

Testing microsatellite loci and preliminary genetic study for Eurasian otter in South Korea

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We used a non-invasive technique with microsatellite primers to investigate genetic variation among Eurasian otters *Lutra lutra* in eastern South Korea. We collected twenty two otter spraints in January and six in August 2008. We used spraints from five dead otters from five different river systems for the present genetic analysis. We extracted DNA from 20 spraints from the January sample. Ten microsatellite primers (Lut435, Lut453, Lut457, Lut604, Lut615, Lut701, Lut715, Lut717, Lut733, and Lut832) for Eurasian otters were tested, and four loci were successfully amplified for further analyses. The results of genotyping the otter population with microsatellite loci lead to the identification of 9 individuals from the Ungokcheon Stream. The Ungokcheon population also showed a genetic structure represented by the Hardy-Weinberg equilibrium.

Keywords: Eurasian otter, genotyping, microsatellite, non-invasive, South Korea

INTRODUCTION

Eurasian otter *Lutra lutra* has a distribution from Western Europe to far Eastern Asia (Wilson and Reeder, 2005). Due to excessive fur trapping and habitat destruction, most Eurasian populations have declined over last two centuries (Mason and Macdonald, 1986; Won and Smith, 1999). Unlike European populations, few Asian populations have been actively studied (Reuther *et al.*, 2000). Through extensive researches, the Japanese otter was determined to be a separate species (Imaizumi and Yoshiyuki, 1989), however, systematic and phylogenetic studies on otter populations in Korea have rarely been conducted.

Because the Korean otter has been designated as an endangered species and a natural monument, non-invasive methods are necessary for genetic studies (Taberlet *et al.*, 1999). Fortunately, non-invasive techniques are well suited to otter research. First, feces provide the best material for non-invasive DNA extraction compared to hair and saliva (Sugimoto *et al.*, 2012). Second, otters deposit their spraints on prominent rocks, which facilitates detection of samples (Brown *et al.*, 1992). As a result of the DNA in feces being easily fragmented by enzymes, short length genes (i.e., microsatellites) are suitable for analysis (Wasser *et al.*, 1997). Microsatellite DNA can be used to identify individuals, thus, helping to improve estimates of population and home range (Goldstein and Schlötterer, 1999).

Although the Korean otter population is protected by

the Natural Environment Conservation Act in Korea, more ecological information about the Eurasian otter is needed before a comprehensive conservation strategy can be organized. To enable a greater understanding of the Eurasian otter in Korea we examined the size of the population, the genetic healthiness and mating conditions of the otter.

This preliminary research in non-invasive techniques will help set the guidelines for future otter surveys and augment ongoing conservation efforts by providing vital ecological information on the status of the Korean otter.

MATERIALS AND METHODS

Sample collection

We collected otter spraints along the Ungok River, Gyeongsangbuk-do on January and August 2008 (Fig. 1). In order to collect fresh samples we removed all spraints in the study area on first day and collected spraints the following 3 days before sunrise. Each spraint was stored in 50 mL tube with 98% ethanol. We placed tubes in a cooler with dry ice at each collecting site (Hajkova *et al.*, 2006). The samples were kept below -70°C for DNA analysis.

We also collected 5 otter caracasses from 5 different water systems (Fig. 2), and used muscle tissue for DNA extraction. The skins and skeletons were made into specimens and stored at the National Institute of Biological Resources (NIBR), Korea.

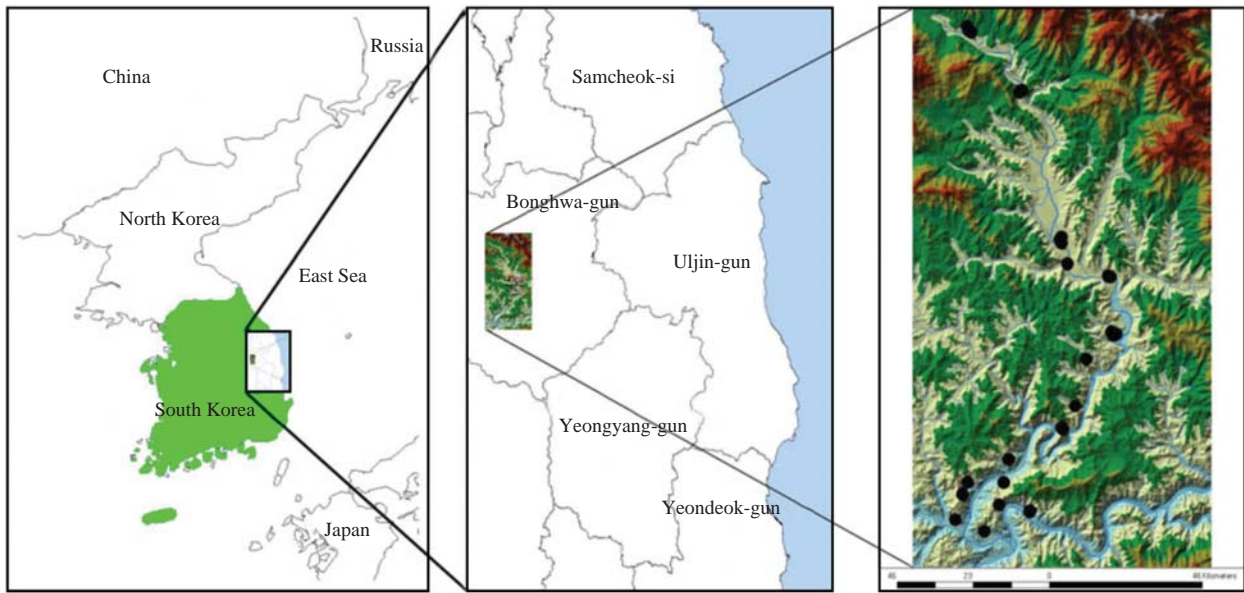


Fig. 1. Spraints collection sites along Ungokcheon Stream, Bonghwa-gun, Gyeongsangbuk-do.

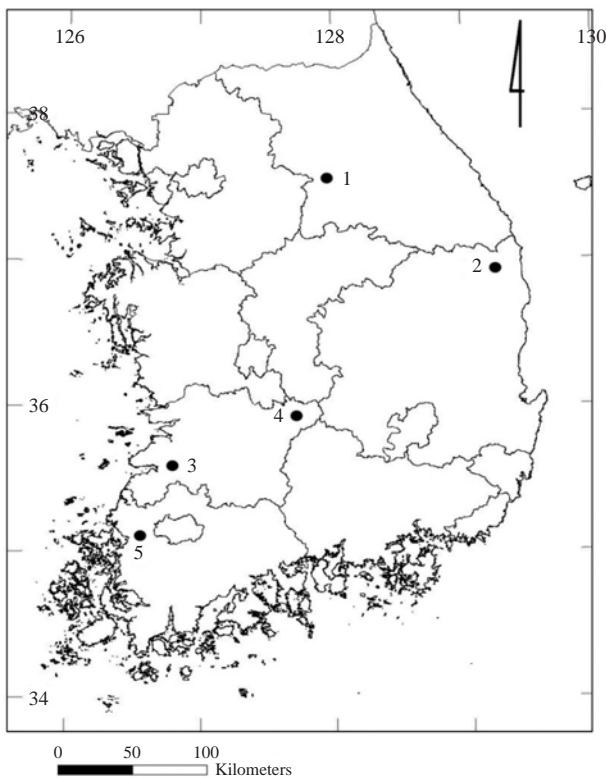


Fig. 2. Locations of sampling for tissue (1. Hoengseong-gun, Gangwon-do, 2. Uljin-gun, Gyeongsangbuk-do, 3. Jeongeup-si, Jeollabuk-do, 4. Muju-gun, Jeollabuk-do, 5. Hampyeong-gun, Jeollanam-do).

Polymerase chain reactions (PCRs) and sequencing

We used a QIAmp DNA Stool Mini Kit (Qiagen, Valen-

cia, California) on the spraints for DNA extraction. We used a DNeasy Blood & Tissue Kit (Qiagen, Valencia, California) to extract DNA from muscle samples. We tested 10 microsatellite primers (Lut435, Lut453, Lut457, Lut604, Lut615, Lut701, Lut715, Lut717, Lut733 and Lut832) from Dallas and Piertney (1998). From the results we chose the most efficient loci (Lut435, Lut457, Lut717 and Lut701) by using a primer pair to amplify each locus with a fluorescent dye. We conducted PCR amplification under the following conditions: (a) initial denaturation at 95°C for 5 min; (b) 30 cycles in which each cycle is composed of denaturation at 95°C for 30s, annealing at 55-60°C depending on each primer set for 30 s, and elongation at 72°C for 30 s; and (c) final elongation at 72°C for 5 min. The reaction mixtures (total 25 µL) were composed of 5 µL of template DNA, 0.5 µL of each primer (10 µM), 1.0 µL of dNTP solution (10 mM), 2.5 µL of *Taq* buffer (10X), 0.2 µL of *Taq* DNA polymerase (Promega, Madison, WI), and 12.8 µL of distilled water (DW). PCR amplification was performed using GeneAmp 9700 (Applied Biosystems, Foster City, CA). Cytochrome oxidase subunit I (COI) and control region and 16S ribosomal RNA gene region in mitochondrial genome were amplified using primer set of LCO-1490 and HCO-2198 (Folmer *et al.*, 1994), 16SAR-SF and 16SBR-SF (Palumbi *et al.*, 1991), and mtCONF and mtCONR (Jung *et al.*, 2009). The total of 25 µL of reaction mixtures were composed of 1 µL of template DNA, 0.5 µL of each primer (10 µM), 1.0 µL of dNTP solution (10 mM), 2.5 µL of *Taq* buffer (10X), 0.2 µL of *Taq* DNA polymerase (Promega, Madison, WI), and 16.8 µL of distilled water (DW). We used a QIAquick PCR purification kit (Qiagen, Val-

encia, CA) to purify amplified products from the PCR mixtures. We used the amplifying primers and the BigDye Terminator Sequencing Kit (Applied Biosystems, Foster City, CA) to sequence the PCR products. We used Gene Amp 9700 to conduct PCRs and sequencing reactions (Applied Biosystems, Foster City, CA), and analyzed purified products of sequencing reaction on a Genetic Analyzer 3730 (Applied Biosystems).

Data analysis

We used ARLEQUIN 3.11 (Excoffier *et al.*, 2005) to calculate observed and expected heterozygosity, and tested the Hardy-Weinberg equilibrium by using GENEPOP 3.4 (Raymond and Rousset, 1995). Mitochondrial sequences (COI, 16S rDNA, and control regions) were aligned using CLUSTAL X (Thompson *et al.*, 1997), and we used DnaSP 5.10.01 (Librado and Rozas, 2009) to calculate haplotype and nucleotide diversity, and average number of pairwise differences.

RESULTS

We extracted otter DNA from 20 of 22 spraints collected in January, and 0 from 6 spraints collected in August.

Nine out of the 10 microsatellite primers functioned on the Korean otters. Of these, Lut435, Lut457, Lut717 and Lut701 were amplified the most from the specimens. The results of the microsatellite genotype are shown in Table 1. The allelic diversity ranged from 3 to 4. The observed heterozygosities of loci varied from 0.28571 to

Table 1. Results of microsatellite genotyping from otter spraints along Ungokcheon Stream.

Spraints	Lut435	Lut457	Lut717	Lut701
1	-/-	182/182	184/188	204/204
2	122/122	182/182	184/184	204/208
3	118/122	182/182	184/184	204/208
4	118/122	182/182	-/-	-/-
5	118/122	182/186	184/188	196/208
6	120/122	180/182	-/-	-/-
7	122/122	-/-	-/-	-/-
8	122/122	-/-	184/188	-/-
9	118/122	-/-	184/192	-/-
10	122/122	182/182	188/192	200/204
11	122/122	-/-	-/-	-/-
12	122/122	182/182	-/-	-/-
13	118/122	180/180	184/192	204/208
14	122/122	180/182	188/192	200/204
15	118/122	180/180	184/192	-/-
16	122/122	182/182	184/188	204/204
17	118/122	180/180	184/184	204/208
18	-/-	-/-	-/-	-/-
19	118/122	182/182	184/192	-/-
20	118/122	182/186	184/188	196/208

0.80000, and did not represent significant deviation from heterozygosity values expected under Hardy-Weinberg equilibrium (Table 2).

The COI gene sequences acquired from 4 individuals are 658 bp in length and show no genetic variation (see aligned sequence data set in Appendix 1). The mean GC-content of the COI gene sequences was 0.407. The 16S ribosomal RNA gene sequences from 5 individuals represented no genetic variation (see Appendix 2). The 16S ribosomal RNA gene sequences are 550 bp in length and no sequence length variability was observed. The mitochondrial control regions are 1,096 bp in length. Unlike the former 2 regions, the mitochondrial control region sequences from 5 individuals were genetically variable. Three haplotypes were observed with a haplotype diversity score of 0.800 (± 0.164). Nucleotide diversity (per site) was 0.00292 (± 0.00107) and the mean number of pairwise differences was 3.2. The summary statistics of genetic polymorphism on these regions are summarized in Table 3.

When we performed a BLAST search, the sequences showed a high similarity with those of mustelid species. The COI sequences of the yellow throated marten (*Martes flavigula*) and the sea otter (*Enhydra lutris*) represented about 84% of identity with that of our study. For the 16S ribosomal RNA gene sequences, the American mink (*Neovison vison*) and the sea otter (*Enhydra lutris*) showed 96% and 95% similarity, respectively. The mitochondrial control region sequences of our study showed 98%, 88% and 84% identity with Italian specimens of the same spe-

Table 2. Results of Hardy-Weinberg equilibrium test from otter spraints along Ungok river.

Locus	No. allele	No. genotype	H_O	H_E	P -value
Lut435	3	3	0.66667	0.52414	0.31590
Lut457	3	4	0.28571	0.56349	0.07060
Lut717	3	4	0.80000	0.72105	0.37048
Lut701	4	3	0.78571	0.62698	0.79835

No. Allele, number of alleles; No. Genotype, number of genotypes; H_O , observed heterozygosity; H_E , expected heterozygosity; P -value, significance of departure from Hardy-Weinberg equilibrium

Table 3. Summary statistics of mitochondrial sequence data.

Gene	COI	16S rRNA	Control region
Number of individuals	4	5	5
Size of sequences (bp)	658	550	1094-1095
Number of segregating sites	0	0	7
Number of haplotypes	1	1	3
Haplotype diversity	0	0	0.800 \pm 0.164
Nucleotide diversity (per site)	0	0	0.00292 \pm 0.00107
Average number of pairwise difference	0	0	3.200

cies of (GenBank accession number: AY860320) sea otter (*Enhydra lutris*) and American mink (*Neovison vison*), respectively.

DISCUSSION

We collected a lesser number of spraints during our summer investigation than in the winter, which we attribute to a seasonal difference. This conforms with previous studies showing that the number of spraints is inversely proportional to temperature (Jo *et al.*, 2006; Mason and Macdonald, 1986; Macdonald and Mason, 1987; Kruuk, 1995). As in previous studies (Hajkova *et al.*, 2006), our DNA extraction success rate was higher for winter samples compared to summer samples. We recommend collecting spraint samples in winter regardless of the more challenging weather conditions.

Nine microsatellite loci were amplified from 10 loci. However, only 4 loci were suitable for further analysis. This result could be explained by 2 factors. First, some primer sites might be modified in their sequences. The primers used in this study were designed from the European population by Dallas and Piertney (1998). Because regional variation was observed in the mitochondrial control region sequences in our study, it is probable that the Korean population has sequences of primer sites that differ from its European counterpart. We recommend a new set of microsatellite loci should be developed for different populations. Second, unlike muscle tissue, it is not easy to obtain high quality DNA from spraints. Failure of DNA extraction from summer spraints suggests that the DNA is easily degraded and DNA stability may be highly dependent on sampling and treatment conditions. Therefore, DNA extracted from fecal samples, if not treated properly, might not be suitable for microsatellite analyses. Despite these challenges we amplified 4 loci of microsatellite: Lut435, Lut453, Lut701 and Lut717.

Based on the result of microsatellite genotypes, otters inhabiting Ungokcheon Stream may be in Hardy-Weinberg equilibrium (Table 2). This indicates that individuals from Ungokcheon formed a population (i.e. a reproductive unit). A study on the genetic structure of *Lutra lutra* populations in Korea has not been attempted even though this species has been designated by the Korean government as an endangered species. Investigating the genetic structure of populations is of utmost importance in the study of population genetics. A study of population genetics will provide valuable information in establishing a conservation measurement on vulnerable and endangered species (Wagner *et al.*, 2005).

No genetic variation in COI and 16S rRNA gene sequences was found among individuals of the Korean population even though we investigated 4 and 5 individuals,

respectively. This suggests that these 2 gene regions are not suitable for population genetic studies. Rather, these regions would be appropriate for species identification not only with no intraspecific genetic variation but with interspecific genetic variation. In contrast to these 2 regions, 3 haplotypes were observed from 5 individuals in sequences of mitochondrial control regions. Such considerable genetic variability makes this region useful for population genetic study.

Even though genotyping failed in some individuals we can identify at least 9 individual otters along Ungokcheon Stream. This count is less than the estimation by traditional methods of observing spraints, which counted about 12 individuals. Fecal shape examination is the least expensive method but is prone to errors in population size estimation. DNA fingerprinting technique provides a more accurate and firm estimation and, therefore, should be used for endangered species.

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Appendix 1. An aligned sequence set of COI gene from 4 otter tissue specimens.

Uljin CACCCTTACCTTTTATTTGGTGCATGAGTCAGAATGGTAGGCACTGCTCTTAACCTATT
 Jeongeup CACCCTTACCTTTTATTTGGTGCATGAGTCAGAATGGTAGGCACTGCTCTTAACCTATT
 Hampyeong CACCCTTACCTTTTATTTGGTGCATGAGTCAGAATGGTAGGCACTGCTCTTAACCTATT
 Muju CACCCTTACCTTTTATTTGGTGCATGAGTCAGAATGGTAGGCACTGCTCTTAACCTATT

Uljin AATCTGTGCTGAATTGGGCCAACCCAGGTGCTCTACTAGGAGATGACCACATCTACAACCT
 Jeongeup AATCTGTGCTGAATTGGGCCAACCCAGGTGCTCTACTAGGAGATGACCACATCTACAACCT
 Hampyeong AATCTGTGCTGAATTGGGCCAACCCAGGTGCTCTACTAGGAGATGACCACATCTACAACCT
 Muju AATCTGTGCTGAATTGGGCCAACCCAGGTGCTCTACTAGGAGATGACCACATCTACAACCT

Uljin AATTGTAACCCTTATGCATTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGG
 Jeongeup AATTGTAACCCTTATGCATTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGG
 Hampyeong AATTGTAACCCTTATGCATTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGG
 Muju AATTGTAACCCTTATGCATTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATTGG

Uljin AGGGTTCAGAAACTGATTAGTACCCTTAATAATTGGCACATGCGACATAGCATTTCACG
 Jeongeup AGGGTTCAGAAACTGATTAGTACCCTTAATAATTGGCACATGCGACATAGCATTTCACG
 Hampyeong AGGGTTCAGAAACTGATTAGTACCCTTAATAATTGGCACATGCGACATAGCATTTCACG
 Muju AGGGTTCAGAAACTGATTAGTACCCTTAATAATTGGCACATGCGACATAGCATTTCACG

Uljin AATAAGTAATATAAGCTTTTGACTTCTGCCCCCTCCTTTCCTCCCCTTCTAGCTTCTTC
 Jeongeup AATAAGTAATATAAGCTTTTGACTTCTGCCCCCTCCTTTCCTCCCCTTCTAGCTTCTTC
 Hampyeong AATAAGTAATATAAGCTTTTGACTTCTGCCCCCTCCTTTCCTCCCCTTCTAGCTTCTTC
 Muju AATAAGTAATATAAGCTTTTGACTTCTGCCCCCTCCTTTCCTCCCCTTCTAGCTTCTTC

Uljin CATGGTAGAAGCAGGTGCAGGAACAGGATGAACTGTGTACCCTCCTCTAGCAGGAAATTT
 Jeongeup CATGGTAGAAGCAGGTGCAGGAACAGGATGAACTGTGTACCCTCCTCTAGCAGGAAATTT
 Hampyeong CATGGTAGAAGCAGGTGCAGGAACAGGATGAACTGTGTACCCTCCTCTAGCAGGAAATTT
 Muju CATGGTAGAAGCAGGTGCAGGAACAGGATGAACTGTGTACCCTCCTCTAGCAGGAAATTT

Uljin AGCACATGCAGGAGCATCCGCAAACCTTAACAATCTTCTCTACACCTGGCAAGTGCTC
 Jeongeup AGCACATGCAGGAGCATCCGCAAACCTTAACAATCTTCTCTACACCTGGCAAGTGCTC
 Hampyeong AGCACATGCAGGAGCATCCGCAAACCTTAACAATCTTCTCTACACCTGGCAAGTGCTC
 Muju AGCACATGCAGGAGCATCCGCAAACCTTAACAATCTTCTCTACACCTGGCAAGTGCTC

Uljin ATCCATCCTAGGAACCATTAACCTTTATTACTACCATTATCAACATAAAAACCCCTGCAAT
 Jeongeup ATCCATCCTAGGAACCATTAACCTTTATTACTACCATTATCAACATAAAAACCCCTGCAAT
 Hampyeong ATCCATCCTAGGAACCATTAACCTTTATTACTACCATTATCAACATAAAAACCCCTGCAAT
 Muju ATCCATCCTAGGAACCATTAACCTTTATTACTACCATTATCAACATAAAAACCCCTGCAAT

Uljin ATCACAATATCAAACCTCCTCTATTTGTATGATCTGTCCTTATTATGGCTGTACTTCTACT
 Jeongeup ATCACAATATCAAACCTCCTCTATTTGTATGATCTGTCCTTATTATGGCTGTACTTCTACT
 Hampyeong ATCACAATATCAAACCTCCTCTATTTGTATGATCTGTCCTTATTATGGCTGTACTTCTACT
 Muju ATCACAATATCAAACCTCCTCTATTTGTATGATCTGTCCTTATTATGGCTGTACTTCTACT

Uljin CTTATCATTACCAGTTCTATCAGCCAGTATTACCATACTACTCACAGACCGAAATCTAAA
 Jeongeup CTTATCATTACCAGTTCTATCAGCCAGTATTACCATACTACTCACAGACCGAAATCTAAA
 Hampyeong CTTATCATTACCAGTTCTATCAGCCAGTATTACCATACTACTCACAGACCGAAATCTAAA
 Muju CTTATCATTACCAGTTCTATCAGCCAGTATTACCATACTACTCACAGACCGAAATCTAAA

Uljin TACTACCTTCTTTGACCCAGCTGGAGGAGGAGACCCTATTCTGTATCAACACCTATTCT
 Jeongeup TACTACCTTCTTTGACCCAGCTGGAGGAGGAGACCCTATTCTGTATCAACACCTATTCT
 Hampyeong TACTACCTTCTTTGACCCAGCTGGAGGAGGAGACCCTATTCTGTATCAACACCTATTCT
 Muju TACTACCTTCTTTGACCCAGCTGGAGGAGGAGACCCTATTCTGTATCAACACCTATTCT

Appendix 2. An aligned sequence set of 16S ribosomal RNA gene from 5 otter tissue specimens.

Hoengseong CACCTCCAGCATATCTAGTATTGGAGGCACTGCCTGCCAGTGACGCTAGTTCAACGGCC
 Uljin CACCTCCAGCATATCTAGTATTGGAGGCACTGCCTGCCAGTGACGCTAGTTCAACGGCC
 Jeongeup CACCTCCAGCATATCTAGTATTGGAGGCACTGCCTGCCAGTGACGCTAGTTCAACGGCC
 Hampyeong CACCTCCAGCATATCTAGTATTGGAGGCACTGCCTGCCAGTGACGCTAGTTCAACGGCC
 Muju CACCTCCAGCATATCTAGTATTGGAGGCACTGCCTGCCAGTGACGCTAGTTCAACGGCC

Hoengseong GCGGTATCCTGACCGTGCAAAGGTAGCATAATCATTGTCTCTAAATAGGGACTTGTAT
 Uljin GCGGTATCCTGACCGTGCAAAGGTAGCATAATCATTGTCTCTAAATAGGGACTTGTAT
 Jeongeup GCGGTATCCTGACCGTGCAAAGGTAGCATAATCATTGTCTCTAAATAGGGACTTGTAT
 Hampyeong GCGGTATCCTGACCGTGCAAAGGTAGCATAATCATTGTCTCTAAATAGGGACTTGTAT
 Muju GCGGTATCCTGACCGTGCAAAGGTAGCATAATCATTGTCTCTAAATAGGGACTTGTAT

Hoengseong GAATGGCCACACGAGGGTTAACTGTCTTACTTCCAATCAGTGAAAATTGACCTTCCC
 Uljin GAATGGCCACACGAGGGTTAACTGTCTTACTTCCAATCAGTGAAAATTGACCTTCCC
 Jeongeup GAATGGCCACACGAGGGTTAACTGTCTTACTTCCAATCAGTGAAAATTGACCTTCCC
 Hampyeong GAATGGCCACACGAGGGTTAACTGTCTTACTTCCAATCAGTGAAAATTGACCTTCCC
 Muju GAATGGCCACACGAGGGTTAACTGTCTTACTTCCAATCAGTGAAAATTGACCTTCCC

Hoengseong TGAAGAGGCGGGAATACACCAATAAGACGAGAAGACCCTATGGAGCTTAAATTAAC
 Uljin TGAAGAGGCGGGAATACACCAATAAGACGAGAAGACCCTATGGAGCTTAAATTAAC
 Jeongeup TGAAGAGGCGGGAATACACCAATAAGACGAGAAGACCCTATGGAGCTTAAATTAAC
 Hampyeong TGAAGAGGCGGGAATACACCAATAAGACGAGAAGACCCTATGGAGCTTAAATTAAC
 Muju TGAAGAGGCGGGAATACACCAATAAGACGAGAAGACCCTATGGAGCTTAAATTAAC

Hoengseong CCATAAATACTTACTAAATCACCGATCAGGCCTAACACAATCCTATTAATGGGTTAGCAA
 Uljin CCATAAATACTTACTAAATCACCGATCAGGCCTAACACAATCCTATTAATGGGTTAGCAA
 Jeongeup CCATAAATACTTACTAAATCACCGATCAGGCCTAACACAATCCTATTAATGGGTTAGCAA
 Hampyeong CCATAAATACTTACTAAATCACCGATCAGGCCTAACACAATCCTATTAATGGGTTAGCAA
 Muju CCATAAATACTTACTAAATCACCGATCAGGCCTAACACAATCCTATTAATGGGTTAGCAA

Hoengseong TTTAGGTTGGGGCGACCTCGGAGAACAACAACTCCGAGTGATTTAATCACAGACAAA
 Uljin TTTAGGTTGGGGCGACCTCGGAGAACAACAACTCCGAGTGATTTAATCACAGACAAA
 Jeongeup TTTAGGTTGGGGCGACCTCGGAGAACAACAACTCCGAGTGATTTAATCACAGACAAA
 Hampyeong TTTAGGTTGGGGCGACCTCGGAGAACAACAACTCCGAGTGATTTAATCACAGACAAA
 Muju TTTAGGTTGGGGCGACCTCGGAGAACAACAACTCCGAGTGATTTAATCACAGACAAA

Hoengseong CCAGTCGAAGCATTCTATCATTGATCCAATAACTTGATCAACGGAACAAGTTACCC
 Uljin CCAGTCGAAGCATTCTATCATTGATCCAATAACTTGATCAACGGAACAAGTTACCC
 Jeongeup CCAGTCGAAGCATTCTATCATTGATCCAATAACTTGATCAACGGAACAAGTTACCC
 Hampyeong CCAGTCGAAGCATTCTATCATTGATCCAATAACTTGATCAACGGAACAAGTTACCC
 Muju CCAGTCGAAGCATTCTATCATTGATCCAATAACTTGATCAACGGAACAAGTTACCC

Hoengseong TAGGGATAACAGCGCAATCCTATTTGAGAGTCCATATCAACAATAGGGTTTACGACCTCG
 Uljin TAGGGATAACAGCGCAATCCTATTTGAGAGTCCATATCAACAATAGGGTTTACGACCTCG
 Jeongeup TAGGGATAACAGCGCAATCCTATTTGAGAGTCCATATCAACAATAGGGTTTACGACCTCG
 Hampyeong TAGGGATAACAGCGCAATCCTATTTGAGAGTCCATATCAACAATAGGGTTTACGACCTCG
 Muju TAGGGATAACAGCGCAATCCTATTTGAGAGTCCATATCAACAATAGGGTTTACGACCTCG

Hoengseong ATGTTGGATCAGGACATCCTAATGGTGCAGCAGCTATTAAGGGTTCGTTTGTTCACGAT
 Uljin ATGTTGGATCAGGACATCCTAATGGTGCAGCAGCTATTAAGGGTTCGTTTGTTCACGAT
 Jeongeup ATGTTGGATCAGGACATCCTAATGGTGCAGCAGCTATTAAGGGTTCGTTTGTTCACGAT
 Hampyeong ATGTTGGATCAGGACATCCTAATGGTGCAGCAGCTATTAAGGGTTCGTTTGTTCACGAT
 Muju ATGTTGGATCAGGACATCCTAATGGTGCAGCAGCTATTAAGGGTTCGTTTGTTCACGAT

Hoengseong TAAAGTCCTA
 Uljin TAAAGTCCTA
 Jeongeup TAAAGTCCTA
 Hampyeong TAAAGTCCTA
 Muju TAAAGTCCTA

Appendix 3. Aligned sequence set of mitochondrial control region from 5 otter tissue specimens.

Hoengseong TTCCCTGATTCTCTCACCCACATTTCAATTCATATATTCAACGACATTTACTGTGCCTG
Hampyeong TTCCCTGATTCTCTCACCCACATTTCAATTCATATATTCAACGACATTTACTGTGCCTG
Jeongeup TTCCCTGATTCTCTCACCCACATTTCAATTCATATATTCAACGACATTTACTGTGCCTG
Muju TTCCCTGATTCTCTCACCCACATTTCAATTCATATATTCAACGACATTTACTGTGCCTG
Uljin TTCCCTGATTCTCTCACCCACATTTCAATTCATATATTCAACGACATTTACTGTGCCTG

Hoengseong CCCAGTATGTATTCCGCGCACCGCCCCCTATGTATATCGTGCATTAATGGTTTGCCCCA
Hampyeong CCCAGTATGTATTCCGCGCACCGCCCCCTATGTATATCGTGCATTAATGGTTTGCCCCA
Jeongeup CCCAGTATGTATTCCGCGCACCGCCCCCTATGTATATCGTGCATTAATGGTTTGCCCCA
Muju CCCAGTATGTATTCCGCGCACCGCCCCCTATGTATATCGTGCATTAATGGTTTGCCCCA
Uljin CCCAGTATGTATTCCACGCACCGCCCC-TATGTATATCGTGCATTAATGGTTTGCCCCA

Hoengseong TGCATATAAGCATGTACATACTATGGTTGATTTTACATGTATCCACCTCACCTAGATCAC
Hampyeong TGCATATAAGCATGTACATACTATGGTTGATTTTACATGTATCCACCTCACCTAGATCAC
Jeongeup TGCATATAAGCATGTACATACTATGGTTGATTTTACATGTATCCACCTCACCTAGATCAC
Muju TGCATATAAGCATGTACATACTATGGTTGATTTTACATGTATCCACCTCACCTAGATCAC
Uljin TGCATATAAGCATGTACATACTATGGTTGATTTTACATGTATCCACCTCACCTAGATCAC

Hoengseong GAGCTTGATCACCATGCCTCGAGAAACCATCAATCCTTGCGCGACGTGTACCTTTCTCG
Hampyeong GAGCTTGATCACCATGCCTCGAGAAACCATCAATCCTTGCGCGACGTGTACCTTTCTCG
Jeongeup GAGCTTGATCACCATGCCTCGAGAAACCATCAATCCTTGCGCGACGTGTACCTTTCTCG
Muju GAGCTTGATCACCATGCCTCGAGAAACCATCAATCCTTGCGCGACGTGTACCTTTCTCG
Uljin GAGCTTGATCACCATGCCTCGAGAAACCATCAATCCTTGCGCGACGTGTACCTTTCTCG

Hoengseong CTCCGGGCCATCACATGTGGGGTTTCTACCGTGAAACTATATCTGGCATCTGGTTCTT
Hampyeong CTCCGGGCCATCACATGTGGGGTTTCTACCGTGAAACTATATCTGGCATCTGGTTCTT
Jeongeup CTCCGGGCCATCACATGTGGGGTTTCTACCGTGAAACTATATCTGGCATCTGGTTCTT
Muju CTCCGGGCCATCACATGTGGGGTTTCTACCGTGAAACTATATCTGGCATCTGGTTCTT
Uljin CTCCGGGCCATCACATGTGGGGTTTCTACCGTGAAACTATATCTGGCATCTGGTTCTT

Hoengseong ACTTCAGGGTCATAACAATCCTCAATCCAATCCTACTAACCTCTCAAATGGGACATCTCG
Hampyeong ACTTCAGGGTCATAACAATCCTCAATCCAATCCTACTAACCTCTCAAATGGGACATCTCG
Jeongeup ACTTCAGGGTCATAACAATCCTCAATCCAATCCTACTAACCTCTCAAATGGGACATCTCG
Muju ACTTCAGGGTCATAACAATCCTCAATCCAATCCTACTAACCTCTCAAATGGGACATCTCG
Uljin ACTTCAGGGTCATAACAATCCTCAATCCAATCCTACTAACCTCTCAAATGGGACATCTCG

Hoengseong ATGGACTAGTGGCTAATCAGCCCATGATCACACATAACTGTGGTGTGCATGCATTTGGTAT
Hampyeong ATGGACTAGTGGCTAATCAGCCCATGATCACACATAACTGTGGTGTGCATGCATTTGGTAT
Jeongeup ATGGACTAGTGGCTAATCAGCCCATGATCACACATAACTGTGGTGTGCATGCATTTGGTAT
Muju ATGGACTAGTGGCTAATCAGCCCATGATCACACATAACTGTGGTGTGCATGCATTTGGTAT
Uljin ATGGACTAGTGGCTAATCAGCCCATGATCACACATAACTGTGGTGTGCATGCATTTGGTAT

Hoengseong CTTTAATTTTTGGGGGGGAGAAATTGGTATCACTCAACTATGGCCAGGTGTGGCCTCGT
Hampyeong CTTTAATTTTTGGGGGGGAGAAATTGGTATCACTCAACTATGGCCAGGTGTGGCCTCGT
Jeongeup CTTTAATTTTTGGGGGGGAGAAATTGGTATCACTCAACTATGGCCAGGTGTGGCCTCGT
Muju CTTTAATTTTTGGGGGGGAGAAATTGGTATCACTCAACTATGGCCAGGTGTGGCCTCGT
Uljin CTTTAATTTTTGGGGGGGAGAAATTGGTATCACTCAACTATGGCCAGGTGTGGCCTCGT

Hoengseong AGCAGTCAAATAACTTGTAGCTGGACTTATCCTTCATCATTTATCCCCGCCGCGTAGCTC
Hampyeong AGCAGTCAAATAACTTGTAGCTGGACTTATCCTTCATCATTTATCCCCGCCGCGTAGCTC
Jeongeup AGCAGTCAAATAACTTGTAGCTGGACTTATCCTTCATCATTTATCCCCGCCGCGTAGCTC
Muju AGCAGTCAAATAACTTGTAGCTGGACTTATCCTTCATCATTTATCCCCGCCGCGTAGCTC
Uljin AGCAGTCAAATAACTTGTAGCTGGACTTATCCTTCATCATTTATCCCCGCCGCGTAGCTC

Hoengseong TAAGGTGCTATTAGTCAATGGTACAGGACATACACACATAGATCCACCCCGTGCACG
Hampyeong TAAGGTGCTATTAGTCAATGGTACAGGACATACACACATAGATCCACCCCGTGCACG
Jeongeup TAAGGTGCTATTAGTCAATGGTACAGGACATACACACATAGATCCACCCCGTGCACG
Muju TAAGGTGCTATTAGTCAATGGTACAGGACATACACACATAGATCCACCCCGTGCACG
Uljin TAAGGTGCTATTAGTCAATGGTACAGGACATACACACATAGATCCACCCCGTGCACG

Appendix 3. Continued.

Hoengseong TACGTATACACGCACACTCACGTACGCATACACGCACACCCACGTACGCATACACGCACA
 Hampyeong TACGTATACACGCACACTCACGTACGCATACACGCACACCCACGTACGCATACACGCACA
 Jeongeup TACGTATACACGCACACTCACGTACGCATACACGCACACCCACGTACGCATACACGCACA
 Muju TACGTATACACGCACACTCACGTACGCATACACGCACACCCACGTACGCATACACGCACA
 Uljin TACGTATACACGCACACTCACGTACGCATACACGCACACCCACGTACGCATACACGCACA

Hoengseong CCCACGTACGCATACACGCACACCCACGTACGTATACACGCACACCCACGTACGCATACA
 Hampyeong CCCACGTACGCATACACGCACACCCACGTACGTATACACGCACACCCACGTACGCATACA
 Jeongeup CCCACGTACGTATACACGCACACCCACGTACGCATACACGCACACCCACGTACGCATACA
 Muju CCCACGTACGTATACACGCACACCCACGTACGCATACACGCACACCCACGTACGCATACA
 Uljin CCCACGTACGCATACACGCACACCCACGTACGCATACACGCACACCCACGTACGCATACA

Hoengseong CGCACACCCACGTACGCATACACGCACACCCACGTACGCATACACGCACACGCACGTACG
 Hampyeong CGCACACCCACGTACGCATACACGCACACCCACGTACGCATACACGCACACGCACGTACG
 Jeongeup CGCACACCCACGTACGCATACACGCACACCCACGTACGCATACACGCACACGCACGTACG
 Muju CGCACACCCACGTACGCATACACGCACACCCACGTACGCATACACGCACACGCACGTACG
 Uljin CGCACACCCACGTACGTATACACGCACACCCACGTACGTATACACGCACACGCACGTACG

Hoengseong TATACACGCACACGCACGTACGCACACACGCACACGCACGTATTCAACAGATATGAAACT
 Hampyeong TATACACGCACACGCACGTACGCACACACGCACACGCACGTATTCAACAGATATGAAACT
 Jeongeup TATACACGCACACGCACGTACGCACACACGCACACGCACGTATTCAACAGATATGAAACT
 Muju TATACACGCACACGCACGTACGCACACACGCACACGCACGTATTCAACAGATATGAAACT
 Uljin TATACACGCACACGCACGTACGCACACACGCACACGCACGTATTCAACAGATATGAAACT

Hoengseong AGCTTAATCAAACCCCCCTTACCCCCCGTAACTTCAAAAAGTATACAAAATACCTGTATTGT
 Hampyeong AGCTTAATCAAACCCCCCTTACCCCCCGTAACTTCAAAAAGTATACAAAATACCTGTATTGT
 Jeongeup AGCTTAATCAAACCCCCCTTACCCCCCGTAACTTCAAAAAGTATACAAAATACCTGTATTGT
 Muju AGCTTAATCAAACCCCCCTTACCCCCCGTAACTTCAAAAAGTATACAAAATACCTGTATTGT
 Uljin AGCTTAATCAAACCCCCCTTACCCCCCGTAACTTCAAAAAGTATACAAAATACCTGTATTGT

Hoengseong CCTGCCAAACCCCAAAAACAGAACTAGGCACATGCAACGTATATGAGAAGTCACTTACAC
 Hampyeong CCTGCCAAACCCCAAAAACAGAACTAGGCACATGCAACGTATATGAGAAGTCACTTACAC
 Jeongeup CCTGCCAAACCCCAAAAACAGAACTAGGCACATGCAACGTATATGAGAAGTCACTTACAC
 Muju CCTGCCAAACCCCAAAAACAGAACTAGGCACATGCAACGTATATGAGAAGTCACTTACAC
 Uljin CCTGCCAAACCCCAAAAACAGAACTAGGCACATGCAACGTATATGAGAAGTCACTTACAC

Hoengseong TAGCGCCACGCATGCTAATCTCATTCACTGATTTCATTAATAAATTCATTAGAAATTCCT
 Hampyeong TAGCGCCACGCATGCTAATCTCATTCACTGATTTCATTAATAAATTCATTAGAAATTCCT
 Jeongeup TAGCGCCACGCATGCTAATCTCATTCACTGATTTCATTAATAAATTCATTAGAAATTCCT
 Muju TAGCGCCACGCATGCTAATCTCATTCACTGATTTCATTAATAAATTCATTAGAAATTCCT
 Uljin TAGCGCCACGCATGCTAATCTCATTCACTGATTTCATTAATAAATTCATTAGAAATTCCT

Hoengseong ATCCAAAAGAAGCTATCTATAGATGTTATTTATATCTCCTACTACCCCCGTCAAAAACGC
 Hampyeong ATCCAAAAGAAGCTATCTATAGATGTTATTTATATCTCCTACTACCCCCGTCAAAAACGC
 Jeongeup ATCCAAAAGAAGCTATCTATAGATGTTATTTATATCTCCTACTACCCCCGTCAAAAACGC
 Muju ATCCAAAAGAAGCTATCTATAGATGTTATTTATATCTCCTACTACCCCCGTCAAAAACGC
 Uljin ATCCAAAAGAAGCTATCTATAGATGTTATTTATATCTCCTACTACCCCCGTCAAAAACGC

Hoengseong TTCTCTCTCCACCAC
 Hampyeong TTCTCTCTCCACCAC
 Jeongeup TTCTCTCTCCACCAC
 Muju TTCTCTCTCCACCAC
 Uljin TTCTCTCACATCAC
