Studies on genetic diversity of marine invertebrates in Korea

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- Marine invertebrates are essential members in the marine ecosystem.

- Korean people have used a variety of marine invertebrates such as

- crabs, shrimps, clams, gastropods, cephalopods

- mainly as food resources.

- Nowadays, we are experiencing the continuous and rapid loss of these marine invertebrates.

- To insure their sustainable use

- we must be fully aware of the current situations on the marine invertebrate biodiversity.

- In Korea, biological research on marine invertebrates is limited only to the faunal studies in most taxa.

- Therefore, it is very difficult to follow the concept of biodiversity conservation such as

- the sustainable use of natural resources.

- The continuous collections, inventory, faunal and systematic studies, and population genetic studies on

- all marine invertebrates are essential requirement to understand our marine ecosystem as a whole.

- It has become a widespread practice to define biodiversity in terms of genes, species and ecosystem.

- Biodiversity is very commonly used as a synonym of species diversity, in particular of 'species richness', which is the number of species in a site or habitat.

- Therefore, correct identification of component species is the first step to study the ecosystem.

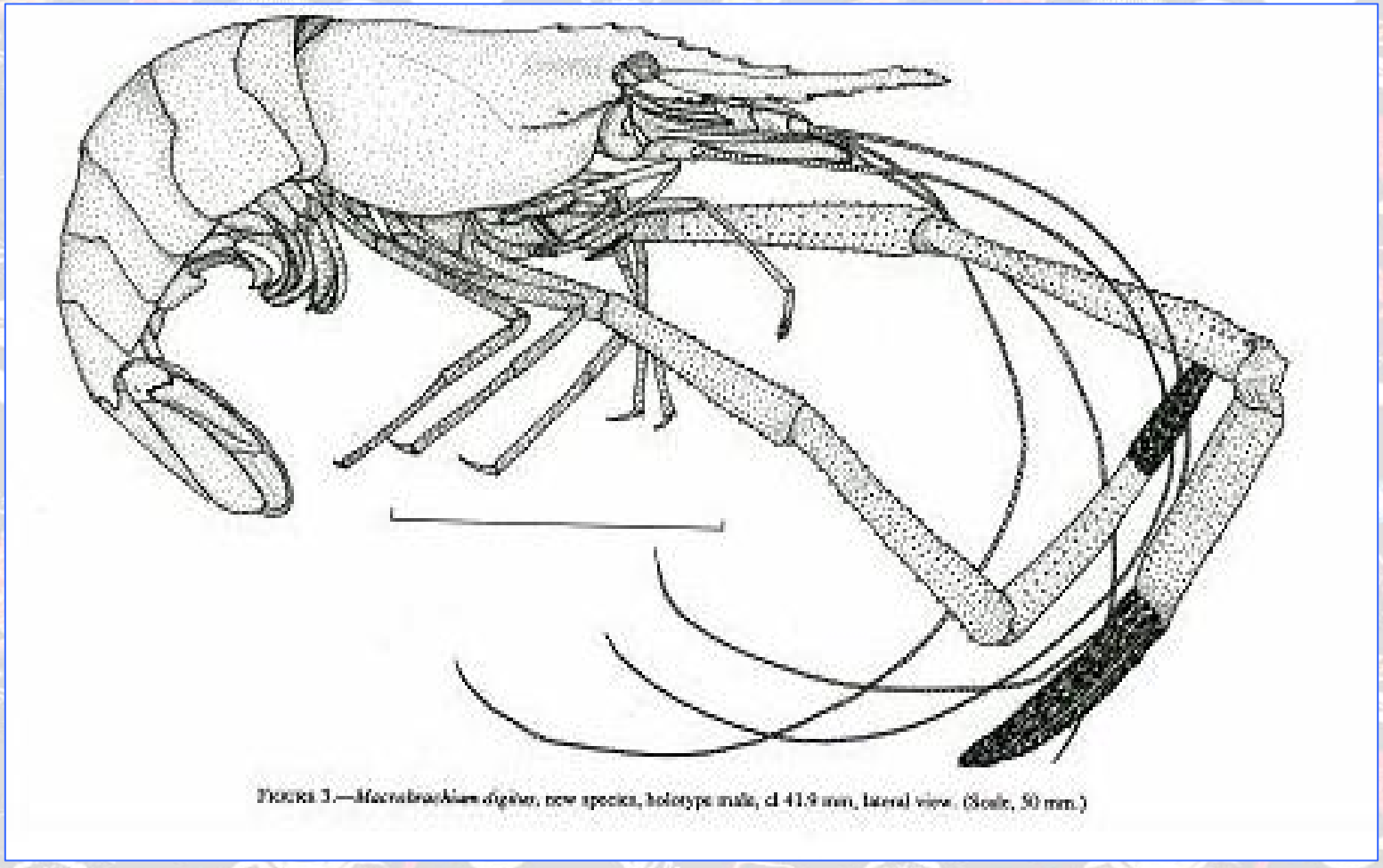


FIGURE 3.—*Macrobrachium digitus*, new species, holotype male, cl 41.9 mm, lateral view. (Scale, 30 mm.)

- In Korea, the most serious problem we are now faced with

- a shortage of these experts, especially taxonomists.

Genetic Diversity

- The genetic diversity inherent in most species provides the raw material to respond rapidly to changed circumstances.

- Genetic diversity is the variety of alleles and genotypes present in the group under study (population, species or group of species).

- Loss of genetic diversity reduces evolutionary potential and is also associated with reduced reproductive fitness.

- Therefore we must continuously make a correct diagnosis of the extent of genetic diversity of species.

- There is an array of techniques available for directly, or indirectly measuring DNA base sequence variation (RFLP, RAPD, AFLP, SSCP, SNP, etc.).

- DNA sequencing is now routinely conducted, especially for systematic studies.

Use of macromolecules in the study of biological diversity

- In Korea, molecular systematic study was introduced in 1989 by the present author.

- Since 1986, my colleagues and I have studied the various aspects of nuclear rRNA genes and mtDNA of selected animal groups in terms of systematics and molecular evolution.

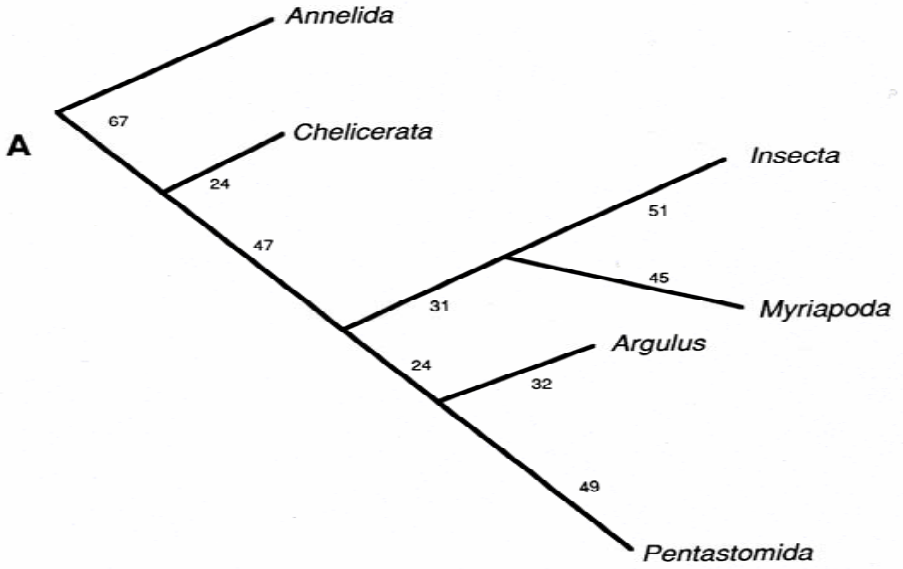
Phylum Pentastomida (tongue worm)

- *Q :* phylogenetic position

- *DATA :* partial nucleotide sequences of 18S rRNA.

- *Results :* the tongue worms are highly modified crustaceans closely related to fish lice (Branchiura, Crustacea, Arthropoda).

(Abele, L.G, W. Kim, and B.E. Felgenhauer, 1989, Mol. Biol. Evol.)



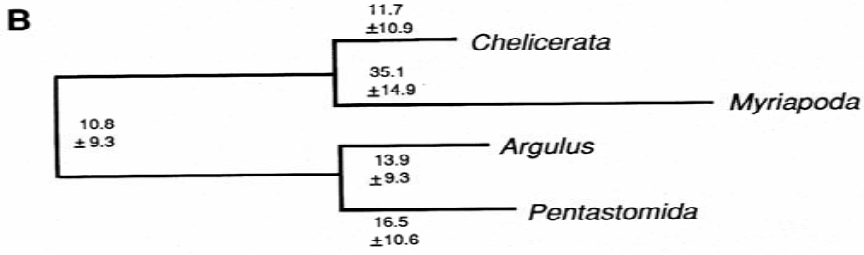


FIG. 2.—A, Relationships of the groups as suggested by the ALLTREES option of PAUP; branch lengths are indicated; total length = 370, CI = 0.681, on the basis of 164 informative sites. The next shortest tree is 374 steps and has *Argulus* and the Pentastomida coming off the main branch adjacent to each other. In the next five shortest trees (two at 375 steps and three at 376 steps), *Argulus* and the Pentastomida either come off the main branch adjacent to each other or share a common node. B, Relationships as suggested by the method of invariants/operator metrics. For this invariant ($X - E + u - H - J = 8 + 2 - 0 - 2$), the hypothesis that $E + u = H + J$ is a two-tailed binomial, where $P(E+u;E+u+H+J,0.5) = P(10;12,0.5) = 0.038$ (see Holmquist et al. 1988). Branch lengths indicated are number of transversions/1,000 nucleotides and are to scale.

FIG. 1.—Anterior, ventral surface of *Argulus nobilis* (*top*) and anterior portion of *Porocephalus crotali* (*bottom*). Both photomicrographs are by SEM.

Selected decapod crustaceans

Q : relationships among infraorders within Decapoda

Data : Partial nucleotide sequences of 18S rRNA

Results:

1) Nucleotide sequences of 3 species of procambarus are virtually identical (differ in only 3 of more than 1,500 nucleotides)
2) Variation is not evenly distributed across the molecule (conserved-variable-highly variable)
3) transversion: transition ratio with a mean 0.987

4) *Penaeus aztecus* (Dendrobrachiata) differs from the other species(Pleocyemata) in the sequence of a highly conserved region

5) Variation is phylogenetically informative to infraorder or possibly superfamily level

6) Suborder Dendrobrachiata are separated from suborder pleocyemata. Within pleocyemata, Caridea are separated from Stenopodids and Brachyura.

(Kim, W. and L. G. Abele, 1990, J. Crustacean Biol.)

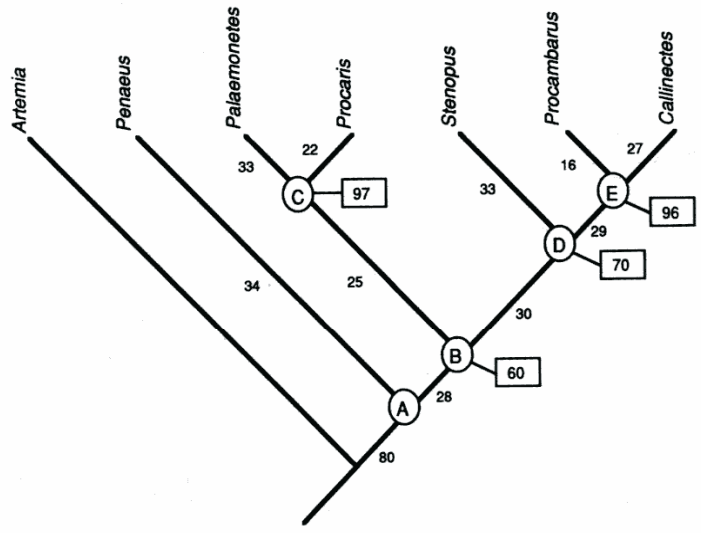


Fig. 2. Relationships among the taxa considered as estimated by PAUP using the ALLTREES option. Length = 357, CI = 0.706, based on 156 characters. □ = An estimate of the confidence intervals of the tree by the bootstrap method based on 100 replicates.

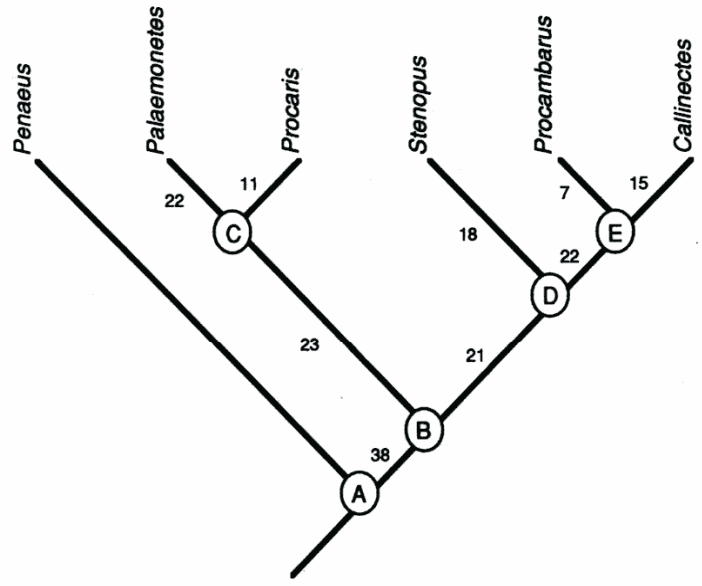


Fig. 3. Relationship among the taxa indicated as estimated by PAUP using the ALLTREES option. Length = 177, CI = 0.740, based on 96 characters.

Rapid progress in molecular systematics and molecular evolution

Rate of nucleotide substitution differs among

(1) the different genes
(2) the different region of same gene
(3) different lineage

Researchers began to search adequate molecular markers in their taxonomic groups.

Molecular Phylogeny

* Data: Partial nucleotide sequences of 18S rRNA

· Molecular phylogeny of some decapod crustaceans based on 18S rRNA nucleotide sequences.

(Kim, W., and L.G. Abele, 1990. Journal of Crustacean Biology)

· Nucleotide analysis of 18S rRNA and molecular phylogeny of the Korean decapods.

(Kim, W. and G.J. Bae, 1992. Korean J. Zool.)

Molecular phylogeny

- Phylogeny of selected ***maxillopodan and other crustacean taxa*** based on 18S ribosomal nucleotide sequences: a preliminary analysis.
  (Abele, L.G., T. Spears, W. Kim, and M. Applegate, 1992. Acta Zoologica)

- The monophyly of ***Brachyuran crabs***: A phylogenetic study based on 18S rRNA.
  (Trisha, S., L.G. Abele, and W. Kim, 1992. Syst. Biol.)

- Molecular phylogeny of ***anthozoans (phylum Cnidaria)*** based on the nucleotide sequences of 18S rRNA gene.
  (Song, J.-I., W. Kim, E.K. Kim, and J. Kim, 1994. Korean J. Zool.)

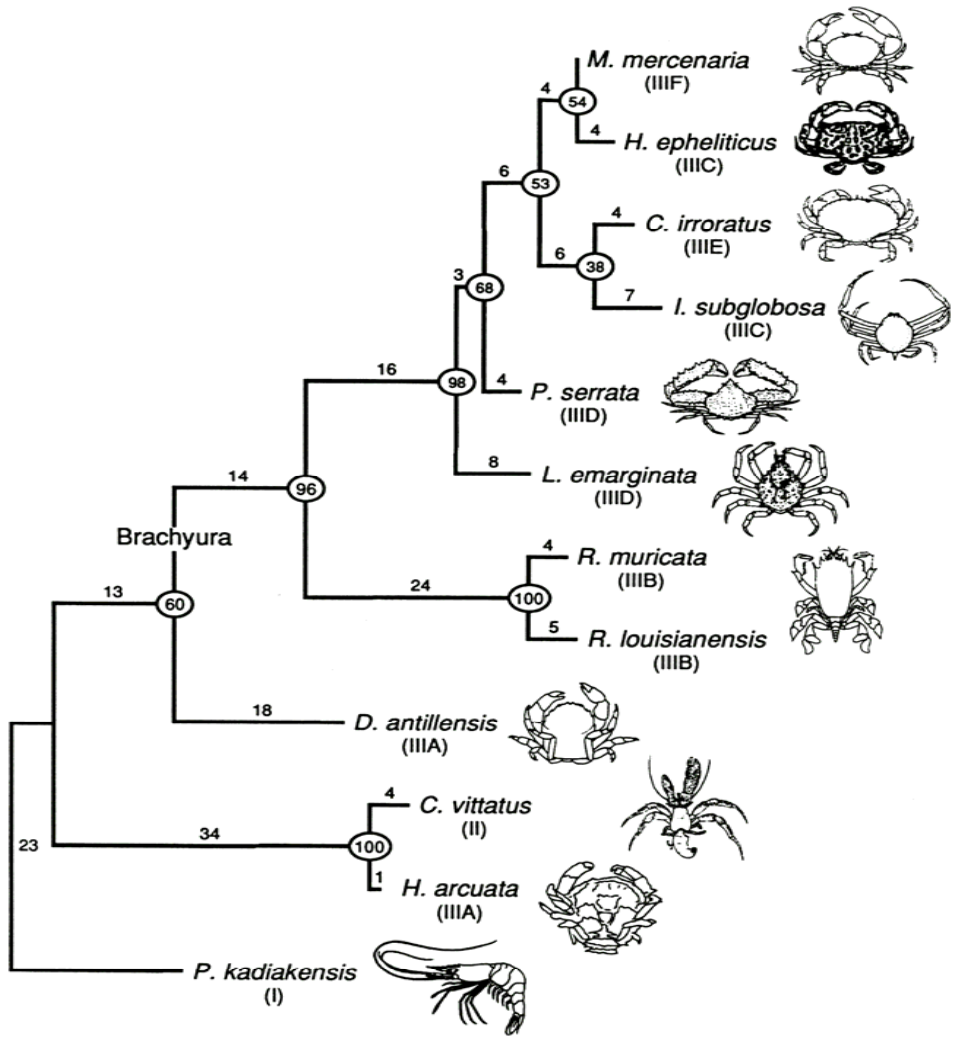


FIGURE 2. Relationships inferred by parsimony analysis of 12 taxa based on 104 informative nucleotide positions (including gaps) using the caridean shrimp *Palaemonetes kadiakensis* as an outgroup. Roman numerals and capital letters in parentheses refer to classification scheme given in Table 1. One of three equally most-parsimonious alternative phylogenies is shown; total length = 202 steps; consistency index (Kluge and Farris, 1969) = 0.748; retention index (Farris, 1989) = 0.715. Circled numbers indicate the percentage of 1,000 bootstrapped replicates that support the nodal relationships shown; our view of the lower limit of the Brachyura is indicated. (Drawings modified from Smith, 1886; Rathbun, 1925, 1933, 1937; Garth, 1939; Holthuis, 1955; Williams, 1965; Hart, 1982; Abele and Kim, 1989.)

Nucleotide Analyses: complete sequence

- Kim, W., G.S. Min, and S.H. Kim, 1992. A study of the nucleotide analysis of 18S rRNA and the molecular evolution of the ***Decapods*** (II). Korean J. Syst. Zool. Special Issue No. 3: 139-146.

- Kim, W., G.S. Min, and S.H. Kim, 1992. The 18S ribosomal RNA gene of a crustacean decapod *Oedignathus inermis*: a comparison with *Artemia salina* gene. Nucleic Acids Res., 20(17): 4658.

- Kim, W., J.I. Song, E.K. Kim, and J. Kim, 1993. The 18S ribosomal RNA gene of an ***anthozoan*** *Anthopleura kurogane*: a comparison with

Nucleotide Analyses

- Kim, W., S.M. Yoon, and J. Kim, 1993. The 18S ribosomal RNA gene of a *crustacean branchiopod Bosmina longirostris*: comparison with another branchiopod *Artemia salina*. Nucleic Acids Res. 21(15): 3583.

- Kim, W., C.B. Kim, G.S. Min, and S.H. Kim, 1993. The nucleotide sequences of 18S ribosomal RNA gene of a *crustacean cumacean*, *Diastylis* sp. Nucleic Acids Res. 21(11): 2767.

Nucleotide Analyses: Taxon specific insertion/deletion of 18S rDNA nucleotide sequences

- Sequence of the 18S ribosomal RNA-encoding gene of the crustacean *Philyra pisum*: *longer sequences of decapods in the V9 region*

  (Moon, S.Y., G.S. Min, S.H. Kim, and W. Kim, 1994. Gene)

- Sequences of the 18S rDNAs from two Collembolan insects: *shorter sequences in the V4 and V7 regions*.

  (Hwang, U.W., B.H. Lee, and W. Kim, 1995. Gene)

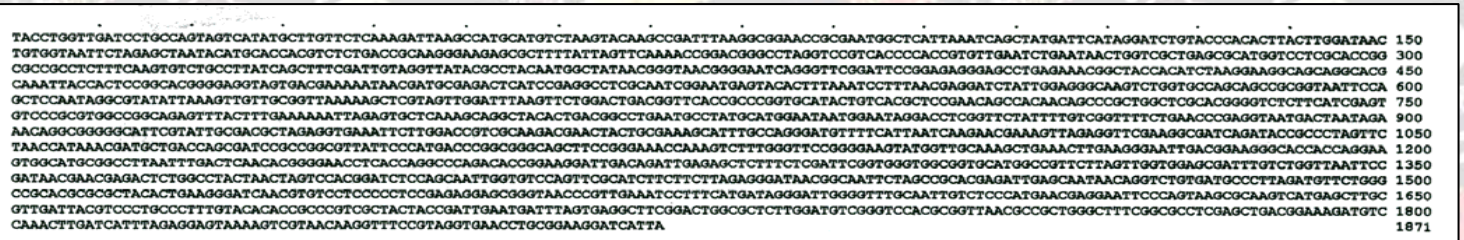


Fig. 1. Sequence of the 18S rRNA gene of *Ph. pisum*. Sequencing was conducted by means of PCR cloning and *Taq* sequencing. The PCR products were obtained using two primers located at both ends of the molecule (5′-TACCTGGTGATCCTGCC, 5′-TAATGATCCTTCCGCAGGTT). For blunt-ended ligation, both ends of PCR products were modified using T4 kinase and T4 polymerase (Kim et al., 1992a).This sequence is deposited with GenBank (accession No. Z25817).

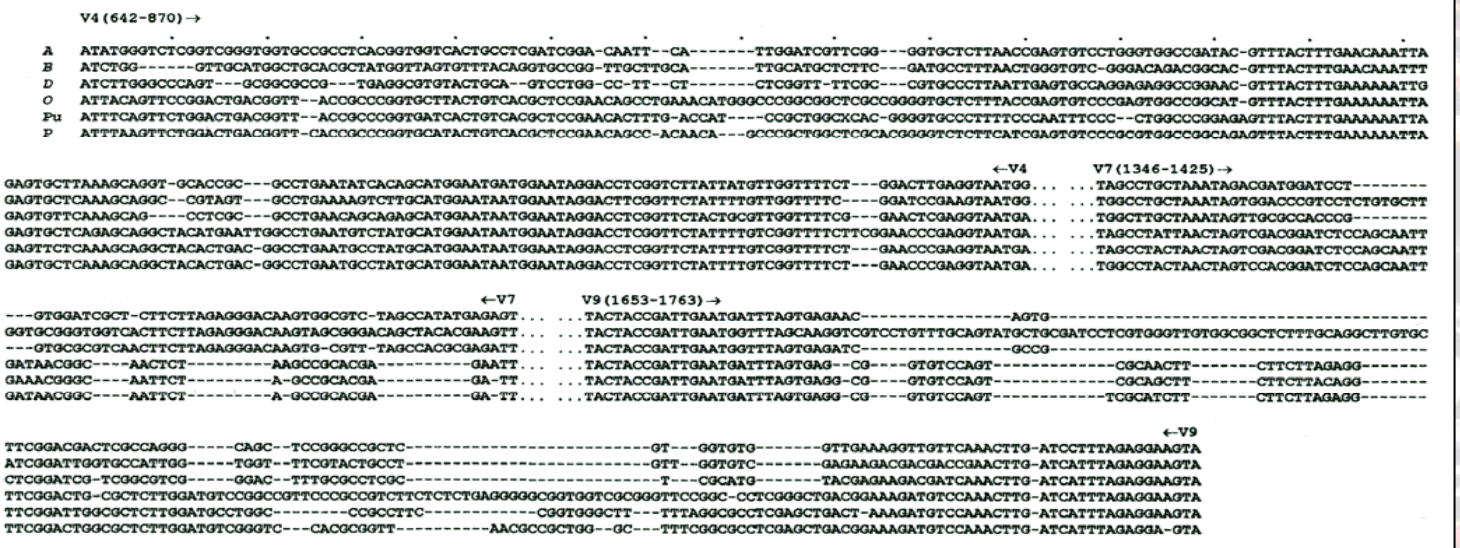


Fig. 2. Sequence comparison among six crustacean species in the V4, V7 and V9 regions of the 18S rRNA gene. The position number is the nt numbering of *A. salina*. A dash marks the absence of a nucleotide. *A*, *A. salina*; *B*, *B. longirostris*; *D*, *Diastylis* sp.; *O*, *O. inermis*; *Pu*, *Pu. quadridens*; *P*, *Ph. pisum*.

Molecular Phylogeny

- Phylogenetic study of the suborder Arthropleona (insecta: Collembola) based on morphological characters and 18S rDNA sequence analysis. (Lee, B.H., U.W., Hwang, W. Kim, K.H., Park, and J.T., Kim 1995. Polskie Pismo Entomologiczne)

- Systematic position of cave Collembola *Gulgastrura reticulosa* (insecta) based on morphological characters and 18S rDNA nucleotide analysis. (Lee, B.H., U.W. Hwang, W. Kim, K.H. Park, and J.T. Kim, 1995. Memories de Biospeologie)

※Data: Morphological characters and complete nucleotide sequences of 18S rDNA

- Moon, S.Y., C.B., Kim, S.R., Gelder, and W. Kim, 1996. Phylogenetic positions of the aberrant branchiobdellidans and aphanoneurans within the Annelida as derived from 18S ribosomal RNA gene sequences. Hydrobiologia 324: 229-236.

※Data: Complete nucleotide sequences of 18S rDNA

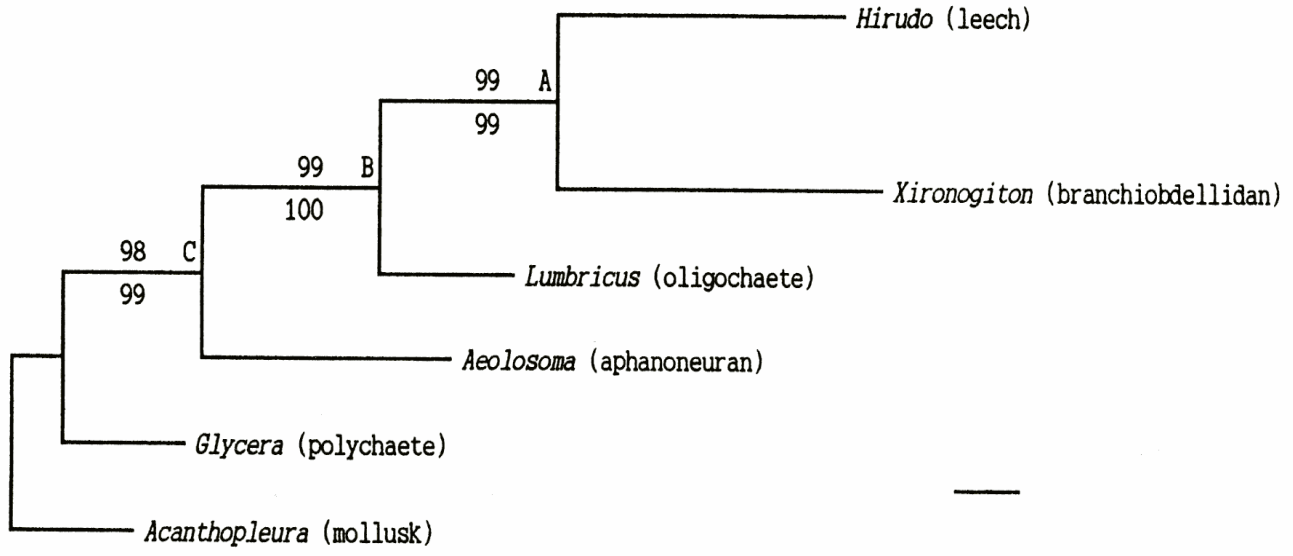


Figure 2. Phylogenetic relationship of selected annelid taxa. The numbers next to the nodes represent the bootstrap proportions for the parsimony (top) and neighbor-joining analyses (bottom). Branch nodes are indicated by A, B, C respectively. Scale bar = 10 steps.

Molecular Phylogeny

- **Moon, S.Y., and W. Kim, 1996. Phylogenetic position of the Tardigrada besed on the 18S ribosomal RNA gene sequences. Zoological Journal of the Linnean Society, 116: 61-69.**

  * Data: Morphological characters and complete nucleotide sequences of 18S rDNA

- **Sequence divergence of 18S ribosomal DNA of gastropods (molluscs).**

  (Yoon, S.H., S.Y. Moon, B.L. Choe, and W. Kim, 1996. Korean J. Malacol.)

- **Molecular Phylogeny of Arthropods and Their Relatives: Polyphyletic Origin of Arthropodization.**

  (Min, G.S., S.H. Kim, and W. Kim, 1998. Mol. Cells)

- **Phylogenetic position of the ciliates *Phacodinium* (Order Phacodiniida) and *Protocruzia* (Subclass Protocruziidia) and systematics of the spirotrich ciliates examined by small subunit ribosomal RNA gene sequences.**

  (Shin, M.K., U.W. Hwang, W. Kim, A.-D.G. Wright, C. Krawczyk, and D.H. Lynn, 2000. Europ. J. Protistol.)

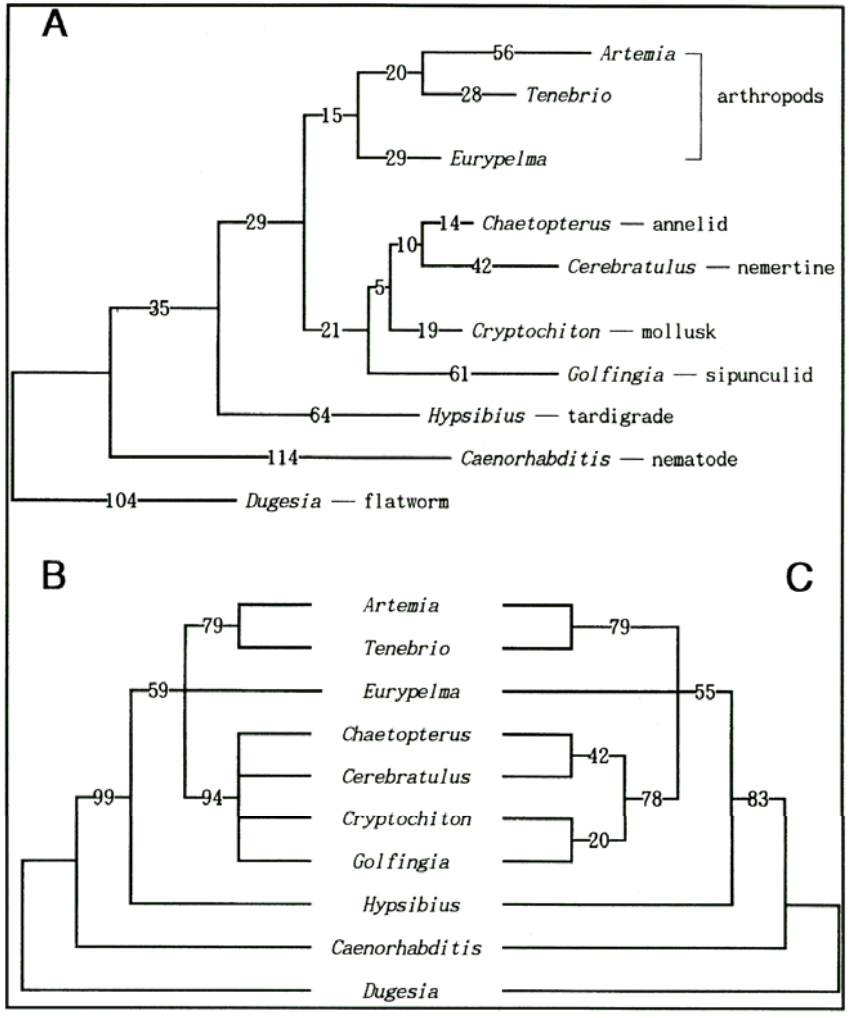


Figure 1. Phylogenetic relationships generated from maximum parsimony analysis using PAUP. A, Minimum-length phylogenetic tree. Numbers indicate the branch lengths at each node. B, Bootstrap 50% majority-rule consensus tree. Numbers at nodes represent the bootstrap percentages from 1000 samples. C, 50% majority-rule consensus tree of 101 trees lying within 1% of the length of the shortest tree. Numbers at nodes represent the frequency with which clades descending from nodes were found among the 101 trees saved.

Combined data set: morphological and molecular characters

- Phylogenetic relationships of ***Annelids, Molluscs, and Arthropods*** evidenced from molecules and morphology.

(Kim, C.B., S.Y. Moon, S.R. Gelder, and W. Kim, 1996. J. Mol. Evol.)

Molecular Phylogeny

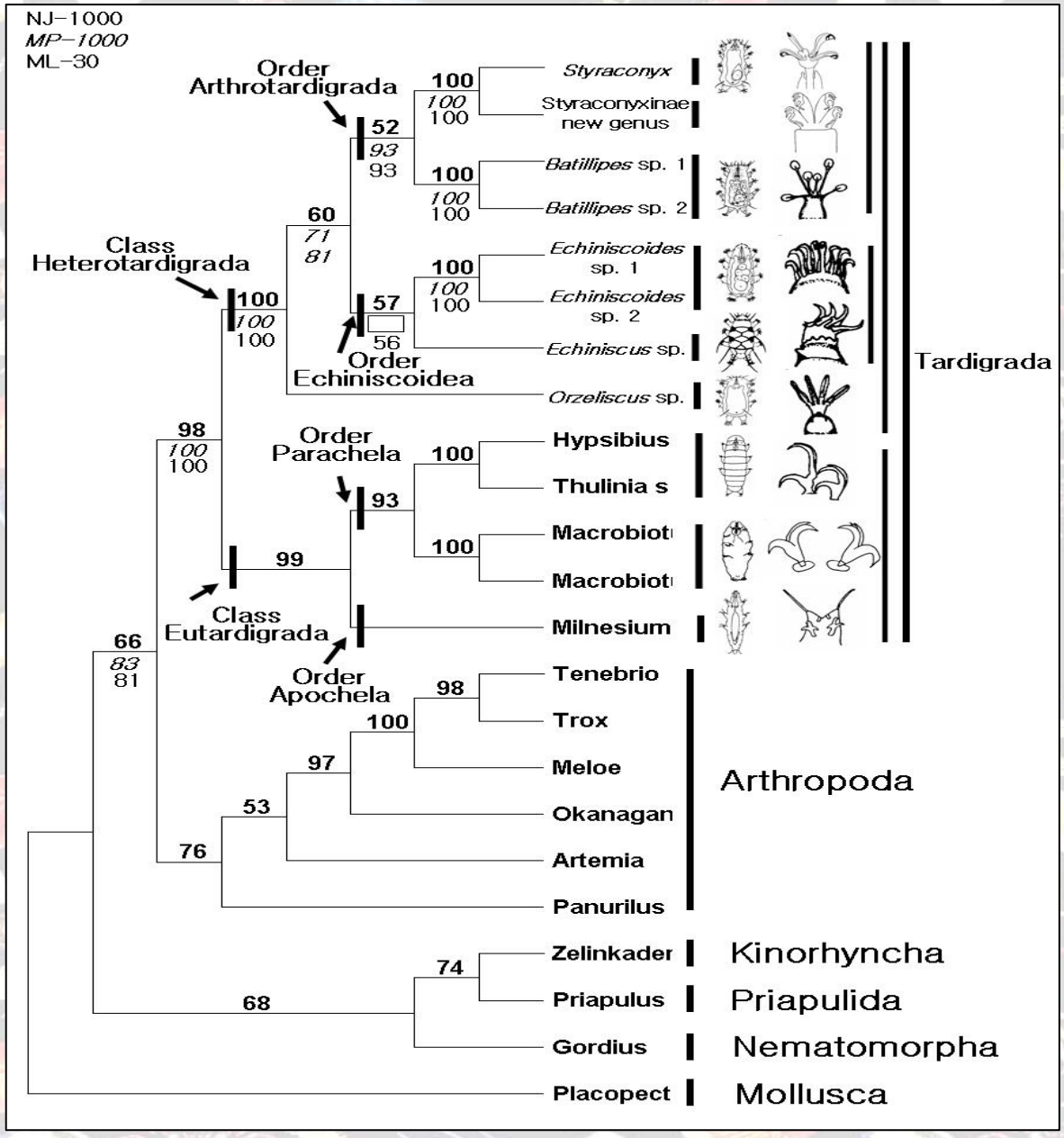
- Molecular Phylogenetics at the Felsenstein Zone: Approaching the Strepsiptera Problem Using 5.8S and 28S rDNA Sequences. (Hwang, U.W., W. Kim, D. Tautz, and M. Friedrich, 1998. Mol. Phylogenet. Evol.)

- A New Perspective on Lower Metazoan Relationships from 18S rDNA Sequence. (Kim, J., W. Kim, and C.W. Cunningham. 1999, Mol. Biol. Evol.)

- Phylogeny of some gastropod mollusks derived from 18S rDNA sequences with emphasis on the Euthyneura. (Yoon, S.H., and W. Kim, 2000. Nautilus)

- Molecular phylogeny of poecilostome Copepods based on the 18S rDNA sequences. (Kim, J.H., and W. Kim, 2000. Korean J. Biol. Sci.)

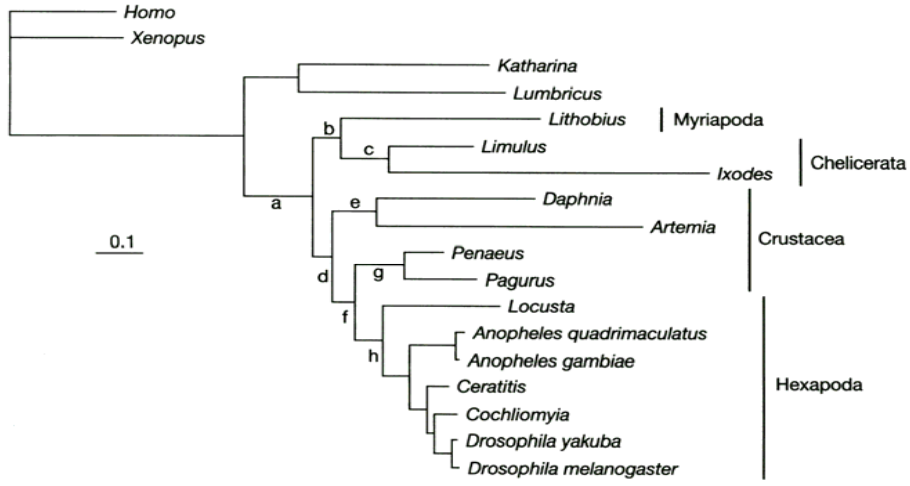
- Phylogenetic Relationships among Tardigrades Based on the Analysis of 18S RNA Gene Sequences: Molecular Evidence for Polyphyly of Arthrotardigrada. (Rho H. S., J. K. Park, C. Y. Chang, and W. Kim, 15th Annual Meeting of the Korean Society for Molecular and Cellular Biology)


NJ-1000
MP-1000
ML-30
Order Arthrotardigrada
Class Heterotardigrada
Order Echiniscoidea
Order Parachela
Class Eutardigrada
Order Apochela
Styraconyx
Styraconyxinae new genus
Batillipes sp. 1
Batillipes sp. 2
Echiniscoides sp. 1
Echiniscoides sp. 2
Echiniscus sp.
Orzeliscus sp.
Hypsibius
Thulinia s
Macrobiot
Macrobiot
Milnesium
Tenebrio
Trox
Meloe
Okanagan
Artemia
Panurilus
Zelinkader
Priapulus
Gordius
Placopect
Tardigrada
Arthropoda
Kinorhyncha
Priapulida
Nematomorpha
Mollusca

Molecular Phylogeny: complete nucleotide sequence of mitochondrial DNA

- Mitochondrial protein phylogeny joins myriapods with chelicerates

  (U.W. Hwang, M. Friedrich, D. Tautz, C.J. Park, and W. Kim, 2001. Nature)



	a	b	c	d	e	f	g	h
MP	100/97	83/84	65/54	40[1]/92	70/92	57/38[4]	100/100	91/98
NJ	100/100	100/96	100/81	17[2]/88	99/86	100/97	100/100	100/100
QP	91/94	99/99	100/99	46[3]/57	99/97	95/84	100/97	99/96

Figure 3 Phylogram of best maximum-likelihood tree with 18P2560 alignment (ln(likelihood) = −42925.32). Bar represents 0.1 substitutions per site. Branches with letters have branch support values (BP) given below the tree for maximum parsimony (MP), neighbour-joining (NJ) and the maximum-likelihood-based quartet puzzling method (QP)[28]. Left numbers refer to 18P2560 alignment, right numbers to 18P1528 alignment. Superscript numbers indicate branches that are not included in bootstrap majority rule consensus trees: 1, Branchiopoda placed at the base of arthropods with BP = 57; 2, Branchiopoda placed at the base of the arthropods with BP = 82; 3, Branchiopoda placed at the base of the arthropods with BP = 53; 4, monophyletic Crustacea supported with BP = 53.

Genome Analysis

- Ribosomal DNA intergenic spacer of the swimming crab, *Charbdis japonica*.
  (Ryu, S.H., Y.K. Do, U.W. Hwang, C.P. Choe, and W. Kim, 1999. J. Mol. Evol.)

- Intragenomic length variation of the ribosomal DNA intergenic spacer in a malaria vector, *Anopheles sinensis*.
  (Whang, I.J., J.W. Jung, J.K. Park, G.S. Min and W. Kim, 2002. Mol. Cells.)

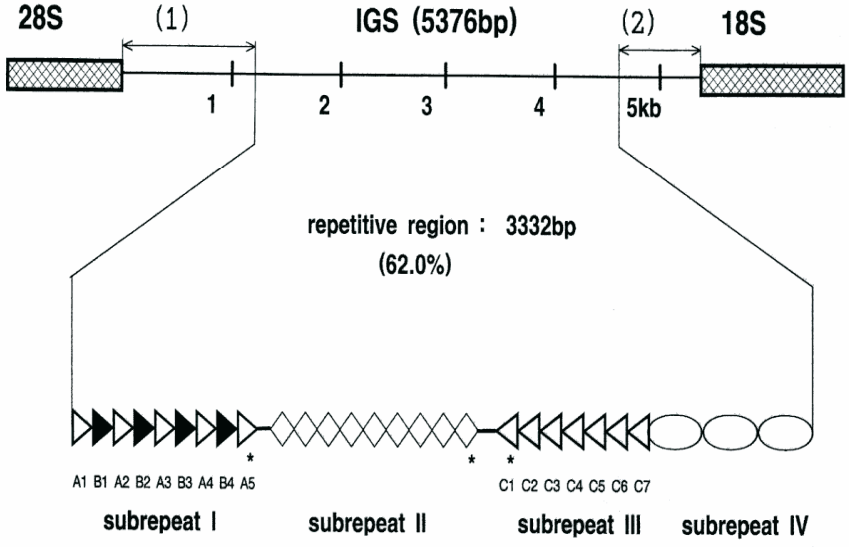


Fig. 1. Structural organization of the *C. japonica* rDNA repeat. The 1195-bp nonrepetititive region 1 and the 813-bp nonrepetitive region 2 are marked by the *numbers in parentheses* at the *top.* The 3332-bp repetitive region is composed of four subrepeats. The tandem array of nine 60-bp repeat units is represented by *arrowheads: open arrowheads,* 60 bp-a; *filled arrowheads,* 60 bp-b. The *letters above* mark each type of 60-bp repeat unit. The array of nine *open diamonds* represents the 142-bp repeat units. The *short vertical lines* located between the subrepeats represent flanked sequences. The open arrowheads reversed in relation to subrepeat I represent seven 60 bp-c repeat units. As indicated by the direction of the heads, the 60-bp repeat units of subrepeats I and III are complementary to each other. Without flanked sequences, the array of the three 391-bp repeat units is represented by *open ovals.* The *asterisks* indicate the truncated repeat unit.

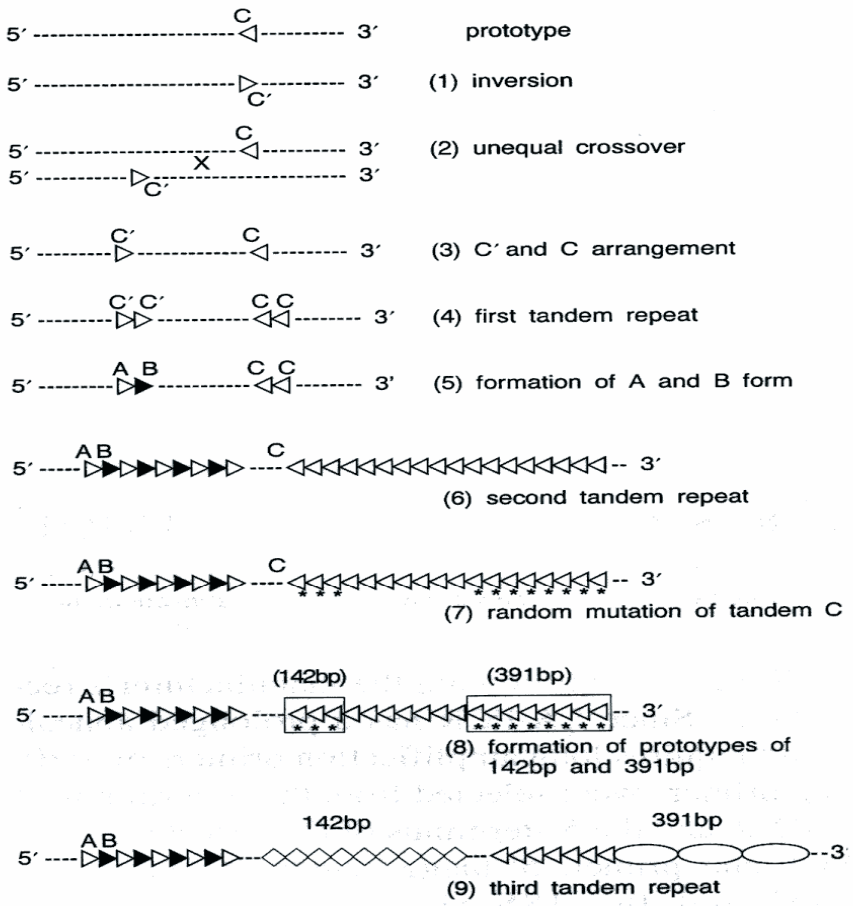


Fig. 2. A model demonstrating how the subrepeats in the IGS region of the swimming crab could be derived from a common ancestor. The *numbers in parentheses* indicate each step for completing the structure. The X in step 2 indicates a crossover between two strands; C′ represents a complementary sequence to C. The *asterisks* in step 7 represent random mutations and the *rectangles* in step 8, which encompass three and eight copies of C, represent prototypes for the 142- and the 391-bp subrepeats respectively. For the complete IGS structure, symbols, and numbers of the elements, see Fig. 1.

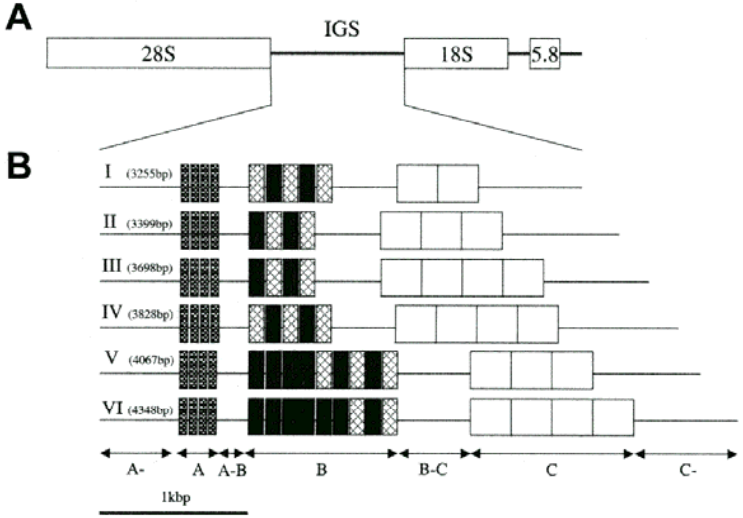


Fig. 1. Comparison of the IGS structural organization of six size variants from one individual. **A.** Schematic diagram of ribosomal DNA unit. **B.** Subrepeat pattern of six size variants of IGS. The repeat region A is indicated by shaded box. Cross-hatching boxes and filled boxes represent b and b′ subrepeat units, respectively. Subrepeats in the repeat region C are marked by blank boxes. Scale bar (1 kbp length) is provided at the bottom.

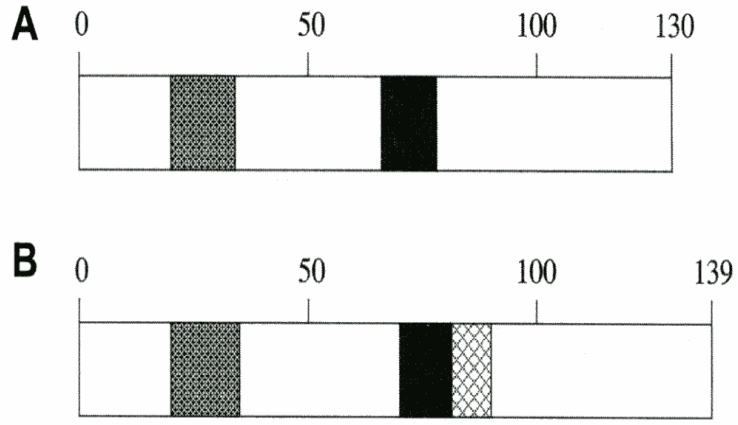


Fig. 2. Schematic representations of the subrepeats b (A) and b′ (B) in the repeat region B. The highly conserved 14, 11, and 10 bp motif sequences are represented by the shaded, filled, and cross-hatched boxes repectively. Numerals mark base position from the 5′ end.

- Our current interests include research fields of speciation, molecular phylogeography, molecular species identification, and

- population genetic studies using different molecular markers such as microsatellites, rDNA, and mtDNA.

Molecular Phylogeography

- A single mitochondrial lineage is shared by morphologically and allozymatically distinct freshwater *Corbicula* clones.

  (Park, J.K., J.S. Lee, and W. Kim, 2002. Mol. Cells.)

- Two *Corbicula* (Corbiculidae: Bivalvia) mitochondrial lineages are widely distributed in Asian freshwater environment.

  (Park, J.K. and W. Kim, 2003. Molecular Phylogenetics and Evolution)

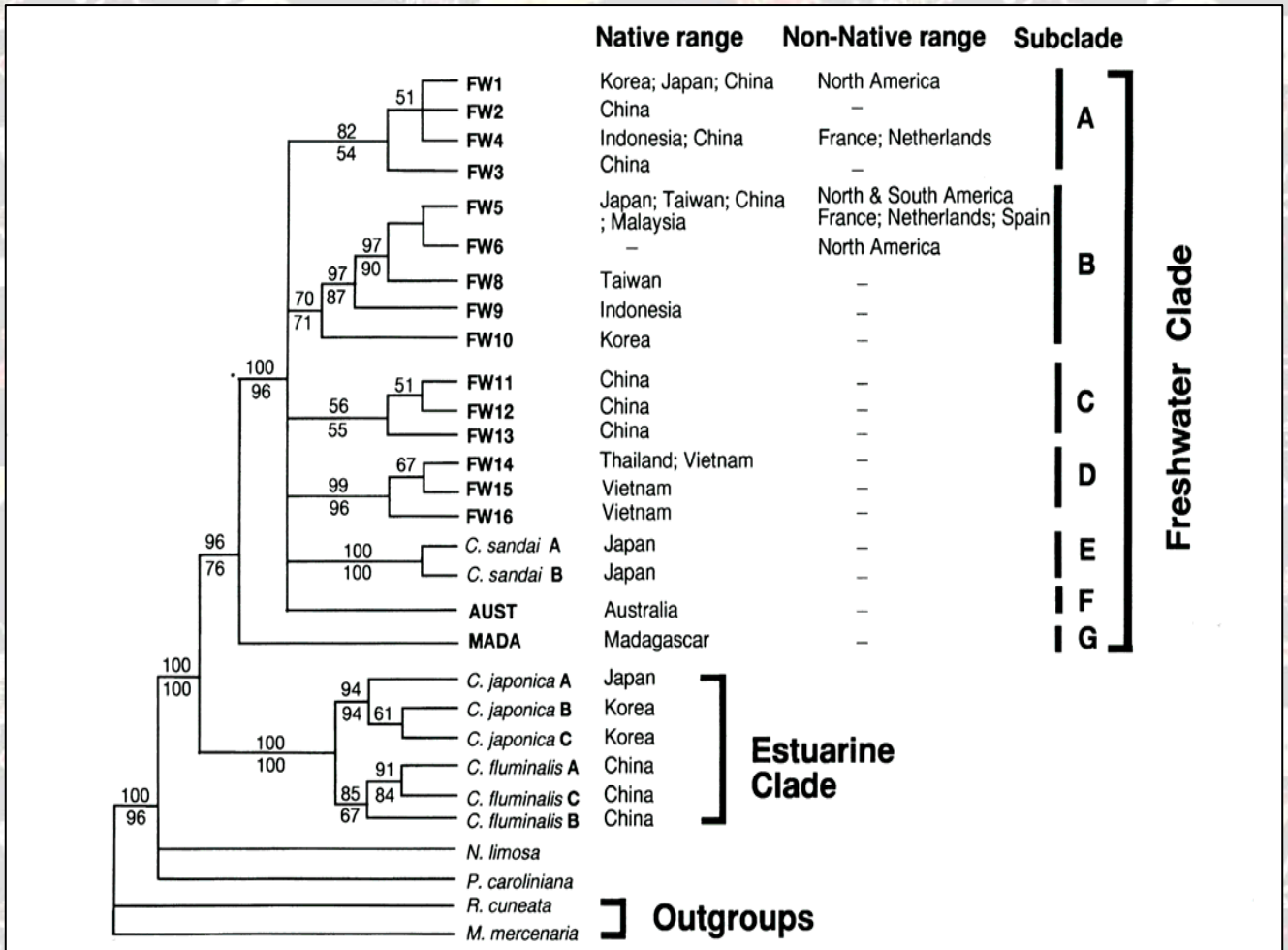
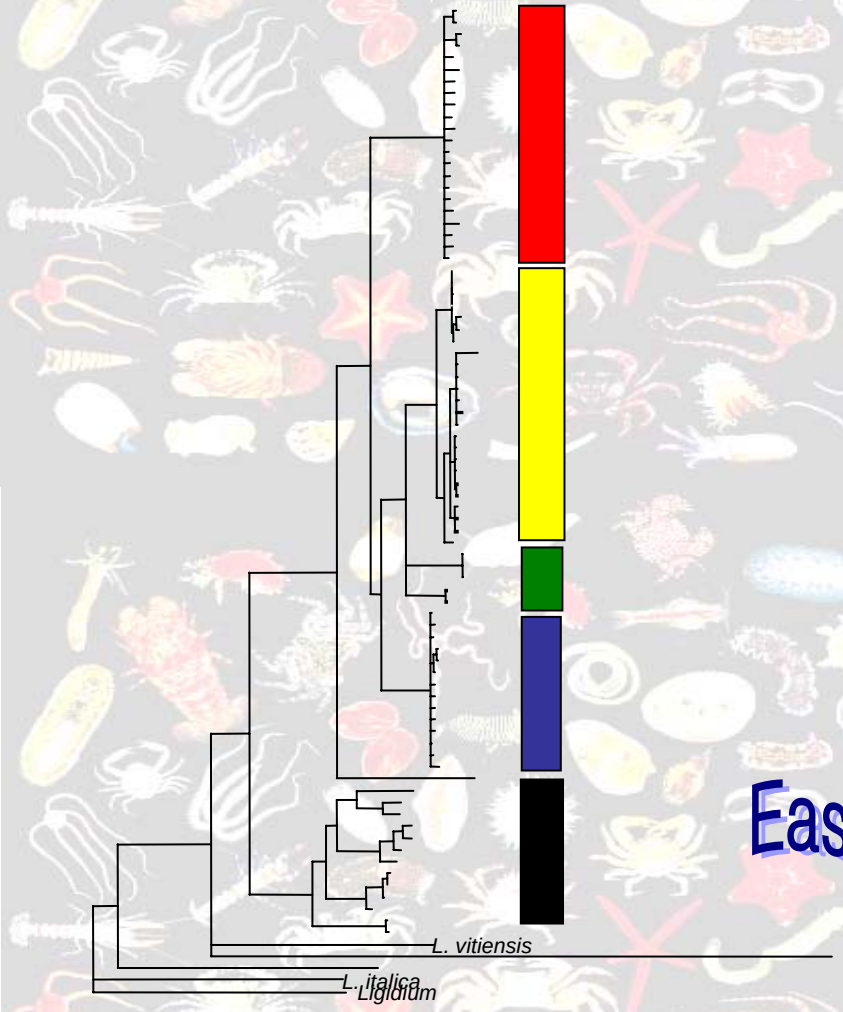


Fig. 2. Bootstrap majority-rule consensus tree obtained from NJ and MP methods. The numbers above (NJ) and below (MP) the branches indicate bootstrap support values (⩾ 50%). Application of MP criterion yielded the same results with the NJ tree, with minor changes in terminal branches.

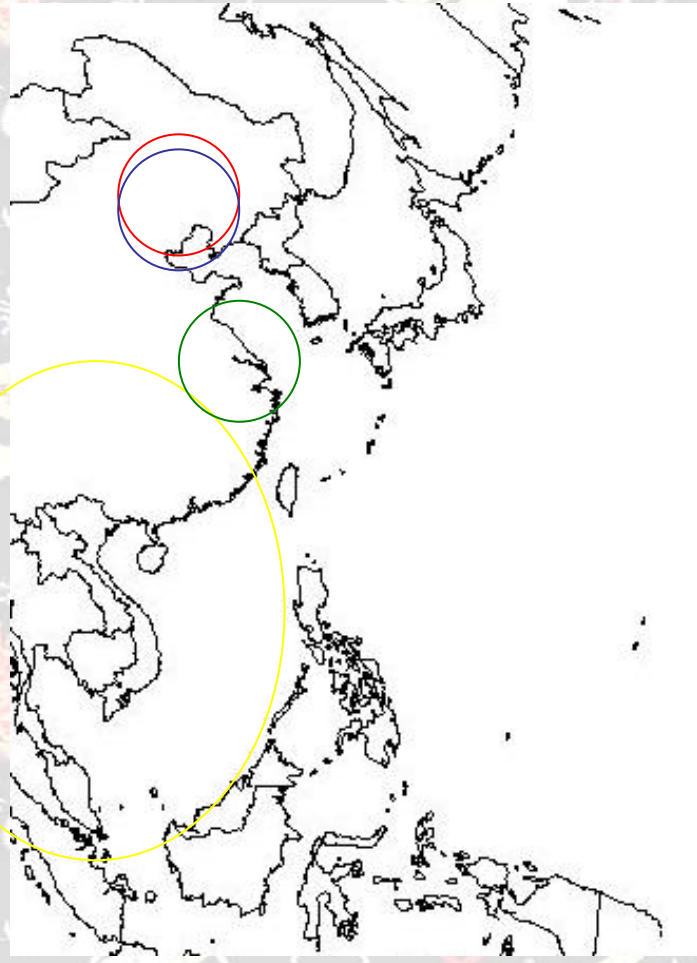
Molecular Phylogeography

- Phylogenetic analysis of some ***ligiid isopods*** based on 16S rRNA and 18S rRNA gene sequences: molecular evidence for two species within *Ligia exotica* populations from South Korea

(Rho, H.S. J.W. Jung, H.S. Eo, and W. Kim, 2004, The 15th Annual Meeting of the Koran Society for Molecular and Cellular Biology)



East Pacific



- In general, very few molecular systematic studies concerning marine invertebrates have been conducted so far.

- Recently, a couple of studies introduced the molecular species identification (DNA barcode).

- Development of species-specific molecular marker as a tool for discrimination between crucian carp gengorobuna (*Carassius cuvieri*) introduced from Japan and Korean native one (*C. auratus*)

  (Song, K.-H., J. Jung, H. Koo. and W. Kim, 2007, Korean J. Limnol.)

Molecular Identification

(DNA Barcode)

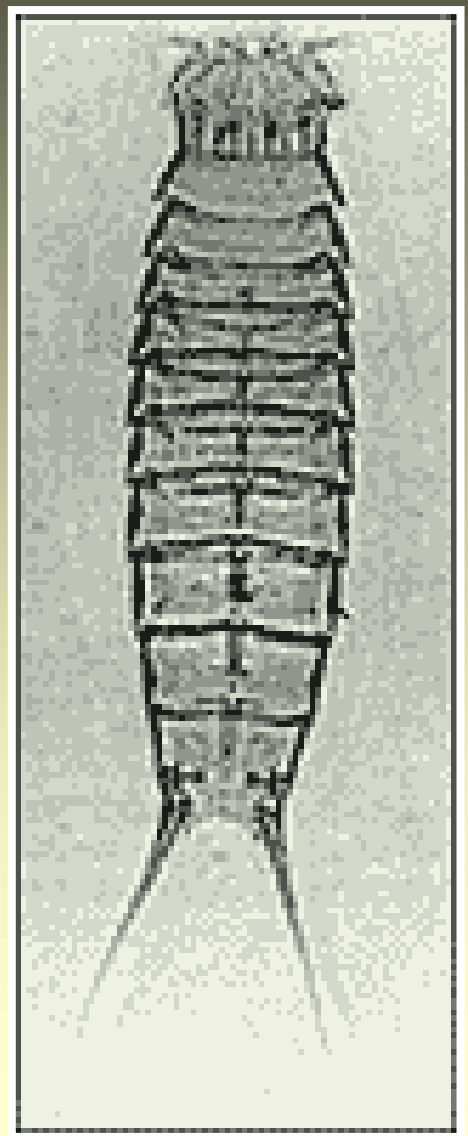
Why?

Meiobenthology?

Meiobenthology is the study of small benthic metazoans that pass through a 0.500 mm sieve and are retained on a 0.063 (or 0.045 mm) sieve. The majority of recognized phyla have meiofaunal representatives.

Porifera, Placozoa, Cnidaria, Ctenophora, Platyhelminthes, Orthonectida, Rhombozoa, Cycliophora, Acanthocephala, Nemertea, Nematomorpha, *Gnathostomulida*, *Kinorhyncha*, *Loricifera*, Nematoda, Rotifera, *Gastrotricha*, Entoprocta, Priapulida, Pogonophora, Echiura, Sipuncula, Annelida, Arthropoda, (Copepoda, Halacaroidea, Ostracoda, Mystacocarida, Tantulocarida), *Tardigrada*, Onychophora, Mollusca, Phoronida, Bryozoa, Brachiopoda, Echinodermata, Chaetognatha, Hemichordata, Chordata

Kinorhyncha
(동문동물)



18S rDNA Sequences Do Not Support the Monophyly of the Suborder Cyclorhagae (Kinorhyncha: Cyclorhagida)

Hyun Soo Rho, Cheon Young Chang[1], Joong-Ki Park[2], and Won Kim

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ABSTRACT

The Kinorhyncha, which is the microscopic marine animal group, currently comprises about 130 species. Although the traditional classification scheme of the phylum Kinorhyncha has been proposed, the phylogenetic relationships among its subgroups has never been tested by molecular analysis. We present here the results of phylogenetic analyses for 18S sequence dataset of six kinorhynch species, comparing with those of some other ecdysozoan representatives. The resulting phylogenetic trees showed the Kinorhyncha is a monophyletic group with respect to the other major ecdysozoan groups. Priapulids, in particular, appeared to be a sister group to kinorhynchs, which is well consistent with the traditional morphology-based phylogenetic hypotheses. Our sequence dataset also provides a new insight into the phylogenetic relationships among major subgroups of kinorhynchs. The phylogenetic trees supported the reciprocal monophyly of the orders Cyclorhagida and Homalorhagida, however, *Campyloderes* and *Echinoderes* in Cyclorhagida did not form a single clade in all phylogenetic methods employed in this study, indicating that the suborder Cyclorhagae is not monophyletic.

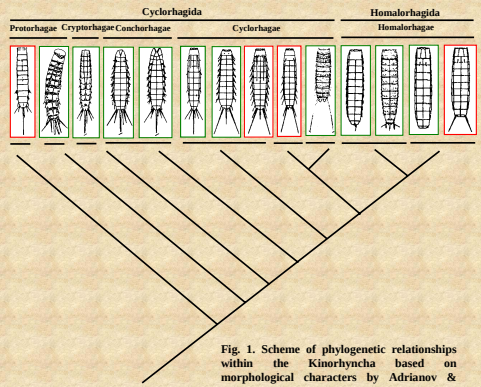


Fig. 1. Scheme of phylogenetic relationships within the Kinorhyncha based on morphological characters by Adrianov & Malakhov (1996)

Table 1. The classification of the Kinorhyncha

Authors	Order	Suborder	Family	Genus
Zelinka (1896)	Echinodera	Cyclorhagae	Echinoderidae	*Echinoderes*
				Echinoderella
				Habroderes
				Habroderella
			Centroderidae	*Centroderes*
				Centropsis
				Hapaloderes
			Meisteroderidae	*Campyloderes*
		Conchorhagae	Pentacentidae	*Semnoderes*
		Homalorhagae	Trachydemidae	*Trachydemus*
				Lepidodemus
			Pycnophyidae	*Pycnophyes*
				Hyalophyes
Higgins (1990)	Cyclorhagida	Cyclorhagae	Echinoderidae	*Echinoderes*
			Zelinkaderidae	*Zelinkaderes*
			Centroderidae	*Centroderes*
				Campyloderes
				Condyloderes
			Dracoderidae	*Dracoderes*
		Conchorhagae	Semnoderidae	*Semnoderes*
				Sphenoderes
		Cryptorhagae	Cateriidae	*Cateria*
	Homalorhagida	Homalorhagae	Pycnophyidae	*Pycnophyes*
				Kinorhynchus
			Neocentrophyidae	*Neocentrophyes*
				Parafcentrophyes
Adrianov & Malakhov (1996)	Cyclorhagida	Protorhagae	Zelinkaderidae	*Zelinkaderes*
			Antigomonidae	*Antigomonas*
		Cryptorhagae	Cateriidae	*Cateria*
		Conchorhagae	Semnoderidae	*Sphenoderes*
				Semnoderes
		Cyclorhagae	Centroderidae	*Centroderes*
				Condyloderes
				Campyloderes
			Echinoderidae	*Echinoderes*
			Dracoderidae	*Dracoderes*
	Homalorhagida	Homalorhagae	Neocentrophyidae	*Neocentrophyes*
				Paracentrophyes
			Pycnophyidae	*Kinorhynchus*
				Pycnophyes

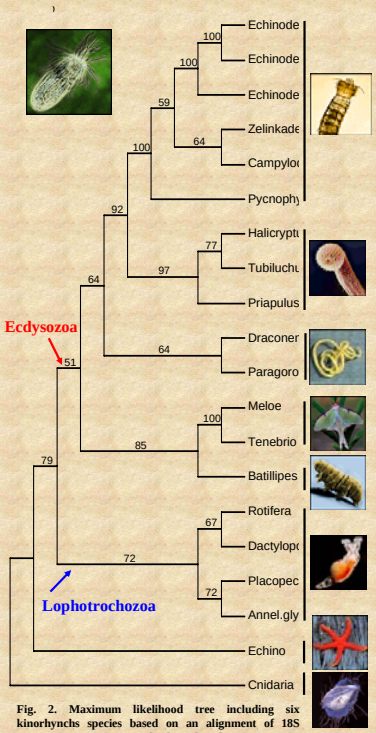


Fig. 2. Maximum likelihood tree including six kinorhynchs species based on an alignment of 18S rRNA gene sequences. The kinorhynchs appear as a monophyletic sister group to priapulids.

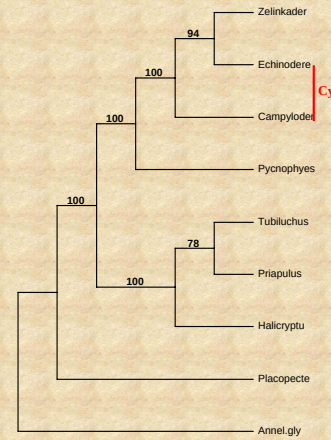


Fig. 3. Maximum likelihood tree reconstruction based on 10,000 puzzling quartets. The same topology was recovered with Neighbor Joining methods. The numbers at the nodes represent estimates of support for each internal branch.

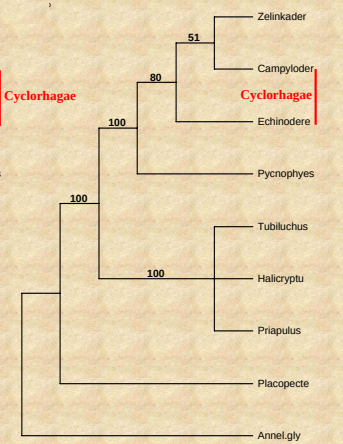


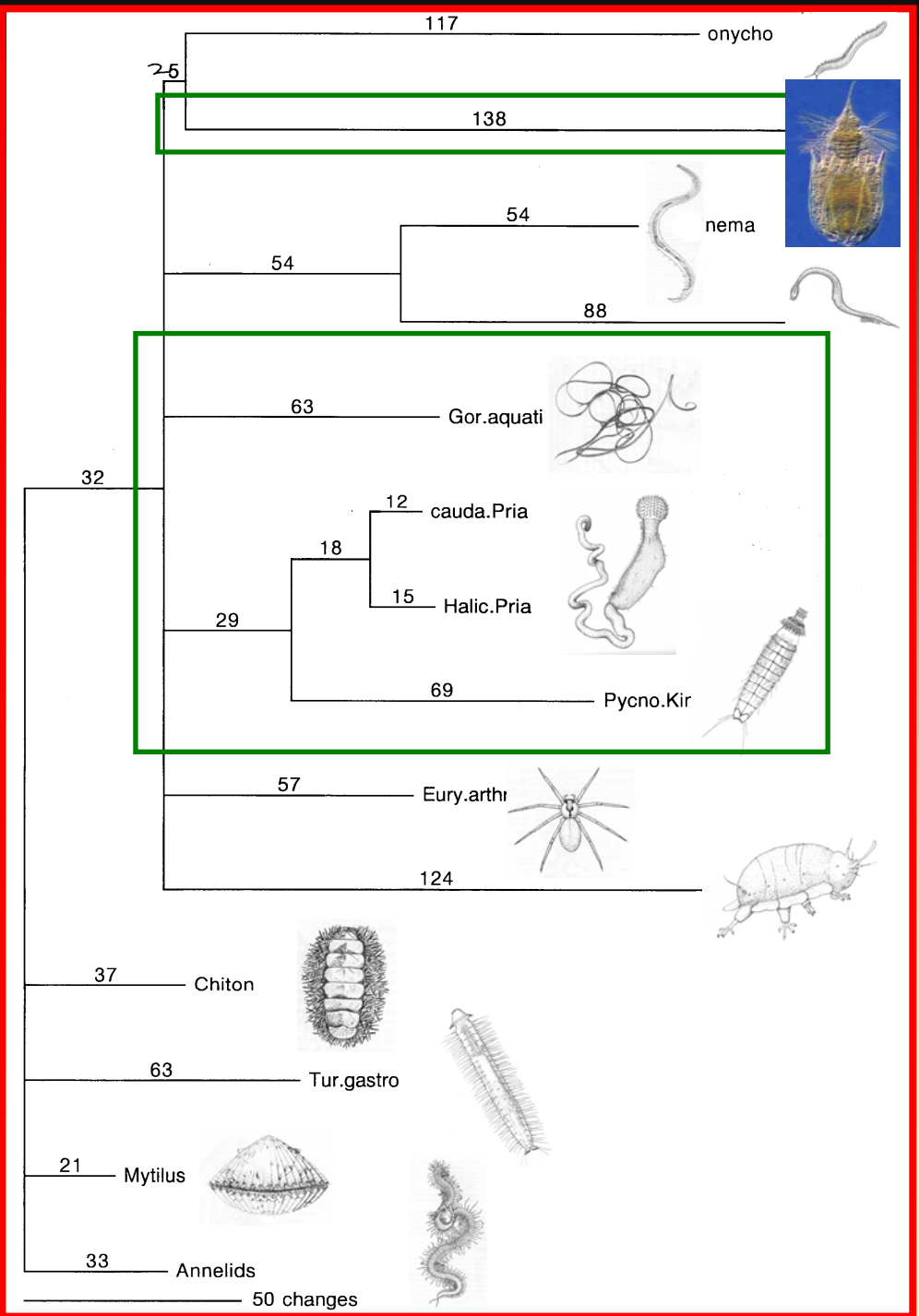
Fig. 4. Maximum parsimony tree constructed from nearly complete 18S rRNA gene sequences. The numbers at the bifurcations represent the percentage of times the group occurred out of 1,000 trees.

RESULTS AND DISCUSSION

The 18S rRNA gene sequences for five species of kinorhynchs taxa were determined and were combined with preexising kinorhynch 18S dataset for phylogenetic analyses. Phylogenetic analyses for the combined 18S kinorhynch dataset using other meiobenthic ecdysozoan taxa as outgorups supported the long-held hypotheses. Our data suggest that:

- The results agree with the currently accepted hypothesis that the phylum Kinorhyncha is monophyletic group.
- The phylum Kinorhyncha is closely related to the priapulid group, which is well consistent with the traditional morphology-based phylogenetic hypotheses.
- Our sequence dataset also provides a new insight into the phylogenetic relationships among major subgroups of kinorhynchs.
- The phylogenetic trees supported the reciprocal monophyly of the orders Cyclorhagida and Homalorhagida, however, *Campyloderes* and *Echinoderes* in Cyclorhagida did not form a single clade in all phylogenetic methods employed in this study, indicating that the suborder Cyclorhagae is not monophyletic.

Loricifera (동갑동물)



Tardigrada (완보동물)

Phylogenetic Relationships among Tardigrades Based on the Analysis of 18S rRNA Gene Sequences: Molecular Evidence for Polyphyly of Arthrotardigrada

Hyun Soo Rho, Joong-Ki Park[1], Cheon Young Chang[2], and Won Kim

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ABSTRACT

The phylum Tardigrada is one of the bilaterally symmetrical micrometazoan groups that have four pairs of lobopod legs terminating into claws or sucking disks. This report presents the result of molecular phylogenetic analyses of the phylum Tardigrada using 18S rRNA gene sequences. The 18S rRNA gene sequences for seven species mostly representing marine tardigrade taxa were determined and were combined with preexising tardigrade 18S dataset for phylogenetic analyses. Phylogenetic analyses for the combined 18S tardigrade dataset using other meiobenthic ecdysozoan taxa as outgorups supported the long-held hypotheses that the tardigrade is closely related to the arthropod group, and that each of Eutardigrada and Heterotardigrada is monophyletic group. Among the heterotardigrades, however, all the phylogenetic methods performed in the present study supported the basal position of *Orzeliscus* in the Arthrotardigrada+Echiniscoidea clade, being inconsistent with morphology-based heterotardigrade phylogeny. This result suggests that the claw type of the echiniscoidean groups has been derived from 4-digit toe system during the heterotardigrade evolutionary process.

INTRODUCTION

The first tardigrade was discovered in 1773. Since then, about 900 species have been described. The phylum Tardigrada is an enigmatic group of lobopodous micrometazoans whose phylogenetic position has been debated for years (Ramazzotti & Maucci, 1983; Kinchin, 1994). Recent molecular studies (Garey et al., 1996; Giribet et al., 1996) indicated that tardigrades are most often allied with the arthropods and onychophorans within Panarthropoda, in aggrement with most morphological studies (Eernisse et al., 1992; Nielsen et al., 1996). The evidence for a clade of molting animals provides additional support for the close relationships between tardigrades and arthropods (Aguinaldo et al., 1997). Within the phylum, two major classes, Heterotardigrada and Eutardigrada, are well established by morphological characters (Ramazzotti and Maucci, 1983; Nelson, 1991; Kinchin, 1994). The heterotardigrades, including marine and terrestrial armored species, are assumed to be the ancestral group, with the greatest number of plesiomorphic charactrers in the marine species. Within eutardigrades, the order Parachela is considered more derived than Apochela. Garey et al. (1999) found close agreement between molecular and morphology based phylogenies that included six species of tardigrades, suggesting that the characters for the current morphological studies are appropriate. Garey et al. (1999) also suggested that heterotardigrades, the marine and terrestrial armored species, are the most basal group with the greatest number of plesiomorphic characters. However, the limitation of Garey et al.' study is incomplete taxon sampling. The Heterotardigrades are composed of two orders divided into eight families, but only a single heterotardigrade (*Echiniscus viridissimus*) is represented. To clarify phylogenetic relationships and positions of major heterotardigrade groups within the phylum, we analyzed 18S rRNA gene sequences from thirteen species of tardigrades, including seven heterotardigrade, mostly representing marine tardigrade and five eutardigrade in the order Apochela and Parachela.

OBJECTIVES

The objectives of this project are to investigate phylogenetic relationships within Tardigrada in order to specifically test (1) the monophyly of Heterotardigrada; (2) the phylogenetic relationships of Arthrotardigrada and Echiniscoidea; (3) to investigate the pattern of claw morphology in light of tardigrade phylogeny.

MATERIALS AND METHODS

Sample Collection
⬇
Identification of Species
⬇
Extraction of Genomic DNA
⬇
PCR Amplification
⬇
Direct Sequencing of 18S rRNA
⬇
Sequence Alignment
⬇
Phylogenetic Analysis

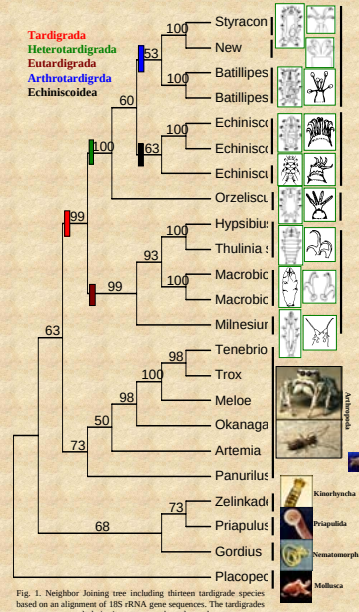

Fig. 1. Neighbor Joining tree including thirteen tardigrade species based on an alignment of 18S rRNA gene sequences. The tardigrades appear as a monophyletic sister group to the arthropods.

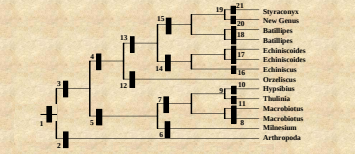

Fig. 2. Topology of a portion of the molecular tree from Fig. 1 mapped with diagnostic morphological characters.

1. Arthropoda + Tardigrada: supraesophageal or preoral position of the frontal appendages and their neuromeres (Dewel & Dewel 1997).
2. Arthropoda: body with articulated exoskeleton; protocerebrum with compound eyes (Nielsen 1995).
3. Tardigrada: connective between protocerebrum and ganglion of first pair of legs (Dewel & Dewel 1997).
4. Heterotardigrada: Cephalic appendages, legs with digits and/or claws (Barnes & Harrison 1993).
5. Eutardigrada: Lack of cephalic appendages; legs with claws but not digits (Barnes and Harrison 1993).
6. Apochela: With cephalic papillae and with double claws with well-separated primary and secondary branches (Schuster et al. 1980).
7. Parachela: Without cephalic papillae and with double claws in which primary and secondary branches are joined (Schuster et al. 1980).
8. *Macrobiotus*: Claw branches with sequence: secondary, primary, primary, secondary; buccal tube with ventral lamina and 10 peribuccal lamellae (Schuster et al. 1980).
9. *Thulinia* + *Hypsibius*: Claw branches with sequence: secondary, primary, secondary, primary; buccal tube without ventral lamina (Schuster et al. 1980; Bertolani 1982).
10. *Thulinia*: twelve peribuccal lamellae (Bertolani 1982).
11. *Hypsibius*: peribuccal lamellae absent (Schuster et al. 1980).
12. *Orzeliscus*: expansion of the toe elongated, to form a spatula, longer than wide; 4th toe equal on each leg (Ramazzotti and Maucci, 1983).
13. Arthrotardigrada + *Orzeliscus*
14. Echiniscoidea: The claws are inserted on minuscule papillae positioned at the end of the legs, which are not digitate; median cirri absent (Ramazzotti and Maucci, 1983).
15. Arthrotardigrada: Median cirri usually present; extremities of the legs digitate or not digitate, but in such cases the claws fixed directly onto ends of legs and not on papillae (Ramazzotti and Maucci, 1983).
16. *Echiniscus*: A dorsal armor composed of various formed of plates (cephalic, scapular, median, and terminal plates); legs with four claws (Ramazzotti and Maucci, 1983).
17. *Echiniscoides* (Echiniscoididae): Marine species, nor armored; cephalic papillae dome-shaped or indistinct; the cephalic appendices are reduced; cirri A and E similar to each other (Ramazzotti and Maucci, 1983).
18. *Batillipes* (Batillipedidae): Legs end in six digits of equal or differing lengths; each digit expanded distally into adhesive disk (Ramazzotti and Maucci, 1983).
19. Styraconyx + Styraconyxinae new genus: Claws with two or four peduncles on four digits; either internal peduncles ansent or heart-shaped proximal pad present; three or four hooks present on each claw, sometimes secondarily reduced to only one or two hooks (Kristensen & Higgins, 1984).
20. Styraconyxinae new genus: Cephalic appendages short and stout.
21. Styraconyx: Digits end in claws with 2 spurs; median cirri short and thin; clava present or absent (Ramazzotti and Maucci, 1983)

RESULTS AND DISCUSSION

The 18S rRNA gene sequences for seven species mostly representing marine tardigrade taxa were determined and were combined with preexising tardigrade 18S dataset for phylogenetic analyses. Phylogenetic analyses for the combined 18S tardigrade dataset using other meiobenthic ecdysozoan taxa as outgorups supported the long-held hypotheses. Our data suggest that:

- The phylum Tardigrada is closely related to the arthropod group.
- The results agree with the currently accepted hypothesis that Eutardigrada and Heterotardigrada are each monophyletic group.
- Among the heterotardigrades, however, all the phylogenetic methods (Maximun parsimony, Neighbor joining, and Maximum likelihood) performed in the present study supported the basal position of *Orzeliscus* in the Arthrotardigrada+Echiniscoidea clade.
- The phylogenetic position of *Orzeliscus* is inconsistent with morphology-based heterotardigrade phylogeny.
- Therefore, the order Arthrotardigrada appears to be polyphyletic and the order Echiniscoidea appears to be monophyletic.
- Among the heterotardigrade, Halechiniscidae (*Styraconyx* sp.) was found to be a sister group to Batillipedidae (*Batillipes* spp.).
- These results suggest that the claw type of the echiniscoidean groups has been derived from 4-digit toe system during the heterotardigrade evolutionary process

SUMMARY AND SIGNIFICANCE

- The 18S rRNA gene sequences contain sufficient phylogenetic information to outline the evolutionary relationships among tardigrade orders, among tardigrade families, and even among tardigrade genera, and will prove to be a usefull tool in more detailed future studies.
- As more molecular data are obtained for the different genera of heterotardigrades and eutardigrades, we will be able to combine morphological and molecular characters to ascertain phylogenetic relationships within the phylum more clearly.

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Nematoda
(선형동물)

A Systematic Study on Korean Desmodorid Nematodes Based on Morphological Characters and 18S rDNA Sequences

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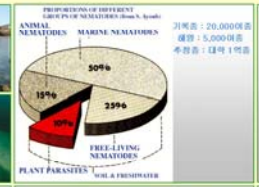
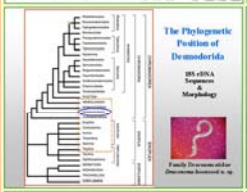
ABSTRACT

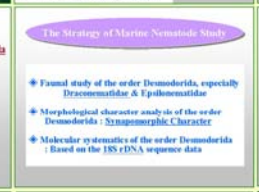
The specimens belonging to the order Desmodorida were collected from various marine habitats in South Korea. As a result of the examination of these specimens, 30 species belonging to 18 genera in three families of two superfamilies were identified. Of these, it was revealed that three species belonged to three different new genera and additional three species were new to science. The photomicrographs of the new species by scanning electron microscopy (SEM) and differential interference contrast (DIC) microscopy are presented. The phylogenetic position of order Desmodorida and the relationships among its families and genera were examined on the basis of 18S rDNA sequences. In the order Desmodorida, sequences of 10 species from two families were determined by direct PCR sequencing techniques. In addition, sequences of 32 species from NCBI were used in the analysis. Three different methods of phylogenetic reconstruction showed strong supports for the monophyly of order Desmodorida and family Draconematidae, respectively. Family Epsilonematidae was located basally among the three Desmodoroidea superfamilies.

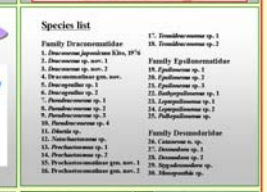
MATERIALS AND METHODS

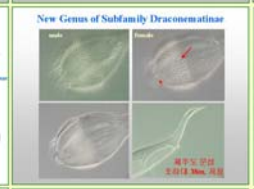
The nematodes were collected from subtidal bottom sediments, or from the rinsings of subtidal benthic invertebrates, which were collected by the fishing net of South Korea. Samples were filtered in the field through nylon net (67㎛ in pore diameter) after rinsed with freshwater for less than a minute for osmotic shock (Kristensen, 1989), and then fixed in 4% formalin and examined with light microscope (LM) equipped with DIC (differential interference contrast) instruments and scanning electron microscope (SEM). For LM, ten males, twelve females and five juveniles were mounted in anhydrous glycerin between two coverslips on H-S slides (Shirayama et al., 1993), and five males were mounted in lactic acid for the examination of the spicules and gubernaculum, and vulval region, and measured and photographed under a DIC microscope. Several specimens were prepared for SEM examination. Seven males, ten females, and five juveniles selected for SEM were treated with hot (about 96℃) 60% ethanol immediately after extraction, and fixed again overnight at 4℃ in a 2.5% buffered glutaraldehyde, then followed by postfixation with 1% cold buffered osmium tetroxide. After dehydration through a graded series of ethanol (60%, 70%, 80%, 90%, 100%, 100%) for 30 minutes each, the materials were dried, and coated with gold-palladium in a high evaporator, and then examined in a Hitachi S-520 scanning electron microscope operated at 20KV. All measurements presented here are from mounted materials.

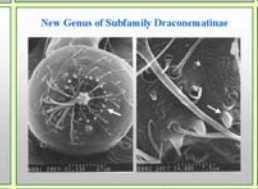


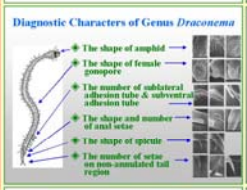


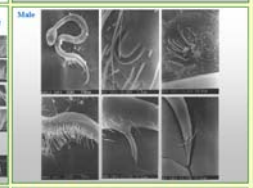


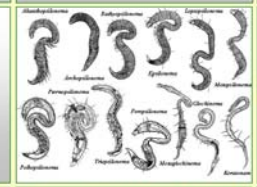


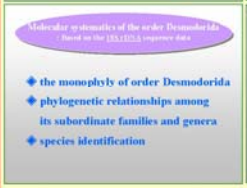


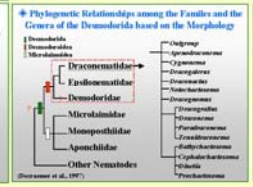


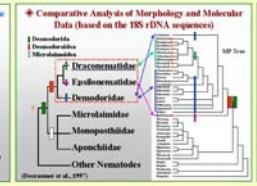


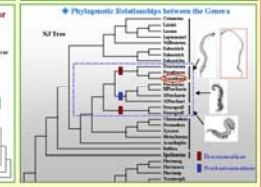












RESULTS

1. We identified 30 species belonging to 18 genera in three families of two superfamilies.
2. Of these, it was revealed that three species belonged to three different new genera and additional three species were new to science.
3. Three different methods of phylogenetic reconstruction showed strong supports for the monophyly of order Desmodorida and family Draconematidae, respectively. Family Epsilonematidae was located basally among the three Desmodoroidea superfamilies.

References

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Molecular Identification System of Decapoda

MISDs: Molecular Identification System of Decapoda - Microsoft Internet Explorer

http://210.125.138.245/~casper/test/

IRBK 유용무척추동물 자원은행 Invertebrate Resources Bank of Korea

MISDs: Molecular Identif

Submit a Sequence

- DNA Sequence Name [optional]
- DNA Sequence Name: Decapoda / Crab / Hermit Crab / Shrimp
- Paste DNA Sequence (Cytochrome Oxydase I)
- Or Upload Sequence File

찾아보기...

Submit sequence | Reset

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완료

MISD: Molecular Identification System of Decapoda - Microsoft Internet Explorer

http://210.125.138.245/~casper/test/result.php

IRBK 유용무척추동물 자원은행 Invertebrate Resources Bank of Korea

MISD: Molecular Identification System of Decapoda

MISDs Prediction Results

- DNA Sequence Name
- Input Sequence

```
GTTGGAACCTCATTGAGATTAATTATTCGAGCTGAATTAGGTCAACCTGG
AACTTTAATTGGTAATGATCAAATTTATAATGTTGTAGTTACTGCTCATG
CATTTGTTATAATTTTCTTTATAGTAATACCAATTATAATTGGTGGATTT
GGAAATTGACTTGTTCCTTTAATGTTAGGAGCTCCTGATATAGCATTCCC
GCGTATAAACAACATAAGATTTTGACTTCTTCCTCCATCTTTAACGCTAT
TACTAATAAGAGGAATAGTTGAAAGAGGTGTGGGTACAGGGTGAACTGTA
TACCCACCTTTGGCGGCAGCTATTGCACACTTTGGTGCTTCGGTAGATCT
TGGTATTTTCTCTCTTCACTTAGCTGGTGTATCTTCTATTTTAGGAGCGG
TTAATTTTATAACTACTGTTATTAATATACGATCTTTCGGTATAAAAATA
```

- Length of Sequence: 450
- Identification approach: BLASTN

- Identified Species of Decapoda: Thalamita sima
- Korean name: 두갈래민꽃게
- Details: Identities = 99.3

Dorsal | Ventral

한국과학재단 | 서울대학교 | 한국동물학회 | 서울대학교 본자진화연구실 | 동물분류학회

서울대학교 관악구 신림동 산 56-1 서울대학교 자연과학대학 생명과학부 20동 318호 유용무척추동물자원은행

인터넷

NJ

● 모두 26 MOTU로 추정됨

MOTU (Molecular Operational Taxonomic Unit)

: COI barcoding

CH4 Woopo
CH7 Woopo
CH3 Woopo
CH6 Woopo
24
CH2 Woopo 21
CH5 Woopo 21
21

CDKOJ3 2
CDKOJ3 1
CDKJ6 1
CDKJ6 2
CDKOJ5 2
CDKOJ5 1
CDDW1
CDDW2
CDKOJ4

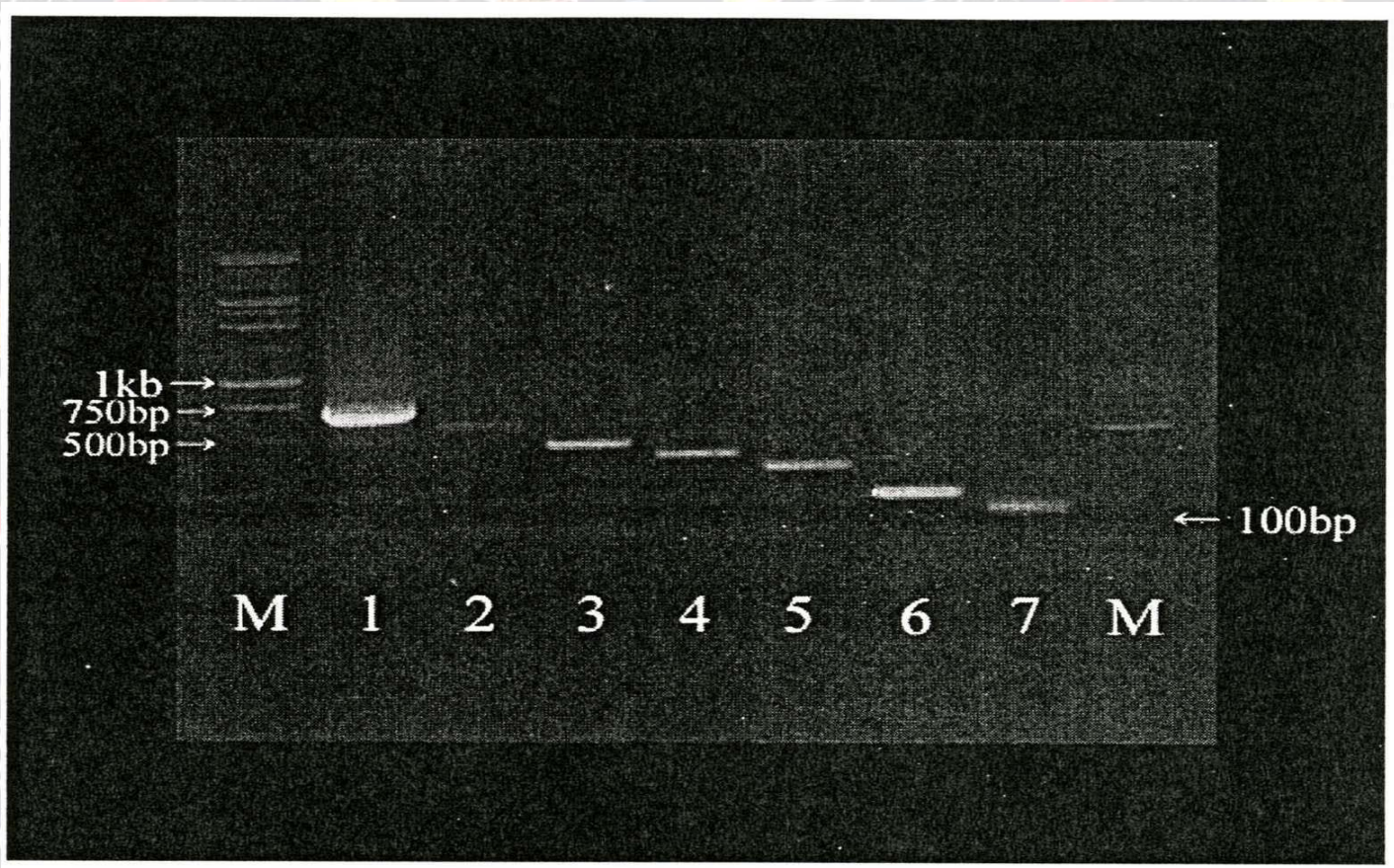
28 4
28 2
28 1
28 5
28 3

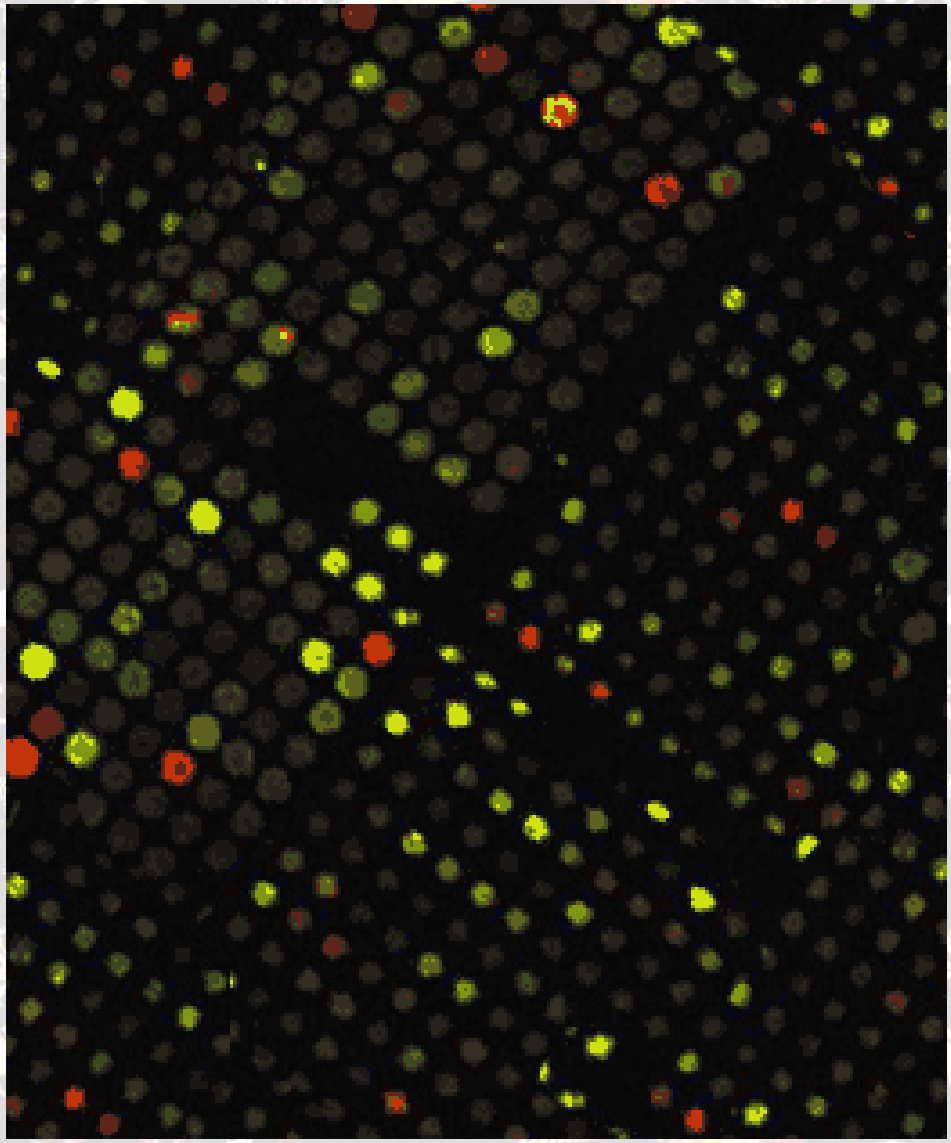
?

새뱅이류에 관한 부분은 꼼꼼한 연구가 필요함

실험상의 오류로 판단됨?

10 changes


1kb
750bp
500bp
100bp
M 1 2 3 4 5 6 7 M



Population Genetic Study

- Population genetic studies can help us to understand why invertebrate/fish populations are dwindling so rapidly, and can assist us with the evaluation of stock enhancement programs.

- Captive breeding and wildlife management programs typically recognize the importance of minimizing loss of genetic diversity and inbreeding.

- Levels of genetic diversity are analyzed and monitored in wild populations of dangered/vulnerable species, and

- gene flow between isolated wild populations may be augmented.

- Microsatellites have advantages over all other methods to measure DNA variation as
- they are highly variable,
- individual genotypes can be directly inferred, and
- individuals can be typed following non-invasive sampling.
- They have the disadvantage that the primers must be developed anew for each species.

Population Genetic Studies

- Evidences for invasion of wharf roach, *Ligia exotica* and competition with endemic species based on mitochodrial 16S ribosomal data

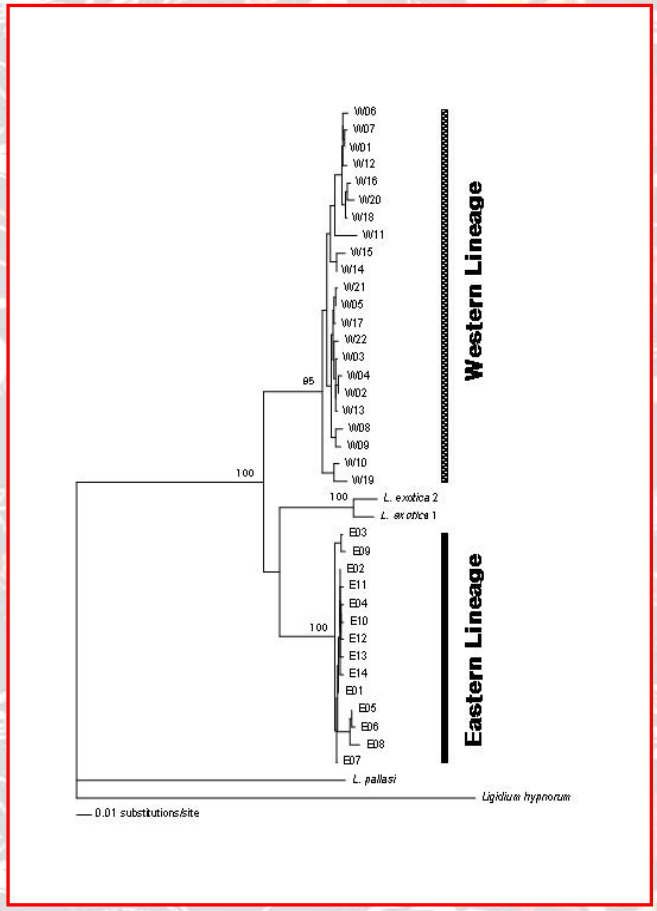
  (Jung, J. W., H. S. Eo, and W. Kim, 2004, The 15th Annual Meeting of the Koran Society for Molecular and Cellular Biology)

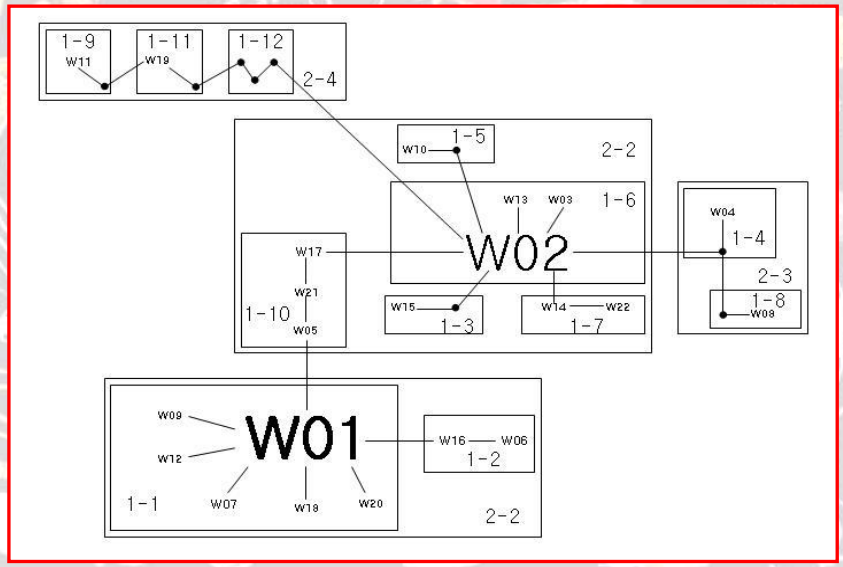
- Genetic variations of the golden orb-web spider *Nephila clavata* (Araneae: Tetragnathidae) in Korea, using AFLP markers.

  (Jung, J., J.-W. Lee, J.-P. Kim and W. Kim. 2006, Korean J. Genet.)

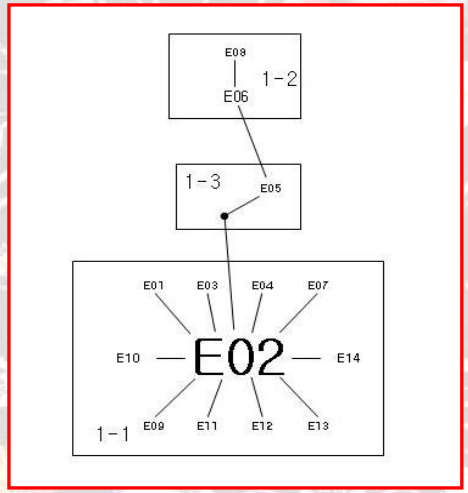
- Analysis of the population structure of the malaria vector *Anopheles sinensis* in South Korea based on mitochondrial sequences.

  (Jung, J., Y. Jung, G.-S. Min and W. Kim., 2007, Am. J. Trop. Med. Hyg.)





Restricted gene flow



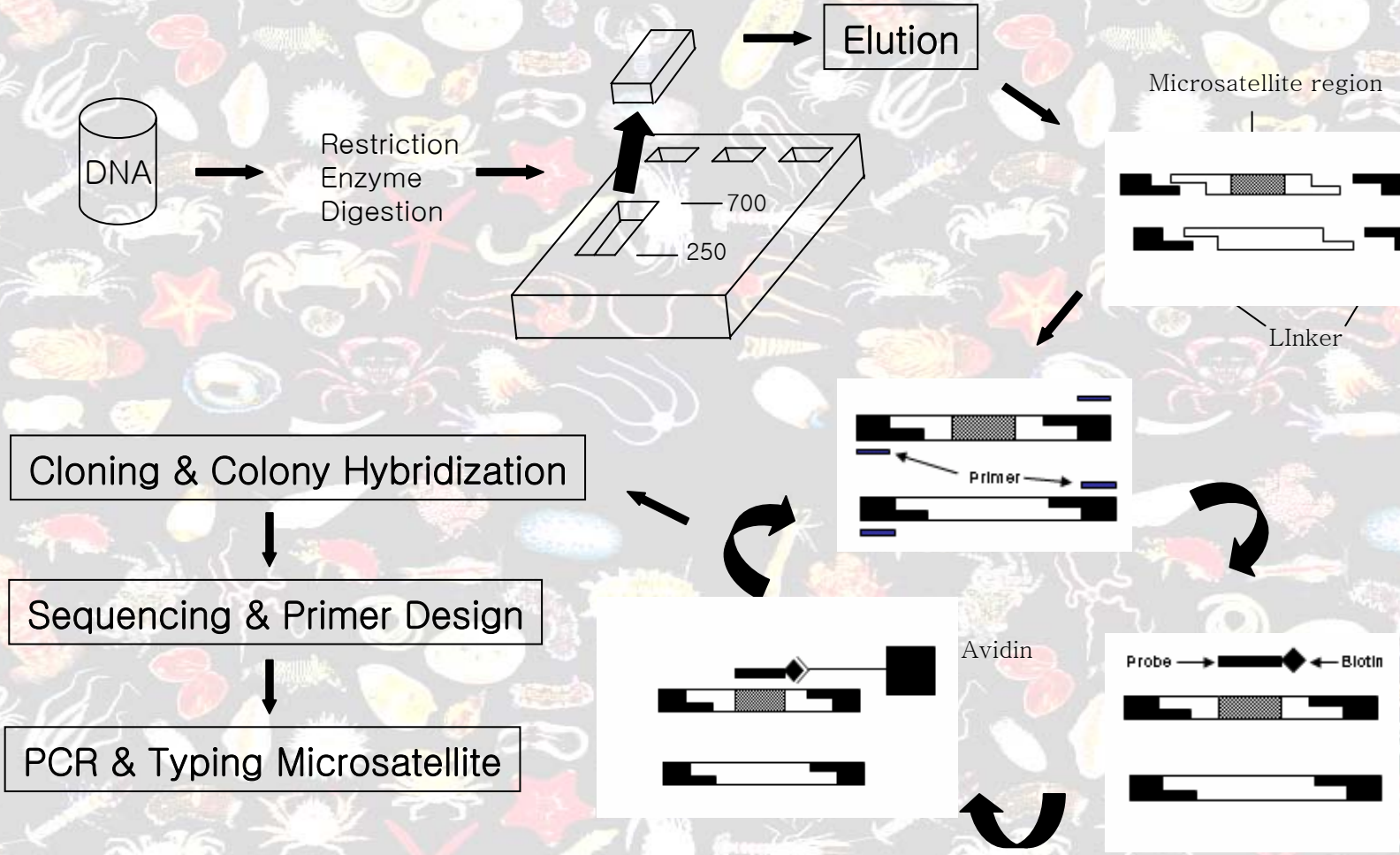
Range expansion

Molecular Marker: Microsatellite

- The present author has been developing several microsatellite markers for population genetic studies of shrimp, crab, sea slater, carp, mosquito, nematodes, etc..

- Isolation and characterization of polymorphic microsatellite markers of *Anopheles sinensis*, a malaria vector mosquito in the East Asia region.
  (Jung, J., E. Lee and W. Kim, 2006, Mol. Ecol. Notes)

- Isolation and characterization of polymorphic trinucleotide microsatellites of the polyploid crucian carp (*Carassius auratus*).
  (Jung, J., E. Lee and W. Kim, 2006, Mol. Ecol. Notes)


DNA
Restriction Enzyme Digestion
700
250
Elution
Microsatellite region
LInker
Primer
Probe
Biotin
Avidin
Cloning & Colony Hybridization
Sequencing & Primer Design
PCR & Typing Microsatellite

Conclusion

- Very few taxonomists in the field of marine invertebrates
- No real extensive population genetic studies on genetic diversity in the field of marine invertebrates
- Microsatellites provide one of the most powerful and practical means currently available for surveying genetic diversity in threatened species of marine invertebrates/fishes
- Need to develop Microsatellite as a molecular marker and to survey genetic diversity of marine