

ANALYSIS OF MITOCHONDRIAL DNA RESTRICTION FRAGMENT PATTERNS IN TWO SUBSPECIES OF STRIPED FIELD MICE *Apodemus agrarius* FROM NORTHERN CHINA AND KOREA^{*}

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Abstract

One hundred and seven samples of two subspecies of striped field mice, *Apodemus agrarius pallidior* from Northern China and *A. agrarius coreae* from Korea, were used for the analysis of mitochondrial DNA (mtDNA) fragment patterns resulted from the digestion with eight restriction enzymes by blot hybridization technique.

All 35 fragments and 12 mtDNA haplotypes were recognized. The 12 haplotypes were grouped into two major subgroups with the mean divergence of 1.01%. One subgroup with four haplotypes consisted of 51 samples from four localities in Northern China, which is *A. agrarius pallidior*, and the other subgroup with eight haplotypes is composed of 56 samples from four localities in Korea, *A. agrarius coreae*.

Subspecies *coreae* appeared to be somewhat diverged in their mtDNA genotypes from subspecies *pallidior*, and taxonomic status of these two subspecies is confirmed in this molecular study. It becomes necessary to carry out further researches with samples of striped field mice from other localities to clarify subspecies classification of *A. agrarius*.

Key words Taxonomy; Mitochondrial DNA (mtDNA); *Apodemus agrarius*; Northern China; Korea

All 14 species of genus *Apodemus* is confined to the Palaearctic and northern parts of the Oriental region; *A. agrarius pallas* (striped field mice) is a sole member of subgenus *Apodemus* and inhabits from central Europe to China and Korea (Corbet et al., 1991).

The subspecies classification of *A. agrarius*, however, is still in confusion. Corbet (1978) noted that most subspecies of this species were designated by the differences in pelage colour and/or mean body size. He summarized 23 nominal subspecies into three

* This research was funded by a grant to the second author from Korea Science and Engineering Foundation (951-0509-036-2) and from KSEF (95 Visiting Program to Foreign Institutes). It was also funded by a grant from Shandong University to the first author.

Received 5 August 1996, Accepted 5 March 1997

subspecies (*agrarius*, a western subspecies including 12 named subspecies; *ningpoensis*, an eastern subspecies including *pallidior*, *manchuricus*, *coreae*, *chejuensis*, and other four named subspecies; *chevrieri*, a southern Chinese subspecies). From the analysis of penial bones, papilla lingulis, and serum proteins of striped field mice, Wang (1985) concluded that subspecies *chevrieri* is a distinct species. In the morphometric analyses of eight subspecies of striped field mice (Koh, 1991), four major subgroups were revealed: 1) *Chejuensis*, 2) *chevrieri*, 3) *coreae*, 4) other five subspecies including *manchuricus*, *pallidior*, *ningpoensis*, *insulaenus*, and *agrarius*. Moreover, in the analyses with mtDNA fragment patterns of two subspecies of *A. agrarius* in Korea, subspecies *chejuensis* appeared to be distinct from subspecies *coreae* (Koh et al., 1992).

Mitochondrial DNA studies are important to infer maternal lineages among closely related species and infraspecific populations (Avice, 1986). The analysis of Restriction Fragment Length Polymorphisms (RFLPs) is simple, and it is possible to analyze more loci per individual by RFLPs analysis than by sequencing (Dowling et al., 1990).

The purpose of this paper is to analyze the degree of differentiation in mtDNA restriction fragments among the samples of two subspecies of striped field mice from Northern China and Korea (*Apodemus agrarius pallidior* and *A. agrarius coreae*, respectively) in order to clarify their subspecific status.

MATERIALS AND METHODS

One hundred and seven samples of two subspecies of striped field mice, *Apodemus agrarius pallidior* from Northern China and *A. agrarius coreae* from Korea, were utilized (see Table 1 and Fig. 1). The Chinese samples studied are in the collection of Department of Biology, Shandong University (ZB9501-15; XC9501-15; TS9501-10; BJ9501-11), and Korean samples studied in the collection of the second author.

Mitochondrial DNA was visualized from Southern blots (Southern, 1975) of total cellular DNA. DNA isolation from liver, electrophoresis, Southern transfer and hybridization were described by Davis (1986). All samples were individually digested using the following eight restriction endonucleases: *Pvu* II, *Bam* HI, *Pst* I, *Dra* I, *Stu* I, *EcoR* I, *EcoR* V, and *Hind* III. The mtDNA probe used for all hybridization was pP1d, which contains 10 kb *Pst* I fragment of *Peromyscus leucopus* mtDNA genome in the vector pUC (courtesy of Dr. S. K. Davis in Texas A&M University).

In mtDNA analysis, each different restriction fragment produced by a particular enzyme is given a different (but arbitrary) letter. Sequence divergence in base substitutions per nucleotide (p in per cent) was estimated from the restriction site data by the method of Upholt (1977). Phenogram was constructed from this data by Unweighted Pair Group Method with Arithmetic means, UPGMA (Sneath et al., 1973).

RESULTS

A total of 35 fragments and 12 mitochondrial haplotypes were recognized (see table

1 and fig. 1). The fragment patterns digested with *Pvu* II, *Bam* HI, *Pst* I, and *Dra* I were not different among the samples of two subspecies (OTU s 1 to 4, Korea, *A. agrarius coreae*; OTU s 5 to 8, Northern China, *A. agrarius pallidior*). However, in the patterns with *Stu* I, type (A) was found only in subspecies *coreae*; type (B) was common in the samples of two subspecies; type (C) was only in subspecies *pallidior*. With

Table 1 Mitochondrial DNA genotypes and haplotypes of two subspecies of striped field mice, *Apodemus agrarius pallidior* from Northern China and *A. agrarius coreae* from Korea. Mitochondrial DNA (mDNA) genotypes are based on the fragment patterns resulted from the digestion with eight restriction enzymes, *Pvu* II, *Bam* HI, *Pst* I, *Dra* I, *Stu* I, *EcoR* I, *EcoR* V, and *Hind* III, in order

Subspecies	Locality	OTU	No. of sample	M tDNA genotype	haplotype
<i>coreae</i>	Korea, Cheongju	1	7	A A A A A A A A	1
	Korea, M t Weolak	2	5	"	1
	Korea, Cheongju	1	3	A A A A A A AB	2
	Korea, Yeoncheon	3	2	"	2
	Korea, M t Chiri	4	12	"	2
	Korea, M t Weolak	2	7	A A A A A A B A	3
	Korea, Yeoncheon	3	5	A A A A A A B B	4
	Korea, M t Chiri	4	3	"	4
	Korea, M t Weolak	2	4	A A A A A B A A	5
	Korea, M t Chiri	4	5	A A A A A B A B	6
<i>pallidior</i>	Korea, M t Weolak	2	2	A A A A B A A A	7
	Korea, Cheongju	1	1	A A A A B B A A	8
	China, Zibo	5	12	A A A A B A B B	9
	China, Zibo	5	1	A A A A B B B B	10
	China, Zibo	5	2	A A A A C A B B	11
	China, Xuecheng	6	15	"	11
	China, M t Tai	7	10	"	11
	China, Beijing	8	10	"	11
China, Beijing	8	1	A A A A C B B B	12	

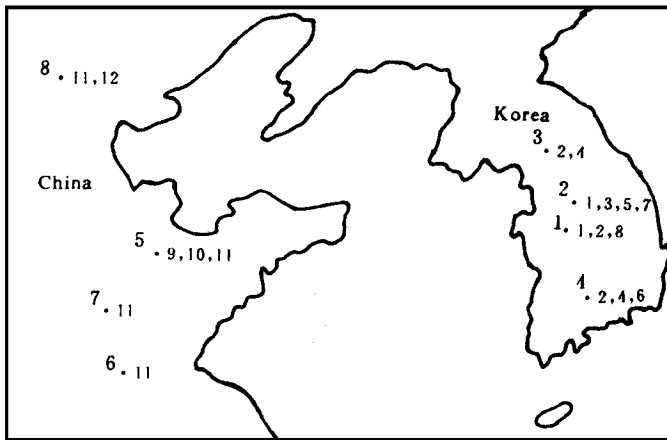


Fig. 1 A map showing eight localities (OTU s) of two subspecies of striped field mice, *Apodemus agrarius pallidior* from Northern China and *A. agrarius coreae* from Korea, with their mitochondrial DNA haplotypes. Large numerals indicate OTU numbers, and small numerals, haplotypes. For locality of each OTU and their haplotypes see Table 1.

EcoR I, two types (A and B) were shown among the samples of two subspecies. With — 210 —

EcoR V and *Hind III* one type (A) was revealed only in subspecies *coreae*, and the other type (B), which was unique in subspecies *pallidior*, was rare in subspecies *coreae*. Moreover, the haplotypes 1 to 8 were shown in the samples of subspecies *coreae*, and the haplotypes 9 to 12 were in the samples of subspecies *pallidior*, indicating that the constitution of mtDNA haplotypes in the samples of each subspecies is different from each other.

Table 2 Nucleotide-sequence divergence among 12 mtDNA haplotypes in two subspecies of striped field mice, *Apodemus agrarius pallidior* from northern China and *A. agrarius coreae* from Korea. For locality and subspecies name of each haplotype (see table 1)

Haplotype	1	2	3	4	5	6	7	8	9	10	11
2	0.003										
3	0.003	0.006									
4	0.006	0.003	0.003								
5	0.003	0.007	0.006	0.012							
6	0.007	0.003	0.013	0.007	0.003						
7	0.001	0.004	0.004	0.008	0.004	0.008					
8	0.005	0.009	0.009	0.013	0.002	0.006	0.002				
9	0.010	0.006	0.005	0.005	0.014	0.011	0.008	0.013			
10	0.014	0.011	0.009	0.006	0.011	0.007	0.012	0.009	0.003		
11	0.010	0.006	0.006	0.008	0.014	0.011	0.009	0.014	0.004	0.008	
12	0.009	0.011	0.010	0.006	0.011	0.007	0.011	0.013	0.006	0.003	0.003

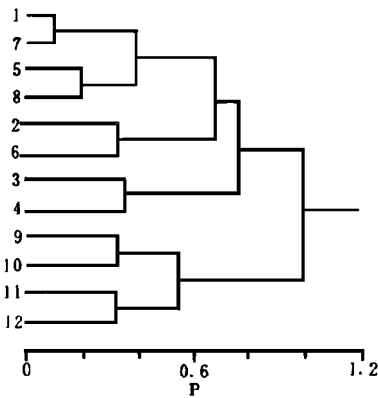


Fig. 2 Grouping of 12 mitochondrial DNA haplotypes in two subspecies of striped field mice, *Apodemus agrarius pallidior* from Northern China and *A. agrarius coreae* from Korea. The nucleotide-sequence divergences, given in Table 2, were used for UPGMA cluster analysis. Numerals refer to the haplotypes in Table 1.

Among 56 samples of subspecies *coreae* from Korea, 12 samples from Cheongju and Mt. Weolak (OTUs 1 to 2) are haplotype 1; 17 samples from Cheongju, Yeoncheon, Mt. Chiri (OTUs 1, 3, 4) are haplotype 2; eight samples from Yeoncheon and Mt. Chiri (OTUs 3 and 4) are haplotype 4. Among 51 samples of subspecies *pallidior* from Northern China, 37 samples from Zibo, Xuecheng, Mt. Tai, and Beijing (OTUs 5 to 8) are haplotype 11. The samples of each subspecies appeared to be more or less similar in their mtDNA genotypes.

The nucleotide-sequence divergence (p in per cent) among 12 mtDNA haplotypes is given in Table 2. The grouping of 12 haplotypes by UPGMA is shown in Fig. 2. Two major subgroups with the mean divergence value of 1.01% were revealed (I, haplotypes 1 to 8, OTUs 1 to 4 from Korea, subspecies *coreae*; II, haplotypes 9 to 12, OTUs 5 to 8 from Northern China, subspecies *pallidior*). Representative fragment patterns of mtDNA in haplotypes 1, 7, 10, and 12 with *Pvu II*, *Sst I*, and *Hind III* are shown in Fig. 3.

In summary, the samples of *Apodemus agrarius coreae* from Korea appears to be different in their mtDNA genotypes from the samples of *A. agrarius pallidior* from Northern China, although samples of each subspecies are more or less related among them selves

DISCUSSION

Kobayashi (1985) noted that it is necessary to reexamine the subspecies classification of striped field mice, *Apodemus agrarius*, which is widely distributed in Eurasia. In China, five subspecies of *A. agrarius* are recognized (Xia, 1984): *agrarius*, from Omin, Tacheong, and northern Xinjiang; *ningpoensis*, middle and lower part of Yangtze Valley, Guizhou, and northern Fujian; *pallidior*, Northern China, eastern part of Northwestern China, Sichuan, and northern Jiangsu; *manchuricus*, Northeastern China and eastern InnerMongolia; *insulaenus*, Taiwan. Zhao et al (1986) analyzed biochemical characters of samples in two subspecies of striped field mice from Shandong, Jiangsu, and Anhui provinces and concluded that *A. agrarius pallidior* from Shandong is distinct from *A. agrarius ningpoensis* from Jiangsu and Anhui provinces. Based on the comparison of black dorsal stripe and colour of their dorsal hair, Liu et al (1991) noted that subspecies *pallidior* from northern part of its distribution is the synonym of *manchuricus* and that subspecies *pallidior* from southern part of its distribution is the synonym of *ningpoensis*.

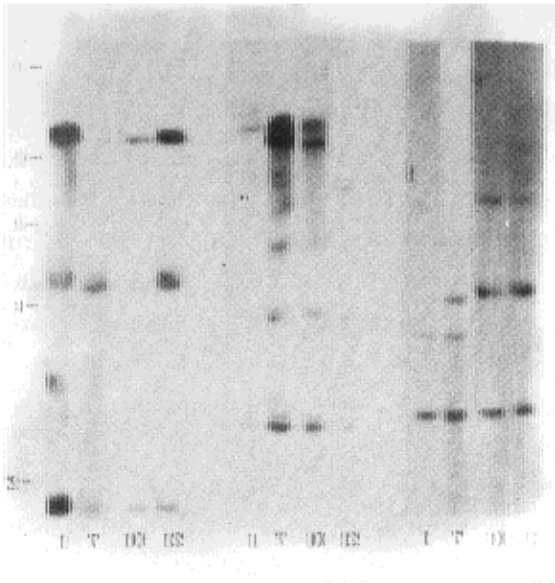


Fig. 3 Representative fragment patterns with *Pvu* I, *Stu* I, and *Hind* III in four mitochondrial DNA haplotypes of two subspecies of striped field mice, *Apodemus agrarius pallidior* from Northern China and *A. agrarius coreae* from Korea. Numerals indicate haplotypes and far left contains size markers in kilo base-pairs

In Korea, Jones et al (1965) reported four subspecies of *A. agrarius*: *manchuricus* in the extreme northern part, *palescence* in the coastal lowlands of southern and southwestern Korea, *coreae* throughout the major portion of the peninsula, and *chejuensis* in Cheju island. In morphometric analyses, Koh (1986) concluded that *A. agrarius palescence* is the synonym of *A. agrarius coreae* and Koh (1987) stated that *A. agrarius chejuensis* is distinct from *A. agrarius coreae*. Moreover, *A. agrarius coreae* of medium size form is also different from other five subspecies of small-size from China and Turkey, i.e., *manchuricus*, *pallidior*, *ningpoensis*, *insulaenus*, and *agrarius* (Koh, 1991).

Wang et al (1993) reported that karyotype of *Apodemus agrarius pallidior* is eight metacentric and 38 telocentric autosomes with large telocentric X and small telocentric Y chromosomes. Koh (1982) reported that the karyotype of *A. agrarius coreae* from Korea is diploid number of 48, i.e., 38 acrocentric autosomes, four pair of small metacentric autosomes, large acrocentric X chromosome, and small acrocentric Y chromosome, indicating that in chromosome karyotype, subspecies *pallidior* is identical with subspecies *coreae*.

Sequence divergence of mtDNA estimated in *Rattus norvegicus* ranged from 0.2% to 0.8% (Brown et al, 1981). The maximum sequence divergence in *Clethrionomys rutilus* was 0.89% (Tegelstrom et al, 1988). The mtDNA sequence divergence between Santa Catalina island and mainland harvest mice, *Reithrodontomys megalotis*, was less than 0.2% (Ashley, 1989). On the other hand, sequence divergence between *Peromyscus maniculatus* mtDNA haplotypes from continental America was as high as 7% and seven assemblages were revealed (Lansmann et al, 1983).

The greatest infraspecific sequence divergence among mtDNA haplotypes of *Apodemus sylvaticus* was 1.4% and that of *A. flavicolis* was 1.0% (Tegelstrom et al, 1989). In this study, with two subspecies of *Apodemus agrarius*, two major mtDNA subgroups with the mean divergence value of 1.01% were revealed (Table 1 and 2; Fig 1, 2, and 3): species name of the samples of one subgroup from Korea is *A. agrarius coreae*, and that of the samples of the other subgroup from Northern China is *pallidior*, indicating that these two subspecies differ in their mtDNA genotypes with each other. Moreover, assuming 2% divergence value per million years (Brown et al, 1979), these two subspecies were diverged before 500 000 years ago. Xia (1984) noted that among the species of genus *Apodemus*, *A. agrarius* might be evolved later than other species.

Developments in the areas of molecular, cyto-, and numerical taxonomy are enormous (Quicke, 1993) and there have been a conflict between biologists and morphologists about the merits of their data (Ferguson, 1980). However, modern molecular techniques have not yet pushed comparative morphology into the shadows (Patterson, 1987). It was advocated that a classification should be the product of all available characters distributed as widely and evenly as possible over the organisms studied (Mayr et al, 1991; Huelsenbeck et al, 1996).

Apodemus agrarius coreae appeared to be different in their mtDNA genotypes and morphology from *A. agrarius pallidior*, but they are identical in their chromosomal karyotype. And further studies using samples from other regions of China and Europe, especially from Northeastern China, seem to be necessary to clarify subspecies classification of species *A. agrarius*.

REFERENCES

- Ashley M. V. 1989. Absence of differentiation in mitochondrial DNA of island and mainland harvest mice, *Reithrodontomys megalotis*. *J Mamm*, 70: 383~ 386.

- A vise J C. 1986 Mitochondrial DNA and the evolutionary genetics of higher animals. *Phil Trans R. Soc Lond* 312: 325~ 342
- Brown G G, Simpson M V. 1981. Intra- and interspecific variation of the mitochondrial genome in *Rattus norvegicus* and *Rattus rattus*: restriction enzyme analysis of variant mitochondrial DNA molecules and their evolutionary relationships. *Genetics*, 97: 125~ 143
- Brown W M, George M, Wilson A C. 1979. Rapid evolution of animal mitochondrial DNA. *Proc Natl Acad Sci U S A*. 76: 1967~ 1971.
- Corbet G B. 1978. The mammals of the Palaearctic region: a taxonomic review. British Museum (Nat Hist). Cornell Univ. Press, London.
- Corbet G B, Hill J E. 1991. A world list of mammalian species. Oxford Univ. Press, Oxford
- Davis S K. 1986. Evolutionary relationships in *Geomys*: an analysis using mitochondrial DNA. Ph. D. Diss. Washington Univ., St. Louis, Mo.
- Dowling, T E, Moritz C, Palmer J D. 1990. Nucleic acids II: restriction site analysis, In: Hills D. M, Moritz C, editors. *Molecular systematics*. Sinauer Associates, Inc. Mass., 250~ 317.
- Ferguson A. 1980. *Biochemical systematics and evolution*. John Wiley and Sons, New York
- Huelsenbeck J P, J Bull J, Cunningham C W. 1996. Combining data in phylogenetic analysis. *Tree*, 11: 152~ 157.
- Jones J K, Johnson D H. 1965. Synopsis of the lagomorphs and rodents of Korea. Univ. Kansas Publ Mus (Nat Hist), 16: 357~ 407.
- Kobayashi T. 1985. Taxonomic problem in the genus *Apodemus* and its allies, In: Kawamichi T, editor: *Contemporary mammalogy in China and Japan*. Mamm. Soc. Japan, 76~ 79.
- Koh H S. 1982. G- and C-banding pattern analyses of Korean rodents: I. Chromosome banding patterns of striped field mice, *Apodemus agrarius coreae*, and black rats, *Rattus rattus rufescens*. *Korean J Zool*, 25: 81~ 92
- Koh H S. 1986. Geographic variation of morphometric characters among three subspecies of striped field mice, *Apodemus agrarius* Pallas from Korea. *Korean J Zool*, 29: 272~ 282
- Koh H S. 1987. Morphometric and chromosomal analyses of striped field mice, *Apodemus agrarius chejuensis* Jones and Johnson, from Cheju-Do. *Korean J Syst Zool*, 3: 24~ 40
- Koh H S. 1991. Multivariate analysis with morphometric characters of samples representing eight subspecies of striped field mice, *Apodemus agrarius* Pallas, in Asia: taxonomic status of subspecies *chejuensis* at Cheju island, Korea. *Korean J Syst Zool*, 7: 179~ 188
- Koh H S, Yoo B S. 1992. Variation of mitochondrial DNA in two subspecies of striped field mice, *Apodemus agrarius coreae* and *A. agrarius chejuensis*, from Korea. *Korean J Zool*, 35: 332~ 338
- Lansmann R A, A vise J C, A quadro C F, Shapira J F, Daniel D W. 1983. Extensive genetic variation in mitochondrial DNA among geographic populations of deer mice, *Peromyscus maniculatus*. *Evolution*, 37: 1~ 16
- Liu C, Wu W, Guo S, Meng J. 1991. A study of the subspecies classification of *Apodemus agrarius* in eastern continental China. *Acta Theriol Sinica*, 11 (4): 294~ 299.
- Mayer E, Ashlock P D. 1991. *Principles of systematic zoology*. McGraw-Hill Inc., New York
- Patterson C. 1987. Introduction, In: Patterson C, editor: *Molecules and morphology in evolution*. Cambridge Univ. Press, Cambridge, 1~ 22
- Quicke D L J. 1993. *Principles and techniques of contemporary taxonomy*. Blackie Academic & Professional Co., London
- Sneath P H A, Sokal R R. 1973. *Numerical taxonomy*. Freeman W H and Co., San Francisco
- Southern E M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Evolution*, 98: 503~ 517.
- Tegelstrom H, Jaarola M. 1989. Genetic divergence in mitochondrial DNA between wood mice (*Apodemus sylvaticus*) and the yellow necked mice (*A. flavicollis*). *Hereditas*, 111: 49~ 60
- Tegelstrom H, Yoni P, Gelter H, Jaarola M. 1988. Concordant divergence in proteins and mitochondrial DNA between two vole species in the genus *Clethrionomys*. *Biochem Genetics*, 26: 223~ 237.

- Upholt W P. 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. *Nucleic Acids Res*, 4: 1257~ 1265.
- Wang Y. 1985. Subspecific classification and distribution of *Apodemus agrarius* in Sichuan, China. In: Kawamichi T, editor. Contemporary mammalogy in China and Japan. Mamm. Soc. Japan, 76~ 79.
- Wang J, Zhao X, Wang X, Tian J. 1993. Studies of chromosome of striped field mouse, *Apodemus agrarius pallidior* (Rodentia). *Acta Theriol Sinica*, 13 (4): 283~ 287.
- Xia W. 1984. A Study on Chinese *Apodemus* with a discussion of its relations to Japanese species. *Acta Theriol Sinica*, 4 (2): 93~ 98.
- Zhao X, Lu H. 1986. Comparative observations of several biochemical indexes of *Apodemus agrarius pallidior* and *Apodemus agrarius ningpoensis* of the striped backed field mice. *Acta Theriol Sinica*, 6 (1): 57~ 62.

中文摘要

中国和韩国黑线姬鼠两亚种的线粒体DNA 限制性片段分析

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本文利用印迹杂交技术对中国华北地区黑线姬鼠 *Apodemus agrarius pallidior* 和韩国黑线姬鼠 *A. agrarius coreae* 共107号标本的线粒体DNA (mitochondrial DNA, mtDNA), 通过8种限制性内切酶的消化, 进行了限制性片段的分析。

共检出35种限制性片段和12种单倍体类型。12种单倍型在平均离散度为1.01%时聚合为两个亚群: 一个亚群为黑线姬鼠华北亚种, 由来自中国4个不同地区的51号标本的4种单倍型所组成; 另一个亚群为黑线姬鼠朝鲜亚种, 由来自韩国4个不同地区的56号标本的8种单倍型所组成。

黑线姬鼠华北亚种和朝鲜亚种在mtDNA表型上表现出一定差异, 这在分子水平上确立了两亚种的分类地位。为了进一步澄清黑线姬鼠种下分类的混乱, 很有必要对中国其他地区的标本进行该项研究。

关键词 分类学; 线粒体DNA; 黑线姬鼠; 中国华北地区; 韩国