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Molecular phylogeny of moon jellyfish *Aurelia aurita* Linnaeus collected from Yeosu waters in Korea based on nuclear and mitochondrial DNA sequences

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This study presents the molecular phylogenetic analysis of Korean *Aurelia aurita* Linnaeus collected from Yeosu in the southern waters of Korea using nuclear ITS1 region and mitochondrial COI gene sequences. The use of oligonucleotide primers F5 (forward) and R5 (reverse) targeted to ITS1 and LCO1490 (forward) and HCO2198 (reverse) targeted to COI amplified 267 bp and 643 bp fragments, respectively. The shortest genetic distance towards the ITS1 region is estimated at 0.023 when comparing Korean *A. aurita* to *Aurelia* sp. collected from California, USA. In particular, Korean and American/Swedish *A. aurita* were located far away in terms of genetic distance, ranging from 0.393 to 0.395. On the other hand, the genetic distance between Korean and English/Turkish/Swedish/American *A. aurita* regarding the mitochondrial DNA COI gene ranged from 0.201 to 0.205. However, a sister-ship with Korean and American *A. aurita* showed an extremely high bootstrap value (100%). The predicted secondary RNA structure of the mitochondrial DNA COI gene showed many different folding structures with a similar energy between Korean and American *A. aurita*. These results suggest that ITS1 and the mitochondrial DNA COI gene could be used as genetic markers for identification of the biogeographic populations.

Key words – aquaculture, ark shell, gene flow, geographic characteristic, mitochondrial DNA, population structure

Introduction

The genus *Aurelia* has a typical bipartite scyphozoan life history in which benthic scyphopolyps asexually strobilate ephyrae that grow into sexual medusae, the females of which brood larvae that settle into the shallow coastal benthos within a few days of being released [25]. Of these life stages, the medusa probably is the principal dispersal phase because only the medusa is both long and planktonic-lived [1,14,22]. At present, the genus *Aurelia* is believed to have as many as 12 *Aurelia* species by taxonomists, which is caused by its high morphological variability in the medusa stage and has resulted in many discussions regarding all other species as varieties [1,11,17,19, 23,30]. In particular, *Aurelia aurita* Linnaeus (moon jellyfish) is found to be the most common inhabitant of inshore waters and has a wider geographic distribution than other species [1,2]. Due to higher ecological characteristics and adaptability in *A. aurita*, this species has undergone extensive research and discussion in the field of chemistry, development, and ecology over the past century [30].

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The significance of its taxonomy, biogeography, phylogeography, and evolution are yet poorly known because of the drastic underestimation of species diversity [8,13,16]. More recently, much variation in cryptic species in many marine taxa based on molecular analysis may be the outcome of genetic variability [5,6,16,32]. In this sense, some researchers have attempted to distinguish between the populations and to address biogeography in the cosmopolitan marine moon jellyfish based on nuclear and mitochondrial DNA sequences [5,7,32]. Genetic characterization studies of *A. aurita* collected from Korean waters have been limited to phylogenetic relationships between strains and geographic areas. So far, the nuclear ribosomal RNA genes with evolving internal transcribed spacer regions (ITS-1 and ITS-2) surrounding 5.8S, are candidates for the clarification of taxonomic levels and phylogenetic comparisons. Here, we report DNA sequences from the nuclear and mitochondrion DNA sequences of Korean moon jellyfish (*A. aurita*) and their phylogenetic relationships.

Material and Methods

Sampling collection

The moon jellyfish, *Aurelia aurita*, was collected from

Yeosu coastal waters of the South Sea of Korea on August 22, 2005. Examination of the shape of gonads and canal branching patterns described by Kramp [17] identified this medusa sample to *A. aurita*.

Genomic DNA

Live specimen of *A. aurita* was done for DNA isolation. Total DNA was extracted from the gonad by the method of Asahida et al. [3]. Briefly, 50 mg gonad tissue was preserved in 800 μl TNES-Urea buffer (8M urea; 10 mM Tris-HCl; 125 mM NaCl; 10 mM EDTA; 1% SDS, pH 7.5) and 3 μl Proteinase K (20 mg ml^{-1}) incubate the mixture for 3-4 h at 37℃. The supernatant was extracted with phenol:chloroform:isoamyalchol (25:24:1, v/v/v). To purify solution, CTAB (Cetyltrimethylammonium bromide) at 65℃ for 10 min was treated [26]. The DNA pellet was washed with 70% ethanol and then dried by air. The DNA was dissolved in 100 μl dH_2O and was kept at -20℃ until required.

Amplification

The region of ITS (Internal Transcribed Space) was amplified from genomic DNA using PCR with the primers F5 and R5 [32]. Amplification of the mitochondrial DNA (mtDNA) COI gene was conducted using primers LCO1490 and HCO2198 [10]. The primer sequences are as follows: F5, 5'-TAACAAGGTTTCCGTAGG; R5, 5'-CTCAG ACAGACATGCTCC-3'; LCO1490, 5'-GGTCAACAAATCA TAAAGATATTGG-3', HCO2198, 5'-TAAACTTCAGGGTG ACCAAAAAATCA-3'. PCR amplification was performed with 0.2-0.5 μg of template DNA in a reaction mixture of 25 μl containing 1.25 units of *Taq* DNA polymerase (FastStar *Taq* DNA polymerase, Roche Co.), 0.5 mM of each dNTPs, 10 × PCR reaction buffer (Roche Co.) and 20 pmol of each primer. First, targeted to ITS rDNA was initially denatured at 95℃ for 10 min followed 35 cycles (denaturation at 95℃ for 1 min, annealing at 55℃ for 30 s, and extension at 72℃ for 1 min). The final extension step was increased to 5 min [20,21]. Second, targeted to mtDNAs, the isolated DNAs were initially denatured at 95℃ for 10 min followed by 35 cycles (denaturation at 95℃ for 1 min, annealing at 50℃ for 90 s, and extension 72℃ for 1 min). Products from specific PCR amplification were analyzed using 2% agarose run at 50V for 50 min and visualized after staining in 0.5 μg ml^{-1} ethidium bromide. The PCR product was purified using PCR Purification kit (NucleoSpin® Extract) by following manufacture's instruction. Purified DNA fragment was stored at -20℃ until used.

Sequencing

The purified DNA using an Applied Biosystem model ABI 3730XL automated sequencer and a Big Dye terminator cycle sequencing kit (Perkin-Elmer Applied Biosystems, UK). For the sequencing reaction, 30 ng of purified PCR products, 2.5 pmol of primer, and 1 μl of Big Dye terminator were mixed and adjusted to a final volume of 7 μl with dH_2O. The reaction was run with 5% DMSO for 30 cycles of 15 s at 95℃, 5 s at 50℃, and 4 min at 60℃. Both strands were sequenced for crosscheck.

Phylogenetic analysis

Sequences were aligned using multiple alignment program Clustal W [33] and were determined by parsimony, distances and maximum likelihood (ML) methods. To understand the possible genetic relationships, PHYLIP (Phylogenetic Inference Package) ver. 3.573c [9] was used in this study. PHYLIP was used *Aurelia* species derived from GenBank data (*Aurelia* sp. AY319839, *Aurelia* sp. AY319838, *Aurelia* sp. AY319837, *Aurelia* sp. AY319836, *Aurelia* sp. AY319835, *Aurelia* sp. AY319834, *Aurelia* sp. AY319846, *Aurelia labiata* AY319843, *A. labiata* AY319842, *A. labiata* AY319844, *Aurelia* sp. AY319841, *Aurelia* sp. 319840, *A. aurita* AY319849, *A. aurita* AY319848, *Aurelia* sp. AY319851, *Aurelia* sp. AY319850, *A. aurita* AY903095, *A. aurita* AY903093, *A. aurita* AY903211, *A. aurita* AY903209, *A. aurita* AY903094, *A. aurita* AY903212, *A. aurita* AY903208, *A. aurita* AY903117, *A. aurita* AY903118, *A. aurita* AY903210). This search for parsimony analysis was repeated several times from different random staring points using the stepwise addition option to make certain the most parsimonious tree was found. For distance analysis, subprogram DNADIST in PHYLIP was used to obtain a matrix of Kimura's two-parameter distance [15]. Distance matrix was analyzed by subprogram NEIGHBOR in PHYLIP with algorithms based on Saitou and Nei's neighbor-joining (NJ) method [31]. All nucleotide substitution was equally weighted and unordered alignment gaps were treated as missing information. The data set was iterated 100 times using a subprogram SEQBOOT. Reliability of the tree was constructed using subprogram CONSENSE in PHYLIP after pairwise sequence distances were estimated by Kimura's two-parameter method, which attempts to

correct observed dissimilarities for multiple substitution in sequences evolving with a transition bias.

Prediction of RNA folding

The secondary structure prediction program MulFold ver. 2.0 [34] and LoopViewer program [12] were used to predict the secondary structures of mtDNA COI gene. The free energy of folding structures of mtDNA COI gene sequences was calculated by MulFold to generate suboptimal folding. The output file was introduced into LoopViewer to draw the predicted secondary structures.

GenBank accession number

The determined ITS region and mtDNA COI gene sequences were deposited at the NCBI (National Center for Biotechnology Information) data library. Their accession numbers are EF010536 and EF010537, respectively.

Results

Electrophoresis and direct sequencing of each PCR reaction confirmed that single products were amplified in accordance with each PCR reaction, and the size of each product corresponded to the expected ITS and mtDNA COI gene.

Nuclear ITS sequences

Amplified gene sequences targeted to the ITS1 region (267 bp) using the primers of F5 as forward and R5 as reverse from Korean *A. aurita* are shown in Fig. 1a. The alignment of nucleotide sequences for Korean and American/Swedish *A. aurita* contained considerable nucleotide variations in the ITS1 regions, although the length of the ITS1 regions appeared to be similar regarding nucleotides (287 bp in USA, 274 bp in Sweden). Likewise, genetic distances obtained between Korean and American/Swedish *A. aurita* were 0.395 and 0.393, respectively (Table 1). On the other hand, the genetic distance between Korean *A. aurita* and *Aurelia* sp. collected from California was 0.023, being the smallest value in this study. The phylogenetic tree inferred from the distance analysis of the aligned data (NJ method) is shown in Fig. 2. Korean *A. aurita* did not form a sister-ship with American and Swedish *A. aurita*, showing the same topology in the analyses of parsimony and ML methods. This indicates that the genetic position and relationship between Korean and American/Swedish *A. aurita* were very different, although they have the same morphological features.

Mitochondrial COI sequences

We obtained an amplified PCR product targeted to the COI gene using the primers of LCO1490 and HCO2198 (643 bp) that was similar to the PCR product of *A. aurita* (658 bp). Fig 1b shows the aligned nucleotide sequences between Korean and Turkish/English/Swedish/American *A. aurita*. The genetic distances between Turkish and English/Swedish/American *A. aurita* ranged from 0.005 to 0.012, while the distances between Korean and Turkish/English/Swedish/American *A. aurita* were from 0.201 to 0.205 (Table 2). The phylogenetic tree using NJ method was congruent with the parsimonious and ML trees, and high support was found in the cluster of Korean and American *A. aurita* (100% in NJ, Fig. 3).

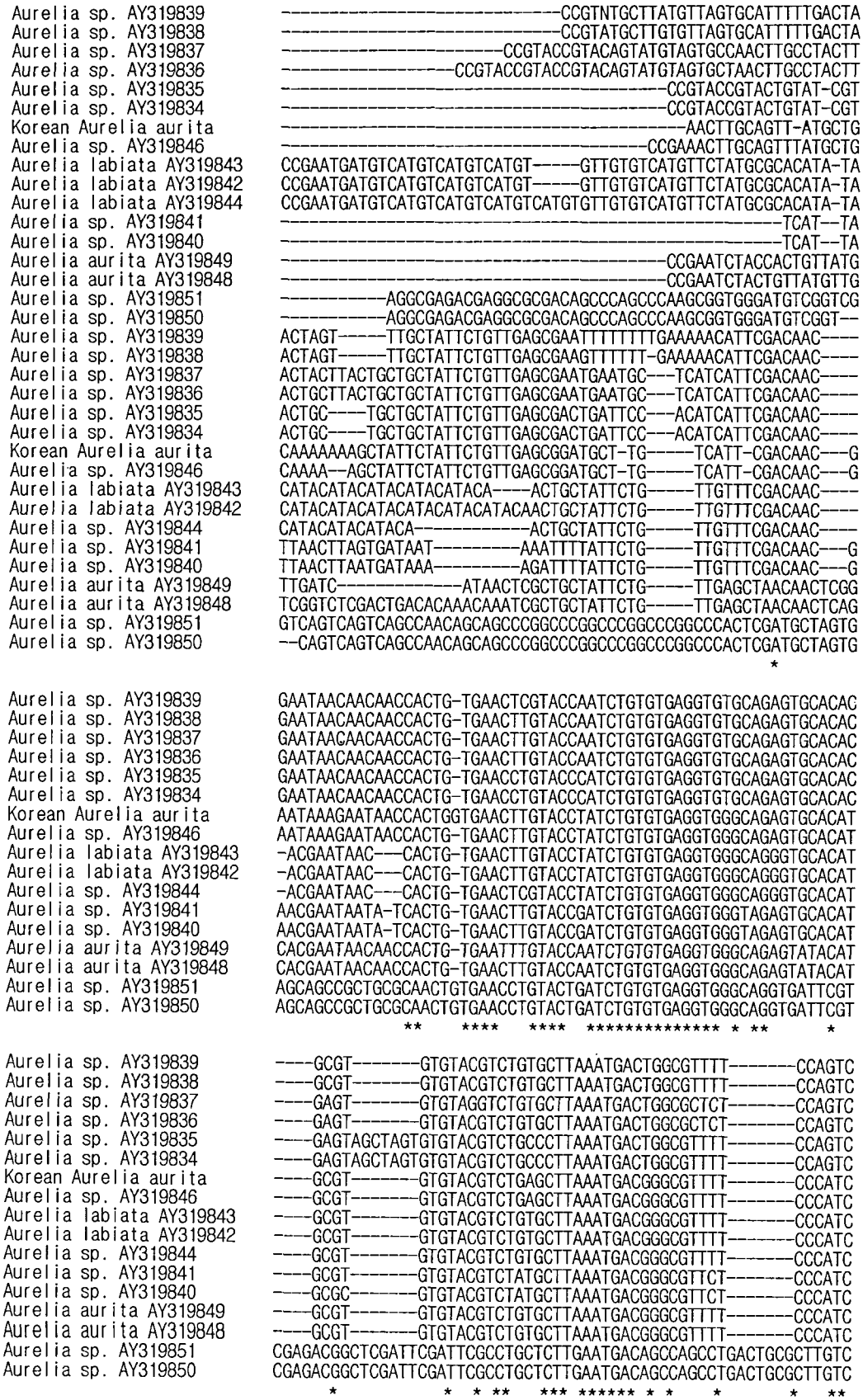
RNA structures COI gene

The secondary structures of the mitochondrial COI RNAs from Korean and American *A. aurita* are shown in Fig. 4. The mtDNA COI secondary structures displayed many different folding structures with a similar energy. In addition, many domains having ambiguous hairpin structures were found.

Discussion

Our results show that Korean *A. aurita* collected from Yeosu reveals a high level sequence divergence (>10%) of the ITS1 region and mtDNA COI gene. In addition, the phylogenetic placement generated by NJ, parsimony, and ML methods are located far away in terms of genetic distance between American and Swedish *A. aurita*. The major reason of the molecular difference was explained by reproductive isolation [5]. A recent study showed that this species was characterized by a great diversity in population and life history connecting to environmental conditions [22]. Likewise, Park [28] reported morphological variations of characters of Korean *A. aurita* according to bell diameter. Only one species of *A. aurita* among morphologically similar taxa [1,28,29] belonging to the genus *Aurelia* has been reported to date in Korea. In contrast to taxonomy, physiological characteristics (e.g., life history, reproduction, feeding, growth), as reported by Bamstedt et al. [4] and Mutlu [27] for *A. aurita* have not yet been studied

(A)


Aurelia sp. AY319839
Aurelia sp. AY319838
Aurelia sp. AY319837
Aurelia sp. AY319836
Aurelia sp. AY319835
Aurelia sp. AY319834
Korean Aurelia aurita
Aurelia sp. AY319846
Aurelia labiata AY319843
Aurelia labiata AY319842
Aurelia labiata AY319844
Aurelia sp. AY319841
Aurelia sp. AY319840
Aurelia aurita AY319849
Aurelia aurita AY319848
Aurelia sp. AY319851
Aurelia sp. AY319850

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Aurelia sp. AY319839        GGCCTCACATGGAGTTTGCTCTTGTTGCAAATTTTTCC-----AAATGACTTTATTTCCA
Aurelia sp. AY319838        GGCCTCACATGGAGTTTGCTCTTGTTGCAAATTTTTGC-----AAATGACTTATTATC--
Aurelia sp. AY319837        GGCCTCACATGGAGTTTGCTCCTGTTGCAAATTTTTTCTCTCGAAATGACATTATTGC--
Aurelia sp. AY319836        GGCCTCACATGGAGTTTGCTCCTGTTGCAAATTTTTTCTCTCGAAATGACATTATTGC--
Aurelia sp. AY319835        GGCCTCACATGGAGTCTGCTCTTGTTGCAAATTTCT-CTCTCGAAATGACCTGAT-----
Aurelia sp. AY319834        GGCCTCACATGGAGTCTGCTCTTGTTGCAAATTTCT-CTCTCGAAATGACCTGAT-----
Korean Aurelia aurita       GGCCTCACATGGAGTTT-TTTATGTTA----------AACAACTTGTGTAAAA
Aurelia sp. AY319846        GGCCTCACATGGAGTTT-TTTTCATTGT----------TAATAACTTGTGTAAAA
Aurelia labiata AY319843    GGCCTCACATGGAGTTTGCTTATGTTT-----TCTT-----CAAAACTACCTGTT-----
Aurelia labiata AY319842    GGCCTCACATGGAGTTTGCTTATGTTT-----TCTT-----CAAAACTACCTGTT-----
Aurelia sp. AY319844        GGCCTCACATGGAGTTTGCTTATGTTT-----TCTT-----CAAAACTACCTGTT-----
Aurelia sp. AY319841        GGCCTCACATGGAGTTTGCTTGTGTTCGTTTATTTT-----CATTAC-ACTTG----ATA
Aurelia sp. AY319840        GGCCTCACATGGAGTTTGCTTGTGTTCGTTTATTTT-----CATTAC-ACTTGCTTGATA
Aurelia aurita AY319849     GGCCTCATATGGAGCTTGCTTATTGTTT---ACTAT-----TATAAATACTTATA---AA
Aurelia aurita AY319848     GGCCTCACATGGAGCTTGCTTATTGTTT---ACTAT-----TATAACAACTTATA---AA
Aurelia sp. AY319851        GGCCTCACATGGAGTTTGTTTGCTCCCGTGATGTTT----TTACTACTACGT--------
Aurelia sp. AY319850        GGCCTCACATGGAGTTTGTTTGCTCCCGTGATGTTT----TTACTACTACGT--------
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Aurelia sp. AY319839        AATTATTCCTGATCATAA-----TA---ATTGAACTCAA---AAGAGTTCGAT-TAAAGT
Aurelia sp. AY319838        -ATTATTCCTGATCGTTA-----TA---ATTGAACTCA-----AGAGTTCGAT-TAAAGT
Aurelia sp. AY319837        TATTATTCCTGATGATGAG-AATCAAGTATTGAGTAAGACTTACTTACTTGTTTCTGAGT
Aurelia sp. AY319836        TATTATTCCTGATCATGAG-AATCAAGTATTGAGTGAGACTTACTTACTTGTTTCTGAGT
Aurelia sp. AY319835        TCTTATTCCTGATCTTGA-----TAGATATCAAGTGCGG-TCGAGCATTTGATGTTGAGT
Aurelia sp. AY319834        TCTTATTCCTGATCTTGA-----TAGATATCAAGTGCGG-TCGAGCATTTGATGTTGAGT
Korean Aurelia aurita       ATTA-TTCCTGAAAATG----GTCAAAC---TTTTTTGG---T----------TTGGCT
Aurelia sp. AY319846        ATTAATTCCTGAAAATG----ATCAAAC---TTTTTTGG---T----------TTGGCT
Aurelia labiata AY319843    -TTA-TTCCTGATCATA----ATCGAACCT-TTTTTTAT---CAAGAAAAAGT-TTGGTT
Aurelia labiata AY319842    -TTA-TTCCTGATCATA----ATCGAACCT-TTTTTTAT---CAAGAAAAAGT-TTGGTT
Aurelia sp. AY319844        -TTA-TTCCTGATCATA----ATCGAACCT-TTTTTTAT---CAAGAAAAAGT-TTGGTT
Aurelia sp. AY319841        ATTA-TTCCAGACTATAGGTAATCGAACGTGCTTTTTAG---TGTGTTTGGTTATTTATA
Aurelia sp. AY319840        ATTA-TTCCAGACTATAGGTAATCGAACGTGCTTTTTAG---TGTGTTTGGTTATTTATA
Aurelia aurita AY319849     TTTT-TTCCTGAAAATTT---ATTGTGTTATTCATTTAG---CGCAA--------TGAAG
Aurelia aurita AY319848     ATTA-TTCCTGAAAATTT---ATTGTGTTATTCATTTAG---CGCAA--------TGAAC
Aurelia sp. AY319851        ------------------------------------------------------------
Aurelia sp. AY319850        ------------------------------------------------------------

Aurelia sp. AY319839        GTAGTAGAAAAATGAGATA
Aurelia sp. AY319838        GTAGTAGAAAAATGAGAT-
Aurelia sp. AY319837        TGCGAAGAGAAATTAGATA
Aurelia sp. AY319836        TGCGAAGAGAAATTAGATA
Aurelia sp. AY319835        TGCGAAGAGAATTTAGATA
Aurelia sp. AY319834        TGCGAAGAGAATTTANATA
Korean Aurelia aurita       AAGTTGCAAAAATGAGAT-
Aurelia sp. AY319846        AAGTTGCAAAAATGAGATA
Aurelia labiata AY319843    AAGTTACAAAAATGAGATA
Aurelia labiata AY319842    AAGTTACAAAAATGAGATA
Aurelia sp. AY319844        AAGTTACAAAAATGAGATA
Aurelia sp. AY319841        AAGTTGAAAAAATGAGATA
Aurelia sp. AY319840        AAGTAGAAAAAATGAGATA
Aurelia aurita AY319849     ATGTT--AAAAATGAAATA
Aurelia aurita AY319848     ATGTT--AAAAATGAAATA
Aurelia sp. AY319851        ------------------
Aurelia sp. AY319850        ------------------
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(B)

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Aurelia aurita AY903095     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Aureila aurita AY903093     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Aurelia aurita AY903211     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Aurelia aurita AY903209     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Aurelia aurita AY903094     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Aurelia aurita AY903212     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Aurelia aurita AY903208     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Aurelia aurita AY903117     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Aurelia aurita AY903118     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Aurelia aurita AY903210     AACACTATACTTAATATTTGGTGCTTTTTCCGCCATGGTGGGAACTGCCTTCAGTATGAT
Korean Aurelia aurita       ----------AATTCGGTGCTTTCTCTGCTATGGTAGGAACTGCCTTTAGTATGAT
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Aurelia aurita AY903095     TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGGACGATCAGCTATATAACG
Aurelia aurita AY903093     TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGCACGATCAACTATATAACG
Aurelia aurita AY903211     TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGGACGATCAACTATATAACG
Aurelia aurita AY903209     TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGGACGATCAACTATATAACG
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Aurelia aurita AY903094          TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGGACGATCAACTATATAACG
Aurelia aurita AY903212          TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGGACGATCAACTATATAACG
Aurelia aurita AY903208          TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGGACGATCAACTATATAACG
Aurelia aurita AY903117          TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGGACGATCAACTATATAACG
Aurelia aurita AY903118          TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGGACGATCAACTATATAACG
Aurelia aurita AY903210          TATAAGACTGGAACT-ATCAGGCCCAGGATCCATGTTGGGGGACGATCAACTATATAACG
Korean Aurelia aurita            TATAAGATTGGAACT-ATCAGGACCTGGATCTATGCTAGGAGATGACCAATTATACAATG
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Aurelia aurita AY903095          TTGTAGTAACCGCTCATGCTCTTATAATGATTTTCTTTTTCGTAATGCCCGTTTTGATAG
Aurelia aurita AY903093          TTGTAGTAACCGCTCATGCTCTTATAATGATTTTCTTTTTCGTAATGCCCGTTTTGATAG
Aurelia aurita AY903211          TTGTAGTGACCGCTCATGCTCTTATAATGATTTTCTTTTTCGTAATGCCCGTTTTGATAG
Aurelia aurita AY903209          TTGTAGTGACCGCTCATGCTCTTATAATGATTTTCTTTTTCGTAATGCCCGTTTTGATAG
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Aurelia aurita AY903117          TTGTAGTGACCGCTCATGCTCTTATAATGATTTTCTTTTTCGTAATGCCCGTTTTGATAG
Aurelia aurita AY903118          TTGTAGTGACCGCTCATGCTCTTATAATGATTTTCTTTTTCGTAATGCCCGTTTTGATAG
Aurelia aurita AY903210          TTGTAGTAACCGCTCATGCTCTTATAATGATTTTCTTTTTCGTAATGCCCGTTTTGATAG
Korean Aurelia aurita            TTGTAGTAACTGCACATGCTCTAATAATGATTTTCTTTTTTGTTATGCCCGTCTTAATAG
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Aurelia aurita AY903095          GGGGATTTGGAAACTGGCTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCAA
Aurelia aurita AY903093          GGGGATTTGGAAACTGGCTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCAA
Aurelia aurita AY903211          GGGGATTTGGAAACTGACTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCAA
Aurelia aurita AY903209          GGGGATTTGGAAACTGACTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCAA
Aurelia aurita AY903094          GGGGATTTGGAAACTGACTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCAA
Aurelia aurita AY903212          GAGGATTTGGAAACTGGCTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCAA
Aurelia aurita AY903208          GGGGATTTGGAAACTGGCTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCAA
Aurelia aurita AY903117          GGGGATTTGGAAACTGGCTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCAA
Aurelia aurita AY903118          GGGGATTTGGAAACTGGCTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCCAA
Aurelia aurita AY903210          GGGGATTTGGAAACTGGCTAGTTCCCCTATATATAGGAGCTCCAGATATGGCCTTTCCAA
Korean Aurelia aurita            GGGGGTTTGGAAATTGACTGGTTCCATTATATATTGGAGCCCCAGATATGGCTTTCCCAA
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Aurelia aurita AY903095          GGCTTAACAATATTAGTTTCTGATTATTACCTCCAGCTTTATTACTATTATTAGGGTCTT
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Aurelia aurita AY903211          GGCTTAACAATATCAGTTTCTGATTATTACCTCCAGCTTTATTACTATTATTAGGGTCTT
Aurelia aurita AY903209          GGCTTAACAATATCAGTTTCTGATTATTACCTCCAGCTTTATTACTATTATTAGGGTCTT
Aurelia aurita AY903094          GGCTTAACAATATCAGTTTCTGATTATTACCTCCAGCTTTATTACTATTATTAGGGTCTT
Aurelia aurita AY903212          GGCTTAACAATATCAGTTTCTGATTATTACCTCCAGCTTTATTACTATTATTAGGGTCTT
Aurelia aurita AY903208          GGCTTAACAATATCAGTTTCTGATTATTACCTCCAGCTTTATTACTATTATTAGGGTCTT
Aurelia aurita AY903117          GGCTTAACAATATCAGTTTCTGATTATTACCTCCAGCTTTATTACTATTATTAGGGTCTT
Aurelia aurita AY903118          GGCTTAACAATATCAGTTTCTGATTATTACCTCCAGCTTTATTACTGTTATTAGGGTCTT
Aurelia aurita AY903210          GGCTTAACAATATCAGTTTCTGATTATTACCTCCAGCTTTATTACTATTATTAGGGTCTT
Korean Aurelia aurita            GGCTTAACAATATTAGTTTTTGATTACTACCCCCAGCTCTGCTTTTACTATTAGGGTCTT
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Aurelia aurita AY903095          CTCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCTCCTTTAAGTTCAATAC
Aurelia aurita AY903093          CTCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCTCCTTTAAGTTCAATAC
Aurelia aurita AY903211          CCCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCTCCTTTAAGTTCAATAC
Aurelia aurita AY903209          CCCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCTCCTTTAAGTTCAATAC
Aurelia aurita AY903094          CCCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCTCCTTTAAGTTCAATAC
Aurelia aurita AY903212          CCCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCTCCTTTAAGTTCAATAC
Aurelia aurita AY903208          CCCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCTCCTTTAAGTTCAATAC
Aurelia aurita AY903117          CCCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCCCCTTTAAGTTCAATAC
Aurelia aurita AY903118          CCCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCTCCTTTAAGTTCAATAC
Aurelia aurita AY903210          CTCTTATAGAACAAGGAGCAGGTACTGGTTGAACCATTTACCCTCCTTTAAGTTCAATAC
Korean Aurelia aurita            CTCTTATAGAACAGGGAGCAGGTACTGGATGAACAATTTATCCCCCCCTAAGCGCAATTC
                                 * *********** ************** ***** ***** ** **  ****  **** *

Aurelia aurita AY903095          AAGCTCATTCCGGAGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Aurelia aurita AY903093          AAGCTCATTCCGGAGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Aurelia aurita AY903211          AAGCTCATTCTGGAGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Aurelia aurita AY903209          AAGCTCATTCTGGAGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Aurelia aurita AY903094          AAGCTCATTCTGGAGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Aurelia aurita AY903212          AAGCTCATTCTGGGGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Aurelia aurita AY903208          AAGCTCATTCTGGGGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Aurelia aurita AY903117          AAGCTCATTCTGGGGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Aurelia aurita AY903118          AAGCTCATTCTGGGGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Aurelia aurita AY903210          AAGCTCATTCTGGAGGTTCAGTAGATATGGCCATATTTAGTCTTCATTTAGCAGGAGCTT
Korean Aurelia aurita            AGGCCCATTCCGGTGGTTCAGTAGATATGGCTATATTTAGTTTGCATCTGGCAGGGGCCT
                                 * ** ***** ** ***************** ********* * *** * ***** ** *
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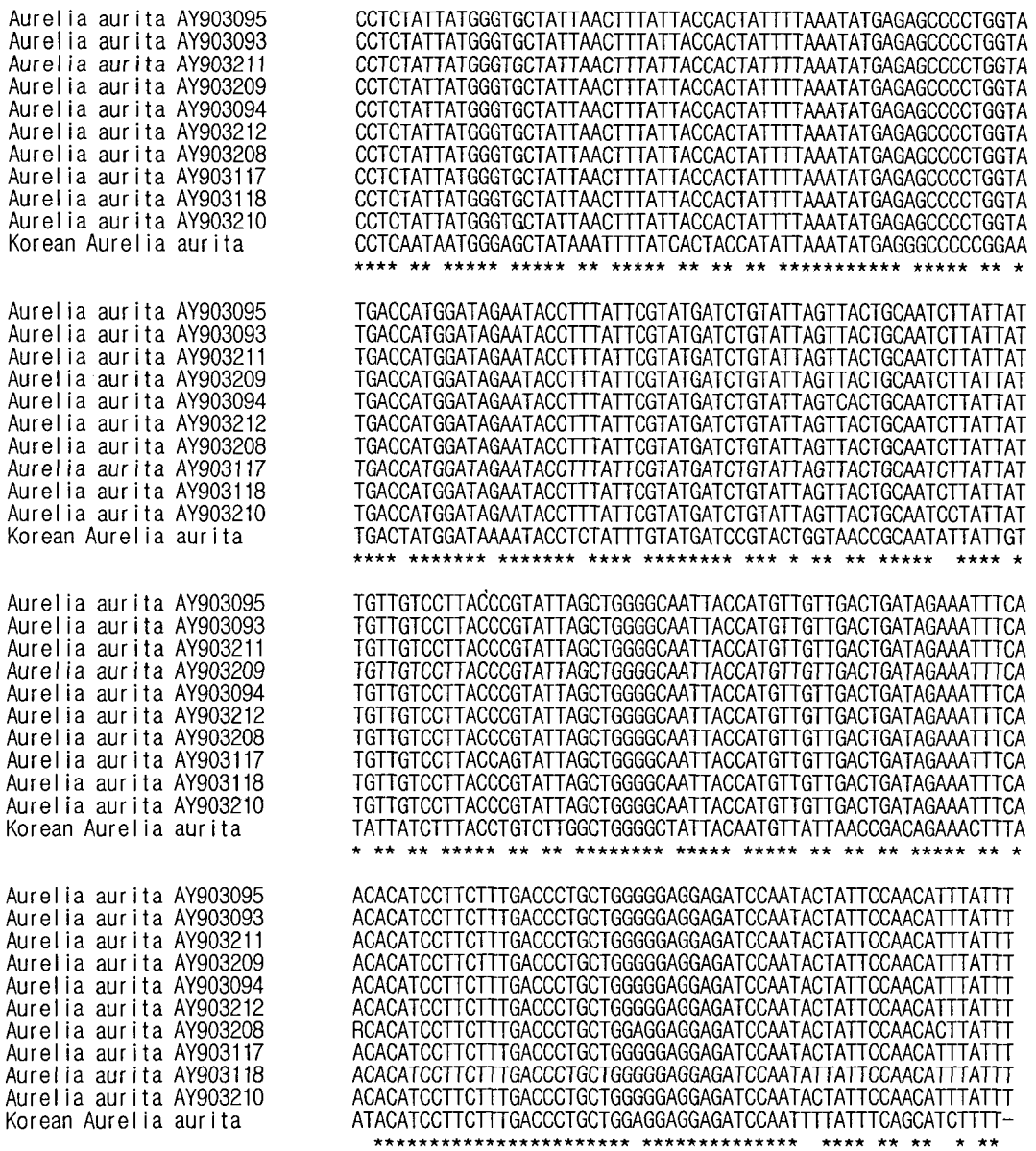

Fig. 1. Sequence alignment of the partial portion in *Aurelia aurita* isolates including relative taxa targeted to ITS1 region (A) and mtDNA COI gene (B). The alignment was generated by the multiple alignment program CLUSTAL W. A hyphen represents a gap and a period represents a base identical to that of the top sequence. An asterisk represents an identical sequence on vertical lines. Sequences have been deposited in GenBank (accession numbers EF010536-EF010537).

in Korea. Based on geographic characteristics, Yeosu in Korea is much influenced by offshore water currents, which often cause changes in environmental conditions. Furthermore, it will be possibly composed of heterogeneous individuals and unstable genetic structure of the population over time.

Possibly, variation in Yeosu population traits is an outcome of certain events that may allow them to better adapt to environmental changes. Charlestown in the U.S.A. and Bjornsund in Sweden, each having the presence of a semi-closed magnitude [5], have limitation for effectively exchanging with offshore waters and these populations may have evolved independent monophyletic groups and accumulated genetic differentiation from Korean *A. aurita* with time. On a micro-geographical scale, water currents play an important role in the distribution of the homogeneous and heterogeneous subpopulations. The reason why the *Aurelia* has geographically wide distribution was consistent

Table 1. Genetic distances between pairs of *Aurelia* species calculated by the Kimura two-parameter model from the sequence data for ITS1.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	0.000	0.032	0.271	0.259	0.242	0.243	0.403	0.415	0.503	0.511	0.469	0.497	0.524	0.604	0.599	1.086	1.070
2	0.031	0.000	0.294	0.282	0.237	0.238	0.399	0.413	0.514	0.522	0.494	0.508	0.543	0.563	0.573	1.059	1.043
3	0.273	0.294	0.000	0.016	0.189	0.189	0.510	0.512	0.645	0.652	0.633	0.568	0.564	0.674	0.733	1.130	1.106
4	0.259	0.282	0.016	0.000	0.175	0.175	0.502	0.504	0.647	0.655	0.636	0.561	0.558	0.683	0.724	1.112	1.088
5	0.242	0.237	0.189	0.175	0.000	0.000	0.459	0.467	0.581	0.589	0.550	0.611	0.613	0.667	0.705	1.106	1.069
6	0.243	0.238	0.189	0.175	0.000	0.000	0.462	0.470	0.585	0.593	0.554	0.615	0.618	0.664	0.709	1.106	1.069
7	0.403	0.399	0.510	0.502	0.459	0.462	0.000	0.023	0.258	0.267	0.230	0.235	0.267	0.393	0.395	0.872	0.851
8	0.415	0.413	0.512	0.504	0.467	0.470	0.023	0.000	0.294	0.302	0.267	0.244	0.276	0.399	0.403	0.852	0.832
9	0.503	0.514	0.645	0.648	0.582	0.585	0.258	0.294	0.000	0.000	0.003	0.219	0.233	0.336	0.333	1.082	1.050
10	0.511	0.522	0.652	0.655	0.589	0.593	0.267	0.303	0.000	0.000	0.003	0.218	0.232	0.357	0.346	1.110	1.076
11	0.469	0.494	0.633	0.637	0.550	0.554	0.230	0.266	0.003	0.003	0.000	0.214	0.234	0.341	0.339	1.110	1.076
12	0.497	0.508	0.568	0.561	0.611	0.615	0.235	0.244	0.219	0.218	0.214	0.000	0.019	0.358	0.357	0.731	0.715
13	0.524	0.543	0.564	0.558	0.613	0.618	0.267	0.276	0.233	0.232	0.234	0.019	0.000	0.375	0.373	0.715	0.698
14	0.604	0.563	0.674	0.683	0.667	0.663	0.393	0.399	0.336	0.357	0.341	0.358	0.375	0.000	0.070	0.951	0.950
15	0.599	0.571	0.733	0.724	0.709	0.705	0.395	0.403	0.333	0.346	0.339	0.357	0.373	0.070	0.000	0.930	0.917
16	1.086	1.059	1.130	1.113	1.106	1.106	0.872	0.852	1.082	1.110	1.110	0.731	0.715	0.951	0.930	0.000	0.000
17	1.070	1.043	1.106	1.088	1.069	1.069	0.851	0.832	1.050	1.076	1.076	0.715	0.698	0.950	0.917	0.000	0.000

1, *Aurelia* sp. AY319839; 2, *Aurelia* sp. AY319838; 3, *Aurelia* sp. AY319837; 4, *Aurelia* sp. AY319836; 5, *Aurelia* sp. AY319835; 6, *Aurelia* sp. AY319834; 7, Korean *Aurelia aurita* 8, *Aurelia* sp. AY319846; 9, *Aurelia labiata* AY319843; 10, *Aurelia labiata* AY319842; 11, *Aurelia labiata* AY319844; 12, *Aurelia* sp.

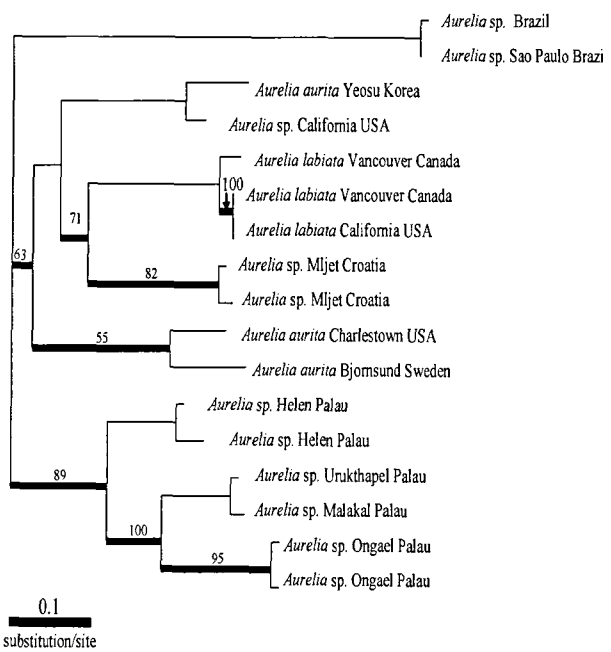

Fig. 2. PHYLIP analysis of 17 species obtained from GenBank database including Korean *Aurelia aurita*. Phylogram was constructed by inferring from nucleotide sequences of ITS1 region. The tree was generated using subprogram NEIGBOR in PHYLIP with the option of Kimura's two-parameter method. Bootstrap values (100 replications) are given above the internal nodes using the subprogram CONSENSE. Bootstrap of <50% represents a hypen on node. This is an unrooted tree.

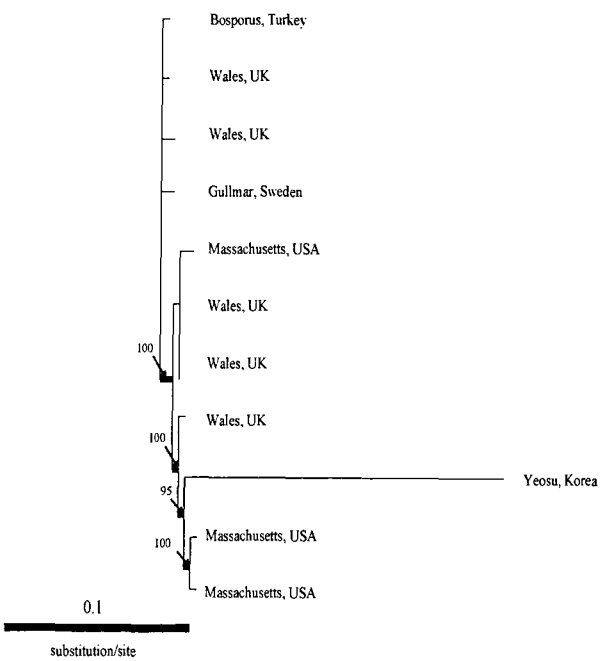

Fig. 3. PHYLIP analysis of 11 *Aurelia aurita* isolates including Korean *A. aurita*. Phylogram was constructed by inferring from nucleotide sequences of partial mitochondrial DNA COI gene. The tree was generated using subprogram NEIGBOR in PHYLIP with the option of Kimura's two-parameter method. Bootstrap values (100 replications) are given above the internal nodes using the subprogram CONSENSE. Bootstrap of <50% represents a hypen on node. This is an unrooted tree.

Table 2. Genetic distances between pairs of Aurelia aurita calculated by the Kimura two-parameter model from the sequence data for COI gene.

	1	2	3	4	5	6	7	8	9	10	11
1	0.000	0.003	0.009	0.009	0.012	0.011	0.012	0.012	0.014	0.006	0.197
2	0.003	0.000	0.009	0.009	0.012	0.011	0.012	0.012	0.014	0.006	0.197
3	0.009	0.009	0.000	0.000	0.003	0.005	0.006	0.006	0.008	0.006	0.201
4	0.009	0.009	0.000	0.000	0.003	0.005	0.006	0.006	0.008	0.006	0.201
5	0.012	0.012	0.003	0.003	0.000	0.008	0.009	0.009	0.011	0.009	0.203
6	0.011	0.011	0.005	0.005	0.008	0.000	0.005	0.005	0.006	0.008	0.205
7	0.012	0.012	0.006	0.006	0.009	0.005	0.000	0.006	0.008	0.009	0.203
8	0.012	0.012	0.006	0.006	0.009	0.005	0.006	0.000	0.008	0.009	0.201
9	0.014	0.014	0.008	0.008	0.011	0.006	0.008	0.008	0.000	0.011	0.201
10	0.006	0.006	0.006	0.006	0.009	0.008	0.009	0.009	0.011	0.000	0.201
11	0.197	0.197	0.201	0.201	0.203	0.205	0.203	0.201	0.201	0.201	0.000

1, *Aurelia aurita* AY903095; 2, *Aurelia aurita* AY903093; 3, *Aurelia aurita* AY903211; 4, *Aurelia aurita* AY903209; 5, *Aurelia aurita* AY903094; 6, *Aurelia aurita*

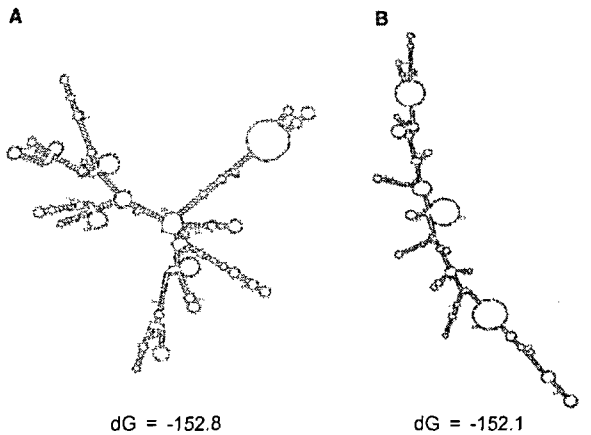

Fig. 4. Secondary structure calculated for the mtDNA COI gene of Korean (A) and American (B) *Aurelia aurita*.

with the current classification described by Kramp [18] and Russell [30]. This is the result of a process shaped by more external factors to the species than intrinsic acts upon the degree of genetic diversity in a population through its effect on the spatial distribution. More recently, some researchers have suggested that phenotypic variation and molecular differences caused by geographical variation contribute to a species-complex existing in *A. aurita* [6,7]. Although our present study collects data from only one sampling site, genetic divergence within even Korean *A. aurita* may occur, caused by heterogeneous environments.

In conclusion, the ITS1 region and the COI gene sequence analysis are useful method to resolve the systematic relationships of Korean *A. aurita*. In addition, they can determine the high resolution of genetic branches with an excellent degree of confidence for diverse assemblages and the degree of phylogenetic signal loss of organisms.

Acknowledgement

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초록 : 여수해역에서 채집한 보름달 둥근 물해파리의 핵과 미토콘드리아 DNA 염기서열을 이용한 유연관계 분석

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본 연구는 여수연안해역에서 채집한 보름달 둥근 물해파리를 대상으로 ITS 부위와 미토콘드리아 유전자 염기서열을 이용하여 계통유연관계를 보았다. ITS 부위를 증폭시키기 위하여 F5와 R5 primer, 미토콘드리아 COI 유전자 증폭을 위하여 LCO1490과 HCO2198 primer를 사용했다. 증폭은 ITS에서 267 bp, COI에서 643 bp로 나타났다. 한국산 물해파리와 미국 캘리포니아에서 채집한 Aurelia sp.가 유전적 거리가 가장 짧은 0.023을 보인 반면에, 한국산과 미국산, 스웨덴산 물해파리는 동일한 종이지만 유전적 거리가 0.393에서 0.395로 매우 먼 것으로 나타났다. COI 유전자의 경우 한국산과 영국산, 터어키산, 스웨덴산, 미국산 물해파리의 유전적 거리 범위는 0.201에서 0.205로 나타났다. 그러나 한국산과 미국산의 bootstrap은 100% 자매군으로 보였다. COI 유전자에 대한 한국산과 미국산 2차 RNA folding 구조를 볼 때 동일한 에너지 하에서도 상이한 2차 folding을 보였다. 따라서 ITS1과 COI 유전자는 보름달 둥근 물해파리 개체군의 생물지리학적 분포 조사를 위하여 유용한 도구로 활용될 것으로 추측된다.