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# Genetic Diversity among Local Populations of the Gold-spotted Pond Frog, *Rana plancyi chosenica* (Amphibia: Ranidae), Assessed by Mitochondrial Cytochrome *b* Gene and Control Region Sequences

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## ABSTRACT

The Gold-spotted pond frog, *Rana plancyi chosenica*, designated as a vulnerable species by IUCN Red list. This species is a typical example facing local population threats and extinction due to human activities in South Korea. A strategic conservation plan for this endangered species is urgently needed. In order to provide information for future conservation planning, accurate information on the genetic diversity and taxonomic status is needed for the establishment of conservation units for this species. In this study, we used a molecular genetic approach using the mitochondrial cytochrome *b* gene and control region sequences to find the genetic diversity of gold-spotted pond frogs within South Korea. We sequenced the mitochondrial DNA cytochrome *b* gene and control region of 77 individuals from 11 populations in South Korea, and one from Chongqing, China. A total of 15 cytochrome *b* gene haplotypes and 34 control region haplotypes were identified from Korean gold-spotted pond frogs. Mean sequence diversity among Korean gold-spotted pond frogs was 0.31% (0.0-0.8%) and 0.51% (0.0-1.0%), respectively. Most Korean populations had at least one unique haplotype for each locus. The Taean, Ansan and Cheongwon populations had no haplotypes shared with other populations. There was a sequence divergence between Korean and Chinese gold-spotted pond frogs (1.3% for cyt *b*; 2.9% for control region). Analysis of genetic distances and phylogenetic trees based on both cytochrome *b* and control region sequences indicate that the Korean gold-spotted pond frog are genetically differentiated from those in China .

**Key words:** *Rana plancyi chosenica*, gold-spotted pond frog, cytochrome *b*, control region, genetic diversity, conservation

## INTRODUCTION

Amphibian populations are declining globally due to various factors including habitat destruction, introduced species, climate change, ultraviolet radiation, and the introduction of exotic diseases (Alfords and Richards, 1999; Houlahan et al., 2000; Alford et al., 2001; Stuart et al., 2004; Cushman, 2006). As populations decline and become more isolated from one another, it is predicted that increased inbreeding and decreased genetic diversity will further accelerate the loss of populations or species.

The gold-spotted frog, *R. plancyi*, is distributed in Eastern China and Korea. Throughout its range, the species shows considerable variation in morphology, coloration, and accordingly, some subspecies have been described (Zhao and Adler, 1993; AmphibiaWeb, 2008). This species is a habitat specialist, living in pond, wetlands, and rice paddies. In China, there are two dominant color morphs (subspecies) present with regional variations in frequency. The Korean populations are treated as another subspecies.

The Korean gold-spotted pond frog (*Rana plancyi chosenica*) were once abundant in paddy fields and marshes throughout the western and southern Korean peninsula (Kang and Yoon, 1975; Yang et al., 2001), but recent surveys have shown that the species is rapidly declining in much of their previously known habitats (Lee, 2003). The range of Korean gold-spotted frog is highly discontinuous at a regional scale and remaining populations are usually composed of less than 50 individuals, with infrequent instances of dispersal over long distances (unpublished data). We expected that restricted movement and habitat specificity would limit

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**Table 1.** Sampling localities and number of haplotypes and GenBank accession numbers

| Collection locality | No. of samples | No. of haplotype | | GenBank accession no. | |
|---|---|---|---|---|---|
| | | Cyt *b* | CR | Cyt *b* | CR |
| 1. Pyeongtaek, Gyeonggi-do (PT) | 11 | 10 | 11 | EU386971-72,74<br>EU386977<br>EU386984,86-87<br>EU387001<br>EU387053-55 | EU387069,76,83,86<br>EU387104-06,24-26<br>EU387129 |
| 2. Paju, Gyeonggi-do (PJ) | 4 | 3 | 3 | EU386988-89<br>EU387009<br>EU387014 | EU387085<br>EU387111-12,18 |
| 3. Goyang, Gyeonggi-do (GY) | 4 | 1 | 1 | EU387010-13 | EU387119-22 |
| 4. Ansan, Gyeonggi-do (AS) | 4 | 1 | 3 | EU387004-05,07-08 | EU387114-17 |
| 5. Gangwha, Incheon (GH) | 5 | 1 | 5 | EU386990-92,94,98 | EU387084<br>EU387107-10 |
| 6. Ongjin-A, Incheon (OJ-A) | 8 | 5 | 6 | EU386975-76<br>EU386981<br>EU387002-03<br>EU387016-17<br>EU387047 | EU387064,66,75,81<br>EU387113,23,27-28 |
| 7. Ongjin-B, Incheon (OJ-B) | 16 | 2 | 8 | EU387018-28<br>EU387048-52 | EU387061-63,65,67-<br>68,70-74,77-80,82, |
| 8. Cheongwon, Chungcheongbuk-do (CW) | 8 | 1 | 1 | EU386961-68 | EU387130-37 |
| 9. Taean-A, Chungcheongnam-do (TA-A) | 7 | 1 | 1 | EU387029-35 | EU387087,90-92,96-97<br>EU387102 |
| 10. Taean-B, Chungcheongnam-do (TA-B) | 5 | 1 | 1 | EU387036-40 | EU387095,98<br>*Eu387100-01,03* |
| 11. Taean-C, Chungcheongnam-do (TA-C) | 5 | 1 | 1 | EU387041-43,45-46 | EU387088-89,93,94,99 |
| 12. Chongqing, China (CQ) | 1 | 1 | 1 | EU387060 | – |
| 13. *R. plancyi*-GenBank (GB) | 1 | 1 | 1 | EF196679 | EF196679 |
| 14. *R. nigromaculata*-GenBank (RN) | 1 | 1 | 1 | NC002805 | NC002805 |

gene flow and that small population sizes would further enhance genetic differentiation by a bottle neck effect whithin isolated populations.

Given the conservation concern for this species, we surveyed range-wide genetic variability of Korean gold-spotted pond frog based on a molecular genetic approach using mitochondrial cytochorme *b* gene and control region sequences.

## MATERIALS AND METHODS

### Samples

We collected 77 individuals of *R. plancyi chosenica* from 11 populations distributed across four provinces of South Korea including Incheon, Gyeonggi-do, Chungcheongbuk-do, and Chungcheongnam-do. One sample was obtained from Chongqing, China (Table 1). These specimens are deposited at CGRB, Seoul National University.

### Amplification and sequencing

Genomic DNA was extracted from toe clipping samples using a QIAamp Tissue Kit (Qiagen). Partial cytochrome *b* gene (1,046 bp) and control region gene (1,465 bp) of mtDNA were amplified using six oligonucleotide primers by PCR. Two primers were designed based on a sequence of Black-spotted pond frog (*Rana nigromaculata*) and the new sequence generated in the present study. The universal primers of F981 (Irwin et al., 1991), L15275 (Groves and Shields, 1996), ControlP-H and 12sZ-H (Goebel et al., 1999) were also used. Reactions were performed in a total volume of 25 μL, each containing 100 ng template DNA, 1 × PCR buffer, 0.75 mM dNTPs, 1.5 mM $MgCl_2$, 1 μM primer, and 0.5 units of Taq polymerase. PCR conditions were the same for all three fragments: 95°C initial denaturation for 5 min, 35 amplification cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min, extension at 72°C for 1 min, and a final 5 min extension at 72°C. The PCR products were purified using gel purification kit (Qiagen) and each gene

**Table 2.** Mitochondrial cytochrome *b* haplotype distribution of gold spotted pond frog in South Korea

| Locality | PT | PJ | GY | AS | GH | OJ-A | OJ-B | CW | TA-A | TA-B | TA-C | CQ | GB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 11 | 4 | 4 | 4 | 5 | 8 | 16 | 8 | 7 | 5 | 5 | 1 | 1 |
| Hap 1 | 4 | 2 | | | 5 | | | | | | | | |
| Hap 2 | | 1 | | | | | | | | | | | |
| Hap 3 | | | | | | 2 | | | | | | | |
| Hap 4 | | | | 4 | | | | | | | | | |
| Hap 5 | 2 | | | | | | | | | | | | |
| Hap 6 | | | | | | | | | 7 | 5 | 5 | | |
| Hap 7 | | | | | | 1 | 5 | | | | | | |
| Hap 8 | | 1 | 4 | | | | | | | | | | |
| Hap 9 | 1 | | | | | | | | | | | | |
| Hap 10 | | | | | | 2 | 11 | | | | | | |
| Hap 11 | 2 | | | | | | | | | | | | |
| Hap 12 | 1 | | | | | | | | | | | | |
| Hap 13 | 1 | | | | | 2 | | | | | | | |
| Hap 14 | | | | | | 1 | | | | | | | |
| Hap 15 | | | | | | | | 8 | | | | | |
| Hap 16 | | | | | | | | | | | | 1 | |
| Hap 17 | | | | | | | | | | | | | 1 |
| No. of Hap | 6 | 3 | 1 | 1 | 1 | 5 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |

**Table 3.** Base composition at first, second, and third positions in mitochondrial cytochrome *b* DNA sequences of Korean gold-spotted pond frog, *Rana plancyi chosenica*

| Codon position | Nucleotide | | | |
|---|---|---|---|---|
| | A | T | C | G |
| First | 25.3 | 24.4 | 26.2 | 24.1 |
| Second | 19.8 | 41.0 | 25.8 | 13.5 |
| Third | 25.9 | 22.4 | 46.1 | 5.6 |
| Average | 23.6 | 29.3 | 32.7 | 14.4 |

was directly sequenced in both directions to avoid base-calling ambiguities with ABI PRISM 310 Genetic Analyzer (Applied Biosystem). The sequences obtained in this study have been deposited in GenBank under accession numbers given in Table 1.

### Sequence alignment and analysis

Contiguous sequences from two overlapping fragments in each region were constructed in Sequence Analysis 3.7 (Applied Biosystem). All regions were sequenced in both directions. Mitochondrial DNA sequences were edited and aligned using AlignIR V2.0 (LI-COR). Multiple sequence alignments were obtained using the CLUSTAL X (Thompson et al., 1997) and visually inspected. Haplotype diversity and nucleotide diversity were calculated using DnaSP 4.0 (Rozas et al., 2003). The phylogenic tree was constructed using MEGA 4 (Tamura et al., 2007), based on the Kimura-2 parameter method. Bootstrap values were obtained through 1,000 replications. For the comparison, *Rana nigromaculata* (NC002805) was used as outgroup and the reference sequences of *Rana plancyi* were obtained from GenBank (EF196679).

### Estimation of divergence times

We used PHYLTEST 2.0 (Kumar, 1996) to apply a relative rate test (Takezaki et al., 1995) to our data. PHYLTEST examines the constancy of the molecular clock for two lineages when an outgroup is given. We conducted several tests for pairwise comparisons of the clades/subclades observed in phylogenetic trees. The averages of observed numbers of substitutions per site from the common ancestor of lineages 1 and 2 are assumed to be equal under the constancy of the molecular clock. The variance of this value can be estimated and tested for its deviation from zero by a two-tailed normal deviation test. If the null hypothesis was not rejected, we estimated dates of divergence for a pairwise comparison of the clades/subclades by calculating the uncorrected percent sequence divergences between samples in Mega 4 (Tamura et al., 2007). Because there is no reliable temporal calibration directly applicable to our data, we tentatively used the evolutionary rate for amphibian (1.28% per MY).

## RESULTS

### Cytochrome *b* sequences and variation

Analysis of the 1,046 bp cytochrome *b* sequences detected 15 haplotypes among 77 Korean gold-spotted pond frogs (Table 2). Nucleotide substitutions of the cytochrome *b* gene were detected at 64 (6.3%) positions and 23 (2.3%) of the sites were parsimony informative. Among the 64 vari-

**Table 4.** Matrix of pairwise sequence divergence of mitochondrial cytochrome *b* gene between different populations of gold-spotted pond frog and *R. nigromaculata* by Kimura-2-parameter distance

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Pyeongtaek | | | | | | | | | | | | | |
| 2. Paju | .003 | | | | | | | | | | | | |
| 3. Goyang | .004 | .001 | | | | | | | | | | | |
| 4. Ansan | .004 | .001 | .002 | | | | | | | | | | |
| 5. Ganghwa | .003 | .000 | .001 | .001 | | | | | | | | | |
| 6. Ongjin-A | .004 | .004 | .004 | .004 | .003 | | | | | | | | |
| 7. Ongjin-B | .004 | .001 | .002 | .002 | .001 | .004 | | | | | | | |
| 8. Cheongwon | .005 | .007 | .008 | .008 | .007 | .005 | .008 | | | | | | |
| 9. Taean-A | .004 | .001 | .002 | .002 | .001 | .004 | .002 | .008 | | | | | |
| 10. Taean-B | .004 | .001 | .002 | .002 | .001 | .004 | .002 | .008 | .000 | | | | |
| 11. Taean-C | .004 | .001 | .002 | .002 | .001 | .004 | .002 | .008 | .000 | .000 | | | |
| 12. China | .013 | .013 | .013 | .013 | .012 | .013 | .013 | .014 | .013 | .013 | .013 | | |
| 13. *R. nigromaculata* | .038 | .039 | .040 | .040 | .039 | .039 | .040 | .038 | .040 | .040 | .040 | .039 | |

**Table 5.** Mitochondrial control region haplotype distribution of gold spotted pond frog in South Korea

| Locality | PT | PJ | GY | AS | GH | OJ-A | OJ-B | CW | TA-A | TA-B | TA-C | GB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | 11 | 4 | 4 | 4 | 5 | 8 | 16 | 8 | 7 | 5 | 5 | 1 |
| Hap 1 | 1 | | | | | 2 | 8 | | | | | |
| Hap 2 | | | | | | | 1 | | | | | |
| Hap 3 | | | | | | | 1 | | | | | |
| Hap 4 | | | | | | | 1 | | | | | |
| Hap 5 | 1 | | | | | | | | | | | |
| Hap 6 | | | | | | 1 | | | | | | |
| Hap 7 | | | | | | | 1 | | | | | |
| Hap 8 | | | | | | | 1 | | | | | |
| Hap 9 | | | | | | 1 | 1 | | | | | |
| Hap 10 | | | | | | | 2 | | | | | |
| Hap 11 | 1 | | | | | | | | | | | |
| Hap 12 | | 1 | | | | | | | | | | |
| Hap 13 | | | | | 1 | | | | | | | |
| Hap 14 | 1 | | | | | | | | | | | |
| Hap 15 | | | | | | | | | 7 | 5 | 5 | |
| Hap 16 | 1 | | | | | | | | | | | |
| Hap 17 | 1 | | | | | | | | | | | |
| Hap 18 | 1 | | | | | | | | | | | |
| Hap 19 | | | | | 1 | | | | | | | |
| Hap 20 | | | | | 1 | | | | | | | |
| Hap 21 | | | | | 1 | | | | | | | |
| Hap 22 | | | | | 1 | | | | | | | |
| Hap 23 | | 2 | | | | | | | | | | |
| Hap 24 | | | | | | 1 | | | | | | |
| Hap 25 | | | | 1 | | | | | | | | |
| Hap 26 | | | | 1 | | | | | | | | |
| Hap 27 | | | | 2 | | | | | | | | |
| Hap 28 | | 1 | | | | | | | | | | |
| Hap 29 | | | 4 | | | | | | | | | |
| Hap 30 | | | | | | 1 | | | | | | |
| Hap 31 | 2 | | | | | | | | | | | |
| Hap 32 | 1 | | | | | | | | | | | |
| Hap 33 | 1 | | | | | 2 | | | | | | |
| Hap 34 | | | | | | | | 8 | | | | |
| Hap 35 | | | | | | | | | | | | 1 |
| No. of Hap | 10 | 3 | 1 | 3 | 5 | 6 | 8 | 1 | 1 | 1 | 1 | 1 |

**Table 6.** Matrix of pairwise sequence divergence of mitochondrial control region gene between different populations of gold-spotted pond frog and *R. nigromaculata* by Kimura-2-parameter distance

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Pyeongtaek | | | | | | | | | | | | | |
| 2. Paju | .006 | | | | | | | | | | | | |
| 3. Goyang | .007 | .005 | | | | | | | | | | | |
| 4. Ansan | .007 | .005 | .007 | | | | | | | | | | |
| 5. Ganghwa | .006 | .003 | .005 | .004 | | | | | | | | | |
| 6. Ongjin-A | .006 | .007 | .008 | .008 | .006 | | | | | | | | |
| 7. Ongjin-B | .005 | .004 | .005 | .005 | .004 | .005 | | | | | | | |
| 8. Cheongwon | .006 | .009 | .010 | .010 | .009 | .005 | .009 | | | | | | |
| 9. Taean-A | .005 | .003 | .004 | .004 | .003 | .005 | .002 | .008 | | | | | |
| 10. Taean-B | .005 | .003 | .004 | .004 | .003 | .005 | .002 | .008 | .000 | | | | |
| 11. Taean-C | .005 | .003 | .004 | .004 | .003 | .005 | .002 | .008 | .000 | .000 | | | |
| 12. China | .027 | .030 | .031 | .029 | .029 | .026 | .029 | .023 | .028 | .028 | .028 | | |
| 13. *R. nigromaculata* | .056 | .058 | .058 | .055 | .056 | .055 | .056 | .055 | .056 | .056 | .056 | .057 | |

able sites, 64 transitions and no transversions or indels (insertions and deletions) were found. The mean values of the nucleotide compositions were adenine (23.6%), thymine (29.2%), cytosine (32.7%) and guanine (14.4%) (Table 3).

Ansan, Taean, Ganghwa, Goyang, and Cheongwon populations showed no intrapopulation variation with different haplotypes in each population and the rest of populations (Pyeongtaek, Ongjin-A, B and Paju) had more than two haplotypes within each population. The Pyeongtaek population was the most variable with six haplotypes (Table 2).

The haplotype 1 consisted of four individuals from Pyeongtaek, five individuals from Ganghwa and two individuals from Paju. Five of the haplotypes (Hap. 1, 7, 8, 10 and 13) were shared among more than two populations. There were no shared haplotypes between Korea and China. Interpopulational sequence divergence among Korean gold-spotted pond frogs ranged from 0.0% to 0.8% (Table 4). Overall haplotype diversity ($h$) and nucleotide diversity ($\pi$) for Korea and China were 0.866 and 0.003, respectively.

### Control region sequences and variation

One hundred twenty nine variable sites (8.8%) out of 1,470 bp were observed in control region sequences from 77 individuals, resulting in 34 haplotypes (Table 5). Mean haplotype diversity of the control region was 0.92. Taean, Cheongwon, and Goyang populations showed no variation whereas the other six populations had variable individuals within the populations. As in cytochrome *b* sequences, Pyeongtaek was the most variable population with ten haplotypes. Most populations had at least one unique haplotypes (Table 5). Interpopulational variation of nucleotide sequence in Korean gold-spotted pond frogs ranged from 0.0% to 1.0% (Average, 0.5%) (Table 6).

Based on the result of cytochrome *b* gene and control region sequence comparison, the mean sequence divergence

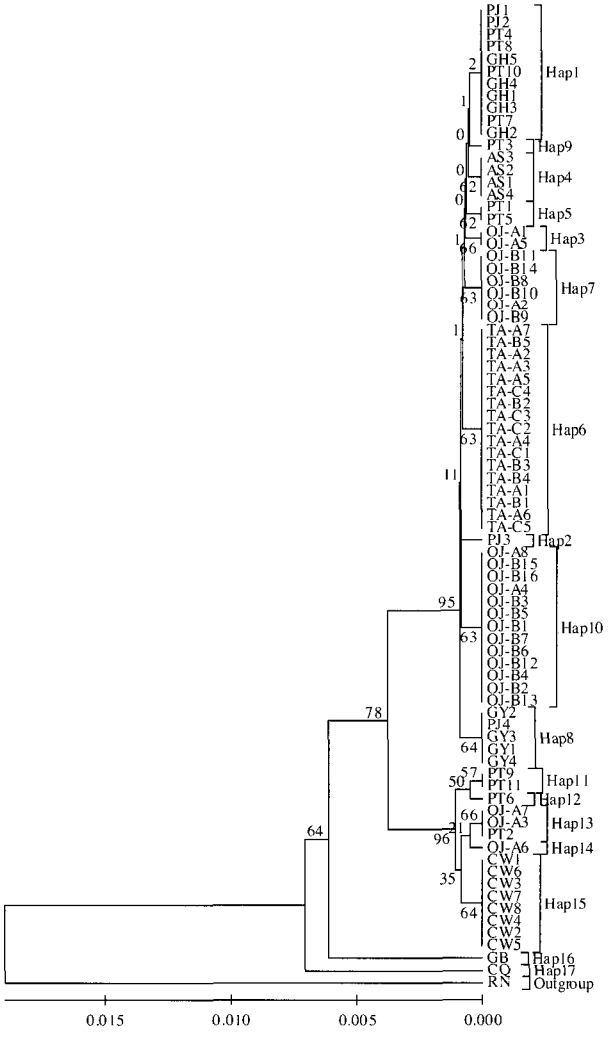


**Fig. 1.** Phylogenetic tree of gold-spotted pond frogs using cytochrome *b* gene based on UPGMA method. Numbers above branches are for percent supports in 1,000 bootstrap replications.

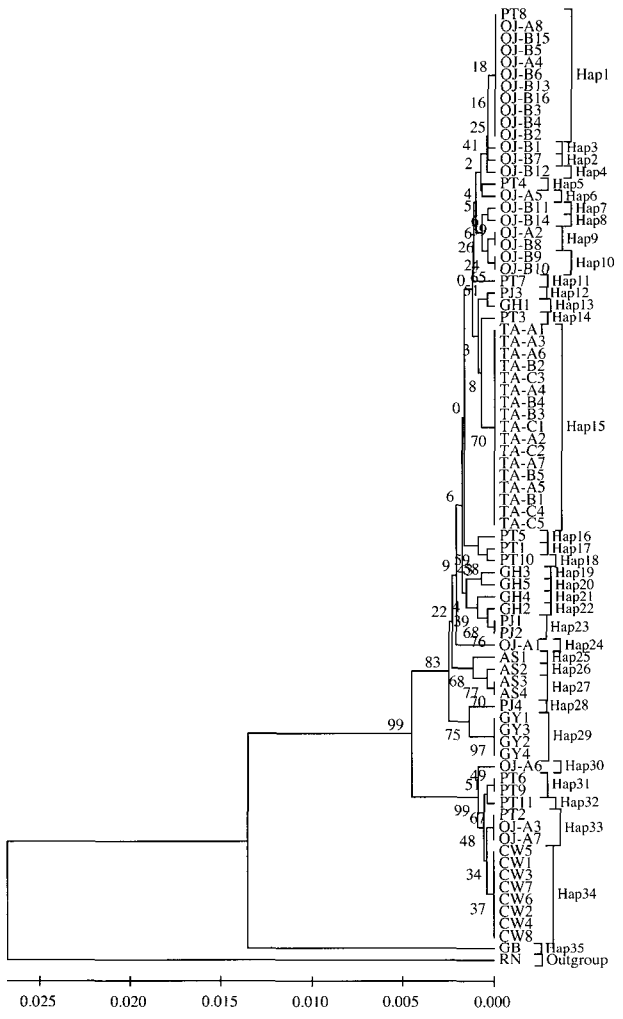


**Fig. 2.** Phylogenetic tree of gold-spotted pond frogs using control region gene based on UPGMA method. Numbers above branches are for percent supports in 1,000 bootstrap replications.

among Korean gold-spotted pond frogs by Kimura-2-parameter distance was 0.3% (range: 0.0-0.8%) and 0.5% (range: 0.0-1.0%), respectively. The mean sequence divergence between Chinese and Korean gold-spotted pond frogs was 1.3% for the cytochrome *b* gene and 2.7% for the control region (Table 4, 6).

The UPGMA construction of phylogenetic relationships of the cytochrome *b* gene and the control region between haplotypes are shown in Fig. 1 and Fig. 2, which had identical topologies with two major clades. The UPGMA trees constructed with haplotypes from cytochrome *b* and the control region clearly show that Korean gold-spotted pond frogs are slightly divided into two clades. Clades I and II included 10 and 5 haplotypes at cytochrome *b*, 29 and 5 haplotypes at the control region comprising 62 individuals and 15 individuals, respectively, for Korean specimens (Figs. 1, 2). These clades are supported with a bootstrap value (BP) of 79% at cytochrome *b* and 99% at the control region and are separated by a mean pairwise distance of 0.3% at cytochrome *b* and 0.6% of K2P between clade I and II. As with the results of the phylogenetic tree, clade I was found mostly in the western part of South Korea (7 populations) and clade II was found in Chongwon and some parts of Pyeongtaek and Onjin-A populations. The cytochrome *b* gene and control region sequence differences between Korean and Chinese populations were 1.3% and 2.9%, respectively. As an outgroup taxon, the true pond frog, *Rana nigromaculata*, was completely separated from Gold spotted pond frogs, *R. plancyi* (3.9% at cytochrome *b* and 5.6% at control region of K2P genetic distance) (Table 4, 6).

## DISCUSSION

The Korean gold-spotted pond frog is a typical example of a species facing local population threats and extinction due to human activities in South Korea. To protect this species, the Korean Ministry of Environment has listed them as an endangered species since 1975 and the IUCN (International Union for the Conservation of Nature and Natural Resources) listed it in the 2006 Red Book of Threatened Species. A prerequisite of the conservation planning for the is accurate information on the genetic diversity and taxonomic status and establishment of conservation units of the species.

In the nucleotide composition of each codon for the cytochrome *b* gene of gold-spotted pond frogs, guanine in the third codon position was the least quantity (5.6%), which is similar to other Korean *Rana* species and other vertebrates. Nucleotide diversity of the assayed fragment of cytochrome *b* gene in gold-spotted pond frog was less than the value reported in the same locus for other Korean Rana species (1.5% for *Rana amurensis*, Lee et al., 1999a; 14.3% for *R. dybowskii*, Kim et al., 1999a; 3.1% for *R. rugosa*, Lee et al., 1999b; 1.2% for *R. nigromaculata*, Kim et al., 1999b).

High sequence similarities in Korean gold-spotted pond frogs indicates a past gene flow among populations, and the presence of individuals with identical sequences suggests that the exchange of genetic materials had continued until recently. But most populations have their own major unique haplotypes for both sequences of cytochrome *b* and control region. Taean (Hap 7), Ansan (Hap 5), and Cheongwon (Hap 17) populations at the cytochrome *b* gene, and Taean, Ansan, Goyang, Ganghwa, and Cheongwon populations at the control region have unique haplotypes in each population, indicating that now there is most likely little or no exchange of genes between populations which are geographically

separated.

The UPGMA trees constructed with haplotypes from the cytochrome *b* gene and control region clearly show that Korean gold-spotted pond frogs are slightly divided into two clades, I and II, which are supported with a bootstrap value (BP) of 78% at the cytochrome *b* gene and 99% at the control region. As with the result of the phylogenetic tree, clade I was found mostly in the western part of South Korea (7 populations) and clade II was found in Chongwon and some parts of the Pyeongtaek and Ongjin-A populations. Pyeongtaek and Ongjin-A populations were more variable than other populations. The phylogenetic trees and genetic distances showed that Korean gold-spotted pond frogs were distinct from Chinese gold-spotted pond frogs (Figs. 1, 2; Table 4, 6). As an outgroup taxon, the true pond frog, *Rana nigromaculata*, was completely separate from gold spotted pond frogs (3.9% at cytochrome *b* and 5.6% at control region of K2P genetic distance) (Table 4, 6).

Avise et al. (1992, 1998) gave the general rate of 0.5-1.4 % divergence per MY (million years) for ectothermic vertebrate mtDNA. We are not sure what value within this range can be properly applied to our data, but Weisrock et al. (2001) provided estimates of approximately 0.64% change per MY per lineage in Amphibians. We adopted Weisrock et al.'s formula to estimate the divergence time between Korean and Chinese gold-spotted pond frogs which would be about 1.7 MYA (late Pleistocene), this would suggest that the two groups separated relatively recently. We acknowledge a potentially large error associated with this estimate as also discussed by Edwards and Beerli (2000).

In our results, we have to consider the evolutionary significant unit (ESU, Crandall, 2000; Frankham et al., 2002) to decide which populations are the best candidates to be included in a conservation project. Larger populations have greater adaptation abilities in variable environments. Nevertheless, we should remain aware of possible historic population characteristics. At present, there is insufficient phylogenetic data to determine a definite ancestral population structure, but our results for the cytochrome *b* gene and control region are indicative of the probable population structure. The sequence variation observed in this study suggest that there are several recognizable populations in Korea. The mitochondrial cytochrome *b* gene and control region used as genetic markers in this study are not sufficient for defining the ESU even though both genes show significant genetic diversity among Korean populations. With more comprehensive sampling from diverse localities in Korea and China and the use of more highly variable genetic markers such as microsatellites, the ESU of gold-spotted pond frogs can be better determined to more accurately define population structure and direct the needs of conservation projects.

Meanwhile, we suggest that the two clades in South Korea recognized in this study be managed separately.

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