

## Molecular Phyogeny of Anthozoans (Phylum Cnidaria) Based on the Nucleotide Sequences of 18S rRNA Gene

Jun-Im Song<sup>1</sup>, Won Kim<sup>2</sup>, Eun Kyoung Kim<sup>1</sup>, and Jihee Kim<sup>2</sup>

<sup>1</sup>Department of Biology, College of Natural Sciences, Ewha Womans University, Seoul 120-750, Korea;

<sup>2</sup>Department of Molecular Biology, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea

The partial nucleotide sequences of 18S ribosomal RNA gene were compared for six cnidarian species (one belongs to class Hydrozoa: *Coryne pusilla*. Five belong to clas Anthozoa: *Bellonella rigida* from Octocorallia; *Anemonia sulcata*, *Anthopleura kurogane*, *Anthopleura midori* from Hexacorallia; *Cerianthus filiformis* from Ceriantipatharia). The aligned sequence data were used to test the hypothesis on the anthozoan phylogeny by using the distance matrix method and parsimony method. The phylogenetic inferences resulting from these methods indicate that the anthozoan is a monophyletic group and support the three subclass scheme (Octocorallia, Hexacorallia, Ceriantipatharia) within class Anthozoa. The result also indicates that ceriantipatharian is more primitive than the other groups and that family Actiniidae is a monophyletic group within the anthozoan. However, the present analysis does not clearly indicate the phylogenetic relationships of species among genera.

**KEY WORDS:** Molecular phylogeny, Anthozoans, 18S rRNA gene

The nucleotide sequence data of the macromolecules have allowed the systematists to test the hypothesis on the phylogenies among the various organisms. Of the macromolecules, the 18S ribosomal RNA has been widely used for the phylogenetic relationships among the remotely related groups in recent years, though the studies within a certain group are relatively few (Field *et al.*, 1988; Hendriks *et al.*, 1990; Abele *et al.*, 1989; Kim and Abele, 1990; Kelly-Borges *et al.*, 1991; Abele *et al.*, 1992; Spears *et al.*, 1992).

In the molecular phylogenetic studies of anthozoans, the 18S rRNA of *Anemonia sulcata* was first fully sequenced and its evolutionary position among other eukaryotes was discussed (Hendricks *et al.*, 1990). However, to date there have been no studies concerning the phylogenetic relationships among groups of anthozoans on the

basis of the nucleotide sequences of 18S rRNA gene. Here we use the partial nucleotide sequences of 18S rRNA gene to test the ideas on the phylogenetic relationships among groups of anthozoans.

### Material and Methods

One hydrozoan species and four anthozoan species were collected from the southern part of Korea (Table 1). The partial nucleotide sequences of 18S rRNA gene of these species were determined by means of PCR cloning and Taq sequencing. PCR-primer sites are located at both ends of the molecule (TACCTGGTTGATC CTGCC, TAATGATCCTTCCGCAGGTT). A PCR-reaction was performed for 30 cycles

(annealing 2', 52°C; extension 3', 72°C denaturation 1', 94°C). For blunt-ended ligation, the resulting fragments were modified as the blunt-end using T4 Kinase and T4 Polynucleotide Polymerase by means of double GeneClean method (BIO 101). They were cloned in pUC 118. Sequencing was conducted by using twelve primers (Table 2) and Taqtrack kit (Promega Co.).

The sequencing data were aligned by using FASTA program (Pearson and Lipman, 1988) and MULTALIN program (Corpet, 1988). The molecular phylogenetic trees were constructed by two different tree making methods, distance matrix (Fitch and Margoliash's method) and parsimony methods (maximum and evolutionary parsimony methods). In the distance matrix method, estimates of the number of nucleotide

substitutions per site ( $d$  values) between pairs of species after excluding alignment gaps and unreadable regions were calculated by the method of Jukes and Cantor (1969). PHYLIP (Felsenstein, 1988) was used in this method. In the parsimony methods, the ALLTREES option and the method of invariant/operator metrics of PAUP (Swofford and Olsen, 1990) were used to construct the phylogenetic trees.

## Results

The partial nucleotide sequences of each species range in length from 953 to 977 (Table 1). The total aligned set of nucleotides of six species is shown Appendix 1.

**Table 1.** A list of species compared in this study.

Systematic résumé	Number of Nucleotides
Phylum Cnidaria	
Class Hydrozoa	
Order Hydrozoidea	
Suborder Athecata	
Family Corynidae	
1. <i>Coryne pusilla</i> Gaertner, 1774	966
Class Anthozoa	
Subclass Octocorallia	
Order Alcyonacea	
Family Alcyoniidae	
2. <i>Bellonella rigida</i> Pütter, 1900	977
Subclass Hexacorallia	
Order Actiniaria	
Family Actiniidae	
* 3. <i>Anemonia sulcata</i> Pennant, 1766	964
(= <i>Anthea (Actinia) cereus</i> Ellis and Solander, 1786)	
(= <i>Anemonia vagans</i> Rosso, 1826)	
** 4. <i>Anthopleura kurogane</i> Uchida & Muramatsu, 1958	966
5. <i>A. midori</i> Uchida & Muramatsu, 1958	974
Subclass Ceriantipatharia	
Order Ceriantharia	
Family Cerianthidae	
6. <i>Cerianthus filiformis</i> Carlgren, 1924	953

\* The sequence data from Hendricks *et al.* (1990)

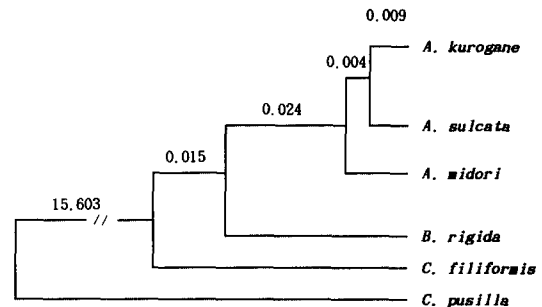
\*\* The sequence data from EMBL accession No. Z21671

**Table 2.** A list of oligonucleotide primers used in this study.

Human c-DNA of 18S rRNA position and vector primer	primer sequences
32-46	5' ACCTTGTTACGACTT 3'
162-176	5' ACGGGCGGTGTGTAC 3'
257-274	5' CCCGMTCCYCCTCTCGGA 3'
361-375	5' TCTAAGGGCATCACA 3'
481-495	5' TCTCGTTCGTTATCG 3'
662-682	5' CCGTCAAWTYCYTTDRRKT 3'
779-793	5' GCATCGTTAHGGTY 3'
897-911	5' TCCAAGAATTCACC 3'
1053-1067	5' TAATTTTTTCAAAGT 3'
1237-1254	5' GWATTACCGCGGCKGCTG 3'
1453-1467	5' ATTCCCCGTTACCCG 3'
1700-1714	5' ACGTCTAGAATTACC 3'
M13 forward(-40)	5' GTTTTCCCAGTCACGAC 3'
M13 reverse(-24)	5' AACAGCTATGACCATGA 3'

Symbols from Bishop *et al.* (1987).

Fig. 1 shows the tree resulting from the Fitch and Margoliash's method using the data of distance matrix (Table 3). *Anthopleura kurogane* combines with *Anemonia sulcata* and these two species are clustered with *Anthopleura midori*, suggesting that family Actiniidae comprise a monophyletic group. *Bellonella rigida* combines with this family and then *Cerianthus filiformis* and *Coryne pusilla* are attached to the cluster one by one. The maximum parsimony analysis yielded a single minimum-length tree (Fig. 2). *Coryne pusilla* was used as an outgroup in this analysis. *Cerianthus filiformis* came off the tree first and *Bellonella rigida* next. The remaining three species belong to family Actiniidae, suggesting that this family is a monophyletic group. The topology of this tree is same as that of the tree resulting from the distance matrix method. Bootstrap 50% majority-rule method was employed to estimate confidence intervals. The resulting consensus tree also suggest that the three species of family Actiniidae comprise a monophyletic group (Fig. 3). The invariant/ operator method (evolutionary parsimony method) was applied to infer the phylogenetic relationships of three species of family Actiniidae. The result shows that *Anemonia sulcata* is more related to *Anthopleura midori* than to *Anthopleura kurogane* (Fig. 4). This result is different from those of the distance matrix



**Fig. 1.** Tree obtained by the PHYLIP program using KITSCH.exe option. This option is accomplished by Fitch-Margoliash method. The numbers are relative length of tree's branches.

method and maximum parsimony method and from the traditional generic classification.

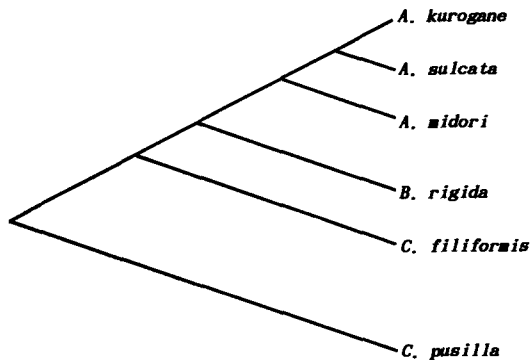
## Discussion

There have been discussions of the classification and phylogeny of the class Anthozoa during the past years. The Anthozoa has been classified as two subclasses according to the shape and number of tentacles and mesenteries (Hyman, 1940; Barnes, 1987; Manuel, 1988). On the other hands, the class has also been classified into three

**Table 3.** The values of pairwise comparison from the nucleotide sequences of the species (Table 1) used in this study.

	<i>C.p.</i>	<i>B.r.</i>	<i>A.s.</i>	<i>A.k.</i>	<i>A.m.</i>	<i>C.f.</i>
<i>C.p.</i>	-	0.117	0.096	0.095	0.102	0.141
<i>B.r.</i>	0.891 (0.109)	-	0.099	0.092	0.102	0.134
<i>A.s.</i>	0.910 (0.090)	0.907 (0.093)	-	0.021	0.034	0.113
<i>A.k.</i>	0.911 (0.089)	0.914 (0.086)	0.980 (0.020)	-	0.036	0.110
<i>A.m.</i>	0.905 (0.095)	0.905 (0.095)	0.967 (0.033)	0.965 (0.035)	-	0.117
<i>C.f.</i>	0.872 (0.128)	0.877 (0.123)	0.895 (0.105)	0.898 (0.102)	0.891 (0.109)	-

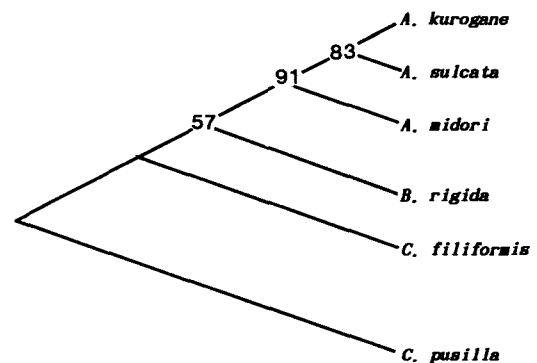
Upper right are the *d* values by the Jukes and Cantor's method (1969). Lower left are the similarities by the FASTA program. Parenthesis are the *p* values (1 - similarity). The taxa are indicated by the initials of genus and species name (from left to right): *Coryne pusilla*, *Bellonella rigida*, *Anemonia sulcata*, *Anthoplura kurogane*, *Anthoplura midori*, *Cerianthus filiformis*.



**Fig. 2.** Tree obtained by the PAUP program (version 2.4) using the ALLTREES option. HYPANC (hypothetical ancestor) = *Coryne pusilla*; Informative character No. = 66; Tree length = 124; Consistency

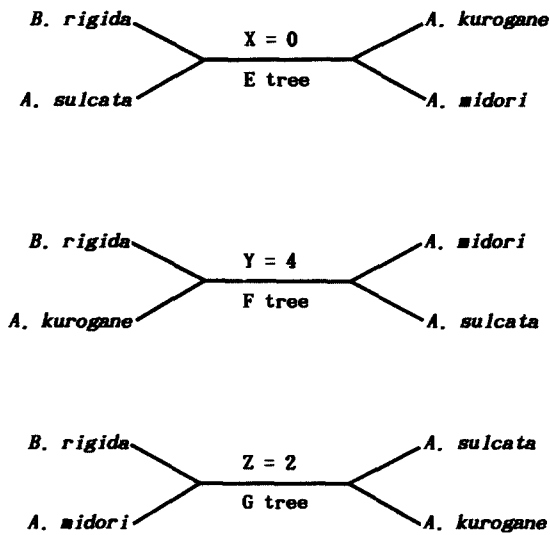
subclasses largely based on the arrangement of the septa (Wells and Hill, 1956; Dunn, 1982; Brusca and Brusca, 1990).

The present results show that class Anthozoa is a monophyletic group as indicated by Fig. 1. The relative length leading from node Hydrozoa to Anthozoa is very long compared with the lengths leading to the nodes within Anthozoa. Within the Anthozoa, *Cerianthus filiformis* branches off



**Fig. 3.** The consensus tree obtained by bootstrap 50% majority-rule. *Coryne pusilla* was used as an outgroup. N = The values of confidence intervals of tree by the bootstrap method based on 100 replicates. Tree length = 316; Consistency Index (CI) = 0.880; Homoplasy Index (HI) = 0.120; *f* value = 106; *f*-ratio = 0.0928.

from the rest species by relative length, 0.015. *Bellonella rigida* branches off from the remaining three species by relative length, 0.024. However, the three species comprising family Actiniidae are grouped by relatively short length (0.004, 0.009). Therefore these results support the three subclass scheme (Octocorallia, Hexacorallia, Cerianti-



**Fig. 4.** Analyses with the Lake's method applied to four taxa. Total counts of invariant (X, Y, Z) are indicated.

patharia) rather than two subclasses (Octocorallia, Hexacorallia).

Fig. 2 shows that *Cerianthus filiformis* comes off first and then *Bellonella rigida* branches off next, and then *Anemonia sulcata* - *Anthopleura kurogane* - *Anthopleura midori* comprise a monophyletic group and branches off latest. This result is same as that of the distance matrix method.

On the phylogenetic relationships among the groups of Anthozoa, several authors had different ideas. The hypotheses are Ceriantipatharia-Alcyonaria-Zoantharia (Hyman, 1940; Wells and Hill, 1956), Alcyonaria-Zoantharia-Ceriantipatharia (Dunn, 1982; Brusca and Brusca, 1990) and Alcyonaria-Ceriantipatharia-Zoantharia (Manuel, 1987). As indicated by Figs. 1 and 2, the present results support the phylogenetic relationships of Ceriantipatharia-Alcyonaria-Zoantharia within class Anthozoa.

As stated in the introduction, the nucleotide sequences of 18S rRNA have been largely used for the phylogenetic relationships among the remotely related organisms. However, the exact categorical rank to which the sequence data of 18S rRNA can apply has not been determined. In the molecular phylogeny of crustacean decapods, Kim and Abele (1990) noted that there are only 3

nucleotide differences among the species of same genus. Therefore the sequence data of the 18S rRNA gene might not be adequate for the phylogenetic studies among the lower categorical ranks. In the present study, the relationships among genera were examined. The results from the distance matrix method and maximum parsimony method are different from that of the evolutionary parsimony method (Figs. 1, 2, 4). These results from the sequence data are also different from the traditional classification based on the morphological data. Morphologically the two genera are clearly distinguished and *Anemonia* is considered to be more primitive than *Anthopleura* (Cargren, 1949). Therefore it is premature to discuss the phylogenetic relationships among the genera until more species are included in analysis and the variations of nucleotide differences of 18S rRNA among the organisms of different categorical ranks are investigated.

Additional data of many species representing subclasses are necessary to determine in greater detail the anthozoan relationships examined in this study. The analysis of the complete nucleotide sequences is also necessary to understand the nature of the 18S rRNA molecule and to construct more reliable phylogenetic trees with the proper tree making method.

## Acknowledgements

This work was supported by funds from the Korean Research Institute for Better Living, Ewha Womans University and grants from the Korea Sciences and Engineering Foudation through the Research Center for Cell Differentiation (93-4-3).

**APPENDIX 1.** Nucleotide sequences of 18S rRNA of the taxa studied. 'N' = unknown seq., '.' = alignment gap (written from 5' to 3' end). KUR = *Anthopleura kurogane*, SUL = *Anemonia sulcata*, MID = *Anthoplura midori*, COR = *Coryne pusila*, FIL = *Cerianthus filiformis*, BEL = *Bellonella rigida*.

KUR TCCCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT  
 SUL TATCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT  
 MID TCCCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT  
 COR XXCCTGGTTGATCCTGCCAGTAACGATATGCTTGTCTGAAAGATTAAGCCATGCATGCTG  
 FIL TCCCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT  
 BEL NNCCTGGTTGATCCTGCCAGTAT.CATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT

KUR ..AAGTATAAGCACTTGTACTGTGAAACTGCG.AATGGCTCATTAATCAGTTATCGTTT  
 SUL ..AAGTATAAGCACTTGTACTGTGAAACTGCG.AATGGCTCATTAATCAGTTATCGTTT  
 MID ..AAGTATAAGCACTTGTACTGTGAAACTGCG.AATGGCTCATTAATCAGTTATCGTTT  
 COR TCAAGTATAAGCACTAGTACTGTGAAACTGCG.AATGGCTCATTAATCAGTTATCGTTT  
 FIL ..AAGTATAAGCACTTGTACTGTGAAACCTCCGAATGGCTCATTAATCAGTTATCGTTT  
 BEL ..AAGTATAAGCACTTGTACTGTGAAACTGCG.AATGGCTCATTAATCAGTTATCGTTT

KUR ATTTGATTGTACGTTTAC.T.ACTTGGG.TAACCGTGGTAATTCTAGAGCTAATACATGC  
 SUL ATTTGATTGTACGTTTAC.T.ACTTGGG.TAACCGTGGTAATTCTAGAGCTAATACATGC  
 MID ATTTGATTGTACGTTTAC.CTACTTGGG.TAACCGTGGTAATTCTAGAGCTAATACATGC  
 COR ATTTGATTGTAACCTTAC.T.ACTTGGG.TAACCGTAGTAATTCTAGAGCTAATACATGC  
 FIL ATTTGATTGATTGT.....ACTTTTACTAATGGXXXXXXXXXXXXXXXXXXXXXXXXXXXX  
 BEL ATTTGATTGTACTCTCATACTACTTGGG.TAACCGTAGTAATTCTAGAGCTAATACATGC

KUR GAAAAGTCCCGACTTCTGG.....AAGGGATGTATTTATTAGATTCAAAA  
 SUL GAAGAGTCCCGACTTCTGG.....AAGGGATGTATTTATTAGATTCAAAA  
 MID GAAACGTCCCGACTGTTAGGC.....AAGGCATGTATTTATTAGATTCAAAA  
 COR GAAAAGTCCCGACTTGCCGCGTCTCGCGTGGCGGAAGGGACGTATTTATTAGATTAAAAA  
 FIL XXX  
 BEL GTAAAAGCCCGACTCTCGTCC.....AAGGGATGTATTTATTAGATTAAAAA

KUR CCAATGCGGGTTCCGCC.....CGGTGTGTTGGTGATTTTCATAGT.AACTGTT  
 SUL CCAATGCGGGTTCTGCC.....CGGTGCTTTGGTGATT.CATAGT.AACTGAT  
 MID CCAGTXXXXXXXXXXXXXX.....XTTTTGGTG...ATTTCAATCGTAACTGAT  
 COR CCAATGCGAC..CTGCCTTCGCGGGCGGCTTACTTGGTGATTCATGAT.AACTTGT  
 FIL XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXTGTCTGGTGATTCATGAT.AACTTTT  
 BEL CCAATGCCGGCTTAACGGCCCTCTAACCAC.....CTTGGTGATTCATAGT.AACTGT.

KUR CG..AAT.CGCAGGGCCTT.....GGCGCTGGCGGATGTTTCATTCAAATTTCTGCC  
 SUL CG..AAT.CGCATGGCCTT.....G.CGCTGGCG.ATGTTTCATTCAAATTTCTGCC  
 MID CGCCAATTCGCATGGCCAA.....C...CTGGCGGATGTTTCATTCAAATTTCTGCC  
 COR GG..AAT.CGCATGCCCTC.....GAGGCCGCG..ATGTTTCATTCAAATTTCTGCC  
 FIL CG..AAT.CGCATGGCCTT.....G...CCGGCGGATGTTTCATTCAAATTTCTGCC  
 BEL X..CGAAT.CGCTGGCCTTCTCTGTTTCGTGCCGGCG.ATGTTTCATTCAAATTTCTGCC

KUR TATCAACTGT.CGATGGTAAGGTATTGG..CTTACCATGGTTACAACGGGTGACGG.AGA  
 SUL TATCAACTGT.CGATGGTAAGGTATTGG..CTTACCATGGTTACAACGGGTGACGG.AGA  
 MID TATCAACTGGTCGATGGTAAGGCTATTGGCCTTACCATGGTTACAACGGGTGACAGGAGA  
 COR TATCAACCTTTTCGATGGTAAGGTAGTGG..CTTACCATGGTGATAACGGGTGACGG.AGA  
 FIL TATCAACTGT.CGATGGTAAGGTAGTGG..CTTACCATGGTTGGAACGGGTGACGG.AGA

BEL CTATCAACTGT.CGATGGTAAGGTAGTGG..CTTACCATGGTTGCAACGGGTGACGG.AG

KUR ATTAGGGTTTCGATTCCGGAGAGGGAGCCTGAGAGACGGCTACCACATCCAAGGAAGGCAG  
SUL ATTAGGGTTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAG  
MID ATTAGGGTTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAG  
COR ATTAGGGTTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAG  
FIL ATTAGGGTTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGGAAGGCAG  
BEL AATTAGGGTTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCA

KUR CAGGCGCGCAAATTACCCAATCCTGACTCAGGGAGGTAGTGACAAGAAATAACAATACAG  
SUL CAGGCGCGCAAATTACCCAATCCTGACTCAGGGAGGTAGTGACAAGAAATAACAATACAG  
MID CAGGCGCGCAAATTACCCAATCCTGACTCAGG.AGGTAGTGACAAGAAATAACAATACAG  
COR CAGGCGCGCAAATTACCCAATCCG.ACTCGGGGAGGTAGTGACAAGAAATAACAATACGG  
FIL CAGGCAACGAAATTACCCAATCCG.ACTCGGGGAGGTAGTGACAAGAAATAACGGTACGG  
BEL GCAGGCGCGCAAATTACCCAATCCTGACGTGGG.AGGTAGTGACAAAAATAACAATACA

KUR GGCTTTTCTAAGTCTTGTAATTGGAATGAGTACAACCTTAAAT.....CCTTTAACGAGGA  
SUL GGCTTTTCTAAGTCTTGTAATTGGAATGAGTACAACCTTAAAT.....CCTTTAACGAGGA  
MID GGCTTTTCTAAGTCTTGTAATTGGAATGAGTACAACCTTAAAT.....CCTTTAACGAGGA  
COR GGCTTTGACAAGTCTCGTAATTGGAATGAGTAGAATTTGAAG.....CTCTTAACGAGGA  
FIL GGTCTTGATAAGTCTCGTAATTGGAATGAGTACAATTTGAATTAATTCCTT..ACGGC.A  
BEL GGCTTTTTTGTAGTCTTGTAATTGGAATGAGTACAATTTAAAT.....CTCTTAACGAGG

KUR TCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATA  
SUL TCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATA  
MID TCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATA  
COR TCGATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATG  
FIL TCAATTGGAGGCCAAGTGGTG..CCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATA  
BEL ACCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTAT

KUR TTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTCGGGGTGGGCG..CCGGTCCG.  
SUL TTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGACTTCGGGGTGGCACGGCCGGTCCG.  
MID TTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATT.CGGGGTGGCACGGCCGGTCCG.  
COR TTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCCGGATGGGTTGTCCGGTGCC.  
FIL TTAAAGTTTGT.CAGTTAAAAAGCTCGTAGTTGGATTTCCGGACGGGTGAGTCCGGTCTG.  
BEL TGTAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCCGGTCCG..ACGGCGAGGTCA

KUR CCGCAAGTGTG..CACTGGCCGGGCGCTCTTCTCGCAAAGACTGCGTGTGCTCTTGAC  
SUL CCGCAAGTGTG.CACTGGCCGGGCGCTCTTCTCGCAAAGACCGCGTGTGCTCTTGAC  
MID CCGCAAGGTGTGCTCACTGGCCGGGCGCTCTTCTCGCAAAGACTGCGTGTGCTCTTAAC  
COR GC.CAAGGTGCGCTACTGGGCGGCTCGTCCTTCTCGTTCCGACCGCGCGTGTGCTCTG.AC  
FIL CCGCAAGGTATGTTACTGGCTTGCCTGTTCTTCTCGCAAAGACTGCGCATGGCTTCAAT  
BEL GCCGCAAGGTATGCCACTGTCGACGTTGGCCTTCTCGCGCAGACTTCGCGTGTCTTTAA

KUR TGAGTGTGCGCAGGTAAGTCTGCGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC  
SUL TGAGTGTGCGCGGG.AGTTGCGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC  
MID TGAGTGTGCGTAGG.ACTTGCGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC  
COR TGAGTG..CTCGGGGACGAGCGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGC  
FIL GCACGTG.CGTAAG.ATTTACGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAG.C  
BEL CTGAGTGTGGCGTTGGATACGCGACGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGG

KUR CAGCG...CTTGAATACATAAGCATGGAATAATGGAATAGGACTTGGGTTCT.ATTTTTGT

SUL CAGCG...CTTGAATACATAAGCATGGAATAATGGAATAGGACTTGGGTTCT.ATTTTGT  
 MID CAGGCGGCCTTGAATACATAAGCATGGAATAATGGAATAGGACTTGGTTCCT.ATTTTGT  
 COR CTTGG...CTTGAATGCATAAGCATGGAATAATGGAATAGGACTCGGXTTCT.ATTTTGT  
 FIL TATCG...CTTGAATACATGAGCATGGAATAATGGAATAGGACTTTGGTTCTTATTTTAT  
 BEL CTTGTG...CTTGGATACATAAGCATGGAATAATGGAATAGGACTTTGGTTCTATTTTCC

KUR TGGTTTCGTGGAACCGAAGTAATGATTAAGAGGGACAGTTGGGGG.CATTTCGTATTTTCGT  
 SUL TGGTTTCGTGGAACCTGAAGTAATGATTAAGAGGGACAGTTGGGGG.CATTTCGTATT.CGT  
 MID TGGTTTCGTGGAACCG.AAGTAATGATTAAGAGGGACAGTTGGGGT.CATTTCGTATTTTCGT  
 COR TGGTTTCGTGAGCCGGAGTGATG.ATGAAGAGGGACAGTTGGGGG.CATTTCGTATTTTCGT  
 FIL TGGTTTCT..AGACCGAAGTAATGATTAAGAGGGACAATTGGGCG.CATCCGTATTTTCGG  
 BEL GTTGGTTTCGTGGAACCGAAGTAATGATTAATAGGGACAGTTGGGGGTCATTTCGTATTTTCG

KUR TGTCAGAGGTGAAATTCTTGGATTTACGAAAGACGAACTACTGCGAAAG.CATTTGCCAA  
 SUL TGTCAGAGGTGAAATTCTTGGATTTACGAAAGACGAACTACTGCGAAAG.CATTTGCCAA  
 MID TGTCAGAGGTGAAATTCTTGGATTTACGAAAGACGAACTACTGCGAAAG.CATTTGCCAA  
 COR TGTCAGAGGTGAAATTCTTGGATTTGCGAAAGACGAACTAATGCGAAAG.CATTTGCCAA  
 FIL TGTCAGAGGTGAAATTCTTGGATTTACGAAAGGCGGACAACACTGCGAAAG.CATTTGCCAA  
 BEL TTGTCAGAGGTGAAATTCTTGGATTTACGAAAGACGAACTAATGCGAAAGTCATTTGCCA

KUR GAATGTTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATACCGTCCT  
 SUL GAATGTTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATACCGTCCT  
 MID GAATGTTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATACCGTCCT  
 COR GAATGTTTTTCATTAATCAAGAACGAAAGTTAGAGGCTCGAAGACGATCAGATACCGTCCT  
 FIL GAGTGTTCATTAATCAAGAACGAAAGTTAGAGGATCGAAGACGATCAGATACCGTCCT  
 BEL AGAATGTTTTCAATAATCAAGAACGAAAGTTAGAGGATCGAAGACGATCAGATACCGTCC

KUR AGTTCTAACCATAAACGATGCCGACTAGGGATCAGAGAGTGTTATTGGATGACCT  
 SUL AGTTATAACCATAAACGATGCCGACTAGGGATCAGAGAGTGTTATTGGATGACCT  
 MID AGTTCTAACCATAAACGATGCCGACTAGGGATCAGAGAGTGTTATTGGATGACCT  
 COR AGTTCTAACCATAAACGATGCCGACTAGGGATCGGAGGGTGTTATTTCATGACCC  
 FIL AGTTCTAACCATAAACGATGTCGACTAGG.ATCAXXCGGCGTTATTGTACGACCT  
 BEL TAGTTCTAACCATAAACGATGCCGACTAGGGATC.....AGCG

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(Accepted April 12, 1994)

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18S rRNA 유전자의 염기서열에 근거한 산호충류 (자포동물 문)의 분자계통  
 송준임<sup>1</sup> · 김 원<sup>2</sup> · 김은경<sup>1</sup> · 김지희<sup>2</sup>(<sup>1</sup>이화여자대학교 자연과학대학 생물학과, <sup>2</sup>서울대학교  
 자연과학대학 분자생물학과)

산호충류의 분자계통을 밝히기 위하여 자포동물에 속하는 6종에 대한 18 rRNA 유전자의 염기서열을 비교하였다. 본 연구에 사용된 종들은 산호충 강에 속하는 5종(팔방산호충 아강의 *Bellonella rigida*, 육방산호충 아강의 *Anemonia sulcata*, *Anthopleura kurogane*, *Anthopleura midori* 및 꽃말미잘 아강의 *Cerianthus filiformis*)과 히드로충 강에 속하는 1종 *Cornyme pusilla* 이었다. 정렬된 염기서열은 Distance matrix method와 parsimony method를 사용하여 기존의 산호충류 계통을 검증해 보았다.

그 결과 산호충류는 단일계통군이었고, 산호충 강을 3아강으로 나누는 기존의 체제는 지지되었지만, 꽃말미잘 아강은 다른 아강보다 더 원시적인 군인 것으로 나타났다. 또한, 해변말미잘과는 산호충 강내의 단일계통군이지만, 속간의 계통유연관계는 명확하지 않았다.