

Genetic Characterization based on a rDNA Spacer, ITS2 and mtDNA, mtCOI Gene Sequences of Korean Venus Clam, *Ruditapes philippinarum*

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Introduction

The venus clam, *Ruditapes philippinarum*, is an aquaculture shellfish mainly distributed in an intertidal zone of East Asia including Korea, China and Japan. The morphological variation of this species is great. In fact, two of the most popular markers used in molecular evolution, mitochondrial DNA (mtDNA) and nuclear ribosomal DNA (rDNA), have quite different properties, which could translate into different consequences of mutation, drift, migration and selection on patterns of geographical variation and molecular divergence.

One way to address the question of the influence of geographical isolation is to study in the same species both mtDNA and rDNA variation, using the former to infer historical patterns of gene flow. In this paper, we studied geographical variation of the mitochondrial cytochrome c oxidase subunit I gene (COI) and the second nuclear ribosomal internal transcribed spacer (ITS2) in the venus clam, *R. philippinarum* in Korea.

Materials and Methods

The clams of *R. philippinarum* used in this study were collected at three different localities, Puan, Dongbaksum and Cheju-do in Korea. Total DNA was extracted from fresh or frozen individual adult clams. The samples were lyophilized and lysed with lysis buffer, proteinase K, and RNase. DNA was extracted in phenol/chloroform and precipitated in ethanol as reported by Sambrook et al. (1989). The PCR amplification was conducted over 40 cycles using the following conditions: 1 minute at 95°C, 1 minute at 54°C, and 1.5 minutes at 72°C, with a final extension step of 7 minutes at 72°C.

Phylogenetic analysis of the mtCOI and ITS2 sequences was performed with several programs.

Results and Summary

Identification of species (and subspecies) and phylogenetic studies have been the main uses to which genetic information has been put. Mitochondrial sequences are likely to be of value in phylogenetic studies and in distinguishing among species or subspecies. Sequences of mtCOI gene have been obtained for several species on bivalvia from Genbank. A partial sequence (564bp) of the ITS2 gene in this study has been deposited in Genbank, but no journal publication has appeared as yet. The mtCOI exhibits no intra-individual variation and little or no intra-specific variation. This is in line with the expectations for such gene regions, which are expected to undergo homogenisation through concerted evolution. Consequently, mtCOI has been used in a number of phylogenetic studies at the species level.

We compared patterns of interspecific polymorphism of two makers with contrasted modes of evolution, nuclear ribosomal DNA and mitochondrial DNA, in the venus clam *Ruditapes philippinarum*. The second internal transcribed spacer 2 of rDNA and a fragment in the mtDNA gene coding for cytochrome c oxidase subunit I, were PCR-amplified and sequenced in samples of three geographical regions distributed in Korea. The nucleotide sequences of the ITS2 (564bp) and mtCOI (607bp) gene region were identical for three geographically separated. The mtCOI exhibits no intra-individual variation and little or no intra-specific variation.

References

- Sambrook, J., Fritsch, T. and Maniatis, T. 1989. Molecular cloning: a laboratory manual, 2nd ed Cold Spring Harbor Laboratory Press, New York. 9.16-9.19.