

Geographic Variation of *Granulilittorina exigua* (Littorinidae, Gastropoda) in Korea Based on the Mitochondrial Cytochrome *b* Gene Sequence

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Partial sequence of the mitochondrial cytochrome *b* gene was analyzed to investigate genetic variation from 10 geographic populations of *Granulilittorina exigua* in Korea. The sequence of 282 base pairs was determined by PCR-directed silver sequencing method. The sequences of two species within the genus *Littorina* reserved in NIH blast search were utilized to determine geographic variations of species referred. The levels of mtDNA sequence differences were 0.00-2.54% within populations and 0.71-4.43% between populations. There were four amino acid differences between representative species of the genera *Granulilittorina* and *Littorina*, but no differences within populations of the genus *Granulilittorina*. The UPGMA and the N-J trees based on Tamura-Nei genetic distance matrix were constructed, which showed that the genus *Granulilittorina* was divided into three groups such as eastern (even exception for Tokdo population), southern, and western regional populations. The degrees of genetic divergence within populations of each group were $p=0.021$, $p=0.019$, and $p=0.018$, respectively. The divergence between the eastern and southern populations was $p=0.032$, showing closer relationship than with the western populations ($p=0.052$). Based on the diverged time estimation, the eastern and southern populations of *Granulilittorina exigua* in Korea diverged from the western populations about 2.1 MYBP, and the eastern and southern populations diverged from each other about 1.3 MYBP.

Most species with an extensive geographic range, including planktonic and nonplanktonic taxa, exhibit abundant geographic variations in both morphology and gene frequencies. The degree of genetic differentiation among local populations provides important indirect evidence, reflecting pattern and scale of effective local dispersal (Reid, 1996; Heipel et al., 1999; Bohonak, 1999). In some gastropods, the range of distribution of species is related to the type of larval development. Especially, these species with planktonic larvae have wider ranges because of the potential for widespread dispersal (Hansen, 1980; Kohn and Perron, 1994).

The degree of genetic similarity between two shell types of planktonic *Littorina* species and their micro- or macro-geographic spatio-temporal genetic structures were previously investigated by allozyme electrophoresis (Johannesson et al., 1995; Tatarencov, 1995; Parsons, 1996; de Wolf et al., 1998a). Recent studies have been performed to investigate inter- and intra-specific differentiation based on mole-

cular techniques (Crossland et al., 1993; de Wolf et al., 1998b). Mitochondrial DNA (mtDNA) is particularly useful for examination of phylogenetic relationships among taxa of recent origin, because it is haploid and usually shows simple matrilineal transmission (Reid et al., 1996; Heipel et al., 1998, 1999). Moreover, it has frequently demonstrated a sharp genetic discontinuity between animal populations collected from two distinctive regions (Karl and Avise, 1992).

There are four species, *Littorina brevicula*, *L. (Palustorina) articulata*, *G. exigua*, and *Peasiella infracostata* in the family Littorinidae (Mesogastropoda, Gastropoda) in Korea (Choe, 1992). To clarify the genetic variation of *L. brevicula* between polluted and unpolluted sites, amylase polymorphism has been previously carried out by Park et al. (1999). The study on geographic variation of *G. exigua*, regarded as one of marine bio-indicators (Song, personal description), would provide fundamental data for searching spatio-temporal changes of its genetic structure or gene pool between polluted and unpolluted areas. Therefore, in this work, we present data on the degree of genetic divergence determined by mtDNA cytochrome *b* (cyt *b*) gene sequence analyses on local populations of *G. exigua* in Korea.

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Materials and Methods

For investigation of genetic variation among *G. exigua*, the specimens were collected from 10 localities along the coasts of South Korea from 1998 to 2000 (Table 1, Fig. 1).

The samples preserved in pure ethanol were repeatedly rinsed with sterile distilled water before procedure. DNA was extracted from the foot of each individual. The tissue was homogenized in extraction buffer of the same volume, and then the genomic DNA was extracted using modified protocols of Micheli and Bova (1997).

Portions of the mt *cyt b* gene were amplified using polymerase chain reaction (PCR) with the following scheme. Double-stranded fragments were amplified with *Taq* (*Thermus aquaticus*) DNA polymerase in 30 cycles (94°C for 1 min, 52°C for 1 min, and 72°C for 1 min). The mt *cyt b* gene primers used were GES1 (5'-TTTTGGTTCTTTACTAGGCC-3') and GEAS1 (5'-AATCCTAAGGGATTATTCGA-3'). Directed sequencing electrophoresis was conducted with silver staining methods.

Alignment of nucleotide sequences was performed by the multisequence editing program CLUSTAL W (Thompson et al., 1994). Two relative species, *L. littorea* and *L. obtusata*, were aligned simultaneously as out-groups.

The DNA sequences were analyzed using the MEGA program (Kumar et al., 1993) and phylogenetic trees were constructed based on Tamura-Nei distances (Tamura and Nei, 1993) using the unweighted pair-group method with arithmetic means (UPGMA: Sneath and Sokal, 1973) and the Neighbor-Joining (N-J: Saitou and Nei, 1987) method. In order to obtain precise

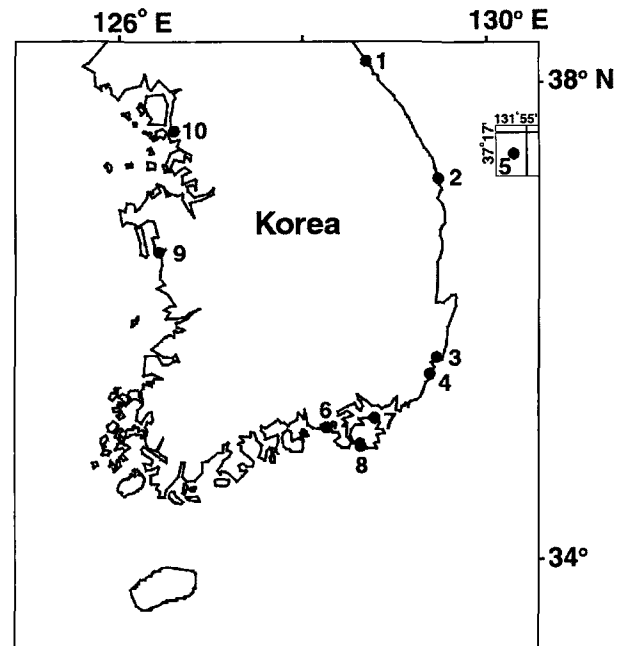


Fig. 1. Map of sampling sites of *Granulittorina exigua*. 1. Sokcho; 2. Uljin; 3. Ulsan; 4. Onsan; 5. Tokdo; 6. Kosong; 7. Heungnam 8. Hammok; 9. Poryong; 10. Incheon.

bootstrap confidence estimates (Felsenstein, 1985) for the statistical support of species clusters in the tree topology, 1,000 bootstrap replications were applied. The bootstrap *p*-value was the proportion (0-100%) of resampled trees in which a particular group was defined and we treated *p*-values above 0.95 (95%) as significance and those above 0.90 (90%) as strongly supported. Particularly, we believed that the N-J algorithm

Table 1. Collection localities of *Granulittorina exigua*

| No. | Specimens | Localities | Dates |
|--------------------------------------|-----------|---|------------------|
| Eastern regional populations | | | |
| 1 | Sokcho1 | Mulchi port, Sokcho-shi, Kangwon-do | May 1- 2, 1999 |
| 2 | Sokcho2 | | |
| 3 | Uljin1 | Uljin-gun, Kyongsangbuk-do | May 1- 2, 1999 |
| 4 | Uljin2 | | |
| 5 | Ulsan1 | Tangsa-ri, Ulsan-shi | Dec. 18-20, 1998 |
| 6 | Ulsan2 | | |
| 7 | Onsan1 | Jinha-ri, Onsan-shi, Kyongsangnam-do | Dec. 18-20, 1998 |
| 8 | Onsan2 | | |
| 9 | Tokdo1 | Tokdo Is., Ulneung-gun, Kyongsangbuk-do | May 15-16, 1999 |
| 10 | Tokdo2 | | |
| Southern regional populations | | | |
| 11 | Kosong1 | Udupo-ri, Kosong-gun, Kyongsangnam-do | Dec. 18-20, 1998 |
| 12 | Kosong2 | | |
| 13 | Heungnam1 | Heungnam-ri, Koje-shi, Kyongsangnam-do | Dec. 18-20, 1998 |
| 14 | Heungnam2 | | |
| 15 | Hammok1 | Hammok-ri, Koje-shi, Kyongsangnam-do | Dec. 18-20, 1998 |
| 16 | Hammok2 | | |
| Western regional populations | | | |
| 17 | Poryong1 | Poryong-shi, Chungchongnam-do | May 1- 2, 1999 |
| 18 | Poryong2 | | |
| 19 | Inchon1 | Jakyakdo Is., Ongjin-gun, Incheon-shi | Apr. 15-16, 2000 |
| 20 | Inchon2 | | |

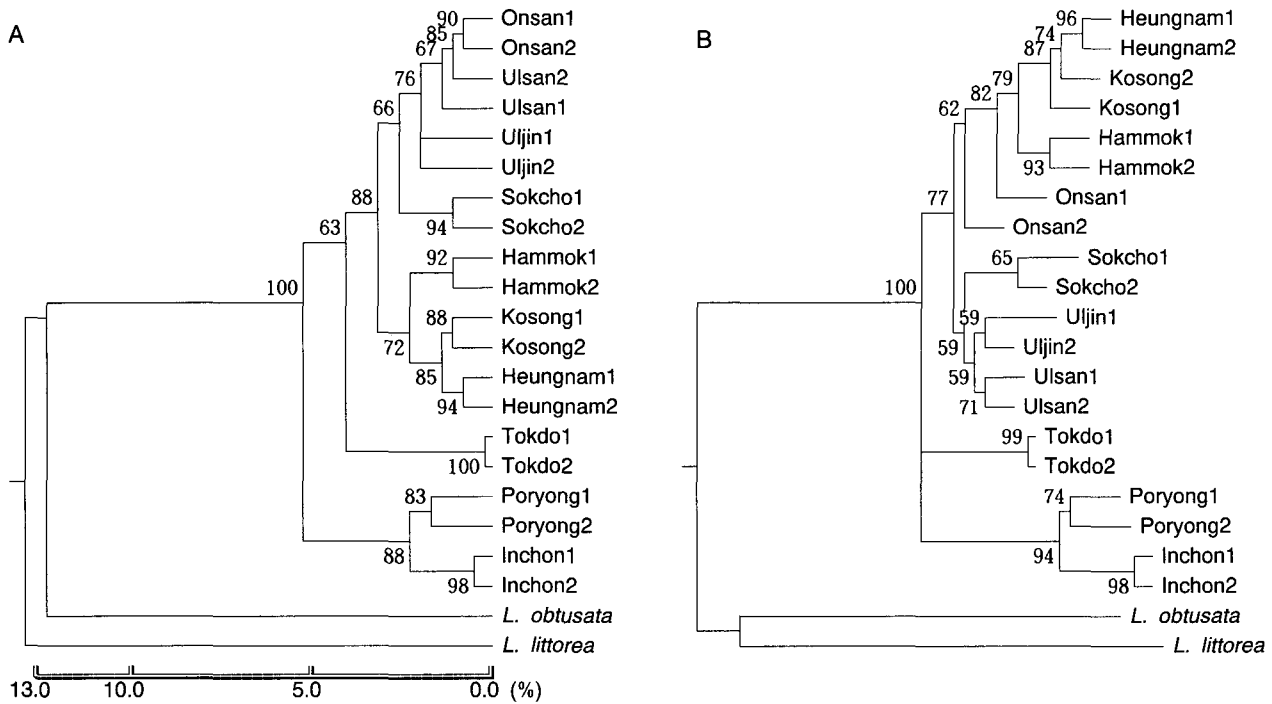


Fig. 2. UPGMA phenogram (A) and N-J phylogenetic tree (B) of *Granuliittorina exigua*. Branch lengths in UPGMA phenogram are drawn proportiona to the sequence divergence estimated by the Tamura-Nei algorithm. The nodal values indicate percent support in 1,000 bootstrap replications.

had been shown to be more efficient in computer simulations (Nei, 1991; Nei et al., 1995) and was the most appropriate analytical method to use for recovering a phylogeny.

Results

Genetic relationships among the 10 populations of *G. exigua* were estimated using nucleotide sequences of

the 282 bp region on the mt cyt *b* gene which were then compared with GenBank data of *L. littorea* and *L. obtusata* (Appendix I). Neither deletion nor insertion was found within any populations. Transitions were more frequent than transversions at synonymous sites and were dominated at the third position of the codon.

The mean values of the nucleotide compositions were adenine (23.8%), thymine (38.6%), cytosine (19.9%), and guanine (17.7%). Interpopulational variations of the

Table 2. Matrix of pairwise sequence divergence (*p*) of mt cyt *b* gene of *Granuliittorina exigua* by Tamura-Nei distance. Sequence divergences are in upper-right matrix and standard errors in lower-left matrix

| | <i>L. P</i> | <i>L. o</i> | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|-------------|-------------|-------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| <i>L. P</i> | - | 0.1289 | 0.1349 | 0.1307 | 0.1297 | 0.1297 | 0.1301 | 0.1163 | 0.1250 | 0.1254 | 0.1297 | 0.1297 | 0.1338 | 0.1457 | 0.1337 | 0.1291 | 0.1344 | 0.1209 | 0.1469 | 0.1560 | 0.1560 | 0.1517 |
| <i>L. o</i> | 0.0242 | - | 0.1261 | 0.1220 | 0.1222 | 0.1353 | 0.1276 | 0.1169 | 0.1128 | 0.1179 | 0.1129 | 0.1129 | 0.1298 | 0.1386 | 0.1334 | 0.1289 | 0.1387 | 0.1288 | 0.1377 | 0.1418 | 0.1418 | 0.1414 |
| 1 | 0.0236 | 0.0226 | - | 0.0108 | 0.0253 | 0.0291 | 0.0254 | 0.0366 | 0.0329 | 0.0254 | 0.0440 | 0.0440 | 0.0327 | 0.0216 | 0.0253 | 0.0327 | 0.0217 | 0.0291 | 0.0636 | 0.0479 | 0.0555 | 0.0554 |
| 2 | 0.0233 | 0.0223 | 0.0060 | - | 0.0293 | 0.0328 | 0.0144 | 0.0253 | 0.0217 | 0.0144 | 0.0327 | 0.0327 | 0.0365 | 0.0327 | 0.0290 | 0.0364 | 0.0253 | 0.0253 | 0.0517 | 0.0439 | 0.0517 | 0.0516 |
| 3 | 0.0226 | 0.0222 | 0.0090 | 0.0101 | - | 0.0180 | 0.0144 | 0.0254 | 0.0146 | 0.0220 | 0.0408 | 0.0408 | 0.0216 | 0.0327 | 0.0402 | 0.0327 | 0.0327 | 0.0440 | 0.0482 | 0.0640 | 0.0558 | 0.0596 |
| 4 | 0.0226 | 0.0235 | 0.0098 | 0.0104 | 0.0073 | - | 0.0180 | 0.0107 | 0.0253 | 0.0254 | 0.0443 | 0.0443 | 0.0180 | 0.0217 | 0.0291 | 0.0217 | 0.0143 | 0.0253 | 0.0443 | 0.0523 | 0.0599 | 0.0637 |
| 5 | 0.0229 | 0.0233 | 0.0093 | 0.0068 | 0.0071 | 0.0075 | - | 0.0181 | 0.0144 | 0.0072 | 0.0328 | 0.0328 | 0.0291 | 0.0327 | 0.0327 | 0.0364 | 0.0480 | 0.0556 | 0.0556 | 0.0594 | | |
| 6 | 0.0210 | 0.0212 | 0.0111 | 0.0091 | 0.0093 | 0.0055 | 0.0078 | - | 0.0144 | 0.0107 | 0.0290 | 0.0290 | 0.0256 | 0.0368 | 0.0328 | 0.0253 | 0.0291 | 0.0216 | 0.0366 | 0.0441 | 0.0520 | 0.0518 |
| 7 | 0.0220 | 0.0209 | 0.0106 | 0.0084 | 0.0074 | 0.0090 | 0.0071 | 0.0071 | - | 0.0254 | 0.0253 | 0.0253 | 0.0366 | 0.0480 | 0.0440 | 0.0364 | 0.0402 | 0.0327 | 0.0404 | 0.0479 | 0.0558 | 0.0556 |
| 8 | 0.0222 | 0.0219 | 0.0093 | 0.0068 | 0.0090 | 0.0093 | 0.0051 | 0.0058 | 0.0048 | - | 0.0253 | 0.0253 | 0.0368 | 0.0403 | 0.0364 | 0.0365 | 0.0404 | 0.0327 | 0.0480 | 0.0477 | 0.0556 | 0.0554 |
| 9 | 0.0226 | 0.0209 | 0.0119 | 0.0100 | 0.0121 | 0.0091 | 0.0104 | 0.0089 | 0.0123 | 0.0096 | - | 0.0000 | 0.0480 | 0.0517 | 0.0477 | 0.0478 | 0.0517 | 0.0439 | 0.0365 | 0.0364 | 0.0518 | 0.0517 |
| 10 | 0.0226 | 0.0209 | 0.0119 | 0.0100 | 0.0121 | 0.0091 | 0.0104 | 0.0089 | 0.0123 | 0.0096 | 0.0000 | - | 0.0480 | 0.0517 | 0.0477 | 0.0478 | 0.0517 | 0.0439 | 0.0365 | 0.0364 | 0.0518 | 0.0517 |
| 11 | 0.0229 | 0.0225 | 0.0100 | 0.0109 | 0.0080 | 0.0111 | 0.0098 | 0.0114 | 0.0075 | 0.0096 | 0.0127 | 0.0127 | - | 0.0107 | 0.0180 | 0.0107 | 0.0180 | 0.0290 | 0.0480 | 0.0637 | 0.0556 | 0.0594 |
| 12 | 0.0239 | 0.0235 | 0.0080 | 0.0102 | 0.0100 | 0.0127 | 0.0102 | 0.0116 | 0.0084 | 0.0114 | 0.0130 | 0.0130 | 0.0057 | - | 0.0071 | 0.0144 | 0.0216 | 0.0180 | 0.0517 | 0.0556 | 0.0593 | 0.0555 |
| 13 | 0.0230 | 0.0226 | 0.0087 | 0.0093 | 0.0121 | 0.0119 | 0.0100 | 0.0107 | 0.0098 | 0.0104 | 0.0122 | 0.0122 | 0.0075 | 0.0048 | - | 0.0071 | 0.0217 | 0.0181 | 0.0594 | 0.0479 | 0.0515 | 0.0478 |
| 14 | 0.0223 | 0.0219 | 0.0100 | 0.0106 | 0.0100 | 0.0107 | 0.0102 | 0.0109 | 0.0084 | 0.0090 | 0.0124 | 0.0124 | 0.0057 | 0.0068 | 0.0048 | - | 0.0144 | 0.0255 | 0.0596 | 0.0520 | 0.0516 | 0.0555 |
| 15 | 0.0233 | 0.0235 | 0.0084 | 0.0090 | 0.0100 | 0.0114 | 0.0102 | 0.0116 | 0.0066 | 0.0098 | 0.0130 | 0.0130 | 0.0073 | 0.0082 | 0.0084 | 0.0068 | - | 0.0108 | 0.0478 | 0.0482 | 0.0557 | 0.0595 |
| 16 | 0.0217 | 0.0220 | 0.0098 | 0.0089 | 0.0119 | 0.0099 | 0.0107 | 0.0100 | 0.0090 | 0.0081 | 0.0116 | 0.0116 | 0.0096 | 0.0076 | 0.0078 | 0.0093 | 0.0060 | - | 0.0439 | 0.0402 | 0.0516 | 0.0478 |
| 17 | 0.0242 | 0.0229 | 0.0150 | 0.0133 | 0.0130 | 0.0116 | 0.0127 | 0.0127 | 0.0123 | 0.0111 | 0.0109 | 0.0109 | 0.0127 | 0.0130 | 0.0141 | 0.0143 | 0.0126 | 0.0118 | - | 0.0144 | 0.0218 | 0.0255 |
| 18 | 0.0252 | 0.0233 | 0.0128 | 0.0120 | 0.0152 | 0.0126 | 0.0136 | 0.0124 | 0.0137 | 0.0121 | 0.0106 | 0.0106 | 0.0149 | 0.0136 | 0.0126 | 0.0134 | 0.0131 | 0.0115 | 0.0068 | - | 0.0218 | 0.0217 |
| 19 | 0.0252 | 0.0233 | 0.0137 | 0.0133 | 0.0138 | 0.0139 | 0.0136 | 0.0136 | 0.0145 | 0.0134 | 0.0132 | 0.0132 | 0.0136 | 0.0139 | 0.0129 | 0.0130 | 0.0139 | 0.0131 | 0.0087 | 0.0087 | - | 0.0036 |
| 20 | 0.0249 | 0.0231 | 0.0135 | 0.0131 | 0.0143 | 0.0136 | 0.0141 | 0.0135 | 0.0149 | 0.0132 | 0.0130 | 0.0130 | 0.0141 | 0.0135 | 0.0124 | 0.0135 | 0.0143 | 0.0126 | 0.0093 | 0.0085 | 0.0032 | - |

*Out groups (*L. l.*: *L. littorea*, *L. o.*: *L. obtusata*), Numbers indicate populations referred to Table 1

nucleotide sequences ranged from 0.00% to 2.54%. The sequence variations of five eastern, three southern, and two western regional populations were 1.07-4.43%, 0.71-2.90%, and 2.17-2.55%, respectively. Among these variations, the eastern and southern populations indicated the highest and the lowest variabilities, respectively.

The 94 amino acids encoded by the 282 bp nucleotide sequence were analyzed. There were no amino acid variations among the populations because all of the nucleotide substitutions were silent mutations. However, compared with outgroups, replacements of four amino acids (4.26%) have occurred. The 26th amino acid was changed from tryptophan to cysteine, the 45th and 47th from glutamine to leucine and histidine, respectively, and also the 60th from threonine to alanine.

Based on the pairwise matrix of sequence divergence (p) calculated by Tamura-Nei distance, interpopulational sequence divergences of *G. exigua* were ranged from $p=0.000$ to $p=0.064$, whereas the divergences between *G. exigua* and *L. littorea* and between *G. exigua* and *L. obtusata* were $p=0.134$ and $p=0.128$, respectively (Table 2).

For phylogenetic analysis, our data and GenBank data on *L. littorea* and *L. obtusata*, as outgroups, were combined. In the UPGMA and the N-J trees (Fig. 2A, B), *L. littorea* and *L. obtusata* were completely separated from the Korean *G. exigua*. Moreover, *G. exigua* were diverged trichotomous (the eastern, southern, and western populations with one exception of the Tokdo population) at the divergent level of $p=0.052$ with 100% bootstrap iterations. The sequence divergence between the eastern and southern populations was $p=0.032$. Especially, the Tokdo population, one of the eastern populations, was followed with $p=0.040$. Thereafter, the western populations were clustered finally ($p=0.052$). In the N-J tree, the Onsan population was clustered with the southern populations, but the nodal value of bootstrap iteration was relatively lower than that of the UPGMA tree.

Discussion

G. exigua occurs in a wide range of conditions, on exposing rocky shores and pebble beaches with low salinity. It is distributed widely at all regions of Korean coastal waters because of its planktonic larvae and temperature tolerance (Habe, 1956; Choe, 1992). Since the major four regions of Korean Waters (East Sea, Yellow Sea, Korea Strait, and Cheju Island areas) exhibit substantial differences in ecological conditions such as current, salinity, transparency, topography, and temperature (Song, 1991; Seo, 1996), these environmental heterogeneities provide valuable motivation for us to evaluate geographic variability in the species which inhabit all Korean coasts.

As shown in the matrix of sequence divergence (p), genetic divergence of Tokdo population (although it is

located in East Sea) was higher ($p=0.040$) than that between the eastern and southern populations ($p=0.032$), and this population clustered separately with the high nodal value of bootstrap iteration (88%) (Table 2, Fig. 2A). Even the species with planktonic larvae has the potential for widespread dispersal and distributes over relatively wider geographic ranges than those of nonplanktonic larvae (Hansen, 1980; Kohn and Perron, 1994; Tatarenkov, 1995). The dispersal ability of planktonic larvae might be affected by the physical or biological barriers, such as patterns of coastal circulation and larval behavior (Bohonak, 1999). For this reason, we consider that Tokdo population could be affected by one of three branches of Tshushima Warm Current influencing Ulneungdo and Tokdo Islands as a physical barrier. As shown in Fig. 2A and 2B, the eastern and southern populations are clustered first, followed by the western populations. We considered that these clustering orders may be related to the current. The eastern and southern populations are affected by the same branch of Tshushima Warm Current, and the western populations by a different branch. The average genetic divergent levels between genera (*G. exigua* - *L. obtusata* and *G. exigua* - *L. littorea*) and within genus (*L. obtusata* - *L. littorea*) are very similar ($p_{G.e.-L.o.}=0.128$, $p_{G.e.-L.l.}=0.134$, and $p_{L.o.-L.l.}=0.129$, respectively). The two genera presented in this study are so closely related to what they have been regarded as the same genus (Reid, 1996). The analysis of amino acid sequences showed, however, that four amino acids were changed between the two genera whereas only one amino acid was substituted between the two outgroup species. The silent mutations, not affecting the amino acid sequence, were more frequent within the genus than those between genera.

The evolutionary dynamics of the *cyt b* gene is better characterized with sister species, congeneric species, and confamilial genera than most other molecular systems (Johns and Avise, 1998). The mean rate of nucleotide substitution (r) can be calculated by dividing the number of substitutions (K) between two homologous sequences by $2T$, where T is the time of divergence between two sequences (Graur and Li, 2000).

Because the evolutionary rate of mtDNA is generally estimated to be 2-4% nucleotide sequence divergences per a million year (Brown et al., 1979; Brown, 1985), we can assume the time of divergence among OTUs. This calculation has been widely applied to studies of genetic relationships using mtDNA RFLP and *cyt b* gene (Brown et al., 1979; Wilson et al., 1985; Irwin et al., 1991; Sumida et al., 1998). According to our calculation, two genera *Littorina* and *Granulittorina* diverged about 3.5 to 9.8 million years before present (MYBP). The eastern and southern populations of Korean *G. exigua* seemed to diverge from the western population about 2.1 MYBP while the eastern and southern populations might have diverged from each other about 1.3 MYBP. Thus, our estimation is similar

to the recent geological study that the first fossil record of the genus *Granulilittorina* is in the middle Miocene Period (5-23 MYBP; Reid et al., 1996).

The phylogenetic relationships of *G. exigua* was shown well in the N-J tree (Fig. 2B). Three regional populations of *Granulilittorina* showed a monophyletic relationship. The Tokdo population branched off distinctly from the eastern populations, indicating that different currents between the two regions might have influenced the genetic diversity. Future studies on *G. exigua* will include related species as well as additional conspecific populations, i.e. west-southern populations and Cheju population to clarify phylogenetic relationships.

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Geographic Variation of *Granulittorina exigua*

Appendix I. Nucleotide sequences of mt cyt *b* gene of *G. exigua*. Dots designate the same sequence as *Littorea littorea*

| | | | | |
|--------------------|--|--|-----|-----|
| | 1 | 50 | 51 | 100 |
| <i>L. littorea</i> | TCCGTTGTCATATTAGCCGTGACGTCAGCTATGGCTGACTCCTCCGCTC | GCTACACGGGAACGGCGGCTCATGGTTTTTTTATTGCATCTATTTTCATA | | |
| <i>L. obtusata</i> |T.....T.....T.....T..... | AT.....T.....T..... | | |
| <i>G. exigua</i> | | | | |
| Sokcho1 | ..T..C..T.....A.....T..TTCT.....T..T.....T.....T.....C..... | | | |
| Sokcho2 | ..T..C..T.....T..TTCT.....T.....T..T.....T.....T.....C..... | | | |
| Uljjin1 | ..T..C..T.....T..TTC.....T.....T..T.....T.....T.....T..... | | | |
| Uljjin2 | ..T..C..T.....A.....T..TTC.....T.....T..T.....T.....T.....T..... | | | |
| Ulsan1 | ..T..C..T.....T..TTC.....T.....T..T.....T.....T.....T..... | | | |
| Ulsan2 | ..T..C..T.....T.....TC.....T.....T..T.....T.....T.....T..... | | | |
| Onsan1 | ..T..C..T.....T.....TC.....T.....T..T.....T.....T.....T..... | | | |
| Onsan2 | ..T..C..T.....T.....TC.....T.....T..T.....T.....T.....T..... | | | |
| Tokdo1 | ..T..C..T.....T.....T.....T.....T..T.....T.....T.....C.....T..... | | | |
| Tokdo2 | ..T..C..T.....T.....T.....T.....T..T.....T.....T.....C.....T..... | | | |
| Kosong1 | ..T..C..T.....T.....TTCT.....T.....T..T.....T.....T.....T..... | | | |
| Kosong2 | ..T..C..T.....A.....T.....TCT.....T.....T..T.....T.....T.....T..... | | | |
| Heungnam1 | ..T..C..T.....A.....T.....TTCT.....T.....T..T.....T.....T.....C..... | | | |
| Heungnam2 | ..T..C..T.....A.....T.....TTCT.....T.....T..T.....T.....T.....C..... | | | |
| Hammok1 | ..T..C..T.....A.....T.....TTCT.....T.....T..T.....T.....T.....C..... | | | |
| Hammok2 | ..T..C..T.....A.....T.....TTCT.....T.....T..T.....T.....T.....T..... | | | |
| Poryong1 | ..T..C..T.....T.....TCT.....T.....T..T.....T.....T.....C.....T..... | | | |
| Poryong2 | ..T..C..T.....A.....T.....TCT.....T.....T..T.....T.....T.....C..... | | | |
| Inchon1 | ..T..C..T.....G.....T.....TCT.....T.....T..T.....T.....T.....C..... | | | |
| Inchon2 | ..T..C..T.....G.....T.....TCT.....T.....T..T.....T.....T.....C..... | | | |
| | 101 | 150 | 151 | 200 |
| <i>L. littorea</i> | TTGGTCGAGGTATATATTACGGCTCATATCAAACCAACACACTTGAAT | ATTGGTGTAATTTTATTATTTTAAACCATAGGAACAGCATTCTAGGCTA | | |
| <i>L. obtusata</i> | .C..C..C.....T.....T..C..... |A.....C.....G.....C.....A..... | | |
| <i>G. exigua</i> | | | | |
| Sokcho1 |C.....T.....C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Sokcho2 |C.....T.....C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Uljjin1 |T.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Uljjin2 |T.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Ulsan1 |T.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Ulsan2 |C.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Onsan1 |C.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Onsan2 |C.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Tokdo1 |C.....T..A..T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Tokdo2 |C.....T..A..T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Kosong1 |T.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Kosong2 |T.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Heungnam1 |T.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Heungnam2 |T.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Hammok1 |T.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Hammok2 |T.....T.....T..C..T.....T..... |A.....C.....C..T.G..A.....T..... | | |
| Poryong1 |C.....T.....T..A..G..C..T.....T..T..... |A.....C.....C..T.G..A.....T..... | | |
| Poryong2 |C.....C.....T..A..G..C..T.....T..T..... |A.....C.....C..T.G..A.....T..... | | |
| Inchon1 |C.....T..C.....T..A..G..C..T.....T..T..... |A.....C.....C..T.G..A.....T..... | | |
| Inchon2 |C.....C.....T..A..G..C..T.....T..T..... |A.....C.....C..T.G..A.....T..... | | |
| | 201 | 250 | 251 | 282 |
| <i>L. littorea</i> | CGTCTTACCTTGAGGACAAATGTCCTTCTGGGGAGCCACTGTTATTACTA | ACCTTCTTTCAGCTGTGCCTTATTTAGGTA | | |
| <i>L. obtusata</i> | T.....C..C..... |A..T..T.....T..... | | |
| <i>G. exigua</i> | | | | |
| Sokcho1 | T..T.....A.....T.....T..... |G.....A..C..... | | |
| Sokcho2 | T..T.....A.....T.....T..... |G.....A..C..... | | |
| Uljjin1 | T..T.....A..G..T.....T..... |G.....A..C..... | | |
| Uljjin2 | T..A.....A.....T.....T..... |G.....A..C..... | | |
| Ulsan1 | T..T.....A.....T.....T..... |G.....A..C..... | | |
| Ulsan2 | T..A.....A.....T.....T..... |G.....A..C..... | | |
| Onsan1 | T..T.....A..G..T.....T..... |G.....A..C..... | | |
| Onsan2 | T..T.....A.....T.....T..... |G.....A..C..... | | |
| Tokdo1 | T..A.....A..G..T.....T..... |G.....A..C..... | | |
| Tokdo2 | T..A.....A..G..T.....T..... |G.....A..C..... | | |
| Kosong1 | T..A.....A.....T.....T..... |G.....A..C..... | | |
| Kosong2 | T..A.....A.....T.....T..... |G.....A..C..... | | |
| Heungnam1 | T..A.....A.....T.....T..... |G.....A..C..... | | |
| Heungnam2 | T..A.....A.....T.....T..... |G.....A..C..... | | |
| Hammok1 | T..A.....A.....T.....T..... |G.....A..C..... | | |
| Hammok2 | T..A.....A.....T.....T..... |G.....A..C..... | | |
| Poryong1 | T..A.....C.....A..G..T.....A..... |G.....A..C..... | | |
| Poryong2 | T..A.....C.....A..G..T.....A..... |G.....A..C..... | | |
| Inchon1 | T..A.....C.....A..G..T.....A..... |G.....A..C..... | | |
| Inchon2 | T..A.....C.....A..G..T.....A..... |G.....A..C..... | | |