
PL	0.970	4.212	1	135	0.042*
DL	0.974	3.653	1	135	0.058
UTR	0.966	4.793	1	135	0.030*
BAM	0.998	0.244	1	135	0.622
BZP	1.000	0.003	1	135	0.954
BOO	0.964	5.037	1	135	0.026*
ZW	0.979	2.834	1	135	0.095
IB	0.993	1.009	1	135	0.317
BB	0.969	4.358	1	135	0.039*
LLD	0.998	0.294	1	135	0.589
LTR	0.950	7.101	1	135	0.009*

The variable codes are given in the text and in Figure 1. Asterisk means significant difference.

Table 2 Factor loadings and percentage of variance explained for principal component analysis on *A. latronum* male samples

Variables	Principal component (PC)		
	1	2	3
GLS	0.907	0.303	0.168
CBL	0.924	0.260	0.190
BL	0.929	0.225	0.193
ONL	0.914	0.244	0.171
PL	0.866	0.345	0.228
DL	0.899	0.233	0.237
UTR	0.889	0.345	0.221
BAM	0.662	0.458	0.185
BZP	0.734	0.367	-0.307
BOO	0.276	0.643	0.218
ZW	0.749	0.487	0.179
IB	0.156	0.783	-0.133
BB	0.343	0.766	0.151
LLD	0.258	0.006	0.853
LTR	0.710	0.216	0.461
Eigenvalues	10.24	1.23	0.86
Variance explained (%)	68.26	8.21	5.76

The variable codes are given in the text and in Figure 1. The extraction method used is the principal component analysis. The rotation method is Varimax with Kaiser Normalization.

Results

Sexual dimorphism and principal component analyses

Of the 142 total samples that were used for cranial measurements, 138 contained sexual information on their labels, with 64 male specimens and 74 female specimens (one without skull). The results from the Tests of Equality of Group Means indicated that 8 of the measured 15 cranial variables exhibited significant differences ($p < 0.05$) between males and females (Table 1). Since 8 out of 15

cranial variables show significant differences and some marked sexual dimorphism, we conducted principal component analyses and found that among the male group ($N = 64$), the eigenvalues for the first three principal components were 10.24, 1.23, and 0.86, respectively, thereby accounting for 82.22% of the total variance. Most of the measured characteristics had high positive loadings on the first principal component, suggesting that this component (68.26% of the total variance) represents size

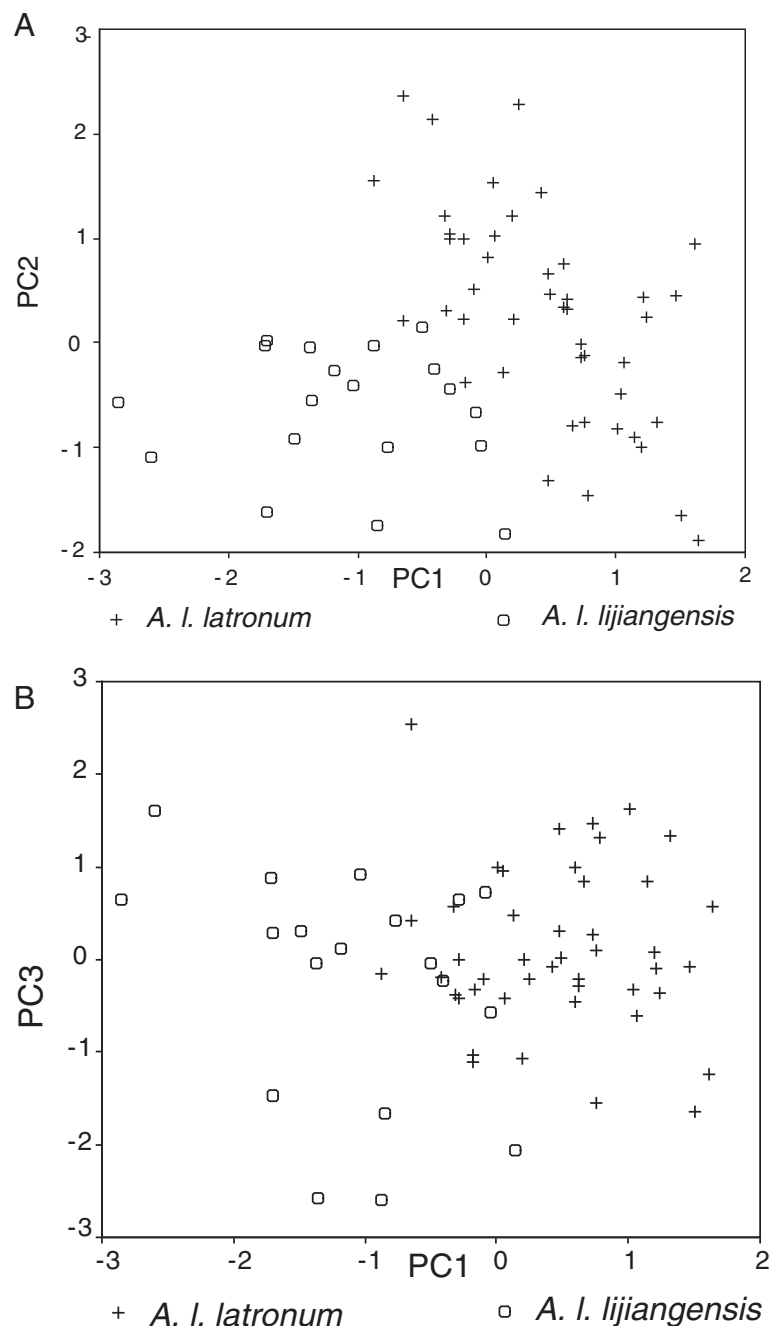


Figure 2 Regional distribution of *A. latronum* male samples by plotting on principal components factors. (A) PC1 by PC2. (B) PC1 by PC3.

variation among the samples. The second principal component (8.21% of variance) was strongly correlated with IB and BB (factor loadings > 0.70), while the third principal component (5.76% of variance) is correlated primarily with LLD (factor loadings > 0.70) (Table 2). Figure 2A,B shows the plots of *A. latronum* male samples on principal component factors 1 vs. 2 and 1 vs. 3, respectively.

Similar results were found among the female group. For the 73 skull specimens in the female group, The PCA analysis showed that the eigenvalues for the first three principal components were 11.49, 0.89, and 0.67, respectively, accounting for 86.93% of the total variance. Most characteristics had high positive loadings on the first principal component, suggesting that this component (76.57% of the total variance) likewise represents size variation, while the second principal component (5.92% of variance) was also strongly correlated with LLD (factor loadings > 0.70); the third principal component (4.44% of variance) was correlated primarily with IB (factor loadings > 0.70) (Table 3). Scatter plots of *A. latronum* female samples on PC1 by PC2 and PC1 by PC3 are shown in Figure 3A,B, respectively.

The collective results from both the male and female groups together (Figures 2 and 3) indicate that the samples

are actually composed of two different groups, and the results of multiple comparisons show significant difference between *A. latronum latronum* and *A. latronum lijiangensis* (Table 4); also, sustaining the samples could be distinguished into two different groups by skull variables. Paired with the data on the geographic distributions of the collection localities (Figure 4), we found that the samples from the Lijiang, Weixi, and Binchuan areas of the Yunnan province form a distinct group which we have named (*A. latronum lijiangensis*, subsp. nov.). The other samples from the Sichuan province (including Markam, Baoxing, Yajiang, Luhuo, Baiyu, Batang, Danba, Daocheng, and Muli areas), northwestern Yunnan province (including Xiaozhongdian and Deqin areas), and southeastern Tibet (including Mangkang, Zuogong, and Bomi areas) form the other absolute group (*A. latronum latronum*).

Coefficient of difference analysis

The comparisons on the CD for the 15 cranial measurable variables between the two distinct groups, *A. latronum lijiangensis* subsp. nov. and *A. latronum latronum* (see Table 5) showed that the CDs of GLS, ONL, DL, and UTR are larger than 1.28 between *A. latronum latronum* and *A. latronum lijiangensis*. External and cranial measurements of *A. latronum latronum* and *A. latronum lijiangensis* subsp. nov. are given in Table 6.

Table 3 Factor loadings and percentage of variance explained for principal component analysis on *A. latronum* female samples

Variables	Principal component (PC)		
	1	2	3
GLS	0.792	0.408	0.396
CBL	0.792	0.412	0.409
BL	0.784	0.419	0.411
ONL	0.759	0.427	0.425
PL	0.783	0.410	0.382
DL	0.703	0.556	0.397
UTR	0.832	0.385	0.350
BAM	0.848	0.260	0.121
BZP	0.583	0.291	0.569
BOO	0.722	0.157	0.293
ZW	0.808	0.330	0.350
IB	0.170	0.005	0.931
BB	0.809	0.007	0.107
LLD	0.265	0.917	0.008
LTR	0.794	0.420	0.123
Eigenvalues	11.49	0.89	0.67
Variance explained (%)	76.57	5.92	4.44

The variable codes are given in the text and in Figure 1. The extraction method use is the principal component analysis. The rotation method is Varimax with Kaiser Normalization.

Description of one new subspecies, *Apodemus latronum lijiangensis* Li et Liu, subsp. nov.

Holotype: KIZ 016518, ♂, adult, collected 19 October 2007, from Yulong Snow mountain, Lijiang, Yunnan Prov., China, elevation 3,305 m.

Paratype: KIZ 016549, ♀, adult, collected 19 October 2007, from Yulong Snow mountain, Lijiang, Yunnan Prov., China, elevation 3,305 m.

Specimens examined: 17♂♂, 41♀♀, and one (no sexual recording) from Lijiang, Weixi, and Binchuan areas of the Yunnan province (Appendix).

Etymology: This new subspecies was named according to the type locality.

Diagnosis: General dorsal color is brown, mixed with black more or less, and from the back to the flank, it changes from fawn brown to clay brown. The muzzle and its sides are mainly grayish mixed black, while the forehead, the central area of the crown, and nape fawn brown are thinner, but largely the same as that at the back.

Description: *A. latronum lijiangensis* subsp. nov. exhibits several distinct and apparently fairly stable characteristics: (1) general dorsal color is brown, mixed with black more or less, and from the back center to the flank, it changes from fawn brown to clay brown, apart from *A. latronum latronum*, which is ochraceous or ochraceous buff, clearly; (2) underparts gray at base and tipped with dull white; (3)

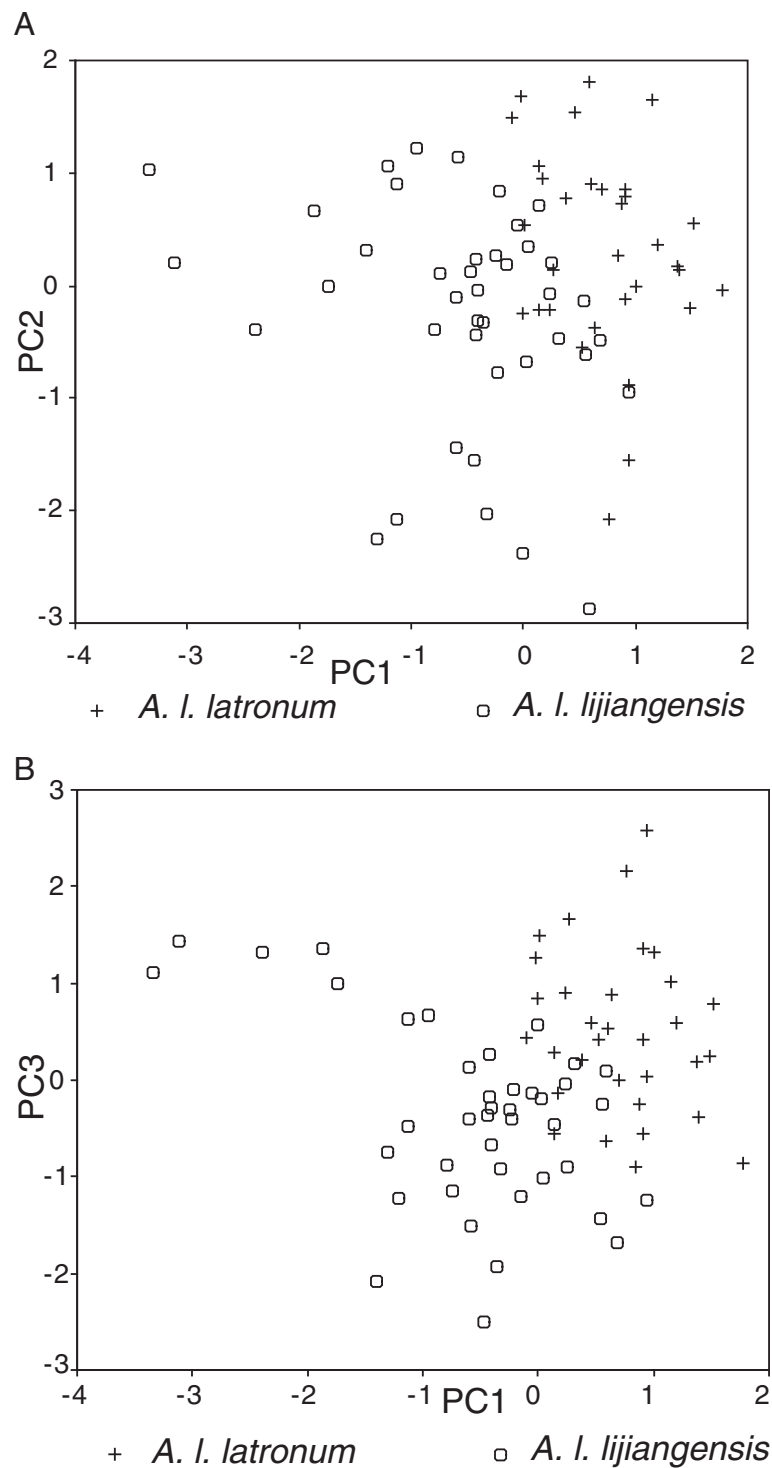


Figure 3 Regional distribution of *A. latronum* female samples by plotting on principal components factors. (A) PC1 by PC2. (B) PC1 by PC3.

the muzzle and its sides mainly grayish mixed black; the forehead, the central area of the crown, and nape fawn brown, just the same as the back, but thinner more or less; apart from *A. latronum latronum*, which the muzzle mainly black brown, and its sides mainly yellow brown;

(4) flanks and sides of cheeks and neck are clay brown; (5) orbits dark brownish; (6) the upper surfaces of the front and hind feet dull white; (7) the tail dark brown above and the undersurface brown in the base but more and more thinner to the twig, apart from *A. latronum latronum*,

Table 4 Multiple comparisons on all 15 cranial variables between *A. latronum latronum* and *A. latronum lijiangensis*

Variables	Mean difference	p
GLS	0.036*	0.000
CBL	0.037*	0.000
BL	0.039*	0.000
ONL	0.034*	0.000
PL	0.037*	0.000
DL	0.049*	0.000
UTR	0.037*	0.000
BAM	0.025*	0.000
BZP	0.052*	0.000
BOO	0.024*	0.000
ZW	0.027*	0.000
IB	0.015*	0.000
BB	0.013*	0.000
LLD	0.024*	0.000
LTR	0.033*	0.000

The variable codes are given in the text and in Figure 1. Asterisk means significant difference.

which the whole tail undersurface deep brown and mixed with black in its twig more or less.

Synonyms: *Apodemus latronum latronum*, Thomas 1911, Abstr. Proc.Zool. Soc. Lond., 100: 49.

Measurements (see Table 7 for external and skull of type specimens of *A. latronum lijiangensis* subsp. nov).

Remarks: This subspecies is known to presently dwell in Lijiang, Weixi, and Binchuan, Yunnan province, and its distribution apparently does not overlap with the other known subspecies. *A. latronum latronum* occurs both in Xiaozhongdian and Deqin of the Yunnan province as well as in southeastern Tibet and Sichuan province. According to the theory of subspecies differentiation (Mayr 1969), when the CD of any parameter of variables within two samples exceeds 1.28, the samples can be regarded as different subspecies. The results of CD analysis indicate that among these 15 variables, 4 of the 15 CDs exceeded 1.28 between *A. latronum lijiangensis* subsp. nov. and *A. latronum latronum* (Table 5). On the pelage characteristics, *A. latronum lijiangensis* subsp. nov. can clearly be distinguished from *A. latronum latronum* by the following combinations of characteristics described above: (1), (3), and (7), further confirming the validity of this

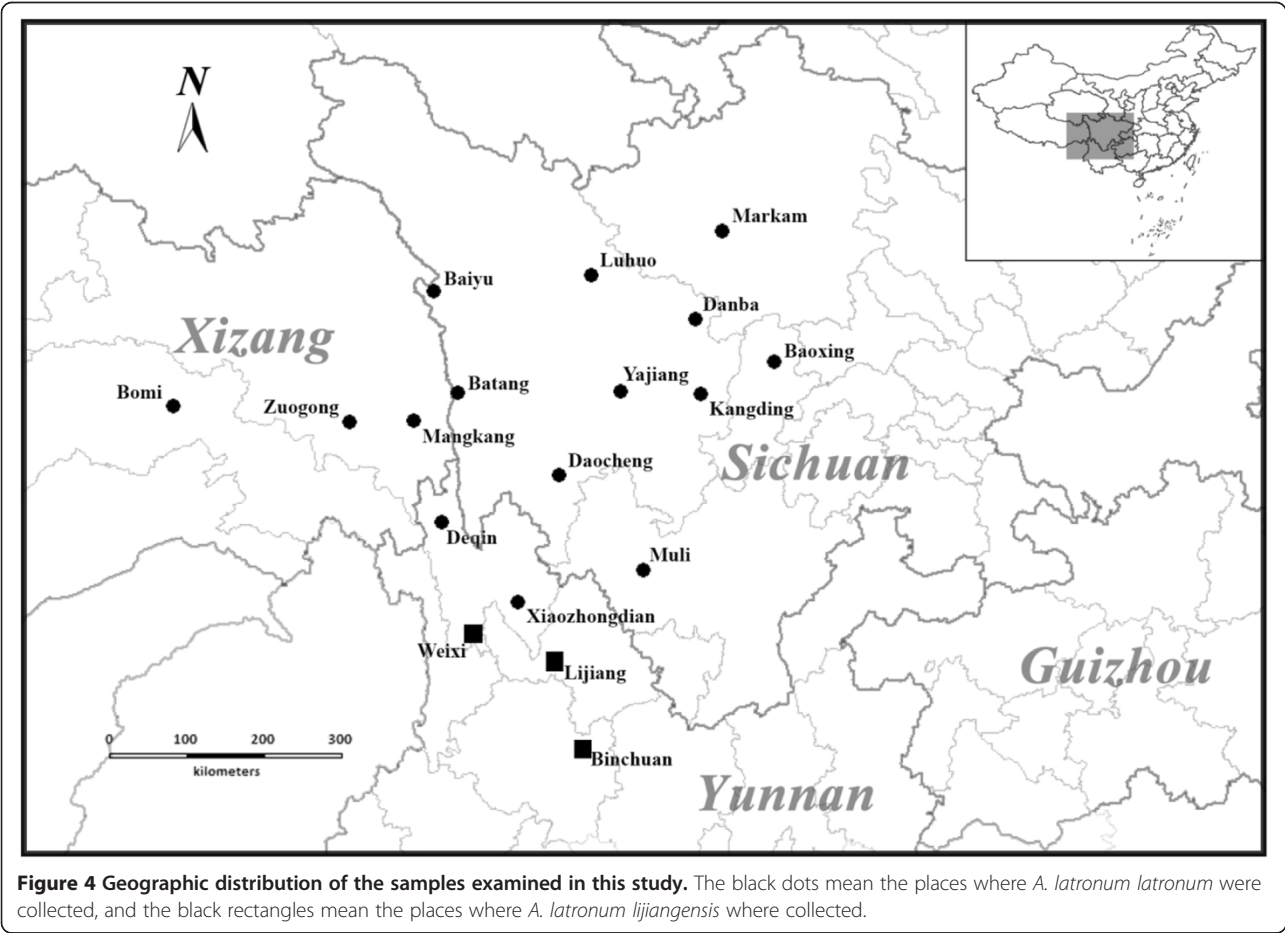


Figure 4 Geographic distribution of the samples examined in this study. The black dots mean the places where *A. latronum latronum* were collected, and the black rectangles mean the places where *A. latronum lijiangensis* were collected.

Table 5 Comparison of coefficient of difference (CD) between *A. latronum latronum* and *A. latronum lijiangensis*, subsp. nov.

Variables	CD
GLS	1.40
CBL	1.27
BL	1.21
ONL	1.29
PL	1.21
DL	1.29
UTR	1.41
BAM	0.85
BZP	0.88
BOO	0.94
ZW	1.04
IB	0.54
BB	0.62
LLD	0.45
LTR	1.02

Italicized values denote those four measurements greater than 1.28. The variable codes are given in the text and in Figure 1.

new subspecies. Figure 5 indicates the pelage differences between *A. latronum lijiangensis* and *A. latronum latronum*.

Discussion

A. latronum has previously been regarded as a valid species (Allen 1940; Zimmermann 1962; Corbet and Hill 1992; Musser and Carleton 1993; Musser et al. 1996; Musser and Carleton 2005), and in 2010, based on molecular data, Sakka et al. (2010) found that 'the phylogeographic pattern observed in *A. latronum* demonstrated strong differentiated populations in the Sichuan and Yunnan regions' (Sakka et al. 2010). In the present study, the results of principal component analyses (Figures 2 and 3) showed that the specimens of *A. latronum* (obtained from 18 sampling areas in southwestern China) cluster into two distinct geographic groups: *A. latronum lijiangensis* subsp. nov. which includes the specimens from Weixi, Lijiang, and Binchuan areas of Yunnan Province and *A. latronum latronum* which includes the specimens from Sichuan (Markam, Baoxing, Yajiang, Luhuo, Baiyu, Batang, Danba, Daocheng, Kangding, and Muli areas), southeastern Tibet (Mangkang, Zuogong, and Bomi areas), and northwestern Yunnan (including Deqin and Xiaozhongdian areas) (Figure 4). The multiple comparisons of all 15 cranial variables also indicate significant difference between

Table 6 Measurements (mm) of external and skull variable measurements (mm) of subspecies of *Apodemus latronum*

Variables	Subspecies	
	<i>A. latronum latronum</i>	<i>A. latronum lijiangensis</i>
HB (n = 139)	102.72 ± 7.72 (85.00~118.00)	90.62 ± 8.44 (74.00~106.00)
TL (n = 136)	108.55 ± 12.32 (90.00~195.00)	94.12 ± 7.45 (78.00~115.00)
HFL (n = 139)	24.30 ± 1.16 (21.00~26.90)	23.25 ± 1.40 (21.00~28.00)
EL (n = 139)	20.33 ± 1.24 (18.00~23.00)	19.51 ± 1.02 (18.00~22.00)
GLS (n = 141)	28.82 ± 0.72 (27.26~30.67)	26.56 ± 0.90 (24.51~28.08)
CBL (n = 141)	25.92 ± 0.77 (24.13~27.90)	23.79 ± 0.92 (21.65~25.30)
BL (n = 141)	23.86 ± 0.79 (22.15~25.67)	21.80 ± 0.91 (19.52~23.33)
ONL (n = 141)	28.18 ± 0.76 (26.30~30.52)	26.06 ± 0.89 (24.31~27.71)
PL (n = 141)	14.44 ± 0.45 (13.42~15.74)	13.27 ± 0.51 (12.31~14.30)
DL (n = 141)	7.64 ± 0.30 (7.01~8.52)	6.83 ± 0.33 (5.99~7.39)
UTR (n = 141)	13.85 ± 0.33 (13.20~14.71)	12.73 ± 0.47 (11.82~13.52)
BAM (n = 141)	5.95 ± 0.19 (5.54~6.37)	5.62 ± 0.19 (4.99~5.90)
BZP (n = 141)	2.96 ± 0.17 (2.60~3.32)	2.63 ± 0.21 (1.85~3.13)
BOO (n = 141)	6.42 ± 0.23 (5.68~6.83)	6.07 ± 0.14 (5.69~6.36)
ZW (n = 141)	13.78 ± 0.39 (12.72~14.63)	12.96 ± 0.40 (11.96~13.60)
IB (n = 141)	4.51 ± 0.15 (4.16~4.90)	4.35 ± 0.14 (4.01~4.68)
BB (n = 141)	12.49 ± 0.26 (11.96~13.33)	12.13 ± 0.30 (11.26~12.61)
LLD (n = 141)	3.97 ± 0.23 (3.43~4.51)	3.76 ± 0.23 (3.12~4.13)
LTR (n = 141)	11.57 ± 0.34 (10.74~12.43)	10.74 ± 0.48 (9.44~11.74)

The variable codes are given in the text and in Figure 1. The values are in mean ± std. dev. with the range given in parentheses. HB, head and body length; TL, tail length; HFL, hind foot length; EL, ear length.

Table 7 Measurements (mm) of external and skull of type specimens of *Apodemus latronum lijiangensis* subsp. nov.

	Specimen	
	Holotype	Paratype
Sex	Male	Female
HB	103	102
TL	97	100
HFL	25	23
EL	20	20
GLS	27.62	28.04
CBL	24.83	24.81
BL	22.57	22.93
ONL	27.21	27.71
PL	13.74	13.51
DL	7.35	7.00
UTR	13.36	13.07
BAM	5.83	5.64
BZP	2.68	2.79
BOO	6.29	6.08
ZW	13.34	13.39
IB	4.32	4.41
BB	12.36	12.23
LLD	3.98	3.91
LTR	11.36	10.46

The variable codes are given in the text and in Figure 1. HB, head and body length; TL, tail length; HFL, hind foot length; EL, ear length.

A. latronum latronum and *A. latronum lijiangensis* (Table 4). The results of our analyses indicate that aside from the difference in pelage characteristics, apparent geographic variations also exist in cranial morphology.

The results of the CD analyses indicate that four CDs exceeded 1.28 between *A. latronum lijiangensis* subsp. nov. and *A. latronum latronum* among these 15 cranial measurements (Table 5). According to the results of Mayr (1969), the CD on subspecific differentiation should be equal to or larger than 1.28 (Mayr 1969); thus, we accept that *A. latronum lijiangensis* subsp. nov. and *A. latronum latronum* should be two valid subspecies within *A. latronum*. Furthermore, pelage comparison between *A. latronum lijiangensis* subsp. nov. and *A. latronum latronum* also sustains their valid subspecies status (Figure 5). Most interestingly thought is that the geographic distribution of the specimens examined in this study (Figure 4) indicates that the *A. latronum lijiangensis* subsp. nov. and *A. latronum latronum* are allopatric populations. Given this finding, we strongly suggest that *A. latronum lijiangensis* subsp. nov. should be considered as a valid subspecies of *A. latronum*.

The locality of the specimens examined in the study nearly covers the distribution areas of *A. latronum* (Figure 4). In terms of biogeography, this area is often ringed by high mountains and deep gorges as a result of the collision of the Indian and Eurasian plates. This area is thought to have the highest species diversity



Figure 5 Representative pictures of *A. latronum latronum* and *A. latronum lijiangensis*. Dorsal (upper left), ventral (upper right), and lateral (lower left). (A) *A. latronum lijiangensis* (Holotype). (B) *A. latronum lijiangensis* (paratype). (C) *A. latronum latronum* (topotype).

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