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Intraspecific Variation of the Korean *Rana nigromaculata* (Amphibia: Ranidae) Based on Morphometric and Sequence Comparison

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The aim of this study was to clarify the intraspecific variation of Korean *R. nigromaculata* based on a morphometric and sequence comparison. Each population was collected from geographically separated areas and examined using an independent samples test and ANOVA after being separated according to whether they were a normal or abnormal type. In addition, a 518 bp DNA fragment of the mitochondrial cytochrome b gene was analyzed by PCR amplification. The two *R. nigromaculata* types were identical because all *p*–values of the characteristics were larger than a significance value, with the exception of HL/SVL. Furthermore, despite the morphological difference, the genetic distance of the populations was quite close ($D=0.002$). Therefore, it is believed that the abnormal type is an ecophenotype as a result of geological, altitude isolation because these frogs are identical to the normal type.

INTRODUCTION

The Family Ranidae first appeared in the Oligocene period, which is approximately 3.6 million years ago, and it is the largest taxon in salientia containing 47 genus with 667 species worldwide (Duellman and Trueb, 1986). Korean Ranidae contains 1 genus and 6 species, which are separated into brown frogs, pond frogs and wrinkle frogs (Kang and Yoon, 1975; Zhao and Adler, 1993; Sengoku *et al.*, 1996; Maeda and Matsui, 1999, Yang *et al.*, 2000; Yang *et al.*, 2001). *R. nigromaculata* is distributed all over Korea, China, Japan and Russia etc. (Kang and Yoon, 1975; Zhao and Adler, 1993; Sengoku *et al.*, 1996; Maeda and Matsui, 1999).

Previous studies on the Korean *R. nigromaculata* involved an examination of the distribution by Yang and Yu (1978), the genetic variations by Yang (1983), the structure and variations in the mating call by Park and Yang (1997), the reproductive isolation mechanism and natural hybrid by Yang *et al.* (1998), the genetic diversity and population structure by Yang *et al.* (1999), and the geographical variation of the mitochondrial cytochrome b gene by Kim *et al.* (1999) in Korea.

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Fig. 1. Morphological variation of Korean *Rana nigromaculata* collected in Jeju, Korea.

This species is identified by the presence (normal) or absence (abnormal) of a middorsal line, by a point pattern of the dorsal or hindlimb, and by the length of the hind limb (Stejneger, 1907; Kang and Yoon, 1975; Nakamura and Ueno, 1976; Yang *et al.*, 2001). However, a species *R. nigromaculata* with no middorsal line was recently discovered in Jeju Island (Fig. 1).

The sequence of the mitochondrial DNA (mtDNA) is widely used in taxonomic analysis (Brown *et al.*, 1982; Smith and Patton, 1991; Moritz *et al.*, 1992: Tan and Wake, 1995; Lee *et al.*, 1997). The mitochondrial DNA has a small genome size (15.0–20.0 kb) and evolves more rapidly than the nuclear DNA, perhaps 5–10 times faster than a typical single–copy nuclear DNA and is maternally inherited. mtDNA mutations that arise in different individuals do not recombine during sexual reproduction, unlike the nuclear DNA. Therefore, the mtDNA of higher animals is an ideal molecular system for phylogenetic analysis among close taxa (Avise *et al.*, 1987).

The aim of this study was clarify the taxonomic status by a comparison of the important morphometric characteristics such as the presence or absence of a middorsal line using identification, and a comparison of the mitochondrial cytochrome b gene.

MATERIALS AND METHODS

Samples of *Rana nigromaculata* were collected from different areas in Korea (Table 1). The small muscles of the hindlimb were extracted after being anesthetized with chloroform. These specimens were kept in 70% ethanol in the Ecological laboratory of Kyonggi University (KUEL).

The method reported by Matsui (1984) was used to analyze the following morphometric characteristics, but these method was modified: 1. Snout–Vent Length (SVL) 2. Head Length (HL) 3. Nostril–Eyelid Length (N–EL) 4. Snout Length (SL) 5. Eye Length (EL) 6. Tympanum–Eye Length (T–EL) 7. Tympanum Diameter (TD) 8. Head Width (HW) 9. Internarial Distance (IND) 10. Distance between anterior tips of upper eyelid (DAU) 11. Radioulna Length (RUL) 12. Hand Length (HAL) 13. Tibia length (TL) 14. Foot length (FL). Digital calipers measured to 0.01 mm were used in all the measurements, and each population was examined by an independent samples test and ANOVA

Table 1. Collected site of Korean *Rana nigromaculata*.

	Address	Remark
1	Mulchat–orum, Jocheon–eup, Bukjeju–gun, Jeju–do	
2	Geumun–orum, Sunhul–ri, Jocheon–eup, Bukjeju–gun, Jeju–do	A
3	Haksan–ri, Dundeuk–myeon, Geoje, Gyeonsangnam–do	
4	Jeonryangji (wetland) hapcheon–eup, Hapcheon–gun, Gyeongsangnam–do	
5	Bongwhagyo, Hwaam–ri, Samdong–myeon, Namhae–gun, Gyeongsangnam–do	
6	Mt. Suryeong, Sumang–ri, Namwon–eup, Namjeju–gun, Jeju–do	
7	Mt. Seongju, Seongju–ri, Seongju–myeon, Boryeong, Chungcheongnam–do	
8	Sampo–ri, Samho–myeon, Youngam–gun, Jeollanam–do	
9	Hakgok–ri, Baekhak–myeon, Yeoncheon–gun, Gyonggi–do	
10	Shinsido–ri, Okdo–myeon, Gunsan, Jeollabuk–do	
11	Woncheong–ri, Nam–myeon, Taean–gun, Chungcheongnam–do	
12	Uishin–ri, Illo–eup, Muan–gun, Jeollanam–do	
13	Sugok–ri, Amtae–myeon, Sinan–gun, Jeollanam–do	
14	Sowhang–ri, Ungcheon–eup, Boryeong, Chungcheongnam–do	
15	Jangsin–ri, haseo–myeon, Buan–gun, Jeollabuk–do	
16	Jangsan–ri, Sushin–myeon, Cheonan, Chungcheongnam–do	
17	Mt. Odae, Dongsan–ri, Jinbu–myeon, Pyeongchang–gun, Gangwon–do	
18	Mt. Bukhan, Jingwannae–dong, Eunpyeong–gu, Seoul	
19	Kyogok–ri, Gunduck–myeon, Samchuk, Gangwon–do	
20	Mt. Gwanggyo, Sanggwnggyo–dong, Youngtong–gu, Suwon, Gyeonggi–do	

A, Abnormal type

using the SPSS software package (Statistical package of the social science) after being separated according to whether they were the normal or abnormal type.

The total DNA was extracted from frozen (–70 °C) tissue samples of the hindlimb muscle of each specimen, using an extraction buffer (150 mM Nacl, 10 mM Tris–HCl (pH 8.0), 10 mM EDTA, 1% sodium dodecyl sulfate), proteinase K and phenol (Sambrook and Rusell, 2001).

The purified total DNA was used to amplify approximately 518 bp of the mitochondrial cytochrome b gene (mt Cytb) fragments. The sequence of the oligonucleotide primers used for PCR and sequencing were CB1 (5'–ccatccaacatctcagcatgatgaaa–3') and 560A (5'–gtctttgtaggagaagtatgg–3').

The total volume in the PCR tube was consisted of 100 μl as the template DNA (10 ng/μl) 1 μl, dNTP (2.5 mM) 1 μl, Taq polymerase (5 Unit/μl) μl, PCR buffer 10 μl (GeneClone Co, Korea), each primer (100 pmole) 1 μl, and distilled water 85 μl. In addition, the mt Cytb was amplified by 30 cycles, each cycle consisting of denaturation for 1 min at 94 °C, annealing for 1 min at 58 °C, polymerization for 1 min at 72 °C. The 518 bp sequences were aligned by CLUSTAL W1.4, and the genetic divergence was estimated based on the Kimura's two–parameter distance. The sequence (NC_001573) of *Xenopus laevis* was used to compare the base composition.

A dendrogram was constructed using the UPGMA method with the PHYLIP package (Felsenstein, 1993), and a bootstrapping test was also carried out using 1,000 replications (Felsenstein, 1985).

RESULTS AND DISCUSSION

The two populations were classified according to whether they were the abnormal (Jeju group Male $n=9$, Female $n=3$) or normal type (Jeju group Male $n=15$, Female $n=10$; Inland group Male $n=28$, Female $n=4$) of a total 68 samples by their morphological characteristics. All the specimens were measured using 13 characteristics, analyzed by an Independent Samples test (T–test and Mann–Whitney U–test) using the SPSS program.

The p–value appeared larger (HL/SVL 0.711, N–EL/SVL 0.398, SL/SVL 0.264, EL/SVL 0.516, T–EL/SVL 0.873, TD/SVL 0.952, IND/SVL 0.601, DAU/SVL 0.462, RUL/SVL 0.9932, HAL/SVL 0.641, TL/SVL 0.581, FL/SVL 0.224) than $p=0.05$ except for HW/SVL using the Levene's test for the equality of variances.

In independent samples test, the p–values were; HL/SVL 0.592, N–EL/SVL 0.190, SL/SVL 0.645, EL/SVL 0.884, T–EL/SVL 0.828, TD/SVL 0.473, IND/SVL 0.993, DAU/SVL 0.273, RUL/SVL 0.772, HAL/SVL 0.162, TL/SVL 0.348, FL/SVL 0.092 and HW/SVL 0.884.

All p–values of all the morphometirc characteristics were $p>0.05$ according to an independent samples test. Therefore, these characteristics are considered identical regardless of whether they were the normal or abnormal type. The samples were separated into three populations, two normal (Jeju, Inland) and one abnormal type (Jeju) according to the local and morphological differences.

In One–Way ANOVA analysis, N–EL/SVL ($F_{2,65}=2.530$, $p=0.087$), EL/SVL ($F_{2,65}=0.824$, $p=0.443$), T–EL/SVL ($F_{2,65}=1.018$, $p=0.367$), TD/SVL ($F_{2,65}=0.318$, $p=0.729$), IND/SVL ($F_{2,65}=0.254$, $p=0.776$), HAL/SVL ($F_{2,65}=0.996$, $p=0.375$), FL/SVL ($F_{2,65}=2.434$, $p=0.096$) were all $p>0.05$ indicating that these characteristics were not significantly different. However, SL/SVL ($F_{2,65}=10.493$, $p=0.000$), DAU/SVL ($F_{2,65}=4.691$, $p=0.013$) and TL/SVL ($F_{2,65}=6.313$, $p=0.003$) were lower than a significant difference.

SL/SVL, DAU/SVL and TL/SVL were examined with a Post–hoc test using the Tukey method, which showed that there was a significant difference between the normal and abnormal type ($p<0.05$). However, the three groups appear to be identical because which is identical between normal and abnormal type in Jeju, even if the normal type of Jeju and Inland were differed by Post Hoc test.

In addition, in the kruskal–wallis test, the HW/SVL and RUL/SVL of the local populations were identified because these characteristics appeared larger than the significance value. However, the HL/SVL ($p=0.043$) in one of the three populations was the lowest. Nevertheless, the HL/SVL was quite close, even if the p–value was lower than the significance value ($p=0.05$).

The alignments for the DNA sequences were determined based on the maximum nucleotide similarity using CLUSTAL W 1.4. The align sequences from mt Cytb gene of 14 local populations are present in APPENDIX 1. The partial sequence of the mt Cytb gene consisted of a total of 518 sites, 44 sites of which were variable.

Genetic distance (D) ranged from a Kimura–2–parameter of 0.002 to 0.016. The genetic distance using the Kimura–2–parameter of one group (Yeosu, Namhae–do, Mokpo and Hamyang) was $D<0.010$. The other group (Normal and abnormal in Jeju–do, Shinshi–do, Boryeong, Anmyeon–do, Gimpo, Yeoncheon, Amtae–do, Hwaseong) was $D<0.010$. However, there appeared to be a genetic difference between the groups because the genetic distance was $D > 0.010$.

In previous studies, the genetic variation of *R. nigromaculata* was reported to be closer than that of other amphibians, but the genetic relationship between the local populations of *R. nigromaculata* was longer even if these species were identical species (Yang, 1983). The mt Cytb gene results concur with Yang (1983). Moreover, the genetic distance within the inland groups was similar to that reported by Yang (1983).

However, the genetic distance ranged from 0.002 to 0.014 compared with the normal type in Jeju Island and the samples obtained from the Inland regions. Therefore, the abnormal type was similar to the other populations because the genetic distance was within the distance of the normal type. On the other hand, the genetic distance of the Korean and Japanese *R. nigromaculata* ranges from 0.061 to 0.072; the Japanese population being longer than that of the Korean population.

In UPGMA analysis using the Phylip package (Fig. 2), the Korean *Rana nigromaculata* formed two clusters, one of the clusters included a population of Yeosu, Namhae–do, Mokpo and Hamyang, the other cluster was included the normal, abnormal population of Jeju–do, Shinshi–do, Boryeong, Anmyeon–do, Gimpo, Yeoncheon, Amtae–do and Hwaseong. In addition, two clusters were strongly supported by the 100% bootstrap iterations. However, the Japanese *Rana nigromaculata* do not form a cluster with the Korean populations even if they are identical species.

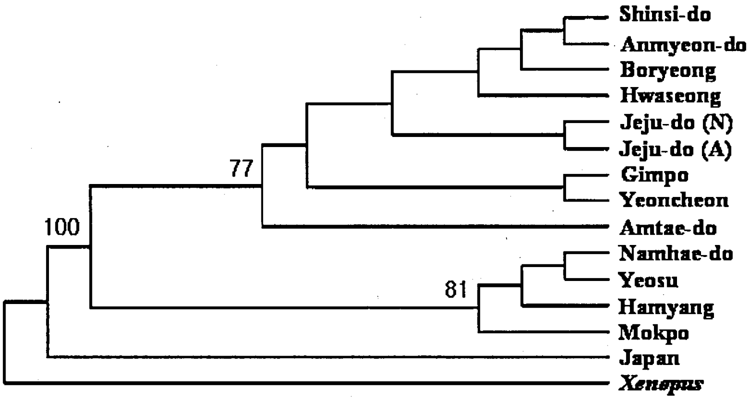

Fig. 2. A UPGMA analysis for 14 local populations of *Rana nigromaculata* based on partial sequence of Cytochrome b gene (518 bp).

In this study, the genetic difference between local populations using of Cytb gene was similar to a previous study, e.g. the Jeju and central Korea populations formed one cluster. Nevertheless, Jeju is a further central than south Korea (Song and Chung, 2003). The common ancestor of *R. nigromaculata* was originally distributed in the eastern area of China from which these frogs became separated into two groups during the Ice–Age; the southern and northern groups. These two groups then differentiated into

R. nigromaculata in the Korean peninsula and to *R. porosa porosa* in the Japanese island. The Korean *R. nigromaculata* dispersed into the Japanese island because the Korean peninsula and the Japanese island were connected as a result of the low sea levels during the second Ice–Age (Matsui, 1996).

In addition, the dispersal of *R. nigromaculata* is assumed to be affected by the distribution of a paleo–river because these frogs are usually distributed by rivers, lowland puddles, rice paddy fields, swamps etc. (Kang and Yoon, 1975). In particular, Ichthyofauna of the Korean peninsula is assumed to be affected by connection with the paleo–Hwangho and Han river, and the connected rivers run from the Yellow sea to Jeju Island (Kim, 1997). It appears that *R. nigromaculata* were spread in Korea by this connected river.

It is believed that the abnormal type is ecophenotype of the normal type as a result of geographical and altitude isolation. However, the taxonomic status needs to be clarified by a comparison of the ecophenotypes in Korea and Japan because the abnormal type was discovered in Fukui, Japan (Kawauchi *et al.*, 2003).

APPENDIX 1. Nucleotide sequence alignment (518 bp) of the mt Cytb genes in local populations of *Rana nigromaculata* and reference species, *Xenopus laevis*.

X. laevis	CTAGGGGTCTGTTTAATTGCCCAAATCATTACAGGATTATTCTTAGCTATACATTATACAGCAGACACATCTATAGCCTTCTCATCAGTAGCCCATATTTGTTTTGACGTTAACTATGGA
Yeosu	CTCGGAGTCTGCTTAATTGTCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCATTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Namhae-do	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCATTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Mokpo	CTCGGAGTCTGCTTAATTACCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCATTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Hamyang	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCATTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Jeju-do (N)	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTTCTAGCCATGCATTACACAGCCGATACATCCCTTGCGTTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Jeju-do (A)	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTTCTAGCCATGCATTACACAGCCGATACATCCCTTGCGTTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Shinshi-do	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCGTTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Boryeong	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCGTTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Anmyeondo	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCGTTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Gimpo	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCGTTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Yeoncheon	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCGTTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Amtae-do	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCGTTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Hwaseong	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGATACATCCCTTGCGTTCTCATCTATCGCTCACATCTGCCGAGACGTTAACAACGGC
Japan	CTCGGAGTCTGCTTAATTGCCCAAATCGCCACCGGCTTATTCCTAGCCATGCATTACACAGCCGACACATCCCTTGCATTCTCATCTATTGCCCACATCTGTCGAGATGTTAATAACGGC

X. laevis	TTATTAATTCGCAATCTCCATGCCAATGGACTCTCATTCTTCTTCATTTGCATCTACCTTCACATCGGACGAGGGTTGTACTACGGCTCTTTCTTATATAAAGAAACATGAAATATTGGT
Yeosu	TGACTCCTACGTAACCTCCATGCTAACGGTGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGGAACATTGGC
Namhae-do	TGACTCCTACGTAACCTCCATGCTAACGGTGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGGAACATTGGC
Mokpo	TGACTCCTACGTAACCTCCATGCTAACGGTGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGGAACATTGGC
Hamyang	TGACTCCTACGTAACCTCCATGCTAACGGTGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGGAACATTGGC
Jeju-do (N)	TGACTCCTACGTAACCTCCATGCTAACGGCGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGAAACATTGGC
Jeju-do (A)	TGACTCCTACGTAACCTCCATGCTAACGGCGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGAAACATTGGC
Shinshi-do	TGACTCCTACGTAACCTCCATGCTAACGGCGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGAAACATTGGC
Boryeong	TGACTCCTACGTAACCTCCATGCTAACGGCGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGAAACATTGGC
Anmyeondo	TGACTCCTACGTAACCTCCATGCTAACGGCGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGAAACATTGGC
Gimpo	TGACTCCTACGTAACCTCCATGCTAACGGCGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTACAAAGAAACCTGAAACATTGGC
Yeoncheon	TGACTCCTACGTAACCTCCATGCTAACGGCGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTACAAAGAAACCTGAAACATTGGC
Amtae-do	TGACTCCTACGTAACCTCCATGCTAACGGCGCATCTTTTTTTTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGAAACATTGGC
Hwaseong	TGACTCCTACGTAACCTCCATGCTAACGGCGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGAGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGAAACATTGGC
Japan	TGACTCCTACGAAACCTTCACGCCAACGGCGCATCTTTTTTCTTTATCTGCATCTACTTCCACATTGGCCGGGGTCTTTACTATGGCTCCTACCTCTATAAAGAAACCTGAAACATTGGT

X. laevis	GTGATCCTCCTATTTTTAGTTATAGCTACAGCATTTGTAGGATATGTTCTACCATGAGGACAAATATCTTTTTGAGGGGCTACAGTAATTACTAATCTTCTTTCTGCTAAACCGTACATC
Yeosu	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTGTCAGCTGCCCCCTACATC
Namhae-do	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTGTCAGCTGCCCCCTACATC
Mokpo	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTGTCAGCTGCCCCCTACATC
Hamyang	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTGTCAGCTGCCCCCTACATC
Jeju-do (N)	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTATCAGCTGCCCCCTACATC
Jeju-do (A)	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTATCAGCTGCCCCCTACATC
Shinshi-do	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTATCAGCTGCCCCCTACATC
Boryeong	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTATCAGCTGCCCCCTACATC
Anmyeondo	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTATCAGCTGCCCCCTACATC
Gimpo	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTCCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTATCAGCTGCCCCCTACATC
Yeoncheon	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTCCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTATCAGCTGCCCCCTACATC
Amtae-do	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTATCAGCTGCCCCCTACATC
Hwaseong	GTCATTCTATTGTTCCTAGTTATAGCCACCGCCTTTGTAGGCTATGTTCTCCCTTGAGGCCAAATATCCTTCTGAGGCGCCACAGTCATCACTAACCTCCTATCAGCTGCCCCCTACATC
Japan	GTCATTCTGTTGTTCTTAGTCATAGCCACTGCCTTTGTGGGCTATGTCCTCCCTTGAGGCCAAATATCTTTCTGAGGCGCCACAGTCATTACCAATCTCCTATCAGCCGCCCCCTACATC

X. laevis	GGAAACGTACTAGTCCAATGAAGTTTAGGAGGATTCTCTGTAGATAACGCCACTTTAACCCGATTCTTCGCATTTCACTTCCTCCTTCCTTTTATTATTGCCGGAGCTAGCATTCTCCAT
Yeosu	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTTATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Namhae-do	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTTATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Mokpo	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Hamyang	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTGTTATTGCAGCAGCAAGCATAATCCAC
Jeju-do (N)	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Jeju-do (A)	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Shinshi-do	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Boryeong	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Anmyeondo	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Gimpo	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Yeoncheon	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Amtae-do	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATCCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Hwaseong	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGATAACGCCACCCTAACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGCATAATCCAC
Japan	GGCCCAGACCTAGTTCAATGAATCTGAGGAGGCTTCTCAGTAGACAACGCCACCCTCACCCGTTTCTTTACATTTCACTTCATTCTCCCCTTTATTATTGCAGCAGCAAGTATGATCCAC

X. laevis	CTTTTATTTCTCCACGAAACTGGATCAACAAACCCAAC
Yeosu	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Namhae-do	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Mokpo	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCGAC
Hamyang	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Jeju-do (N)	CTCCTGGTTCTTCACCAAACCGGATCCTCTAACCCAAC
Jeju-do (A)	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Shinshi-do	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Boryeong	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Anmyeondo	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Gimpo	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Yeoncheon	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Amtae-do	CTCCTGTTTCTTCACCAAACCGGATCCTCTAACCCAAC
Hwaseong	CTCCTGTTTCTTCACCAAACTGGATCCTCTAACCCAAC
Japan	CTACTCTTTCTTCACCAAACTGGATCCTCTAACCCAAC

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