



Genetic analysis of the purplish Washington clam (*Saxidomus purpuratus* Sowerby) of Korean coastal waters

Eun-Seob Cho¹, Young-Il Seo² and Young-Sang Suh^{1*}

¹Fishery and Ocean Information Division, National Fisheries Research and Development Institute, Busan-619705, Republic of Korea

²Fisheries Resources Research Division, National Fisheries Research and Development Institute, Yeosu-556823, Republic of Korea

*Corresponding Author email : yssuhkorea@korea.kr

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Abstract

To investigate the genetic structure of the purplish Washington clam population, *Saxidomus purpuratus* Sowerby, in Korea. A portion of mitochondrial COI gene sequences (605 bp) for phylogenetic comparison was determined. Sequence analysis of 62 individuals collected from six regions revealed 13 haplotypes. Phylogenetic analysis using Phylogeny Inference Package (PHYLIP) subdivided the purplish Washington clam into two clades (termed clade A and B), weak supported groups (<65 of bootstrap value). This haplotype subdivision was also in accordance with geographic separation; one each at Masan, Yeosu, Samcheonpo, Jubyeon and Geojedo, and the other at Sineju. Population genetic analysis subdivided these two population groups with a geographic distance ($d=0.431$, $p=0.379$). Furthermore, in the Sineju population, the maximum sequence divergence (2.67%) and minimum nucleotide diversity (0.0012426) were shown in which might be reflective of a relatively small population size and the geographical isolation of the population as compared with other populations. However, a very high migration rate ($N_m=59.62$ -infinite) and a very low level of geographic distance ($F_{ST}=-0.076$ -0.055) were noted to exist among the South and East Sea populations, suggesting that individuals between populations should show a significantly active genetic mixing and migration regardless of geography. These findings allowed us to conclude that the purplish Washington clam populations occurring in the South and East Sea were formed with randomly dispersed individuals.

Key words

Purplish washington clam, mitochondrial DNA, genetic differentiation, biogeography

Introduction

The purplish Washington clam (*Saxidomus purpuratus* Sowerby) is belong to the family Veneridae and with a wide distribution across the whole of Korea to Japan and is one of the most important shellfish resources in Korea (Yoo, 1976). In this sense, various studies have conducted to population ecological characteristics over the past decades (Chung and Kim, 1994; Chung *et al.*, 1999; Kim *et al.*, 2003) and fisheries management implications (Zhang *et al.*, 2004).

Data on the genetic structure of populations are of particular interest because marine organisms may reveal evidence of restricted gene flow or genetic isolation that is

undetectable through traditional ecological studies (Avisé, 1994). However, the purple Washington clam is poorly understood in studies of fisheries management based on genetics and relatively non-abundant compared with other marine organisms. Consequently, our goals were to quantify genetic population structure and estimate levels of gene flow in the purplish Washington clam.

Materials and Methods

A total of 62 specimens of the purplish Washington clam at four localities on the south coast (location 2, 4, 5 and 6), one locality on the west coast (location 3) and one locality on the east coast (location 1) of Korea were obtained from April to May 2010 (Fig. 1). Amplification and sequencing

of the partial region of the mtDNA COI gene were conducted using primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). After an initial denaturation step at 95°C for 5 min, 35-cycle amplification (94°C for 1 min, 50-55°C for 90 sec, and 72°C for 2 min) was conducted. The final extension step was continued for 8 min at 72°C. The PCR was carried out by iCycler Thermocycle (Bio-Rad). The purified DNA was directly sequenced using an Applied Biosystem model ABI 3730XL automated sequencer and a Big Dye terminator cycle sequencing kit (Perkin-Elmer Applied Biosystems). Sequence data were aligned using the multiple alignment program Clustal W (Thomson *et al.*, 1994).

Phylogenetic analysis was performed by the parsimony method incorporated in PHYLIP (Phylogeny Inference Package) ver. 3.5c (Felsenstein *et al.*, 1993) as a subprogram NEIGHBOR. To obtain the genetic tree, the data set was iterated 1,000 times using a subprogram SEQBOOT. Individual trees from each iterated data set were obtained using the subprogram DNAMLK with the option of Kimura's 2-parameter method (Kimura, 1980). A consensus tree representing reliability at each branch in the tree was obtained

using the subprogram CONSENSE. Genetic diversity and mean number of pairwise differences among haplotypes, gene diversity, and nucleotide diversity were calculated using Arlequin ver 1.1 (Schneider *et al.*, 1996). Nucleotide diversity was calculated by estimating the probability that two randomly chosen homologous sequences will be different (Nei, 1987). Genetic distance (F_{ST}), coefficient of coancestry (D), and female migration rate (N_m) were estimated by subroutines in Arlequin ver 1.1 (Schneider *et al.*, 1996). Statistical significance and migration rate were conducted by the methods of Excoffier and Reynolds (Reynolds *et al.*, 1983; Excoffier *et al.*, 1992). Hierarchical genetic relationships among populations and sets of populations were assessed by the Holsinger and Mason-Gamer (H-MG) method (Holsinger and Mason-Gamer, 1996). This method provides the proportion of diversity in the sample differences among populations after a bias correction to Nei's formula (Nei, 1982).

Results and Discussion

The use of the primer combination of LCO1490 and HCO2198 for amplifying the mtDNA COI gene in the purple

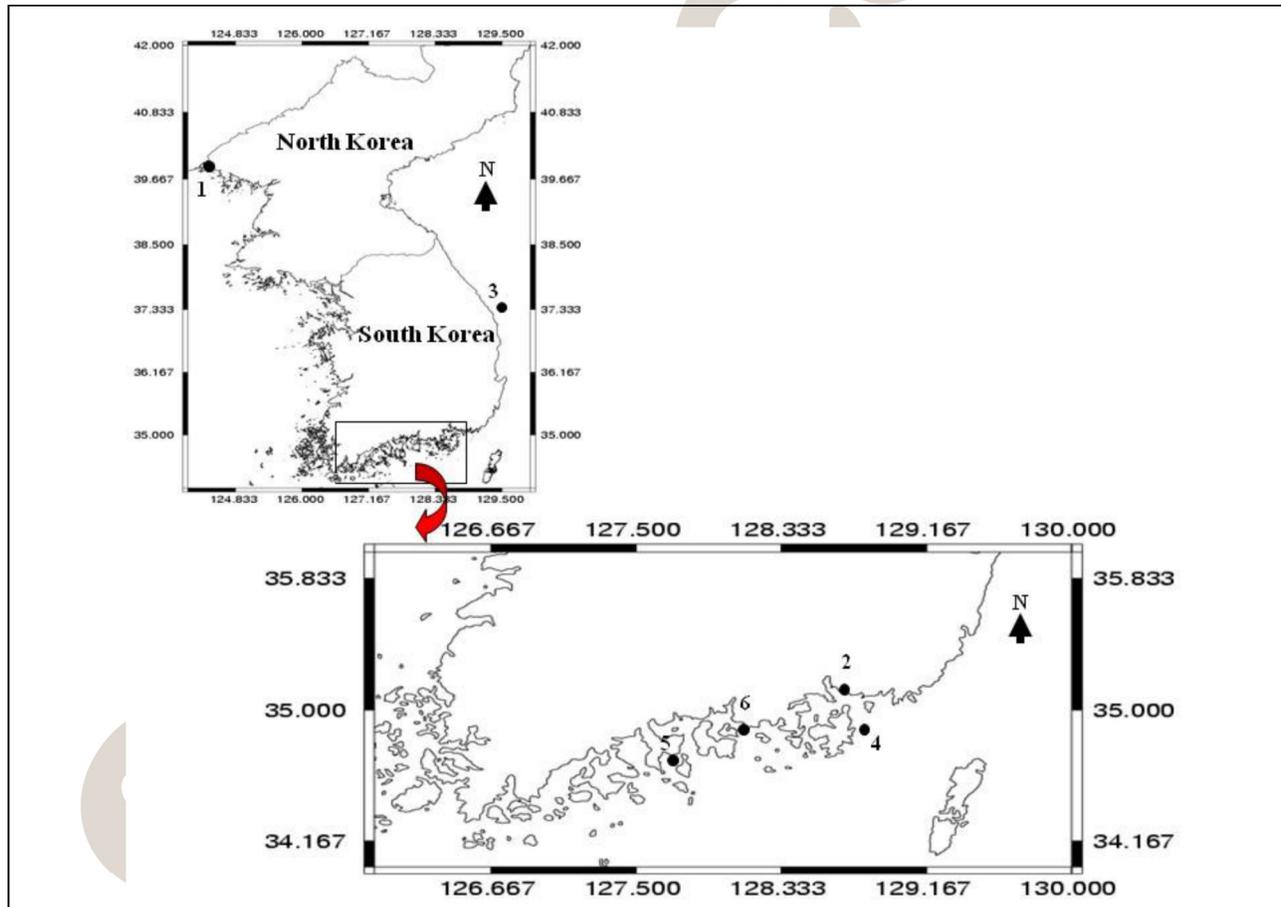


Fig. 1 : Sampling locations of the purplish Washington clam (*Saxidomus purpuratus*) in Korea. General locality names are as follows: 1, Sineuju, North Korea; 2, Masan, South Korea; 3, Jubyeon, South Korea; 4, Geojedo, South Korea; 5, Yeosu, South Korea; 6, Samcheonpo, South Korea

Washington clam had a high resolution of PCR product (Fig. 2). A PCR product was obtained at 605 bp. The sequence alignment showed 39 variable nucleotides (Fig. 3). In nucleotide position 452 and 601, transitional substitutions (G⇔A and T⇔G) were shown. The rest of them were transversion (G⇔C and A⇔T). Nucleotide position ranging from 360 to 420 bp had many variable transversions at 10 sites.

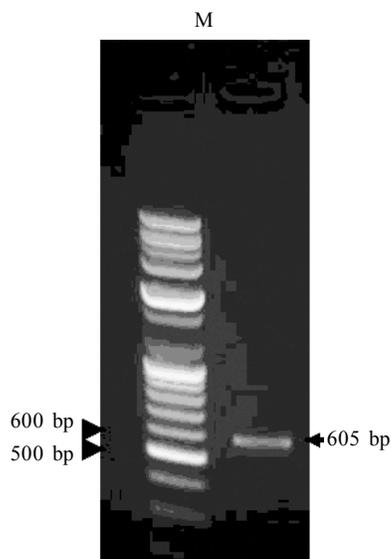


Fig. 2 : PCR product using the primers LCO1490 and HCO2198 for the purplish Washington clam, *Saxidomus purpuratus*. 100 bp DNA ladder was used as molecular size

A total of 13 haplotypes was obtained from 62 individuals. Regional distribution is shown in Table 1. Three different haplotypes among 10 individuals were observed from samples of the Sineju population, whereas the populations in the South Sea had similar numbers of haplotypes, ranging from 5-8 haplotypes among 10-12 individuals. The Sineju population accounted for $\geq 50\%$ of relative frequency of haplotypes compared with these of the South Sea and East Sea populations. As shown in relative haplotypes frequency (Table 2), the rest of the 10 haplotypes, excluding P4, P5 and P9 haplotypes, were found in the South Sea and East Sea populations. It indicates that P1, P2, P3, P6, P7, P8, P10, P11, P12 and P13 haplotypes were extensively distributed on the coast of the South Sea and East Sea. However, P4, P5 and P9 haplotypes were only found in the Sineju and Jubyeon populations, indicating geographical restriction. A pairwise comparison between P5/P4 and the remainder showed a divergence of around 1.5%.

To illustrate the genetic relationships among haplotypes, we used the NJ method and MLK method

Table 1 : Frequencies of haplotypes at each population

Population (Total no. specimen)	COI haplotypes (Corresponding no. specimen)
Sineju (10)	P4(3), P5(5), P13(2)
Masan (10)	P1(2), P3(2), P6(1), P8(3), P10(2)
Jubyeon (12)	P1(1), P2(2), P7(3), P9(1), P11(2), P13(3)
Geojedo (10)	P2(3), P3(1), P6(1), P7(2), P10(1), P11(1), P12(1)
Yeosu (10)	P2(2), P3(1), P7(1), P10(3), P12(1), P13(2)
Samchenpo (10)	P1(2), P2(1), P3(1), P7(2), P8(1), P10(1), P11 (1), P13(1)

Table 2 : Relative frequencies of mtDNA COI gene haplotype according to populations

Haplotype	1 (10)	2 (10)	3 (12)	4 (10)	5 (10)	6 (10)
P1	0	0.20	0.08	0	0	0.20
P2	0	0	0.16	0.30	0.20	0.10
P3	0	0.20	0	0.10	0.10	0.10
P4	0.30	0	0	0	0	0
P5	0.50	0	0	0	0	0
P6	0	0.10	0	0.10	0	0
P7	0	0	0.25	0.20	0.10	0.20
P8	0	0.30	0	0	0	0.10
P9	0	0	0.08	0	0	0
P10	0	0.20	0	0.10	0.30	0.10
P11	0	0	0.16	0.10	0	0.10
P12	0	0	0	0.10	0.10	0
P13	0.20	0	0.25	0	0.20	0.10

Note: 1, Sineju; 2, Masan; 3, Jubyeon; 4, Geojedo; 5, Yeosu; 6, Samchenpo. Numbers in parentheses indicate sample size of each population

incorporated in PHYLIP, respectively. These methods generated more branches than the parsimonious analysis, but the bootstrap values at the nodes were similar to those of the parsimonious tree. The 13 total haplotypes were clearly divided into Clade A and Clade B based on the analysis of NJ and MLK (Fig. 4). Clade A consisted of a large number of haplotypes (P1, P2, P3, P6, P7, P8, P9, P10, P11, P12 and P13), which formed an extremely monophyletic relationship with strong bootstrap values. Clade B consisted of only two kind of haplotypes P4 and P5, with a strong bootstrap value of 100%. However, the relationship between Clade A and Clade B was weakly supported by bootstrap analysis. Although the treatments of several transitions: transversion weightings of 1:0, 1:4, 1:10 and 1: 20 were conducted, the topology of the genetic tree was not affected.

Within-locality diversity was estimated in terms of maximum sequence divergence (MSD) and nucleotide diversity (Table 3). In terms of MSD, the estimates were ranked in the following order (lowest and highest): The Masan population as 0.004321, the Yeosu population as 0.004567, the Samcheonpo population as 0.005692, the

P1 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P2 ATTGGGACTTTATATTTTGTAAATGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P3 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P4 ATTGCCACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P5 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P6 ATTGGGACTATATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P7 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P8 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACC
P9 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGATCTGGATTGATGGGAACAGCTTTTACG
P10 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P11 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P12 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG
P13 ATTGGGACTTTATATTTTGTCTTTTGGCTTTTGGAGCTGGATTGATGGGAACAGCTTTTACG

P1 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P2 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P3 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P4 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P5 GTAGTAATTCGTTTAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P6 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P7 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P8 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P9 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P10 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P11 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P12 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT
P13 GTAGTAATTCGAATAGAGTTAGCTCAACCTGGCACTATTTTAGATGACGCTCATTTATAT

P1 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P2 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P3 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P4 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P5 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P6 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P7 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P8 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P9 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P10 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P11 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P12 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA
P13 AATTTAATTGTTACTTCTCATGGGTTAGTTATAATTTTTTTTTCTAGTGATGCCTATGATA

P1 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P2 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P3 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P4 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P5 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P6 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P7 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P8 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P9 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P10 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P11 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P12 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT
P13 ATAGGGGGTTTTGGTAATTGGTTGGTACCATTAATGCTGACTGCTCCGGATATGGCTTTT

P1 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P2 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P3 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P4 GCCCCAGTAAACAACCTTGAGGAATTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P5 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P6 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P7 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P8 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P9 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P10 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P11 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P12 CCCCAGTAAACAACCTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
P13 CCCCAGTAAACAAGTTGAGGTTTTGGTTATTAGGTGTTTCTGTTTTATTCTTCTTAGGG
* *****

P1 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P2 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P3 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P4 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P5 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P6 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P7 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P8 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P9 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P10 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P11 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P12 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT
P13 TCTGCTTACGTGGATGCTGGTGC GGGGACCGGATGAACAATTTATCCTCCGCTGTCAAGT

P1 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P2 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P3 TGATTACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P4 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTACCCGGT
P5 TGAAAACATCAAATTTGGGGTTTCTGTAGATTATTTAAAAATATCTTTACATTTAGGGGGT
P6 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P7 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P8 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P9 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P10 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P11 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P12 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
P13 TGAAAACATCATTTTGGGGTTTCTGTAGATTATTTAATTTTATCTTTACATTTAGGGGGT
*** *****

P1 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P2 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P3 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P4 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P5 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P6 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
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P8 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P9 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P10 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P11 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P12 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT
P13 GCTTCTTCTATTATGTCTGGTATTAATTTTAGTACGACAGCCTTATGTATGCGGCCGGAT

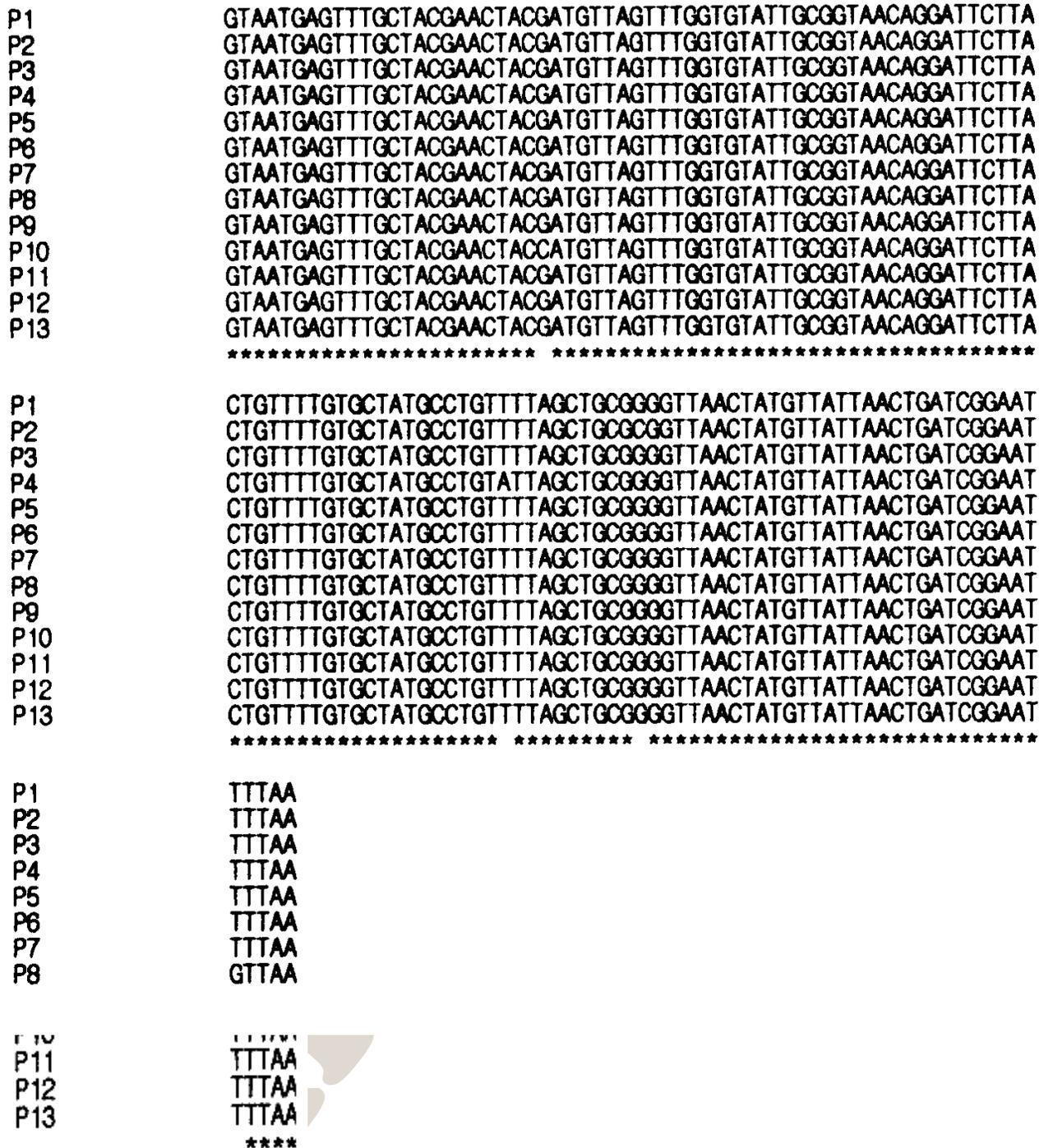


Fig. 3 : Multiple sequence alignment of mitochondrial DNA COI gene (605 bp) for 13 haplotypes of the purplish Washington clam, *Saxidomus purpuratus*. An asterisk represents an identical sequence on vertical lines. Blank shows transversion of G ↔ C or A ↔ T. Only positions that differ from the haplotype P1 are indicated.

Jubyeon population as 0.006017 and the Geojedo population as 0.006128.

Genetic distance (F_{ST}), coancestry coefficients (D) and per-generation migration rates (N_m) are shown in Table 4. The greatest genetic distance ($F_{ST}=0.127$) was found in a

comparison between the geographically remote Sineju population and Samchenpo population. Estimate of the Masan/Geojedo/Yeosu populations showed a similar range of genetic distance (0.05-0.07) from the Jubyeon population. The test of statistical significance of pairwise F_{ST} estimates showed that the Sineju population was significantly

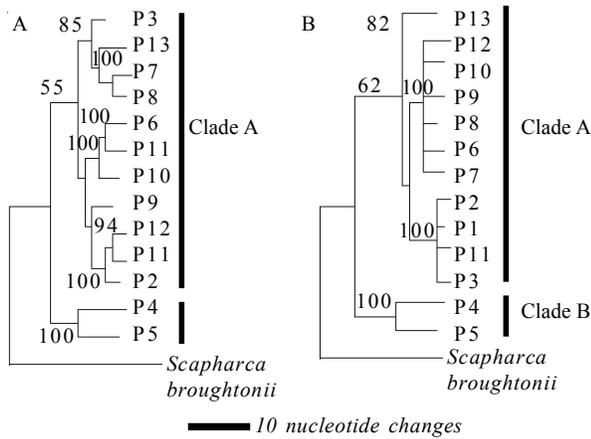


Fig. 4 : PHYLIP analysis of mitochondrial DNA COI gene sequences using mtDNA sequences of 13 *Saxidomus purpuratus* haplotypes. The tree was generated using the subprogram NEIGHBOR (A) and DNAMLK (B) incorporated in PHYLIP with the option of Kimura's 2-parameter method (Kimura, 1980). The tree was rooted using *Scapharca broughtonii*. The numbers shown on branches, which represent bootstrap values for 100 replications, were obtained using the subprogram CONSENSE.

differentiated from five populations ($p < 0.05$). The increase in genetic isolation was not observed in proportion to the increase in geographical distance. Estimates of coancestry coefficients were also consistent with F_{ST} estimates.

The hierarchical relationship among populations analyzed by Holsinger and Mason-Gamer (HM-G) method is shown in Fig. 5. Genetic distance between the Masan population and Yeosu population ($d = -0.157$) and between the Samcheonpo population and Geojedo population ($d = -0.094$), was negative. Also, genetic distance between the Jubyeon population and the group consisting of South Sea populations ($d = -0.246$) was zero. The hierarchical partition of genetic variance and fixation index (F) of each hierarchical level is presented in Table 5. In the analysis, the within sample variance component was 82.34% and the estimate was statistically significant ($p < 0.01$), indicating that most

Table 3 : Genetic variability estimates for southern, western and eastern purplish Washington clam

Population	Sample size	Number of haplotypes	Maximum sequence divergence (%)	Nucleotide diversity
West Sea				
Sineju	10	3	2.6	0.012426
South Sea				
Masan	10	5	1.3	0.004321
Geojedo	10	7	1.4	0.006128
Yeosu	10	6	1.3	0.004567
Samcheonpo	10	8	1.4	0.005692
East Sea				
Jubyeon	12	6	1.4	0.006017

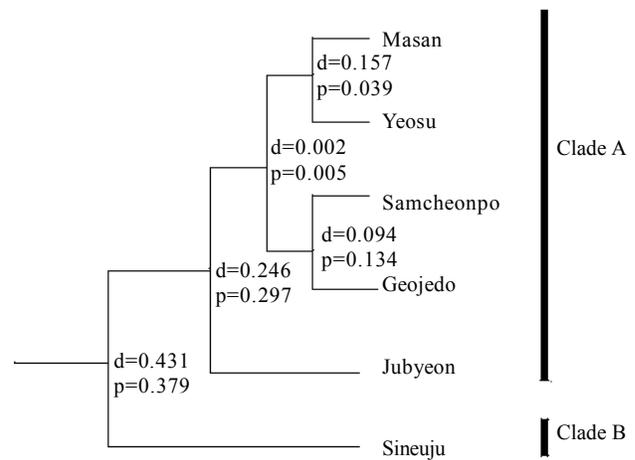


Fig. 5 : Hierarchical relationships among populations using Holsinger and Mason-Gamer method (Holsinger and Mason-Gamer, 1996). The value at each node is the distance between two daughter nodes and the p value is the significance of differentiation based on 10,000 random re-samplings

genetic diversity in the purple Washington clam was shown.

As a result of mtDNA analysis, distribution pattern of mtDNA haplotypes of the purplish Washington clam obtained from six coasts in Korea seemingly represent such pattern, but it is somewhat more complex than expected. This is because haplotypes P4 and P5 found in eight individuals at the Sineju population were phylogenetically distinguished and genetically distant from the rest of the haplotypes. An existence of fairly large genetic distance between haplotypes P4/P5 and the rest of the haplotypes (1.3% to 2.2%), an independent separation, formed another clade for itself. Thus, the rest of haplotypes except for haplotypes P4 and P5 shows phylogenetic continuity with a strong relationship and are randomly distributed over geographic barriers. The purplish Washing clam in Korea seems to sustain more than one independent phylogenetic group. It has been known that mitochondrial lineages of two independent clades are unlikely to survive within a single population for a long period of time, because stochastic processes enable the population to lose haplotypes over time (Aranishi and Okimoto, 2005).

The present overall result therefore supports the general observation that the purplish Washington clam with planktotropic larval stage such as marine invertebrates shows a high genetic relationship because of potential larval dispersal (Burton and Tegner, 2000).

An interesting feature of passive larval movement for high genetic relatedness may be as follows: one is swimming ability based on ecological aspects of the purplish Washington clam and the other is environmental difference

Table 4 : Estimates of genetic differentiation (F_{ST}), coancestry coefficients (D) and migration rate (N_m) between pairs of populations

	1	2	3	4	5	6
1						
2	$F_{ST}=0.073^*$ D=0.059 $N_m=12.49$					
3	$F_{ST}=0.095^*$ D=0.078 $N_m=6.78$	$F_{ST}=0.052$ D=0.051 $N_m=59.82$				
4	$F_{ST}=0.066^*$ D=0.085 $N_m=8.12$	$F_{ST}=-0.025$ D=0.000 $N_m=inf$	$F_{ST}=0.055$ D=0.054 $N_m=92.33$			
5	$F_{ST}=0.089^*$ D=0.082 $N_m=9.48$	$F_{ST}=-0.034$ D=0.000 $N_m=inf$	$F_{ST}=0.073$ D=0.056 $N_m=85.34$	$F_{ST}=-0.076$ D=0.000 $N_m=inf$		
6	$F_{ST}=0.127^*$ D=0.025 $N_m=2.41$	$F_{ST}=-0.019$ D=0.000 $N_m=inf$	$F_{ST}=-0.042$ D=0.000 $N_m=inf$	$F_{ST}=-0.036$ D=0.000 $N_m=inf$	$F_{ST}=-0.017$ D=0.000 $N_m=inf$	

Note: 1, Sineju; 2, Masan; 3, Jubyeon; 4, Geojedo; 5, Yeosu; 6, Samchenpo. * $p<0.05$. inf, infinite.

Table 5 : Analysis of molecular variance (AMOVA) of temporal replicates within six populations

(M+G+Y+S+J) vs (Sin)				
Source of variation	d.f.	%	F	p
Among populations	2	37.12	0.492	***
Among temporal samples within populations	3	-1.75	-0.043	NS
Within temporal samples	49	82.34	0.137	**

M, Masan; G, Geojedo; Y, Yeosu; S, Samchenpo; J, Jubyeon; Sin, Sineju; d.f., degree of freedom; %, percentage of variation; F, fixation index; p, significant of percentage variation and fixation indices estimated from permutation tests (1,000 permutations); NS, non-significant; ** $p<0.01$; *** $p<0.001$

after long dispersal. Del Rio-Portilla and Beaumont (2001) suggested that high dispersal potential of gametes or larvae of the marine organisms may not always translate into spatial population genetic homogeneity and the attendant high estimate of gene flow. This is because larvae may not always be the passive dispersal, but rather may adopt more active migrational behaviors and settlement choice in some species (Avisé, 1994). In the case of the blue mussel species, the veliger larvae with a ciliated swimming velum allow mussels to possess high dispersal ability (Kartavtsev et al., 2005). The larvae of the purplish Washington clam have no swimming organ and they are carried by only ocean currents. In fact, it has been suggested that mollusk larvae moved by ocean currents and subjected to pelagic dispersion display a high death rate (Ladoukakis et al., 2002). However, the longer spawning season means that the purplish Washington clam larvae will experience a broader range of oceanographic conditions and might have greater realized dispersal (Kim et al., 2003). It is thought that adults

from Sineju may have a shorter spawning period than those of South and East Sea adults and they experiences a more limited range of oceanographic conditions and consequently realizes reduced dispersal, resulting in more population differentiation. Moreover, even if the larvae passively drifted over a long distance, they should overcome environmental difference to become successfully matured adults. In the case of mussels, differential selection pressure caused by tidal level, salinity variation, density and the resultant degree of exposure to the air exists within even among neighboring populations (Matsumoto, 2003). Consequently, environmental conditions play an important role in the long dispersed larvae and effective source of recruits for the enhancement or re-establishment of distant populations.

It is concluded that the purplish Washington clam populations in the Korean coast except for the Sineju population are forming a large, close genetic group regardless of their geographic distance.

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