Geographic Variations between Jedo Venus Clam (*Protothaca jedoensis*, Lischke) Populations from Boryeong and Wonsan of Korea

Gi-Sik Park[1] and Jong-Man Yoon[2*]

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

ABSTRACT

GDNA was isolated from the jedo venus clam (*Protothaca jedoensis, Lischke*) from Boryeong (jedo venus clam from Boryeong JVCB) and Wonsan (jedo venus clam from Wonsan; JVCW) located in the West Sea and the East Sea of Korean Peninsula, respectively and we performed clustering analyses, DNA polymorphisms and the populations genetic variations. In the present study, the seven decamer primer generated the one hundred and eleven major/minor specific bands in JVCB population and ninety four-specific bands in JVCW population. Seven primers generated the unique shared bands to each population of one hundred and seventy-six, on average of 25.1, in JVCB population from Boryeong and three hundred thirty, on average of 47.1, in JVCW population from Wonsan, respectively. The dendrogram obtained by the seven oligonucleotides primers, indicates two genetic clusters. Especially, two *Protothaca* between the individual WONSAN no. 12 and BORYEONG no. 10 showed the longest genetic distance (0.537) in comparison with other individuals used. Accordingly, RAPD analysis showed that the JVCB was a little more genetically diverse than the JVCW population. This result implies the genetic similarity owing to rearing in the same and/or similar circumstances or inbreeding within the JVCW population. So to speak, JVCB population may have high levels of genomic DNA variability owing to the introduction of the wild individuals from the other sites to sampling sites although it may be the geographically diverse distribution of this species. However, it was confirmed that it did not appear like that really in this study. We feel convinced that RAPD analysis discovered a significant genetic distance between two *Protothaca* population pairs (P〈0.001). The existence of population discrimination and genetic diversity between two *Protothaca* populations was identified by RAPD analysis.

Key words: DNA variability, Genetic distance, Jedo venus clam, Korean peninsula, *Protothaca jedoensis*

INTRODUCTION

The random amplified polymorphic DNAs (RAPD)-PCR method for investigation of genomic

DNAs in a population in addition to the no prior knowledge of the genome (Callejas and Ochando, 1998; Kim *et al.*, 2000; Diaz-Jaimes and Uribe-Alcocer, 2003; Yoon and Kim, 2003b; Kim *et al.*, 2004; Kim *et al.*, 2006; Yoon, 2006). Particularly, the specific markers peculiar to genus, species, and geographic population have been applied for individual/species/population discrimination, and for the screening of DNA markers for marker-assisted selection and genotype-assisted selection (Tassanakajon *et al.*, 1998; Huang *et al.*, 2000; Geng *et al.*, 2002). Until now, polymorphic bands generated by RAPD-PCR using arbitrary primers had good merits for detecting DNA diversity between life organisms (Yoon and Kim, 2001). The potential of RAPD analysis to identify diagnostic markers for stock, species and population identification in teleosts (Callejas and Ochando, 1998; Siti Azizah *et al.*, 2005; Yoon *et al.*, 2007), in shellfish (Huang *et al.*, 2000; Yoon and Kim, 2003a; Kim *et al.*, 2004), and in crustacean (Yoon and Kim, 2003b; Park *et al.*, 2005; Kim *et al.*, 2006) has been demonstrated.

Jedo venus clam (*Protothaca jedoensis*, Lischke), which belongs to the family Veneridae, and the order Veneroida, constitutes an economically important bivalve species. This species is widely distributed in the estuary flat, the intertidal zone and the 10-meter depth of seawater areas of the East Sea, the West Sea and the Southern Sea in Korean Peninsula. In general, the color, type, and size of this bivalve vary in accordance with water depth, water temperature, turbidity, nutrition, growth period, and other environmental aspects. Research for clam artificial production has progressed steadily in many aspects, over-catching, and water pollution by industries and city sewage. As the clam preservation increases, the understanding of the genetics of this clam species

becomes more necessary for the evaluation of the potential genetic effects induced by clam artificial reproduction. The reproductive cycle, age and growth, sexual maturation and feeding selectivity in jedo venus clams have been assessed by various biological methods (Kim *et al.*, 2002; Kim *et al.*, 2003; Kim *et al.*, 2003; Jo *et al.*, 2004). As the clam culture industry grows, so doe's interest into the genetics of this shellfish species. However, only a little information presently exist with reference to the genetics of jedo venus clam or other clam (Yoon and Kim, 2003a; Jung *et al.*, 2004a; Jung *et al.*, 2004b).

Accordingly, we performed clustering analyses, DNA polymorphisms and the genetic variations of two jedo venus clam (*P. jedoensis*) populations from the Boryeong and Wonsan of Korea, respectively.

MATERIALS AND METHODS

Sample collection and extraction of genomic DNA

Two jedo venus clam populations of *P. jedoensis* were gained from the Boryeong and Wonsan located in the West Sea and the East Sea of Korean peninsula, respectively, as shown in Fig. 1. Especially, individuals of jedo venus clam from Wonsan were imported from North Korea. RAPD analysis was performed on the foot muscle extracts from 22 individuals, using seven different decamer primers.

The extraction of genomic DNA was performed under conditions as described (Yoon and 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
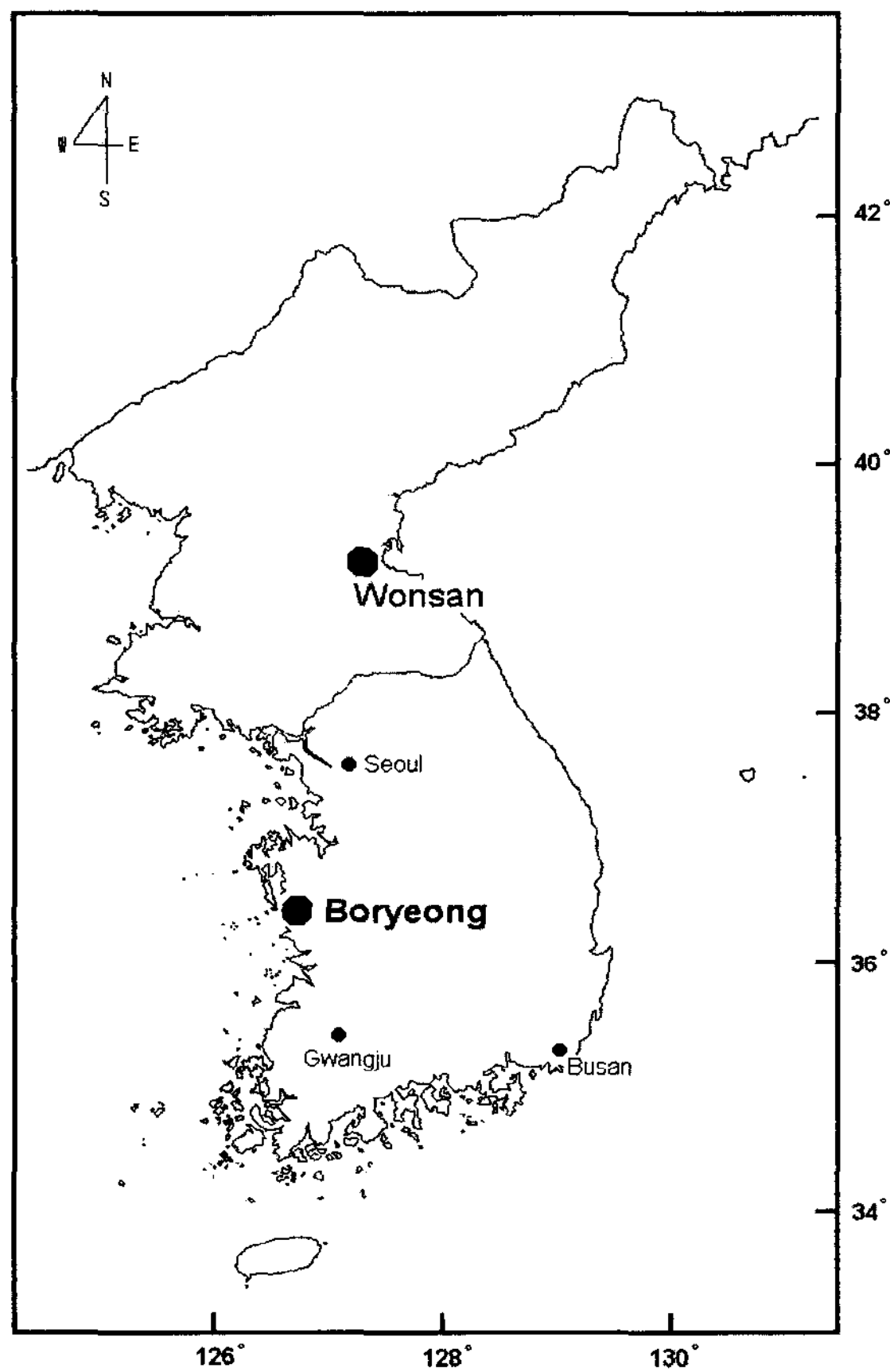
Fig. 1. Map illustrating sampling localities of jedo venus clam (*P. jedoensis*). Individuals from Wonsan were imported from North Korea.

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 solution (10 mg/ml). After incubation, there was added 300 μl of 3 M NaCl and gently pipetted for a few of min. 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 concentration of the extracted genomic DNA was calculated based on the absorbance at 260 nm by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK).

The oligonucleotides decamer primers and the amplification condition

The decamer primer chosen arbitrarily were obtained from Bioneer Corporation, Korea. The G+C content of the primers is between 60~70%. Seven primers, OPA-04 (5'-AATCGGGCTG-3'), OPA-07 (5'-GAAACGGGTG-3'), OPA-08 (5'-GTGACGTAGG-3'), OPA-11 (5'-CAATCGCCGT-3'), OPD-08 (5'-GTGTGCCCCA -3'), OPD-05 (5'-TGAGCGGACA-3') and OPD-18 (5'-GAGAGCCAAC-3') were exposed to generate the bands observed per primer, shared bands by each population, specific bands, unique shared bands to each population and shared bands by the two populations that can be scored clearly and reproducibly. I thus used the primers to identify the genetic similarity and/or variations of two jedo venus clam populations. RAPD-PCR 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. 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 step DNA Ladder (Bioneer Corp., Daejeon, Korea) was used as DNA molecular weight marker. The agarose gels expressed by electrophoresis were stained with ethidium bromide. The bands were illuminated with ultraviolet ray and then photographed by photoman

direct copy system (PECA Products, Beloit, WI, USA).

The statistical analysis

The primers that generate minor bands were excluded from the analysis. Only the bands ranged from 100 bp to 2,000 bp that were readily visible were scored for the statistical analysis. The bandsharing (BS) values were calculated according to Jeffreys and Morton (1987), 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 (Jeffreys and Morton, 1987; Yoon and Kim, 2003b; Yoke-Kqueen and Radu, 2006).

The average of within-populations similarity is calculated by the pairwise comparison between individuals within a population. The relatedness among different individuals of jedo venus clam from Boryeong (BORYEONG 01 ~ BORYEONG 11) and jedo venus clam from Wonsan (WONSAN 12 ~ WONSAN 22) generated in reference to the bandsharing 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). The genetic difference and Euclidean genetic distance within- and between-populations were calculated with hierarchical dendrogram program Systat version 10. All data were expressed as mean ± SE. Significance was accepted for all tests at P⟨0.001.

RESULTS AND DISCUSSION

The variation within-and between populations

Seven decamer primers produced a total of 707 bands in JVCB population and 960 in JVCW population with DNA band size from 100 bp to 2,000 bp, as summarized in Table 1. The banding patterns generated by seven decamer primers of individual in JVCB population varied widely, as shown in Fig. 2C. Particularly, the OPA-08 primer gave DNA profiles with more bands than the other six primers in the JVCB population and the OPA-08 also in JVCW population (Table 1). Inversely, the decamer primer, OPA-04, generated the less bands (a total of 62), with an average of

Table 1. The number of bands observed, number of shared bands by each population and number of specific bands generated by RAPD analysis using 7 random primers in *P. jedoensis* from Boryeong and Wonsan of Korea, respectively.

Item	No. of bands observed per primer		No. of shared bands by each population		No. of specific bands	
Primer	Boryeong	Wonsan	Boryeong	Wonsan	Boryeong	Wonsan
OPA-04	62(5.6)	114(10.4)	11	88	9	6
OPA-07	131(11.9)	136(12.4)	77	22	14	27
OPA-08	201(18.3)	207(18.8)	143	110	18	6
OPA-11	102(9.3)	11.2(10.2)	55	77	11	21
OPD-08	63(5.7)	113(10.3)	22	33	21	12
OPD-05	75(6.8)	144(13.1)	11	66	26	13
OPD-18	73(6.6)	134(12.2)	11	88	12	9
Total no.	707(9.2)	960(12.5)	330	484	111	94
Average no.per primer	101	137.1	47.1	69.1	15.9	13.4

The average number of bands per lane generated by a primer in Boryeong and Wonsan population is shown in parentheses.
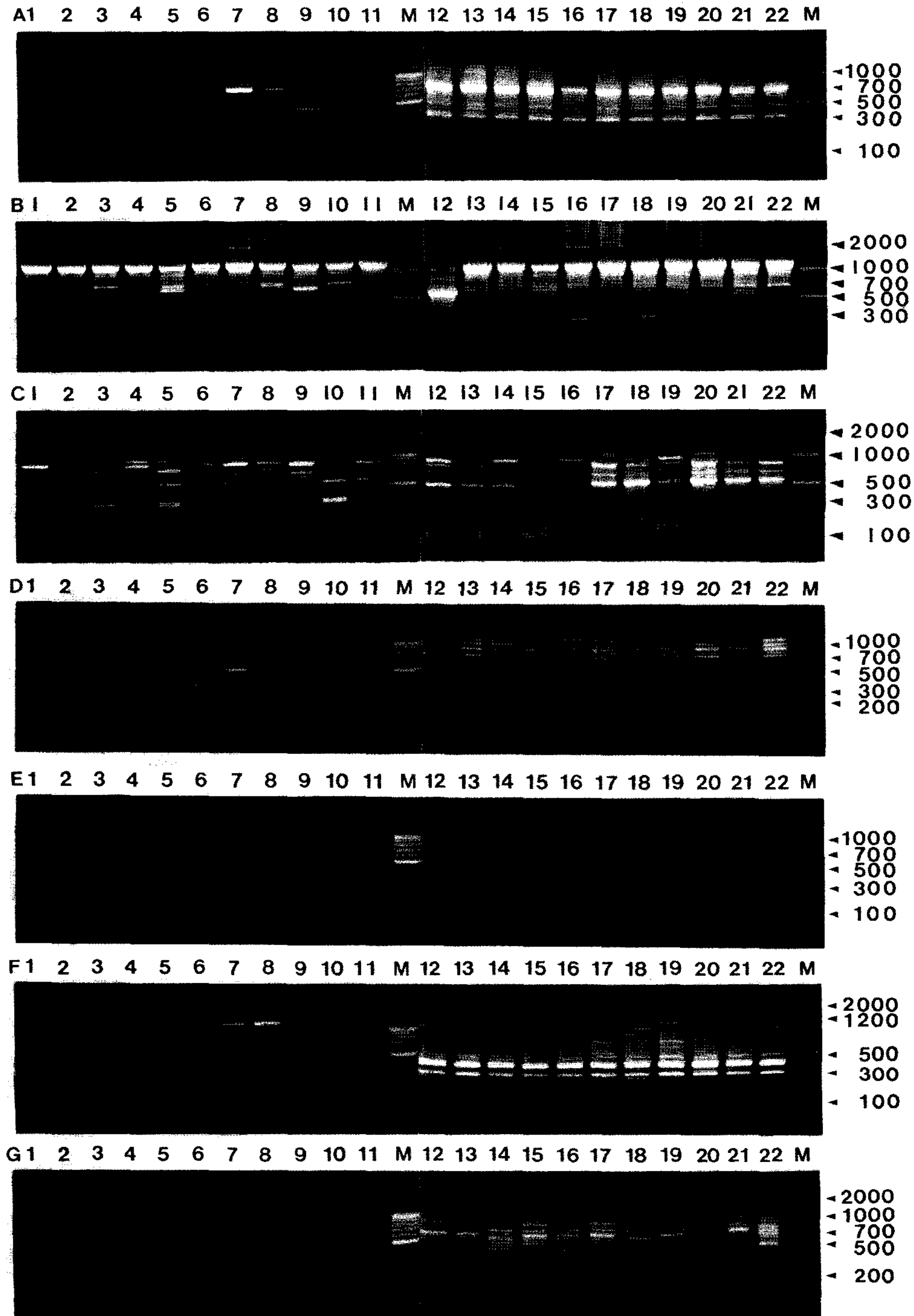
Fig. 2. PCR-founded electrophoretic outlines of two jedo venus clam (*P. jedoensis*) populations. DNAs isolated from two geographical populations collected at Boryeong (lane 1 ~ 11) and Wonsan (lane 12 ~ 22), respectively, were amplified by random primer OPA-04 (A), OPA-07 (B), OPA-08 (C), OPA-11 (D), OPD-08 (E), OPD-05 (F) and OPD-18 (G). Amplified products were electrophoresed on a 1.4% agarose gel and detected by staining with ethidium bromide. The electrophoresed agarose gels were illuminated by ultraviolet rays, and photographed using a photoman direct copy system. Each lane shows DNA samples from 22 individuals (44 individuals are specimen numbers for duplicate experiments). 100 bp step DNA ladder markers are used as a molecular weight.

5.6, in comparison to the other primers used in JVCB population (Table 1). OPA-11 primer also generated the less bands (a total of 112), with an average of 10.2, in JVCW population.

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). 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). The primer OPF-10 produced 11 amplified fragments in the Eastern Pacific yellowfin tuna (*Thunnus albacares*), with sizes ranging from 200 to 600 bp (Diaz-Jaimes and Uribe-Alcocer, 2003). In the marsh clam from Gochang (*Corbicula* sp.), 7 out of 20 primers generated 585 major and minor RAPD bands from three geographic sites, producing approximately 6.6 fragments per primer on average (Yoon and 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 and Kim, 2003b). In other vertebrates and invertebrates, 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-based RAPD markers from the silkworm, ranging from 200 bp to 4,000 bp (*Bombyx mori*) (Hwang *et al.*, 1995).

Here, we first assessed genetic variation in JVCB population. The banding patterns of the bands by each population showed a total of 330, with an average of 47.1, as illustrated in Table 1. Especially, 143 bands were generated by the dec-

amer primer OPA-08, as illustrated in Table 1. Secondly, in JVCW population, seven primers generated the RAPD profile of the shared bands by each population with four hundred eighty-four DNA bands, as illustrated in Table 1.

Interestingly, the primer OPA-07 generated the less shared bands by each population with twenty-two bands. Especially, the 480 bp and 1,200 bp bands, respectively, generated by the primer OPA-07 were identified commonly in JVCW populations, which identified individuals, as shown in Fig. 2B.

The primer generated the one hundred eleven major/minor specific bands in JVCB population and ninety four-specific bands in JVCW population, as illustrated in Table 1. I have identified 250 bp RAPD-PCR amplified specific bands (lanes 2 and 6), 300 bp (lanes 4, 5, 7, 8 and 9), 650 bp (lane 9) and 900 bp (lane 7) in JVCB population from Boryeong, as shown in Fig. 2A. Interestingly, the primer also generated six major/minor specific bands in JVCW population: 150 bp (lane 12), 400 bp (lanes 13, 14 and 17) and 1,000 bp (lanes 13 and 14), as shown in Fig. 2A. Six major/minor specific bands were generated by the primer OPA-08 in JVCW population: 300 bp (lanes 12, 15 and 18) and 400 bp (lanes 12, 18 and 20), as shown in Fig. 2C. Especially, the twenty-six specific bands in JVCB population and twenty-seven specific bands in JVCW population generated by the primer OPD-05 and OPA-07, respectively, exhibited inter-individual-specific characteristics, thus revealing DNA polymorphisms. Here, 12 specific bands generated by the decamer primer OPD-18 exhibited the inter-individual-specific characteristics and DNA polymorphisms in JVCB population, as shown in Fig. 2G and Table 1. 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 on the nucleotide sequence of the primer used and on the source of the template DNA, resulting in a genome-specific DNA band (Welsh and McClelland, 1990).

In this study, 7 primers generated 111 specific bands (111/707 bands, 15.70%) in JVCB population and 94 (94/960 bands, 9.79%) in JVCW population, as illustrated in Table 1. The results display that a primer detects plenty of specific loci. This also suggests the genetic variation in JVCB population is higher than in JVCW population. 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. Generally speaking, PCR-based RAPD analyses have been applied to identify specific markers particular to species, genus and geographical population, as well as genetic diversity/similarity in various organisms (Kim et al., 2000; McCormack et al., 2000; Park et al., 2005). Polymorphisms are determined by the banding patterns of primer-amplified products

at specific positions (Smith et al., 1997; Tassanakajon et al., 1998; Yoon and Kim, 2001). 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 (Callejas and Ochando, 1998; Yoon and Park, 2002; Siti Azizah et al., 2005; Yoon, 2006).

Bandsharing values and the genetic distances

The number of unique shared bands to each population and number of shared bands by the two populations were generated by RAPD-PCR using 7 random primers in *Protothaca* populations from Boryeong and Wonsan, respectively, as shown in Table 2. Seven decamer primers generated the unique shared bands to each populations of one hundred seventy-six, on average of 25.1, in JVCB population from Boryeong and three hundred thirty, on average of 47.1, in JVCW population from Wonsan, respectively, as illustrated in Table 2. Especially, in JVCB population from Boryeong, the decamer primer OPD-08 generated 11 unique shared bands to

Table 2. The number of unique shared bands to each population and number of shared bands by the two populations produced by PCR analysis using seven oligonucleotides primers in *P. jedoensis* from Boryeong and Wonsan of Korean Peninsula, respectively

Item	No. of unique shared bands to each population		No. of shared bands by the two populations
Primer / Population	Boryeong	Wonsan	Two localities
OPA-04	0	77	11
OPA-07	55	0	22
OPA-08	77	44	66
OPA-11	22	44	33
OPD-08	11	22	11
OPD-05	0	55	11
OPD-18	11	88	0
Total no.	176	330	154
Average no. per primer	25.1	47.1	22.0

Table 3. Similarity matrix including bandsharing values calculated using Nei and Li's index of the similarity of jedo venus clam from Boryeong and Wonsan

Bandsharing values of jedo venus clam																						
Boryeong											Wonsan											
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
-	0.690	0.868	0.785	0.749	0.681	0.757	0.747	0.796	0.798	0.773	0.501	0.567	0.586	0.529	0.555	0.582	0.567	0.551	0.561	0.573	0.504	1
	-	0.776	0.713	0.679	0.735	0.741	0.764	0.731	0.743	0.734	0.592	0.632	0.638	0.594	0.609	0.605	0.622	0.597	0.618	0.612	0.617	2
		-	0.871	0.830	0.706	0.737	0.779	0.825	0.835	0.771	0.555	0.628	0.630	0.581	0.593	0.586	0.607	0.583	0.572	0.566	0.556	3
				0.822	0.728	0.774	0.792	0.835	0.824	0.753	0.521	0.617	0.601	0.536	0.551	0.565	0.566	0.539	0.568	0.547	0.524	4
					0.651	0.735	0.717	0.800	0.757	0.730	0.528	0.579	0.554	0.535	0.532	0.553	0.537	0.528	0.547	0.528	0.494	5
					-	0.682	0.662	0.758	0.726	0.656	0.479	0.582	0.556	0.510	0.531	0.558	0.530	0.493	0.554	0.523	0.495	6
							0.839	0.813	0.743	0.756	0.539	0.591	0.609	0.555	0.584	0.591	0.609	0.560	0.604	0.588	0.584	7
							-	0.813	0.796	0.776	0.564	0.606	0.638	0.608	0.626	0.621	0.602	0.604	0.607	0.617	0.595	8
								-	0.891	0.820	0.513	0.591	0.608	0.560	0.567	0.596	0.596	0.561	0.552	0.572	0.534	9
										0.857	0.463	0.545	0.577	0.505	0.521	0.532	0.536	0.507	0.533	0.516	0.479	10
										-	0.493	0.593	0.583	0.527	0.556	0.516	0.545	0.522	0.551	0.563	0.521	11
											-	0.713	0.753	0.767	0.763	0.752	0.774	0.784	0.753	0.767	0.736	12
												-	0.872	0.786	0.813	0.825	0.842	0.819	0.838	0.811	0.840	13
													-	0.819	0.878	0.856	0.867	0.867	0.847	0.866	0.860	14
														-	0.867	0.825	0.804	0.847	0.801	0.804	0.824	15
															-	0.875	0.878	0.923	0.857	0.882	0.872	16
																-	0.877	0.881	0.874	0.882	0.870	17
																	-	0.881	0.871	0.886	0.866	18
																		-	0.874	0.896	0.873	19
																			-	0.902	0.856	20
																				-	0.881	21
																						22

each population, 600 bp, as shown in Fig. 2E (Table 2). In JVCW population from Wonsan, the decamer primer OPD-08 also generated 22 unique shared bands to each population, 300 bp and 450 bp, as shown in Fig. 2E.

One hundred fifty four shared bands by the two populations, with an average of 22.0 per primer, were observed in two populations. Especially, sixty-six numbers of shared bands by the two populations were observed in the two

Protothaca populations. The decamer primer OPA-08 generated the shared bands by the two populations, approximately 120 bp, 180 bp, 270 bp, 450 bp, 500 bp and 550 bp, between the two *Protothaca* populations (Fig. 2C, Table 2). The oligonucleotide primer OPA-11 also generated the shared bands by the two populations, approximately 300 bp, 500 bp and 1,200 bp in JVCB population from Boryeong and JVCW population from Wonsan, as shown in Fig. 2D. The

other primers, OPA-04, OPA-07, OPD-08, and OPD-05 generated the shared bands by two *Protothaca* populations. The results demonstrate that JVCB population from Boryeong is genetically similar to JVCW population from Wonsan.

In this 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 0.766 ± 0.008 for JVCB population from Boryeong and 0.838 ± 0.007 for JVCW population from Wonsan. Bandsharing value between two *Protothaca* populations ranged from 0.463 to 0.638 with the average 0.563 ± 0.004. As compared with JVCB population or JVCW population, the bandsharing values are also higher than previously reported results in which the average bandsharing value obtained by five random primers was in the cultured population (0.69 ± 0.08) (Yoon and Park, 2002), in oyster population (0.282 ± 0.008) (Kim *et al.*, 2004), in bullhead population (0.504 ± 0.115) (Yoon and Kim, 2004), and Indian Ocean lobster (0.742 ± 0.009) (Park et al., 2005), and in Hwanghae rockfish (0.690 ± 0.012) (Yoon et al., 2007). Other reports have showed the average bandsharing value ranging from 0.71 to 0.81 in Spanish barbel species (Callejas and Ochando, 1998). Compared individuals separately, the bandsharing value of individuals for JVCW population was higher than that for JVCB population. Even if seaweed, our bandsharing values between two *Protothaca* populations are similar to the result of Park *et al.* (1998), 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.

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. 3. The dendrogram obtained by the seven oligonucleotides primers, indicates two genetic clusters. The longer genetic distance displaying significant molecular differences was between the individual WONSAN no. 12 and WONSAN no. 13 between two Protothaca populations (0.287). Especially, two *Protothaca* between the individual WONSAN no. 12 and BORYEONG no. 10 showed the longest genetic distance (0.537) in comparison with other individuals used, as illustrated in Fig. 3. The shortest genetic distance displaying significant molecular difference was between individuals WONSAN no. 19 and WONSAN no. 16 from Wonsan (genetic distance = 0.077). The genetic distance between the two geographical populations ranged from 0.053 to 0.605. The genetic similarity for the six isolates of seaweed *Hizikia fusiformis* ranged from 0.23 to 0.59 (Park *et al.*, 1998).

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). 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).
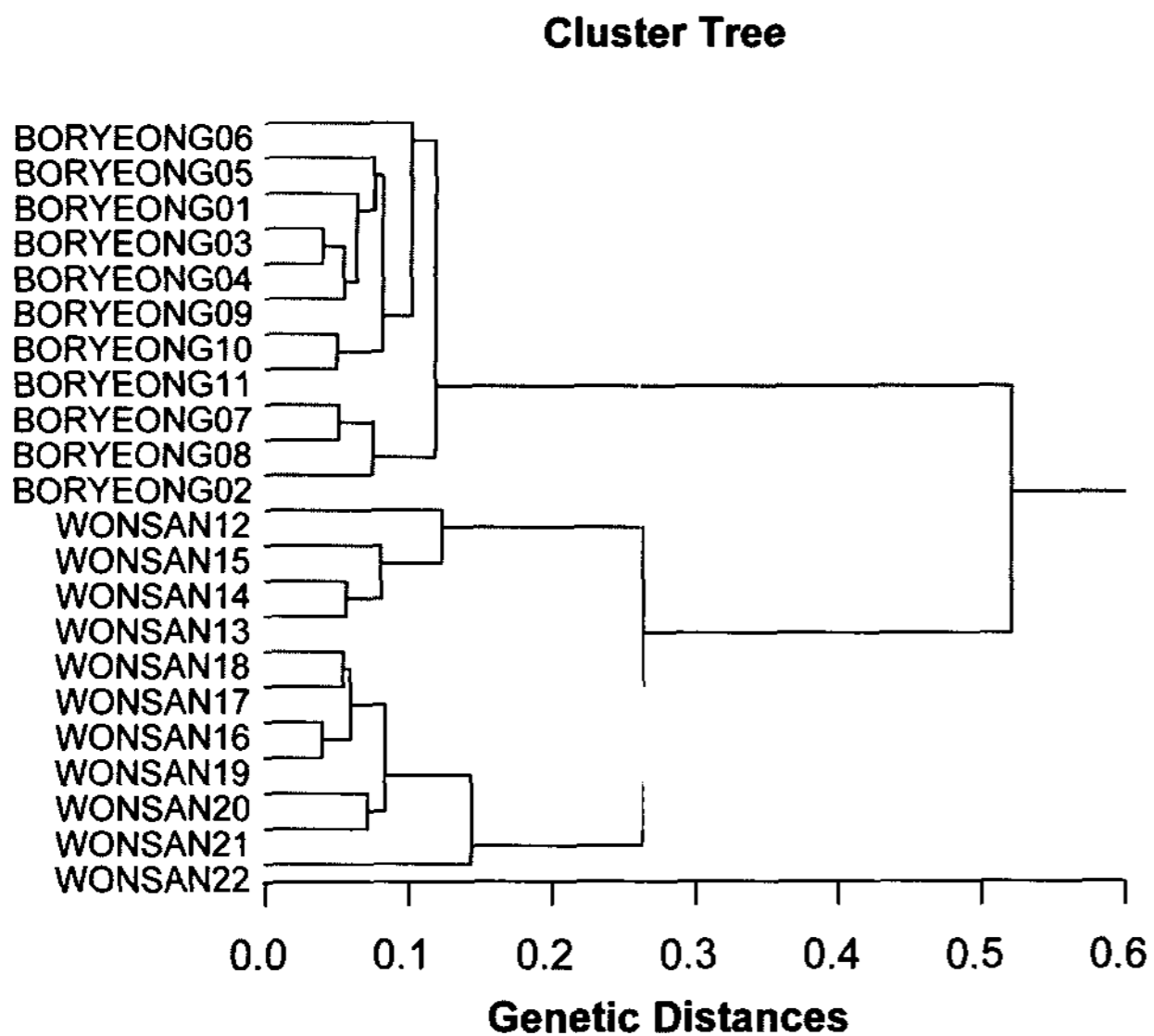
Cluster Tree

Fig. 3. Hierarchical dendrogram of genetic distances obtained from two jedo venus clam (*P. jedoensis*) populations. The relatedness among different individuals of jedo venus clams from Boryeong (BORYEONG 01 ~ BORYEONG 11) and jedo venus clam from Wonsan (WONSAN 12 ~ WONSAN 22) populations. Bar scale represents the coefficient of Nei's genetic distance.

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*. This study showed 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 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 Korean lobster species and DEEPSEA no. 16 of the Indian Ocean lobster species (genetic distance = 0.612).

Consequently, as mentioned above, RAPD analysis showed that the JVCB was a little more genetically diverse than the JVCW population. This result implies the genetic similarity owing to rearing in the same and/or similar circumstances or inbreeding within the JVCW population. So to speak, JVCB population may have high levels of genomic DNA variability owing to the introduction of the wild individuals from the other sites to sampling sites although it may be the geographically diverse distribution of this species. It is thought to have further more genetic difference between the jedo venus clam populations of South Korea and North Korea because of segregation for a old time. However, it was confirmed that it did not appear like that actually.

In this study, we convinced that RAPD analysis revealed a significant genetic distance between two *Protothaca* population pairs (P < 0.001). The existence of population discrimination and genetic diversity between two *Protothaca* populations was identified by RAPD analysis. This shows that the research method can be an adequate tool to compare DNA in individuals, species and/or populations. Additionally, the basic knowledge of DNA polymorphisms and molecular markers of Protothaca populations may contribute significantly to the seedling production and the selective breeding program of jedo venus clam. The genetic identification of black tiger shrimp (*Penaeus monodon*), oyster (*Crassostrea* sp.), crayfish (*Cambaroides similis*) and freshwater crab (*Eriocheir sinensis*) populations is a necessary step for invertebrate breeding programs (Tassanakajon *et al.*, 1998; Kim *et al.*, 2004; Park *et al.*, 2005; Yoon *et al.*, 2006).

The classification of geographical populations/

species of *Protothaca* needs to be based on the morphological variation in shell shape, shell size 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-PCR markers (Jung *et al.*, 2004a, and b).

As stated above, the potential of RAPD to identify diagnostic markers for stock, species and population identification in clams (Klinbunga *et al.*, 2000; Yoon and 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 arrange entirely the specificity of bands to particular taxa and subsequent inter-specific gene flow in the genus *Protothaca*. Additional sampling sites and isolates will be necessary to determine precisely the area where the phylogeographic break occurs. In the future, PCR-based RAPD markers will be necessary for genetic traits of the different geographical jedo venus clam species to connect with the morphological traits and for clarification of the obscurity among species and/or geographic populations. Both more time, and further more research methods, will also be necessary to identify the differentially expressed genes between/among populations and species, using an annealing control primer system and real-time PCR method.

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