

## Sequence Divergence of 18S ribosomal DNA of Gastropods (Molluscs)

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=국문초록=

### 복족류 (연체동물)의 18S ribosomal DNA의 염기서열 분화

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3종의 복족류(*Rapana venosa*, *Reishia bronni*, *Anthosiphonaria sirius*)와 1종의 다관류, *Lepidosona* (*Lepidosona*) *coreanica*에 대한 18S ribosomal DNA의 염기서열을 밝히고 이들을 이미 보고된 18종의 이매패류, 2종의 복족류 그리고 1종의 다관류의 염기서열과 비교분석하였다. 그 결과 복족류는 V4 region에서 다른 연체동물과 구별되는 독특한 inserted sequences를 가지고 있었으며, V2 region에서는 복족류(Prosobranchia와 Pulmonata)와 이매패류(Pteriomorpha와 Heterodonta) 각각의 두 야강들이 서로 다른 특징적인 insertions 또는 deletions으로 구분되었다

### INTRODUCTION

The phylum Mollusca includes the second largest species and shows a dominant structural diversity. Traditional morphological characters have led to several controversial hypotheses on molluscan phylogeny (e.g. Milburn, 1960; Götting, 1980; Scheltema, 1988; von Salvini-Plawen, 1985; Nielsen, 1995), and the phylogenetic relationships of molluscs have not been answered up to now. Recently, molecular data have been introduced as a new source of phylogenetic information. Particularly, the 18S ribosomal DNA sequences are well suited for resolving relationships among distantly related as

well as more closely related organisms (e.g. Sogin *et al.*, 1989; Field *et al.*, 1988). These 18S rDNA have been used to infer the phylogenetic relationship of the phylum Mollusca, particularly among higher taxonomic ranks (Rice *et al.*, 1993; Winnepennickx *et al.*, 1992; 1994; 1995). However, the complete or nearly complete sequences of the 18S rRNA genes of molluscs have been mostly restricted to the species of the Bivalvia, and only two sequences from the Gastropoda and one from the Polyplacophora are known for other classes of the Mollusca. Here, we determined the 18S rRNA gene sequences from the three gastropods representing two different subclasses, the Prosobranchia (*Rapana venosa*, *Reishia bronni*) and Pulmonata (*Antho-*

*siphonaria sirius*) and one chiton species, *Lepidosona* (*Lepidosona*) *coreanica*, and investigated the sequence divergence of the molluscan 18S rRNA genes.

## MATERIALS AND METHODS

The gastropod specimens were collected in Cheju Island, and the chiton specimen was provided by Dr. Seung Shic Yum (National Institute of Genetics in Japan). Total genomic DNA was isolated from live and ethanol preserved individuals by using a modified standard procedure (Sambrook *et al.*, 1989). The 18S rRNA coding region was amplified in polymerase chain reaction (PCR) with oligonucleotide primers that recognize conserved sequences proximal to 5' and 3' termini of eukaryotic 18S rRNAs (Medlin *et al.*, 1988). PCR amplifications were performed with Taq polymerase for 30 cycles (94°C for 1min, 52°C for 2min, and 72°C for 3min). The ends of the amplified DNA fragments were modified for blunt-ended ligation using T4 kinase and T4 polymerase. The blunt-ended 18S rRNA genes were inserted into the Sma I site of the pGEM-3zf(-) plasmid vector (Promega) and transformed to DH5- $\alpha$  cell lines. The 18S rRNA coding regions were sequenced both strands. The DNA sequencing was performed by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977), with two vector primers and additional sequencing primers (Moon *et al.*, 1996). Sequencing reaction mixtures were electrophoresed on buffer-gradient 6% polyacrylamide gels and visualized by autoradiography. The nucleotide sequences were aligned with the CLUSTAL V multiple alignment program (Higgins *et al.*, 1992). The sequences determined in this study are deposited in the EMBL nucleotide sequence library (accession numbers, X98826, X98827, X98828, and X98829).

## RESULTS AND DISCUSSION

The multiple alignment of the sequences of the molluscan species shows that there exist taxon specific insertions and/or deletions in the V2 and V4 regions (Nomenclature of variable regions is after Neef *et al.*, 1991) (Fig. 1). In the V2 region (sequence position number 232-260), the two subclasses of the Gastropoda (Prosobranchia and Pulmonata) are distinguished by the insertions and/or deletions of 16-17 base pairs, and the two subclasses of the Bivalvia (Pteriomorpha and Heterodonta) are distinguished by the insertions and/or deletions of 12 base pairs. All the gastropod sequences were distinguished from the other molluscs by a portion of 7-9 inserted sequences in the V4 region (sequence position number 717-736).

The extant molluscs appeared in the fossil record around Cambrian boundary and subsequently diversified explosively within the short time span (Runnegar and Pojeta, 1985). Since most of the fast-evolving nucleotide sequences of variable regions changed further, the original informative substitutions can be obliterated. Nevertheless, the conserved taxon-specific insertions and deletions can be used as reliable taxonomic signals. As shown in the previous studies on the structural analyses of 18S rRNA gene sequences in crustaceans and insects (Moon *et al.*, 1994; Hwang *et al.*, 1995), the present study suggest that there exist taxonomic markers within the 18S rRNA gene sequences in molluscs and their markers will definitely elucidate evolutionary relationships of the molluscan major groups. Further analysis with more expanded taxa covering the taxonomic diversity might help to find out more information on the taxon specific markers in the rRNA genes.

## SUMMARY

The nucleotide sequences of the 18S ribosomal DNA of three gastropods (*Rapana venosa*, *Reishia bronni*, *Anthosiphonaria sirius*) and one chiton species, *Lepidosona* (*Lepidosona*) *coreanica* were deter-

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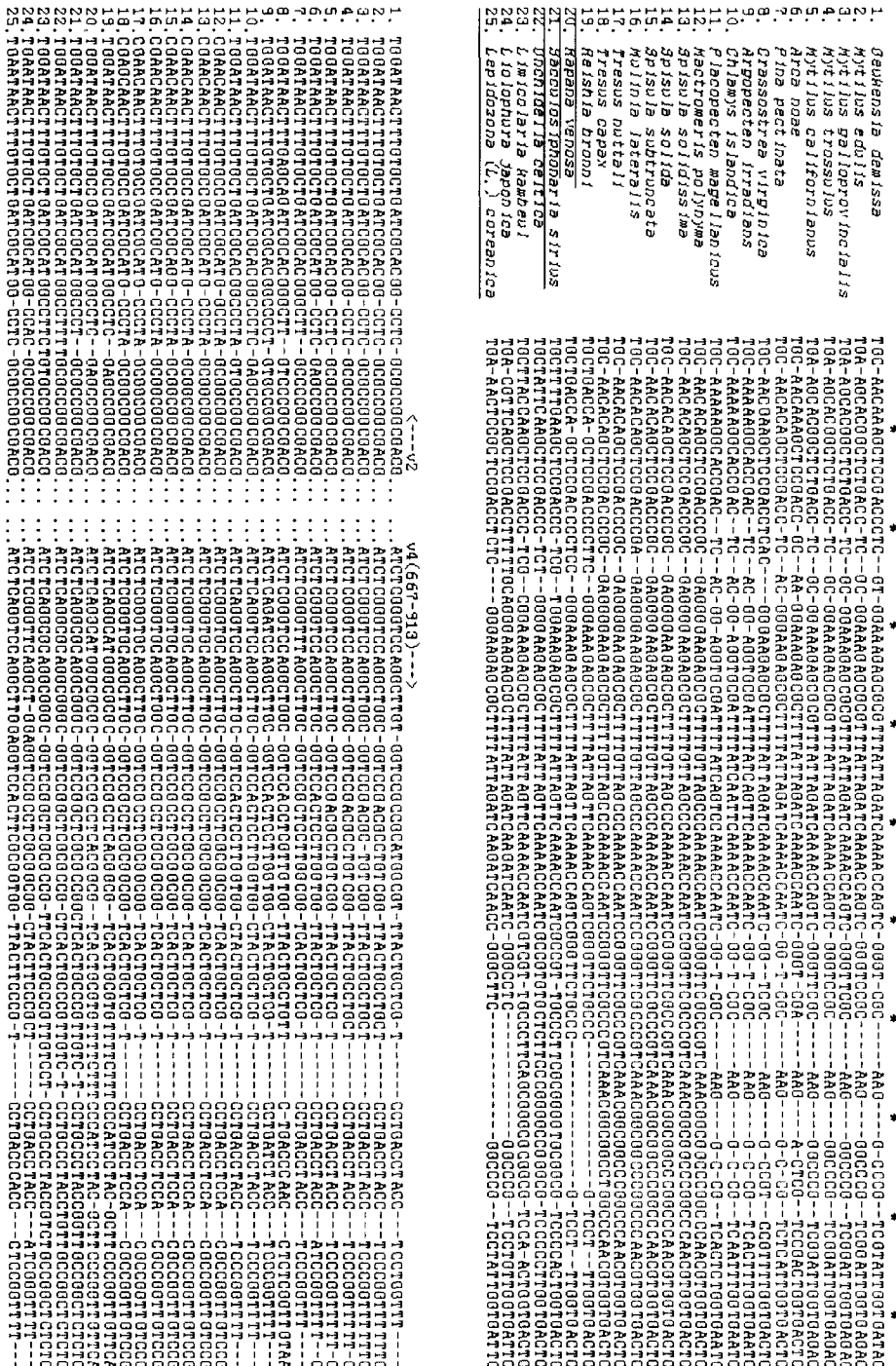


Fig. 1. (to be continued)

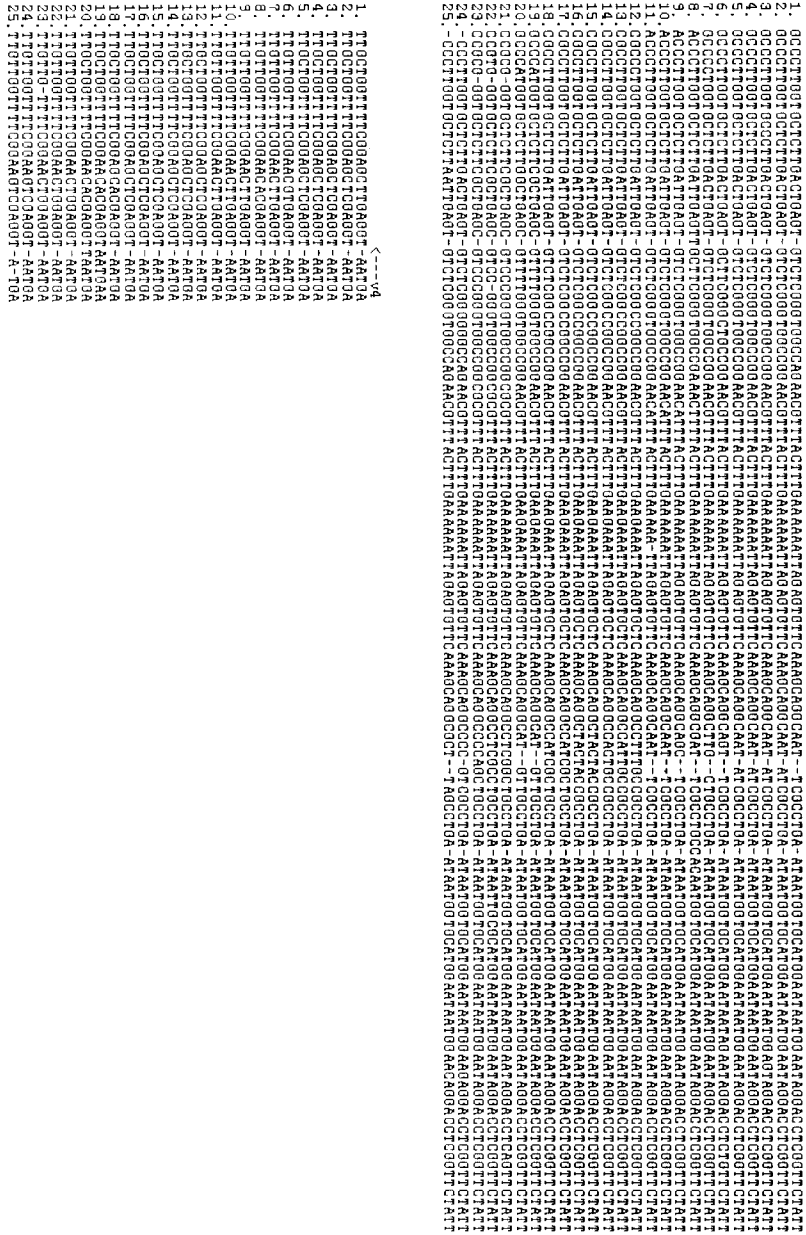


Fig. 1. Sequence comparison of the 18S rDNA of 25 molluscan species in the V2 and V4 regions. The nucleotide positions were numbered from the 5'-terminus. A dash marks an alignment gap. Species numbers are: 1-11, Pteriomorpha (Bivalvia); 12-18, Heterodonta (Bivalvia); 19-20, Prosobranchia (Gastropoda); 21-23, Pulmonata (Gastropoda); 24-25, Polyplacophora. New sequences marked with underline.

mined. These sequences were compared with those published from other molluscs which include eighteen bivalves, two gastropods, and one chiton. Comparison of the sequences shows that the gastropods are distinguished from the other molluscs by a portion of inserted sequences in the V4 region. The two subclasses of the the Gastropoda (Prosobranchia and Pulmonata) and the two subclasses of the Bivalvia (Pteriomorpha and Heterodonta) are distinguished by the insertions and/or deletions in the V2 region.

### ACKNOWLEDGEMENT

We thank Dr. Chang Bae Kim and Ui Wook Hwang for laboratory assistance. We also thank Dr. Seung Shic Yum (National Institute of Genetics in Japan) for providing us with one chiton. This work was supported by the grants from Non Directed Research Fund, Korea Research Foundation (01-D-0169).

### REFERENCES

- Field, K.G., Olsen, G.J., Lane, D.J., Giovannoni, S.J., Ghiseline, M.T., Raff, E.C., Pace, N.R. and Raff, R.A. (1988) Molecular phylogeny of the animal kingdom. *Science*, **239**: 748-753.
- Götting, K.J. (1980) Origin and relationships of the Mollusca. *Z. Zool. Syst. Evolut.-forsch.*, **18**: 24-27.
- Higgins, D.G., Bleasby, A.J. and Fuchs, R. (1992) CLUSTAL V: Improved software for multiple sequence alignment. *Comput. Appl. Biosci.* **8**: 189-191.
- Hwang, U.W., Lee, B.H. and Kim, W. (1995) Sequences of the 18S rDNAs from two Collembolan insects: Shoter sequences in the V4 and V7 regions. *Gene*, **154**: 293-294.
- Medlin, L., Elwood, H.J., Stickel, S. and Sogin, M.L. (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA coding regions. *Gene*, **71**: 491-499.
- Milburn, P.W. (1960) Further remarks on the interpretation of the Mollusca. *Veliger*, **3**: 43-48.
- Moon, S.Y., Min, G.S., Kim, S.H. and Kim, W. (1994) Sequence of the 18S ribosomal RNA coding gene of the crustacean *Philyra pisum*: longer sequences of decapods in the V9 region. *Gene*, **149**: 379-380.
- Moon, S.Y., Kim, C.B., Gelder, S.R. and Kim, W. (1996) Phylogenetic positions of the aberrant branchiobdellidans and aphanoneurans within the Annelida as derived from 18S ribosomal RNA gene sequences. *Hydrobiologia*, **324**: 229-236.
- Neefs, J.M., van de Peer, Y., De Rijk, P., Goris, A. and de Wachter, R. (1991) Compilation of small ribosomal subunit RNA sequences. *Nucleic Acids Res.*, **19**: 1987-2015.
- Nielsen, C. (1995) Animal evolution (Interrelationships of the living phyla), pp. 110-123, *Oxford University Press, Oxford*.
- Rice, E.L., Roddick, D. and Singh R.K. (1993) A comparison of molluscan (Bivalvia) phylogenies based on palaeontological and molecular data. *Mol. Mar. Biol. Biotech.* **2**: 137-146.
- Runnegar, B. and Pojeta, J. (1985) Origin and diversification of the Mollusca. *In: The Mollusca*, Vol. 10, Evolution (eds. by Wilbur, K.M., Truman, E.R. and Clarke, M.R.), pp. 1-57, *Academic Press, New York and London*.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular cloning: a laboratory manual. *Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York*.
- Sanger, F., Nicklen, S. and Coulson, A.R. (1977). DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci., USA*, **74**: 5463-5467.
- Scheltema, A.H. (1988) Ancestors and descendants: Relationships of the Aplacophora and Polyplacophora. *Amer. Malac. Bull.*, **6**(1): 57-68.
- Sogin, M.L., Edman, U. and Elwood, M. (1989) A single kingdom of eukaryotes. *In: The hierarchy of life*. (eds. by Fernholm, B., Bremer, K. and Jörnvall, H.), pp. 133-143.
- von Savini-Plawen, L. (1985) Early evolution and the primitive groups. *In: The Mollusca*, Vol. 10, Evolution. (eds. by Wilbur, K.M., Truman, E.R. and Clarke, M.R.), pp. 59-150, *Academic Press, New York and London*.

- Winnepenninckx, B., Backeljau, T., van de Peer, Y. and de Wachter, R. (1992) Structure of the small ribosomal subunit RNA of the pulmonate snail, *Limacolaria kameul*, and phylogenetic analysis of the Metazoa. *FEBS*, **309**(2): 123-126.
- Winnepenninckx, B., Backeljau, T. and De Wachter, R. (1994) Small ribosomal subunit RNA and the phylogeny of Mollusca. *Nautilus Supplement 2*: 98-110.
- Winnepenninckx, B., Backeljau, T. and De Wachter, R. (1995) Phylogeny of protostome worms derived from 18S rRNA sequences. *Mol. Bio. Evol.*, **12**(4): 641-649.