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Genetic Variations in Geographic Venus Clam(*Gomphina aequilatera*, Sowerby) Populations from Samcheok and Wonsan

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삼척과 원산의 지리적 민들조개(*Gomphina aequilatera*, Sowerby) 집단의 유전적 변이

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ABSTRACT : Genomic DNAs(gDNAs) were isolated from the venus clam(*Gomphina aequilatera*) from Samcheok(venus clam from Samcheok; VCS) and Wonsan(venus clam from Wonsan; VCW) located in the East Sea of the Korean Peninsula. The amplified products were generated by agarose gel electrophoresis(AGE) with oligonucleotides primer, detected by staining with ethidium bromide and viewed by ultraviolet ray. The seven arbitrarily selected primers BION-21, BION-23, BION-25, BION-27, BION-29, BION-31 and BION-33 generated the shared loci, polymorphic, and specific loci, with the molecular sizes ranging from 150 bp to 2,400 bp. In this study, 147 polymorphic loci(147/954 loci, 15.41%) in VCS population and 274(274/996 loci, 27.51%) in VCW population were generated with seven primers. These results suggest the genetic variation in VCW population is higher than in VCS population. Especially, the 700 bp bands generated by the primer BION-21 were identified commonly in two *Gomphina* populations, which identified populations and/or species. This specific primer was found to be useful in the identification of individuals and/or population, resulting from the different DNA polymorphism among individuals/species/population. Two *Gomphina* populations between the individual SAMCHEOK no. 03 and WONSAN no. 22 showed the longest genetic distance(0.696) in comparison with other individuals used. The complete linkage cluster analysis indicating three genetic groupings and dendrogram revealed close relationships among individual identities within two geographical populations of venus clam(*G. aequilatera*) from the Samcheok and Wonsan. The intra-species classification and clustering analyses inferred from molecular markers supported the traditional taxonomy of the species based on morphological characters such as shell size, shape and color. Accordingly, as mentioned above, RAPD analysis showed that VCS population was more or less separated from VCW population.

Key words : Dendrogram, Genetic distance, *Gomphina aequilatera*, Intra-species classification, Venus clam.

요 약 : 한반도의 동쪽에 위치해 있는 삼척(venus clam from Samcheok; VCS) 과 원산(venus clam from Wonsan; VCW) 지역에서 채취된 민들조개(*Gomphina aequilatera*)에서 genomic DNAs(gDNAs) 를 분리 추출하였다. 증폭산물은 primer와 agarose 전기영동법에 의해서 생성되었고, EtBr에 의해서 염색된 이후에 자외선에 의해서 확인되었다. 150 bp에서 2,400 bp에 해당되는 shared loci, polymorphic 및 specific loci를 얻기 위해서 BION-21, BION-23, BION-25, BION-27, BION-29, BION-31 및 BION-33와 같은 7개의 primer를 사용하였다. 본 연구에서 7개의 primer는 VCS 민들조개 집단에서 147개의 polymorphic loci(147/954 loci, 15.41%)와 VCW 집단에서 274 개의 polymorphic loci(274/996 loci, 27.51%) 를 확인하였다. 이것은 VCS 민들조개 집단에서 보다 VCW 집단에서 더 높은 유전적 변이를 나타내고 있다는 것을 제시하고 있다. 특히 BION-21 primer에 의해서 나타난 700 bp는 민들조개 2개 집단에서 공통적으로 확인되었으며, 이러한 것은 집단이나 종을 확인할 수 있는 marker로서 활용이 가능할 것이다. 이러한 특이한 primer는 개체, 종 및 집단에서 서로 다른 DNA 다형성을 나타내며, 개체나 집단을 확인하는데 유용하다는 것을 알 수 있다. 2개 민들조개 집단의 개체들을 비교해 보았을 때 SAMCHEOK no. 03와 WONSAN no. 22에서 가장 긴 유전적 거리(0.696)를 나타내었다. 3개의 genetic groupings and dendrogram을 포함한 complete linkage cluster analysis을 통해서 볼 때 지리적 거리가 있었지만 삼척과 원산 2 민들조개 집단의 개체

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정체성과 다소 가까운 친척관계를 확인시켜 주었다. 분자적인 표지인자로부터 얻어진 종내 분류와 clustering analyses은 패각 크기, 패각 형태 및 패각 색깔과 같은 형태적인 형질을 기초한 재래적인 종 분류를 지원하고 있다. 따라서 위에서 언급된 바와 같이 RAPD 분석은 VCS 민들조개 집단이 VCW 집단과 어느 정도 차이가 있다는 것을 확인시켜 주었다.

INTRODUCTION

Various diagnostic skills, as reported earlier by many workers, have been applied to analyze the heredity of organisms such as the morphological trait(Orozco-Castillo *et al.*, 1994; Adams, 2000), the allozyme differentiation(Mamuris *et al.*, 1999; Diaz-Jaimes & Uribe-Alcocer, 2003), and various PCR-based molecular techniques including the restriction fragment length polymorphisms(RFLPs)(Jaiswal *et al.*, 1998), the amplified fragment length polymorphisms (AFLPs)(Das *et al.*, 1999), and the random amplified polymorphic DNAs(RAPD)(Callejas & Ochando, 1998; Diaz-Jaimes & Uribe-Alcocer, 2003; Kim & Choi, 2003; Kim *et al.*, 2006; Martínez *et al.*, 2006; Yoon, 2006).

Among them, RAPDs are the most frequently used molecular markers for taxonomic and systematic analyses of organisms(Adams, 2000). Generally, RAPD-PCR is one of fast and simple research methods to identify genetic difference and the polymorphism in various organisms, which does not require the prior knowledge of the genomic DNA (Iyengar *et al.*, 2000). Particularly, the polymorphic and/or specific markers peculiar to genus, species, breed and geographic population have been applied for individual/species/population discrimination, pedigree analysis and for the screening of DNA markers for marker-assisted selection and genotype-assisted selection, as reported earlier by many workers(Liu *et al.*, 1998; Tassanakajon *et al.*, 1998; Huang *et al.*, 2000; Geng *et al.*, 2002; Ramesha *et al.*, 2002). The outcome of RAPD analysis to identify diagnostic markers for breed, species and population identification in shellfish(Huang *et al.*, 2000; Yoon & Kim, 2003a; Kim *et al.*, 2004) and in crustacean(Klinbunga *et al.*, 2000b; Yoon & Kim, 2003b; Park *et al.*, 2005; Kim *et al.*, 2006; Yoon, 2006) has been established.

Venus clam(*Gomphina aequilatera*, Sowerby), which belongs to the family Veneridae, and the order Veneroida, constitutes an economically important bivalve mollusk. Venus clam is widely distributed in the mouth of a river, brackish-water habitats, a field of reeds and seawater areas of the East Sea, the West Sea and the southern sea in the Korean Peninsula. The clams inhabit in the estuary flats consisting of a lot of sand, mud and slime in the coastal tidal wetland. By the way, recently, the consumption of this bivalve species has also seen a considerable upshift as restaurants have begun to specialize in various clam recipes, including steamed stuffed clam in a pan.

The environmental requirements and tolerances of clam from different geographic areas remain unknown, as does its population structure. However, in general, the size, color and type of this bivalve mollusk vary according to environmental factors, such as topography, water depth, water temperature, nutrition, growth period and other common factors. 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 the artificial reproduction of the clam. Research in the artificial production of the clam has progressed steadily in many aspects, excessive hunting and water contamination by trade, industrial sewage and living 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.

As the clam culture industry grows, so doe's interest into the genetics of this shellfish species. However, little information currently exists regarding the genetics of venus clam. Until now, the gonadal development and reproductive cycle and the age, rickettsia-like organisms, cytochemical characteristics, and morphological characters in clams have been assessed by manifold ecological and biochemical researches, as reported earlier by many workers (Lee *et al.*, 1999; Park *et al.*, 2002; Jung *et al.*, 2004a).

We performed similarity matrix and clustering analyses to analyze genetic variations and DNA polymorphisms of

two venus clam(*G. aequilatera*) populations from the Samcheok and Wonsan of the Korean Peninsula.

MATERIALS AND METHODS

1. Sample Collection and gDNA Extraction

Two venus clam(*G. aequilatera*) populations were obtained from the Samcheok and Wonsan located in the East Sea of the Korean peninsula. The venus clam was collected, placed in the sterile tubes on dry ice immediately, and stored at −40℃ until the gDNA extraction. PCR analysis was performed on muscle extracts of the foot from 22 individuals, using seven different decamer primers. The extraction of gDNA was performed under conditions as described previously(Yoon & Kim, 2003b). After several washing, the lysis buffer I [155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA] was added to samples, and the mixture tubes were gently inverted. The precipitates obtained were centrifuged and resuspended with lysis buffer II [10 mM Tris-HCl(pH 8.0), 10 mM EDTA, and 100 mM NaCl, 0.5% SDS] and added 15 μL proteinase K solutions(10 mg/mL). After incubation, there was added 300 μL of 3 M NaCl and lightly pipetted for several minutes. Added not phenol, 600 μL of chloroform were added to the mixture and then inverted. DNA from the lysates was extracted by adding ice-cold 70% ethanol and centrifuged at 19,621 g for 5 min. The DNA pellets were incubation-dried for more than 2 hrs, held at −40℃ until needed and then dissolved in the pure water(JABA KOREA, Korea). The concentration of the extracted gDNA was measured with the optical density proportion at 260 nm by a spectrophotometer(Beckman Coulter, Buckinghamshire, UK).

2. The Oligonucleotides Primers, Molecular Markers and Amplification Circumstances

The oligonucleotides decamer primers chosen arbitrarily were purchased from Bioneer Corporation, Korea. Seven oligonucleotide primers showing sequences such as BION-21(5'-AATCGGGCTG-3'), BION-23(5'-CAGGCGGCGT-3'), BION-25(5'-CTGGCGGCTG-3'), BION-27(5'-TGCGCCGCGG-3'), BION-29(5'-GACATCTCGC-3'), BION-31(5'-AACGCGTAGA-3') and BION-33(5'-ACATCCTGCG-3') were used to generate the shared loci, polymorphic loci and specific loci that can be counted clearly and reproducibly. RAPD-PCR was performed using two Programmable DNA Thermal Cyclers(Perkin Elmer Cetus, Norwalk, CT, USA; MJ Research Inc., Waltham, MA, USA) as described previously (Yoon & Kim, 2004). 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. The DNA amplification was performed in 25 μL containing 10 ng of template DNA, 20 μL premix(Bioneer Corp., Daejeon, Korea) and the 1.0 unit primer. Amplification products were separated by 1.4% agarose(VentechBio, Korea) gel electrophoresis with TBE [90 mM Tris(pH 8.5), 90 mM borate, 2.5 mM EDTA]. The 100 bp DNA Ladder(Bioneer Corp., Daejeon, Korea) was used as DNA molecular weight marker and the bands electrophoresed were stained with ethidium bromide. The bands were illuminated with UV-ray and then photographed by photoman direct copy system(PECA Products, Beloit, WI, USA).

3. The Data Analyses

Only the unambiguously-counted bands ranged from 150 bp to 2,400 bp that were readily visible were scored for the statistical analyses. The bandsharing(BS) value was calculated by the presence/absence of amplified products at the specific positions in the same gel from the RAPD profiles. The degree of variability was calculated by use of the Dice coefficient, which is given by the formula: $BS = 2\ n_{ab}/(n_a+n_b)$, where n_{ab} is the number of bands shared between the samples a and b, n_a is the total number of bands for sample a and n_b is the total number of bands for sample b, as reported earlier by many workers(Jeffreys & Morton, 1987; Yoon & Kim, 2004; Yoke-Kqueen & Radu, 2006). A BS value of 1.000 indicates that the two samples are identical and a BS value if 0 indicates that the samples are different.

The average of within-populations similarity is calculated by the pairwise comparison between individuals within a population. The relevance among different individuals

of venus clam from Samcheok(SAMCHEOK 01~SAMCHEOK 11) and venus clam from Wonsan(WONSAN 12~WONSAN 22) generated according to the BS values and similarity matrix. 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). Significance was accepted for all tests at $p<0.001$. All data were expressed as mean±SE. The genetic difference and Euclidean genetic distance within- and between-population were also calculated with hierarchical dendrogram program Systat version 10.

RESULTS AND DISCUSSION

1. The Variation within-and between Populations

In spite of the variation in the RAPD profiles and the difference in reproducibility, many genetic researchers used the techniques because RAPD-PCR is a relatively rapid, reliable and conveniently useful method to investigate numerous genomic DNAs for the genetic diversity in a population as well as it does not require the prior knowledge of the genome, as reported earlier by many workers(Orozco-Castillo *et al.*, 1994; Iyengar *et al.*, 2000; Klinbunga *et al.*, 2000a & b; Kim & Choi, 2003). The polymorphisms are determined by the banding patterns of amplified products at the specific positions by primers(Tassanakajon *et al.*, 1998; Yoon & Kim, 2001). RAPD and/or RAPD-based techniques have been applied to the identification of the genetic features of diverse species of Veneridae(Jung *et al.*, 2004b). Genomic DNA isolated from two populations of *Gomphina* obtained from the East Sea was amplified by PCR reaction. The amplified products were separated by AGE with oligonucleotides decamer primer and stained with ethidium bromide.

In this study, seven decamer primers generated a total of 954 loci in VCS population and 996 in VCW population, with molecular sizes from 150 bp to 2,400 bp, as summarized in Table 1. In particular, the BION-27 primer gave DNA profiles with more loci than the other six primers in the VCS population and the BION-31 from VCW population(Table 1). DNA fragments ranging from 350 to 700 bp were also detected in the RAPD-PCR profile of barramundi(*Lates calcarifer*)(Partis & Wells, 1996). A tandemly repeated satellite DNA containing 290 bp~291 bp was identified by the *Sal*I digestion of the genomic DNA from five species of Eastern Pacific abalone using another molecular method(genus *Haliotis*)(Muchmore *et al.*, 1998). Eighty fragments ranging from 200 bp to 2,200 bp were unambiguously counted in the black tiger shrimp(*Penaeus monodon*)(Tassanakajon *et al.*, 1998). One to eight DNA bands were amplified, ranging from approximately 240 bp to 1,200 bp in seaweed *Hizikia fusiformis*(Park *et al.*, 1998).

Table 1. The number of loci observed per primer, shared loci, specific loci and polymorphic loci produced by RAPD analysis using 7 decamer oligonucleotide primers in *G. aequilatera* from Samcheok and Wonsan of Korea

Primer	No. of loci observed per primer		No. of shared loci by each population		No. of specific loci		No. of polymorphic loci	
	Samcheok	Wonsan	Samcheok	Wonsan	Samcheok	Wonsan	Samcheok	Wonsan
BION-21	124(11.3)	137(12.5)	11	33	2	9	13	5
BION-23	118(10.7)	99(9.0)	99	66	12	8	14	7
BION-25	144(13.1)	145(13.2)	66	88	4	14	18	23
BION-27	147(13.4)	137(12.5)	22	55	28	9	20	33
BION-29	136(12.4)	162(14.7)	33	22	16	16	30	18
BION-31	143(13.0)	168(15.3)	33	55	8	9	44	41
BION-33	142(12.9)	148(13.5)	22	33	5	5	8	47
Total no.	954(12.4)	996(12.9)	286	352	75	70	147	274
Average no. per primer	136.3	142.3	40.9	50.3	10.7	10.0	21.0	39.1

The average number of loci per lane generated by an oligonucleotide primer in Samcheok and Wonsan population, respectively, is shown in parentheses.

The total amplified products from the six isolates were: 143 bands from the Chungmu sample, 135 bands from Haenam, 72 bands from Kijang, 120 bands from Pusan, 136 bands from Wando, and 109 bands from Yosu. The size of the fragments varied from 220 bp to 1,700 bp in four species of the Mullidae family(Mamuris *et al.*, 1999).

The DNA fragments obtained using the four primers ranged from 100 bp to 2,300 bp in the brittle star(*Amphiura filiformis*)(McCormack *et al.*, 2000). In wild and cultured species of crucian carp, five primers generated a total of 1,084 distinct fragments ranging in size from 120 to> 4,270 bp(Yoon & Park, 2002). In the marsh clam from Gochang(*Corbicula* spp), 7 out of 20 primers generated 585 major and minor RAPD bands from three geographic sites, producing approximately 6.6 products per primer on average(Yoon & Kim, 2003a). Seven primers generated 317 bands in the cultured shrimp population and 385 in the wild population, ranging 100 bp to 1,800 bp(Yoon & Kim, 2003b). The primer OPF-10 produced 11 amplified fragments in the eastern Pacific yellowfin tuna(*Thunnus albacares*), with sizes ranging from 200 bp to 600 bp(Diaz-Jaimes & Uribe-Alcocer, 2003). Six primers were used to generate a total of 602 and 195 scorable bands in the catfish and bullhead populations, respectively, with DNA fragments ranging in size from<100 bp to>2,000 bp(Yoon & Kim, 2004). In livestock and insects, all 141 primers generated from Zebu cattle breeds ranged from 270 bp to 1,350 bp (Gwakisa *et al.*, 1994). Geng *et al.*(2002) also reported that 8 random primers generated 176 bp to 2,937 bp fragments in 3-goat populations from the China Chaidamu Basin. Finally, twenty-six primers produced a total of 137 polymorphic PCR-generated RAPD markers from the silkworm, ranging from 200 bp to 4,000 bp(*Bombyx mori*)(Hwang *et al.*, 1995).

In the present study, a decamer primer generated 136.3 of average number of per primer in VCS population from Samcheok. A RAPD primer generated 12.4 loci on average observed per sample and ranged from 10.7 to 13.4 loci in this VCS population from Samcheok. In VCS population, the primer BION-21 generated various sized bands, ranging from 200 bp to 1,200 bp, as summarized in Fig. 1. In VCS population, the banding patterns of the shared loci, 700 bp bands, were generated by the decamer primer BION-21, as shown in Fig. 1A. The banding patterns generated by decamer primer BION-25 of individual in VCS population varied widely, as shown in Fig. 1C. Moreover, the banding patterns also generated by decamer primer BION-33 of individual in VCW population varied widely, as shown in Fig. 1G. The complexity of the banding pattern varied widely between primers and/or geographically locales. Generally, the size and the number of the bands generated unbiased depend upon the nucleotide sequence of the primer used and on the source of the template DNA, resulting in a genome-specific DNA band(Welsh & McClelland, 1990).

In VCW population from Wonsan, the oligonucleotide decamer primer BION-25 generated 44 unique shared loci to each population, 350 bp, 400 bp, 500 bp and 700 bp, respectively, as shown in Fig. 1C(Table 2). The result in-

Table 2. The number of unique shared loci to each species and the number of shared loci by the two populations generated by RAPD PCR using 7 decamer oligonucleotide primers in *G. aequilatera* from Samcheok and Wonsan of Korea

Item	No. of unique shared loci to each population		No. of shared loci by the two populations
Primer\Population	Samcheok	Wonsan	Two localities
BION-21	0	22	11
BION-23	33	0	66
BION-25	22	44	44
BION-27	0	33	22
BION-29	11	22	22
BION-31	11	33	22
BION-33	0	11	22
Total no.	286	352	209
Average no. per primer	40.9	50.3	29.9

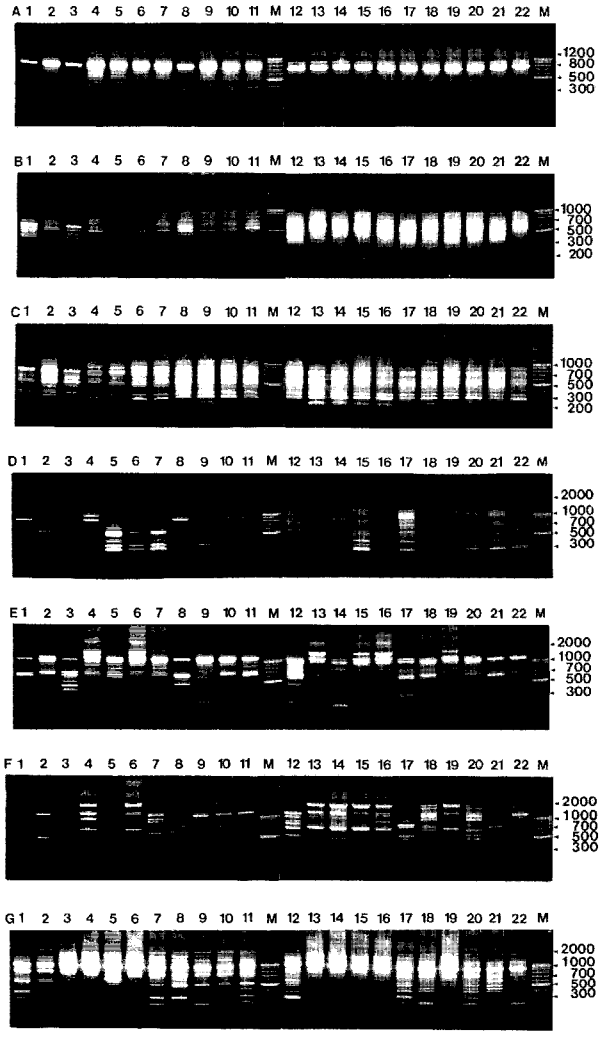

Fig. 1. PCR-based agarose gel electrophoretic outlines of the RAPD analysis on two venus clam(*G. aequilatera*) populations. DNAs isolated from twp populations collected at Samcheok(lane 1~11) and Wonsan(lane 12~22), respectively, were amplified by decamer primer BION-21 (A), BION-23 (B), BION-25 (C), BION-27 (D), BION-29 (E), BION-31 (F) and BION-33 (G). The 100 bp Ladder (M) was used as a DNA molecular marker.

dicates that the genome sizes of VCS population from Samcheok are similar to those in VCW population from Wonsan. The number of unique shared loci to each population and number of shared loci by the two populations were generated by RAPD-PCR using 7 random primers in *Gomphina* populations from Samcheok and Wonsan, respectively, as shown in Table 2. Two hundred eighty six unique shared loci to each population, with an average of 40.9 per primer, were observed in VCS population from Samcheok. Three hundred fifty two unique shared loci, with an average of 50.3 per primer, were identified in the VCW population from Wonsan. Especially, 209 numbers of shared loci by the two populations, with an average of 29.9 per primer, were observed in the two *Gomphina* populations. The decamer primer BION-23 generated the shared loci by the two populations, approximately 200 bp, 300 bp, 380 bp, 550 bp, 750 bp and 1,000 bp, between the two *Gomphina* populations(Fig. 1B, Table 2). The oligonucleotide primer BION-25 also generated the shared loci by the two populations, approximately 350 bp, 400 bp, 500 bp and 700 bp, in VCS population from Samcheok and VCW population from Wonsan, as shown in Fig. 1C. The other primers, BION-21, BION-27, BION-29, BION-31 and BION -33 generated the shared loci by two *Gomphina* populations. The results demonstrate that VCS population from Samcheok is genetically similar to VCW population from Wonsan.

Using numerous arbitrary primers, PCR-generated RAPD analyses have been applied to identify specific/polymorphic markers particular to breed, line, species and geographical population as well as genetic polymorphism/diversity in organisms(Partis & Wells, 1996; Callejas & Ochando, 1998; Klinbunga *et al.*, 2000a; Yoon & Kim, 2004; Yoon, 2006). In this study, 7 primers generated 147 polymorphic loci(147/954 loci, 15.41%) in VCS population and 274(274/996 loci, 27.51%) in VCW population, as illustrated in Table 1. The results demonstrate that a primer detects a great deal of polymorphic loci. This also suggests the genetic variation in VCW population is higher than in VCS population.

A total of 88 polymorphic fragments were scored from 24 primers after excluding the bands that were monomorphic for the five clones of silver crucian carp, *Carassius auratus gibelio* Block(Zhou *et al.*, 2000). Iyengar *et al.* (2000) used a RAPD-based technique to identify several microsatellite repeats in the turbot(*Scophthalmus maximus*) and Dover sole(*Solea solea*) and report the characterizations of six novel polymorphic microsatellite markers for Dover sole. 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. Fifty-three RAPD markers obtained from 7 primers were common to all breeds, 22 were specific to individuals, and 18 were polymorphic in the different breeds of zebu cattle (Ramesha *et al.*, 2002). Four of the fragments were polymorphic for all samples in eastern Pacific yellowfin tuna(*Thunnus albacares*)(Diaz-Jaimes & Uribe-Alcocer, 2003).

Upon RAPD analysis of genetic differences and characteristics in wild and cultured crucian carp populations, the pattern of polymorphic fragments of fifty individuals in the wild population was reported to be different(Yoon & Park, 2002). Six primers generated 47 polymorphic fragments (24% of 195 fragments) in a bullhead population(Yoon & Kim, 2004). 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). Islam *et al.* (2005) reported that 32 fragments were yielded from four primers in the Indian major carp(*Catla catla*) of which 24 (75%) were polymorphic. Eight primers generated 27 polymorphic fragments(27/510 fragment, 5.3%) in the Korean lobster(*Ibacus ciliatus*), and 42(42/526 fragments, 8.0%) in the Indian Ocean lobster(*Puerulus sewelli*)(Park *et al.*, 2005).

Here, 28 specific loci generated by the decamer primer BION-27 exhibited the inter-individual-specific characteristics and DNA polymorphisms, as shown in Fig. 1D and Table 1. We have also identified 200 bp RAPD-PCR-amplified specific loci(lane 3) and 500 bp(lane 3) in VCS population from Samcheok, as shown in Fig. 1A. Especially, the 700 bp bands generated by the primer BION-21 were identified commonly in two *Gomphina* populations, which identified populations and/or species, as shown in Fig. 1A. The specific primer was found to be useful in the identification of individuals and/or population, resulting from the different DNA polymorphism among individuals/species/population(Yoon & Park, 2002; Yoon & Kim, 2003a; Yoon, 2006). Although the main disadvantage of the RAPD method is its reproducibility, the method was considered suitable for the identification of a species and/or population. Three out of 40 nanomer primers that amplified DNA bands specific to a grass species or to a durum cultivar were identified(Bommineni *et al.*, 1997). The primer PR21 amplified DNA bands specific to five individual durum cultivars. The primers PR 22 amplified bands specific to a grass species. Similarly, the primer PR23 amplified bands specific to a grass species. The amplified bands ranging from 1,000 bp to 1,200 bp were specific to *Thinopyrum junceiforme*. The DNA band approximately 1.5 kb was specific to *Lophopyrum elongatum*. Generally, polymorphic loci generated by RAPD-PCR using arbitrary primers were suitable to detect genetic similarity/diversity among various organisms(Bommineni *et al.*, 1997).

Generally speaking, using a variety of oligonucleotide primers, RAPD-PCR has been applied to identify polymorphic/specific markers particular to line, breed, species, genus and geographical population, as well as genetic similarity/polymorphism in various organisms(Muchmore *et al.*, 1998; Kim *et al.*, 2000; McCormack *et al.*, 2000; Park *et al.*, 2005). The specific primer was found to be useful in the identification of individuals and/or populations, resulting from variations in DNA polymorphisms among individuals/populations, as described earlier by many researchers(Liu *et al.*, 1998; Yoon & Park, 2002; Yoon & Kim, 2003b; Yoon & Kim, 2004).

2. The Genetic Distances and Hierarchical Clustering Analysis

In the present study, the bandsharing value based on the presence or absence of amplified bands was utilized to calculate the similarity indices, as illustrated in Table 3. The similarity matrix based on the average bandsharing value was 0.859±0.004 for VCS population from Samcheok and 0.916±0.006 for VCW population from Wonsan. Bandsharing value between two *Gomphina* populations ranged from 0.250 to 0.415 with the average 0.340±0.003. The value difference between the two *Gomphina* populations is statistically significant($p<0.001$). Compared individuals separately, the bandsharing value of individuals for VCW population was higher than that for VCS population. Even if seaweed, our bandsharing values between two *Gomphina* populations re similar to the result of Park *et al.*(1998)

Table 3. Similarity matrix calculated using the Dice coefficient, which is given by the formula: $BS=2n_{ab}/(n_a+n_b)$, of venus clam from Samcheok and Wonsan. Bandsharing values of venus clam from two geographical regions are above the diagonal and genetic differences are below the diagonal

		Bandsharing values of venus clam from Samcheok											Bandsharing values of venus clam from Wonsan										
		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 venus clam from Samcheok	1		0.781	0.808	0.608	0.764	0.725	0.562	0.667	0.712	0.673	0.589	0.344	0.292	0.243	0.335	0.359	0.339	0.288	0.325	0.412	0.323	0.304
	2	0.219		0.720	0.636	0.687	0.669	0.591	0.623	0.638	0.601	0.613	0.370	0.340	0.279	0.279	0.384	0.356	0.333	0.321	0.354	0.337	0.334
	3	0.192	0.280		0.669	0.753	0.783	0.626	0.597	0.733	0.687	0.672	0.390	0.344	0.255	0.382	0.427	0.356	0.360	0.349	0.396	0.340	0.367
	4	0.392	0.364	0.331		0.697	0.684	0.717	0.690	0.719	0.620	0.682	0.506	0.438	0.405	0.386	0.451	0.407	0.401	0.433	0.386	0.421	0.454
	5	0.236	0.313	0.247	0.303		0.805	0.679	0.650	0.733	0.681	0.649	0.433	0.355	0.268	0.382	0.397	0.364	0.356	0.430	0.423	0.380	0.319
	6	0.275	0.331	0.217	0.316	0.195		0.722	0.615	0.744	0.728	0.609	0.367	0.297	0.294	0.359	0.408	0.375	0.370	0.396	0.361	0.376	0.355
	7	0.438	0.409	0.374	0.283	0.321	0.278		0.608	0.791	0.702	0.675	0.384	0.323	0.317	0.359	0.433	0.376	0.398	0.393	0.325	0.414	0.482
	8	0.333	0.377	0.403	0.310	0.350	0.385	0.392		0.665	0.741	0.713	0.395	0.376	0.347	0.380	0.437	0.431	0.403	0.465	0.417	0.374	0.387
	9	0.288	0.362	0.267	0.281	0.267	0.256	0.209	0.335		0.764	0.710	0.446	0.390	0.401	0.443	0.486	0.453	0.456	0.458	0.411	0.376	0.430
	10	0.327	0.399	0.313	0.380	0.319	0.272	0.298	0.259	0.236		0.776	0.467	0.363	0.357	0.469	0.489	0.467	0.415	0.450	0.381	0.461	0.488
	11	0.411	0.387	0.328	0.318	0.351	0.391	0.325	0.287	0.290	0.224		0.457	0.426	0.402	0.420	0.441	0.452	0.450	0.437	0.360	0.443	0.462
Genetic differences of venus clam from Wonsan	12	0.656	0.630	0.610	0.494	0.567	0.633	0.616	0.605	0.554	0.533	0.543		0.807	0.655	0.667	0.603	0.707	0.723	0.750	0.492	0.679	0.635
	13	0.708	0.660	0.656	0.562	0.645	0.703	0.677	0.624	0.610	0.637	0.574	0.193		0.691	0.620	0.622	0.688	0.648	0.731	0.482	0.637	0.610
	14	0.757	0.721	0.745	0.595	0.732	0.706	0.683	0.653	0.599	0.643	0.598	0.345	0.309		0.665	0.660	0.742	0.597	0.685	0.445	0.609	0.588
	15	0.665	0.721	0.618	0.614	0.618	0.706	0.641	0.620	0.557	0.531	0.580	0.333	0.380	0.335		0.760	0.683	0.588	0.696	0.556	0.582	0.630
	16	0.641	0.616	0.573	0.549	0.603	0.592	0.624	0.563	0.514	0.511	0.559	0.397	0.378	0.340	0.240		0.743	0.614	0.673	0.590	0.558	0.669
	17	0.661	0.644	0.644	0.593	0.636	0.625	0.624	0.569	0.547	0.533	0.548	0.293	0.312	0.258	0.317	0.257		0.655	0.714	0.526	0.659	0.682
	18	0.712	0.667	0.640	0.599	0.644	0.630	0.602	0.597	0.544	0.585	0.550	0.277	0.352	0.403	0.412	0.386	0.345		0.727	0.486	0.699	0.626
	19	0.675	0.679	0.651	0.567	0.570	0.604	0.607	0.535	0.542	0.550	0.675	0.250	0.269	0.315	0.304	0.327	0.286	0.273		0.566	0.678	0.583
	20	0.588	0.646	0.604	0.614	0.577	0.639	0.675	0.583	0.589	0.619	0.640	0.508	0.518	0.555	0.444	0.410	0.474	0.514	0.434		0.543	0.546
	21	0.677	0.663	0.660	0.579	0.620	0.624	0.586	0.626	0.624	0.539	0.557	0.321	0.363	0.391	0.418	0.442	0.341	0.301	0.322	0.457		0.640
	22	0.696	0.666	0.633	0.546	0.681	0.645	0.518	0.613	0.570	0.12	0.538	0.365	0.390	0.412	0.370	0.331	0.318	0.374	0.417	0.454	0.360	

who reported that the genetic similarity for the six isolates of seaweed *Hizikia fusiformis* ranged from 0.23 to 0.59. Our bandsharing values are also similar to previously reported results of Kim *et al.*(1997) in which similarity values obtained by RnRc primer analysis of nuclear DNA varied from 0.364 to 0.714 between *Porphyra tenera*(wild) and *Porphyra tenera*(Ariake). On the contrary, the bandsharing values are lower than the results of Kim *et al.* (1997) reported that similarity values of *Porphyra* chloroplast DNA were high and ranged from 0.727 to 1.000.

As compared with VCS population or VCW population, the bandsharing values are also higher than previously reported results in which the average bandsharing value obtained by five random primers was in Spanish barbel species(0.71~0.81)(Callejas & Ochando, 1998), in the cultured population(0.69±0.08)(Yoon & Park, 2002), between the species common carp and Israeli carp(0.57±0.03)(Yoon, 2001) and in bullhead population(0.504±0.115)(Yoon & Kim, 2004).

The average genetic difference was 0.141±0.004 within VCS population from Samcheok and 0.084±0.006 within VCW population from Wonsan. As compared individuals separately, the average genetic difference was higher in VCS population than in VCW population. The difference between the two *Gomphina* populations is statistically significant($p<0.001$). Accordingly, as mentioned above, RAPD

analysis showed that VCS population was more or less separated from VCW population.

Based on the similarity matrix generated by bandsharing values and genetic distances, hierarchical clustering analysis was performed to obtain the dendrogram, as shown in Fig. 2. In this study, the genetic distance between the two geographical venus clam populations ranged from 0.053 to 0.605. The dendrogram obtained by the seven primers, indicates two genetic clusters. The longer genetic distance displaying significant molecular differences was between the individual WONSAN no. 22 and WONSAN no. 20 between two *Gomphina* populations(0.454). Especially, two *Gomphina* between the individual SAMCHEOK no. 03 and WONSAN no. 22 showed the longest genetic distance (0.696) in comparison with other individuals used, as illustrated in Fig. 2. The shortest genetic distance displaying significant molecular difference was between individuals

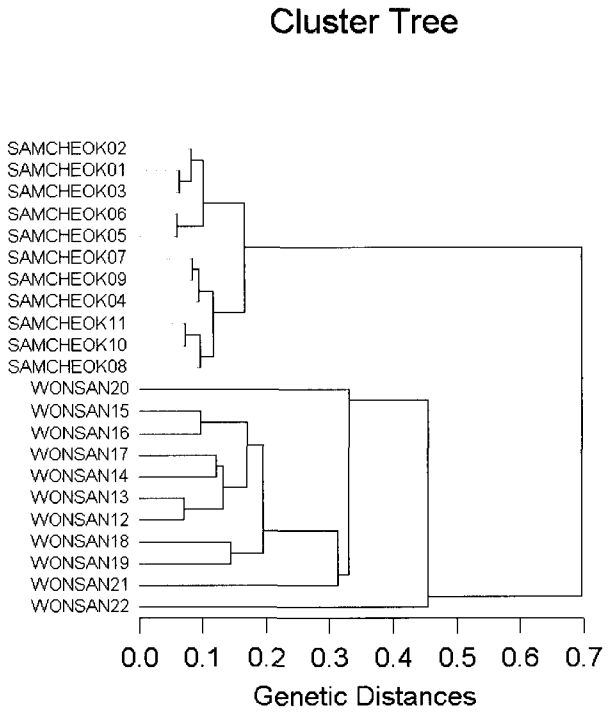

Fig. 2. Representation of hierarchical dendrogram of genetic distances obtained from two venus clam populations. The relevance among different individuals of venus clams from Samcheok(SAMCHEOK 01~SAMCHEOK 11), venus clams from Wonsan(WONSAN 12~WONSAN 21) and venus clam from Wonsan(WONSAN 22) generated by Systat program using the similarity matrix demonstrated in Table 3.

SAMCHEOK no. 06 and SAMCHEOK no. 05 from Samcheok(genetic distance=0.060). Our cluster analysis showed the similar pattern illustrated by Kim *et al.*(2006) in which the complete linkage cluster analysis indicating four genetic groupings and dendrogram revealed close relationships between individual identities within two geographical populations of crayfish(*Cambaroides similis*) from Jeongup and Jeonju. The genetic similarity for the six isolates of seaweed *Hizikia fusiformis* ranged from 0.23 to 0.59(Park *et al.*, 1998). Especially, isolates collected from Kijang and Chungmu showed that they are most distantly related to the others based on genetic similarity(0.23).

In shellfishes and crustaceans, 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). 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. 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). Ultimately, they 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 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 shortest genetic distance displaying significant molecular differences, 0.080, was found to exist between individuals' no. 09 and no. 07 from Buan. The genetic distance between the Indian Ocean lobster and the Korean Slipper lobster species ranged between 0.040 and 0.612(Park *et al.*, 2005). In particular, the longest genetic distance displaying significant molecular differences was determined to exist between individuals in the two lobster species, namely between individuals SLIPPER no. 04 of the Korea lobster species and DEEPSEA no. 16 of the

Indian Ocean lobster species(genetic distance=0.612). In their study, the dendrogram obtained with the eight primers also indicates two genetic clusters, designated cluster 1(SLIPPER 01~SLIPPER 11), and cluster 2(DEEPSEA 12~DEEPSEA 22).

Consequently, as stated above, RAPD analysis showed that the VCS were more genetically diverse than the VCW population. This result implies the genetic similarity owing to rearing in the same and/or similar circumstances or inbreeding within the VCW species. So to speak, KPS species may have high levels of gDNA variability owing to the introduction of the wild individuals from the other sites to sampling sites although it may be the geographically diverse distribution of this species.

In our study, RAPD-PCR analysis revealed a significant genetic distance between two *Gomphina* population pairs ($p<0.001$). The existence of population discrimination and DNA polymorphism between two *Gomphina* populations was detected by RAPD analysis. This shows that the method can be an adequate tool to compare DNA in individuals, species and/or populations. Furthermore, the basic knowledge of DNA polymorphisms and molecular markers of *Gomphina* populations may contribute significantly to the seedling selection and the selective clam-breeding program. The genetic identification of black tiger shrimp(*Penaeus monodon*), penaeid shrimp(*Penaeus chinensis*), oyster(*Crassostrea* sp.), crayfish(*Cambaroides similis*) and freshwater crab(*Eriocheir sinensis*) populations is a necessary step for invertebrate/crustacean breeding programs(Tassanakajon *et al.*, 1998; Yoon & Kim, 2003a; Kim *et al.*, 2004; Kim *et al.*, 2006; Yoon, 2006).

The classification of geographical populations/species of *Gomphina* needs to be based on the morphological variation in shell shape, shell size, shell type, and shell color. Siti Azizah *et al.*(2005) identified the RAPD markers using the morphology and external features in wild and cultured populations of eel-loach(*Pangio* sp.). Phylogenetic relationships of the five Korean clams(Bivalvia, Veneroida) have been demonstrated by morphological characters and RAPD markers(Jung *et al.*, 2004a & b).

From what has been said above, the potential of RAPD to identify diagnostic markers for stock, species and population identification in clams(Klinbunga *et al.*, 2000a; Yoon & Kim, 2003a; Jung *et al.*, 2004b; Kim *et al.*, 2004) has also been demonstrated. Nevertheless, further analysis with more individuals, primers and species will be required to establish fully the specificity of loci to particular taxa and subsequent inter-specific gene flow in the genus *Gomphina*. Further sampling sites and isolates will be necessary to determine precisely the area where the phylogeographic break occurs. In the future, PCR-generated RAPD markers will be necessary for characterization of the different geographical venus clam species to correlate with the morphological characters and for clarification of the ambiguity among species and/or geographic populations. Both more time and more researches, will also be necessary to identify the differentially expressed genes(DEG) between/among populations and species, using an annealing control primer (ACP) system.

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