

Genetic origin identification of Siberian chipmunks (*Tamias sibiricus*) in pet shops of South Korea

Seo-Jin Lee^{a†}, Gila Jung^{b†}, Mi-Sook Min^a, Chuel-Kyu Kim^c, Hang Lee^a, Chang Bae Kim^{b*} and Mu-Yeong Lee^{a*}

^aConservation Genome Resource Bank for Korean Wildlife and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, South Korea; ^bDepartment of Life Science, Sangmyung University, Seoul, South Korea; ^cDepartment of Toxicological Evaluation and Research, National Institute of Food and Drug Safety Evaluation, Korea Food & Drug Administration, Seoul 122-704, South Korea

(Received 22 October 2010; received in revised form 19 January 2011; accepted 19 January 2011)

Siberian chipmunks, *Tamias sibiricus*, are one of several popular companion animals found in the pet shops of South Korea. At present, however, there have been no studies done in South Korea examining their origin even though they could be potential carriers of zoonotic diseases, and are a species of concern for efficient conservation and management strategies. Sequences of the mitochondrial cytochrome *b* gene (1140 bp) were determined to investigate the origin of Siberian chipmunks sold in four South Korean pet shops through comparison with sequence data from animals of known locality. Nine Siberian chipmunks were collected from pet shops in South Korea, which resulted in nine haplotypes. One (AR) of these coincided with the haplotype previously described. Phylogenetic and network analyses using 53 haplotypes including 45 haplotypes from GenBank showed three phylogenetic groups in South Korea, almost concordant to locality, designated as northern, central, and southern parts as described in a previous study. Of the nine individuals examined from the pet shops, eight were clustered into the northern phylogroup but one (cgrb9153) was grouped with the southern phylogroup, implying that at least the Siberian chipmunks examined in this study did not originate from other countries. It is likely that most individuals sold in the pet shops of Seoul were caught in the wild in Gyeonggi-do and Gangwon-do, or are maternal descendants of captive-bred individuals originating from the northern part of South Korea. It is recommended that conservation and management units of Korean chipmunks should be examined in further detail.

Keywords: Siberian chipmunk; genetic tracking; cytochrome *b* gene; South Korea; pet shop

Introduction

Recently, there has been a growing interest in genetic tracking and monitoring of wildlife to improve conservation and management strategies by tracing the origin of plants and animals in markets suspected to have been illegally harvested or transferred by the illegal wildlife trade, the second biggest threat to wildlife biodiversity (Baker 2008; Baker et al. 2010). For instance, Baker et al. (2010) showed that DNA profiling makes it possible to identify the individual source of a product or track the distribution of products; this largely contributes to the establishment of an information base for effective conservation and management strategies. In addition, species identification and sex determination using DNA molecular techniques provide critical evidence in forensic cases where the origin of confiscated samples such as a mass of muscle or a piece of hair could not be identified by sight (An et al. 2007). DNA-based techniques have even enabled the discrimination of a questionable wild boar individual that was illegally caught (Lorenzini 2005). These

findings rigorously demonstrate the importance and effectiveness of genetic tracking and monitoring.

The Siberian chipmunk, *Tamias sibiricus*, has been one of the most popular companion animals because of its attractive appearance and unique behavior. It is known that Korean chipmunks have been exported to other countries such as Japan and Europe. Consequently, this species was introduced into some parts of eastern and central Europe (Vourc'h et al. 2007). In South Korea, the Siberian chipmunk is currently a relatively popular companion animal and is sold in pet shops. However, little is known about their origins.

Recently, several researchers have studied Siberian chipmunks by DNA molecular approaches using the mtDNA cytochrome *b* gene in particular as a genetic marker. Siberian chipmunks not native to Taiwan that are sold in Taiwanese pet shops have been found to be highly polymorphic and belong to several distinct phylogroups (Chang 2008). However, the origin of the Siberian chipmunk in these pet shops was not determined due to a lack of sequence data from samples across the species range. Moreover, it was reported that

*Corresponding authors. Emails: evodevo@smu.ac.kr; muyeong@gmail.com

†S.-J. Lee and G. Jung contributed equally to this article.

there are two highly divergent lineages with several sublineages: one consisting of only individuals from South Korea, and the other composed of individuals from Japan, Russia, Mongolia, and China (Lee et al. 2008 and unpublished data; Koh et al. 2009; Obolenskaya et al. 2009). Furthermore, the Siberian chipmunk of South Korea has three clades that correspond to geographic locations, each with high genetic variation (Lee et al. 2008). Koh et al. (2009) also described substantial polymorphisms and several phylogroups of Siberian chipmunks in South Korea. These findings thus provide an opportunity to trace the origin of Siberian chipmunks sold in South Korean pet shops using a genetic approach.

The aim of the present study was to trace the origin of Siberian chipmunks sold in South Korean pet shops using genetic techniques to determine whether these animals were imported from abroad or originated from domestic populations. In addition, we ascertained from what part of South Korea the domestic populations originated. Our results will contribute to the establishment of a conservation and management unit of Siberian chipmunks.

Materials and methods

Sample collection and laboratory work

To trace the origin of Siberian chipmunks from pet shops in South Korea, nine individuals were collected from four different pet shops located in Seoul, South Korea. It was not known whether the samples collected in this study were captive-bred or caught in the wild. We utilized mtDNA cytochrome *b* gene sequences from GenBank for the analyses (Accession nos. EU754751-EU754822). *Tamias striatus* (AY292715) was used as an outgroup. The sequences generated in this study were deposited in GenBank (Accession nos. HQ214034 – HQ214042) (Table 1).

Whole genomic DNA was isolated from hair samples using a DNeasy Blood & Tissue Kit (Qiagen, USA) according to the manufacturer's protocol. The complete sequences of mtDNA cytochrome *b* gene were amplified and sequenced with the previously described primers, L14724 and H15915 (Kocher et al. 1989). PCR amplifications were conducted in a 30 μ L volume containing 3 μ L of 10 \times rTaq reaction buffer, 0.2 mM of each dNTP, 0.5 μ M of each primer, and 1 unit of Takara rTaqTM polymerase (Takara, Japan). PCR products were checked on a 1% agarose gel. Additional internal primers, KTS-F (5'-CTG AGG AGC AAC AGT TAT TAC-3') and KTS-R (5'-TGC GAA GAA TCG TGT TAG AGT-3'), were used for the sequencing (Lee et al., unpublished data in preparation). All samples were directly sequenced on an Applied Biosystems 3730 XL

DNA sequencer according to the manufacturer's instructions.

Data analysis

The sequences generated here were compared and modified manually using Geneious v5.0 (Drummond et al. 2010). The sequences were aligned by ClustalX v2 (Thompson et al. 1997) and novel haplotypes were identified with DnaSP v5 (Rozas et al. 2003). These haplotypes were further compared to the previously published mtDNA cytochrome *b* gene sequences (Lee et al. 2008). Phylogenetic reconstruction was performed using Paup 4.0b10 (Swofford 2002). The best-fit model was selected by Modeltest v3.7 (Posada and Crandall 1998). In addition, we constructed a median-joining network using the program Network to investigate the possible relationships among the haplotypes of Siberian chipmunks (Bandelt et al. 1999).

Results and discussion

The complete sequences (1140 bp) of the mtDNA cytochrome *b* gene were successfully determined. No indels were detected in nine sequences of the mtDNA cytochrome *b* gene, yielding nine different haplotypes with 124 polymorphic nucleotides of which 66 were parsimony informative sites (Table 2). A summary of the haplotypes is provided in Table 2. Among the nine haplotypes, only one specimen (AR) was identical to the SKC2 haplotype from a previous study by Lee et al. (2008).

Comparing the haplotypes in our study with previously published molecular data on Siberian and Korean chipmunks (Lee et al. 2008), the neighbor-joining tree (Figure 1) reconstructed with 37 known haplotypes of Korean chipmunks and nine haplotypes from the pet shops revealed the Korean haplotypes described by Lee et al. (2008). The nine haplotypes of Siberian chipmunks from the pet shops were genetically distinct from animals in neighboring countries (Russia and China). In addition, three well-structured phylogroups (southern, central, and northern) in South Korea were observed (Figure 1), which coincide with the results of Lee et al. (2008). The outcome of phylogenetic analysis showed that the haplotypes of Siberian chipmunks from the pet shops were significantly different from those of animals with Russian and Chinese haplotypes, and were grouped together with the Korean haplotypes (Figure 1). This implied that the Siberian chipmunks sold in the South Korean pet shops were not from neighboring countries such as China and Russia.

The results of a median-joining network (Figure 2) and phylogenetic tree demonstrated that the majority

Table 1. Sample ID numbers, collection localities, cytochrome *b* haplotype designations, and accession numbers of the Siberian chipmunks.

No.	CGRB ID	Collection locality	Cytochrome <i>b</i> haplotype	Accession no.	
1	9146	Pet shop B*	South Korea	PC1	HQ214034
2	9147	Pet shop C*	South Korea	PC2	HQ214035
3	9148	Pet shop A*	South Korea	PC3	HQ214036
4	9149	Pet shop C*	South Korea	PC4	HQ214037
5	9150	Pet shop C*	South Korea	PC5	HQ214038
6	9151	Pet shop C*	South Korea	PC6	HQ214039
7	9152	Pet shop A*	South Korea	PC7	HQ214040
8	9153	Pet shop B*	South Korea	PC8	HQ214041
9	AR [†]	Pet shop D	South Korea	SKC2	HQ214042
10	288 [#]	Chuncheon, Gangwon-do	South Korea	SKN1	EU754807
11	289 [#]	Hwacheon, Gangwon-do	South Korea	SKN2	EU754808
12	290 [#]	Yanggu, Gangwon-do	South Korea	SKN3	EU754809
13	2389 [#]	Cheolwon, Gangwon-do	South Korea	SKN4	EU754822
14	2605 [#]	Cheolwon, Gangwon-do	South Korea	SKN5	EU754811
15	3478 [#]	Gapyeong, Gyeonggi-do	South Korea	SKN6	EU754816
16	3480 [#]	Gapyeong, Gyeonggi-do	South Korea	SKN7	EU754818
17	3481 [#]	Gapyeong, Gyeonggi-do	South Korea	SKN8	EU754820
18	3494 [#]	Cheolwon, Gangwon-do	South Korea	SKN9	EU754819
19	3894 [#]	Cheolwon, Gangwon-do	South Korea	SKN10	EU754821
20	4098 [#]	Teaback, Gangwon-do	South Korea	SKN11	EU754812
21	4099 [#]	Teaback, Gangwon-do	South Korea	SKN12	EU754814
22	4102 [#]	Teaback, Gangwon-do	South Korea	SKN13	EU754810
23	4103 [#]	Teaback, Gangwon-do	South Korea	SKN14	EU754803
24	1567 [#]	Cheolwon, Gangwon-do	South Korea	SKN15	EU754801
25	1276 [#]	Okcheon, Chungcheongbuk-do	South Korea	SKC1	EU754800
26	3103 [#]	Chungju, Chungcheongbuk-do	South Korea	SKC2	EU754815
27	2125 [#]	Goesan, Chungcheongbuk-do	South Korea	SKC3	EU754806
28	3510 [#]	Goesan, Chungcheongbuk-do	South Korea	SKC4	EU754798
29	3750 [#]	Danyang, Chungcheongbuk-do	South Korea	SKC5	EU754802
30	1975 [#]	Cheongsong, Gyeongsangbuk-do	South Korea	SKC6	EU754804
31	2096 [#]	Hapcheon, Gyeongsangnam-do	South Korea	SKC7	EU754799
32	3407 [#]	Suncheon, Jeollanam-do	South Korea	SKS1	EU754805

Table 1 (Continued)

No.	CGRB ID	Collection locality	Cytochrome <i>b</i> haplotype	Accession no.	
33	1129 [#]	Gurye, Jeollanam-do	South Korea	SKS2	EU754795
34	1144 [#]	Gurye, Jeollanam-do	South Korea	SKS3	EU754785
35	1145 [#]	Namwon, Jeollabuk-do	South Korea	SKS4	EU754792
36	1151 [#]	Namwon, Jeollabuk-do	South Korea	SKS5	EU754796
37	1213 [#]	Gurye, Jeollanam-do	South Korea	SKS6	EU754784
38	1267 [#]	Gurye, Jeollanam-do	South Korea	SKS7	EU754789
39	2134 [#]	Gurye, Jeollanam-do	South Korea	SKS8	EU754793
40	3345 [#]	Gurye, Jeollanam-do	South Korea	SKS9	EU754787
41	3346 [#]	Gurye, Jeollanam-do	South Korea	SKS10	EU754794
42	3653 [#]	Gwangju, Jeollanam-do	South Korea	SKS11	EU754788
43	3672 [#]	Gwangju, Jeollanam-do	South Korea	SKS12	EU754786
44	3714 [#]	Namwon, Jeollanam-do	South Korea	SKS13	EU754790
45	3814 [#]	Sangcheong, Gyeongsangnam-do	South Korea	SKS14	EU754790
46	3815 [#]	Sangcheong, Gyeongsangnam-do	South Korea	SKS15	EU754791
47	4111 [#]	Heilongjiang Province	China	CH1	EU754778
48	4113 [#]	Heilongjiang Province	China	CH2	EU754779
49	83 [#]	Chita Region, Transbaikalia	Russia	RU1	EU754772
50	84 [#]	Chita Region, Transbaikalia	Russia	RU2	EU754769
51	157 [#]	Magadan vicinity	Russia	RU3	EU754751
52	165 [#]	Magadan vicinity	Russia	RU4	EU754753
53	2710 [#]	Lazo Reserve, Primorye Territory	Russia	RU5	EU754764
54	2711 [#]	Lazo Reserve, Primorye Territory	Russia	RU6	EU754765

*All specimens were collected from four different pet shops located in Seoul, South Korea. The pet shops' names are omitted for anonymity and are instead referred to by letters (A, B, C, and D).

[†]Hair samples of the specimen (AR) were donated by an anonymous contributor who was taking care of a pet Siberian chipmunk.

[#]GenBank accession number of the published cytochrome *b* sequences of Lee et al. (2008) used in our study.

of Siberian chipmunks from the pet shops fell into the northern phylogroup of Korean chipmunks, except for one haplotype (cgrb9153) which clustered with the haplotypes of the southern phylogroup (Figures 1 and 2). This implies that most of the Korean chipmunks sold in pet shops were imported from the northern part of South Korea (primarily Gyeonggi-do or Gangwon-do) or were from captive populations whose maternal ancestors originated from these locations. Despite the small sample sizes, this hypothesis is supported not only by the fact that there is abundant suitable habitat for Siberian chipmunks in Gangwon-do consisting of many well-conserved mountains and national parks, but also by a report that Siberian chipmunks have been caught in those regions (National Institute of Environmental Research in South Korea 2004). In addition, it is generally known that some

chipmunks sold in South Korean pet shops might be illegally harvested from Gangwon-do although they are presented as captive-bred by the merchants. Our results support this possibility, but should be confirmed by further studies.

Our results showed the presence of three genetic groups of chipmunks in South Korea. These genetic groups may be separately managed until solid data are generated using multiple neutral loci to define more concrete management units. Currently, Siberian chipmunks in South Korea are being sold in pet shops and distributed to urban areas not only as pets, but also as experimental animals even though their origin is ambiguous and their genetic composition is complex. This could result in genetic pollution of local populations of Korean chipmunks genetically adapted to the local environment. Therefore, we suggest formulating

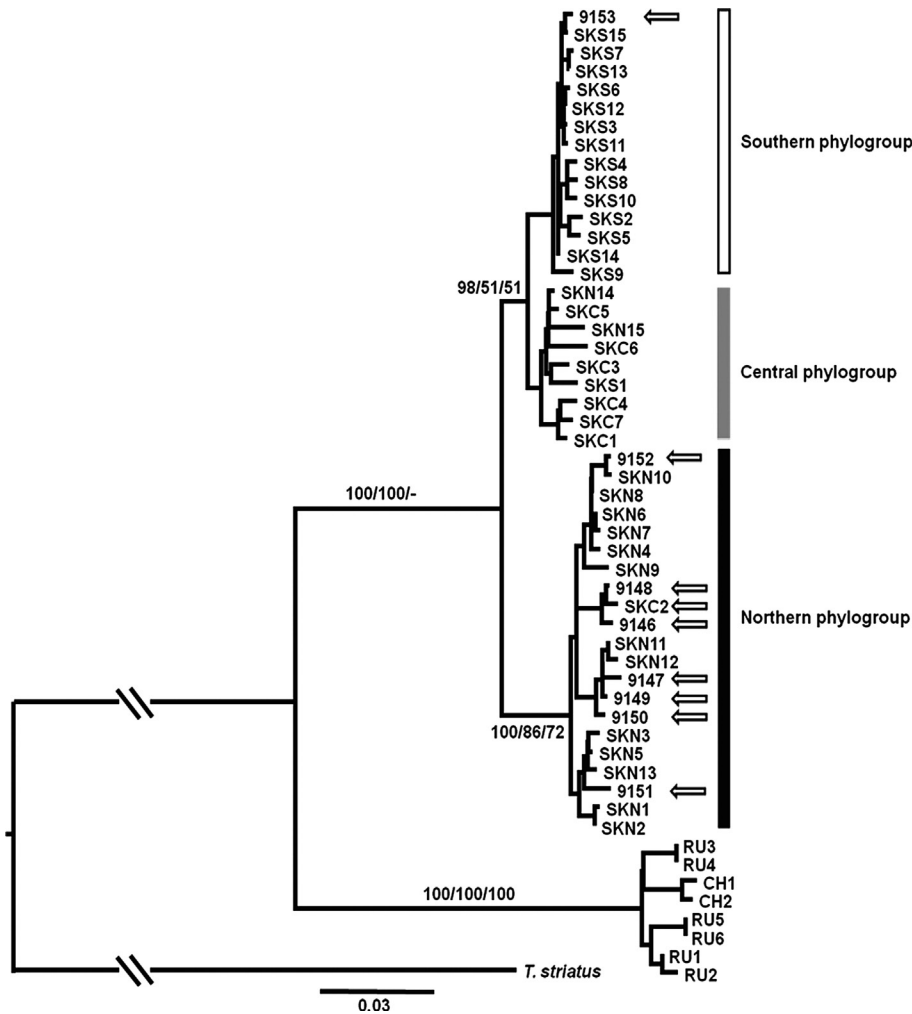


Figure 1. Neighbor-joining tree of 53 haplotypes of Siberian chipmunks. The numbers on the branch are the bootstrap values of NJ, MP, and ML, respectively. The arrows represent the animals collected from South Korean pet shops. SKC2 includes the AR specimen.

measures to regulate the movement of Korean chipmunks between different localities to prevent disturbances of the natural genetic structure among chipmunk populations in South Korea. In addition, special attention is needed to prevent the movement of Siberian chipmunks between countries to avoid genetic pollution of the species as the South Korean population is genetically distinct from Russian and Chinese populations, which can be considered at the level of different species (Lee et al. 2008).

The invasion of alien species from other countries can cause indigenous species to become endangered or threatened as well as lead to major environmental damage and economic losses (Pimentel et al. 2000; Sandro 2008). For instance, the Eurasian red squirrel, *Sciurus vulgaris*, is endangered in some European countries such as the United Kingdom partially due

to habitat fragmentation and loss, and the introduction of North American gray squirrels, *Sciurus carolinensis*, which led to high competition for food and habitat (Wauters et al. 2000; Gunnell et al. 2004). It is also known that the red squirrel parapox virus introduced to the United Kingdom with the gray squirrel resulted in high red squirrel mortality rates (Tompkins et al. 2002). Recently, it was determined that the Siberian chipmunks in South Korea are remarkably distinct from individuals of the same species in countries neighboring the Korean peninsula (Lee et al., unpublished data), implying that the Siberian chipmunk in South Korea is endemic. Furthermore, the origin of Siberian chipmunks sold in Taiwanese pet shops was determined to most likely be China (Lee et al., unpublished data). These findings suggest that more attention concerning conservation and management of

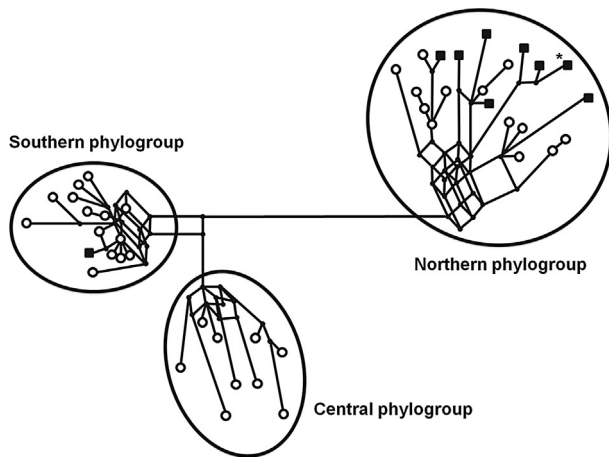


Figure 2. Median-joining network of 45 mtDNA haplotypes of the Siberian chipmunks in South Korea. Haplotypes found in pet shops are indicated by closed squares, and open circles represent known origins of haplotypes. The asterisk (*) means that the AR specimen and SKC2 are together.

the Siberian chipmunks inhabiting South Korea is needed. Our study showed that mtDNA as a genetic marker can be used as a tool to help identify the origins of animals in pet shops and contribute to the establishment of efficient management strategies for wildlife resources.

The current study showed that genetic tracking of chipmunks in South Korea using mtDNA is possible and was used to identify three genetic groups of Siberian chipmunks in South Korea. However, mtDNA analysis only allows examination of maternal genealogy. Therefore, it is necessary to establish biparentally inherited genetic markers to overcome this shortcoming to understand a complete population structure. Additionally, it should be noted that the molecular approach used in this study does not prove whether or not the samples were from animals captured from the wild. In summary, the genetic tracking applied in the current study confirmed that Siberian chipmunks sold in South Korean pet shops are not imported from abroad, and that most of the individuals originated in or were caught in the northern part of South Korea.

Acknowledgements

We wish to thank all individuals who made contributions to the CGRB sample collection. We also thank Aram An, who contributed to this experiment. This study was supported by a grant (550-20080074) from the Korea Food and Drug Administration (KFDA), and a grant from the Basic Science Research Program (2010-0025751) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology.

References

- Aguirre AA, Ostfeld RS, Tabor GM, House C, Pearl MC. 2002. Conservation medicine: ecological health in practice. 1st ed. New York: Oxford University Press.
- An JH, Lee MY, Min MS, Lee MH, Lee H. 2007. A molecular genetic approach for species identification of mammals and sex determination of birds in a forensic case of poaching from South Korea. *Forensic Sci Int.* 167:59–61.
- Baker CS. 2008. A truer measure of the market: the molecular ecology of fisheries and wildlife trade. *Mol Ecol.* 17:3985–3998.
- Baker CS, Steel D, Choi Y, Lee H, Kim KS, Choi SK, Ma Y-U, Hambleton C, Psihoyos L, Brownell RL, Funahashi N. 2010. Genetic evidence of illegal trade in protected whales links Japan with the US and South Korea. *Biol Lett.* 14: online version.
- Bandelt HJ, Forster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol.* 16:37–48.
- Chang SW. 2008. Identification of chipmunks sold in pet shops of Taiwan. *Teyou Shengwu Yanjiu.* 10:25–34 (in Chinese).
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A. 2010. Geneious v5.0, available from <http://www.geneious.com>.
- Gunnell J, Wauters L, Lurz PWW, Tosi G. 2004. Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. *J Anim Ecol.* 73:26–35.
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc Natl Acad Sci USA.* 86:6196–6200.
- Koh HS, Wang J, Lee BK, Yang BG, Heo SW, Jang KH, Chun TY. 2009. A phylogroup of the Siberian chipmunk from Korea (*Tamias sibiricus barberi*) revealed from the mitochondrial DNA cytochrome b gene. *Biochem Genet.* 47:1–7.
- Lee MY, Lisovsky AA, Park SK, Obolenskaya EV, Dokuchav NE, Zhang Y, Yu L, Kim YJ, Voloshina I, Choi TY, Min MS, Lee H. 2008. Mitochondrial cytochrome b sequence variations and population structure of Siberian chipmunk (*Tamias sibiricus*) in Northeastern Asia and population substructure in South Korea. *Mol Cells.* 26:566–575.
- Lorenzini R. 2005. DNA forensics and the poaching of wildlife in Italy: a case study. *Forensic Sci Int.* 153:218–221.
- National Institute of Environmental Research in South Korea. 2004. The second National Ecosystem Survey.
- Obolenskaya EV, Lee MY, Dokuchaev NE, Oshida T, Lee MS, Lee H, Andrey AL. 2009. Diversity of Palaearctic chipmunks (*Tamias, Sciuridae*). *Mammalia.* 73: 281–298.
- Pimentel D, Lach L, Zuniga R, Morrison D. 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience.* 50:53–65.
- Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics.* 14:817–818.
- Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics.* 19:2496–2497.

- Sandro B. 2008. Introduction of the American grey squirrel (*Sciurus carolinensis*) in Europe: a case study in biological invasion. *Curr Sci.* 95:903–906.
- Swofford DL. 2002. PAUP*: Phylogenetic analysis using parsimony (and Other Methods) 4.0 Beta. Sinauer Associates, Massachusetts.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25: 4876–4882.
- Tompkins DM, Sainsbury AW, Nettleton P, Buxton D, Gunnell J. 2002. Parapox virus causes a deleterious disease in red squirrels associated with UK population declines. *Proc Biol Sci.* 269:529–533.
- Vourc'h G, Marmet J, Chassagne M, Bord S, Chapuis J-L. 2007. *Borrelia burgdorferi* sensu lato in Siberian chipmunks (*Tamias sibiricus*) introduced in suburban forests in France. *Vector Borne Zoonotic Dis.* 7:637–641.
- Wauters LA, Lurz PWW, Gunnell J. 2000. Interspecific effects of grey squirrels (*Sciurus carolinensis*) on the space use and population demography of red squirrels (*Sciurus vulgaris*) in conifer plantations. *Ecol Res.* 15:271–284.