Korean J Biol Sci 3: 79-87, 1999

Unusual Mitochondrial DNA Polymorphism of the Blue Mussel (*Mytilus edulis*) Species Complex on the Southern Coast of Korea

Iksoo Kim*, Byung-Yoon Min, Myung-Hee Yoon[1], Myong-Suk Yoo[2], and Doh-Hoon Kim[3]

Department of Environmental Protection, College of Natural Sciences, Kyungnam University, Masan 630-701, Korea; [1]Department of Biology, Faculty of Natural Sciences, Kyungsung University, Pusan 608-736, Korea; [2]Department of Marine Biology, College of Fisheries Sciences, Pukyong National University, Pusan 608-737, Korea; [3]Department of Agronomy, Faculty of Natural Resources and Life Science, Dong-A University, Pusan 604-714, Korea

Key Words:
Mytilus species complex
Mytilus galloprovincialis
Mytilus edulis
Cytochrome oxidase III
mtDNA polymorphism
Southern coast of Korea
mtDNA introgression

Mitochondrial DNA (mtDNA) from 54 specimens of the blue mussel (*Mytilus edulis*) species complex sampled from the southern coast of Korea was assayed for polymorphism with a portion of the COIII gene (336 bp). Fifteen haplotypes were found. PAUP, one-step networks, and PHYLIP analyses revealed the presence of two clearly differentiated mitochondrial clades (termed clades B and E), separated by 3.6% of minimum sequence divergence. The distribution pattern of the species appears to be consistent with category II of the phylogeographic pattern *sensu* (Avise et al., 1987); the presence of two discontinuous and distinct mtDNA genotypes in the same geographic region. This unusual mitochondrial polymorphism was explained by the presence of the Mediterranean species, *M. galloprovincialis*, possessing mtDNA of both *M. galloprovincialis* and *M. edulis*.

Mytilus galloprovincialis Lamarck, 1819, *M. edulis* Linnaeus, 1758, and *M. trossulus* Gould, 1850 are morphologically similar mussels that have been collectively referred to as the "*M. edulis* species complex" (McDonala and Koehn, 1988; McDonald et al., 1991; Seed, 1992). *M. galloprovincialis* is known to be native to southern Europe and the Mediterranean Sea while *M. edulis* is to northern Europe and Atlantic coast of North America (McDonald et al., 1991). The center of origin of *M. galloprovincialis* is believed to be the Mediterranean Sea, where *M. galloprovincialis* diverged from *M. edulis* when the Mediterranean Sea was separated from the Atlantic during the Pleistocene ice age, about 1-2 million years ago (Barsotti and Meluzzi, 1968). As the glaciers retreated to the north and the climate became warmer, a northward expansion of the species range may have occurred. Presently the historical range of *M. galloprovincialis* has been greatly changed world-wide by the accidental introduction and culture for food (McDonald et al., 1991; Gosling, 1992). Thus, recent reports often show the presence of *M. galloprovincialis* in place where *M. edulis* was believed to exist (Gosling, 1992).

Although *M. trossulus* is widely accepted as a distinct taxon on the basis of genetic and biogeographic patterns, the taxonomic status of *M. galloprovincialis* and *M. edulis* is unclear (McDonal et al., 1991; Geller et al., 1993). Much of the confusion stems from the fact that these two species were classified primarily on the basis of shell morphology, which exhibits enormous plasticity influenced by habitat environment, such as tidal level, salinity variation, density, and habitat type (Seed, 1968; Yoo and Kang, 1974). Further confusion stems from the hybridization of the two species and, therefore, the presence of intermediate forms (e.g. Atlantic coasts of Ireland and Northwest France) makes accurate identification almost impossible (Seed, 1974; Coustau et al., 1991). Even in the Mediterranean Sea, where pure populations of *M. galloprovincialis* have been known to occur, the percentage of misidentification based wholly on shell morphology was 20%-60% (Seed, 1972, 1974). To overcome these difficulties, the taxonomic identification of the species is largely dependent upon several molecular markers (Geller et al., 1994; Heath et al., 1995).

Given these circumstances, the scientific name of blue mussels in Korea has been questioned. The blue mussel populations on the coasts of Korea and Japan were known as *M. edulis* until recently, but reproductive patterns (Yoo and Kajihara, 1983) and shell morphology of the populations (Yoo, 1992) were reported to be similar to the Mediterranean species, *M. galloprovincialis*. Furthermore, using allozyme and morphometric characters, McDonald et al. (1990) reported the pre-

* To whom correspondence should be addressed.
Tel: 82-551-79-0012, Fax: 82-551-79-0019
E-mail: iksookim@hanmail.net

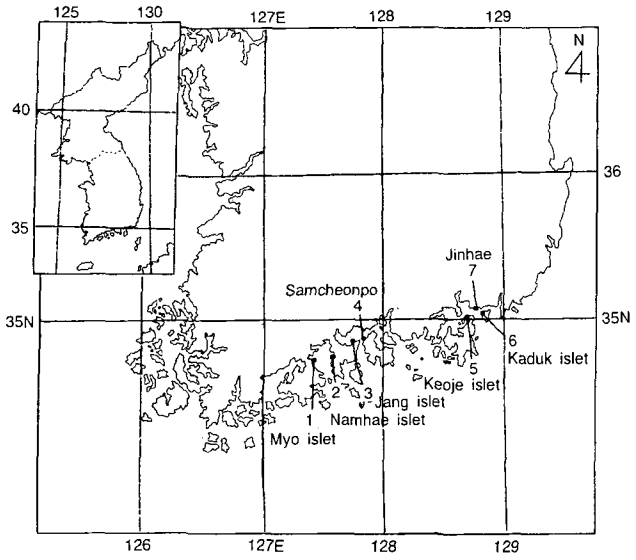

Fig. 1. *Mytilus* spp. sampling sites on the southern coast of Korea.

sence of *M. galloprovincialis* northward along East China as far as northern Korea. In this study, we sequenced a partial mitochondrial cytochrome *c* oxidase subunit III (COIII gene) of the blue mussel (*M. edulis*) species complex, collected from the southern coast of Korea to answer the following questions: (1) What is the taxonomic status of the blue mussel species distributed in the southern coast of Korea? (2) What is the genetic relationship among samples of the *M. edulis* species complex?

Materials and Methods

We obtained a total of 54 specimens of the blue mussel (*Mytilus edulis*) species complex at seven localities from the southern coast of Korea from May to November 1997 (Fig. 1). Each individual mussel was collected from spots at least 1-2 meter apart from each other to cover various microhabitats. Generalized locality and individual sample names are given in Table 1.

Mitochondrial DNA (mtDNA)

Samples were transported to Kyungnam University and placed in 70% ethanol. DNA was extracted from a piece of mantle tissue by the method of Proteinase K which requires treatment using phenol-chloroform-isoamyl alcohol (Kocher et al., 1989).

Although mtDNA in animals has been known to be maternally inherited for over twenty years, Hoeh et al. (1991) reported biparental inheritance in *Mytilus edulis*, *M. trossulus*, and *M. galloprovincialis*. More precisely, female mussels normally possess one type of mtDNA that they transmit to both female and male, but male mussels have two types of mtDNA and transmit only the male type mtDNA to sons. This system of mtDNA transmission has been termed "doubly uniparental inheritance" (DUI) (Zouros et al., 1994). Thus, a successful design of a "maternal" primer set was essential for the purpose of this study. We were able to design a primer set which specifically amplifies a partial female mitochondrial cytochrome *c* oxidase subunit III (COIII) gene by utilizing male and female sequences of *M. edulis* and *M. trossulus* (Hoffmann et al., 1992; Stewart et al., 1996). The primer sequences are as follows: *CO31, 5′-AACGGAATAGCGGTAGGGTT-3′ and CO32,* 5′-TCTTATGGGCTTGAGTTAC-3′ (COIII gene, nucleotide position 508-527 and 883-865, respectively, of

Table 1. A list of trapping localities, animal numbers, and mitochondrial COIII haplotypes

Trapping locality	Animal number	COIII haplotypes
1. Myo islet, Cheonranamdo (12)	ME7	F
	ME8	A
	ME9	L
	ME10	E
	ME11	L
	ME12	E
	ME13	B
	ME14	B
	ME29	L
	ME30	E
	ME31	A
	ME32	E
2. Namhae islet, Kyungsangnamdo (6)	ME17	B
	ME18	E
	ME21	E
	ME22	A
	ME52	E
	ME53	B
3. Jang islet, Kyungsangnamdo (2)	ME19	A
	ME20	C
4. Samcheonpo, Kyungsangnamdo (3)	ME1	E
	ME2	O
	ME3	B
5. Keoje islet, Kyungsangnamdo (5)	ME4	B
	ME5	B
	ME6	N
	ME27	E
	ME28	E
6. Kaduk islet, Pusan (18)	ME15	G
	ME16	L
	ME23	A
	ME24	M
	ME25	B
	ME26	A
	ME33	B
	ME34	K
	ME35	B
	ME36	I
	ME37	I
	ME38	A
	ME39	H
	ME40	C
	ME50	B
	ME51	J
	ME54	A
	ME55	D
7. Jinhae, Kyungsangnamdo (8)	ME41	L
	ME42	B
	ME43	A
	ME44	L
	ME45	J
	ME47	C
	ME48	B
	ME49	B

Table 2. Pairwise distances among 15 haplotypes in the *Mytilus edulis* species complex based on DNA sequences of a fragment of the COIII gene and each two homologous sequences of *M. edulis* and *M. trossulus* obtained from Hoffmann et al. (1992) and Stewart et al. (1996)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 A (ME8)	-	0.003	0.006	0.009	0.039	0.042	0.042	0.042	0.042	0.045	0.039	0.045	0.042	0.051	0.054	0.024	0.027	0.167	0.182
2 B (ME13)	1	-	0.003	0.006	0.036	0.039	0.039	0.039	0.039	0.042	0.042	0.042	0.039	0.048	0.051	0.021	0.024	0.164	0.179
3 C (ME20)	2	1	-	0.009	0.039	0.042	0.042	0.042	0.042	0.045	0.045	0.045	0.042	0.051	0.054	0.024	0.027	0.167	0.182
4 D (ME55)	3	2	3	-	0.042	0.045	0.045	0.045	0.045	0.048	0.048	0.048	0.045	0.054	0.057	0.027	0.027	0.167	0.182
5 E (ME1)	13	12	13	14	-	0.003	0.003	0.003	0.003	0.006	0.006	0.006	0.009	0.012	0.015	0.027	0.03	0.164	0.179
6 F (ME7)	14	13	14	15	1	-	0.006	0.006	0.006	0.009	0.009	0.009	0.012	0.015	0.018	0.03	0.033	0.167	0.182
7 G (ME15)	14	13	14	15	1	2	-	0.006	0.006	0.009	0.009	0.009	0.012	0.015	0.018	0.03	0.033	0.164	0.179
8 H (ME39)	14	13	14	15	1	2	2	-	0.006	0.009	0.009	0.009	0.012	0.015	0.018	0.03	0.033	0.167	0.182
9 I (ME37)	14	13	14	15	1	2	2	2	-	0.003	0.003	0.003	0.006	0.009	0.012	0.03	0.033	0.167	0.182
10 J (ME5)	15	14	15	16	2	3	3	3	1	-	0.006	0.003	0.006	0.009	0.012	0.033	0.036	0.167	0.182
11 K (ME34)	13	14	15	16	2	3	3	3	1	2	-	0.006	0.009	0.012	0.015	0.033	0.036	0.17	0.185
12 L (ME44)	15	14	15	16	2	3	3	3	1	1	2	-	0.003	0.006	0.009	0.033	0.036	0.164	0.179
13 M (ME24)	14	13	14	15	3	4	4	4	2	2	3	1	-	0.009	0.012	0.036	0.039	0.161	0.176
14 N (ME6)	17	16	17	18	4	5	5	5	3	3	4	2	3	-	0.003	0.039	0.042	0.164	0.179
15 O (ME2)	18	17	18	19	5	6	6	6	4	4	5	3	4	1	-	0.042	0.045	0.167	0.182
16 U50216-EF	8	7	8	9	9	10	10	10	10	11	11	11	12	13	14	-	0.009	0.158	0.173
17 M83760-EF	9	8	9	9	10	11	11	11	11	12	12	12	13	14	15	3	-	0.155	0.17
18 U50213-TF	56	55	56	56	55	56	55	56	56	56	57	55	54	55	56	53	52	-	0.018
19 U50218-TF	61	60	61	61	60	61	60	61	61	61	62	60	59	60	61	58	57	6	-

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute values

segment 5 in Hoffman et al., 1992). The condition for PCR amplification is as follows: the extracted DNA was denatured initially at 94℃ for 7 min followed by 35 cycles (denaturation at 93℃ for 1 min, annealing at 50 ℃ for 1 min, and extension at 72℃ for 2 min). The final extension step was later increased from two to nine minutes. The PCR amplification product was then purified using PCR Purification Kit (QIAGEN). DNA sequencing was performed using ABI 310 Genetic Analyzer with a long capillary (PE Applied Biosystems). Each strand was sequenced twice to minimize errors. Sequence alignments among four sequences in each individual were performed using IBI MacVector version 4.1.1. When homologous sequences from two different animals differed by ≥ 1 nucleotide base, the sequences were considered as different haplotypes. Haplotype designations (A, B, C and so forth) were applied to new sequences as they were discovered.

Phylogenetic analyses with PAUP, networks, PHYLIP

PAUP (version 3.1, Swofford, 1990) was used to infer relationships among the matralines identified through mtDNA sequencing from this study and two homologous sequences of *M. edulis* obtained from Hoffmann et al. (1992) and Stewart et al. (1996), respectively. Two homologous mtDNA sequences from *M. trossulus* (Stewart et al., 1996) were used as outgroups. The analyses were performed using equal weighting of transitions and transversions as well as several ratios up to and including 1:20. Heuristic searches were performed, and reliability was tested by bootstrapping (1,000 iterations). With intraspecific mtDNA sequence data it often happens that parsimony analyses provide limited resolution because of polytomies. One solution, which we employed, is to prepare one-step median networks, which can provide insight into probable relationships among closely related lineages (Bandelt et al., 1995). In addition, we used subprograms DNAMLK, NEIGHBOR, and KITSCH in PHYLIP version 3.5c (Felsenstein, 1993). Reliability of the tree was tested by bootstrapping (1,000 iterations) using subprogram CONSENSE in PHYLIP after pairwise sequence distances were estimated by Kimuras two-parameter method (1980), which attempts to correct observed dissimilarities for multiple substitutions in sequences evolving with a transition bias.

Results

MtDNA sequence analysis

From the sequences of 336 base pairs (bp) of COIII gene from 54 individuals over seven geographic localities, 15 haplotypes were obtained and designated as A-O (Table 1). Among these 15 haplotypes, 24 variable nucleotide sites occurred in the 336-bp segment of mtDNA which we analyzed. Among the 24 variable sites, 19 (79%) were transition substitutions and four (17%) were transversion substitutions. Nucleotide position 201 was the only position that showed parallel mutations (C, A, and T) (Fig. 2).

The sequence divergence in pairwise comparisons ranged from 0.3% to 5.7% (one to 19 bp) and the largest sequence divergence occurred in a comparison between haplotypes D and O (Table 2). The sequence divergence between the two *M. edulis* sequences obtained from Hoffmann et al. (1992) and Stewart et al. (1996) was 0.9% (three bp) and ranged from 2.1% to 4.5% (7-15 bp) when these two sequences were compared to haplotypes obtained from this study.

PAUP, PHYLIP, and network analysis for phylogeny

The genetic relationships among haplotypes and *M. edulis* sequences were depicted in the PAUP phylogeny presented in Fig. 3. Because analyses run with transition: transversion weightings of 1:0, 1:5, 1:10 and 1:20 did not affect the topology of trees, the results of

```
                                            30                    60
A (ME8)      ATTCTGTGAC TGCATCGAAC CCCCAGATTT TTATTAATAG GTATGAGTTT GGTTTGCATA
B (ME13)     ...T...... .......... .......... .......... .......... ..........
C (ME20)     ...T...... .......... .......... .......... ...C...... ..........
D (ME55)     ...T...... .......... .......... .......... .......... ..........
E (ME1)      ...T...... .......... .......... G......... ........C. ..........
F (ME7)      ...T...T.. .......... .......... G......... ........C. ..........
G (ME15)     ...T...... .......... .......... G......... ........C. ..........
H (ME39)     ...T...... .......... .......... G......... ........C. ..........
I (ME37)     ...T...... .......... .......C.. G......... ........C. ..........
J (ME5)      ...T...... .......... .......C.. G......... ........C. ..........
K (ME34)     .......... .......... .......C.. G......... ........C. ..........
L (ME44)     ...T...... .......... .......C.. G......... ........C. ..........
M (ME24)     ...T...... .......... .......C.. G......... .......... ..........
N (ME6)      ...T...... .......... T.....GC.. G......... ........C. ..........
O (ME2)      ...T...... .......... T.....GC.. G......... ........C. ..........
U50216-EF    ...T...... .......... .......... .......... ........C. ..........
M83760-EF    ...T...... .......... .......... .......... ........C. ..........
U50213-TF    ...T...... .......... T......... C......... .A.....G.. ..GG..T...
U50218-TF    ...T...... .......... T......... C......... .A.....G.. ..GG..T...

                                            90                   120
A (ME8)      TTATTGAGAA CTTTTAGATG GTGACGCGAT TTAATTCGTG AAGGAGACAT TGGGTTTCAC
B (ME13)     .......... .......... .......... .......... .......... ..........
C (ME20)     .......... .......... .......... .......... .......... ..........
D (ME55)     ....C..... .......... .......... .......... .......... ..........
E (ME1)      .......... .......... .......... .......... .......... ....C.....
F (ME7)      .......... .......... .......... .......... .......... ....C.....
G (ME15)     .......... .......... .......... .......... .......... ....C.....
H (ME39)     .......... .......... .......... ....A..... .......... ....C.....
I (ME37)     .......... .......... .......... .......... .......... ....C.....
J (ME51)     .......... .......... .......... .......... .......... ....C.....
K (ME34)     .......... .......... .......... .......... .......... ....C.....
L (ME44)     .......... .......... .......... .......... .......... ....C.....
M (ME24)     .......... .......... .......... .......... .......... ....C.....
N (ME6)      .......... .......... .......... .......... .......... ....C.....
O (ME2)      .......... .......... .......... .......... .......... ....C.....
U50216-EF    ...C...... .......... .......... .......... .......... ..........
M83760-EF    .......... .......... .......... .......... .......... ..........
U50213-TF    C......... .......... A..G..A..C .......... .G.....T.. .........T
U50218-TF    C......... .......... A..G..A..C .......... .G.....T.. .........T

                                           150                   180
A (ME8)      ACTCGTTTTG TAATTAAAAG ATTCCGAGAT GGTGTTGCCT TGTTTATTCT GTCTGAAGTG
B (ME13)     .......... .......... .......... .......... .......... ..........
C (ME20)     .......... .......... .......... .......... .......... ..........
D (ME55)     .......... .......... .......... .......... .......... ..........
E (ME1)      .......... .G..C..... ...T...... ..C....... .......... ..........
F (ME7)      .......... .G..C..... ...T...... ..C....... .......... ..........
G (ME15)     .......... .G..C..... ...T...... ..C....... .......... ..........
H (ME39)     .......... .G..C..... ...T...... ..C....... .......... ..........
I (ME37)     .......... .G..C..... ...T...... ..C....... .......... ..........
J (ME51)     .......... .G..C..... ...T...... ..C....... .......... ..........
K (ME34)     .......... .G..C..... ...T...... ..C....... .......... ..........
L (ME44)     .......... .G..C..... ...T...... ..C....... .......... ..........
M (ME24)     .......... .G..C..... ...T...... ..C....... .......... ..........
N (ME6)      .......... .G..C..... ...T...... ..C....... .......... ..........
O (ME2)      .......... .G..C..... ...T...... ..C....T.. .......... ..........
U50216-EF    .......... ....C..... ...T...... ..C....... .......... ..........
M83760-EF    .......... ....C..... ...T...... T.C....... .......... ..........
U50213-TF    .....C..C. ....C..G.. ...T..T... ..A......C .T........ .........A
U50218-TF    .....C..C. ....C..G.. ...T..T... ..A......C .T........ .........A
```

Fig. 2. Sequence alignment of 15 mitochondrial haplotypes (designated as A-O) obtained from 336-bp COIII sequences and homologous sequences of *M. edulis* (U50216-EF and M83760) and *M. trossulus* (U50213-TF and U50218-TF) obtained from Hoffmann et al. (1992) and Stewart et al. (1996). Only positions that differ from haplotype A are indicated. Sequences have been deposited in GenBank (accession numbers AF127464-AF127478) and the Korean Sequence Database, GenNuri (accession numbers KS101661-KS101675).

Fig. 2. Continued.

```
                                               210                                            240
A (ME8)      ATATTTTTCT TCACTTTTTT CTGGACTTTT TTCCATAATG CTTTAAGGCC CTCATGTGAG
B (ME13)     .......... .......... .......... .......... .......... ..........
C (ME20)     .......... .......... .......... .......... .......... ..........
D (ME55)     .......... .A........ .......... .......... .......... ..........
E (ME1)      .......... .......... .......... .......... ..C....... T..G......
F (ME7)      .......... .......... .......... .......... ..C....... T..G......
G (ME15)     .......... .......... .......... .......... ..C....... T..G......
H (ME39)     .......... .......... .......... .......... ..C....... T..G......
I (ME37)     .......... .......... .......... .......... ..C....... T..G......
J (ME51)     .......... .......... A......... .......... ..C....... T..G......
K (ME34)     .......... .......... .......... .......... ..C....... T..G......
L (ME44)     .......... .......... T......... .......... ..C....... T..G......
M (ME24)     .......... .......... T......... .......... ..C....... T..G......
N (ME6)      .......... .......... T......... .......... ..C....... T..G......
O (ME2)      .......... .......... T......... .......... ..C....... T..G......
U50216-EF    .......... .......... .......... .......... .......... ...G......
M83760-EF    .......... .T........ .......... .......... .......... ...G......
U50213-TF    ..G..C.... .TT....... T........C .......... .CC....A.. ...G.....A
U50218-TF    ..G..C.... .TT....... T........C .......... .CC....A.. ...G.....A

                                               270                                            300
A (ME8)      CTAGGAATAC GGTGGCCTCC TCCTGGAATT CGTACACCAA ACCCGTCATC TACTAGTCTG
B (ME13)     .......... .......... .......... .......... .......... ..........
C (ME20)     .......... .......... .......... .......... .......... ..........
D (ME55)     .......... .......... .......... .......... .......... ..........
E (ME1)      T......... .......... .......... .......... .......... ..........
F (ME7)      T......... .......... .......... .......... .......... ..........
G (ME15)     T......... .......... .......... .......... .......... ......C...
H (ME39)     T......... .......... .......... .......... .......... ..........
I (ME37)     T......... .......... .......... .......... .......... ..........
J (ME51)     T......... .......... .......... .......... .......... ..........
K (ME34)     T......... .......... .......... .......... .......... ..........
L (ME44)     T......... .......... .......... .......... .......... ..........
M (ME24)     T......... .......... .......... .......... .......... ..........
N (ME6)      T......... .......... .......... .......... .......... ..........
O (ME2)      T......... .......... .......... .......... .......... ..........
U50216-EF    .......... .......... .......... .....G.... .......... ..........
M83760-EF    .......... .......... .......... .....G.... .......... ..........
U50213-TF    .....G..G. .A..A..C.. ...A..G..C ..C..G.... .......G.. G..A..G...
U50218-TF    .....G..G. .A..A..C.. ...A..G..C ..C..G.... .......G.. G..A..G...

                                                    336
A (ME8)      TTTGAGACAG GTCTTCTAAT TAGAAGAGGG CTGTTT
B (ME13)     .......... .......... .......... ......
C (ME20)     .......... .......... .......... ......
D (ME55)     .......... .......... .......... ......
E (ME1)      .......... .......... .......... T.....
F (ME7)      .......... .......... .......... T.....
G (ME15)     .......... .......... .......... T.....
H (ME39)     .......... .......... .......... T.....
I (ME37)     .......... .......... .......... T.....
J (ME51)     .......... .......... .......... T.....
K (ME34)     .......... .......... .......... T.....
L (ME44)     .......... .......... .......... T.....
M (ME24)     .......... .......... .......... T.....
N (ME6)      .......... .......... .......... T.....
O (ME2)      .......... .......... .......... T.....
U50216-EF    .......... .......... .......... ......
M83760-EF    .......... .......... .......... ......
U50213-TF    ..C....... .....T.... ...G...... T.A...
U50218-TF    ..C....... .....T.... ...G..G..T TAT.CC
```

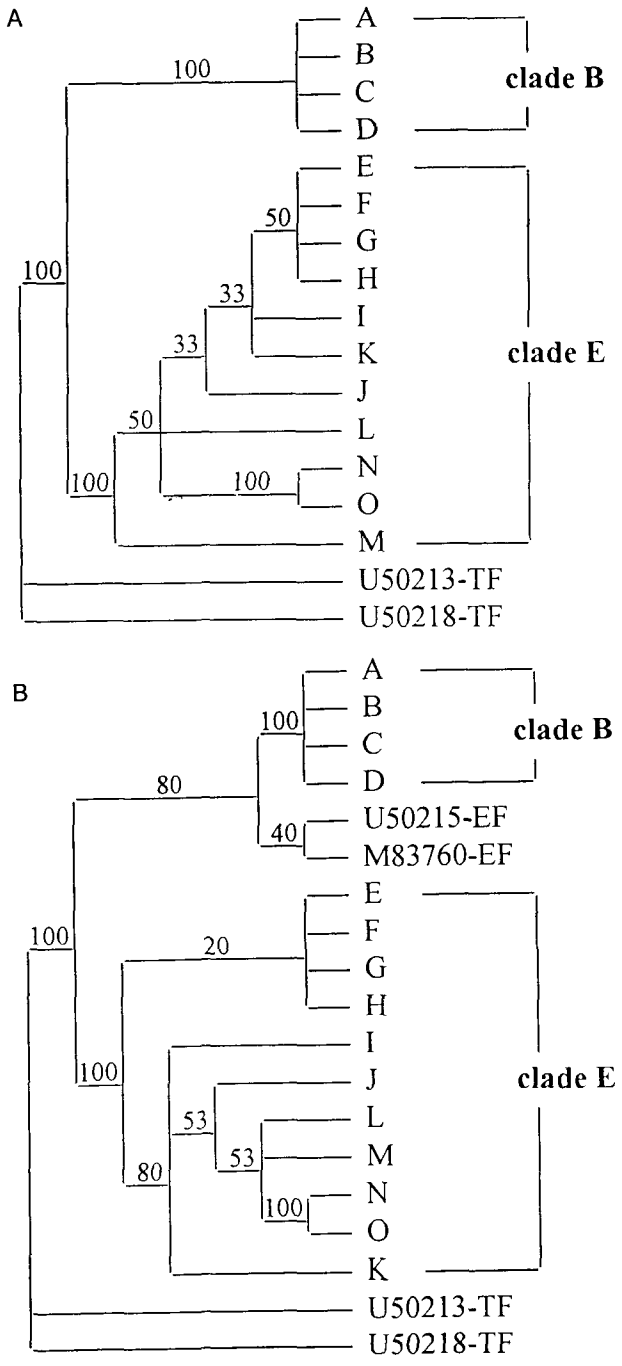

Fig. 3. PAUP analyses of mitochondrial COIII sequences presented in Fig. 2. A, Result of a heuristic search for the two clades of haplotypes (clade B and clade E) obtained on the southern coast of Korea. The three shown is a majority-rule consensus of six equally parsimonious trees obtained using two homologous sequences of *M. trossulus* as outgroups. The tree was obtained with transitions and transversions weighted equally. The numbers shown on branches are frequencies of observed partitions. Tree length is 83 steps, Consistency Index (CI) is 0.952, and Retention Index (RI) is 0.964. B, Result of PAUP analysis for all haplotypes obtained on the southern coast of Korea and two homologous sequencies of *M. edulis* obtained from Hoffmann et al.(1992) and Stewart et al. (1996). The three shown is a majority-rule consensus of fifteen equally parsimonious trees. Except for the two additional *M. edulis* sequences, other conditions for the analysis was the same as those in A. Tree length is 88 steps, CI is 0.92, and RI is 0.945.

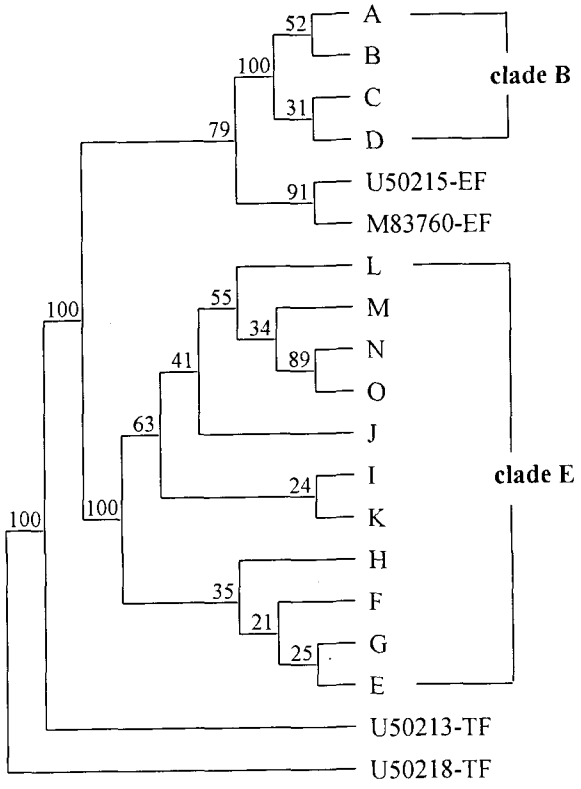

Fig. 4. PHYLIP analysis of 15 mitochondrial COIII haplotypes and two homologous sequences of *M. edulis* obtained from Hoffmann et al. (1992) and Stewart et al. (1996). The tree was obtained using subprogram DNAMLK incorporated in PHYLIP with the option of Kimura's 2-parameter method (1980). The numbers shown on branches, which represent bootstrap values for 1,000 replications, was obtained using subprogram CONSENSE.

unordered analyses were presented. For the first phase of our analysis (Fig. 3A), we tested the genetic relationships among 15 haplotypes found on the south coast of Korea. In this analysis the haplotypes formed two clades, which we arbitrarily labeled B (consisting of A, B, C, D) and E (consisting of all but members of clade B). Each clade was supported by a high bootstrap value (100%), indicating presence of two mtDNA-based genetic populations of the blue mussel (*Mytilus edulis*) species complex on the south coast of Korea. For the second phase of our analysis (Fig. 3B), we included two *M. edulis* sequences obtained from Hoffmann et al. (1992) and Stewart et al. (1996) to test the genetic relationship among the two clades and two *M. edulis* sequences. Newly added *M. edulis* sequences formed relatively strong monophyletic group (80% of bootstrap replications) with members of clade B, indicating the genetic similarity of the two sequences of *M. edulis* to clade B. Except for this aspect, the overall tree topology remained unchanged. To further elucidate the data set, we compared unrooted maximum likelihood PHYLIP trees (Fig. 4) to the PAUP analysis. The essential aspects of topology were retained. For example, in the PHYLIP tree members of each clade exactly included their own clades, forming strong

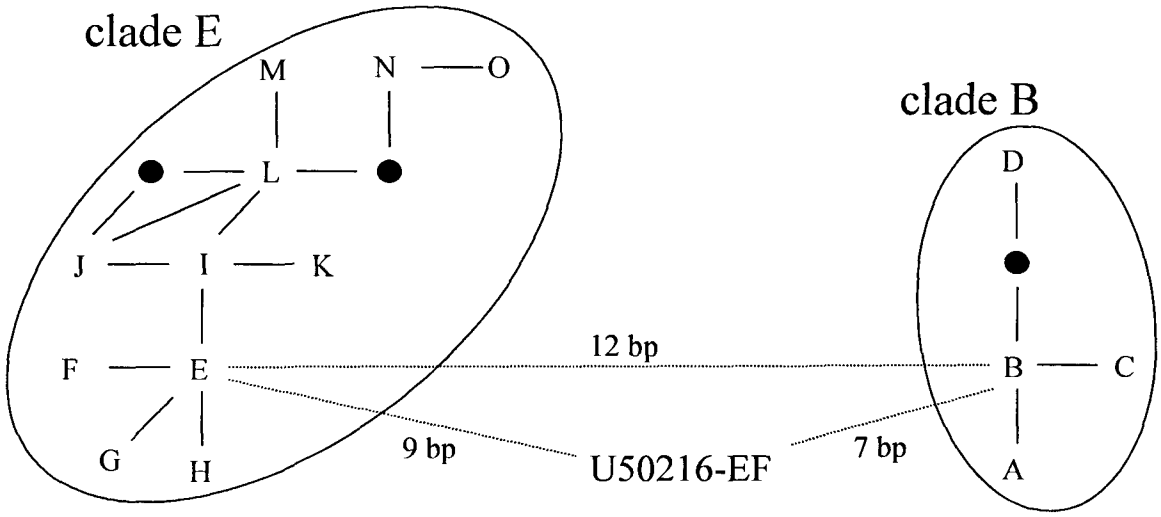

Fig. 5. Parsimonious one-step median networks analysis among 15 mitochondrial COIII haplotypes. Each bar indicates one nucleotide difference from the neighboring haplotype and dark circles indicate the hypothetical haplotypes, which were not found in this study. Haplotype E in clade E and haplotype B in clade B requires 12 mutational steps to connect to each other. A homologous sequence of *M. edulis* (U50126-EF) obtained from the interent shows a minimum of seven and nine mutational steps to connect to clade B and E, respectively.

monophyletic groups (100% of bootstrap replications both in clades B and E; Fig. 4). Furthermore, *M. edulis* sequences also grouped together with clade B haplotypes, forming a relatively strong monophyletic group (79% of bootstrap replications). The unrooted phylogenetic trees obtained from the other two methods (Neighbor-Joining/UPGMA and Kitsch distance matrices) were similar to the result obtained from maximum likelihood PHYLIP tree and were not present.

To further illustrate the genetic relationships among haplotypes, we used an unrooted one-step median network, which visualizes a possible evolutionary pathway among them (Fig. 5). Although we expected more resolution among closely related haplotypes, it provided us with limited information. For example, the network suggested that haplotypes belonging to clade B could have been derived from a single founder, labeled haplotype B, but it did not provide much information on the genetic relationships among the members of clade E. The complexity of clade E could have been stemmed from back mutations and parallel mutations. Nevertheless, the network confirmed the results obtained from PAUP and PHYLIP, in that haplotypes were separated exactly into two clades, separated by 3.6% (12 mutational steps) of minimum sequence divergence.

Distribution and genetic structure of each clade

Among 54 individuals sampled from seven localities on the southern coast of Korea, 27 individuals possessed haplotypes belonging to clade B. In all but the Jang islet, where two individuals were sampled (locality 3; Fig. 1), haplotypes of both clades were found (Table 1). The frequencies over six localities ranged from 33%-63% in clade B and 37%-67% in clade E, indicating an overall even distribution of members of each clade on the southern coast of Korea.

Discussion

Interpretation of geographic distribution pattern of mtDNA clade is a complex issue that requires understandings of phylogenetics, population genetics, and biogeography. Avise et al. (1987) proposed five different distribution patterns of mtDNA clones. Among them category II is one that possesses distinct clones in the same geographic region. The blue mussel species complex sampled on the southern coast of Korea revealed two distinct mtDNA clades (clades B and E) with a minimum sequence divergence of 3.6% (Table 2; Fig. 5). Thus, our data appear to represent the category II of the phylogeographic pattern *sensu* Avise (Avise et al., 1987; Avise 1989): discontinuous mtDNA genotypes which occur concurrently in the same geographic region. Because of stochastic extinction of mitochondrial lineages two distinct clades are very unlikely to survive within a single population for a long time (Neigel and Avise, 1986). Thus, Avise et al. (1987) ascribed the occurrence of class II category to secondary admixture of once geographically isolated populations or inclusion of sympatric sibling species that possesses intrinsic barriers to the experimental organism. Although the co-occurrence of two very distinct mitochondrial clades within the same populations has been previously reported for many organisms, interpretations of the observation were very diverse (Taberlet et al., 1992; Kim et al., 1998). For the Korean blue mussel two different interpretations may be proper for the observed results. The first interpretation could be that all of the blue mussels dwelling on the south coast of Korea are *M. edulis*, possessing two distinct mtDNA clades with unknown reason. The second interpretation could be

that the blue mussels belonging to clade B are *M. edulis*, but members of clade E possess mtDNA of other species within the genera (e.g. *M. galloprovincialis*). Originally the scientific name of the blue mussel species found on the coast of Korea was thought to be *M. edulis*, but some authors preferred to use the scientific name of *M. edulis galloprovincialis* by following the scientific name of the blue mussel in Japan (Paboo et al., 1977). Recently, the blue mussels dwelling on the coasts of Korea and Japan were regarded as the Mediterranean species, *M. galloprovincialis*, on the basis of shell morphology (Yoo and Kajihara, 1983; Yoo, 1992). This revision is consistent with the evidence of worldwide extension of the Mediterranean species including Asia. For example, it is believed that *M. galloprovincialis* has been accidentally introduced into California in the 1900s (McDonald and Koehn, 1988), South Africa in the 1960s (Grant and Cherry, 1985), and Hong Kong in 1981 and 1982 (Lee and Morton, 1985). In Japan, this species is thought to be introduced in the 1930s, and a high density of the mussel on the coast of Honshu and Hokkaido was reported (Wilkins et al., 1983). Furthermore, Geller et al. (1994) reported that all the mtDNA haplotypes of *M. galloprovincialis* were found in the ballast seawater of ocean-going Japanese ship. Considering these worldwide, circumstantial instances, one can speculate about the expansion of the species to the Korean coast. In fact, Gosling (1992) depicted that all the Korean and Japanese coasts are occupied by *M. galloprovincialis* in the global distribution map of the *Mytilus* species. Thus, the morphological study and the circumstantial information appear to weaken the first interpretation of all *M. edulis*. Instead, individuals of clade E, which were excluded from the monophyletic group of clade B and *M. edulis* sequences could be *M. galloprovincialis* in consideration with other studies (McDonald et al., 1990; Yoo, 1992).

We intentionally did not specify the members of clade B, which formed a relatively strong monophyletic group with the homologous sequences of *M. edulis* dwelling in Canada as *M. edulis* (Figs. 3B and 4). The prime reason for that is because there is no supportive evidence from other researchers and no study has thoroughly tested the possibility of the co-existence of *M. edulis* and *M. galloprovincialis* in Korea yet (Yoo and Kajihara, 1983; Yoo, 1992). In spite that the taxonomy of *Mytilus* utilizes shell morphology, ecology, and environmental conditions (e.g. water temperature and salinity), taxonomic identification is largely dependent upon the patterns of allelic frequencies of enzyme-coding loci (Edwards and Skibinski, 1987; Coustau et al., 1991; Geller et al., 1994; Wilhelm and Hilbish, 1998). Therefore, although this and other studies can not directly provide an answer for the existence of *M. edulis* in the Korean coast, it would be possible to attempt to discuss some plausible explanations by utilizing all possible information. First, *M. edulis* might truly occur in Korea, forming clade B (Fig. 3B and 4). This explanation is inconsistent with a morphological study done by Yoo (1992). She sampled a substantial amount of specimens (920 individuals) from six localities on the south and east coasts of Korea, but no supportive evidence for the existence of *M. edulis* was obtained. Although the first explanation did not gain support by a morphological study, we currently can not be completely sure of the absence of *M. edulis* on the south coast of Korea, because the taxonomic study of *Mytilus* in Korea has been limited. For example, there has been no direct hypothesis relevant to this issue, no allozyme data comparable to worldwide samples is available, and no wide geographic sample was obtained. The second possibility involves introgression of the mitochondrial genome of *M. edulis* into *M. galloprovincialis*. That is, Korean coasts are occupied by morphologically *M. galloprovincialis*-like mussels, and the mitochondrial genome of *M. edulis* might have been introgressed into *M. galloprovincialis* when the species was introduced to Korea. A cross between a female *M. edulis* and a male *M. galloprovincialis* would produce a hybrid offspring carrying a *M. edulis* mtDNA haplotype. A repeated backcrossing of such hybrid females with male *M. galloprovincialis* will produce offspring with predominantly *M. galloprovincialis* nuclear DNA which determines shell morphology and *M. edulis* mitochondrial DNA. This scenario may explain the existence of a single morphological species with two distinct mtDNA clades on the southern coast of Korea. In fact, there is evidence of introgression of mtDNA from *M. edulis* to *M. galloprovincialis*. Edwards and Skibinski (1987) analyzed allozyme and mtDNA of mussels collected from northeast to southwest England and found that the *M. galloprovincialis* population in South West England possessed an allozyme allele of *M. galloprovincialis* only, but both mtDNA of *M. galloprovincialis* and *M. edulis*. If this is the case, *M. galloprovincialis* could be the only species dwelling on the southern coast of Korea as evidenced by a morphological study of Yoo (1992). Because our data set is limited, a future study, which includes samples from more diverse habitats covering a wide geographic region and other molecular markers (e.g. allozyme) will further provide a more detailed understanding on this issue.

Acknowledgements

We thank Do Heon Kwon at Inje University for valuable suggestions. We also thank Sung-Wook Wee and Hae-Kyung Hong at the International Environment Science Institute in IMI for helping to collect mussel samples. We also thank two anonymous reviewers for helpful suggestions.

References

Avise JC (1989) Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* 43: 1192-1208.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, and Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu Rev Ecol Syst* 18: 489-522.

Bandelt H-J, Forster P, Sykes BC, and Richards MB (1995) Mitochondrial portraits of human populations using median networks. *Genetics* 141: 743-753.

Barsotti G and Meluzzi C (1968) Osservazioni su *Mytilus edulis* L. e *Mytilus galloprovincialis* Larmarck. *Conchiglie Milan* 4: 50-58.

Coustau C, Renaud F, and Delay B (1991) Genetic characterization of the hybridization between *Mytilus edulis* and *M. galloprovincialis* on the Atlantic coast of France. *Mar Biol* 111: 87-93.

Edward CA, and Skibinski DOF (1987) Genetic variation of mitochondrial DNA in mussel (*Mytilus edulis* and *M. galloprovincialis*) populations from south west England and south Wales. *Mar Biol* 94: 547-556.

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) Version 3.5c. Department of Genetics, University of Washington, Seattle.

Geller JB, Carleton JT, and Powers DA (1993) Interspecific and intrapopulation variation in mitochondrial ribosomal DNA sequences of *Mytilus* spp. (Bivalvia: Mollusca). *Mol Mar Biol Biotechnol* 2: 44-50.

Geller JB, Carleton JT, and Powers DA (1994) PCR-based detection of mtDNA haplotypes of native and invading mussels on the northeastern Pacific coast: latitudinal pattern of invasion. *Mar Biol* 119: 243-249.

Gosling E (1992) Systematics and geographic distribution of *Mytilus*. In: Gosling E (ed), The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture. Elsevier, New York, pp 1-17.

Grant WS and Cherry MI (1985) *Mytilus galloprovincialis* Lmk. in southern Africa. *J Exp Mar Biol Ecol* 90: 179-191.

Habe M (1977) Systematics of Japanese Mollusca. Fukuryukan, Tokyo, pp 50-51.

Heath D, Rawson P, and Hilbish T (1995) PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp.) genotypes on the west coast of Canada. *Can J Fish Aquat Sci* 52: 2621-2627.

Hoeh WR, Blakley KH, and Brown WM (1991) Heteroplasmy suggests limited bipatental inheritance of *Mytilus* mitochondrial DNA. *Science* 251: 1488-1490.

Hoffman RJ, Boore JL, and Brown WM (1992) A novel mitochondrial genome organization for the blue mussel *Mytilus edulis*. *Genetics* 131: 397-412.

Kim I, Phillips CJ, Monjeau JA, Birney EC, Noack K, Pumo DE, Sikes RS, and Dole JA (1998) Habitat islands, genetic diversity, and gene flow in a Patagonian rodent. *Mol Ecol* 7: 667-678.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J Mol Evol* 116: 111-120.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, and Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc Natl Acad Sci USA* 86: 6196-6200.

Lee SY and Morton BS (1985) The introduction of the Mediterranean mussel *Mytilus galloprovincialis* into Hong Kong. *Malacol Rev* 18: 107-109.

McDonald JH and Koehn RK (1988) The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of the North America. *Mar Biol* 99: 111-118.

McDonald JH, Koehn RK, Balakirev ES, Manchenko GP, Pudovkin AI, Sergiyevsky SO, and Krutovsky KV (1990) On species identity of the common mussel inhabiting Asiatic coasts of the Pacific Ocean. *Biol Morya* 1: 13- 22.

McDonald JH, Seed R, and Koehn RK (1991) Allozyme and morphometric characters of three species of *Mytilus* in the Northern and Southern hemispheres. *Mar Biol* 111: 323-335.

Neigel JE, and Avise JC (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: Nevo E, and Karlin S (eds), Evolutionary Processes and Theory, Academic Press, New York, pp 515-534.

Seed R (1968) Factors influencing shell shape in the mussel *Mytilus edulis*. *J Mar Biol Assoc UK* 48: 561-584.

Seed R (1972) Morphological variations in *Mytilus* from the French coasts in relation to the occurrence and distribution of *Mytilus galloprovincialis* (Lmk.). *Cah Biol Mar* 13: 357-384.

Seed R (1974) Morphological variations in Mytilus from the Irish coasts in relation to the occurrence and distribution and distribution of *Mytilus galloprovincialis* (Lmk.). *Cah Biol Mar* 15: 1-25.

Seed R (1992) Systematics, evolution, and distribution of mussels belonging to the genus *Mytilus*: an overview. *Am alacol Bull* 9: 123-137.

Stewart DT, Kenchington ER, Singh RK, and Zouros E (1996) Degree of selective contraint as explanation of the different rates of evolution of gender-specific mitochondrial DNA lineages in the mussel *Mytilus*. *Genetics* 143: 1349-1357.

Swofford DL (1990) PAUP: Phylogenetic Analysis Using Parsimony, Version 3.0. Illinois Natural History Survey, Champaign.

Taberlet P, Meyer A, and Bouvet J (1992) Unusual mitochondrial DNA polymorphism in two local populations of blue tit *Parus caeruleus*. *Mol Ecol* 1: 27-36.

Wilhelm R and Hilbish TJ (1998) Assessment of natural selection in a hybrid population of mussels: evaluation of exogenous vs endogenous selection models. *Mar Biol* 131: 505-514.

Wilkins NP, Fujino K, and Gosling EM (1983) The Mediterranean mussel *Mytilus galloprovincialis* Lmk. in Japan. *Biol J Linn Soc* 20: 365-374.

Yoo MS (1992) A taxonomical study of the shell morphology of blue mussel, *Mytilus edulis galloprovincialis* Lamarck in Korea and Japan. *Bull Korean Fish Soc* 25: 165-170.

Yoo MS and Kajihara T (1983) Reproductive biology of the marine mussel, *Mytilus edulis galloprovincialis*, in Japan. *Mar Foulding* 4: 11-21.

Yoo SK and Kang YJ (1974) Study on the morphological variation of mussel, *Mytilus coruscus* Gould. *Bull Korean Fish Soc* 7: 87-90.

Zouros E, Ball AO, Saavedra BC, and Freeman, KR (1994) An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. *Proc Natl Acad Sci USA* 91: 7463-7467.

[Received January 4, 1999; accepted January 30, 1999]