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Variability in Two Species of Osmeridae (*Hypomesus nipponensis* and *Mallotus villosus*)

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바다빙어과 2종 (*Hypomesus nipponensis*와 *Mallotus villosus*)의 변이

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ABSTRACT : The variability within and between Korean pond-smelt (*Hypomesus nipponensis*; KPS) and Canadian capelin (*Mallotus villosus*; CCP) were studied in order to clarify the genetic distances and differences. The dendrogram obtained by the seven primers indicates cluster 1 (KOREAN 01~KOREAN 11) and cluster 2 (CANADIAN 12~CANADIAN 22). The longest genetic distance displaying significant molecular differences was found to exist between individuals in the two geographic species of Osmeridae, between individuals' no. 10 of Korean and no. 18 of Canadian (0.686). 121 unique shared loci to each species, with an average of 17.3 per primer, were observed in the KPS species, and 264 loci, with an average of 37.7 per primer, were observed in the CCP species. 77 shared loci by the two species, with an average of 11.0 per primer, were observed in the two fish species. RAPD analysis showed that the KPS species was more genetically diverse than the CCP species. KPS species may have high levels of genomic DNA variability owing to the introduction of the wild individuals from the other sites to sampling sites although it may be the geographically diverse distribution of this species. As stated above, the existence of species discrimination and genetic variability between the KPS and the CCP species was identified by RAPD analysis.

Key words : Canadian capelin, DNA polymorphism, *Hypomesus nipponensis*, Korean pond-smelt, *Mallotus villosus*, Osmeridae, Variability.

INTRODUCTION

Korean pond-smelt (*H. nipponensis* McAllister) (Kim et al., 2005b) is one of economically important aquacultural species, belonging to the family Osmeridae, and the order Clupeiformes. In the natural ecosystem, pond-smelt is widely distributed in the entirety of lakes, marshes, rivers, brackish-water habitats and seawater areas of the Korean Peninsula, as well as in several areas in China, Japan, Russia, and the Americas (Kim et al., 2005b). In general, the color, size and type of the fish in this species were affected by habitat, such as marsh, or river, the depth of the water, nutrition, and various environmental factors (Mamuris et al., 1999; Klinbunga et al., 2000; Yoon & Kim 2003; Siti Azizah et al., 2005; Kim et al., 2006b; Park et al., 2006). The environmental requirements and tolerances of the pond-smelt species from different geographic areas are currently unknown, as is pond-smelt species discrimination. The other species of Canadian capelin (*M. villosus*) (Kim et al., 2005b) is also one of economically important fish species, belonging to the family Osmeridae, and the order Clupeiformes. In the natural ecosystem, capelin is widely distributed in the entirety of seawater areas of the North

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Atlantic Ocean, as well as in several nations in northern Europe, Russia, and Canada. A habitation temperature of 5～15℃ is about optimal (Kim et al., 2005b). Even if Canadian capelin has a close phylogenetic relationship to the pond-smelt, this fish species exhibit a variety of morphological characteristics such as a number of very small scales in the skin and small teeth. Spawning season of the capelin is estimated to be the period from early spring to summer (from March to April in northern Europe; from June to July in the North America). Recently, this species of fish was successfully introduced into many fish markets or supermarkets in Korea from Canada.

Until now, genetic characters such as the pneumatic duct, pyloric caeca, chromatophore, and membrane structure across the nasal cavity also classified the genus *Hypomesus* species from various sites (Youn et al., 1999). Genetic variation, species-specific markers, and region-specific markers in catfish, penaeid shrimp, oysters, bullhead, lobster, and venus clam have already been analyzed by molecular methods (Liu et al., 1998; Yoon & Kim, 2001; Kim et al., 2004; Park et al., 2005; Kim et al., 2006a), proving the feasibility of such a study on other species. Clustering analyses of the genetic distances between populations/species/genera of various invertebrates and fishes from different geographic sites using RAPD-PCR would be useful.

As the pond-smelt culture industry increases, the understanding of the genetics of this fish species becomes more necessary; to evaluate the potential genetic effects induced by pond-smelt production operations. However, notwithstanding their economic and scientific consequences, a little information currently exist regarding the genetics and early development of this family Osmeridae in Korea (Han et al., 1996; Youn et al., 1999; Kim et al., 2006b). Evidence was presented that RAPD markers may be useful for systematic investigations at the level of species and subspecies (Hassanien et al., 2004). In the latest date, because of little information with respect to genetic variability between Canadian capelin imported to Korea and indigenous Korean pond-smelt, the objective of this study was to clarify the genetic variability within and between Korean pond-smelt (*H. nipponensis*) and Canadian capelin (*M. villosus*).

MATERIALS AND METHODS

1. Sample Collection, Extraction of DNA and Development of RAPD Molecular Markers

Korean pond-smelt (*H. nipponensis*) was obtained from a region of Chuncheon, the inland of Korea and Canadian capelin (*M. villosus*) was imported from a region of the Great Lakes of Canada, respectively. RAPD-PCR analysis was performed on the muscle extract of 22 individuals. Pond-smelt and capelin muscles were collected in sterile tubes, placed on ice immediately, and stored under deep-freezing refrigeration (－70℃) until needed for use.

Genomic DNA was extracted and/or purified under the experimental conditions as previously described (Yoon & Park, 2002), with some modifications. 600 μL of chloroform was added to the mixture and then inverted (no phenol). Adding ice-cold 70% ethanol and then centrifuging at 19,621 g for 5 minutes extracted genomic DNA from the lysates. The DNA pellets were incubation-dried for more than 10 hours, held at －40℃ until use and application, and then dissolved in the ultra-pure water generated by a water purification system. The concentration and purity of the extracted genomic DNA was measured by optical density (absorbance ratio) at 260 nm by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK).

Four decamer primers (Operon Technologies, Alameda, CA, USA) and three 20-mer primers (SeouLin Bioscience, Seoul, Korea), respectively, were used to identify informative DNA markers. Selected seven primers, OPB-01 (5'-GTTTCGCTCC-3'), OPB-10 (5'-CTGCTGGGAC-3'), OPB-17 (5'-AGGGAACGAG-3'), OPC-05 (5'-GATGACCGCC-3'), URP-05 (20-mer), URP-07 (20-mer) and URP-09 (20-mer) were shown to generate the unique shared loci to each

species and the shared loci by the two species which could be clearly and reproducibly counted. Whenever bands were faint, reproducibility and repeatability of amplification products were tested for each primer used in the experiments. Polymerase chain reaction amplification was generated using two programmable DNA thermal cyclers (Perkin Elmer Cetus, Norwalk, CT, USA; MJ Research Inc., Waltham, MA, USA) according to the manufacturer's instructions as previously described (Yoon & Kim, 2004). The mixture was followed a pre-denaturation at 94℃ for 5 min. The thermal cycler programmed for 45 cycles at 94℃ for 1 min for denaturation, at 36℃ for 1 min for annealing, at 72℃ for 1 min for extension, at 72℃ for 5 min for post-extension, using the fastest available transition between each temperature. The 100 bp step Ladder DNA markers (Bioneer Corp., Daejeon, Korea) were used as a DNA molecular weight marker. The gels were photographed over ultraviolet light using a Photoman direct copy system (PECA Products, Beloit, WI, USA).

2. Data Analysis

Readily visible DNA amplification products, ranging in molecular size from approximately 80 bp to 2,600 bp in size, were counted manually for statistical analysis. DNA polymorphisms are revealed not only by the banding patterns of amplified products at specific positions but also by primers (Tassanakajon et al., 1998). In this study, the bandsharing value (BS), which is based on the presence or absence of amplified loci, was used to calculate similarity indices. The degree of variability was calculated by use of the Dice coefficient, which is given by the formula: BS band = 2 $n_{ab}/(n_a+n_b)$, where n_{ab} is the number of bands shared between the samples a and b, n_a is the total number of bands for sample a and n_b is the total number of bands for sample b (Yoke-Kqueen & Radu, 2006; Yoon et al., 2007). A BS value of 1.000 indicates that the two samples are identical and a BS value if 0 indicates that the samples are different. A dendrogram was constructed by cluster analysis using the Systat version 10 (SPSS Inc., Chicago, IL, USA) software. Cluster analysis was performed on similarity indices to generate the dendrogram.

RESULTS AND DISCUSSION

1. Genetic Variations within and between Species

The genomic DNA isolated from Korean pond-smelt (*H. nipponensis*) and Canadian capelin (*M. villosus*), respectively, were amplified several times by RAPD-PCR reaction using the seven arbitrarily selected primers OPB-01, OPB-10, OPB-17, OPC-05, URP-05, URP-07 and URP-09 (Tables 1). As shown in Fig. 2, the variety of the banding patterns showed dramatically between the two geographic

Table 1. The number of unique shared loci to each species, and shared loci by the two species generated by RAPD-PCR using 7 random primers in Korean pond-smelt (*H. nipponensis*) and Canadian capelin (*M. villosus*), respectively

Item / Primer\species	No. of unique shared loci to each species		No. of shared loci by the two species
	Korean pond-smelt	Canadian capelin	Two fish species
OPB-01	0	11	0
OPB-10	11	11	0
OPB-17	11	33	0
OPC-05	55	55	33
URP-05	22	66	22
URP-07	11	55	11
URP-09	11	33	11
Total no.	121	264	77
Average no. per primer	17.3	37.7	11.0

locales. The higher fragment sizes are still more observed in the KPS species, but did not in the CCP species. Reversely, the lower fragment sizes are much more observed in the CCP species, but did not in the KPS species.

In this study, the unique shared loci to each species were generated by RAPD-PCR, using 7 random primers in two fish species, as illustrated in Table 1 and Fig. 1. 121 unique shared loci to each species, with an average of 17.3 per primer, were observed in the KPS species, and 264 loci, with an average of 37.7 per primer, were observed in the CCP species. This primer, however, in the CCP species, the banding patterns of the unique shared loci to each species, corresponding to loci of 1,400 bp, were generated by the decamer primer OPB-01, as shown in Fig. 1A. These results demonstrate that the KPS is genetically different from the CPS species. 33 unique shared loci to each species, of approximately 450 bp, 750 bp and 1,200 bp, were identified by the decamer primer OPB-17, which established identifications for populations and/or species, as shown in Fig. 1C. 55 unique shared loci to each species that generated by the 20-mer primer URP-09, of 380 bp, 650 bp, 1,000 bp, 1,100 bp and 1,600 bp, were identified

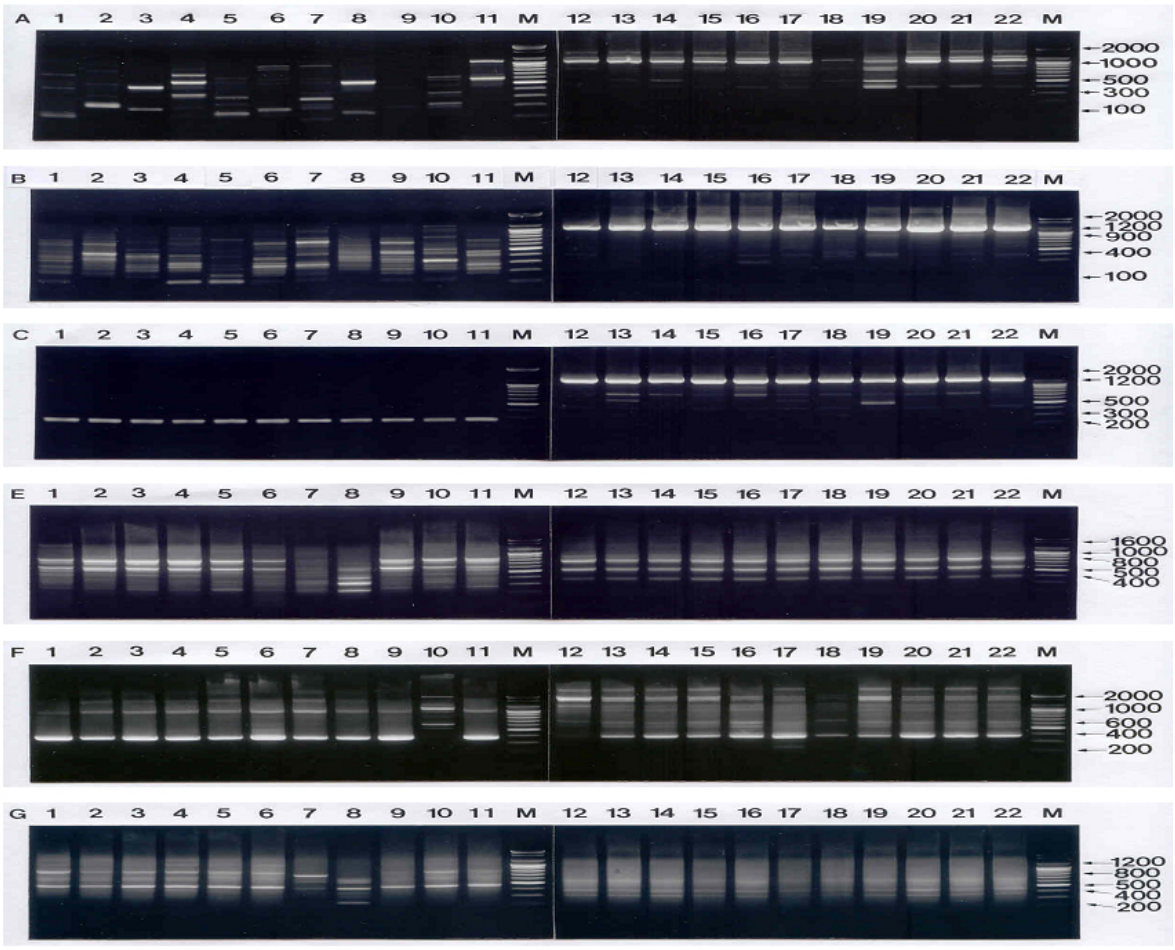

Fig. 1. RAPD-PCR-generated electrophoretic profiles of individual Korean pond-smelt (*H. nipponensis*) and Canadian capelin (*M. villosus*). Each lane shows DNA samples from 22 individuals. DNA isolated from Korean pond-smelt (lane 1~11) and Canadian capelin (lane 12~22) were amplified by random primers OPB-01 (A), OPB-10 (B), OPB-17 (C), URP-05 (E), URP-07 (F) and URP-09 (G). Amplification products were generated via electrophoresis on 1.4% agarose gel containing ethidium bromide. The 100 bp DNA Ladder (M) was used as a DNA molecular weight marker.

in the CCP species (Fig. 1F).

In the present study, the shared loci by the two species were generated by RAPD-PCR, using 7 random primers in two fish species, as illustrated in Table 1 and Fig. 1. Especially, 77 shared loci by the two species, with an average of 11.0 per primer, were observed in the two fish species. The 20-mer primer URP-07 generated the shared loci by the two species DNA loci, approximately 1,000 bp, in both the KPS and CCP species (Fig. 1F). The 11 shared loci by the two species generated by the 20-mer primer URP-09 were, with size of approximately 450 bp, and were identical in both the KPS and CCP species, which were identifying populations and/or species, as shown in Fig. 1G.

Diagnostic markers that are found to be present in two populations of an eel-loach species (*Pangio* sp.) are also considered to be species-specific markers, whereas the other bands were considered to be population-specific markers

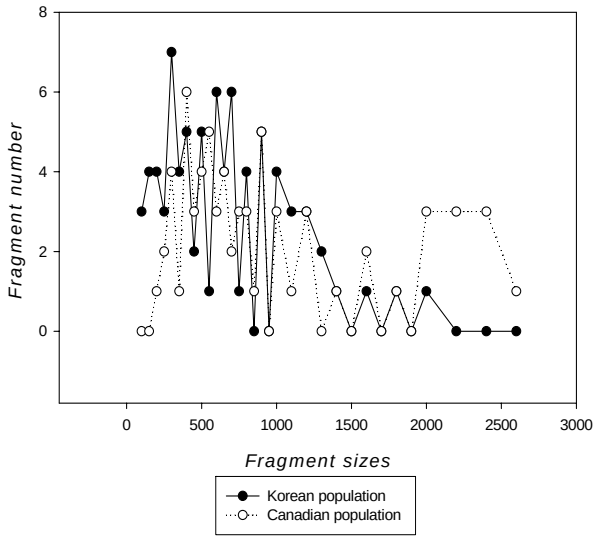

Fig. 2. Distribution of fragment sizes of Korean pond-smelt (*H. nipponensis*) and Canadian capelin (*M. villosus*). Circles: Korean pond-smelt population. Blank circles: Canadian capelin population. The fragment numbers in each size interval have been computed from the pooled fragments obtained with all the primers. The lower fragment sizes are much more observed in the CCP species.

(Siti Azizah et al., 2005). Three diagnostic markers were observed in *P. piperata* and 14 in *P. shelfoldii*, with molecular weights ranging from 300～2,000 bp. Especially, species-related specific loci (approximately 280 bp in KPS; 1,200 bp in CCP) generated by the primer OPB-17 were identified between KPS and CCP species, as shown in Fig. 1C. In future, these specific loci of other sizes are applicable as the indicator, which established identifications for populations and/or species. The RAPD data serve as a baseline analysis of the current genetic diversity found among *Oreochromis niloticus* populations in Egypt (Hassanien et al., 2004). It was reported that species-specific RAPD markers were generated from 0.5 kb to 3.2 kb in red or black seabream, and also in their hybrids (Kim et al., 2005a). In particular, they insisted that with URP6 among the 12 universal rice primers (URP) surveyed, the hybrids exhibited two specific markers at 500 and 850 bp, derived from the red and black seabream, respectively.

2. The Bandsharing Value and Genetic Distances

In this study, the dendrogram, generated by seven trustworthy primers, indicates two genetic clusters, as shown in Fig. 3: cluster 1 (KOREAN 01～KOREAN 11), and cluster 2 (CANADIAN 11～CANADIAN 22). Individual no. 12 from Canada was genetically most closely related to Canadian no. 14 (genetic distance = 0.062). The longest genetic distance displaying significant molecular differences was found to exist between individuals in the two geographic species of Osmeridae, between individual's no. 10 of Korean and no. 18 of Canadian (0.686). Ultimately, upon RAPD analyses of the patterns of genetic distance, these results indicated that KPS species was distantly related to CCP species. Supplementary, two geographic species were distinguishable, especially by a few external morphological characters. Here, hierarchical clustering analysis shown distant relationships between two species revealed a pattern different to the one posited by Yoon & Kim (2004). The present study according to cluster analysis showed that genetic similarity could be found among individuals within

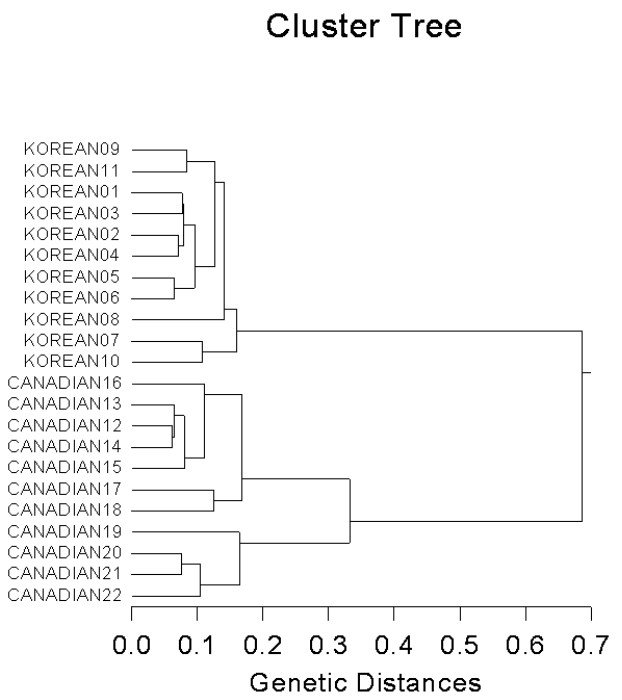

Fig. 3. Cluster analysis was performed on similarity indices obtained from Korean pond-smelt (*H. nipponensis*) and Canadian capelin (*M. villosus*) to generate the dendrogram.

a geographic species, apart from between-species. They reported that single linkage cluster analysis, which indicated four genetic groupings, and a dendrogram revealed close relationships between individual identities within two geographic fish species. However, additional statistical analyses derived from RAPD-PCR data should be needed for the acquisition of more profound and further evaluation of genetic variability among various geographic fish species belonging to the family Osmeridae. The present study suggests that KPS species was distantly related to CCP species according to cluster analysis.

The genetic distances among populations using the RAPD-PCR method, indicates the relatives of three mud crab species (Klinbunga et al., 2000). This study showed that large genetic differences could be found between geographic populations within a species, as well as between species. Kim et al. (2000) insisted that RAPD analysis constitutes a powerful device for the elucidation of phylogenetic relationships, based on their analysis of 6 species of *Haliotis*. The genetic distance between the two geographic populations ranged from 0.039 to 0.284. Among the five Nile tilapia populations, the Delta lake populations exhibited the clear genetic distances from 0.147 to 0.216 (Hassanien et al., 2004). There are relatively higher genetic distances between Quena and Cairo populations (D=0.216) than that obtained between Cairo and Assuit populations (D=0.166). In other fish and invertebrates, the identification of the catfish (*Silurus asotus*), bullhead (*Pseudobagrus fulvidraco*), and rockfish populations was a necessary step in the inauguration and development of invertebrate/teleost breeding programs (Yoon & Park, 2002; Yoon & Kim, 2004; Basavaraju et al., 2007; Yoon et al., 2007).

Information on the genetic makeup of fish species is valuable for species and/or genus identification, breed improvement and species preservation of biodiversity (Tassanakajon et al., 1998; Waldbieser & Wolters, 1999). There are many studies according to correlation between genetic and morphological characters. The classification of geographic populations between KPS and CCP species is founded on external morphological variations in body size, body color, body type, head shape, fin style, eye shape, scale form, and scale number (Salini et al., 2004). The best combination markers affecting body weight demonstrate major differences in the genetic structure between different common carp stocks (Basavaraju et al., 2007). The greater variation among the specimens suggests the presence of structured populations or morphotypes in sympatry at site (Matoso et al., 2004). The association of RAPD groups with fish species of origin, evidence of fish species-specific virulence and constitutive in *Flavobacterium columnare* cell size indicates significant genetic differences between strains (Thomas-Jinu & Goodwin, 2004).

In the present study, RAPD-PCR analysis enabled us to detect the existence of species identification and genetic variation in the pond-smelt species of Korea and the capelin species of Canada. This confirms that the method

is a suitable device for DNA comparisons, both within and between individuals, species, and populations. Moreover, fundamental information of the DNA polymorphisms and molecular markers in Korean pond-smelt (*H. nipponensis*) and Canadian capelin (*M. villosus*) may contribute significantly to broodstock selection and selective fish-breeding programs. The unpredictedly unique gene pools shown by some samples would need new conservation policies, such that much wilder family Osmeridae could be preserved. Accordingly, further analysis with more individuals, primers, fish/shellfish species and sampling sites will be required to fully establish the specificity of loci to particular taxa, and subsequent inter-specific gene flow in the family Osmeridae such as the genus *Hypomesus* and/or *Mallotus*. Especially, additional statistical analyses derived from RAPD-PCR data will also be needed for the acquisition of more profound and further evaluation of genetic differences and relationships among various geographic fish species (Ali et al., 2004).

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REFERENCES

Ali BA, Huang TH, Qin DN, Wang XM (2004) A review of random amplified polymorphic DNA (RAPD) markers in fish research. Review. Fish Biol Fish 14: 443-453.

Basavaraju Y, Prasad DT, Rani K, Kumar SP, Naika UD, Jahageerdar S, Srivastava PP, Penman DJ, Mai GC (2007) Genetic diversity in common carp stocks assayed by random-amplified polymorphic DNA markers. Aquac Res 38:147-155.

Han KH, Lee SJ, Kim YU, Myoung JG (1996) Eggs development and larval development of the ice fish, *Hypomesus transpacificus nipponensis* McAllister. J Korean Fish Soc 29(4):497-502.

Hassanien HA, Elnady M, Obeida A, Itriby H (2004) Genetic diversity of Nile tilapia populations revealed by randomly amplified polymorphic DNA (RAPD). Aquac Res 35:587-593.

Huang BX, Peakall R, Hanna PJ (2000) Analysis of genetic structure of blacklip abalone (*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. Mar Biol 136:207-216.

Kim BS, Kang JH, Kim JH, Kim KK, Lee JU, Lee JY, Park IS (2005a) Production of hybrids between female red seabream and male black seabream in Korea. Korean J Genet 27:133-140.

Kim IS, Choi Y, Lee CL, Lee YJ, Kim BJ, Kim JH (2005b) Illustrated Book of Korean Fishes. Kyo-Hak Pub., Seoul, 150 pp.

Kim JY, Park CY, Yoon JM (2004) Genetic differences and DNA polymorphism in oyster (*Crassostrea* spp.) analysed by RAPD-PCR. Korean J Genet 26:123-134.

Kim SK, Jung YH, Han SH, Oh YS, Ko MH, Oh MY (2000) Phylogenetic relationship among *Haliotis* spp. distributed in Korea by the RAPD analysis. Korean J Genet 22:43-49.

Kim JR, Chung CH, Kim YH, Yoon JM (2006a) Genetic variations in geographic venus clam (*Gomphina aequilatera*, Sowerby) populations from Samcheok and Wonsan. Dev Reprod 10(4):227-238.

Kim YH, Park SY, Yoon JM (2006b) Geographic variation in pond smelt (*Hypomesus nipponensis*) by RAPD analysis. Korean J Ichthyol 18(1):1-11.

Klinbunga S, Boonyapakdee A, Pratoomchat B (2000b) Genetic diversity and species-diagnostic markers of mud crabs (Genus *Scylla*) in Eastern Thailand determined by RAPD analysis. Mar Biotech 2:180-187.

Liu Z, Li P, Argue BJ, Dunham RA (1998) Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their F_1, F_2 and backcross

hybrids. Anim Genet 29:58-62.

Mamuris Z, Stamatis C, Bani M, Triantaphyllidis C (1999) Taxonomic relationships between four species of the Mullidae family revealed by three genetic methods: allozymes, random amplified polymorphic DNA and mitochondrial DNA. J Fish Biol 55:572-587.

Matoso DA, Artoni RF, Galetti PM (2004) Genetic diversity of the small characid fish *Astyanax* sp., and its significance for conservation. Hydrobiologia 527:223-225.

Park SY, Park JS, Yoon JM (2005) Genetic differences and variations in slipper lobster (*Ibacus ciliatus*) and deep sea lobster (*Puerulus sewelli*) determined by RAPD analysis. Korean J Genet 25:307-317.

Park SY, Kim JY, Yoon JM (2006) Geographic variations and DNA polymorphisms in gizzard-shad (*Konosirus punctatus*). Korean J Ichthyol 18(4):300-310.

Salini JP, Milton DA, Rahman MJ, Hussain MG (2004) Allozyme and morphological variation throughout the geographic range of the tropical shad, hilsa *Tenualosa ilisha*. Fish Res 66:53-69.

Siti Azizah, MN, Ruzainah A, Patimah I (2005) Development of RAPD markers in the eel-loach (*Pangio* spp.) for genetic discrimination and monitoring of wild and cultured populations. World Aqua 36(1):37-43.

Tassanakajon A, Pongsomboon S, Jarayabhand P, Klinbunga S, Boonsaeng V (1998) Genetic structure in wild populations of black tiger shrimp (*Penaeus monodon*) using randomly amplified polymorphic DNA analysis. J Mar Biotech 6:249-254.

Thomas-Jinu S, Goodwin AE (2004) Morphological and genetic characteristics of *Flavobacterium columnare* isolates: correlations with virulence in fish. J Fish Dis 27:29-35.

Waldbieser GC, Wolters WR (1999) Application of polymorphic microsatellite loci in a channel catfish *Ictalurus punctatus* breeding program. J World Aqua Soc 30(2): 256-262.

Yoke-Kqueen C, Radu S (2006) Random amplified polymorphic DNA analysis of genetically modified organisms. J Biotechnol 127:161-166.

Yoon JM, Kim GW (2001) Randomly amplified polymorphic DNA-polymerase chain reaction analysis of two different populations of cultured Korean catfish *Silurus asotus*. J Biosci 26:641-647.

Yoon JM, Park HY (2002) Genetic similarity and variation in the cultured and wild crucian carp (*Carassius carassius*) estimated with random amplified polymorphic DNA. Asian-Aust J Anim Sci 15:470-476.

Yoon JM, Kim YH (2003) Wide marsh clam (*Corbicula* spp.) populations from three sites analysed by RAPD-PCR-AGE. Bull Electrochem 19:337-348.

Yoon JM, Kim JY (2004) Genetic differences within and between populations of Korean catfish (*S. asotus*) and bullhead (*P. fulvidraco*) analysed by RAPD-PCR. Asian-Aust J Anim Sci 17:1053-1061.

Yoon JM, Choi Y, Kim JY (2007) Genetic differences and variation in black rockfish (*Sebastes schlegeli*) and Hwanghae rockfish (*S. koreanus*) from the Yellow Sea. Korean J Genet 29(4):437-445.

Youn CH, Kim IS, Kim WO (1999) Taxonomic revision of the genus *Hypomesus* in Korea. Korean J Ichthyol 11(2): 149-154.