Recent moon jelly (*Aurelia* sp.1) blooms in Korean coastal waters suggest global expansion: examples inferred from mitochondrial COI and nuclear ITS-5.8S rDNA sequences

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The moon jelly *Aurelia* was found recently in Korean coastal environments, and its dense blooms caused economic losses for fisheries and power plants. The species is tentatively recognized as *Aurelia aurita*; yet, its identity and origin remain elusive. To find reliable molecular evidence for its identity, we determined the DNA sequence of the mitochondrial cytochrome c oxidase subunit I gene and nuclear ITS-5.8S rDNA of specimens collected from different Korean locations. We compared the nuclear and mitochondrial DNA data among specimens and demonstrated that all Korean *Aurelia* have an identical genotype. BLAST searches demonstrated that the Korean *Aurelia* matched the previously designated *Aurelia* sp.1. Parsimony and relevant phylogenetic analyses of the genus *Aurelia* demonstrated that the genotypes of Korean, Japanese, and Californian *Aurelia* sp.1 were nearly identical (>99.6% similarity), whereas they were significantly different (<84.1% similarity) from other *Aurelia*. This suggests that *Aurelia* sp.1, which occur in the three regions, are descendants of a single population and may have dispersed from one location. However, the dispersal time and origin of *Aurelia* sp.1 still remain uncertain.

Keywords: *Aurelia*, blooms, COI sequence, exotic species, moon jelly, phylogenetic comparison.

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Introduction

The moon jelly (*Aurelia* spp.) belongs in the phylum Cnidaria (Scyphozoa) and is one of the most widely distributed species of jellyfish (Arai, 1996). Traditionally, classification of adult jellyfish has been based on morphological features and body measurements. However, identification of the fragile moon jelly is not easy because the shape of its medusae is highly variable, and the moon jelly has different life stages. Mainly as a result of the high morphological variability in the medusa, ~12 *Aurelia* species or subspecies have been described (Mayer, 1910; Kramp, 1961). However, only two of them are recognized as distinct species: *Aurelia aurita* and *Aurelia limbata* (Russell, 1970).

To resolve morphological ambiguities, molecular tools have been applied to various aquatic organisms (e.g. Dawson and Jacobs, 2001; Collins *et al.*, 2006). These tools allow the differentiation of cryptogenic taxa, the identification of source populations and vectors, and the assessment of the extent and impacts of invasions (Holland, 2000; Wares *et al.*, 2002). The molecular phylogeny of the moon jelly has been studied extensively, based on the nuclear ribosomal DNA (rDNA) and mitochondrial 16S or cytochrome c oxidase subunit I (COI) genes that have led to taxonomic revisions. For example, Schroth *et al.* (2002) suggested historical speciation events and the reconstruction of at least seven different species within *Aurelia*, based on the phylogenetic patterns derived from mitochondrial 16S and nuclear rDNA data from 66 specimens collected worldwide. Dawson and colleagues (Dawson and Jacobs, 2001; Dawson, 2003; Dawson *et al.*, 2005) proposed a more acceptable taxonomic system for the moon jelly by combining its macro-morphology and internal transcribed spacer (ITS), as well as partial COI gene sequences. The global phylogeny of the genus *Aurelia* reveals at least 16 phylogenetic branches, i.e. 13 cryptic species including three recognized species (*A. aurita*, *A. limbata*, and *Aurelia labiata*). Most of them appear to be restricted regionally. However, several species such as *Aurelia* sp.1 and *A. aurita* have disjointed distributions, which may be a characteristic of globally expanded species (Dawson *et al.*, 2005). Based on the molecular data, at least 13 species are distinguishable.

A global issue was recently identified: the rapid expansion of moon jellies' geographic range and spread across oceanic regions from a single origin (e.g. Dawson *et al.*, 2005). This could be by natural as well as anthropogenic means. Moon jellies can be introduced accidentally as organisms growing on ship hulls or in ballast water, or released unintentionally (or intentionally) by the aquarium trade and aquaculture (Holland, 2000; Grosholz, 2002). Several negative impacts caused by the introduction of exotic jellyfish species have been observed, including severe blooms, which can cause decreases in the abundance of endemic gelatinous plankton (Mills, 2001). In addition, jellyfish introductions can be hazardous to bathers because of their sting, and to fishers because they clog fishing nets (Mills, 2001).

In Korean coastal waters, a species of the moon jelly *Aurelia* is recognized as one of the most economically harmful animals to date, and its dense blooms have damaged commercial fisheries when swarms of medusae impeded trawling (data from the National Fisheries Research and Development Institute; http://nfrdi.re.kr/). In addition, these organisms have clogged seawater intakes, shutting down power plants located on the east coast of the Korean peninsula. There is no record of the moon jelly's existence before 2000. It remains therefore unresolved whether the Korean moon jelly is an endemic or an introduced species. Furthermore, based on specific morphological characteristics, it was presumed to be *A. aurita*. However, its identity remains elusive because of morphological similarities with closely related species.

In this paper, we determined the complete DNA sequences of the mitochondrial COI gene and the ITS-5.8S rDNA from a Korean moon jelly specimen, and compared them with previously reported DNA sequences of diverse *Aurelia* species and subspecies to determine their identity. In addition, we investigated partial COI gene and ITS-5.8S rDNA of several Korean *Aurelia* specimens collected from three geographically separated coastal areas at different times of the year. Furthermore, we investigated the biogeographic relationships of *Aurelia* occurring in Korea and other countries, using various molecular characteristics such as sequence similarity, parsimony, and phylogenetic analyses.

Material and methods

Specimens of *Aurelia* were collected from different localities in southern (Geoje-do, 34°59′33″N 128°40′31″E), southeastern (Busan, 35°12′58″N 129°13′41″E), and western (Incheon, 37°26′23″N 126°22′40″E) Korean coastal waters (Figure 1). They were immediately preserved in absolute ethanol to dehydrate, washed several times, and stored at room temperature until use. Genomic DNA was isolated from the stored tissues, using a treatment of proteinase K with chloroform extraction and isopropanol precipitation, followed by the protocol described in Lee (2000). In addition, the manually isolated DNA was purified with the DNeasy tissue kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. To amplify the mitochondrial COI gene from *Aurelia* sp., PCR was used with a set of primers (JF-F1, 5′-AGC AAG CCC ATT ATA CAA AAG GTA C-3′; JF-R1, 5′-CGA CAA ACA TTA TTT GAT CRT GAA G-3′). Primers were designed based on a comparison between *A. aurita* (GenBank number DQ787873) and a coral mtDNA sequence (GenBank number DQ304771). A volume of 25 μl of PCR reactions was carried out in 1× PCR buffer (10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatine, pH 8.3) with <0.1 μg of genomic DNA as a template, 200 μM each of the four dNTPs, 0.5 μM of each primer, and 0.2 units of LA *Taq* polymerase (TaKaRa, Japan), using iCycler (Bio-Rad, CA, USA). PCR thermocycling parameters were as follows: 95°C for 5 min; 35 cycles of denaturation at 95°C for 20 s; annealing at 55°C for 30 s and extension at 72°C for 60 s; and a final extension at 72°C for 5 min. The PCR products (2 μl) were analysed by 1.0% agarose gel electrophoresis according to a standard method.

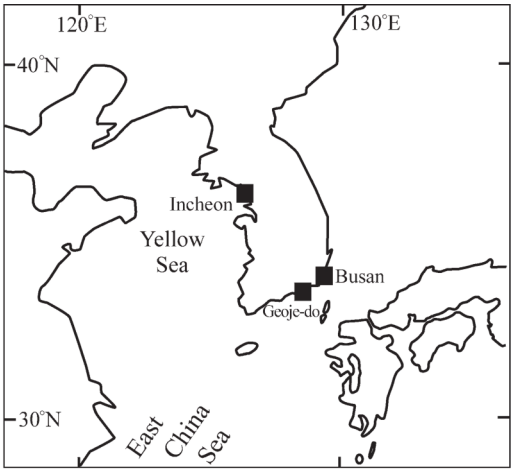

Figure 1. Sampling sites of moon jelly in Korea.

The entire ITS containing 5.8S rDNA was amplified from the same genomic DNA material from the *Aurelia* specimens, followed by conventional PCR protocols with its flanking 18S and 28S rDNA-target primers (JF-18F1750, 5′- AAA GTC GTA ACA AGG TTT CCG-3′; JF-28R765, 5′-TTG GTC CGT GTT TCA AGA CG-3′).

All PCR products of the COI gene were subcloned into pCR2.1 vectors (Invitrogen, CA, USA), and additional PCR products from geographic samples were cloned into identical vectors. All the subcloned DNAs were sequenced according to the manufacturer's suggested protocol with commercial primers (T7 and M13R) and nested flanking sequencing primers for primer walking in both directions. Labelled DNA fragments were analysed on a Model 3700 automated DNA sequencer (Applied Biosystems, CA, USA). All the PCR amplicons of ITS-5.8S rDNA gene were purified with the QIAquick PCR purification kit (Qiagen GmbH, Germany), and were directly sequenced using the identical PCR and additional nested primers (e.g. JF-28R1, 5′-GCG AAT TGT AGT CTC GAG AAG CGT-3′; JF-5.8R1, 5′-GAA TCA TCG AAT CTT TGA ACG C-3′), followed by the sequencing method described above. Editing and contiguous assembly of the DNA sequence fragments were carried out with Sequencher 4.7 (Gene Codes, MI, USA).

For comparative molecular features, complete COI and ITS-5.8S DNA sequences determined in the present study (Table 1) were aligned with other *Aurelia* sequences retrieved from GenBank/DDBJ/EMBL, using Clustal W version 1.84 (Thompson *et al.*, 1994). For precise comparisons within the genus *Aurelia*, the data matrixes were constructed with well-defined DNA sequences, excluding sequence data that contained ambiguous nucleotide readings such as N, W, K, M, or S (designated as degeneracy symbols by the International Union of Pure and Applied Chemistry). Overall, genetic characteristics such as parsimony-informative (P-I), transversion, transition, conserved,

Table 1. Origin of specimens examined and GenBank accession numbers for nuclear rDNA, including ITS and 5.8S, and mitochondrial COI gene sequences.

Species	Isolation locality	Length (bp)		GenBank number	
		COI	rDNA	COI	Nr. rDNA
Aurelia sp.1	Korea: Incheon[a]	1 581	708	EU010386	EU276014
Aurelia sp.1	Korea: Geoje-do	712	858	EU366144	EU332745
Aurelia sp.1	Korea: Busan	712	858	EU366143	EU332744
Aurelia sp.1	Japan: Miyazu Bay, Honshu	658	978	AY903169	AY935214
Aurelia sp.1	Japan: Sakata Bay, Honshu	658	–	AY903185	–
Aurelia sp.1	Japan: Uwa Bay, Inland Sea	640	–	AY903191	–
Aurelia sp.1	Japan: Ondo Strait, Inland Sea	632	–	AY903196	–
Aurelia sp.1	Japan: Tokyo Bay	658	–	AY903116	–
Aurelia sp.1	USA: Marina del Rey, CA	658	994	AY903081	AY935203
Aurelia sp.1	USA: Long Beach, CA	658	–	AY903085	–
Aurelia sp.1	USA: Newport Beach, CA	658	–	AY903088	–
Aurelia sp.1	USA: San Diego, CA	658	–	AY903092	–
Aurelia sp.1	Australia: Millers Point, New South Wales	658	–	AY903131	–
Aurelia sp.1	Australia: Greys Point, New South Wales	658	–	AY903142	–
Aurelia sp.1	Australia: Darling Harbour, New South Wales	658	–	AY903143	–
Aurelia sp.1	Australia: Coila Lake, New South Wales	638	–	AY903149	–
Aurelia sp.1	Australia: Port Jackson, New South Wales	649	–	AY903182	–
Aurelia sp.1	Australia: Lake Illawarra, New South Wales	634	–	AY903154	–
Aurelia sp.1	Australia: Tuggerah Lake, New South Wales	656	–	AY903160	–
Aurelia sp.1	Australia: Lake Macquarie, New South Wales	658	–	AY903166	–
Aurelia sp.1	Australia: Mooloolaba Harbour, Queensland	619	–	AY903167	–
Aurelia sp.1	Australia: Mooloolaba, Queensland	658	–	AY903128	–
Aurelia sp.1	Australia: Huon Estuary, Tasmania	625	–	AY903151	–
Aurelia sp.1	Australia: Perth, Western Australia	658	–	AY903127	–
Aurelia sp.1	United Kingdom: Anglesey, Wales	634	–	AY903213	–
Aurelia sp.2	Brazil: Cananeia, Sao Paulo	658	1 228	AY903121	AY935204
Aurelia sp.3	Palau: near CRRF dock, Koror State	658	1 014	AY903096	AY935209
Aurelia sp.3	Palau: Tab Kukau Cove, Koror State	633	–	AY903113	–
Aurelia sp.3	Palau: Tketau Lake, Koror State	658	–	AY903114	–
Aurelia sp.3	Palau: Risong Cove, Koror State	658	–	AY903115	–
Aurelia sp.4	Palau: Hotwater Lake, Koror State	658	–	AY903098	–
Aurelia sp.4	Palau: Ongeim'l Tketau, Koror State	658	–	AY903101	–
Aurelia sp.4	Palau: Ongael Lake, Koror State	658	971	AY903109	AY935208
Aurelia sp.4	Palau: Uetera Ngermeuangel, Koror State	645	–	AY903111	–
Aurelia sp.4	USA: Ala Wai Marina, HI	658	–	AY903137	–
Aurelia sp.4	Indonesia: Halimeda Lake, Berau	658	–	AY903145	–
Aurelia sp.5	Croatia: Veliko Jezero, Mljet	658	1 146	AY903124	AY935210
Aurelia sp.6	Palau: Ngell Channel, Koror State	658	–	AY903100	–
Aurelia sp.6	Palau: Helen Reef, Southwest Islands	618	1 109	AY903106	AY935207
Aurelia sp.6	Papua New Guinea: New Britain	658	–	AY903129	–
Aurelia sp.7	Italy: North Adriatic Sea	658	1 009	AY903133	AY935212
Aurelia sp.7	Croatia: Bay of Ston	658	1 018	AY903135	AY935213
Aurelia sp.8	Australia: Huon Estuary, Tasmania	658	1 054	AY903141	AY935217
Aurelia sp.8	New Zealand: coastal waters	1 070	1 070	–	AY935218
Aurelia sp.9	USA: Gulf of Mexico, off AL	622	1 165	AY903175	AY935216
Aurelia sp.10	USA: Kachemak Bay, AK	455	1 096	AY903067	AY935211
Aurelia aurita	–	657	–	AY428838	–
Aurelia aurita	USA: Boston Harbor, MA	658	–	AY903095	–
Aurelia aurita	USA: Charlestown, RI	1 119	1 119	–	AY935205

Continued

Table 1. *Continued*

Species	Isolation locality	Length (bp)		GenBank number	
		COI	rDNA	COI	Nr. rDNA
Aurelia aurita	Turkey: Bosphorus	658	–	AY903117	–
Aurelia aurita	Sweden: Gullmar Fjord	658	1 098	AY903118	AY935206
Aurelia aurita	UK: Anglesey, Wales	658	–	AY903212	–
Aurelia aurita	Finland: White Sea	1 581	–	NC_008446	–
Aurelia labiata	Canada: Sooke Basin, British Columbia	658	–	AY903071	–
Aurelia labiata	Canada: Todd Inlet, British Columbia	658	–	AY903073	–
Aurelia labiata	USA: Kachemak Bay, AL	1 166	1 166	–	AY935202
Aurelia labiata	USA: Tomales Bay, CA	658	–	AY903077	–
Aurelia limbata	Japan: Hokkaido	618	1 020	AY903189	AY935215

[a]All DNA sequences of Korean specimens were determined in the present study, and the others were retrieved from the public database.

and variable sites were calculated using MEGA 3.1 (Kumar *et al.*, 2001). Molecular similarities of each gene between subspecies or subgenus of *Aurelia* were measured separately in BioEdit 5.0.6 (North Carolina State University). The uncorrected pairwise (*p*-) distance was calculated when investigating nucleotide substitutions.

Phylogenetic analysis of the genus *Aurelia* was carried out with the COI gene sequences from the data in Table 1. In all, 55 DNA sequences were aligned as described above, and the alignment results were adjusted manually. Only those positions that could be unambiguously aligned were used for the analysis. This yielded 625 of the 658 alignment positions for the partial COI sequence. Modeltest 3.07 (Posada and Crandall, 1998) was used to find the optimal model of DNA substitution for maximum-likelihood (ML) construction. As the best-fit model for this dataset, the GTR+I+G model was chosen by both hierarchical likelihood ratio tests (hLRTs) and the Akaike information criterion (AIC), respectively. The Bayesian analysis was implemented with MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001), using the molecular model selected by AIC, namely a GTR nucleotide substitution model, with among-site rate variation modelled with a proportion of sites being invariable, while the rates for variable sites were drawn from a gamma distribution. The Markov chain Monte Carlo (MCMC) process was set to four chains, and 1 000 000 generations were conducted and sampled every 100 generations. After analysis, the first 1000 trees were deleted as burn-in, and the consensus tree was constructed. Bayesian posterior probabilities (>0.50) were indicated at each branch node. The phylogenetic trees were visualized with TreeView version 1.6.6 (Page, 1996).

Additionally, the phylogeography of conspecific *Aurelia* members was studied with ITS-5.8S rDNA sequences. In all, 20 DNA sequences were available for analysis, using the method described above. In this analysis, 982 of the 1008 alignment positions for the complete ITS-5.8S rDNA sequence were used for the subsequent analysis. Modeltest 3.07 was used to find the optimal model of DNA substitution for ML construction and suggested the GTR+G model with $-\ln L = 6821$ as the best-fit model for the ITS-5.8S dataset by AIC. An ML tree of *Aurelia* was constructed with the selected GTR+G model in PAUP* 4.0b10 (Swofford, 2002), using the following likelihood settings determined from the above Modeltest: base frequencies $A = 0.2558$, $C = 0.2307$, $G = 0.2404$; base substitution rates $AC = 0.8610$, $AG = 1.9527$, $AT = 1.5856$, $CG = 0.7921$, $CT = 2.6291$; assumed proportion of invariable sites = 0.0001; and gamma distribution shape parameter = 0.3406. Bootstrap analyses with 100 replicates were conducted to determine the robustness of the clades. Further Bayesian analysis of the identical ITS-5.8S dataset was implicated with MrBayes version 3.1.2, followed by the above method used in COI analysis.

Results and discussion

Complete COI sequence of *Aurelia* sp.1

The complete sequence DNA of a Korean *Aurelia* COI gene is 1581 bp in length (Table 2). The representative sequence has been deposited in GenBank (Table 1). In this study, we compared Korean *Aurelia* with *A. aurita*, because the latter had been considered previously as being identical with the Korean moon jelly. A BLAST search of the GenBank database revealed the COI sequence determined here was closely matched with those of the *Aurelia* sp.1 COI sequence from Lake Macquarie (GenBank number DQ787873), Perth (AY903163), and Tokyo Bay (AY903126). Comparison of 658 sites revealed that it was nearly identical, with 99.9% DNA similarity. Therefore, we were able to

Table 2. Characteristics of complete COI genes from the moon jelly, *Aurelia aurita* and *Aurelia* sp.1, and comparisons between them.

Characters	*Aurelia* sp.1 (Korean Sea)	*Aurelia aurita* (Atlantic Ocean)
DNA sequence (bp)	1 581	1 581
Amino acid sequence	526	526
%GC content	37.6	36.2
%GC in protein coding		
1	43.7	42
2	40.6	40.7
3	28.3	26.2
Start codon	ATG	ATG
Stop codon	TAA	TAG
Amino acid similarity		
Aurelia sp.1	–	98%
DNA similarity		
Aurelia sp.1	–	84.8%

identify the Korean moon jelly as *Aurelia* sp.1 and used this designation hereafter. The COI gene of the Korean *Aurelia* sp.1 coded 526 amino acids, of which the start and stop codons were recorded as "ATG" and "TAA", respectively. The GC ratio of the COI gene was 37.6%. In addition, GC composition of each first, second, and third codon position in COI gene was 43.7%, 40.6%, and 28.3%, respectively. Interestingly, the third position was significantly lower in GC content in both *Aurelia* sp.1 and *A. aurita* (Table 2).

Until now, the COI sequences of *Aurelia* spp. available in public databases, except for *A. aurita*, are slightly less than 700 bp in length. Here, we are the first to report on a complete COI DNA sequence of *Aurelia* sp.1 from a Korean specimen collected from Incheon coastal waters. It allows us to make more detailed comparisons between *Aurelia* sp.1 and *A. aurita*. By a comparison of COI amino acid sequences, *Aurelia* sp.1 was found to differ from *A. aurita* with 98.0% similarity (Table 2), whereas the DNA sequence comparison had 84.8% similarity. In addition, the stop codon was different from *A. aurita*, "TAG", whereas the start codon, "ATG", in the protein coding sequence was identical in both species (Table 2). As pointed out previously, robust morphological characters are rare in *Aurelia* (Greenberg *et al.*, 1996), making it difficult to distinguish the various *Aurelia* species. However, using molecular signatures (e.g. Dawson and Jacobs, 2001), it is possible to resolve discrepancies between *A. aurita* and *Aurelia* sp.1 and so to distinguish the two species.

In this study, we also included some *Aurelia* specimens collected from geographically distinct areas (Figure 1). Based on our findings, we sequenced partial COI gene sequences (around 500 bp) from five specimens collected in Incheon coastal waters (37°26′23″N 126°22′40″E), where *Aurelia* occurred densely, and found all the sequences were exactly identical with GenBank number EU010386, which suggested that the single bloom of *Aurelia* in Incheon was genetically homogenous. Furthermore, partial COI sequences of some specimens, including two polyps and one ephyra-staged sample, collected from Busan, were also identical with other specimens in our collections. This suggested that all *Aurelia* found in three different areas of Korea might have a mitochondrial COI genotype that is identical with *Aurelia* sp.1.

ITS-5.8S rDNA of Korean *Aurelia*

To further study their genotypes, we sequenced the nuclear ITS-5.8S rDNA genes and the flanking regions from the Korean *Aurelia* specimens, which were used in the COI analyses. All the specimens have identical genotypes. A total length of the ITS-5.8S rDNA from the Korean *Aurelia* spp. was 272 bp (ITS1), 158 bp (5.8S rDNA), and 278 bp (ITS2), respectively. Nucleotide frequencies of complete rDNA were recorded at A, 25.8%; T, 27.4%; G, 24.6%; C, 22.2%, respectively. The present ITS-5.8S rDNA sequence was compared with other *Aurelia* rDNA sequences in the NCBI database. A BLAST search confirmed that the Korean *Aurelia* examined matched the previously designated *Aurelia* sp.1 (GenBank accession number AY935214 and AY935203). This agrees with the present COI analysis and confirms that the Korean moon jelly was *Aurelia* sp.1 among the diverse *Aurelia* members classified by Dawson *et al.* (2005).

In addition, we found that all *Aurelia* blooming in different areas of Korea have an identical genotype (i.e. *Aurelia* sp.1) when compared with ITS-5.8S rDNA sequences (Table 1). This was consistent with the COI gene comparison. These results suggest that the *Aurelia* blooming in Korean coastal areas were identical with *Aurelia* sp.1.

Molecular phylogeny of *Aurelia*

Mitochondrial DNA, which is transferred to descendants through maternal lineages, is useful to estimate genetic diversity and phylogenetic relationships. Particularly, the COI gene is highly conserved within the same species but variable among species, so can be used for phylogenetic studies and serve as DNA barcodes (that is, species identification tags).We examined the phylogenetic relationships of conspecifics of *Aurelia* using their partial COI DNA sequences (Table 1). In an unrooted Bayesian tree (Figure 2a), we detected 13 distinct clades including *A. aurita, A. limbata*, *A. labiata*, and ten unassigned *Aurelia*, with overall well-supported branch confidence. The same tree with unscaled branches (Figure 2b) showed the origins of specimens and that the 13 *Aurelia* spp. were clustered according to their species identities rather than to the isolation localities. For example, *A. aurita* forms a clade that includes specimens from Sweden, Turkey, Finland, the UK, and the USA, and also forms a sister group relationship with *A. labiata* with 0.97 of posterior probability (PP). *Aurelia* sp.1 forms a clade including Japanese, Korean, USA, and Australian specimens with a high PP value (0.99), and has a sister group relationship with both *A. limbata* and *Aurelia* sp.10. Taking into account the branch topology of the Bayesian analysis and the genetic distance between the three identified species (*A. aurita*, *A. limbata*, and *A. labiata*), it is possible to consider the ten unassigned *Aurelia* spp. as truly distinct species, as proposed by Dawson *et al.* (2005). Therefore, existing ecological and systematic descriptions of *Aurelia* may need to be revised in view of these molecular data.

Beyond the taxonomy of *Aurelia*, an additional Bayesian tree of *Aurelia* sp.1 showed that expected changes per site were ~20 times lower in *Aurelia* sp.1 than in the genus *Aurelia* (Figure 2c). Here, we included COI sequences from 23 different sampling sites in four countries (Japan, Korea, USA, and Australia). As noted previously, all the Korean specimens formed a clade because their genotype is identical, and clustered with a Japanese specimen (isolated from Tokyo Bay, GenBank number AY903116; Figure 2d). In this tree, four different Japanese specimens (collected from Ondo Strait, Miyazu Bay, Tokyo Bay, Sakata Bay) differed slightly, based on their origins. The specimen from Sakata Bay clustered with specimens from the US and Australia, with 0.84 PP value (Figure 2c and d). However, phylogenetic resolution in the *Aurelia* sp.1 COI analysis was low because of the conservative nature of the COI gene that sometimes does not reflect the relationships between subspecies.

In contrast, ITS rDNA regions are useful for defining intraspecific differences, because they are less subject to functional constraints, so evolve more rapidly (Bena *et al.*, 1998). The ITS regions are highly variable within *Aurelia* (see parsimony analysis below), so their sequences may be useful in studying the relationships among subspecies. Phylogenetic analysis of the ITS-5.8S rDNA demonstrated that the 13 species included here with 18 sequences from different localities were separated from each other, and all clades within the same species were well supported by bootstrap (BP) and PP values (Figure 3). All the Korean *Aurelia* specimens formed a clade with other *Aurelia* sp.1 collected in Japan and the US (California), and the tree branch was separated from the other *Aurelia* members (1.0 PP, 100 BP).

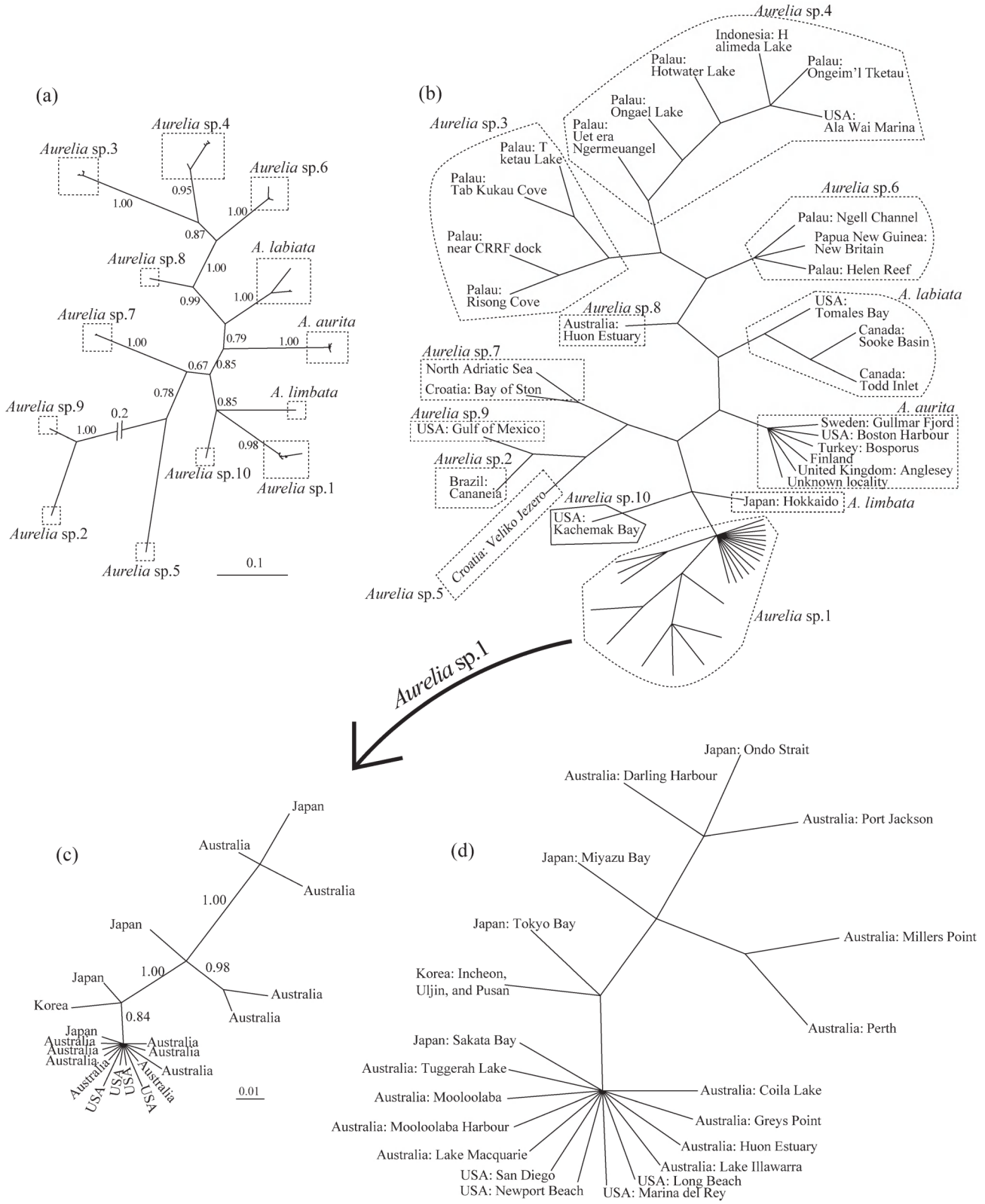

Figure 2. Unrooted Bayesian trees showing the relationships of the genus *Aurelia* (a and b) and *Aurelia* sp.1 (c and d), inferred from partial COI gene sequences (Table 1) in MrBayes version 3.1.2. (b) and (d) were generated from the trees of (a) and (c), with unscaled branches. The numbers at the nodes are posterior probabilities in which PP values above 0.50 are indicated at each node.

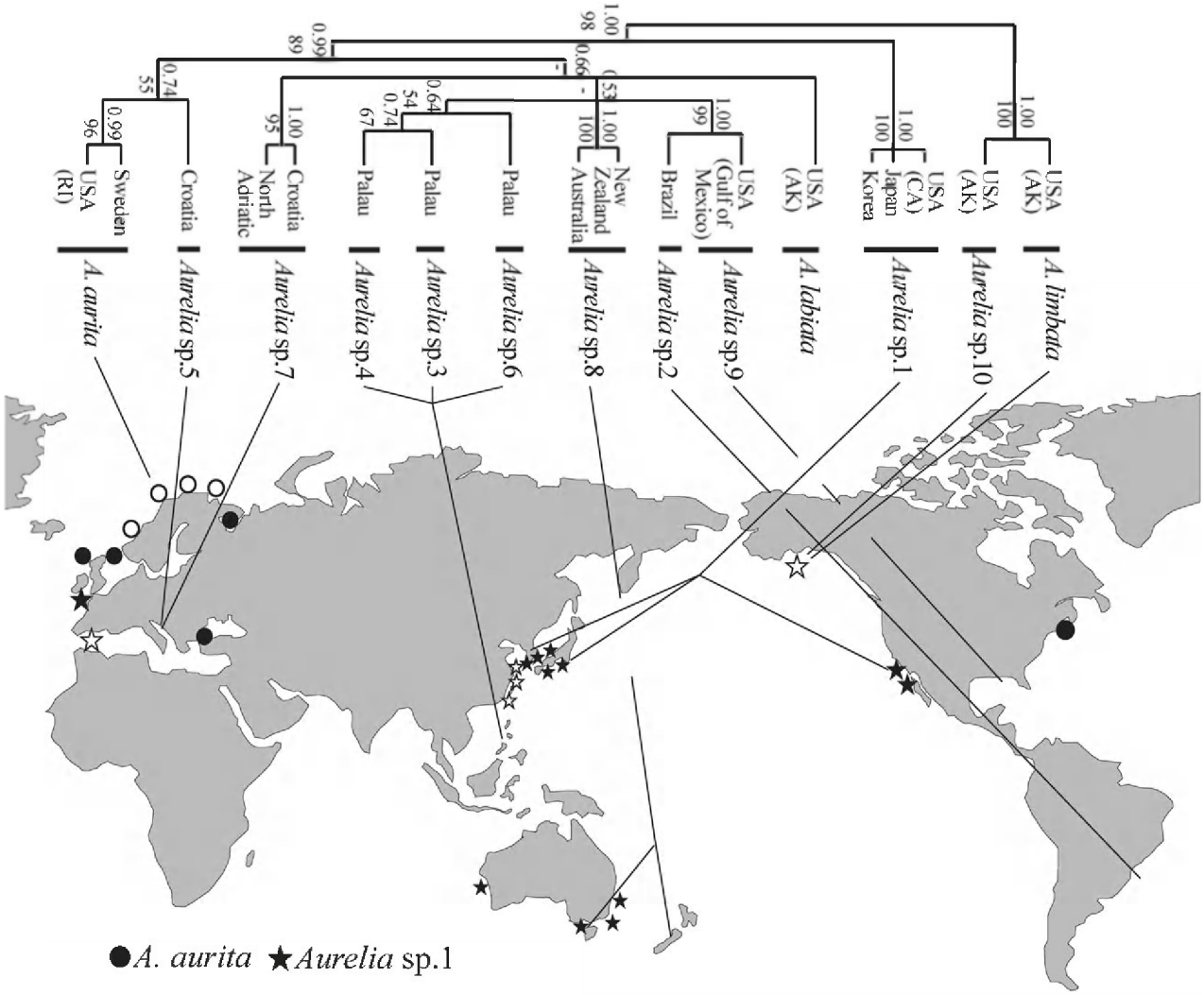

Figure 3. Phylogenetic tree of the genus *Aurelia* and a map showing the geographical distribution of both *Aurelia* sp.1 and *A. aurita*. The tree was inferred from Bayesian and ML methods using ITS-5.8S rDNA of the genus *Aurelia*. The numbers above the nodes are posterior probabilities (>0.50); the numbers below are bootstrap values (percentage) for internal branches of the ML trees as computed by PAUP* 4.0b10. On the map, solid circles and stars represent availability of the COI DNA information of *A. aurita* and *Aurelia* sp.1, respectively. Open symbols show waters in which *Aurelia* sp.1 and *A. aurita* are present, yet no DNA was isolated.

Based on all available information, we find that *Aurelia* sp.1 generally occurs in temperate regions (Korea, Japan, Europe, Australia, and North America) rather than tropical and cold seas, whereas *A. aurita* was frequently found in the cold–temperate North Atlantic, although it also occurs as far as Boston Harbor, US, and the Bosphorus, Turkey (Figure 3). Schroth *et al.* (2002) demonstrated that *A. aurita* occurs in the cold–temperate North Atlantic, *A. labiata* in the cold–temperate eastern North Pacific, and *A. limbata* in the northern polar oceans, respectively. The latter two are present in very restricted areas, whereas *A. aurita* and *Aurelia* sp.1 are dispersed in coastal areas worldwide.

Parsimony analyses of COI and ITS-5.8S rDNA

By numerical comparison of *Aurelia* COI gene sequences (Table 3), we found that similarities within different specimens of the same species were great (*Aurelia* sp.1, >99.3% similarity, <0.0098 genetic distance; *Aurelia* sp.7, 99.6%, 0.0024); similarities between interspecies were significantly smaller (<85.0%, >0.1449). In addition, the Korean, Japanese, and Californian *Aurelia* sp.1 COI sequences are nearly identical (99.6% DNA similarity, <0.0098 genetic distance). In contrast, similarities between our *Aurelia* sp.1 and the other *Aurelia* spp. were generally small (<84.1% similarity). Similarly, comparisons of *Aurelia* ITS-5.8S, which were determined from the same specimens used in COI sequencing listed in Table 1, revealed results similar to the COI comparisons (Table 3). Specifically, the greatest similarity (99.7%) was recorded between our specimens and *Aurelia* sp.1 (AY935214; Miyazu Bay, Japan), followed by 99.4% with *Aurelia* sp.1 (AY935203; California). Overall, DNA sequence similarities between ours and those reported for other *Aurelia* spp. were <80% (mostly <70%). In this context, the current data on *Aurelia* sp.1 were significantly different from those of *A. aurita* (AY935206, 76.4%), *A. labiata* (AY935202, 70.8%), and *A. limbata* (AY935215, 79.5%), as reported previously (Dawson *et al.*, 2005).

Table 3. Similarity scores (percentage—above diagonal) and genetic distance (below diagonal) estimated by the Kimura two-parameter model between 14 pairs of the aligned sequence data of the partial COI gene and nearly complete ITS, including the 5.8S rDNA gene from *Aurelia*.

Number	Species	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]
Partial COI gene																
[1]	*Aurelia* sp.1K		99.6	99.3	79.0	81.4	82.0	82.6	77.5	84.0	84.1	82.2	75.2	60.3	82.2	80.8
[2]	*Aurelia* sp.1J	0.00		99.3	79.0	81.4	82.0	82.6	77.5	84.3	84.4	82.2	75.2	60.3	82.2	80.8
[3]	*Aurelia* sp.1N	0.01	0.01		79.1	81.7	82.3	82.8	77.8	84.3	84.4	82.6	75.3	60.7	82.3	80.6
[4]	*Aurelia* sp.2	0.25	0.25	0.24		77.6	78.2	78.1	75.3	79.6	79.3	78.8	83.5	55.1	78.8	72.3
[5]	*Aurelia* sp.3	0.24	0.24	0.23	0.27		84.6	83.2	79.6	81.7	81.4	84.1	74.1	57.7	83.7	78.1
[6]	*Aurelia* sp.4	0.25	0.25	0.25	0.25	0.19		83.1	78.5	81.4	81.4	84.9	75.8	56.5	82.9	78.1
[7]	*Aurelia* sp.5	0.23	0.23	0.23	0.28	0.20	0.22		77.8	82.9	82.6	86	75.8	57.1	81.9	77.2
[8]	*Aurelia* sp.6	0.23	0.23	0.22	0.23	0.17	0.20	0.22		77.3	77.6	80.8	73.9	51.5	79.6	71.8
[9]	*Aurelia* sp.7	0.22	0.21	0.21	0.25	0.24	0.24	0.23	0.23		99.6	83.5	75.9	57.7	81.9	78.5
[10]	*Aurelia* sp.7	0.22	0.21	0.21	0.25	0.24	0.25	0.23	0.23	0.00		83.2	76.2	57.5	81.9	78.4
[11]	*Aurelia* sp.8	0.25	0.25	0.24	0.25	0.2	0.18	0.17	0.17	0.23	0.24		76.2	57.9	86	77.8
[12]	*Aurelia* sp.9	0.26	0.26	0.25	0.13	0.26	0.25	0.23	0.24	0.24	0.23	0.22		54.4	76.5	75.1
[13]	*Aurelia* sp.10	0.14	0.14	0.14	0.24	0.2	0.21	0.19	0.21	0.19	0.19	0.20	0.26		58	64.5
[14]	*Aurelia aurita*	0.22	0.22	0.21	0.23	0.18	0.21	0.21	0.18	0.22	0.23	0.17	0.21	0.19		78.7
[15]	*Aurelia limbata*	0.18	0.18	0.18	0.26	0.22	0.2	0.21	0.22	0.22	0.23	0.24	0.25	0.14	0.19	
ITS-5.8S rDNA gene																
[1]	*Aurelia* sp.1K		99.7	99.4	50.6	72.8	72.1	69.1	73.3	79.3	78.8	66.8	50.2	79.1	76.4	79.5
[2]	*Aurelia* sp.1J	0.00		99.1	50.5	72.7	72.1	69.0	73.4	79.3	78.8	66.9	50.1	78.9	76.4	79.3
[3]	*Aurelia* sp.1N	0.00	0.00		50.9	72.8	72.1	69.1	73.6	78.9	78.4	66.9	50.6	79.2	76.2	79.8
[4]	*Aurelia* sp.2	0.38	0.38	0.38		49.8	48.0	49.1	48.0	50.2	50.1	48.3	85.9	48.3	50.4	49.0
[5]	*Aurelia* sp.3	0.18	0.18	0.18	0.41		76.1	61.8	73.4	71.5	70.8	66.7	49.2	69.2	69.5	69.3
[6]	*Aurelia* sp.4	0.18	0.18	0.18	0.43	0.15		62.7	73.2	73.6	72.6	67.6	49.0	69.3	69.1	70.2
[7]	*Aurelia* sp.5	0.16	0.16	0.16	0.45	0.25	0.22		64.7	70.2	69.7	61.1	49.2	66.5	71.0	66.1
[8]	*Aurelia* sp.6	0.15	0.15	0.15	0.41	0.14	0.15	0.19		74.0	74.5	68.2	48.1	75.2	69.8	73.7
[9]	*Aurelia* sp.7	0.12	0.12	0.12	0.39	0.18	0.16	0.16	0.12		96.2	66.2	50.2	77.5	74.3	78.0
[10]	*Aurelia* sp.7	0.12	0.12	0.12	0.39	0.17	0.16	0.16	0.11	0.01		66.9	50.2	78.2	74.4	78.4
[11]	*Aurelia* sp.8	0.16	0.16	0.16	0.42	0.20	0.18	0.21	0.15	0.16	0.15		49.5	66.9	65.7	66.8
[12]	*Aurelia* sp.9	0.38	0.38	0.38	0.03	0.41	0.43	0.44	0.40	0.38	0.38	0.41		49.1	50.3	49.0
[13]	*Aurelia* sp.10	0.12	0.12	0.12	0.42	0.2	0.19	0.17	0.14	0.12	0.11	0.18	0.40		70.8	88.2
[14]	*Aurelia aurita*	0.15	0.15	0.15	0.4	0.19	0.19	0.17	0.17	0.14	0.13	0.18	0.40	0.20		70.9
[15]	*Aurelia limbata*	0.11	0.11	0.11	0.4	0.19	0.18	0.17	0.14	0.12	0.12	0.17	0.40	0.10	0.20	

In the comparisons, only COI and ITS gene sequences for specimens from the same locality and the same species were chosen. Note: K, J, and N represent *Aurelia* sp.1 from Korea, Japan, and North America, respectively.

Parsimony analyses of the COI gene and ITS rDNA, including additional 5.8S (Table 4), revealed that the transition:transversion ratio (Ts/Tv) was high in the coding COI gene (2.25) rather than in the non-coding ITS region (0.76). High *p*-distance, in contrast, was recorded at ITSs (0.314) when compared with that in the COI gene (0.211). Additionally, analyses on P-I sites revealed that P-I sites were higher in the ITS rDNA (48.2% in only ITS, 40.7% in ITS-5.8S rDNA) than in the COI gene (33.3%). Variation in the ITS non-coding rDNA was ~1.45 times greater than that of the coding COI genes, as judged by a comparison of the P-I sites. These observations suggest that the variations in COI and ITS DNA sequences are sufficient to allow us to discriminate distinct species, sometimes at the subspecies level.

Table 4. Sequence characteristics of the moon jelly *Aurelia* spp., calculated by parsimony analysis of three datasets from 15 sequences of the genus *Aurelia*, the same data used in the analyses of Table 3.

Gene	Nn	Average *p*-distance	Nc	Nd	Ts	Tv	Ts/Tv	P-I	%P-I
COI	651	0.211	411	247	72	32	2.25	217	33.3
ITS only	823	0.314	229	558	71	93	0.76	397	48.2
ITS, 58S	981	0.210	383	562	72	93	0.77	399	40.7

Nn, total number of nucleotides; Nc, total number of conserved nucleotides; Nd, total number of nucleotide differences.

Implications of global expansion

The morphological identification of the fragile moon jelly and its relatives is not easy because their medusae and other life stages are highly variable. Correct and rapid identification of these species is crucial to understanding their global expansion and to controlling blooms to protect fisheries and the biodiversity of endemic species

(Mills, 2001). To date, molecular tools (e.g. *in situ* hybridization, species-specific PCR, restriction fragment length polymorphism, DNA sequencing) are increasingly being applied to recognize and distinguish particularly cryptic organisms. DNA sequencing generally provides the most accurate means for identification (Dawson and Jacobs, 2001; Dawson, 2003). The complete COI sequence of *Aurelia* sp.1 reported here will provide an additional option of molecular signatures and/or DNA barcodes.

This study demonstrates that the moon jelly blooms in Korean coastal waters were caused by *Aurelia* sp.1. The species identity was strongly supported by a comparison of COI and ITS sequences (Table 3) and phylogenies (Figures 2 and 3). In addition, molecular comparison and parsimony analyses demonstrated that genotypes of the Korean, Japanese, and Californian *Aurelia* sp.1 were nearly identical (>99.6% similarity), whereas they were significantly different (<84.1 similarity) from other *Aurelia*. Phylogeny of the COI sequences demonstrated that slight differences among the *Aurelia* sp.1 were detected according to their geographic origins. However, by comparing the non-coding ITS, which evolves faster than the coding COI gene (Table 4), *Aurelia* sp.1, occurring in the three regions, reveal an identical genotype. Interestingly, we found only one base difference in the ITS-5.8S rDNA sequences between Korean and Californian specimens, and found that the difference was detected in a tract of poly(T), possibly caused by sequencing or PCR errors. This demonstrated that *Aurelia* sp.1 of both regions have exactly the same genotype in this respect. However, we found an insertion–deletion site (nucleotide sequence, TAA) at position 205–206 bp of *Aurelia* sp.1 from Incheon (GenBank number EU276014) in the ITS comparison of Korean and Japanese specimens. As noted previously (Figure 3), the moon jellyfish *Aurelia* sp.1 has the broadest geographic range of all species of *Aurelia* studied to date. It occurs at least in Japan, Australia, and the Atlantic, and along the Mediterranean coast of France, in San Francisco Bay (Greenberg *et al.*, 1996; Dawson and Martin, 2001), and from Los Angeles to San Diego (Dawson and Jacobs, 2001; Schroth *et al.*, 2002; Dawson, 2003). These results suggest that *Aurelia* sp.1 populations may have recently dispersed worldwide.

In eastern Asian coastal waters, the Korean and some Japanese moon jellies were assigned to *Aurelia* sp.1, using ITS and COI sequences. The moon jelly has also been observed in the East China Sea and in Taiwan coastal areas (Figure 3), but its identity remains unclear because its genetic data are not available. The identity of *Aurelia* sp. 1 in Korean and Japanese waters can be explained by the Kuroshio and the Tsushima currents, which flow from the South China Sea to the coasts of Korea and Japan. Dawson *et al.* (2005) used this kind of reasoning in trying to explain how *Aurelia* were spread from Japan northwards and eastwards by the Kuroshio Current in the North Pacific, as well as local mixing in the Yellow Sea, the East China Sea, and Japanese waters. The low genetic variation in ITS coupled with such a large geographic range that includes distinct populations from some of the major warm-temperate seaports of the world would suggest that *Aurelia* sp.1 may have dispersed recently across the Pacific; however, the dispersal time and origin of the *Aurelia* sp.1 remain uncertain.

Recently, Mills (2001) reviewed the worldwide occurrence of blooms and the dispersal of cryptic jellyfish, as well as their ecological and economic impacts. We found no record or monitoring data indicating the presence of moon jelly in Korean waters before 2000. Since then, two dense blooms have occurred. In this study, we found that the moon jelly blooms from different geographic areas of Korea had an identical genotype and should be assigned to *Aurelia* sp.1, based on the characteristics of mitochondrial COI and nuclear ITS-5.8S rDNA sequences. In addition, we demonstrated that Korean *Aurelia* sp.1 has a genotype identical to that of specimens from California and is slightly different from specimens collected in Japan. This suggests that *Aurelia* sp.1 in the three Korean regions is likely to have descended from the same population. This agrees with the conclusion of Dawson *et al.* (2005). To understand the origin and global expansion of *Aurelia* precisely, more detailed studies are necessary in future, including wider geographic sampling and intensive molecular analyses, such as population genetics.

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