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Phylogenetic Relationships among Five Major Arthropod Subphyla Including the Fifth Subphylum Pycnogonida Inferred from Complete Mitochondrial Genome Information

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Up to now, arthropod phylogenetic relationships among five subphyla (Hexapoda, Crustacea, Myriapoda, Chelicerata, and Pycnogonida) still remain a vigorous debate. In relation to the phylogenetic relationships among major arthropod groups, there are two conflicting hypotheses: Hwang *et al.*'s (2001) hypothesis suggesting sister relationships of Myriapoda/Chelicerata and Hexapoda/Crustacea and Giribet *et al.*'s (2001) hypothesis supporting monophyly of Mandibulata and suggesting Pycnogonida phylogenetic position places on the most basal position in arthropod phylogeny. According to the latter, Pycnogonida placed on the most basal position in arthropod phylogeny. However, Hwang *et al.*'s hypothesis based on the complete mitochondrial genome information did not include any pycnogonid, because no complete pycnogonid mitochondrial genome had been published at that time. To elucidate pycnogonid phylogenetic position using complete mitochondrial genome sequences, we planned to determine complete mitochondrial genome sequences from a pycnogonid, *Achelia bituberculata*. Under the current state, nearly complete mitochondrial genome sequences from *Achelia* were determined. Based on the newly determined pycnogonid mitochondrial genome sequences, here, we re-examine pycnogonid phylogenetic position among arthropods.

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Revised Arthropod Phylogeny Based on Tropomyosin Amino Acid Residues

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Even though many systematists have tried to elucidate phylogenetic relationships among five major arthropod groups, Hexapoda, Crustacea, Myriapoda, Chelicerata, and Pycnogonida with many-sided methods including morphological and molecular data, arthropod phylogeny is still unclear in some important lineages. Especially important points of such vigorous debates include the two as followings: arthropod monophyly and phylogenetic positions of Pycnogonida and Myriapoda. According to Giribet *et al.*'s (2001) published most recently, Pycnogonida was the most primitive taxon compared with the other four arthropod groups and Myriapoda was a sister group of Pancrustacea (Hexapoda + Crustacea) supporting Mandibulata theory. To examine this hypothesis, we employed tropomyosin gene which has generally 284 amino acid (852bp) residues as a phylogenetic marker. In the present study, we determined nearly complete tropomyosin cDNA sequences from a sea spider (*Ammothea hilgendorfi*), two millipedes (*Syntelopodeuma* sp. and *Thyrophygus* sp.), and a mantis crab (*Oratosquilla oratoria*). Phylogenetic analysis was performed with 43 metazoan tropomyosin aa residues obtained through the present study and retrieved from the GenBank. It revealed that arthropods are a monophyletic group (100% bootstrap value) and arthropods were divided into two lineages: Hexapoda/Crustacea (BP 35%) and Myriapoda/Cheliceriformes (BP 23%). Cheliceriformes is a group uniting Chelicerata and Pycnogonida. It indicated that Pycnogonida is not a primitive and basal group of arthropods but a sister taxon of Chelicerata. With resurrection of Cheliceriformes group, the resultant tree supported Hwang *et al.*'s (2001) hypothesis insisting a sister group of Myriapoda/Chelicerata although the confidence values were relatively low, rejecting the Giribet *et al.*'s suggestion. In addition, monophyly of Lophotrochozoa and Ecdysozoa were also observed in this result (BP 99% and 98%, respectively).

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Complete Mitochondrial Genome of Cirriped *Pollicipes mitella* and Its Phylogenetic Implication

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In spite of recent morphological and molecular evidence has changed interpretations of crustacean phylogeny and evolution, Crustacean origins are not resolved monophyletic or paraphyletic. In order to resolve this enigmatic problem, the gene arrangement and DNA sequences of the mitochondrial genome of *P. mitella* (Crustacea, Thoracica) were completely determined. The sequence was 14,915 bp in length. The gene arrangement in mitochondrial DNA of *P. mitella* was similar to those in other arthropods, extending the observation that mitochondrial gene arrangement is conserved in Crustacea. Although serious variation in the positions and sequences of the tRNA genes including tRNA^{Leu}(UUR) and tRNA^{Cys} occurred, the relative arrangements of protein and rRNA genes were similar to those of all arthropods investigated so far, and strongly supports arthropod monophyly. In particular, translocation in some tRNAs were observed in the *P. mitella* mitochondrial genome, which suggests *P. mitella* independently constitutes a separate evolutionary lineage as compared with other crustacea. We compared the protein coding nucleotide sequences of the *P. mitella* mitochondrial genome with those of other arthropods. The amino acid compositions of the 13 predicted proteins were also analyzed and compared. A comparative analysis of mitochondrial gene arrangements that are informative for arthropod phylogeny was performed. As mitochondrial DNA gene arrangements and complete sequences of more crustaceans and crustacean relatives (e.g. Amphipoda, Stomatopoda and Remipedia) become known, it will be possible to discern phylogenetic relationships among spiralian groups including crustacean relatives as well as two major crustacean lineages.

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Molecular Phylogeny of the Family Pomacentridae (Pisces, Perciformes) Inferred from Mitochondrial DNA Sequences

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Damselfishes are a diverse and widespread family of primarily marine fishes found throughout the tropical oceans, forming a major component of coral reef communities. Phylogenetic relationships among pomacentrids were investigated by comparing mitochondrial 16S rRNA gene sequences from forty pomacentrid species. Among them, here, we newly determined 16S rRNA genes from four pomacentrid species (*Pomacentrus coelestis*, *Pomacentrus bankaensis*, *Chromis fumea* and *Abudefduf vaigiensis*). The families Cichlidae and Syngnathidae were employed as outgroups for the present phylogenetic analysis. In the three subfamilies of the family Pomacentridae, only one of them, Amphiprioninae appears to be monophyletic. All the putative members of the subfamily Chrominae formed a monophyletic group with the exception of Acanthochromis. Putative chromine genus Acanthochromis was not clustered with other chromine species, but it placed within the Pomacentrinae group II. Although *Mechaenichthys* was grouped with Pomacentrinae members in previous molecular phylogenetic studies on the basis of 16S rDNA sequences from limited number of samples, the present result with large sample size was recovered as a member of the Chrominae supporting traditional classification scheme. The subfamily Pomacentrinae was divided into two major groups, Pomacentrinae group I and II supporting Pomacentridae polyphyly as suggested previously. Group I members of Pomacentrinae (*Pomacentrus*, *Neopomacentrus*, *Neoglyphidodon*, *Chrypsitera*) were grouped with Amphiprioninae indicating paraphyletic origin of Pomacentrinae Group I. In addition, monophyly of Amphiprioninae and monophyly of four genera (*Dascyllus*, *Stegastes*, *Abudefduf* and *Stegastes*) were observed as known before.