Regular Article
pISSN: 2288-9744, eISSN: 2288-9752
Journal of Forest and Environmental Science
Vol. 37, No. 4, pp. 331-337, December, 2021
https://doi.org/10.7747/JFES.2021.37.4.331

JFES
Journal of Forest and Environmental Science

Study on Species Identification Error Caused by Comparing Feces Appearance of *Felis catus* and *Prionailurus bengalensis* in the Ecosystems

Yoon Jeong Lee[1], Jong Hyun Kim[1] and Eui Kyeong Kim[2,*]
[1]*Nature And People Co., Ltd., Daegu 42281, Republic of Korea*
[2]*Korea National Park Research Institute, Wonju 26441, Republic of Korea*

Abstract

This study is designed according to the fact that the feces presumed to be from a *Prionailurus bengalensis* was found in Ulleungdo Island, where *Prionailurus bengalensis* is not known to inhabit, and that visual observation of the feces may cause errors in species identification. The feces observed in Ulleungdo Island on October 21, 2019 and August 29, 2020, in Gyeongju on December 4, 2020, and in Jecheon on December 7, 2020 was found intactly on grass, not buried in the ground. Although it was difficult to distinguish and identify the feces of *Felis catus* and *Prionailurus bengalensis* with visual observation, the feces collected from Ulleungdo Island was closely related to the *Felis catus* according to the genetic analysis whereas the ones collected from Gyeongju and Jecheon was identified from *Prionailurus bengalensis*. Therefore through the gene analysis, this study proved that visual observation of feces with similar appearance, specifically the feces found in Ulleungdo Island, Gyeongju, and Jecheon, may cause errors in species identification. It is judged to be necessary to analyze fields signs and genes for the species identification when using the feces of *Felis catus* and *Prionailurus bengalensis*.

Key Words: *Felis catus*, *Prionailurus bengalensis*, dropping behavior, cytochrome *b* gene, specific identification

Introduction

Felis catus is an animal taxonomically belonged to Felidae in the Carnivora, and it is known as a relatively recently differentiated species from african wild cat (*Felis lybica*). The Invasive Species Specialist Group (ISSG) of the International Union for Conservation of Nature and Natural Resources (IUCN) selected *Felis catus* as one of the world's top 100 worst invasive species, and the Ministry of Environment classified it as feral cat and manages it (Lowe et al. 2000; Ministry of Environment 2001; Wilson and Reeder 2005; Kim et al. 2019).

Prionailurus bengalensis, a wild animal with similar ecological characteristics to the feral cat, is a Class II endangered wild species designated by the Ministry of Environment of Korea. Mainly inhabiting in forests and valleys, it was once distributed throughout the country but is known to disappear in Jeju Island during 1980s (National Institute of Biological Resources 2020).

In general, researchers use methods such as line transect, capture method and camera-trapping depending on the purpose and scale of the research to understand the habitat status of terrestrial mammals (Lee 2019). The field sign investigation is generally used in the survey of wildlife in

Received: October 1, 2021. Revised: November 8, 2021. Accepted: November 8, 2021.

Corresponding author: Eui Kyeong Kim
Korea National Park Research Institute, Wonju 26441, Republic of Korea
Tel: +82-33-769-1653, Fax: +82-33-769-1639, E-mail: keuik98@knps.or.kr

Korea (National Institute of Environmental Research 2006; Kim et al. 2010a; Han et al. 2018; Lee 2019).

The field sign investigation is a method that determines inhabiting or not of medium- and large-sized mammals by examining feces, footprints, diet, trace of rest, migration path, road-kill and territory marks using the ecological characteristics by mammal. It is a method that is most frequently used for the investigation of natural resources in Korea (Korea National Park Research Institute 2017; Kim et al. 2019).

It is known that feral cats have the characteristic of burying their own feces in the ground after excretion. In fact when they are introduced into the wild, they excrete on grass without burying feces on the ground; therefore, there is a high possibility that they are identified as *Prionailurus bengalensis* whose food source and feces are similar (Corbett 1979; Liberg 1984; Ministry of Environment 2001).

Therefore, this study compared the feces of the feral cats collected in Ulleungdo Island, where *Prionailurus bengalensis* does not inhabit, and that of the *Prionailurus bengalensis* found in Gyeongju and Jecheon and analyzed the genes of the feces with similar appearance to prove that there is a possibility to have an error when identifying species by the visual observation of feces. The implication of this study is that a gene analysis must be conducted when it comes to identifying wild cat and *Prionailurus bengalensis*.

Materials and Methods

Observation of feces and area of its collection

Ulleungdo is a small island with warm and humid maritime climate. Since it has ecosystem isolated from the adjacent area, this area is known as very important biogeographically and bio-evolutionary. However, there is a need to preserve the ecosystem due to destruction of nature by tourists and habitat disturbance caused by contamination and wild livestock. It is known that wild mammals do not inhabit on Ulleungdo Island, but wild animals such as Feral cat and goats still inhabit (Chung and Yang 1999).

The feces that appear to have been excreted by a *Prionailurus bengalensis* was observed in Ulleungdo Island twice on October 21, 2019 and once on August 29, 2020. The feces observed on Ulleungdo Island on August 29, 2020, in Gyeongju on December 4, 2020, and in Jecheon on December 7, 2020 were placed in a Falcon tube and transferred to the laboratory. Then, these were stored in a deep freezer (-70°C) (Table 1).

Extraction of genomic DNA

200 mg of the feces collected from Ulleungdo Island was divided into 3 e-tubes while the feces collected from Gyeongju and Jecheon were placed in an e-tube. Then, the genomic DNA was extracted by using the AccuPrep Stool DNA Extraction kit (BIONEER, Korea) and it was kept in a freezer at -20°C.

Analysis of mitochondrial DNA cytochrome b

To analyze the mtDNA cytochrome *b* from the feces,

Table 1. The list of samples

Sample no.	Date	Area	GPS
1	October 21, 2019	Sadong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, Korea (Observation)	37° 28' 45. 65" N 130° 53' 17. 17" E
2	October 21, 2019	Sadong-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, Korea (Observation)	37° 28' 39. 85" N 130° 53' 20. 49" E
3	August 29, 2020	Namseo-ri, Seo-myeon, Ulleung-gun, Gyeongsangbuk-do, Korea (Observation/Gene Analysis)	37° 29' 14. 43" N 130° 49' 24. 50" E
4	December 4, 2020	Geomdan-ri, Angang-eup, Gyeongju-si, Gyeongsangbuk-do, Korea (Observation/Gene Analysis)	35° 55' 42. 87" N 129° 11' 26. 43" E
5	December 7, 2020	Seongnae-ri, Geumseong-myeon, Jecheon-si, Chungcheongbuk-do, Korea (Observation/Gene Analysis)	37° 01' 50. 17" N 128° 10' 33. 10" E

800 bp out of the 1,140 bp cytochrome *b* was divided into three regions according to the method of Patel et al. (2017) and amplified through Polymerase Chain Reaction (PCR: BIO-RAD, T100 Thermal Cycler, USA) using three kinds of primer pairs (Table 2, 3).

Table 2. The primers and sequences for mtDNA cytochrome *b* gene amplification and sequence analysis

Primer	Primer sequence (5'→3')	Size (bp)
cyt*b1*	F: ATGACCAACATTCGAAAATC	269
	R: AATATGGAGGCTCCGTTGG	
cyt*b2*	F: CCAACGGAGCCTCCATATT	282
	R: ATCGTGTTAGGGTGGCTTTG	
cyt*b3*	F: CAAAGCCACCCTAACACGAT	288
	R: TGAGGAGGGGTGTTTAAAGG	

For the PCR reaction, 1 μL of genomic DNA (50 ng/μL), 2 μL of each primer (10 pmol/μL), 0.25 μL of DNA Taq polymerase (DiaStar^{TM}, Solgent, Korea), 5 μL of 10X buffer, 1 μL of 10 mM dNTP, and distilled water were added to have 50 μL for the final volume.

For the PCR condition, an initial denaturation was conducted at 95°C for 3 minutes and then denaturation at 94°C for 30 seconds, 54 to 56°C for 45 seconds, and 72°C for 45 seconds was promoted as one cycle. After repeating it for 40 times, the final extension was conducted at 72°C for 10 minutes and was stored at 8°C (Table 4). The PCR product was electrophoresed on 1.5% agarose gel prepared by mixing DNA staining solution (EcoDye^{TM}, Solgent, Korea) and then identified by LED (Davinch Gel imaging & Small LED Geldoc, Davinch, Korea).

Table 3. The primers activating region for mtDNA cytochrome *b* gene amplification and sequence analysis in AB194817 *Felis catus* mitochondrial cyt*b* gene (1,140 bp)

AB194817 *Felis catus* mitochondrial cyt*b* gene (1,140 bp)

ATGACCAACATTCGAAAATCATACCCCCTTACCAAAATTATTAATCACTCATTCATCGACCTACCCGCCCCATC
→ cyt*b*1 F.

TAACATCTCAGCATGATGAAACTTCGGCTCCCTTCTAGGAGTCTGCCTAATCTTACAAATCCTCACCGGCCTCTTTTT
GGCCATACACTACACATCAGACACAATAACCGCCTTTTCATCAGTTACCCACATCTGTCGCGACGTTAATTATGGCTG
AATCATCCGATATTTACACGCCAACGGAGCTTCTATATTCTTTATCTGCCTGTACATACATGTAG
← cyt*b*1 R.
→ cyt*b*2 F.

GACGGGGAATATACTACGGCTCCTACACCTTCTCAGAGACATGAAACATTGGAATCATACTATTATTTACAGTCATAG
CCACAGCTTTTATGGGATACGTCCTACCATGAGGCCAAATGTCCTTCTGAGGAGCAACCGTAATCACTAACCTCCTG
TCAGCAATTCCATACATCGGGACTGAACTAGTAGAATGGATCTGAGGGGGCTTCTCAGTAGACA
→ cyt*b*3 F.

AAGCCACCCTAACACGATTCTTCGCCTTCCACTTCATCCTTCCATTCATTATCTCAGCCTTAGCAGCAGTACAC
← cyt*b*2 R.

CTCTTATTCCTTCATGAAACAGGATCTAACAACCCCTCAGGAATTACATCCGATTCAGACAAAATCCCATTCCACCCA
TACTATACAATCAAAGACATCCTAGGTCTTCTAGTACTAGTTTTAACACTCATACTACTCGTCCTATTTTCACCAGACC
TGCTAGGAGACCCAGACAACTACATCCCAGCCAACCCTTTAAATACCCCTCCCCATATTAAACCT
← cyt*b*3 R.

GAATGATACTTCCTATTCGCATACGCAATTCTCCGATCCATCCCCAACAAACTAGGAGGAGTCCTAGCCCTAGTACTC
TCCATCCTAGTACTAGCAATCATTCCAATCCTCCACACCTCCAAACAACGAGGAATAATGTTTCGACCACTAAGCCAA
TGTCTATTCTGACTCCTAGTAGCGGATCTCCTAACCCTAACATGAATCGGTGGCCAACCTGTAGAACATCCATTCATC
ACCATCGGCCAACTAGCCTCCATCCTATATTTCTCAACCCTCCTAATCCTAATACCCATCTCAGGCATTATTGAAAACC
GTCTACTCAAATGAAGA

Table 4. The PCR condition for mtDNA cytochrome *b* gene amplification

Step	Temp. (°C)	Time	Cycles
Initial denaturation	95	3 min.	1
Denaturation	94	30 sec.	40
Annealing	54-56	45 sec.	
Extension	72	45 sec.	
Final extension	72	10 min.	1

The PCR product was purified with the Gel and PCR Clean-up kit (LaboPass, Korea), and then the nucleic sequence was analyzed using ABI 3730XL DNA Analyzer (Applied Biosystems, USA).

Preparation of phylogenetic tree

For the nucleotide sequence analysis, multiple alignment was performed using Geneious Prime (version 11.0.4+11), and the results were compared and analyzed after obtaining the nucleotide sequence information of *Prionailurus bengalensis* and *Felis catus* from the GenBank of the National Center for Biotechnology Information (NCBI) (Tamada et al. 2005; Benson et al. 2007; Kim et al. 2010b; Koh and Jang 2012) (Table 5). The genetic relationship of the collected feces samples was analyzed by using the Mega 6.06 program (Kumar et al. 2004), and then a phylogenetic tree was created by implementing the neighbor-joining method (Saitou and Nei 1987; Patel et al. 2017).

Result

Result of the feces' visual observation

The Table 6 shows the state of feces observed in Ulleungdo Island on October 21, 2019 and August 29, 2020, in Gyeongju on December 4, 2020, and in Jecheon on December 7, 2020. All feces that were visually observed were found excreted on grass not buried in the ground.

Result of the feces' gene analysis

The analysis result of the mitochondrial cyt*b* gene for the feces collected in Ulleungdo Island on August 29, 2020, in Gyeongju on December 4, 2020, and in Jecheon on December 7, 2020 is shown in Figs. 1-4. The 800 bp out of the 1,140 bp cytochrome *b* was divided into three regions and PCR using three primer pairs was conducted respectively. The Figs. 1-3 show the relationship after the sequencing promoted after the PCR and the Fig. 4 is the result that expressed and identified the relationship after combining the nucleotide sequence information of the three regions. Although it was difficult to distinguish and identify the *Felis catus* and *Prionailurus bengalensis* by visual observation of the feces but as all the results of the gene analysis show, the feces collected from Ulleungdo Island is closely related to the *Felis catus*, whereas the feces collected from Gyeongju and Jecheon are identified to be from *Prionailurus bengalensis*.

Table 5. Comparison for the *Prionailurus bengalensis* (AB194818, JF693231) and *Felis catus* (AB194817) in the NCBI GenBank nucleotide database

GenBank number	Species	Localities and source	GenBank information
JF693231	*Prionailurus bengalensis*	Korea	Koh and Jang (2012)
AB194818	*Prionailurus bengalensis*	Japan	Tamada et al. (2005)
AB194817	*Felis catus*	Japan	Tamada et al. (2005)

Discussion

This study is designed according to the fact that the feces presumed to be from a *Prionailurus bengalensis* was found in Ulleungdo Island, where *Prionailurus bengalensis* is not known to inhabit, and that visual observation of the feces may cause errors in species identification.

The field sign investigation is generally used in the survey of wildlife in Korea (National Institute of Environmental Research 2006; Kim et al. 2010a; Han et al. 2018; Lee 2019). The field sign investigation is a method that determines inhabiting or not of medium- and large-sized mammals by examining feces, footprints, diet, trace of rest, migration path, road-kill and territory marks using the ecological characteristics by mammal. It is a method that is most frequently used for the investigation of natural resources in Korea (Korea National Park Research Institute 2017; Kim et al. 2019).

Several reports suggest that feces of feral cats may be

Table 6. Feces observed on Ulleungdo Island, Gyeongju, and Jecheon

Sample no.	Date	Area	Image
1	October 21, 2019	Ulleungdo Island (Observation)	
2	October 21, 2019	Ulleungdo Island (Observation)	
3	August 29, 2020	Ulleungdo Island (Observation/Gene Analysis)	
4	December 4, 2020	Gyeongju (Observation/Gene Analysis)	
5	December 7, 2020	Jecheon (Observation/Gene Analysis)	

mistakenly identified as the feces from *Prionailurus bengalensis*. It is known that the wild cats generally bury their own feces in the ground after excretion; however when they are introduced into the wild, they excrete on grass without burying feces, leading to an error in species identification due to similar food source and feces form (Corbett 1979; Liberg 1984; Ministry of Environment 2001).

In this study, all feces observed visually was found on the grass being exposed after excretion, so it was identified as the feces of *Prionailurus bengalensis*. According to the results of gene analysis of the feces, however, the feces collected from Ulleungdo Island except for the ones from Gyeongju and Jecheon was determined to be from a feral cat.

Therefore, this study proved that errors in species identification can occur by visual observation of feces by analyzing the genes of the feces with similar appearance found in Ulleungdo Island, Gyeongju, and Jecheon. It is judged to be necessary to analyze fields signs and genes for the species identification when using the feces of *Felis catus* and *Prionailurus bengalensis*.

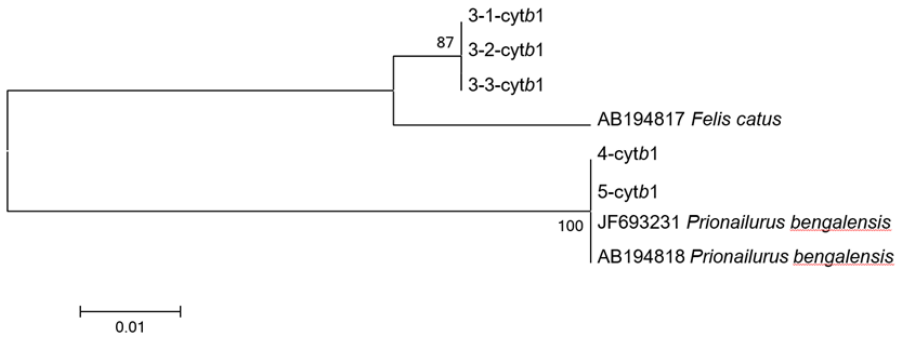

Fig. 1. Neighbor-joining tree for mitochondrial DNA cytochrome *b* (primer: cyt*b*1 F/R) of Ulleungdo Island sample, *Felis catus* and *Prionailurus bengalensis*. Sample No. 3: Ulleungdo Island, Sample No. 4: Gyeongju, Sample No. 5: Jecheon.

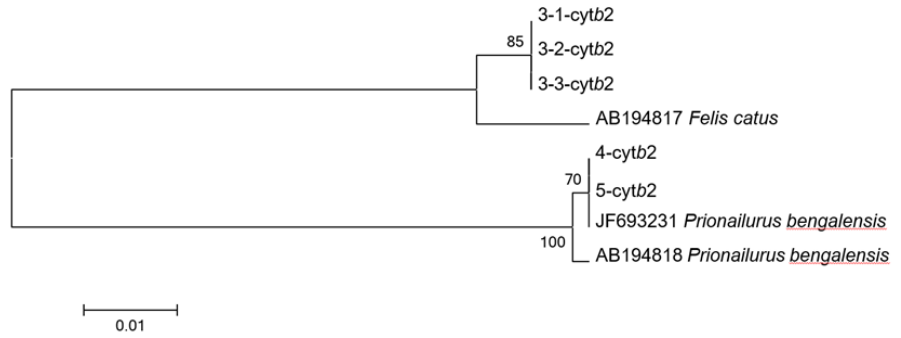

Fig. 2. Neighbor-joining tree for mitochondrial DNA cytochrome *b* (primer: cyt*b*2 F/R) of Ulleungdo Island sample, *Felis catus* and *Prionailurus bengalensis*. Sample No. 3: Ulleungdo Island, Sample No. 4: Gyeongju, Sample No. 5: Jecheon.

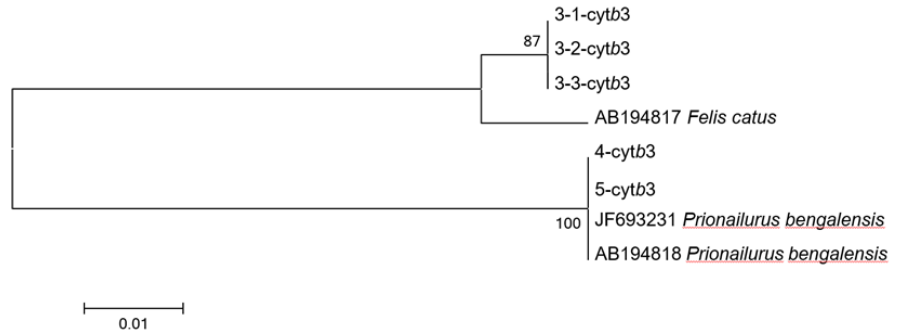

Fig. 3. Neighbor-joining tree for mitochondrial DNA cytochrome *b* (primer: cyt*b*3 F/R) of Ulleungdo Island sample, *Felis catus* and *Prionailurus bengalensis*. Sample No. 3: Ulleungdo Island, Sample No. 4: Gyeongju, Sample No. 5: Jecheon.

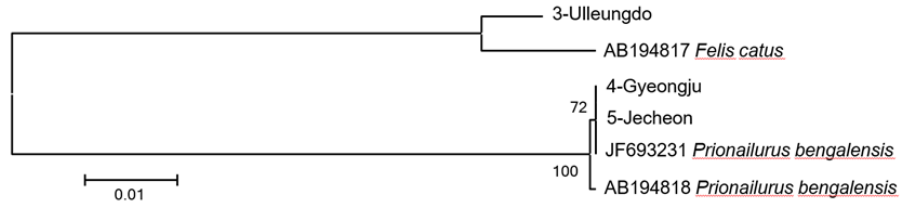

Fig. 4. Neighbor-joining tree for mitochondrial DNA cytochrome *b* (primer: cyt*b* F/R) of Ulleungdo Island sample, *Felis catus* and *Prionailurus bengalensis*. Sample No. 3: Ulleungdo Island, Sample No. 4: Gyeongju, Sample No. 5: Jecheon.

Acknowledgements

This research was supported by the Park Resources Research Project of the National Park Research Institute, Korea National Park Service.

References

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2007. GenBank. Nucleic Acids Res 35(Database issue): D21-D25.

Chung KH, Yang HJ. 1999. A Study on the Fauna and Speciation of the Cheju Island and Ulrung Dagelet in Korea. J Kyonggi Basic Sci 12: 189-200.

Corbett LK. 1979. Feeding ecology and social organization of wildcats (*Felis silvestris*) and domestic cats (*Felis catus*) in Scotland. PhD thesis. University of Aberdeen, Aberdeen, United Kingdom. (in English)

Han CW, Lim SJ, Park HB, Park YC. 2018. Seasonal Characteristics of Fecal Sites of the Siberian Flying Squirrel *Pteromys volans*. J For Environ Sci 34: 184-187.

Kim EK, Kim HR, Park YC. 2010a. Terrestrial Mammal Fauna from the Royal Tombs of the Joseon Dynasty. J Natl Park Res 1: 284-288.

Kim KM, Woo DG, Seo HJ, Park TJ, Song EG, Choi TY. 2019. Korea Road-Kill Observation System: The First Case to Integrate Road-Kill Data in National Scale by Government. J For Environ Sci 35: 281-284.

Kim YS, Yoo MH, Jung BD, Kim JT. 2010b. Genetic diversity in Korean leopard cats (*Prionailurus bengalensis euptilura*), based on mitochondrial DNA cytochrome *b* gene sequence analysis. Korean J Vet Serv 33: 353-359.

Koh HS, Jang KH. 2012. Taxonomic status of *P. iriomotensis*. https://www.ncbi.nlm.nih.gov/nuccore/JF693231.1. Accessed Feb 2021.

Korea National Park Research Institute. 2017. Gyeongju National Park Nature Resource Survey. Korea National Park Service, Wonju.

Kumar S, Tamura K, Nei M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief Bioinform 5: 150-163.

Lee JB. 2019. Studies on the taxonomic review and survey techniques for mammals in the Korean peninsula. PhD thesis. Incheon National University, Incheon, Korea. (in Korean with English abstract)

Liberg O. 1984. Food Habits and Prey Impact by Feral and House-Based Domestic Cats in a Rural Area in Southern Sweden. J Mammal 65: 424-432.

Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. 100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database. Invasive Species Specialist Group, Auckland.

Ministry of Environment. 2001. A Study on the Habitat Condition and Management Plan of Domestic Cats. Ministry of Environment, Sejong.

National Institute of Biological Resources. 2021. Red Data Book of Republic of Korea Volume 4. Mammals. 2nd ed. National Institute of Biological Resources, Incheon.

National Institute of Environmental Research. 2003. Wildlife Survey. No. 2003-07-679.

Patel RP, Wutke S, Lenz D, Mukherjee S, Ramakrishnan U, Veron G, Fickel J, Wilting A, Forster DW. 2017. Genetic Structure and Phylogeography of the Leopard Cat (*Prionailurus bengalensis*) Inferred from Mitochondrial Genomes. J Hered 108: 349-360.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425.

Tamada T, Kurose N, Masuda R. 2005. Genetic diversity in domestic cats *Felis catus* of the Tsushima Islands, based on mitochondrial DNA cytochrome b and control region nucleotide sequences. Zoolog Sci 22: 627-633.

Wilson DE, Reeder DM. 2005. Mammal Species of the World: A Taxonomic and Geographic Reference. 3rd ed. Johns Hopkins University Press, Baltimore, MD.