Molecular classification of *Coptolabrus jankowskii* (Coleoptera: Carabidae) in regions of Korea

Eun Young Choi, Jinyoung Park, Deok Jin Jang, Kun Hyung Kim, Jong Kyun Park

*Department of Ecology Science, College of Ecology and Environmental Science, Kyungpook National University, Sangju, South Korea*

*Department of Ecological Monitoring and Assessment, National Institute of Ecology, Seocheon, South Korea*

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- ND5
- neighbor-joining tree

**Abstract**

This study was carried out to ascertain regional gene differences in the carabid beetle, *Coptolabrus jankowskii*, in Korea. There are eight subspecies of this species in Korea and it is very difficult to identify them due to morphological similarity. The mitochondrial NADH dehydrogenase subunit 5 (ND5) gene of *Coptolabrus jankowskii* from four regions, Sangju, Daegu, Jumbongsan Mountain, and Jejudo Island, was compared. The results showed differences in the base sequence at a total of 57 sites and amino acid variation at 25 sites. The neighbor-joining (NJ) tree and the maximum parsimony (MP) tree were established based on sequence data for ND5. In the NJ tree, the Jeju area (except the Songdangri site) and the Jumbongsan region showed close relationships. In the case of the MP tree, the results were divided into three separate groups: (A) the Songdangri, Jeju area, and the other Korean peninsula regions, except Jumbongsan; (B) the Jeju area, except Songdangri; and (C) the Jumbongsan region.

**Introduction**

Coleoptera is a large taxonomic group that accounts for a quarter of all Hexapoda. A total of 3333 species of 105 families belonging to Coleoptera has been reported from Korea to date (Lee et al., 2005).

*Coptolabrus jankowskii jankowskii* and *Coptolabrus smaragdinus branickii* are the largest types of Coleoptera and are commonly distributed in Korea. In the case of *C. jankowskii*, seven subspecies including *C. jankowskii chinodensis*, *C. jankowskii fallettii*, *C. jankowskii fusanus*, *C. jankowskii kojensis*, *C. jankowskii obtusipennis*, *C. jankowskii quelpartianus*, and *C. jankowskii taebeagasanensis* have been recorded to date (Park, 2004), and they show various regional variations due to restricted mobility attributable to their degenerated hindwings.

Morphological identification has traditionally been the basis for biological classification. However, many molecular biological methods have been introduced in recent times, and these are now widely used for classification and identification. Mitochondrial DNA is commonly employed in these methods, but certain gene sequences such as internal transcribed spacer 2 (ITS2) and ribosomal RNA (rRNA) are also used. Morphological variations among carabid beetles and phylogenetic classification have mostly been achieved through the comparison of NADH dehydrogenase subunit 5 (ND5) gene sequences of mitochondria (Su et al., 2001).

This study compared and analyzed mitochondrial ND5 gene sequences of *C. jankowskii* distributed inland on the Korean Peninsula, Jejudo Island, and Jumbongsan Mountain in order to identify regional molecular differences.

**Materials and methods**

**Experimental materials**

The *C. jankowskii* specimens analyzed in this study were living matter and dried specimens collected from the Sangju and Daegu areas of the Korean Peninsula, dried specimens from Jejudo Island, and dried specimens from Jumbongsan Mountain. The Jumbongsan Mountain (1424 m) is located at 38°02'35"N 128°25'40"E and was designated as a Biosphere Reserve by the United Nations Educational, Scientific, and Cultural Organization (UNESCO) in 1993. This area is defined to be off-limits to civilians by 2026 (Cha, 2012). Jejudo Island is a representative southern island located at 33°20'06.94"N 126°40'05.20"E and is isolated from the Korean Peninsula (Figure 1, Table 1). ND5 gene sequences of *C. jankowskii* registered to the National Center for Biotechnology...
PCR involved a total of 30 reaction (PCR) analysis of onds 72 repeated 35 times in total. Afterwards, extension was carried out at used as a template. One reaction cycle was de

DNA extraction and gene amplification

The genomic DNA was extracted from each C. jankowskii sample using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s guidelines. Nested polymerase chain reaction (PCR) analysis of ND5 sequences was performed. The first PCR involved a total of 30 μl of sample after the addition of distilled water to i-Taq DNA Polymerase (2.5 units; iNtRON Biotechnology, Daejeon, Korea), dNTP 200 μm, 10- Taq buffer 2.5 μL, PCR Primer 20 pmol, and template DNA 1 μL using primer made according to the existing report (Table 2; Su et al., 2001). The PCR conditions were set as follows: going through a metamorphic process at 94°C for 5 minutes. For the second PCR, the first PCR product was used as a template. One reaction cycle was defined as going through a metamorphic process at 94°C for 2 minutes, 72°C for 45 seconds, then 72°C for 45 seconds; this process was repeated 35 times in total. Afterwards, extension was carried out at 72°C for 5 minutes. The resulting sequences from the PCR went through electrophoresis in 1% agarose gel from which ethidium bromide (EtBr) was removed, and the bands were confirmed on a UV transilluminator. The refined sequences of the PCR were inser
ted in a vector using the RBC TA Cloning Kit (RBC) (England, UK) and were transformed into a competent cell by heat shock. The plasmid mini prep process was performed using the Plus Plasmid Mini Kit (NucleoGen Biotechnology, Seoul, Korea). The sequence analysis was performed by Macrogen upon request.

Sequencing and phylogenetic classification

The ND5 sequences were compared with the genes for Coleop
tera registered to the GenBank database of NCBI. Genetic differ
cences were confirmed using the EditSeq program (DNASTAR, Madison, USA) and Clustal X software. To examine genetic dis
tances, phylogenetic classification trees were drawn by the neighbor-joining (NJ) method and the maximum parsimony (MP) method using Molecular Evolutionary Genetics Analysis (MEGA) version 4.0 (Figures 2 and 3). In order to achieve reliability, the bootstrap value was set at 1000.

Results and discussion

The ND5 genomic length of C. jankowskii used in the study was 1066 base pairs (bp) each and the final genomic length confirmed through nested PCR was 898 bp. When comparing samples from different regions, ND5 genes showed sequence variations in 57 sites. When comparing amino acid sequences, it was confirmed that there were amino acid variations in 25 sites.

The genetic divergence of ND5 genes ranged from 0% to 4.4% (Figure 4). When comparing the areas in the Korean Peninsula and the Jeju Island region, Daegu showed the most significant difference compared to Bongseong-ri, Gyorae-ri, Sogil-ri, and Hannam-dong of Jejudo Island while there was no significant difference among Korean Peninsula regions such as Yangpyeong, Daegu, Sangju, and Suwon. In the case of the isolated region, Jumbongsan, there was no significant difference when compared with regions of Jejudo Island, but when compared with regions of the Korean Peninsula, there was a significant difference. In phylo
genetic classification based on genetic distance, the NJ tree was divided into two groups: (A) regions in the Korean Peninsula formed one group; (B) Jumbongsan and regions of Jejudo Island, excluding Hannam-dong, formed the other group (Figure 2). In the case of the MP tree, it was divided into three groups: A, B, and C (Figure 3). Group A showed a similar pattern to the NJ tree. Group B included populations in Jeju areas excluding Songdang-ri. Group C was formed by populations in Jumbongsan that formed a separate group on the NJ tree. According to the experimental results, it was confirmed that the populations in Jeju regions, Jumbongsan, and the Korean Peninsula showed base sequence differences. Additionally, when sequences from Jeju area groups were compared to the sequence of subspecies C. jankowskii quelpartianus registered in

### Table 1

Geographical characteristics of the sites examined in the study.

<table>
<thead>
<tr>
<th>Areas</th>
<th>Localities</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpine isolated area</td>
<td>Jumbongsan Mountain</td>
<td>Hotspot area of biological conservation, civilian restricted area</td>
</tr>
<tr>
<td>Inland area</td>
<td>Suwon, Hanam, Hwaseong, Yangpyeong, Sangju, Daegu</td>
<td>Central inland and capital area</td>
</tr>
<tr>
<td>Island area</td>
<td>Jejudo Island</td>
<td>Isolated area</td>
</tr>
</tbody>
</table>

### Table 2

List of primers used to amplify and sequence the ND5 gene Damaster (Coptolabus) jankowskii.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences (from 5’ to 3’)</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carabi-ND5-F</td>
<td>CCTGTTCCTGTTTAGTCCA</td>
<td>Su et al. (2001)</td>
</tr>
<tr>
<td>Carabi-ND5-R</td>
<td>GTCTACTTAATAATAAGTCA</td>
<td>Su et al. (2001)</td>
</tr>
<tr>
<td>Carabi-ND5-F2</td>
<td>TCACATTGTAACTCTGCGGTCA</td>
<td>This study</td>
</tr>
<tr>
<td>Carabi-ND5-R2</td>
<td>CCGTIGERCECCAAAATCT</td>
<td>This study</td>
</tr>
</tbody>
</table>

Figure 1. Examined sites of the Damaster (Coptolabus) jankowskii in Korea.
Figure 2. The neighbor-joining (NJ) tree of the mitochondrial NADH dehydrogenase subunit 5 (ND5) gene sequences of Damaster (Coptolabrus) jankowskii.


Figure 3. The maximum parsimony (MP) tree of Damaster (Coptolabrus) jankowskii (Jeon, 2009).

Figure 4. Sequence divergences in the NADH dehydrogenase subunit 5 (ND5) genes of Damaster (Coptolabrus) jankowskii.

the NCBI, sequence distances ranging from 0.3% to 0.7% were found with groups of Bongseong-ri, Gyorae-ri, Sogil-ri, and Hannam-dong areas, confirming a close relationship, and a significant difference of 3.7% sequence distance was observed with the Songdangri group. The existing research assumed that most *C. jankowskii* inhabiting Jejudo Island and *C. jankowskii* inhabiting Songdang-ri of Jejudo Island had different origins (Jeon, 2009). However, in this experiment, the population of Jumbongsan was additionally studied, and it was found that the populations of the Jumbongsan and Jeju regions have similarities in terms of phylogenetic classification. We surmise from these results that geographical isolation for long periods of time may have generated the genetic differences in the populations of Jejudo Island and Jumbongsan. The two regions have a common denominator as Jejudo Island is an island surrounded by the sea and Jumbongsan is a restricted area. In the case of the Jeju region, there could be an inflow of *C. jankowskii* populations from the Korean Peninsula associated with the recent movement of large numbers of people to and from the inland. However, this study could not find sufficient proof to support this hypothesis. To obtain reliable findings, additional experiments using *C. jankowskii* distributed in different areas across the Korean Peninsula as well as its seven subspecies should be conducted. Only ND5 gene sequence of subspecies of *C. jankowskii* were registered in the NCBI database; to compare mainland samples, allied information was lacking in the NCBI database. If additional studies are performed, it will be possible to obtain reliable findings on the origins and interregional genetic differences of *C. jankowskii* inhabiting isolated areas such as Jumbongsan and Jejudo Island.

**Conflicts of interest**

All authors declare no conflicts of interest.

**References**


