

Geographic Variations and Genetic Distance of Three Geographic Cyclina Clam (*Cyclina sinensis* Gmelin) Populations from the Yellow Sea

Jong-Man Yoon[†]

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

ABSTRACT : The gDNA isolated from *Cyclina sinensis* from Gochang (GOCHANG), Incheon (INCHEON) and a Chinese site (CHINESE), were amplified by PCR. Here, the seven oligonucleotide decamer primers (BION-66, BION-68, BION-72, BION-73, BION-74, BION-76, and BION-80) were used to generate the unique shared loci to each population and shared loci by the three cyclina clam populations. As regards multiple comparisons of average bandsharing value results, cyclina clam population from Chinese (0.763) exhibited higher bandsharing values than did clam from Incheon (0.681). In this study, the dendrogram obtained by the seven decamer primers indicates three genetic clusters: cluster 1 (GOCHANG 01~GOCHANG 07), cluster 2 (INCHEON 08~INCHEON 14), cluster 3 (CHINESE 15~CHINESE 21). The shortest genetic distance that displayed significant molecular differences was between individuals 15 and 17 from the Chinese cyclina clam (0.049), while the longest genetic distance among the twenty-one cyclina clams that displayed significant molecular differences was between individuals GOCHANG no. 03 and INCHEON no. 12 (0.575). Individuals of Incheon cyclina clam population was somewhat closely related to that of Chinese cyclina clam population. In conclusion, our PCR analysis revealed a significant genetic distance among the three cyclina clam populations.

Key words : Cyclina clam population, *Cyclina sinensis*, Genetic cluster, Genetic distance, Korean peninsula, The unique shared loci to each population

INTRODUCTION

Korean cyclina clam (*Cyclina sinensis* Gmelin) is one species of an economically important bivalve species, belonging to the family Veneridae, and the order Veneroida. The clams inhabit in the estuary flats consisting of a lot of sand, mud and slime in the coastal tidal wetland. Cyclina clam is widely distributed in the field of reeds and seawater habitats of the East Sea, the Yellow Sea and the southern sea such as the coasts of Incheon, Taean, Boryeong, Seocheon, Gunsan, Yeosu, Suncheon, and Namhae in the Korean Peninsula. The clams are silvery white and coarse in the shell surface under natural

conditions. The ribs of the shell surface are compact and yellowish brown or light gray. In general, the size, color and type of this bivalve vary according to environmental factors, such as geography, water depth, water temperature, nutrition, growth period, and other common factors. However, the environmental necessities and forbearances of clam from different geographic areas remain unknown, as does its population structure.

As the clam culture industry grows, so does interest into the genetics of this shellfish species. However, little information currently exists regarding the genetics of cyclina 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 (Yoon & Park, 2002; Jung et al., 2004).

[†] Corresponding author: Jong-Man Yoon, Dept. of Aquatic Life Medicine, College of Ocean Science and Technology, Kunsan National University, Gunsan 573-701, Korea. Phone: +82-63-469-1887, Fax: +82-63-469-1881, E-mail: jmyoon@kunsan.ac.kr

To analyze the genetics of organisms, a number of analytical and molecular techniques have been applied, including morphological standards (Orozco-Castillo et al., 1994), allozyme variation (Smith et al., 1997), and various PCR-based molecular techniques. Polymorphisms are determined from specific positions in the banding patterns of the amplified products (Tassanakajon et al., 1998; Yoon & Kim, 2004). Suitable loci with high reproducibility allow the identification of unambiguously different species (Greig et al., 2005). So, this technique has been used for identification and detection of genetic diversity in various shellfish (Tassanakajon et al., 1998; McCormack et al., 2000; Yoon and Kim, 2003; Kim et al., 2004; Park et al., 2005) and teleost species (Callejas & Ochando, 1998; Yoon & Kim, 2004). Author carried out clustering analyses to clarify the genetic distances among three cyclina clam (*Cyclina sinensis* Gmelin) geographical populations from Gochang, Incheon and Chinese site.

MATERIALS AND METHODS

1. Isolation of genomic DNA, amplification condition and analysis of genetic distances

Three geographical populations of cyclina clam (*Cyclina sinensis*) were obtained from three different regions: Gochang, Incheon regions of Korea and a site of China, respectively. Cyclina clam muscle was collected in sterile tubes, instantaneously placed in liquid nitrogen, and stored at -40°C until the gDNA extraction. The extraction/purification of genomic DNA was performed under the experimental conditions previously described (Yoon & Kim, 2004). After several washings, lysis buffer I (155 mM NH_4Cl ; 10 mM KHCO_3 ; 1 mM EDTA) was added to the samples, and the mixture tubes were gently inverted. Ice-cold 70% ethanol was added, and then the samples were centrifuged at 19,621 g for 5 minutes to extract the DNA from the lysates. The concentration of the extracted genomic DNA was measured

by optical density at 260 nm by a spectrophotometer (Beckman Coulter, Buckinghamshire, UK).

PCR analyses were performed on the muscle extract of 21 cyclina clam individuals using seven primers. Seven decamer primers, BION-66 (5'- TGGTGGACCA-3'), BION-68 (5'-CCTTGACGCA-3'), BION-72 (5'-CTTAGGGCAC-3'), BION-73 (5'-CAGCACCCAT-3'), BION-74 (5'-CCTCTGACTG-3'), BION-76 (5'-GAGGCCCGTT-3') and BION-80 (5'-GGGAGGCAAA-3') were shown to generate the unique shared loci to each population and shared loci by the three cyclina clam populations which could be clearly scored. PCR was performed using two Programmable DNA Thermal Cyclers (Perkin Elmer Cetus, Norwalk, CT, USA; MJ Research Inc., Waltham, MA, USA). DNA amplification was performed in 25 μl samples, which contained 10 ng of template DNA, 20 μl of premix (Bioneer Corp., Daejeon, Korea), and 1 unit of primer. Electrophoresis of the amplification reaction was performed 30 minutes at 100 V in a 1.4% agarose (Bioneer Corp., Daejeon, Korea) gel containing ethidium bromide in TBE buffer (90 mM Tris, pH 8.5; 90 mM borate; 2.5 mM EDTA). The sizes of DNA fragments were estimated by comparison with a commercial 100-bp DNA ladder (Bioneer Corp., Daejeon, Korea) used as the molecular weight marker. A hierarchical clustering tree was constructed using similarity matrices to generate a dendrogram, which was facilitated by the Systat version 10 (SPSS Inc., Chicago, IL, USA). The degree of variability was calculated by use of the Dice coefficient (F), which is given by the formula: $F=2n_{ab}/(n_a+n_b)$, 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; Yoke-Kqueen and Radu, 2006).

RESULTS AND DISCUSSION

DNA fragments obtained by seven decamer primers

ranged in size from 50 to 1,200 bp in cyclina clam, as shown in Fig. 1. At first, the decamer primer BION-66 generated 28 unique loci to each population, approximately 150 bp, 200 bp, 300 bp and 500 bp, respectively,

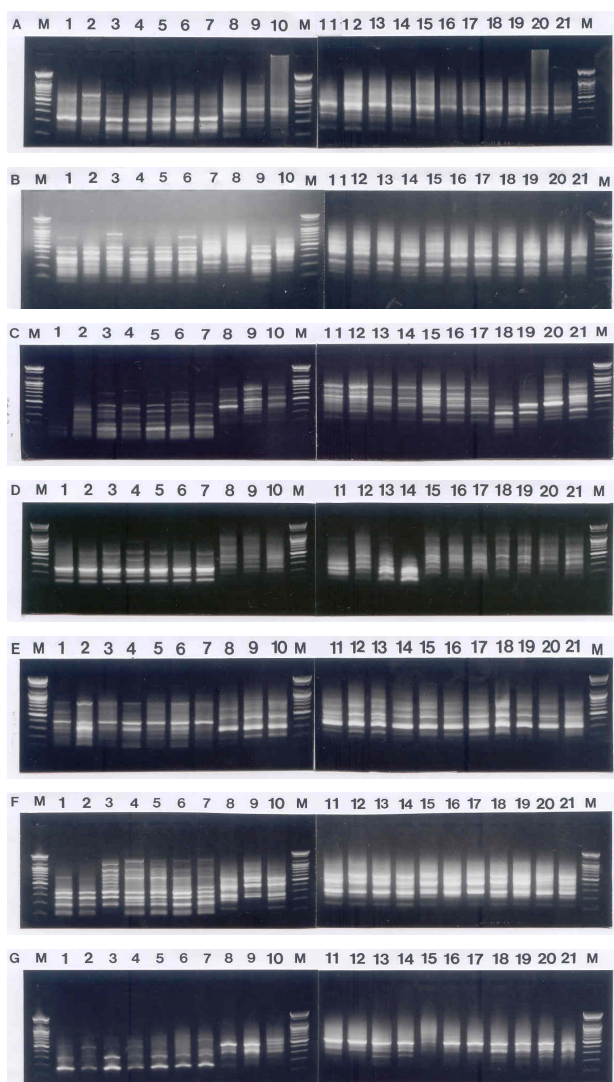


Fig. 1. PCR-based electrophoretic profiles of cyclina clam (*Cyclina sinensis*) DNA isolated from Gochang (lane 01~lane 07), Incheon (lane 08~lane 14) and Chinese clam population (lane 15~lane 21). Each Individual DNA was amplified by decamer primer BION-66 (A), BION-68 (B), BION-72 (C), BION-73 (D), BION-74 (E), BION-76 (F) and BION-80 (G). The PCR products were separated by 1.4% agarose gel electrophoresis and detected by ethidium bromide staining. M, 100 bp ladder DNA markers.

in the Gochang population (Fig. 1A). Entertainingly, the decamer primer BION-68 generated 35 unique loci to each population, which were identifying each population, approximately 150 bp, 200 bp, 250 bp, 400 bp and 500 bp, in Gochang population (Fig. 1B). Particularly, this primer detected 21 shared loci by the three populations, major and/or minor fragments of sizes 500 bp, which were identical in all samples, as illustrated in Table 1. Especially, the primer BION-73 generated 49 unique loci to each population, which were identifying each population, approximately 250 bp, and 350 bp, in Gochang cyclina clam populations (Fig. 1D & Table 1). Ultimately, the decamer primer BION-76 generated 14 unique loci to each population, which were identifying each population, approximately 150 bp and 300 bp in Gochang clam populations (Fig. 1G). As regards multiple comparisons of average bandsharing value results, cyclina clam population from Chinese (0.763) exhibited higher bandsharing values than did clam from Incheon (0.681), as illustrated in Table 2. This average bandsharing value reported by our study is similar to the value reported for Spanish barbel species (0.71~0.81) (Callejas & Ochando, 1998). However, the average bandsharing value recorded in our study is also higher than the average value between the two oyster populations (0.282 ± 0.008) (Kim et al., 2004). In the present study, the dendrogram obtained by the seven decamer primers indicates three genetic clusters: cluster 1 (GOCHANG 01~GOCHANG 07), cluster 2 (INCHEON 08~INCHEON 14), cluster 3 (CHINESE 15~CHINESE 21), as shown in Fig. 2. The shortest genetic distance that displayed significant molecular differences was between individuals 15 and 17 from the Chinese cyclina clam (0.049), while the longest genetic distance among the twenty-one cyclina clams that displayed significant molecular differences was between individuals GOCHANG no. 03 and INCHEON no. 12 (0.575). Relatively, individuals of Incheon cyclina clam population was somewhat closely related to that of Chinese cyclina clam population. In the case of blacklip

Table 1. The number of unique loci to each population and number of shared loci by the three populations generated by PCR analysis using 7 decamer primers in Korean cyclina clam (*Cyclina sinensis*) and Chinese cyclina clam, respectively

Item Primer\Population	No. of unique loci to each population			No. of shared loci by the three populations
	Gochang	Incheon	Chinese	Three populations (7 individuals per population)
BION-66	28	21	14	0
BION-68	35	14	14	21
BION-72	14	28	21	21
BION-73	49	28	56	63
BION-75	49	21	49	21
BION-76	21	21	28	21
BION-80	14	28	42	0
Total no.	210	161	224	147
Average no. per primer	30	23	32	21

Table 2. Multiple comparisons of average bandsharing values among Korean and Chinese cyclina clam (*Cyclina sinensis*) populations from three regions were generated according to the bandsharing values and similarity matrix

Population	GOCHANG	INCHEON	CHINESE
GOCHANG	0.745	0.563	0.509
INCHEON	-	0.681	0.645
CHINESE	-	-	0.763

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).

In this study, PCR analysis revealed a significant genetic distance among three cyclina clam populations. The existence of population differentiation and DNA polymorphisms among three cyclina clam populations were detected by PCR analysis. This shows that the method is one of the adequate tools for comparing the DNA of individuals, species and/or populations of clam. As mentioned above, the potential of this analysis to identify diagnostic markers for the identification of three cyclina clam populations has also been demonstrated (Tassanakajon et al., 1998; McCormack et al., 2000; Yoon & Park, 2002; Jung et al., 2004; Yoon, 2008). It was reported that the species relationships revealed by

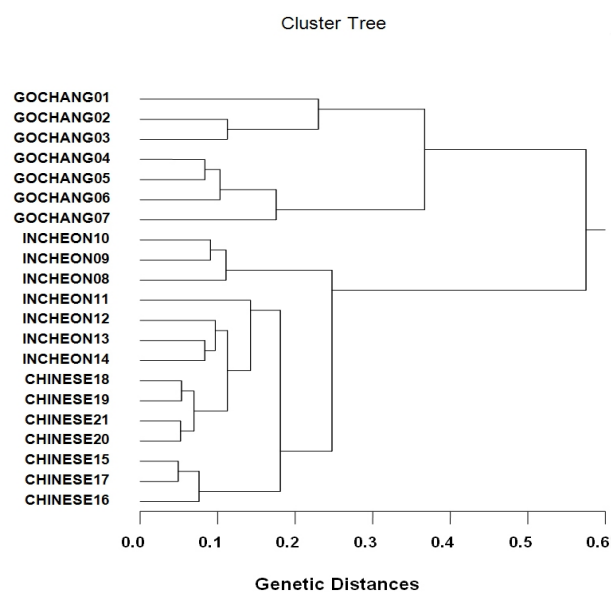


Fig. 2. Hierarchical dendrogram of genetic distances obtained from three geographic cyclina clam (*Cyclina sinensis*) populations. The relatedness among different individuals of cyclina clam from Gochang (GOCHANG 01~GOCHANG 07), Incheon (INCHEON 08~INCHEON 14) and Chinese clam (CHINESE 15~CHINESE 21) generated according to the bandsharing values and similarity matrix.

the PCR approach should be consistent with previously obtained data using morphological portrait (Nebauer et al., 2000). From what has been said above, a dendrogram revealed close relationships between individual identities

within three geographical populations. Thus, this PCR analysis revealed a significant genetic distance among the three cyclina clam populations. High levels of a significant genetic distance among three cyclina clam populations showed this PCR approach is one of the most suitable tools for individuals and/or population biological DNA studies. Therefore, this method can also be applied to other species of Veneridae clam and make technically-convenient the analysis of many samples in a short time.

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REFERENCES

- Callejas C, Ochando MD (1998) Identification of Spanish barbel species using the RAPD technique. *J Fish Biol* 53:208-215.
- Greig TW, Moore MK, Woodley CM, Quattro JM (2005) Mitochondrial gene sequences useful for species identification of western North Atlantic Ocean sharks. *Fish Bull* 103:516-523.
- Huang BX, Peakall R, Hanna PJ (2000) Analysis of genetic structure of blacklip abalone (*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. *Mar Biol* 136:207-216.
- Jeffreys AJ, Morton DB (1987) DNA fingerprints of dogs and cats. *Animal Genet* 18:1-15.
- Jung HT, Kim J, Shin JA, Soh HY, Choi SD (2004) Genetic relationship of the five venerid clams (Bivalvia, Veneridae) in Korea. *J Aquacult* 17:251-257.
- Kim JY, Park CY, Yoon JM (2004) Genetic differences and DNA polymorphism in oyster (*Crassostrea* spp.) analysed by RAPD-PCR. *Korean J Genet* 26:123-134.
- McCormack GC, Powell R, Keegan B (2000) Comparative analysis of two populations of the brittle star *Amphiura filiformis* (Echinodermata: Ophiuroidea) with different life history strategies using RAPD markers. *Mar Biotechnol* 2:100-106.
- Nebauer SG, del Castillo-Agudo L, Segura J (2000) An assessment of genetic relationships within the genus *Digitalis* based on PCR-generated RAPD markers. *Theor Appl Genet* 100:1209-1216.
- Orozco-Castillo C, Chalmers KJ, Waugh R, Powell W (1994) Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. *Theor Appl Genet* 87:934-940.
- Park SY, Park JS, Yoon JM (2005) Genetic differences and variations in slipper lobster (*Ibacus ciliatus*) and deep sea lobster (*Puerulus sewelli*) determined by RAPD analysis. *Korean J Genet* 25:307-317.
- Smith PJ, Benson PG, McVeagh SM (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, Pongsomboon S, Jarayabhand P, Klinbunga S, Boonsaeng V (1998) Genetic structure in wild populations of black tiger shrimp (*Penaeus monodon*) using randomly amplified polymorphic DNA analysis. *J Mar Biotech* 6:249-254.
- Yoke-Kqueen C, Radu S (2006) Random amplified polymorphic DNA analysis of genetically modified organisms. *J Biotechnol* 127:161-166.
- Yoon JM, Park HY (2002) 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 JM, Kim GW (2003) Genetic differences between cultured and wild penaeid shrimp (*Penaeus chinensis*) populations analysed by RAPD-PCR. *Korean J Genet*

25:21-32.

- Yoon JM, Kim JY (2004) Genetic differences within and between populations of Korean catfish (*S. asotus*) and bullhead (*P. fulvidraco*) analyzed by RAPD-PCR. Asian-Aust J Anim Sci 17:1053-1061.
- Yoon JM (2008) Variability in two species of Osmeridae

(*Hypomesus nipponensis* and *Mallotus villosus*). Dev Reprod 12:151-158.

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