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# Genetic Differences and Variation in Two Largehead Hairtail (*Trichiurus lepturus*) Populations Determined by RAPD-PCR Analysis

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**Genomic DNA was isolated from two geographic populations of largehead hairtail (*Trichiurus lepturus*) in Korea and the Atlantic Ocean. The eight arbitrarily selected primers were found to generate common, polymorphic, and specific fragments. The complexity of the banding patterns varied dramatically between primers from the two locations. The size of the DNA fragments also varied widely, from 150 bp (base pairs) to 3,000 bp. Here, 947 fragments were identified in the largehead hairtail population from Korea, and 642 in the largehead hairtail population from the Atlantic Ocean: 148 specific fragments (15.6%) in the Korean population, and 61 (9.5%) in the Atlantic population. In the Korean population, 638 common fragments with an average of 79.8 per primer were observed.; 429 common fragments, with an average of 53.6 per primer, were identified in the Atlantic population. The number of polymorphic fragments in the largehead hairtail population from Korea and the Atlantic Ocean was 76 and 27, respectively. Based on the average bandsharing values of all samples, the similarity matrix ranged from 0.784 to 0.922 in the Korean population, and from 0.833 to 0.990 in the Atlantic population. The bandsharing value of individuals within the Atlantic population was much higher than in the Korean population. The dendrogram obtained by the eight primers indicated two genetic clusters: cluster 1 (KOREAN 01~KOREAN 11), and cluster 2 (ATLANTIC 12~ATLANTIC 22). Individual KOREAN no. 10 from Korea was genetically most closely related to KOREAN no. 11 in the Korean population (genetic distance = 0.038). Ultimately, individual KOREAN no. 01 of the Korean population was most distantly related to ATLANTIC no. 16 of the Atlantic population (genetic distance = 0.708).**

**Key words : Bandsharing value, DNA polymorphism, genetic variability, geographical population, largehead hairtail, RAPD-PCR, *Trichiurus lepturus***

## Introduction

Largehead hairtail (*Trichiurus lepturus*), alias ribbonfish, which belongs to the family Trichiuridae, and the order Perciformes, constitutes an economically important marine fish species. In the natural ecosystem, largehead hairtail is distributed widely throughout the entirety of seawater areas (especially the West Sea and South Sea) of the Korean Peninsula, as well as in the temperate zones, tropical and/or subtropical waters of China (especially East and South China Sea), Japan, Southeast Asia, New Zealand, Australia, the Atlantic, Pacific and Indian oceans (Zhang and Sohn, 1997; Huh, 1999; Park *et al*., 2000; Kim *et al*., 2001). A water temperature of 7~25°C is optimal. The spawning season of the fish is from May to August in the South and West

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Sea of Korean Peninsula. The water temperature during spawning season ranges from 18°C to 20 °C. Large hairtail grow to a maximum length of 2.5 m and approximately 5 kg in weight. The body is very elongated. The fish have a silver, ribbon-like body that ends in a thin whip-like tail and large, slashing teeth that should be avoided under natural conditions. They lack caudal and ventral fins but possess a dominant and elongated dorsal fin and pectoral fins. Scales are very small and embedded in the skin. The color of body surface, a dominant dorsal and caudal fin is silver-white and/or silver-gray. The upper-jaw adorned with two pairs of canine teeth of hook type and lower jaw with two pairs of canine teeth. Hairtail are predatory fish and will eat just about anything. Adult hairtail feed on wide variety of animals including earthworms, shrimps, crabs, shells, fishes and others. Recreational fishers in Korea have traditionally caught this fish by boat fishing and in the tide embankment of Gunsan, Seocheon, Mokpo, Masan and Jeju Island in Korea. Trawling and angling fishing during summer months can catch hairtail. The anglers have caught hairtail on anchovy, squid, scaled sardine, chub mackerel, and Pacific herring.

The polymorphic and/or specific markers have all also been employed in the identification of individuals, species and populations, genetic variability and inbreeding, hybrid parentages and genetic diagnostics for aquaculture breed, species, genus or geographical population (Badarcki and Skibinski, 1994; Smith *et al*., 1997; Kim *et al*., 2000; Liu and Cordes, 2004; Yoon and Kim 2004b). These genetic markers have been used to estimate genetic variation of population structure in fishes, including brown trout (*Salmo trutta*) (Cagigas *et al*., 1999), yellowfin tuna (*Thunnus albacares*) (Diaz-Jaimes and Uribe-Alcocer, 2003), Korean catfish (*Silurus asotus*) and bullhead (*Pseudobagrus fulvidraco*) (Yoon and Kim, 2004a), and eel-loach (*Pangio* sp.) (Siti Azizah *et al*., 2005). Even if the reproducibility of RAPD is somewhat poor and depends upon PCR conditions, polymorphic bands generated by RAPD-PCR using arbitrary primers have classically been considered to constitute a reliable method for the detection of DNA similarity and/or diversity between organisms (Jeffreys and Morton 1987; Liu *et al*., 1998; Kim *et al*., 2004). Many molecular/genetic studies have employed this technique, as RAPD-PCR is an easy, reliable, and relatively speedy method for the investigation of numerous genomic DNAs, which can help in the determination of the degree of genetic diversity in a given population. Another merit of this method is that it requires no prior knowledge of the genome for its efficacy (Welsh and McClelland, 1990; Welsh *et al*., 1991; Koh *et al*., 1998; Iyengar *et al*., 2000; Liu and Cordes, 2004).

Generally, the color, size, and type of fish from this species vary according to habitat, water depth, nutrition, and other common factors. The environmental requirements and tolerances of largehead hairtail from different geographic areas remain unknown, as does its population structure. As the largehead hairtail fishery industry grows, so does interest into the genetics of this fish species. The distribution and migration pattern, stock assessment and management and maturation, feeding habits and spawning of hairtail have been reported by researchers (Zhang and Sohn, 1997; Huh, 1999; Park *et al*., 2000; Park *et al*., 2002; Cha and Lee, 2004). However, little information currently exists regarding the genetics of Korean and/or other geographical largehead hairtails.

Our study attempted to determine the genetic distances and differences within and between geographical largehead hairtail populations. In order to realize this, we performed clustering analyses of two largehead hairtail (*T. lepturus*) populations from the Korea and the Atlantic Ocean. We also employed analysis of random amplified polymorphic DNA (RAPD) to identify genetic variability and DNA polymorphism of these largehead hairtail populations.

## Materials and Methods

### Sample collection and extraction of genomic DNA

Two geographical populations of largehead hairtail (*T. lepturus*) were obtained from two different regions: the West Sea of Korea, and the Atlantic Ocean. Largehead hairtail muscle was collected in sterile tubes, immediately placed on ice, and stored at −40°C until needed. RAPD-PCR analysis was performed on the muscle extracts from 22 individuals, using eight arbitrarily selected primers of two decades of different decamer primers. The extraction/purification of genomic DNA was performed under the conditions

described previously (Yoon and Kim, 2003b). The DNA pellets were then incubation-dried for more than 10 hours, maintained at −40°C until analysis, then dissolved in the ultra-pure water produced by a water purification system (JABA KOREA, Korea). The concentration of the extracted genomic DNA was measured by its absorbance ratio at 260 nm, with a spectrophotometer (Beckman DU 600 series, UK; Shimadzu, Australia).

### Decamer primers, molecular markers and amplification stipulations

The arbitrarily chosen primers were purchased from Operon Technologies, USA and Seoulin Biotechnologies, Korea. All of the primers had a 60∼70 percent G+C content. Among the 20 selected primers, eight primers, OPA-07 (5′-GAAACGGGTG-3′), OPA-20 (5′-GTTGCGATCC-3′), OPB-14 (5′-TCCGCTCTGG-3′), OPB-15 (5′-GGAGGGTGTT-3′), OPB-17 (5′-AGGGAACGAG-3′), OPB-18 (5′-CCACAGCAGT-3′), OPD-16 (5′-AGGGCGTAAG-3′) and URP-07 (20-mer) were shown to generate identical, specific and polymorphic fragments which could be clearly scored. We used these primers to determine the genetic variations, DNA polymorphisms, genetic diversity, and similarity of the largehead hairtail. RAPD-PCR was performed using two Programmable DNA Thermal Cyclers (MJ Research, Inc., USA; Perkin Elmer Cetus, USA). Amplification products were generated via electrophoresis on 1.4% agarose (VentechBio, Korea) gel containing TBE (90 mM Tris, pH 8.5; 90 mM borate; 2.5 mM EDTA). A 100 bp DNA Ladder (Bioneer Co., Korea) was used as size standard. After electrophoresis, agarose gels were stained with ethidium bromide and illuminated by ultraviolet rays. Bands were also photographed by a Photoman direct copy system (PECA products, USA).

### Data analysis

Primers that generated minor bands were excluded from our analyses. Only readily visible fragments between 150 bp and 3,000 bp in size, were scored for statistical analysis. Bandsharing (BS) values were calculated according to the presence/absence of amplified products at specific positions in the same gel from the RAPD profiles. Absence of bands indicates that the priming site is not present, presumably as a result of some alteration in the DNA sequence. The values were calculated according to the protocols developed by Nei (1978) and Jeffreys and Morton (1987). Comparing two lanes, BS values were calculated as follows:

$BS = 2(Nab) / (Na + Nb)$.

Nab: the number of bands shared by the samples b and a

Na: the total number of bands in sample a

Nb: the total number of bands in sample b.

The average of within-population similarity was calculated by pairwise comparison between individuals within a population. The relatedness between different individuals in the largehead hairtail populations of Korea and the Atlantic Ocean was generated according to the bandsharing values and similarity matrix. Using similarity matrices to generate a dendrogram, facilitated by the PC-package program Systat version 10 (SPSS Inc., USA), a hierarchical clustering tree was constructed. Genetic differences and Euclidean genetic distances within and between populations were also calculated using the Systat hierarchical dendrogram program version 10. Systat version 10 was also used to obtain other statistical results, including means, standard errors, and t-test scores.

## Results

### RAPD-PCR variations

Genomic DNA was isolated from two geographical largehead hairtail populations in Korea and the Atlantic Ocean. The amplified products were separated by agarose gel electrophoresis (AGE) with an oligonucleotide primer, and stained with ethidium bromide. The eight arbitrarily selected primers OPA-07, OPA-20, OPB-14, OPB-15, OPB-17, OPB-18, OPD-16 and URP-07 were found to generate common, polymorphic, and specific fragments (Tables 1∼2). The complexity of the banding patterns varied dramatically between the primers from the two locations (Fig. 2). The size of the DNA fragments also varied widely, from 150 bp to 3,000 bp (Fig. 1). All examined primers generated a total of 947 fragments scored from the Korean largehead hairtail population, while a total of 642 fragments were generated from the Atlantic population (Table 1).

We assessed genetic variation in the Korean population first. The primer OPA-07 generated fragments ranging from 180 to 2,400 bp (Fig. 1A). This primer detected 99 identical major and/or

**Table 1.** The total, average, common, specific, and polymorphic fragments generated by RAPD-PCR using 8 random primers in largehead hairtail (*T. lepturus*) from Korea and the Atlantic Ocean

| Item | No. of average fragment per lane | | No. of common fragments | | | No. of specific fragments | | No. of polymorphic fragments | |
|---|---|---|---|---|---|---|---|---|---|
| Primer | Korean | Atlantic | Korean | Atlantic | Between two populations (%) | Korean | Atlantic | Korean | Atlantic |
| OPA-07 | 12.1 (133) | 7.8 (86) | 99 | 44 | 11 (15.4) | 15 | 11 | 22 | 3 |
| OPA-20 | 15.0 (165) | 9.2 (101) | 110 | 66 | 33 (37.5) | 31 | 5 | 9 | 4 |
| OPB-14 | 8.5 (93) | 4.7 (52) | 44 | 44 | 11 (25) | 8 | 10 | 6 | 0 |
| OPB-15 | 10.9 (120) | 5.3 (58) | 110 | 55 | 11 (13.3) | 23 | 8 | 0 | 0 |
| OPB-17 | 13.4 (147) | 6.4 (70) | 55 | 55 | 11 (20) | 24 | 2 | 17 | 0 |
| OPB-18 | 11.6 (128) | 8.8 (97) | 121 | 33 | 11 (14.3) | 10 | 19 | 11 | 16 |
| OPD-16 | 8.7 (96) | 6.4 (70) | 77 | 55 | 22 (33.3) | 10 | 2 | 7 | 4 |
| URP-07 | 5.9 (65) | 9.8 (108) | 22 | 77 | 11 (22.2) | 27 | 4 | 4 | 0 |
| Total no. | 10.8 (947) | 7.3 (642) | 638 | 429 | 121 (22.7) | 148 | 61 | 76 | 27 |
| Average no. per primer | 118.4 | 80.3 | 79.8 | 53.6 | 15.1 | 18.5 | 7.6 | 9.5 | 3.4 |

The total number of fragments generated by a primer in largehead hairtail (*T. lepturus*) obtained from Korea and the Atlantic Ocean is shown in parentheses.

minor fragments of sizes 200 bp, 280 bp, 300 bp, 450 bp, 550 bp, 650 bp, 900 bp, 1,000 bp and 2,000 bp, which were identical in all samples. 15 specific and 22 polymorphic RAPD fragments were also observed in this population. This primer produced an average of 12.1 fragments, and a total of 133 fragments, in comparison to the other primers used. The primer OPA-20 identified 110 common banding patterns in the RAPD products (Fig. 1B). This decamer primer generated the most fragments (a total of 165), with an average of 15.0, as illustrated in Table 1. The 31 specific fragments generated by this primer exhibited inter-individual-specific characteristics, thus revealing DNA polymorphisms. 9 polymorphic fragments, of 1,000 bp, were generated by this primer, and subsequently analyzed. The 44 common fragments generated by the decamer primer, OPB-14, were approximately 300 bp, 400 bp, 800 bp, and 1,200 bp in size, and were found to be identical (Fig. 1C). Interestingly, the 33 common fragments that established population identity were 400 bp, 800 bp and 1,200 bp. This primer produced the fragments (a total of 93), in comparison to the other primers used, with an average of 8.5. Interestingly, this primer detected 8 specific major and/or minor fragments that identified individuals. The polymorphic banding patterns of the RAPD products of approximately 350 bp (lane 3) and 800 bp (lanes 3, 5, 7, 8 and 11) were also detected. The 110 fragments generated by the decamer primer, OPB-15 were found to be identical (Fig. 1D). This primer generated an average of 10.9 fragments, and a total of 120 fragments. Interestingly, this primer detected 23 specific major and/or minor fragments that identified individuals. Polymorphic fragments that identified populations and/or species were not observed here. The 55 common fragments generated by the decamer primer, OPB-17, were approximately 700 bp, 1,400 bp, 1,600 bp, 2,200 bp and 2,400 bp, and were found to be identical (Fig. 1E). Interestingly, the 44 common fragments that established population identity were 700 bp, 1,400 bp, 1,600 bp and 2,400 bp. This primer generated an average of 13.4 fragments, and a total of 147 fragments. Interestingly, this primer detected 24 specific and 17 polymorphic major and/or minor fragments that identified individuals. The 121 common fragments were detected in all of the largehead hairtails from Korean population (Fig. 1F). This primer produced an average of 11.6 fragments, and a total of 128 fragments, in comparison to the other primers used. Interestingly, this primer detected 10 specific and 11 polymorphic major and/or minor fragments that identified individuals. The decamer primer, OPD-16, detected 77 common fragments, of 500 bp, 600 bp, 1,000 bp, 2,000 bp and 2,400 bp (Fig. 1G). Interestingly, the 55 common fragments that established population identity were 500 bp, 600 bp, 1,000 bp, 1,200 bp and 1,400 bp. The primer generated these minor specific fragments: 800 bp (lanes 1, 2, 3, 4, 5, 6, 7, 8, 10 and 11) and 2,200 bp (lanes 7, 8, 9, 10 and 11). Seven polymorphic

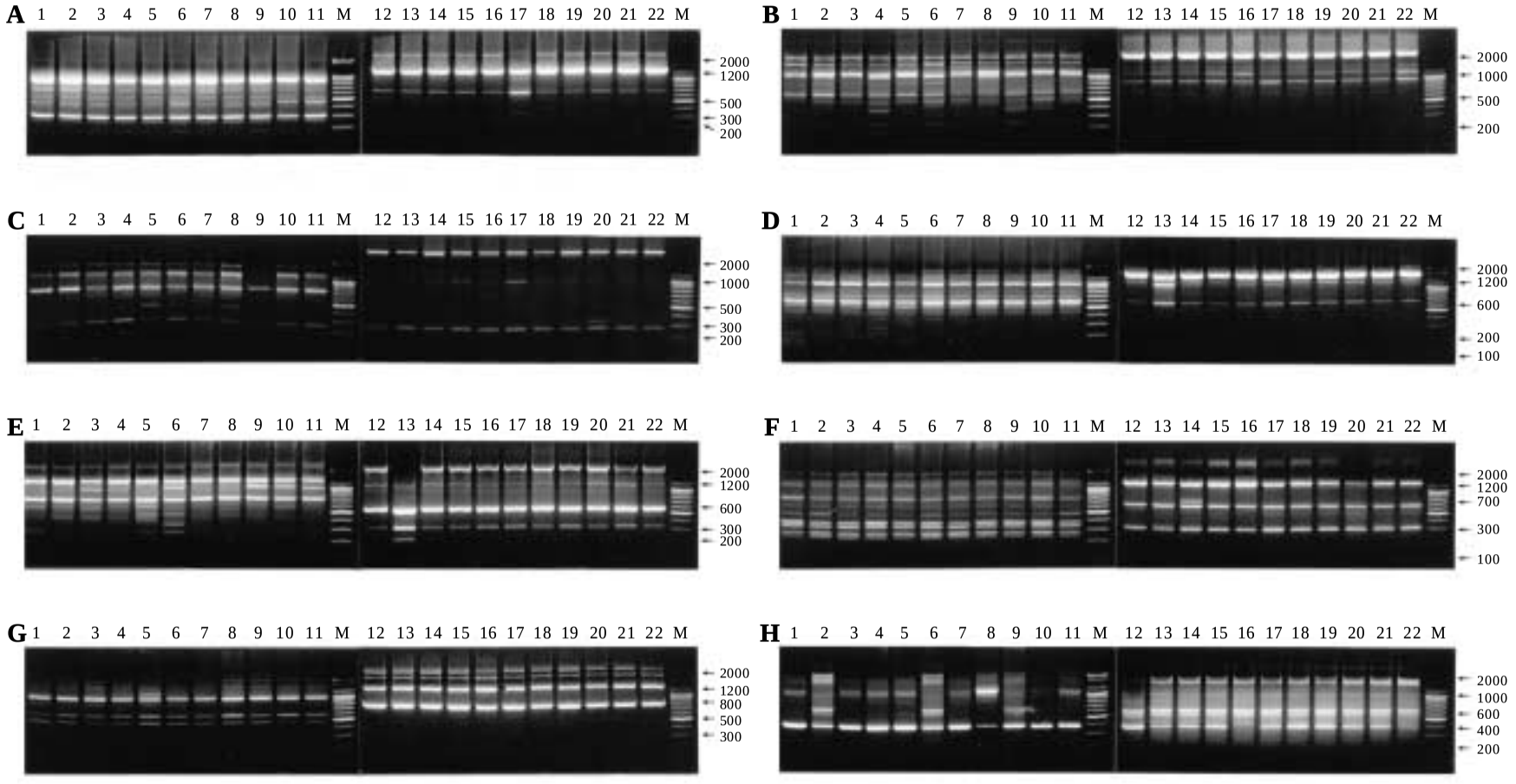


**Fig. 1.** RAPD–PCR–based electrophoretic profiles of individual largehead hairtail (*T. lepturus*). DNAisolated from Korea (lane 1–11) and the Atlantic Ocean (lane 12–22) were amplified by primers OPA–07. (A), OPA–20 (B), OPB–14 (C), OPB–15 (D), OPB–17 (E), OPB–18 (F), OPD–16 (G) and URP–07 (H). Amplified products were electrophoresed on 1.4% agarose gel and detected by staining with ethidium bromide. Each lane shows DNA samples extracted from 22 individuals. M, 100 bp Ladder DNA marker.

major/minor fragments, which were smaller than 1,100 bp, were also detected. This primer generated an average of 8.7 fragments, and a total of 96 fragments, in comparison to the other primers used. The random primer URP−07 detected 22 identical major and/or minor fragments of 400 bp, and 1,100 bp in all samples (Fig. 1H). The 27 specific fragments generated by this primer exhibited inter−individual−specific characteristics, thus revealing DNA polymorphisms. High degrees of RAPD variation were observed in the banding patterns generated by this primer, ranging from 250 bp to 2,400 bp. This primer generated 4 polymorphic major and/or minor fragments of approximately 450 bp (lanes 2, 6 and 9) and 1,000 bp (lane 8) each. This primer generated the least of fragments (a total of 65), in comparison to the other primers used, with an average of 5.9.

Moreover, in the Athletic largehead hairtail population, genetic variation was identified in the banding pattern generated by decamer OPA−07, which ranged from approximately 180 bp to 2,400 bp, as shown in Fig. 1A. This primer generated 86 fragments, with an average of 7.8. The 44 common fragments, of approximately 700 bp, 1,000 bp, 1,200 bp and 2,400 bp, represented the geographical population: these 44 fragments were detected in all of the largehead hairtails obtained from the Korean population. This primer, as illustrated in Table 1, generated 3 polymorphic bands. The 11 specific fragments generated by this primer exhibited inter−individual−specific characteristics, thus revealing DNA polymorphisms. The primer OPA−20 detected 66 identical major and/or minor fragments of sizes 900 bp, 1,200 bp, 1,400 bp, 1,500 bp, 2,200 bp and 3,000 bp, in all samples (Table 1) (Fig. 1B). This decamer primer generated 5 major/minor specific bands, of approximately 800 bp in size (lanes 15, 18, 19, 20 and 22). The primer generated a polymorphic RAPD profile consisting of 4 DNA fragments of 1,200 bp in size (lanes 14, 15, 16 and 22). This primer generated 101 fragments, with an average of 9.2. RAPD variation was observed in the banding patterns, ranging from 300 to 2,400 bp, and was generated by the decamer primer, OPB−14 (Fig. 1C). 44 identical fragments, of 300 bp, 1,000 bp, 2,300 bp, and 2,400 bp, were generated by this primer, and subsequently analyzed. This primer generated the least fragments, with a total of 52 bands, and an average of 4.7 (Table 1). 10 specific RAPD fragments were also observed in this population. Polymorphic fragments that identified populations and/or species were not observed here. The decamer primer, OPB−15, detected 55 common fragments, of 500 bp, 650 bp, 1,200 bp, 1,400 bp and 2,400 bp (Fig. 1D). The primer generated these minor specific fragments: 800 bp (lanes 13, 14, 18, 19, 20 and 21), 1,000 bp (lane 13) and 1,100 bp (lane 13). This decamer primer generated 58 fragments, with an average of 5.3. RAPD−PCR variation was identified in all banding patterns, ranging from approximately 200 bp to 2,100 bp, and generated by the decamer primer, OPB−17 (Fig. 1E). Two specific banding patterns of the RAPD product of approximately 200 (lane 13) and 700 bp (lane 13) were also detected. Especially, one of two specific fragments generated by this primer, exhibited inter−individual−specific characteristics, thus revealing DNA polymorphisms. Polymorphic fragments that identified populations and/or species were not observed here. A high degree of RAPD variation was observed in the banding patterns, ranging from 150 to 2,000 bp, and was generated by the decamer primer, OPB−18. 33 identical fragments were observed, which established the identifications for populations and/or species (Fig. 1F). Interestingly, the 22 common fragments that established population identity were 300 bp and 650 bp. 19 specific and 16 polymorphic bands were generated by this primer, and illustrated in Table 1. This primer generated 97 fragments, with an average of 8.8. The decamer primer, OPD−16, detected 55 common fragments, of 750 bp, 800 bp, 1,300 bp, 2,000 bp and 2,400 bp (Fig. 1G). Interestingly, the 33 common fragments that established population identity were 750 bp, 800 bp, and 1,300 bp. The primer generated these minor specific and polymorphic fragments, respectively: 1,100 bp (lanes 16 and 21) and 800 bp (lanes 17, 18, 19 and 20). This decamer primer generated the fragments (a total of 170), with an average of 6.4, as illustrated in Table 1. RAPD variation was observed in the banding patterns generated by the 20−mer primer, URP−07, ranging from 400 bp to 2,400 bp (Fig. 1H). The primer identified 77 common banding patterns in the RAPD products. 4 specific RAPD fragments were detected in this population: 450 bp (lanes 20, 21 and 22) and 700 bp (lane 22). Interestingly, this primer generated the most fragments, a total of 108, although the average was 9.8.

### Variation within and between populations, and genetic distances

Here, 947 fragments were identified in the largehead hairtail population from Korea, and 642 in the largehead hairtail population from the Atlantic Ocean: 148 specific fragments (15.6%) in the Korean population, and 61 (9.5%) in the Atlantic population (Table 1). 638 common fragments, with an average of 79.8 per primer, were observed in the Korean population. 429 common fragments, with an average of 53.6 per primer, were identified in the Atlantic population. The number of polymorphic fragments in the largehead hairtail population from Korea and the Atlantic Ocean was 76 and 27, respectively. The decamer primer, OPA−20, generated identical DNA fragments, of approximately 1,200 bp, 2,200 bp and 3,000 bp, respectively, in both the Korean and the Atlantic populations (Fig. 1B). The oligonucleotide decamer primer, OPD−16, also generated identical DNA fragments, of approximately 2,000 bp and 2,400 bp each, in the Korean and the Atlantic populations (Fig. 1G).

Based on the average bandsharing values of all samples, the similarity matrix ranged from 0.784 to 0.922 in the Korean population, and from 0.833 to 0.990 in the Atlantic population (Table 2). The average bandsharing value was 0.859± 0.004 within the Korean population, and 0.916± 0.006 within the Atlantic population. The average bandsharing value between the two geographical largehead hairtail populations was 0.340±0.003, ranging from 0.250 to 0.415. The bandsharing value between individuals no. 01 and no. 04 was 0.922, which was the highest value identified within the Korean population. The bandsharing value between individuals no. 01 and no. 08 was 0.784, which was the lowest observed. The bandsharing value between no. 21 and no. 22 was 0.990, which was the highest value observed

**Table 2.** Similarity matrix, including bandsharing values and genetic differences, calculated using Nei and Li's index, of the similarity of largehead hairtail (*T. lepturus*) from Korea and the Atlantic Ocean

| | | Bandsharing values of largehead hairtail from Korea | | | | | | | | | | | Bandsharing values of largehead hairtail from the Atlantic | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| Genetic differences of largehead hairtail from Korea | 1 | | 0.872 | 0.878 | 0.922 | 0.891 | 0.848 | 0.896 | 0.784 | 0.829 | 0.858 | 0.874 | 0.301 | 0.292 | 0.341 | 0.317 | 0.344 | 0.343 | 0.319 | 0.342 | 0.334 | 0.341 | 0.343 |
| | 2 | 0.128 | | 0.875 | 0.872 | 0.884 | 0.909 | 0.863 | 0.876 | 0.866 | 0.855 | 0.853 | 0.319 | 0.360 | 0.414 | 0.385 | 0.381 | 0.396 | 0.392 | 0.415 | 0.407 | 0.414 | 0.399 |
| | 3 | 0.122 | 0.125 | | 0.894 | 0.886 | 0.846 | 0.885 | 0.812 | 0.807 | 0.892 | 0.865 | 0.250 | 0.267 | 0.318 | 0.291 | 0.304 | 0.302 | 0.295 | 0.318 | 0.311 | 0.318 | 0.303 |
| | 4 | 0.078 | 0.128 | 0.106 | | 0.902 | 0.837 | 0.871 | 0.808 | 0.853 | 0.880 | 0.903 | 0.267 | 0.273 | 0.331 | 0.305 | 0.313 | 0.314 | 0.309 | 0.332 | 0.324 | 0.331 | 0.318 |
| | 5 | 0.109 | 0.116 | 0.114 | 0.098 | | 0.877 | 0.880 | 0.804 | 0.843 | 0.837 | 0.844 | 0.280 | 0.291 | 0.344 | 0.318 | 0.329 | 0.328 | 0.323 | 0.346 | 0.337 | 0.344 | 0.330 |
| | 6 | 0.152 | 0.091 | 0.154 | 0.163 | 0.123 | | 0.844 | 0.850 | 0.851 | 0.795 | 0.823 | 0.315 | 0.345 | 0.392 | 0.366 | 0.377 | 0.375 | 0.370 | 0.393 | 0.385 | 0.392 | 0.379 |
| | 7 | 0.104 | 0.137 | 0.115 | 0.129 | 0.120 | 0.156 | | 0.833 | 0.827 | 0.879 | 0.912 | 0.324 | 0.322 | 0.368 | 0.344 | 0.370 | 0.370 | 0.348 | 0.371 | 0.361 | 0.368 | 0.356 |
| | 8 | 0.217 | 0.124 | 0.189 | 0.192 | 0.196 | 0.150 | 0.167 | | 0.834 | 0.844 | 0.843 | 0.301 | 0.314 | 0.380 | 0.355 | 0.363 | 0.363 | 0.344 | 0.367 | 0.373 | 0.380 | 0.367 |
| | 9 | 0.171 | 0.134 | 0.193 | 0.147 | 0.157 | 0.149 | 0.174 | 0.166 | | 0.845 | 0.853 | 0.268 | 0.298 | 0.367 | 0.341 | 0.335 | 0.344 | 0.345 | 0.368 | 0.360 | 0.367 | 0.354 |
| | 10 | 0.142 | 0.145 | 0.109 | 0.120 | 0.163 | 0.205 | 0.121 | 0.156 | 0.156 | | 0.895 | 0.264 | 0.274 | 0.342 | 0.316 | 0.313 | 0.324 | 0.318 | 0.342 | 0.335 | 0.342 | 0.329 |
| | 11 | 0.126 | 0.147 | 0.135 | 0.097 | 0.156 | 0.177 | 0.088 | 0.157 | 0.147 | 0.105 | | 0.300 | 0.314 | 0.365 | 0.339 | 0.347 | 0.348 | 0.342 | 0.365 | 0.358 | 0.365 | 0.352 |
| Genetic differences of largehead hairtail from the Atlantic | 12 | 0.699 | 0.681 | 0.750 | 0.733 | 0.720 | 0.685 | 0.676 | 0.699 | 0.732 | 0.736 | 0.700 | | 0.843 | 0.886 | 0.861 | 0.897 | 0.847 | 0.866 | 0.870 | 0.854 | 0.861 | 0.852 |
| | 13 | 0.708 | 0.640 | 0.733 | 0.727 | 0.709 | 0.655 | 0.678 | 0.686 | 0.702 | 0.726 | 0.686 | 0.157 | | 0.890 | 0.855 | 0.833 | 0.847 | 0.843 | 0.870 | 0.853 | 0.886 | 0.871 |
| | 14 | 0.659 | 0.586 | 0.682 | 0.669 | 0.656 | 0.608 | 0.632 | 0.620 | 0.633 | 0.658 | 0.635 | 0.114 | 0.110 | | 0.954 | 0.905 | 0.920 | 0.940 | 0.967 | 0.958 | 0.988 | 0.978 |
| | 15 | 0.683 | 0.615 | 0.709 | 0.695 | 0.682 | 0.608 | 0.656 | 0.645 | 0.659 | 0.684 | 0.661 | 0.139 | 0.145 | 0.046 | | 0.918 | 0.984 | 0.957 | 0.962 | 0.937 | 0.954 | 0.964 |
| | 16 | 0.656 | 0.619 | 0.696 | 0.687 | 0.671 | 0.623 | 0.630 | 0.637 | 0.665 | 0.687 | 0.653 | 0.103 | 0.167 | 0.095 | 0.082 | | 0.919 | 0.920 | 0.916 | 0.883 | 0.905 | 0.896 |
| | 17 | 0.657 | 0.604 | 0.698 | 0.686 | 0.672 | 0.625 | 0.630 | 0.637 | 0.656 | 0.676 | 0.652 | 0.153 | 0.153 | 0.080 | 0.016 | 0.081 | | 0.940 | 0.944 | 0.918 | 0.933 | 0.924 |
| | 18 | 0.681 | 0.608 | 0.705 | 0.691 | 0.677 | 0.630 | 0.652 | 0.656 | 0.655 | 0.682 | 0.658 | 0.134 | 0.157 | 0.060 | 0.043 | 0.080 | 0.060 | | 0.975 | 0.921 | 0.954 | 0.945 |
| | 19 | 0.658 | 0.585 | 0.682 | 0.668 | 0.654 | 0.607 | 0.629 | 0.633 | 0.632 | 0.658 | 0.658 | 0.130 | 0.130 | 0.033 | 0.038 | 0.084 | 0.056 | 0.025 | | 0.952 | 0.980 | 0.970 |
| | 20 | 0.666 | 0.593 | 0.689 | 0.676 | 0.663 | 0.615 | 0.639 | 0.627 | 0.640 | 0.665 | 0.642 | 0.146 | 0.147 | 0.042 | 0.063 | 0.117 | 0.082 | 0.079 | 0.048 | | 0.972 | 0.963 |
| | 21 | 0.659 | 0.586 | 0.682 | 0.669 | 0.656 | 0.608 | 0.632 | 0.620 | 0.633 | 0.658 | 0.635 | 0.139 | 0.114 | 0.013 | 0.046 | 0.095 | 0.067 | 0.046 | 0.020 | 0.028 | | 0.990 |
| | 22 | 0.657 | 0.601 | 0.697 | 0.682 | 0.670 | 0.621 | 0.644 | 0.633 | 0.646 | 0.671 | 0.648 | 0.148 | 0.129 | 0.022 | 0.036 | 0.104 | 0.076 | 0.055 | 0.030 | 0.037 | 0.010 | |

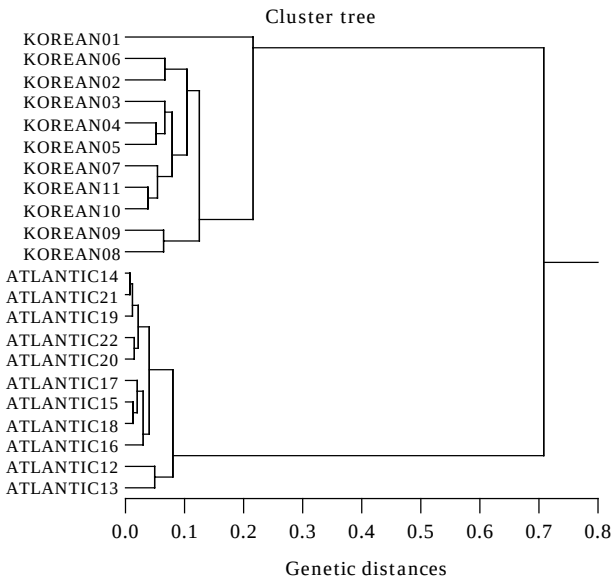


**Fig. 2.** Hierarchical dendrogram of genetic distances, obtained from two geographical populations of largehead hairtail (*T. lepturus*). The relatedness between different individuals in the largehead hairtail populations of Korea and the Atlantic Ocean was generated according to the bandsharing values and similarity matrix (see Table 2).

within the Atlantic population. The value between individuals no. 13 and no. 16 was 0.833, which was the lowest measured. Therefore, the bandsharing value of individuals within the Atlantic population was much higher than in the Korean population. The bandsharing value between individuals no. 02 and no. 19 was 0.415, which was the highest measured between the two geographical populations. The value between individuals no. 03 and no. 12 was 0.250, which was the lowest seen between the two geographical populations.

The genetic difference derived from the bandsharing values varied from 0.078 to 0.217 in the Korean population, and from 0.010 to 0.167 in the Atlantic population (Table 2). The average genetic difference was $0.141 \pm 0.004$ within the Korean population, and $0.084 \pm 0.006$ within the Atlantic population. Compared separately, the average genetic difference was greater in the Korean population than in the Atlantic population. The average genetic difference was $0.667 \pm 0.003$ between the two geographical largehead hairtail populations.

To obtain the dendrogram, we performed a hierarchical clustering analysis, employing the similarity matrix based on the bandsharing values and genetic differences (Fig. 2). The dendrogram obtained by the eight primers indicated two genetic clusters: cluster 1 (KOREAN 01～KOREAN 11), and cluster 2 (ATLANTIC 12～ATLANTIC 22). The genetic distance between the two geographical populations ranged from 0.007 to 0.708. The shortest genetic distance representing a significant molecular difference was between individuals ATLANTIC no. 14 and ATLANTIC no. 21 from the Atlantic Ocean in two geographical populations (0.007). Individual KOREAN no. 10 from Korea was genetically most closely related to KOREAN no. 11 in Korean population (genetic distance = 0.038). Ultimately, individual KOREAN no. 01 of the Korean population was most distantly related to ATLANTIC no. 16 of the Atlantic population (genetic distance = 0.708).

## Discussion

In general, RAPD-PCR is one of the fastest and simplest research methods for the identification of genetic differences and polymorphisms in various organisms. Also, RAPD-PCR analysis requires no prior knowledge of genomic DNA (Welsh *et al*., 1991; Mamuris *et al*., 1999; Iyengar *et al*., 2000; Klinbunga *et al*., 2000a; Liu and Cordes, 2004). Polymorphisms are determined by the banding patterns of primer-amplified products at specific positions (Smith *et al*., 1997; Tassanakajon *et al*., 1998; Yoon and Kim, 2001; Liu and Cordes, 2004). Accordingly, in this study, eight oligonucleotide primers generated a total of 947 fragments in the largehead hairtail population of Korea, and 642 in the Atlantic population, with a DNA fragment size ranging from 150 to 3,000 bp, as summarized in Table 1 and Fig. 1. Researchers have studied the sizes of DNA fragments in the RAPD-PCR profiles of barramundi (*Lates calcarifer*) (Partis and Wells, 1996), brown trout (*Salmo trutta*) (Cagigas *et al*., 1999), four species of the Mullidae family (Mamuris *et al*., 1999), crucian carp (*Carassius carassius*) (Yoon and Park, 2002), marsh clams (*Corbicula* sp.) (Yoon and Kim, 2003a) and yellowfin tuna (*Thunnus albacares*) (Diaz-Jaimes and Uribe-Alcocer, 2003). Particularly, the decamer primer, OPF-10 produced 11 amplified fragments, with sizes from 200 bp to 600 bp (Diaz-

Jaimes and Uribe-Alcocer, 2003). Six primers were also commonly used, generating a total of 602 scorable bands in catfish, and 195 in the bullhead population, respectively, ranging in DNA fragment size from less than approximately 100 bp, to more than 2,000 bp (Yoon and Kim, 2004a).

In this study, on average, a decamer primer generated 118.4 amplified products in the Korean largehead hairtail population. A RAPD primer generated an average of 13.9 amplified fragments per sample, ranging from 5.9 to 15.0 fragments in this population. 947 fragments were identified in the largehead hairtail population from Korea, and 642 in the largehead hairtail population from the Atlantic Ocean: 638 common fragments (67.4%) in the Korean population, with an average of 79.8 per primer, and 429 (66.8 %) in the Atlantic population, with an average of 53.6 per primer, as illustrated in Table 1. Seeing the number of common bands of total bands, as if individuals inbreeded (inbreeders) also raise together in the same rearing tank, a brood of young largehead hairtails would inhabit as a shoal of fish type through life.

In the Korean largehead hairtail population, the OPA-20 primer generated fragments of various sizes, ranging from 200 to 3,000 bp, as shown in Fig. 1B. In the Korean largehead hairtail, the decamer primer, OPB-14, generated 44 identical major and/or minor fragments, of 300 bp, 400 bp, 800 bp and 1,200 bp, respectively, as shown in Fig. 1C. The oligonucleotide primer OPA-20 also generated identical DNA fragments, of approximately 1,200 bp, 2,200 bp and 3,000 bp, in both largehead hairtail populations, as shown in Fig. 1B. The decamer primer OPD-16 also generated identical DNA fragments, of approximately 2,000 bp and 2,400 bp, in both largehead hairtail populations, as shown in Fig. 1G. The other primers also generated identically sized fragments in both largehead hairtail populations, as shown in Fig. 1 and Table 1. These results demonstrate evidence that exists genetically indigenous and identical character between two largehead hairtail groups.

The number of fragments generated per primer varied between 17 and 30, with a mean of 24.2 bands per individual and primer, in three endemic Spanish barbel species (*Barbus bocagei*, *B. graellsii* and *B. sclateri*) (Callejas and Ochando, 1998). It has also been reported that one primer generated 9 to 15 distinct bands in the black tiger shrimp (Tassanakajon *et al*., 1998). The number of scored bands varied from 7 to 12 per primer in four species of the Mullidae family (Mamuris *et al*., 1999). The primers generated 36, 32, and 24 bands in mud crabs from Eastern Thailand (genus *Scylla*) (Klinbunga *et al*., 2000b). 176 common fragments, at an average of 25.1 per primer, were observed in the Buan population, and 99 fragments, at an average of 14.1 per primer, were observed in the Geojedo population (Kim *et al*., 2004). Moreover, in the Atlantic largehead hairtail population, common banding patterns, corresponding to fragments of 400 bp, 420 bp, 550 bp, 650 bp, 1,000 bp, 1,600 bp and 2,000 bp, were generated by the decamer primer, URP-07, as shown in Fig. 1H. The banding patterns generated by the decamer primers OPA-07, OPB-17, and OPD-16 of the individual Korean largehead hairtail varied widely, as shown in Figs. 1A, 1E, and 1F. The banding patterns generated by the decamer primers OPA-20, OPB-18, and OPD-16 of the individual Atlantic largehead hairtail also varied widely, as shown in Figs. 1B, 1F, and 1G. The complexity of the banding patterns varied widely between primers and/or geographic locales. Generally, the size and number of fragments generated depends both on the nucleotide sequence of the primer used, and on the source of the template DNA, resulting in a genome-specific DNA fragment (Welsh and McClelland, 1990; Welsh *et al*., 1991).

Generally speaking, using numerous arbitrary primers, RAPD-PCR has been applied to identify specific/polymorphic markers particular to breed, line, species and geographical population, as well as genetic similarity/polymorphism/diversity in various organisms (Partis and Wells, 1996; McCormack *et al*., 2000; Klinbunga *et al*., 2000a; Diaz-Jaimes and Uribe-Alcocer, 2003). In the present study, 8 primers generated 76 polymorphic fragments (76/947 fragments, 8.0%) in the Korean largehead hairtail population, and 27 (27/642 fragments, 4.2%) in the Atlantic largehead hairtail population, as illustrated in Table 1. It has also been reported that the percentage of polymorphic bands obtained from five geographic populations in black tiger shrimp (*Penaeus monodon*) varied from 51.5 to 57.7% (Tassanakajon *et al*., 1998). Two primers yielded the highest levels of polymorphism, which was 88.9%, in the black tiger shrimp. The results of this analysis also illustrated that 22 out of 80 bands (27.5%) were monomorphic and 58 bands (72.5%) were polymorphic. Of the 46 polymorphic

fragments, only 3 allelic markers were private, distinguishing sample 1 from the rest, within and among four natural Spanish populations of brown trout (*Salmo trutta*) (Cagigas *et al*., 1999). Six primers produced 84 polymorphic bands, out of a total of 90 bands in the blacklip abalone (Huang *et al*., 2000). McCormack *et al*. (2000) reported that a total of 98 individuals were examined in two populations of *A. filiformis*, using these four primers. They reported that the banding patterns showed a high degree of variation, with individual organisms being clearly distinguishable from one another. All four primers generated 111 polymorphic DNA fragments from 70 individuals. The sum of the average polymorphic products, 73.7, was identified in the combination of the common carp and the Israeli carp (Yoon, 2001). Upon RAPD analysis of genetic differences and characteristics in wild and cultured crucian carp populations, the patterns of polymorphic fragments of 50 individuals in the wild population were reported to be different (Yoon and Park, 2002). Six primers generated 47 polymorphic fragments (24% of 195 fragments) in a bullhead population (Yoon and Kim, 2004a). Kim *et al*. (2004) reported 143 polymorphic fragments (29.7%) in the Buan oyster population and 60 (22.7%) in the Geojedo oyster population. Yoon and Kim (2004b) stated that the average of value of total polymorphic bands was approximately 9.08% in shortnecked clam (*Reditapes philippinarum*). Relatively speaking, these results demonstrate that the primers detected comparatively a small number of polymorphic fragments in largehead hairtail.

Here, we have identified 27 specific fragments in the Korean largehead hairtail population, generated by the 20-mer primer URP-07, as shown in Fig. 1H. Especially, two specific fragments of (lane 8) 800 bp and (lane 8) 900 bp specific fragments generated by this primer exhibited inter-individual-specific characteristics and DNA polymorphisms. We have also identified two specific fragments of (lane 13) 200 bp and (lane 13) 700 bp in the Atlantic largehead hairtail population, generated by the decamer primer OPB-17, as shown in Fig. 1E. In general, the polymorphic fragments generated by RAPD-PCR using arbitrary primers were suitable for the detection of genetic similarity/diversity/ polymorphisms among various organisms (Welsh *et al*., 1991; Liu *et al*., 1998; McCormack *et al*., 2000; Kim *et al*., 2004).

The 1,200 bp, 2,200 bp and 3,000 bp bands (33 common bands, 37.5%) produced by the primer OPA-20 were identified as being common to two largehead hairtail populations, as shown in Fig. 1B. Eleven identical fragments (14.3%) generated by the primer OPB-15 were also identified as being common to two populations. These population-related primers proved useful in the identification of species, populations and/or genera, resulting from variations in DNA polymorphisms among species/populations/genera (Yoon and Park, 2002; Yoon and Kim, 2003b; Yoon and Kim, 2004a). Especially, Liu *et al*. (1998) reported that population-related RAPD fragments were identified in the channel catfish (*Ictalurus punctatus*) and the blue catfish (*I. furcatus*), and also in their $F_1$, $F_2$ and backcross hybrids. It has been reported that the silver dory (*Cyttus australis*) has a major 460 bp fragment, and that the mirror dory (*Zenopsis nebulosis*) has a major 422 bp fragment (Partis and Wells, 1996). These major fragments revealed the characteristic profiles of fish species such as the john dory, silver dory and mirror dory. The RAPD-PCR method, using random primers, was applied to the identification of three endemic Spanish barbel species: *Barbus bocagei*, *B. graellsii* and *B. sclateri* (Callejas and Ochando, 1998). Results indicated that *Barbus bocagei* and *B. graellsii* were more closely related to each other than they were to *B. sclateri*.

In the present study, the bandsharing value, which is based on the presence or absence of amplified fragments, was utilized to calculate similarity indices, as illustrated in Table 2. Regarding individual results, individual largehead hairtail from the Atlantic Ocean exhibited higher bandsharing values than did fish from Korea. Our reported bandsharing values between the two geographical largehead hairtail populations are inconsistent with previously reported results (Yoon and Park, 2002). Other reports have indicated that the average bandsharing value obtained using five random primers was $0.40 \pm 0.05$ in the wild crucian carp population, and $0.69 \pm 0.08$ in the cultured crucian carp population. The average bandsharing value recorded in our study is higher than the average value between the common carp and Israeli carp species ($0.57 \pm 0.03$) (Yoon, 2001), the bullhead population ($0.504 \pm 0.115$) (Yoon and Kim, 2004a), and also between the two oyster populations ($0.282 \pm 0.008$) (Kim *et al*., 2004). However, the

average bandsharing value reported by our study is similar to the value reported for Spanish barbel species (0.71～0.81) (Callejas and Ochando, 1998).

The average genetic difference in the Korean largehead hairtail population was approximately 0.141±0.004, and was 0.084±0.006 in the Atlantic population. The average level of genetic difference in the Atlantic largehead hairtail population was lower than in the Korean population. This suggests that genetic variation in the Korean largehead hairtail population is more pronounced than in the Atlantic population. The average genetic difference level between the two largehead hairtail populations was approximately 0.660±0.003. The difference between the two largehead hairtail populations is statistically significant ($P < 0.001$). Accordingly, as stated above, RAPD-PCR analysis indicated that the largehead hairtail population from Korea was more genetically diverse than the Atlantic largehead hairtail population. Two populations were clearly distinguished, especially by their morphological characters such as long body type and large head size of this fish.

In this study, based on the similarity matrix generated by bandsharing values and genetic distances, hierarchical clustering analysis was performed in order to construct a dendrogram, as shown in Fig. 2. The dendrogram, generated by seven reliable primers, indicates two genetic clusters. Particularly, the longest genetic distance representing significant molecular differences, 0.708, was found to exist between individuals KOREAN no. 01 of Korea and ATLANTIC no. 16 of the Atlantic. Our cluster analysis revealed the patterns similar to those posited by Kim *et al*. (2004), Yoon and Kim (2004a) and Yoon and Kim (2004b). They reported that single linkage cluster analysis, which indicated four genetic groupings, and the dendrogram revealed close relationships between individual identities within two geographical populations.

Via the cluster analysis of genetic similarity values obtained from RAPD data, Callejas and Ochando (1998) indicated that Spanish barbel species (*Barbus bocagei* and *B. graellsii*) were more closely related to each other than to *B. sclateri*. As for interspecific similarity, the highest value was found to exist between *B. graellsii* and *B. bocagei* (0.4123); *B. sclateri* exhibited a 0.3827 coefficient of interspecific similarity with *B. graellsii*, and 0.3981 with *B. bocagei*. Nei's genetic distances varied from 0.327 to 0.655 in four species of the Mullidae family (Mamuris *et al*., 1999). The genetic distance ranged from 0.091 to 0.316, with an average of 0.160, within and among four natural Spanish populations of brown trout (*Salmo trutta*) (Cagigas *et al*., 1999). The principal aspect of the dendrogram was also a striking separation of sample 1 from the others, which were closely grouped.

In fish and invertebrates, cluster analysis of the pairwise population matrix, generated from RAPD data, showed that geographically close populations tended to cluster together in the blacklip abalone (Huang *et al*., 2000). Additional principal component analysis, again based on RAPD data, showed that the Point Cook population was clearly separated from the two other central populations. A neighbor-joining tree based on the genetic distances between populations, using the RAPD-PCR method, indicates the relationships of three mud crab species (Klinbunga *et al*., 2000b). This study showed that large genetic differences could be found between geographical populations within a species, as well as between species. The two phylogenetic trees resulting from neighbor-joining and parsimony analyses both showed the same topology in distinguishing Mullidae species (Mamuris *et al*., 1999). The main advantage of the RAPD technique over the two other methods was its largely superior discriminative ability. Phylogenetic relationships were assessed using the neighbor-joining and maximum parsimony methods in killifish, *Fundulus parvipinnis* (Bernardi and Talley, 2000). At a higher level, samples were partitioned into two major clades. These two clades were very robust (99 to 100% of bootstrap replicates) and genetically distant (average sequence divergence 5.8%). Phylogenetic relationships among 5 *Haliotis* species and one hybrid were conducted by calculation of the distance coefficient and construction of a phylogenetic tree based on RAPD data (Kim *et al*., 2000). These branched off into two clusters: cluster I was formed by *H. discus hannai, H. discus, H. gigantea, H. sieboldii*, and the hybrid, which was subsequently re-divided into two sub-clusters. Cluster II contained only *H. diversicolor aquatilis*. Ultimately, Kim *et al*. (2000) insisted that RAPD analysis constitutes a powerful tool for the elucidation of phylogenetic relationships, based on their analysis of 6 species of *Haliotis*. The dendrogram obtained from the Korean oyster popu-

lation by the four primers, indicates three genetic clusters (Kim *et al*., 2004). The genetic distance between the two geographic populations ranged from 0.039 to 0.284. The shortest genetic distance representing significant molecular differences, 0.080, was found to exist between individuals no. 09 and no. 07 from Buan.

The identification of the penaeid shrimp (*Penaeus chinensis*), bullhead (*Pseudobagrus fulvidraco*), and oyster (*Crassostrea gigas*) populations constituted a necessary step in the inception and development of invertebrate/teleost breeding programs (Yoon and Kim, 2003b; Yoon and Kim, 2004a; Kim *et al*., 2004). Molecular genetic markers, including, most notably, microsatellite loci, quantitative trait loci, and genomic mapping, will ultimately prove useful in the selection of broodstocks for multiple reproductive traits, or health- and production-related traits, in fishery science (Waldbieser and Wolters, 1999). The classification of geographical populations of largehead hairtail is based on morphological variations in the color, size, and type of fish. It is assumed that differences in such traits reflect distinct origins or genetic identity (Chenyambuga *et al*., 2004). It seems that there is a clear association between particular goat types and sub-regions of sub-Saharan Africa. As stated above, the potential of RAPD to identify diagnostic markers for breed, stock, species and population identification in teleosts (Partis and Wells, 1996; Iyengar *et al*., 2000; Diaz-Jaimes and Uribe-Alcocer, 2003; Yoon and Kim, 2004a; Siti Azizah *et al*., 2005), and in shellfish (Tassanakajon *et al*., 1998; Klinbunga *et al*., 2000b; Yoon and Kim, 2003b; Kim *et al*., 2004) has also been well established.

In our study, RAPD-PCR analysis has revealed a significant genetic distance between two population pairs. RAPD-PCR enabled us to detect the existence of population discrimination and genetic variation in largehead hairtail populations of Korea and the Atlantic Ocean. This confirms that the method is a suitable tool for DNA comparisons, both within and between individuals, species, and populations. The genetic identification and discrimination of aquaculture stocks is a fundamental requirement in any aquaculture program, whether for color pattern (in aquarium fish), fast-growth (food fish) or fish for restocking into their natural populations (Badarcki and Skibinski, 1994; Siti Azizah *et al*., 2005). Furthermore, basic knowledge of the DNA polymorphisms and molecular markers in largehead hairtail (*T. lepturus*) may contribute significantly to broodstock selection and selective fish-breeding programs. The extraordinarily unique gene pools exhibited by some samples (especially in the case of the photo in Fig. 1B) would require new conservation policies, such that many wilder Korean largehead hairtail populations could be maintained. Accordingly, further analysis with more individuals, primers, and species will be required to fully establish the specificity of loci to particular taxa, and subsequent inter-specific gene flow in the genus *Trichiurus*. Further sampling sites will also be necessary to more precisely determine the area in which the phylogeographic break occurs. Particularly, combined assessments of other research methods, including RFLP, AFLP, microsatellite, genetic sequencing and 2 or more different PCR-based approaches may be required for further investigations of bandmapping and genetic linkage maps.

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## RAPD-PCR 분석에 의해 결정된 갈치 (*Trichiurus lepturus*) 2 집단의 유전적 차이와 변이

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한국과 대서양산 갈치 (Trichiurus lepturus) 2지리적 집단으로부터 genomic DNA를 분리 추출하였다. 선택된 8개의 RAPD primer를 이용하여 common, polymorphic 그리고 specific fragment를 얻어냈다. 2지역으로부터 primer간 banding patterns 의 복잡성이 두드러지게 나타났다. DNA fragment 의 분자적 크기는 150 bp에서부터 3,000 bp까지 커다란 차이를 나타내었다. 본 연구에서 한국산 갈치 집단에서는 947개의 fragment가 나타났고, 대서양산 갈치 집단에서는 642개의 fragment 가 확인되었다. 또한 한국산 집단에서는 148개의 specific fragment (15.6%) 가 확인되었으며, 대서양산 갈치집단에서는 61개의 specific fragment (9.5%)가 발생되었다. 한국산 갈치집단에서는 638개의 common fragment가 나타났으며, 이는 primer 당 평균적으로 79.8개의 fragment로 확인되었다. 또한 대서양산 갈치집단에서는 429개의 common fragment 가 확인되었고, 평균해서 primer 당 53.6개의 common fragment 가 나타났다. 한국산 갈치집단과 대서양산 갈치집단의 polymorphic fragment는 각각 76개와 27개로 확인되었다. 모든 갈치시료의 평균적인 bandsharing value를 기초로 해서 한국산 갈치집단의 similarity matrix를 조사해 본 결과 0.784 로부터 0.922까지 나타났고, 대서양산 갈치집단의 값은 0.833로부터 0.990까지 확인되었다. 결과적으로 대서양산 갈치집단내 개체의 bandsharing value 평균값은 한국산 갈치집단의 평균값보다 높게 나타났다. 8개의 primer를 사용하여 얻어진 dendrogram은 cluster 1 (KOREAN 01～KOREAN 11) 및cluster 2 (ATLANTIC 12～ATLANTIC 22)와 같이 2개의 유전적 클러스터로 나뉘어졌다. 한국산 갈치집단내의 10번째 개체 (KOREAN no. 10)와 11번째 개체 (KOREAN no. 11) 사이가 가장 가까운 유전적 관계 (genetic distance = 0.038)를 나타내었다. 궁극적으로 볼 때 한국산 갈치집단의 1번째 (KOREAN no. 01)와 대서양산 갈치집단의 16번째 (ATLANTIC no. 16) 개체 사이가 가장 먼 유전적 거리 (genetic distance = 0.708)를 나타내었다.