Korean Journal of Malacology, Vol. 22(2): 97-108, December 30, 2006

Genetic Differences and Variation in Two Purple Washington Clam (*Saxidomus purpuratus*) Populations from South and North Korea

Jong-Man Yoon and Su-Young Park

Department of Aquatic Life Medicine, College of Ocean Science and Technology, Kunsan National University, Gunsan 573-701, Korea

ABSTRACT

Genomic DNA samples isolated from geographical purple Washington clam (*Saxidomus purpuratus*) were obtained from two different regions in Korean Peninsula: Gunsan (Gunsan population; GSP), and Haeju (Haeju population; HJP), a collection area in the vicinity of the West Sea. The seven arbitrarily primers, OPA-07, OPA-09, OPA-18, OPA-20, OPC-03, OPC-06 and OPC-09 were shown to generate the total loci, loci observed per primer, shared loci by each population, specific, and polymorphic loci which could be clearly scored. We also generated the unique shared loci to each population and shared loci by the two populations in purple Washington clam. The size of the DNA fragments also varied wildly, from 50 to 2,400 bp. Here, 304 total loci were identified in the GSP purple Washington clam population, and 282 in the HJP: 91 polymorphic loci (29.9%) in the GSP and 47 (16.7) in the HJP. 198 shared loci, with an average of 28.3 per primer, were observed in the GSP population. The decamer primer OPA-07 generated the shared loci by the two populations, approximately 1,000 bp, between the two *Saxidomus* populations. The oligonucleotide primer OPC-03 also generated the shared loci by the two populations, approximately 500 bp and 1,000 bp, in GSP population from Gunsan and HJP population from Haeju. The other primer, OPC-06 generated the shared loci by two *Gomphina* populations (approximately 400 bp). The dendrogram, generated by seven reliable primers, indicates three genetic clusters. The dendrogram obtained by the seven primers indicates three genetic clusters: cluster 1 (GUNSAN 01-GUNSAN 02), cluster 2 (GUNSAN 03-GUNSAN 11), and cluster 3 (HAEJU 12-HAEJU 22). The genetic distance between the two geographical populations ranged from 0.043 to 0.506. Especially, the longest genetic distance displaying significant molecular differences, 0.506, was found to exist between individuals GUNSAN no. 11 of Gunsan and HAEJU no. 17 of Haeju.

Keywords: Dendrogram, Genetic distance, Geographical population, *Saxidomus purpuratus*, Purple Washington clam.

Received November 11, 2006; Accepted December 8, 2006
Corresponding author: Yoon, Jong-Man
Tel: +82 (63) 469-1887 e-mail: : jmyoon@kunsan.ac.kr
1225-3480/22201

INTRODUCTION

Various analytical techniques have been applied to analyze the heredity of organisms such as morphological trait (Orozco-Castillo *et al.,* 1994), the allozyme differentiation (Kang *et al.,* 1996; Bartish *et al.,* 2000), and PCR-based molecular techniques including the restriction fragment length polymorphisms (RFLPs) (Kim *et al.,* 1997), the amplified fragment length polymorphisms (AFLPs) (Eujayl *et al.,* 1998), and the random amplified polymorphic DNAs (RAPD) (Partis and Wells, 1996; Callejas and Ochando, 1998; Esselman *et al.,* 2000; Kim *et al.,* 2000; Yoon and Kim, 2003b; Park *et al.,* 2005).

Although 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; McCormack *et al.,* 2000; Kim *et al.,* 2004). Many molecular/genetic studies have employed this technique, as RAPD-PCR is a reliable, easy, 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 advantage 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; Klinbunga *et al.,* 2000a).

Purple Washington clam (*Saxidomus purpuratus*), which belongs to family Veneridae, and the order Veneroida, constitutes an economically important aquacultural species. Purple Washington clam is widely distributed in the entirety of estuary flat, brackish-water habitats, a field of reeds and seawater areas of the West Sea and the southern Sea in Korean Peninsula, as well as in several regions of China and Japan. The clams inhabit in the estuary flats consisting of a lot of mud, sand and slime in the coastal tidal wetland where the freshwater is flowed temporarily. Like other clams basically, the rate at which the clam grows depends very much on water quality. The consumption of this species has also seen a considerable upshift as restaurants have begun to specialize in various clam recipes, including steamed stuffed clam in a pan with red pepper, boiled with vegetables, fried, *etc.*

In general, the color, size and type of the clam in this species vary according to environmental factors, such as sex, season, habitat, nutrition, life history, and so on. The environmental requirements and tolerances of the clam species from different geographic areas are currently unknown, as is clam species identification. As the clam preservation increases, the understanding of the genetics of this clam species becomes more necessary; to evaluate the potential genetic effects induced by clam artificial reproduction operations. Research in clam artificial production has progressed steadily in many aspects, over-catching, and water pollution by industries and city sewage.

The clams are silvery white and coarse in the shell surface under natural conditions. The ribs of the shell surface are compact and brownish-yellow. Generally, the color, type, and size of shell in this clam species vary according to habitat, water depth, nutrition, growth period, and other habitat factors. The environmental requirements and tolerances of clam from different geographic areas remain unknown. As the clam culture industry grows, however, little information currently exists regarding the genetics of Korean purple Washington clam.

The polymorphic and/or specific markers peculiar to species, genus or geographic population have all also been employed in the discrimination of individuals, species and populations, hybrid parentages and genetic diagnostics (Kang *et al.,* 1996; Partis and Wells, 1996; Smith *et al.,* 1997; Callejas and Ochando, 1998; Muchmore *et al.,* 1998; Yoon and Kim, 2004). The present study aims to determine the genetic distances and differences within and among purple Washington clam populations. In order to accomplish this, we performed clustering analyses of two purple Washington clam (*Saxidomus purpuratus*) populations from the Gunsan and Haeju regions of Korea. We also analyzed genetic variations and DNA polymorphisms of these Korean purple Washington clam populations.

MATERIALS AND METHODS

1. Sampling and genomic DNA extraction

Two geographical populations of purple Washington clam (*Saxidomus purpuratus*) were obtained from two different regions in Korea: Gunsan, in the vicinity of the West Sea, and Haeju, a collection area in the vicinity of the West Sea. Purple Washington clam muscle was collected in sterile tubes, immediately placed on ice, and stored at -40℃ until the genomic DNA extraction. RAPD-PCR analysis was performed on the muscle extracts from 22 individuals, using seven 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). After

several washings, lysis buffer I (155 mM NH_4Cl; 10 mM $KHCO_3$; 1 mM EDTA) was added to the samples, and the mixture tubes were gently inverted. The precipitates obtained were then centrifuged and resuspended in lysis buffer II (10 mM Tris-HCl, pH 8.0; 10 mM EDTA; 100 mM NaCl; 0.5% SDS), and 15 μl of proteinase K solution (10 mg/ml) was added. After incubation, we added 300 μl of 3 M NaCl, and gently pipetted for a few minutes. 600 μl of chloroform was then added to the mixture and inverted (no phenol). Ice-cold 70% ethanol was added, and then the samples were centrifuged at 19,621 g for 5 minutes to extract the DNA from the lysates. The DNA pellets were then incubation-dried for more than 10 hours, maintained at -40℃ 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 Coulter, Buckingham-shire, UK).

2. Decamer primers, molecular markers and amplification stipulations

The arbitrarily chosen primers were purchased from Operon Technologies, USA. Seven primers, OPA-07 (5'-GAAACGGGTG-3'), OPA-09 (5'-GGGTAACGCC -3'), OPA-18 (5'-AGGTGACCGT-3'), OPA-20 (5'-GTTGCGATCC-3'), OPC-03 (5'-GGGGGTCTTT-3'), OPC-06 (5'-GAACGGACTC-3') and OPC-09 (5'-CTCACCGTCC-3') were shown to generate the total loci, loci observed per primer, shared loci by each population, specific, and polymorphic loci which could be clearly scored. We also generated the unique shared loci to each population and shared loci by the two populations in purple Washington clam (*Saxidomus purpuratus*) from Gunsan and Haeju. RAPD analysis was performed using two Programmable DNA Thermal Cyclers (Perkin Elmer Cetus, Norwalk, CT, USA; MJ Research Inc., Waltham, MA, USA). This 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. DNA amplification was performed in 25 μl samples, which contained 10 ng of template DNA, 20 μl of premix (Super-Bio Co., Korea), and 1 unit of primer. 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). Bands were stained with ethidium bromide using 100 bp ladder (Bioneer Corp., Daejeon, Korea) as a DNA molecular weight marker. After electrophoresis, agarose gels were illuminated with ultraviolet rays, and then photographed using a Photoman direct copy system (PECA Products, Beloit, WI, USA).

3. Data 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 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 hierarchical clustering tree was analyzed by the similarity matrices to generate a dendrogram using pc-package program Systat version 10 (SPSS Inc., Chicago, IL, USA). Genetic differences and Euclidean genetic distances within and between populations were also calculated using the Systat hierarchical dendrogram program version 10. Descriptive statistics of Systat version 10 was also used to obtain other statistical results, including means, standard errors, and t-test scores.

RESULTS and DISCUSSION

1. RAPD-PCR variations

RAPD and/or RAPD-based techniques have been widely applied to the identification of genetic characteristics of diverse species of invertebrates and shellfish (Yoon and Kim, 2003a; Yoon and Kim, 2004; Kim *et al.,* 2004; Park *et al.,* 2005). Polymorphisms are determined by the banding patterns of primer-amplified products at specific positions (Smith *et al.,* 1997; Nozaki *et al.,* 2000; Yoon and Kim, 2001; Yoon, 2006). We used various random primers to determine the genetic variations of the purple Washington clam populations.

Genomic DNA was isolated from two geographical purple Washington clam populations in Gunsan and Haeju. The seven arbitrarily selected primers OPA-07, OPA-09, OPA-18, OPA-20, OPC-03, OPC-06 and OPC-09 were found to generate the total, average, shared, specific, and polymorphic loci (Tables 1 and 2). The size of the DNA fragments also varied notably, from 50 to 2,400 bp, as shown in Fig. 1. The complexity of the banding patterns varied dramatically between the primers from the two locations (Fig. 1). All examined primers generated a total of 304 loci scored from the GSP population, while a total of 282 loci were generated from the HJP population (Table 2).

We assessed genetic variation in the GSP purple Washington clam population first. Primer OPA-07 generated loci ranging from 320 to 1,200 bp (Fig. 1A). This primer detected 11 major shared loci of size 1,000 bp, which were identical in all samples, as illustrated in Table 1. Five specific minor loci, which were at approximately 320 bp, 500 bp, 900 bp and 1,200 bp in size, were also detected. This primer produced the least of loci (a total of 13), in comparison to the other primers used, with an average of 1.2, as illustrated in Table 1. This primer, interestingly enough, generated a single monomorphic locus (lanes 1, 2, 3, 4, 5, 6, 7, 8 and 9). The 22 loci obtained with the decamer primer OPA-09, approximately 500 bp and 600 bp in size, were observed in all samples (Fig. 1B). This primer detected four specific and nine polymorphic major and/or minor loci, which were approximately 72 bp and larger than 580 bp, respectively. The primer OPA-18 generated 33 shared loci in all samples, of approximately 500 bp, 600 bp, and 700 bp in size (Fig. 1C). Interestingly, the 11 unique shared loci to each population that established population identity were 700 bp, as illustrated in Table 2. This primer detected seven specific major and/or minor loci that identified

Table 1. The total, average, shared, specific, and polymorphic loci generated by RAPD analysis using seven random primers in purple Washington clam (*Saxidomus purpuratus*) from Gunsan and Haeju.

Item	No. of average loci per lane		No. of shared loci		No. of specific loci		No. of polymorphic loci	
Primer	Gunsan	Haeju	Gunsan	Haeju	Gunsan	Haeju	Gunsan	Haeju
OPA-07	1.2 (13)	2.2 (24)	11	11	5	2	0	0
OPA-09	3.4 (37)	2.3 (25)	22	0	4	9	9	2
OPA-18	4.2 (46)	3.7 (41)	33	22	7	7	0	1
OPA-20	6.3 (69)	3.6 (40)	22	22	15	9	2	6
OPC-03	4.2 (46)	4.3 (47)	22	33	2	6	8	0
OPC-06	2.7 (30)	4.1 (45)	22	22	9	4	0	3
OPC-09	5.8 (63)	5.5 (60)	0	22	23	12	0	2
Total no.	27.6 (304)	25.6 (282)	198	176	65	49	19	14
Average no. per primer	43.4	40.3	28.3	25.1	9.3	7	2.7	2

The total number of loci generated by a primer in purple Washington clam obtained from Gunsan and Haeju is shown in parentheses.

individuals. The primer OPA-20 detected 22 major and/or minor shared loci of sizes 150 and 250 bp, in all samples (Table 1) (Fig. 1D). The primer generated the most of loci, a total of 69, although the average was 6.3, as illustrated in Table 1. The 15 specific loci generated by this primer exhibited inter-individual-specific characteristics, thus revealing DNA polymorphisms. Interestingly, two specific loci that established the identity of two individuals were smaller than 250 bp. The random primer OPC-03 detected 22 major and/or minor shared loci of 500 bp and 1,000 bp in all samples (Fig. 1E). This primer, interestingly enough, generated a major specific locus approximately 550 bp (lane 9) and minor about 300 bp (lane 9). Eight polymorphic loci were observed in 900 bp (lanes 5, 7, 8, 10 and 11) and in 1,200 bp (lanes 8, 10 and 11). The random primer OPC-06 generated 22 major and/or minor shared loci of approximately 50 bp and 400 bp each (Fig. 1F). The primer generated these minor specific loci: 150 bp (lanes 7 and 9), 200 bp (lanes 6, 7, 8, 9, 11) and 300 bp (lanes 7 and 11). The primer OPC-09 produced an average of 5.8 loci, and a total of 63 loci (Fig. 1G). 23 specific and 12 polymorphic RAPD loci were also observed in this population. High degrees of RAPD variation were observed in the banding patterns generated by this primer, ranging from 100 bp to 2,400 bp, in comparison to the other primers used.

Moreover, in the HJP purple Washington clam population, genetic variation was detected in the banding pattern generated by decamer OPA-07, which ranged from approximately 500 bp to 1,600 bp, as illustrated in Table 1 (Fig. 1A). This primer generated 24 loci, with an average of 2.2. The 11 shared loci, of approximately 1,000 bp, represented the geographical population: these 11 loci were detected in all of the purple Washington clams obtained from the Haeju population. RAPD variation was observed in the banding patterns, ranging from 180 bp to 600 bp, and was generated by the decamer primer, OPA-09 (Fig. 1B). The only single locus was also approximately 200 bp (lane 12). Nine specific minor loci, which were at approximately 180 bp, 200 bp, 250 bp and 450 bp in size, were also detected. This decamer primer generated two minor polymorphic loci, of approximately 450 bp in size (lanes 18 and 22). However, shared loci that identified populations and/or species were not identified here. A high degree of RAPD variation was observed in the banding patterns, ranging from 72 to 580 bp, and was generated by the decamer primer, OPA-18. The decamer primer, OPA-18, detected 22 shared loci, of 500 bp and 650 bp (Fig. 1C). Especially, two specific loci were identified approximately 300 bp and 350 bp (lanes 18 and 22). RAPD-PCR variation was identified in all banding patterns, ranging from approximately 300 bp to 1,200 bp, and generated by the decamer primer, OPA-20 (Fig. 1D). The 22 shared loci generated by the decamer primer, were approximately 600 bp and 700 bp, and were found to be identical. Nine specific RAPD fragments were detected in this HJP population. The primer, OPC-03, produced an average of 4.3 loci, and a total of 47 loci, in comparison to the other primers used (Fig. 1E). The twenty-two shared loci, of 500 bp and 1,000 bp, were generated by this primer, and subsequently analyzed. Six specific loci were observed in 1,200 bp (lanes 15, 16, 18, 20, 21 and 22). This primer generated 45 loci, with an average of 4.1 (Fig. 1F). A high degree of RAPD variation was observed in the banding patterns generated by the primer, OPC-09, ranging from 150 bp to 2,400 bp (Fig. 1G). This decamer primer generated the most loci (a total of 60), with an average of 5.5, as illustrated in Table 1. The twenty-two shared loci were identified, which established the identifications for populations and/or specie. This primer, interestingly enough, generated specific loci approximately 100 bp (lanes 18 and 21), 150 bp (lanes 16, 19 and 20), 200 bp (lanes 16, 19 and 20), 450 bp (lanes 16, 19 and 20) and 800 bp (lane 21). Two polymorphic loci were observed in 200 bp (lane 16) and in 320 bp (lane 15).

Researchers have studied the sizes of DNA fragments in the RAPD-PCR profiles of five species of Eastern Pacific abalone (genus *Haliotis*) (Muchmore *et al.,* 1998), black tiger shrimp (*Penaeus monodon*) (Tassanakajon *et al.,* 1998), the brittle star (*Amphiura filiformis*) (McCormack *et al.,* 2000), marsh clams

from Gochang (*Corbicula* spp) (Yoon and Kim, 2003a) and cultured and wild shrimp populations (Yoon and Kim, 2003b). It has also been reported that one primer generated nine to 15 distinct bands in the black tiger shrimp (Tassanakajon *et al.*, 1998). The primers generated 36, 32, and 24 bands in mud crabs

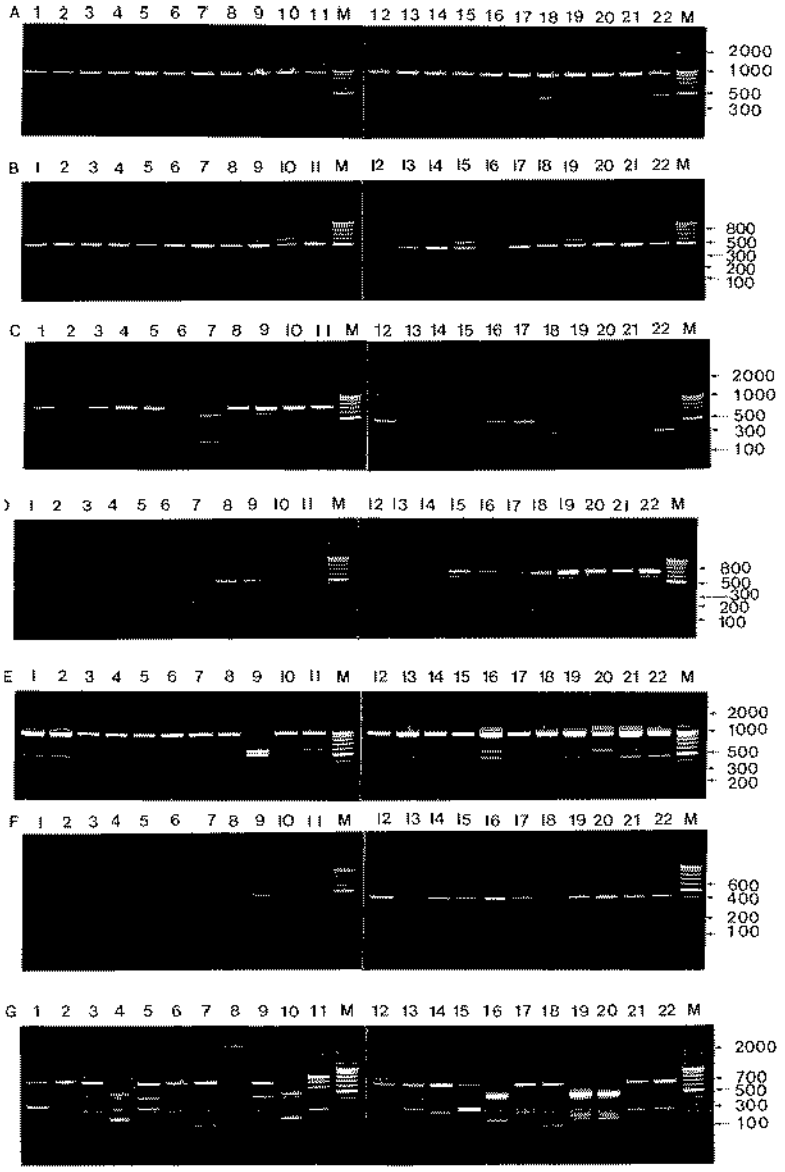

Fig. 1. RAPD-PCR-generated electrophoretic profiles of individual purple Washington clam (*Saxidomus purpuratus*). DNA isolated from Gunsan (lane 1-11) in the West Sea and Haeju of North Korea (lane 12-22) were amplified by random primers OPA-07 (A), OPA-09 (B), OPA-18 (C), OPA-20 (D), OPC-03 (E), OPC-06 (F) and OPC-09 (G). 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 marker.

from Eastern Thailand (genus *Scylla*) (Klinbunga *et al.,* 2000b). One hundred seventy-six 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). Genomic DNA samples isolated from *Fenneropenaeus chinensis* (fleshy prawn; FP) and *Palaemon gravieri* (Chinese ditch prawn; CDP) collected in the West Sea, off the Korean Peninsula, at Buan, were PCR-amplified repeatedly (Yoon and Kim, 2005). The sizes of the DNA fragments generated by seven different primers varied from 50 bp to 1,600 bp. We identified 358 fragments for the FP species and 301 fragments for the CDP species. There were 18 polymorphic fragments (5.03%) for the FP species and 12 (3.99%) for the CDP species. In total, 66 common fragments (average of 9.4 fragments per primer) were observed for the FP species and 44 fragments (average of 6.3 fragments per primer) were observed for the CDP species. The numbers of specific fragments seen for the FP species and CDP species were 38 and 47, respectively. The complexity of the banding patterns varied widely between primers, populations, species 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).

2. Variation within and between populations, and genetic distances

Here, a total of 304 loci were identified in the GSP purple Washington clam population, and 282 in the HJP: 91 polymorphic loci (29.9%) in the GSP and 47 (16.7) in the HJP (Table 1). 198 shared loci, with an average of 28.3 per primer, were observed in the GSP population. 176 shared loci, with an average of 25.1 per primer, were identified in the HJP. The numbers of specific loci in the GSP and HJP were 44 and 75, respectively. The decamer primer, OPA-07, generated shared loci by the two populations, of approximately 1,000 bp, respectively, in both the GSP and HJP populations (Fig. 1A). The oligonucleotide decamer primer, OPC-03, also generated shared loci by the two populations, of approximately 1,000 bp, in the Gunsan and Haeju purple Washington clam populations (Fig. 1E). The number of unique shared loci to each population and number of shared loci by the two populations were generated by RAPD analysis using seven random primers in *Saxidomus* populations from Gunsan and Haeju, respectively, as shown in Table 2. Ninety-nine unique shared loci to each population, with an average of 14.14 per primer, were observed in GSP population from Gunsan. In GSP population from Gunsan, the oligonucleotide decamer primer OPA-09 generated 22 unique shared loci to each population, 500 bp and 600 bp, respectively, as shown in Fig. 1B. Sixty-six unique shared loci, with an average of 9.43 per primer, were identified in the HJP population from Haeju. Especially, 55 numbers of shared loci by the two populations, with an average of 7.86 per primer, were observed in the two *Saxidomus* populations. The decamer primer OPA-07 generated the shared loci by the two populations, approximately 1,000 bp, between the two *Saxidomus* populations (Fig. 1A, Table 2). The oligonucleotide primer OPC-03

Table 2. The number of unique shared loci to each population and number of shared loci by the two populations generated by RAPD analysis using seven random primers in purple Washington clam (*Saxidomus purpuratus*) from Gunsan and Haeju.

Item	No. of unique shared loci to each population		
Primer \ Population	Gunsan	Haeju	Two localities
OPA-07	0	0	11
OPA-09	22	0	0
OPA-18	11	0	11
OPA-20	22	22	0
OPC-03	22	11	22
OPC-06	22	11	11
OPC-09	0	22	0
Total no.	99	66	55
Average no. per primer	14.14	9.43	7.86

also generated the shared loci by the two populations, approximately 500 bp and 1,000 bp, in GSP population from Gunsan and HJP population from Haeju, as shown in Fig. 1E. The other primer, OPC-06 generated the shared loci by two *Gomphina* populations (approximately 400 bp). The result indicates that the genome sizes of GSP population from Gunsan are similar to those in HJP population of Haeju. The results demonstrate also that GSP population from Gunsan is genetically similar to HJP population of Haeju.

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. 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 *Amphiura 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. 481 fragments were identified in an oyster population from Buan, and 264 were identified in an oyster population from Geojedo in Korea: 143 polymorphic fragments (29.7%) in the Buan population, and 60 (22.7%) in the Geojedo population (Kim *et al.,* 2004). Generally speaking, using numerous arbitrary primers, RAPD-PCR has

Table 3. Similarity matrix, including bandsharing values and genetic differences, calculated using Nei and Li's index, of the similarity of purple Washington clam (*Saxidomus purpuratus*) from Gunsan and Haeju. Bandsharing values of purple Washington clam from two regions are above the diagonal and genetic differences are below the diagonal.

		Bandsharing values of purple Washington clam from Gunsan											Bandsharing values of purple Washington clam from Haeju										
		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 purple Washington clam from Gunsan	1		0.733	0.753	0.797	0.857	0.863	0.84	0.721	0.664	0.728	0.715	0.632	0.604	0.711	0.772	0.494	0.678	0.675	0.599	0.622	0.576	0.645
	2	0.267		0.67	0.658	0.654	0.696	0.744	0.554	0.502	0.556	0.561	0.457	0.512	0.633	0.644	0.388	0.593	0.567	0.576	0.543	0.577	0.625
	3	0.247	0.33		0.799	0.841	0.864	0.775	0.678	0.68	0.733	0.719	0.672	0.604	0.611	0.66	0.514	0.622	0.6	0.594	0.535	0.558	0.674
	4	0.203	0.342	0.201		0.802	0.769	0.72	0.631	0.631	0.785	0.692	0.608	0.572	0.614	0.532	0.549	0.617	0.65	0.629	0.634	0.57	0.735
	5	0.143	0.346	0.159	0.198		0.823	0.847	0.673	0.685	0.752	0.693	0.723	0.619	0.623	0.602	0.614	0.695	0.608	0.66	0.678	0.606	0.7
	6	0.137	0.304	0.136	0.231	0.177		0.768	0.657	0.642	0.705	0.673	0.663	0.622	0.666	0.671	0.59	0.672	0.674	0.705	0.635	0.612	0.668
	7	0.16	0.256	0.225	0.28	0.153	0.232		0.691	0.689	0.742	0.704	0.591	0.618	0.636	0.676	0.575	0.636	0.652	0.672	0.654	0.664	0.727
	8	0.279	0.446	0.322	0.369	0.327	0.343	0.309		0.646	0.679	0.68	0.626	0.619	0.648	0.607	0.609	0.623	0.664	0.621	0.685	0.65	0.631
	9	0.336	0.498	0.32	0.369	0.315	0.358	0.311	0.354		0.723	0.627	0.534	0.52	0.561	0.502	0.467	0.568	0.642	0.539	0.574	0.518	0.575
	10	0.272	0.444	0.267	0.215	0.248	0.295	0.258	0.321	0.277		0.761	0.557	0.611	0.587	0.616	0.542	0.681	0.697	0.632	0.675	0.575	0.735
	11	0.285	0.439	0.281	0.308	0.307	0.327	0.296	0.32	0.373	0.239		0.536	0.592	0.599	0.605	0.483	0.64	0.672	0.585	0.62	0.602	0.685
Genetic differences of purple Washington clam from Haeju	12	0.368	0.543	0.328	0.392	0.277	0.337	0.409	0.374	0.466	0.443	0.464		0.887	0.77	0.677	0.796	0.72	0.628	0.541	0.563	0.73	0.65
	13	0.396	0.488	0.396	0.428	0.381	0.378	0.382	0.381	0.48	0.389	0.408	0.113		0.878	0.809	0.81	0.838	0.726	0.652	0.668	0.818	0.668
	14	0.289	0.367	0.389	0.386	0.377	0.334	0.364	0.352	0.439	0.413	0.401	0.23	0.122		0.852	0.706	0.897	0.821	0.76	0.782	0.749	0.684
	15	0.228	0.356	0.34	0.468	0.398	0.334	0.324	0.393	0.498	0.384	0.395	0.323	0.191	0.148		0.638	0.749	0.802	0.7	0.709	0.705	0.686
	16	0.506	0.612	0.486	0.451	0.386	0.41	0.364	0.391	0.533	0.458	0.517	0.204	0.19	0.294	0.362		0.737	0.55	0.665	0.724	0.781	0.578
	17	0.322	0.407	0.378	0.383	0.305	0.328	0.364	0.377	0.432	0.319	0.36	0.28	0.162	0.103	0.251	0.263		0.779	0.748	0.776	0.737	0.694
	18	0.325	0.433	0.4	0.35	0.392	0.326	0.348	0.336	0.358	0.303	0.328	0.372	0.274	0.179	0.198	0.45	0.221		0.752	0.69	0.685	0.765
	19	0.401	0.424	0.406	0.371	0.34	0.295	0.328	0.379	0.461	0.368	0.401	0.459	0.348	0.24	0.3	0.335	0.252	0.248		0.922	0.78	0.783
	20	0.378	0.457	0.465	0.366	0.322	0.365	0.346	0.315	0.426	0.325	0.38	0.437	0.332	0.218	0.291	0.276	0.224	0.31	0.078		0.72	0.663
	21	0.424	0.423	0.442	0.43	0.394	0.388	0.336	0.35	0.482	0.425	0.398	0.27	0.182	0.251	0.295	0.219	0.263	0.315	0.22	0.28		0.738
	22	0.355	0.375	0.326	0.265	0.3	0.332	0.273	0.369	0.425	0.265	0.315	0.35	0.332	0.316	0.314	0.422	0.306	0.235	0.217	0.337	0.262	

been applied to identify polymorphic/specific markers particular to breed, line, species and geographical population, as well as genetic polymorphism/diversity/similarity in various organisms (Partis and Wells, 1996; Callejas and Ochando, 1998; Huang *et al.,* 2000; Klinbunga *et al.,* 2000a; Yoon, 2006).

Based on the average bandsharing values of all samples, the similarity matrix ranged from 0.502 to 0.864 in the GSP purple Washington clam population, and from 0.541 to 0.922 in the HJP population (Table 3). The average bandsharing value was 0.714 ± 0.011 within the GSP population, and 0.733 ± 0.011 within the HJP population, respectively. The average bandsharing value between the two geographical purple Washington clam populations was 0.615 ± 0.006, ranging from 0.388 to 0.772. The bandsharing value between individuals no. 03 and no. 06 was 0.864, which was the highest value identified within the GSP population. The bandsharing value between individuals' no. 02 and no 09 was 0.502, which was the lowest observed. The bandsharing value between no. 19 and no. 20 was 0.922, which was the highest value observed within the HJP population. Therefore, the bandsharing value of individuals within the HJP population was more or less higher than in the GSP population. The value between individuals' no. 2 and no. 16 was 0.388, which was the lowest measured. The bandsharing value between individuals' no. 01 and no. 15 was 0.772, which was the highest measured between the two geographical populations. The value between individuals' no. 02 and no. 16 was 0.388, which was the lowest between the two geographical populations.

The difference between the two purple Washington clam populations is statistically significant ($p < 0.01$). Accordingly, as stated above, RAPD-PCR analysis indicated that the purple Washington clam population from Gunsan was more genetically diverse than the HJP clam population. The bandsharing values between the two geographical purple Washington clam populations are consistent with previously reported result (Yoon and Kim, 2003a). They reported that the bandsharing value in marsh clam population of Gochang ranged from 0.61 to 0.82, with the average of 0.73 ± 0.03 and in Chinese marsh clam population from 0.55 to 0.87, with the average of 0.75 ± 0.04, as calculated by bandsharing analysis. The average bandsharing value recorded in our study is higher than the average value between the freshwater crab species (0.587 ± 0.012) and swimming crab (0.508 ± 0.016) (Yoon, 2006), and also between the two oyster populations (0.282 ± 0.008) (Kim *et al.,* 2004).

In the present study, based on the similarity matrix generated by bandsharing values and genetic distances, hierarchical clustering analysis was performed in order to obtain a dendrogram, as shown in Fig. 2. The dendrogram, generated by seven reliable primers, indicates three genetic clusters. The dendrogram obtained by the seven primers indicates three genetic clusters: cluster 1 (GUNSAN 01-GUNSAN 02), cluster 2 (GUNSAN 03-GUNSAN 11), and cluster 3 (HAEJU 12-HAEJU 22). The genetic distance between the two geographical

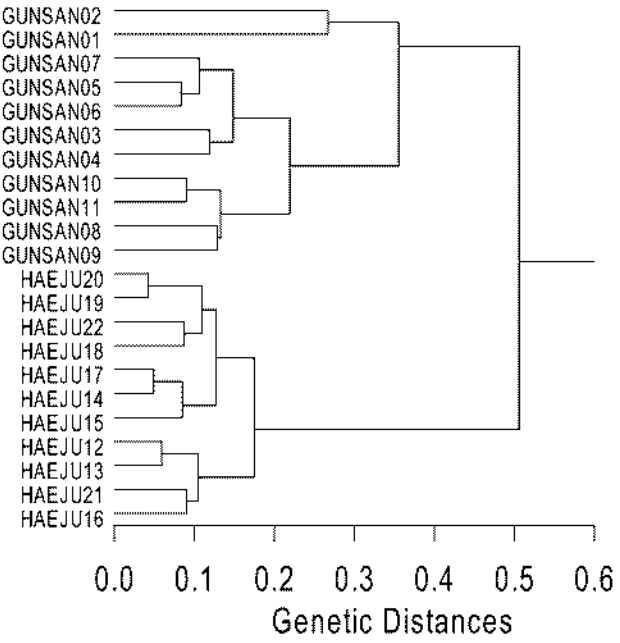

Fig. 2. Hierarchical dendrogram of genetic distances, obtained from two geographical populations of purple Washington clam (*Saxidomus purpuratus*). The relatedness between different individuals in the purple Washington clam populations of Gunsan and Haeju, a collection site of North Korea were generated according to the bandsharing values and similarity matrix (see Table 3).

populations ranged from 0.043 to 0.506. The lowest value of genetic distance displaying significant molecular difference was the one between individuals HAEJU no. 19 and HAEJU no. 20 from Haeju (genetic distance = 0.043). Especially, the highest value of genetic distance displaying significant molecular differences, 0.506, was found between individuals GUNSAN no. 11 of Gunsan and HAEJU no. 17 of Haeju.

In 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, based on RAPD data, showed that the Point Cook population was clearly separated from the two other central populations. Phylogenetic relationships among five *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*. 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), showing that large genetic differences could be found between geographical populations within a species, as well as between species. The dendrogram obtained from the Korean oyster population 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 lowest genetic distance representing significant molecular differences, 0.080, was found to exist between individuals' no. 09 and no. 07 from Buan. The genetic distance between the two prawn species (*Fenneropenaeus chinensis* and *Palaemon gravieri*) ranged from 0.071 to 0.642 (Yoon and Kim, 2005). The dendrogram obtained using the data from the seven primers indicated seven genetic clusters: cluster 1, FLESHY 01, 02, 03, and 04; cluster 2, FLESHY 05, 06, and 07; cluster 3, FLESHY 08, 09, 10, and 11; cluster 4, DITCH 13, 14, 16, and 18; cluster 5, DITCH 12, 15, and 17; cluster 6, DITCH 19, 20, and 21; and cluster 7, DITCH 22. Thus, RAPD-PCR analysis revealed a significant genetic distance between the two prawn species.

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 purple Washington clam is based on morphological variations in shell type, shell length and shell color. It is assumed that differences in such traits reflect distinct origins or genetic identity (Chenyambuga *et al.,* 2004). The genetic variation and molecular discrimination of Korean arkshell *Scapharca* species were investigated via mitochondrial COI gene sequences and PCR-RFLP analyses (Lee and Kim, 2003). Two distinct fragments were produced in *S. broughtonii* and *S. subcrenatta*. It was supposed that this difference in phylogenetic grouping might result from differences in the analyzed regions of the COI genes. Two species were clearly distinguished, especially by their morphological characters. In our study, RAPD-PCR analysis has revealed a significant genetic distance between two purple Washington clam populations. The extraordinarily peculiar gene pools exhibited by some samples (especially in the case of the photo in Fig. 1E and 1G) would require new conservation policies, such that many wilder Korean purple Washington clam populations could be preserved. RAPD-PCR enabled us to detect the existence of population discrimination and genetic variation in purple Washington clam populations of Gunsan and Haeju.

As it is shown in the present paper, the potential of RAPD to identify diagnostic markers for breed, stock, species and population identification in shellfish

(Tassanakajon *et al.,* 1998; Klinbunga *et al.,* 2000b; Yoon and Kim, 2003b; Kim *et al.,* 2004) has also been well established. Furthermore, basic knowledge of the DNA polymorphisms and molecular markers in purple Washington clam (*Saxidomus purpuratus*) may contribute significantly to broodstock selection and selective shellfish-breeding programs. Further sampling sites will be necessary to more precisely determine the area in which the phylogeographic break occurs. Additional statistical analytical methods based on RAPD-PCR data will also be necessary for the acquisition of more profound and further assessments of genetic differences and variations among geographical purple Washington clam populations.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support given by Kunsan National University's Academic Support Program in the year of 2005. Particular thanks go to our laboratory colleagues, for their assistance in sample collection, and their help with the RAPD-PCR techniques. The authors also would like to thank the referees who assisted us with thorough and far-reaching criticisms.

REFERENCES

Bartish, I.V., Garkava, L.P., Rumpunen, K. and Nybom, H. (2000) Phylogenetic relationships and differentiation among and within populations of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPDs and isozymes. *Theoretical and Applied Genetics,* **101**: 554-563.

Callejas, C. and Ochando, M.D. (1998) Identification of Spanish barbel species using the RAPD technique. *Journal of Fish Biology,* **53**: 208-215.

Chenyambuga, S.W., Hanotte, O., Hirbo, J., Watts, P.C., Kemp, S.J., Kifaro, G.C., Gwakisa, P.S., Petersen, P.H. and Rege, J.E.O. (2004) Genetic characterization of indigenous goats of sub-Saharan Africa using microsatellite DNA markers. *Asian-Australasian Journal of Animal Sciences,* **17**: 445-452.

Esselman, E.J., Crawford, D.J., Brauner, S., Stuessy, T.F., Anderson, G.J. and Silva, O.M. (2000) RAPD marker diversity within and divergence among species of *Dendroseris* (Asteraceae: Lactuceae). *American Journal of Botany,* **87**: 591-596.

Eujayl, I., Baum, M., Powell, W., Erskine, W. and Pehu, E. (1998) A genetic linkage map of lentil (*Lens* sp.) based on RAPD and AFLP markers using recombinant inbred lines. *Theoretical and Applied Genetics,* **97**: 83-89.

Huang, B.X., Peakall, R. and Hanna, P.J. (2000) Analysis of genetic structure of blacklip abalone (*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. *Marine Biology,* **136**: 207-216.

Iyengar, A., Piyapattanakorn, S., Dtone, D.M., Heipel, D.A., Howell, B.R., Baynes, S.M. and Maclean, N. (2000). Identification of microsatellite repeats in turbot (*Scophthalmus maximus*) and dover sole (Solea solea) using a RAPD-based technique: Characterization of microsatellite markers in dover sole. *Marine Biotechnology,* **2**: 49-56.

Jeffreys, A.J. and Morton, D.B. (1987) DNA fingerprints of dogs and cats. *Animal Genetics,* **18**: 1-15.

Kang, Y.J., Kim, Y.H., Hong, Y.K., Park, J.Y. and Park, K.Y. (1996) A population genetic analysis of the common squid, *Todarodes pacificus* Steenstrup in the Korean waters. *Journal of Korean Fisheries Society,* **29**(3): 320-331.

Kim, L.G., Jin, H.J., Kim, Y.S., Park, J.Y., Nam, K.W. and Hong, Y.K. (1997) Discrimination of two red algae *Acrosorium polyneurum* and *A. yendoi* using polymerase chain reaction technique. *Journal of Korean Fisheries Society,* **30**: 574-584.

Kim, J.Y., Park, C.Y. and Yoon, J.M. (2004) Genetic differences and DNA polymorphism in oyster (*Crassostrea* spp.) analysed by RAPD-PCR. *Korean Journal of Genetics,* **26**: 123-134.

Kim, S.K., Jung, Y.H., Han, S.H., Oh, Y.S., Ko, M.H. and Oh, M.Y. (2000) Phylogenetic relationship among *Haliotis* spp. distributed in Korea by the RAPD analysis. *Korean Journal of Genetics,* **22**: 43-49.

Klinbunga, S., Ampayup, P., Tassanakajon, A., Jarayabhand, P. and Yoosukh, W. (2000a) Development of species-specific markers of the tropical oyster (*Crassostrea belcheri*) in Thailand. *Marine Biotechnology,* **2**: 476-484.

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

Koh, M.C., Lim, C.H., Chua, S.B., Chew, S.T. and Phang, S.T. (1998) Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. *Meat Science,* **48**: 275-285.

Lee, S.Y. and Kim, S.H. (2003) Genetic variation and discrimination of Korean arkshell *Scapharca* species (Bivalvia, Arcoida) based on mitochondrial COI gene sequences and PCR-RFLP. *Korean Journal of Genetics*, **25**: 309-315.

Liu, Z., Li, P., Argue, B.J. and Dunham, R.A. (1998) Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their F1, F2 and backcross hybrids. *Animal*

Genetics, **29**: 58-62.

McCormack, G.C., Powell, R. and Keegan, B. (2000) Comparative analysis of two populations of the brittle star Amphiura filiformis (Echinodermata: Ophiuroidae) with different life history strategies using RAPD markers. Marine Biotechnology, 2: 100-106.

Muchmore, M.E., Moy, G.W., Swanson, W.J. and Vacquier, V.D. (1998) Direct sequencing of genomic DNA for characterization of a satellite DNA in five species of Eastern Pacific abalone. *Molecular Marine Biology and Biotechnology,* **7**(1): 1-6.

Nozaki, T., Mishiba, K., Mii, M. and Koba, T. (2000) Construction of synteny groups of *Brassica alboglabra* by RAPD markers and detection of chromosome aberrations and distorted transmission under the genetic background of *B. campestris*. *Theoretical and Applied Genetics,* **101**: 538-546.

Orozco-Castillo, C., Chalmers, K.J., Waugh, R. and Powell, W. (1994) Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. *Theoretical and Applied Genetics,* **87**, 934-940.

Park, S.Y., Park, J.S. and Yoon, J.M. (2005) Genetic difference and variation in slipper lobster (*Ibacus ciliatus*) and deep sea lobster (*Puerulus sewelli*) throughout its distribution range determined by RAPD-PCR Analysis. *Korean Journal of Genetics,* **27**: 307-317.

Partis, L. and Wells, R.J. (1996) Identification of fish species using random amplified polymorphic DNA (RAPD). *Molecular and Cellular Probes,* **10**: 435-441.

Smith, P.J., Benson, P.G. and McVeagh, S.M. (1997) A comparison of three genetic methods used for stock discrimination of orange roughy, *Hoplostethus atlanticus*: allozymes, mitochondrial DNA, and random amplified polymorphic DNA. *Fishery Bulletin,* **95**: 800-811.

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

Waldbieser, G.C. and Wolters, W.R. (1999) Application of polymorphic microsatellite loci in a channel catfish *Ictalurus punctatus* breeding program. *Journal of the World Aquaculture Society*, **30**(2): 256-262.

Welsh, J. and McClelland, M. (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research,* **18**: 7213-7218.

Welsh, J., Petersen, C. and McClelland, M. (1991) Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. *Nucleic Acids Research*, **19**: 303-306.

Yoon, J.M. and Kim, G.W. (2001) Randomly amplified polymorphic DNA-polymerase chain reaction analysis of two different populations of cultured Korean catfish *Silurus asotus*. *Journal of Biosciences,* **26**: 641-647.

Yoon, J.M. and Kim, Y.H. (2003a) Wide marsh clam (*Corbicula* spp.) populations from three sites analysed by RAPD-PCR-AGE. *Bulletin of Electrochemistry*, **19**: 337-348.

Yoon, J.M. and Kim, G.W. (2003b) Genetic differences between cultured and wild penaeid shrimp (*Penaeus chinensis*) populations analysed by RAPD-PCR. *Korean Journal of Genetics,* **25**: 21-32.

Yoon, J.M. and Kim, J.Y. (2004) Genetic differences within and between populations of Korean catfish (*S. asotus*) and bullhead (*P. fulvidraco*) analysed by RAPD-PCR. *Asian-Australasian Journal of Animal Sciences,* **17**: 1053-1061.

Yoon, J.M. and Kim, J.Y. (2005) Genetic differences and DNA polymorphisms between the fleshy prawn (*Fenneropenaeus chinensis*) and Chinese ditch prawn (*Palaemon gravieri*). *Journal of Fisheries Science and Technology,* **8**(3): 151-160.

Yoon, J.M. (2006) Genetic differences and variations in freshwater crab (*Eriocheir sinensis*) and swimming crab (*Portunus trituberculatus*). *Development and Reproduction,* **10**(1): 19-32.