J. Fish. Sci. Technol. 8(3), 151-160, 2005

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY
© The Korean Fisheries Society

Genetic Differences and DNA Polymorphisms between the Fleshy Prawn *Fenneropenaeus chinensis* and Chinese Ditch Prawn *Palaemon gravieri*

Jong-Man Yoon[1]* and Jong-Yeon Kim[2]

[1]*Department of Aquatic Life Medicine and*
[2]*Department of Marine Aquaculture and Biotechnology, College of Ocean Science and Technology, Kunsan National University, Gunsan 573-701, Korea*

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. 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 dramatically between the primers and the two species. In the FP species, a specific fragment of approximately 1,200 bp generated by primer OPB-04 exhibited inter-individual-specific characteristics that were indicative of DNA polymorphisms. Moreover, in the CDP species, a major fragment of approximately 550 bp generated by primer OPB-20 was found to be specific for the CDP. The average bandsharing value between the two prawn species was 0.421 ± 0.006, and ranged from 0.230 to 0.611. 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. The genetic distance between the two prawn species ranged from 0.071 to 0.642. Thus, RAPD-PCR analysis revealed a significant genetic distance between the two prawn species. Using various arbitrary primers, RAPD-PCR may be applied to identify specific/polymorphic markers that are particular to a species and geographic population, and to define genetic diversity, polymorphisms, and similarities among shrimp species.

Key words: Bandsharing value, Chinese ditch prawn, DNA polymorphism, *Fenneropenaeus chinensis*, Fleshy prawn, Genetic distance, *Palaemon gravieri*, RAPD-PCR

Introduction

Shrimps and prawns are the most popular marine products in Korea because of their taste and nutritional value, and Koreans consume them in large quantities. Among shrimps and prawns, the fleshy prawn *Fenneropenaeus chinensis* (FP) is an economically important aquacultural species that belongs to the family Penaeidae, which includes the genus Fenneropenaeus. The FP is widely distributed in the West Sea and South Sea, off the Korean Peninsula, and in the Gulf of Pohai off China, as well as in several areas around Japan. In particular, during the summer, this shrimp is widely distributed off the coasts of Incheon, Taean, Boryeong, Kunsan, Yeonggang, Goheung, and Yeosu, in the Korean peninsula. Marine aquaculture may have started with prawns that were cultivated in the West Sea in the early 1980s. The FP is one of the most highly sought after species that is collected from the sea during the late autumn. The consumption of this species has in-

*Corresponding author: jmyoon@kunsan.ac.kr

creased considerably, as restaurants that specialize in serving prawns in various forms, such as raw with lemon, boiled with vegetables, or fried, have recently become established. Basically, the body size of the prawn varies widely according to the environmental conditions. The environmental requirements and the tolerances of prawn species grown at different geographic sites are not known, although prawn species identification is advanced. As the FP culture industry increases rapidly, understanding the genetics of this mollusk species is crucial to an evaluation of the genetic impact of prawn production operations. However, little is known about the genetics of shrimps in Korea (Yoon and Kim, 2003b).

The Chinese ditch prawn *Palaemon gravieri* (CDP) is widely distributed across the West Sea and South Sea, off the Korean Peninsula, as well as in several areas off the coast of China. In particular, this prawn inhabits the seas off Incheon, Boryeong, Kunsan, Yeonggang, Jindo, and Tongyeong, in the Korean Peninsula, which are influenced by warm water currents. Generally, the spawning season for this prawn starts in March and continues through to August. The size and type of the prawn varies according to habitat, and includes such factors as the temperature and depth of the water, and the availability of nutrients. The CDP is another highly desirable species from the sea and is available year round. These prawns are used in combination with Japanese vinegared rice delicacies. However, in spite of their economic and scientific importance, little information currently exists regarding the genetics and early development of the CDP species in Korea.

To analyze the genetics of organisms, a number of analytical and molecular techniques have been applied, including morphological yardsticks (Orozco-Castillo et al., 1994), allozyme variation (Smith et al., 1997), and various PCR-generated biological molecular techniques, which include restriction fragment length polymorphisms (RFLPs) (Dahle, 1991; Kim et al., 1997), amplified fragment length polymorphisms (AFLPs) (Das et al., 1999), random amplified polymorphic DNAs (RAPD) (Hwang et al., 1995; Moeller and Schaal, 1999; Iyengar et al., 2000; Yoon and Kim, 2003a; Yoon et al., 2003c; Jung et al., 2004), and analyses of the microsatellites of mitochondrial and genomic DNAs (Huang et al., 2000; Iyengar et al., 2000). In particular, polymorphic and/or specific markers that are specific to the genus, species, breed or geographical population have been applied to determine individuals, species, and populations; they have also been used for pedigree analysis and for the screening of DNA markers for marker-assisted selection and genotype-assisted selection (Callejas and Ochando, 1998; Tassanakajon et al., 1998; Huang et al., 2000; Nebauer et al., 2000; Ramesha et al., 2002).

The RAPD method requires very small amounts of genomic DNA and can be used to analyze a large number of individuals in a short time (Deragon and Landry, 1992). Polymorphisms are determined from specific positions in the banding patterns of the amplified products (Smith et al., 1997; Tassanakajon et al., 1998; Yoon and Kim, 2001). Thus, RAPD has been applied to the identification of the genetic characteristics of various species of fish and shellfish (Partis and Wells, 1996; Smith et al., 1997; Callejas and Ochando, 1998; Yoon and Kim, 2003b; Yoon et al., 2003c).

The clustering analysis of the genetic distances between various fish and mollusk species, or populations from different geographic sites, which was performed using RAPD-PCR, is of little quantity (Klinbunga et al., 2000; McCormack et al., 2000; Yoon and Park, 2001). In addition, genetic variability, species-specific markers, and region-specific markers in oysters have been assessed by molecular biological methods (Kim et al., 1997; Klinbunga et al., 2000). In the present study, to elucidate the genetic distances and differences among prawn species, we performed a clustering analysis of FPs and CDPs collected in the West Sea (Buan). We also analyzed the genetic diversity of prawns in Korea. Finally, the present study was undertaken to confirm that the species relationships identified by RAPD-PCR are consistent with previously obtained data using morphological affinities.

Materials and Methods

Sample collection and isolation of genomic DNA

Fleshy prawns (*F. chinensis*) and Chinese ditch prawns (*P. gravieri*) were obtained from the West Sea off Buan. The muscle tissues of the prawns were collected in sterile test tubes, which were immediately placed in liquid nitrogen and stored until needed. The RAPD-PCR analysis was performed on the muscle extracts of 22 prawns using seven arbitrarily selected primers of two decades of different decamer primers. DNA extraction was performed as described previously (Yoon and Park, 2001). DNA samples were extracted from the lysates by the addition of ice-cold 70% ethanol and centrifugation at 6,289×g for 5 min. The DNA pellets were incubator-dried for 2 h and maintained at -40°C until analysis, at which time the samples were dissolved in TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA]. The concentrations

of the extracted genomic DNA samples were calculated based on the absorbance at 260 nm in a Beckman DU 600 series spectrophotometer (Beckman Coulter, Buckinghamshire, UK).

Decamer primers, molecular markers and amplification condition

Of the 20 primers (Operon Technologies, Alameda, CA, USA) selected, the seven arbitrarily selected primers: OPA-12 (5'-TCGGCGATAC-3'), OPA-13 (5'-CAGCACCCAC-3'), OPA-19 (5'-CAAACGTCGG-3'), OPB-04 (5'-GGACTGGAGT-3'), OPB-16 (5'-TTTGCCCGGA-3'), OPB-18 (5'-CCACAGCAGT-3') and OPB-20 (5'-GGACCCTTAC-3') were used to identify identical, polymorphic, and specific bands (Tables 1-3). Therefore, we used these primers to study the genetic similarities and diversity of prawns. RAPD-PCR was performed using two DNA Thermal Cyclers (Perkin Elmer Cetus, Norwalk, CT, USA; MJ Research Inc., Waltham, MA, USA). The amplified products were subjected to 1.4% agarose (SeaKem LE; FMC BioProducts, Rockland, ME, USA) gel electrophoresis (AGE) in TBE [90 mM Tris (pH 8.5), 90 mM borate, 2.5 mM EDTA]. The 100-bp

Table 1. The total, average, identical, specific and polymorphic bands generated by RAPD-PCR using 7 random primers in fleshy prawn and Chinese ditch prawn from Buan in Korea

Primer	Number of average band per lane		Number of identical bands		Number of specific bands		Number of polymorphic bands	
	Fleshy prawn	Chinese ditch prawn	Fleshy prawn	Chinese ditch prawn	Fleshy prawn	Chinese ditch prawn	Fleshy prawn	Chinese ditch prawn
OPA-12	6.7 (74)	4.7 (52)	11	0	3	3	7	0
OPA-13	6.1 (67)	6.0 (66)	22	0	5	7	2	3
OPA-19	4.6 (51)	2.9 (32)	11	0	5	7	4	0
OPB-04	4.2 (46)	5.3 (58)	0	22	2	5	3	3
OPB-16	3.5 (39)	2.4 (26)	11	0	16	14	1	0
OPB-18	5.6 (62)	3.2 (35)	11	0	4	6	0	3
OPB-20	1.7 (19)	2.9 (32)	0	22	3	5	1	3
Total number	32.5 (358)	27.4 (301)	66	44	38	47	18	12
Average number per primer	51.1	43.0	9.4	6.3	5.4	6.7	2.6	1.7

The total number of sfragments generated by a primer in fleshy prawn and Chinese ditch prawn is shown in parentheses.

Table 2. Similarity matrix including bandsharing values and genetic differences calculated using Nei and Li's index of the similarity of fleshy prawn and Chinese ditch prawn from Buan in Korea

		Bandsharing values of fleshy prawn											Bandsharing values of Chinese ditch prawn										
		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 fleshy prawn	1	-	0.701	0.797	0.785	0.563	0.544	0.573	0.648	0.573	0.610	0.633	0.418	0.230	0.315	0.382	0.385	0.295	0.242	0.308	0.368	0.497	0.403
	2	0.299	-	0.745	0.712	0.535	0.622	0.462	0.648	0.669	0.675	0.719	0.322	0.367	0.321	0.476	0.380	0.574	0.440	0.450	0.425	0.483	0.423
	3	0.203	0.255	-	0.788	0.590	0.628	0.476	0.663	0.561	0.581	0.712	0.405	0.340	0.340	0.383	0.307	0.504	0.475	0.514	0.348	0.404	0.358
	4	0.215	0.288	0.212	-	0.557	0.655	0.509	0.582	0.577	0.651	0.741	0.394	0.361	0.367	0.439	0.365	0.503	0.364	0.530	0.358	0.477	0.449
	5	0.437	0.465	0.410	0.443	-	0.469	0.606	0.536	0.559	0.463	0.587	0.433	0.386	0.289	0.332	0.343	0.410	0.402	0.449	0.464	0.481	0.371
	6	0.456	0.318	0.372	0.345	0.531	-	0.456	0.487	0.478	0.576	0.713	0.437	0.434	0.383	0.323	0.426	0.420	0.491	0.515	0.412	0.484	0.473
	7	0.427	0.538	0.524	0.491	0.394	0.544	-	0.559	0.592	0.527	0.613	0.563	0.433	0.399	0.359	0.405	0.357	0.423	0.487	0.406	0.452	0.428
	8	0.352	0.352	0.337	0.418	0.464	0.513	0.441	-	0.703	0.687	0.675	0.476	0.402	0.375	0.373	0.401	0.469	0.472	0.453	0.333	0.473	0.361
	9	0.427	0.331	0.439	0.423	0.441	0.522	0.408	0.297	-	0.556	0.649	0.565	0.428	0.413	0.397	0.482	0.459	0.512	0.538	0.505	0.499	0.538
	10	0.390	0.325	0.419	0.349	0.537	0.424	0.473	0.313	0.444	-	0.731	0.517	0.378	0.347	0.354	0.384	0.427	0.454	0.455	0.379	0.592	0.414
	11	0.327	0.281	0.288	0.259	0.413	0.287	0.387	0.325	0.351	0.269	-	0.406	0.452	0.435	0.408	0.396	0.457	0.513	0.468	0.421	0.611	0.424
Genetic differences of Chinese ditch prawn	12	0.582	0.678	0.595	0.606	0.567	0.563	0.437	0.524	0.435	0.483	0.594	-	0.413	0.356	0.490	0.398	0.594	0.540	0.668	0.600	0.568	0.532
	13	0.770	0.633	0.660	0.639	0.614	0.566	0.567	0.598	0.572	0.622	0.548	0.587	-	0.554	0.390	0.646	0.625	0.637	0.423	0.461	0.424	0.478
	14	0.685	0.679	0.660	0.633	0.711	0.617	0.601	0.625	0.587	0.653	0.565	0.644	0.446	-	0.551	0.744	0.601	0.703	0.543	0.532	0.506	0.528
	15	0.618	0.524	0.617	0.561	0.668	0.677	0.641	0.627	0.603	0.646	0.592	0.510	0.610	0.449	-	0.526	0.571	0.548	0.643	0.516	0.561	0.624
	16	0.615	0.620	0.693	0.635	0.657	0.574	0.595	0.599	0.518	0.616	0.604	0.602	0.354	0.256	0.474	-	0.479	0.764	0.573	0.341	0.499	0.550
	17	0.705	0.426	0.496	0.497	0.590	0.580	0.643	0.531	0.541	0.573	0.543	0.406	0.375	0.399	0.429	0.521	-	0.681	0.599	0.658	0.542	0.632
	18	0.758	0.560	0.525	0.636	0.598	0.509	0.577	0.528	0.488	0.546	0.487	0.460	0.363	0.297	0.452	0.236	0.319	-	0.690	0.495	0.603	0.665
	19	0.692	0.550	0.486	0.470	0.551	0.485	0.513	0.547	0.462	0.545	0.532	0.332	0.577	0.457	0.357	0.427	0.401	0.310	-	0.574	0.768	0.595
	20	0.632	0.575	0.652	0.642	0.536	0.588	0.594	0.667	0.495	0.621	0.579	0.400	0.539	0.468	0.484	0.659	0.342	0.505	0.426	-	0.599	0.508
	21	0.503	0.517	0.596	0.523	0.519	0.516	0.548	0.527	0.401	0.408	0.389	0.432	0.576	0.494	0.439	0.501	0.458	0.397	0.232	0.401	-	0.625
	22	0.597	0.577	0.642	0.551	0.629	0.527	0.572	0.639	0.462	0.586	0.576	0.468	0.522	0.472	0.376	0.450	0.368	0.335	0.405	0.492	0.375	-

DNA Ladder (Bioneer Corp., Daejeon, Korea) was used as the DNA molecular weight marker. The agarose gels were stained with ethidium bromide and photo-graphed under ultraviolet illumination using the Photoman Direct Copy system (PECA Products, Beloit, WI, USA).

Data analysis

Amplified fragments that ranged in size from 50 bp to 1,600 bp were analyzed. The primers that generated identical minor bands were excluded from the analysis. The values were calculated according to the methods of Jeffreys and Morton (1987). When comparing two lanes, the bandsharing (BS) value was calculated as follows:

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

where Nab is the number of bands shared by samples a and b; Na is the total number of bands for sample a; and Nb is the total number of bands for sample b.

The average within-population similarity was calculated by pairwise comparison between individuals within a species. The levels of relatedness among different individuals of the FP species (FLESHY 01-11) and CDP species (DITCH 12-22) were generated according to the bandsharing values and similarity matrix. A hierarchical clustering tree was constructed using similarity matrices to generate a dendrogram, which was facilitated by the Systat ver. 10 software (SPSS Inc., Chicago, IL, USA). The Systat software was also used to calculate genetic differences, Euclidean genetic distances within and between populations, means, standard errors, and *t*-test scores.

Results and Discussion

RAPD-PCR variation within and between species

Genomic DNA samples isolated from FP and CDP species were amplified repeatedly by PCR. The amplified products were separated by AGE and stained with ethidium bromide. In the present study, seven decamer primers generated a total of 358 fragments from the FP DNA and 301 fragments from the CDP DNA, with fragment sizes that ranged from 50 bp to 1,600 bp, as summarized in Table 1 and Figure 1. For black tiger shrimp, 80 bands ranging in size from 200 bp to 2,200 bp were unambiguously scored (Tassanakajon et al., 1998). It has been reported that a single primer generates 9 to 15 distinct bands. The primers generated 36, 32, and 24 bands from the DNAs of mud crabs from Eastern Thailand (genus *Scylla*) (Klinbunga et al., 2000). Seven primers generated 317 bands for the cultured shrimp population and 385 fragments for the wild shrimp population, with fragment sizes that ranged from 100 bp to 1,800 bp (Yoon and Kim, 2003b). For the brittle star, the DNA fragments amplified using four primers ranged in size from 100 bp to 2,300 bp (McCormack et al., 2000). In the case of marsh clams from Gochang (*Corbicula* spp) collected at three geographic sites, 7/20 primers generated 585 major and minor RAPD bands, producing an average of 6.6 products per primer (Yoon and Kim, 2003a). For five venerid clams (Bivalvia, Veneridae), of the 20 primers used, 15 primers produced at least two or three fragments (Jung et al., 2004). Barramundi (*Lates calcarifer*) DNA fragments detected by RAPD-PCR ranged in size from 350 bp to 700 bp (Partis and Wells, 1996). In wild and cultured populations of crucian carp, five primers generated 1,084 distinct fragments that ranged in size from 120 bp to larger than 4,270 bp (Yoon and Park, 2001). Eight random primers were used to generate fragments of 176 bp to 2,937 bp for three goat populations in the Chaidamu Basin, China (Geng et al., 2002), while 26 primers generated 137 polymorphic RAPD markers for ten silkworm (*Bombyx mori*) strains (Hwang et al., 1995).

A single decamer primer generated an average of 51.1 amplified products for the FP species from Buan. The RAPD primer generated an average of 4.6 amplified bands per sample (range: 1.7 to 6.7 fragments) for the FP species. The primer OPA-12 amplified fragments of sizes that ranged from 100 bp to 500 bp from the FP species, as summarized in Figure 1. The oligonucleotide decamer primer OPA-12 generated the same DNA fragments of approximately 500 bp for the FP species, as shown in Figure 1A. However, the OPA-12, OPA-13, OPA-19, OPB-16, and OPB-18 primers did not generate fragments of identical size for the CDP species. These results demonstrate that the FP species is genetically distinct from the CDP species. For the FP species, the decamer primer OPA-13 generated 22 identical major and/or minor fragments, of 190 bp and 230 bp, respectively, as shown in Fig. 1B. The random primer OPA-19 amplified 11 identical major and/or minor bands of 150 bp in all the samples (Fig. 1C). Five specific and four polymorphic RAPD fragments were also observed in this population. Two specific and three polymorphic major and/or minor bands of approximately 280 bp, 350 bp, and 1,200 bp, respectively, were generated by the random primer OPB-04 (Fig. 1D). In particular, the fragment of approximately 1,200 bp generated by this primer exhibited the interindividual-specific characteristic that is indicative of DNA

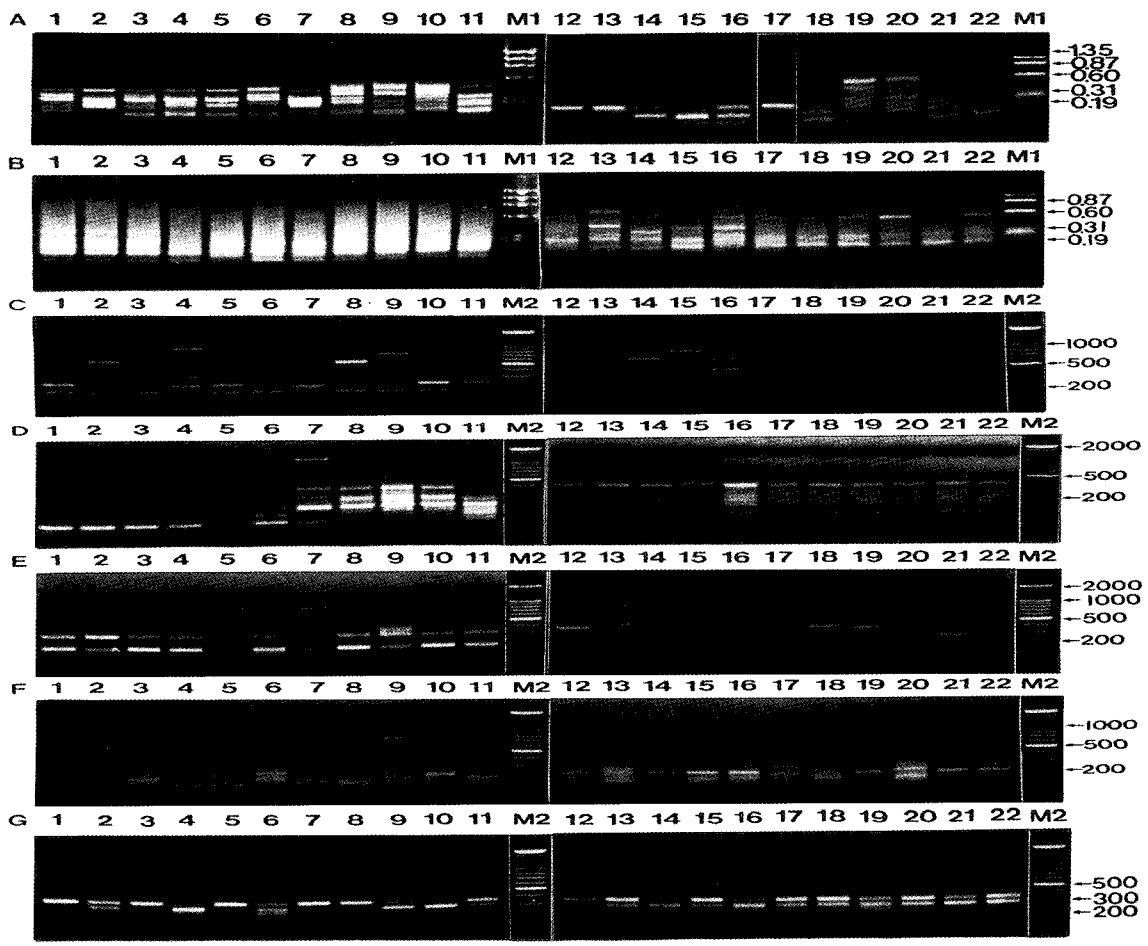

Fig. 1. RAPD-PCR-generated electrophoretic profiles of fleshy prawn (*F. chinensis*) and Chinese ditch prawn (*P. gravieri*). DNA isolated from fleshy prawn population (lane 1-11) and Chinese ditch prawn population (lane 12-22) were amplified by random primer OPA-12 (A), OPA-13 (B), OPA-19 (C), OPB-04 (D), OPB-16 (E), OPB-18 (F) and OPB-20 (G). Amplified products were electrophoresed on a 1.4% agarose gel and detected by staining with ethidium bromide. Molecular size marker 1.35, 1.08, 0.87, 0.60, 0.31, 0.28, 0.23 and 0.19 kb: M1, ΦX174 DNA marker digested with restriction enzyme *Hae*III; M2, 100 bp Ladder DNA markers.

polymorphisms. High degrees of RAPD variation were observed in the banding patterns generated by primer OPB-16, with the fragments ranging in size from 250 bp to 1,600 bp (Fig. 1E). Interestingly, the decamer primer generated the lowest number of fragments (a total of 26 or an average of 2.4 fragments) of all the primers used, as illustrated in Table 1. The 11 bands, of approximately 800 bp, produced by the decamer primer OPB-18 were observed in all the samples (Fig. 1F). This primer detected four specific major and/or minor bands of >100 bp or <300 bp. This primer also generated the highest number of fragments (a total of 62 or an average of 5.6 fragments). OPB-20 detected three specific and polymorphic major and/or minor bands of 250 bp in lanes 3, 6, and 11, and a band of 280 bp in lane 4 (Fig. 1G). This primer produced the lowest number of fragments (a total of 19 or an average of 1.7 fragments). These results indicate that the genome sizes of the FP species from Buan are similar to those of blue catfish (Liu et al., 1998), black tiger shrimp (Tassanakajon et al., 1998), cultured Korean catfish (Yoon and Kim, 2001), and penaeid shrimp (Yoon and Kim, 2003b). Fifty-three RAPD markers obtained from seven primers were common to all breeds, 22 were specific to individuals, and 18 were polymorphic in different breeds of zebu cattle (Ramesha et al., 2002).

In the CDP species from Buan, the decamer primer OPA-13 generated a polymorphic RAPD profile with three DNA fragments (Fig. 1B). Notably, one specific fragment (800-bp band in lane 13) generated by this

primer exhibited the inter-individual-specific characteristic indicative of DNA polymorphism. This primer also produced the highest number of fragments (a total of 66 or an average of 6.0 fragments). The common banding patterns, which contained the 300-bp and 400-bp fragments, were generated by the decamer primer OPB-04, as shown in Figure 1D. The banding patterns generated by decamer primers OPA-12 and OPB-16 for individual prawns from Buan varied significantly, as shown in Figure 1A and E. RAPD variation was observed in the banding patterns, which ranged in size from 100 bp to 400 bp, generated by decamer primer OPB-18 (Fig. 1F). The decamer primer OPB-20 detected 22 common fragments of 300 bp and 350 bp (Fig. 1G). This primer generated four minor specific bands of approximately 250 bp (lane 19), 500 bp (lane 14), and 550 bp (lanes 14 and 20). One major fragment of approximately 550 bp (lane 15), which was specific for prawn individuals from the CDP group, was detected. The complexity of the banding pattern shown varied significantly with the primers used and/or the geographical location. Generally, the size and number of fragments generated in an unbiased manner depend on the nucleotide sequence of the primer used and on the source of the template DNA, resulting in a genome-specific DNA fragment (Welsh et al. 1991).

In the present study, 18 polymorphic fragments (5.03%) were identified for the FP species from Buan and 12 (3.99%) polymorphic fragments were identified for the CDP species, as illustrated in Table 1. Sixty-six common fragments, i.e., an average of 9.4 fragments per primer, were observed for the FP species, and 44 fragments, i.e., an average of 6.3 per fragments per primer, were detected for the CDP species. The numbers of specific fragments identified for the FP species and CDP species were 38 and 47, respectively. These results demonstrate that a primer can detect a large number of specific fragments. This suggests higher genetic variability in the CDP species than in the FP species. Similar results were demonstrated by Kim et al. (2004), who reported that seven primers generated 143 polymorphic fragments (143/481 fragments, 29.72%) for an oyster population from Buan and 60 polymorphic fragments (60/264 fragments, 22.7%) for an oyster population from Geojedo.

The percentage of polymorphic bands obtained from five geographic populations for the black tiger shrimp (*Penaeus monodon*) varied from 51.5 to 57.7% (Tassanakajon et al., 1998). Two primers yielded the highest level of polymorphism, 88.9%, in black tiger shrimp and showed that 22/80 (27.5%) bands were monomorphic and 58 (72.5%) bands were polymorphic. Six primers produced 84 polymorphic bands out of a total of 90 bands for the blacklip abalone (Huang et al., 2000). The RAPD-PCR method using random primers was used to identify three endemic Spanish barbel species: *Barbus bocagei*, *B. graellsii*, and *B. sclateri* (Callejas and Ochando, 1998). This analysis indicated that *Barbus bocagei* and *B. graellsii* were more closely related to each other than *B. sclateri*. Population-related RAPD fragments were identified in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*), and their F_1, F_2, and backcross hybrids. The frequencies of fragments generated by six primers were calculated in various catfish populations, as described previously (Liu et al., 1998). The sum of the average polymorphic products (73.7) was calculated for the combination of common carp and Israeli carp (Yoon, 2001). In an assessment of the genetic differences and characteristics of two oyster populations using the RFLP method, the restriction fragment patterns of twenty individuals in the West Sea have been reported to be identical (Kim et al., 1997). Calculation of sequence divergence between localities using the RFLP method revealed a high level of divergence between samples from coastal and Arctic areas (1.77-5.62%), as opposed to the low intra-population divergence (0.10%) and low divergences between localities along the coast (0.17%) and in the Barents Sea (1.00%) (Dahle, 1991). Geng et al. (2002) have reported 94 amplified fragments for three goat populations from the Chaidamu Basin in China, and shown that the frequencies of DNA polymorphism of CG, CCG, and LCG are 0.8404, 0.8617, and 0.8511, respectively. In addition, they reported that RAPD analysis generated 252 genotypes. The number of amplified fragments generated by a single primer varied from 19 to 27. In the case of *Digitalis*, 98.9% of these fragments were polymorphic (Nebauer et al., 2000). For five venerid clams (Veneridae), 15 unit primers generated one specific or polymorphic band (Jung et al., 2004).

In this study, we have observed 38 RAPD-PCR-amplified specific bands and 18 polymorphic bands generated by seven decamer primers for FP and 47 specific bands and 12 polymorphic bands for CDP. Furthermore, 14 specific fragments generated by the decamer primer OPB-16 exhibited inter-individual-specific characteristics and DNA polymorphisms for CDP, as shown in Figure 1E. The specific primer was found to be useful for the identification of individuals, which were the result of different DNA polymorphisms (Liu et al., 1998; Yoon and Park, 2001; Yoon and Kim, 2003b). Although the main disad-

vantage of the RAPD method is reproducibility, the method was considered suitable for the identification of one out of eight fish species (Partis and Wells, 1996). Three out of forty nanomer primers that amplified DNA fragments specific for a grass species or to durum cultivar have been identified (Bommineni et al., 1997). The PR21 primer amplified DNA fragments that were specific for five individual durum cultivars. The PR22 primer amplified fragments that were specific for a grass species. Similarly, the PR23 primer amplified fragments that were specific for a grass species. The amplified fragments ranged in size from 1,000 bp to 1,200 bp and were specific for *Thinopyrum junceiforme*. A DNA fragment of approximately 1,500 bp was specific for *Lophopyrum elongatum*. Generally, polymorphic fragments generated by RAPD-PCR using arbitrary primers are suitable for the detection of genetic diversity, polymorphisms, and similarity among various organisms (Welsh et al., 1991; Bommineni et al., 1997; Callejas and Ochando, 1998; Liu et al., 1998; McCormack et al., 2000; Nebauer et al., 2000; Ramesha et al., 2002). In particular, Geng et al. (2002) have stated that RAPD markers should have been more effective in analyzing the genetic relationships among three goat populations.

Bandsharing values and genetic distances

In this study, bandsharing values based on the presence or absence of amplified fragments were used to calculate similarity indices, as illustrated in Table 2. Based on the average bandsharing values of all the samples, the similarity matrix ranged from 0.456 to 0.797 for the FP species and from 0.341 to 0.768 for the CDP species. The average bandsharing value was 0.613 ± 0.012 within the FP species and 0.562 ± 0.013 within the CDP species, as illustrated in Table 2. The average bandsharing value between the two prawn species was 0.421 ± 0.006, and ranged from 0.230 to 0.611. This outcome is similar to that reported by Kim et al. (2004), who reported that the similarity matrix based on the average bandsharing value of all the samples ranged from 0.493 to 0.879 for an oyster population from Buan, and ranged from 0.244-0.889 for an oyster population from Geojedo. In addition, they concluded that the bandsharing values of individual oysters from Buan were higher than those from Geojedo (Kim et al., 2004).

The bandsharing values we obtained for our two prawn species are also similar to previously reported results (Yoon and Park, 2001). The average bandsharing value obtained with five random primers was 0.40 ± 0.05 for a wild crucian carp population and 0.69 ± 0.08 for a cultured crucian carp population. The average bandsharing value of our study is lower than that seen between the common carp and Israeli carp (0.57 ± 0.03) (Yoon, 2001), and that between zebu cattle breeds (0.73-0.79) (Gwakisa et al., 1994). The highest value (0.87) was obtained for the comparison of the purplish Washington clam *Saxidomus purpuratus* with the hard clam *Meretrix lusoria*, and the lowest value (0.46) was observed for the comparison of the little clam *Ruditapes philippinarum* with the purplish Washington clam *S. purpuratus* (Jung et al., 2004). The values were similar to those obtained in the present study. The genetic difference obtained by the bandsharing values varied from 0.203 to 0.544 for the FP species and from 0.232 to 0.659 for the CDP, as illustrated in Table 2. The genetic difference varied from 0.389 to 0.770 between the two prawn species. The average genetic difference was 0.385 ± 0.012 within the FP species and 0.438 ± 0.013 within the CDP species. Compared separately, the average genetic difference was higher in the CDP species than in the FP species. The average genetic difference between the two prawn species was 0.578 ± 0.007. Previously, the difference between the two oyster populations was found to be statistically significant. The present results are similar to the previous results of Kim et al (2004), who reported that the average genetic difference in an oyster population from Buan was approximately 0.361 ± 0.013, and in a population from Geojedo the corresponding value was 0.462 ± 0.017. Accordingly, as mentioned above, RAPD-PCR analysis showed that the CDP species from Buan was separate from the FP species. In general, between-breed genetic similarity is an indicator of relatedness between two breeds/populations/strains with respect to the sequences amplified by PCR (Sharma et al., 2004).

Based on the similarity matrix generated by bandsharing values and genetic distances, hierarchical clustering analysis was performed to produce the dendrogram shown in Figure 2. The dendrogram obtained using the seven primers contained 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. In two oyster populations, Kim et al. (2004) reported that a dendrogram generated using four reliable primers contained three genetic clusters. Between the two prawn species, the shortest genetic distance that displayed significant molecular differences was between individuals 04 and 03 from the FP species

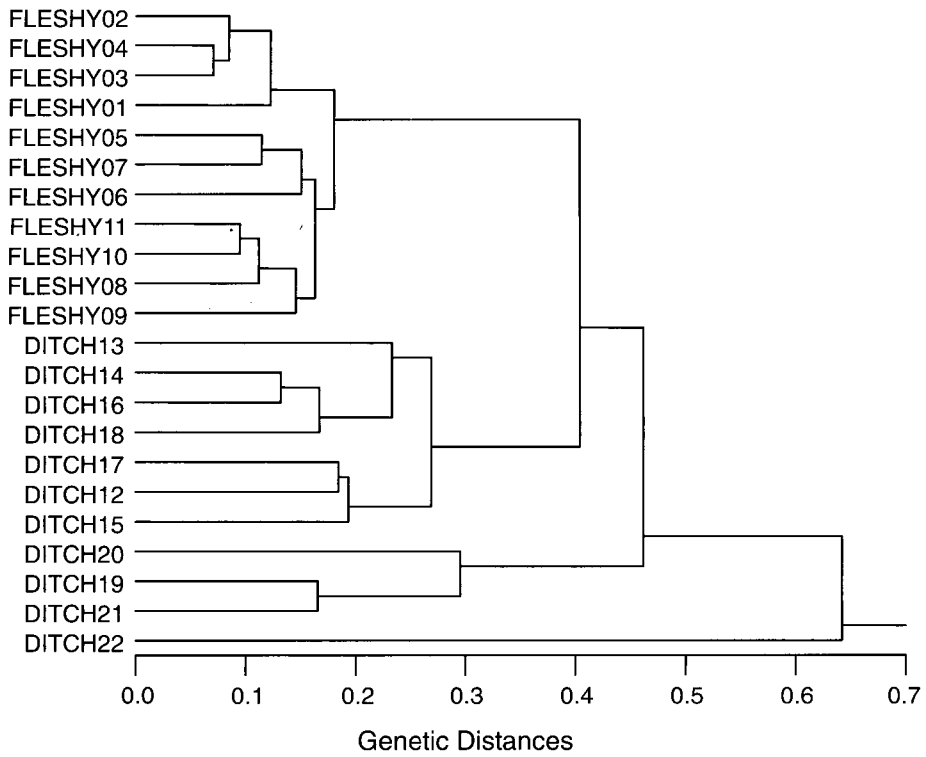

Fig. 2. Hierarchical dendrogram of genetic distances obtained from two geographic prawn species. The relatedness among different individuals of fleshy prawn species (FLESHY 01-FLESHY 11) and Chinese ditch prawn species (DITCH 12-DITCH 22) generated according to the bandsharing values and similarity matrix as in Table 2.

(0.071), as shown in Figure 2, while the longest genetic distance between the two prawn species that displayed significant molecular differences was between individuals 16 and 22 (0.642). Individual 21 of the CDP species was closely related to individual 19 of the CDP species (genetic distance = 0.165).

Based on cluster analysis of the genetic similarity values obtained from RAPD data, Jung et al. (2004) reported that the genetic similarity between *S. purpuratus* and *M. lusoria* was the highest at 0.87, and that the level of similarity between the venus clam *Protothaca jedoensis* and the little clam *R. philippinarum* was 0.84. However, the lowest level of genetic similarity (0.46) was noted between *R. philippinarum* and *S. purpuratus*. The genetic relationships between three goat populations in China were also estimated using the genetic differentiation coefficient and genetic similarity coefficient (Geng et al., 2002) in a manner similar to the way in which we obtained the genetic distance/similarity values by cluster analysis. The results showed that the genetic relationships between LCG and CCG were the closest (genetic distance = 0.0106), followed by CG and CCG (genetic distance = 0.0109). In silkworm (*Bombyx* spp.), ten silkworm strains were classified into two groups at the genetic similarity coefficient of 0.496: the *Bombyx mori* group, consisting of nine domesticated silkworms, and the wild silkworm *Bombyx mandarina* group (Hwang et al., 1995).

Our cluster analysis showed a pattern similar to that described by Orozco-Castillo et al. (1994), who reported that RAPD analysis reflected morphological differences between the subgroups as well as the morphological origin of coffee plants. The phenogram that they generated using RAPD data obtained for all primers revealed close relationships between accession identities for native American maize accessions (Moeller and Schaal, 1999).

In the case of blacklip abalone, cluster analysis of the pairwise population matrix generated from RAPD data showed that geographically close populations tended to cluster together (Huang et al., 2000). Additional principal component analysis based on the RAPD data showed that the Point Cook population was clearly separated from two other central populations. A neighbor-joining tree based on genetic distances between populations using the RAPD-PCR method indicated the relationships between three mud crab species (Klinbunga et al., 2000), among which there were large intraspecies and interspecies diffe-

rences between geographic populations. RAPD data analysis of genetic distance and parsimony methods, family clustering, and analysis of molecular variance were applied to the study of genetic relationships between a few of species within a genus. It was reported that the species relationships revealed by the RAPD-PCR approach should be consistent with previously obtained data using morphological affinities (Nebauer et al., 2000).

In spite of the problems with variability and reproducibility, a large number of genetic studies have used RAPD-PCR because it is a relatively fast, reliable, and simple method for investigating a variety of genomic DNAs for polymorphisms, and for revealing genetic diversity within a population; additionally, it does not require prior knowledge of the genome (Welsh et al., 1991; Orozco-Castillo et al., 1994; Bommineni et al., 1997; Klinbunga et al., 2000; Geng et al., 2002). The potential of RAPD to identify diagnostic markers for breed, line, species, genus, and population identification in teleosts and invertebrates (Partis and Wells, 1996; Tassanakajon et al., 1998; McCormack et al., 2000; Yoon and Kim, 2003b; Jung et al., 2004), in insects (Hwang et al., 1995), in livestock (Jeffreys and Morton, 1987; Gwakisa et al., 1994; Geng et al., 2002; Ramesha et al., 2002; Sharma et al., 2004), and in plants (Moeller and Schaal, 1999) has also been demonstrated. In the present study, RAPD-PCR analysis revealed a significant genetic distance between two prawn species. The existence of population differentiation and DNA polymorphisms between two prawn populations were detected by RAPD-PCR. This shows that the method is an adequate tool for comparing the DNA of individuals, species and/or populations of prawn. Furthermore, the basic knowledge acquired regarding DNA polymorphisms and molecular markers in prawn species may significantly contribute to broodstock selection and the selective invertebrate-breeding program. The characterization of black tiger shrimp (*Penaeus monodon*), penaeid shrimp (*Penaeus chinensis*) and venerid clam (Veneridae) populations is a necessary step for breeding programs (Tassanakajon et al., 1998; Yoon and Kim, 2003b; Jung et al., 2004). In future, increased sample sizes, increased sample sites, additional PCR-based techniques, such as AFLP, microsatellites, genetic sequencing, annealing control primer (ACP) technology, gene fishing technology, and gene chip analysis may be required to advance studies in this area. Additional statistical analyses, such as bootstrapping and band-mapping of shared polymorphic amplification products and principal coordinates, based on the RAPD-PCR data, will be necessary to obtain more precise data.

Acknowledgements

We thank our laboratory colleagues, graduate students, and undergraduate students for their assistance with sample collection, RAPD-PCR techniques, and statistical analyses. The support received from Dr. Y. H. Kim of Kunsan National University is also gratefully acknowledged. Particular thanks go to the anonymous reviewers who assisted us with thorough and far-reaching criticisms. The authors are grateful to the Fisheries Science Institute of Kunsan National University for providing equipment and funding during program year 2005.

References

Bommineni, V.R., P.P. Jauhar, T.S. Peterson, R.N. Chibbar and A.B. Almouslem. 1997. Analysis of hybrids of durum wheat with *Thinopyrum juncelforme* using RAPD markers. Theor. Appl. Genet., 95. 757-763.

Callejas, C. and M.D. Ochando. 1998. Identification of Spanish barbel species using the RAPD technique. J. Fish Biol., 53, 208-215.

Dahle, G. 1991. Cod, *Gadus morhua* L., populations identified by mitochondrial DNA. J. Fish Biol., 38, 295-303.

Das, S., J. Rajagopal, S. Bhatia, P.S. Srivastava and M. Lakshmikumaran. 1999. Assessment of genetic variation within Brassica campestris cultivars using amplified fragment length polymorphism and random amplification of polymorphic DNA markers. J. Biosci., 24, 433-440.

Deragon, J.M. and B.S. Landry. 1992. PCR methods and applications: RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. Cold Spring Harbor Laboratory Press, pp. 175-180.

Geng, S.M., W. Shen, G.Q. Qin, X. Wang, S.R. Hu, Q. Wang and J.Q. Zhang. 2002. DNA fingerprint polymorphism of 3-goat populations from China Chaidamu Basin. Asian-Aus. J. Anim. Sci., 15, 1076-1079.

Gwakisa, P.S., S.J. Kemp and A.J. Teale. 1994. Characterization of zebu cattle breeds in Tanzania using random amplified polymorphic DNA markers. Anim. Genet., 25, 89-94.

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

Hwang, J.S., J.S. Lee, H.A. Kang, S.M. Lee and D.S. Suh. 1995. Analysis of genetic relationships among the

silkworm, *Bombyx Mori*, strains using RAPD-PCR. Korean J. Genet., 17, 291-300.

Iyengar, A., S. Piyapattanakorn, D.M. Dtone, D.A. Heipel, B.R. Howell, S.M. Baynes and N. Maclean. 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. Mar. Biotechnol., 2, 49-56.

Jeffreys, A.J. and D.B. Morton. 1987. DNA fingerprints of dogs and cats. Anim. Genet., 18, 1-15.

Jung, H.T., J. Kim, J.A. Shin, H.Y. Soh and S.D. Choi. 2004. Genetic relationship of the five venerid clams (Bivalvia, Veneridae) in Korea. J. Aquacult., 17, 251-257.

Kim, S.H., M.S. Park, Y.H. Kim and D.W. Park. 1997. Genetic analysis of mitochondrial DNA from Korean oyster, *Crassostrea gigas*. J. Kor. Fish. Soc., 30, 804-808.

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

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

Liu, Z., P. Li, B.J. Argue and R.A. Dunham. 1998. Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. Furcatus*) and their F_1, F_2 and backcross hybrids. Anim. Genet., 29, 58-62.

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

Moeller, D.A. and B.A. Schaal. 1999. Genetic relationships among Native American maize accessions of the Great Plains assessed by RAPDs. Theor. Appl. Genet., 99, 1061-1067.

Nebauer, S.G., L. del Castillo-Agudo and J. Segura. 2000. An assessment of genetic relationships within the genus *Digitalis* based on PCR-generated RAPD markers. Theor. Appl. Genet., 100, 1209-1216.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89, 583-590.

Orozco-Castillo, C., K.J. Chalmers, R. Waugh and W. Powell. 1994. Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. Theor. Appl. Genet., 87, 934-940.

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

Ramesha, K.P., T. Saravanan, M.K. Rao, M.M. Appannavar and A.O. Reddy. 2002. Genetic distance among south Indian breeds of zebu cattle using random amplified DNA markers. Asian-Aust. J. Anim. Sci., 15, 309-314.

Sharma, A.K., B. Bhushan, S. Kumar, P. Kumar, A. Sharma and S. Kumar. 2004. Molecular characterization of rathi and tharparkar indigenous cattle (*Bos indicus*) breeds by RAPD-PCR. Asian-Aust. J. Anim. Sci., 17, 1204-1209.

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

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

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

Yoon, J.M. 2001. Genetic similarity and difference between common carp and Israeli carp (*Cyprinus carpio*) based on random amplified polymorphic DNAs analyses. Kor. J. Biol. Sci., 5, 333-339.

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

Yoon, J.M. and H.Y. Park. 2001. Genetic similarity and variation in the cultured and wild crucian carp (*Carassius carassius*) estimated with random amplified polymorphic DNA. Asian-Aust. J. Anim. Sci., 15, 470-476.

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

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

Yoon, J.M., K.H. Park and S.N. Choe. 2003c. Genetic polymorphism of marsh clam (*Corbicula leana*) identified by RAPD-PCR. J. Fish. Sci. Technol., 6, 13-19.

(Received July 2005, Accepted September 2005)