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Genetic characterization of the scyphozoan jellyfish *Aurelia* spp. in Chinese coastal waters using mitochondrial markers

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ABSTRACT

Blooms of the moon jellyfish *Aurelia* spp. have occurred in the harbors and coastal waters around the world. The phylogenetic relationship and genetic characterization of *Aurelia* spp. was determined along the Chinese coastal waters based on sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene. The molecular analysis confirmed that all samples collected in Chinese coastal waters were *Aurelia* sp.1. We also analyzed the phylogenetic and population genetic structure of *Aurelia* sp.1 using the newly generated sequences supplemented with existing data from previous studies. The phylogenetic analyses of the COI regions did not support geographically restricted groups among the global samples of *Aurelia* sp.1. Analysis of molecular variance (AMOVA) indicated a complex genetic population structure and pattern of connectivity. Populations of *Aurelia* sp.1 were highly structured between most sampling sites over distances as small as 100 km (Rizhao and Qingdao) in certain cases. However, non-significant pairwise F_{ST} values were also observed between short geographic distances (Yantai, Rongcheng and Qingdao) and relatively distant sampling sites (Caofeidian, Rizhao and Japan). The life-cycle characteristics, together with the prevailing ocean currents in this region and possible anthropogenic introduction, were proposed and discussed as the main factors that determined the genetic patterns of *Aurelia* sp.1.

1. Introduction

The moon jellyfish *Aurelia* spp. (Cnidaria: Scyphozoa) is distributed worldwide in coastal and shelf sea marine environments between 70°N and 40°S (Lucas, 2001). Historically, 12 *Aurelia* species have been described based on morphological variability in the medusa (Mayer, 1910; Kramp, 1961). However, only *Aurelia aurita* and *Aurelia limbata* are recognized as distinct species (Russell, 1970). Recently, phylogenetic analysis of the genus *Aurelia* revealed 13 cryptic species that appear to be regionally restricted (Dawson and Jacobs, 2001; Schroth et al., 2002; Dawson, 2005; Ki et al., 2008). For example, *Aurelia labiata* was recognized as native to Pacific North America (Canada and USA) (Wrobel and Mills, 1998), while *Aurelia* sp.2 was distributed in marine environments in Brazil, *Aurelia* sp.3 in Palau, *Aurelia* sp.4 in Indonesia, Palau and Hawaii, *Aurelia* sp.5 in Croatia, *Aurelia* sp.7 in New Zealand and Tasmania, *Aurelia* sp.9 in the Gulf of Mexico, and *Aurelia*

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sp.10 in Alaska (Dawson and Jacobs, 2001; Schroth et al., 2002; Dawson, 2003, 2005; Crawford et al., 2011; Ramšak et al., 2012).

Due to intensive human activity, many marine species are introduced to seawaters beyond their natural geographic range either unintentionally or intentionally. Several cryptic species of *Aurelia* spp. have disjunct distributions thought to be due to the anthropogenic introduction of exotic species (Kideys and Gucu, 1995; Coles et al., 1999; Dawson, 2005). For example, the distribution of *Aurelia* sp.4 in the western Pacific and Pearl Harbor was thought to be based on introductions dating to the Second World War (Coles et al., 1999), while *Aurelia* sp.8 occurred with a Lessepsian distribution in the Adriatic, Mediterranean and Red Seas due to the opening of the Suez Canal (Kideys and Gucu, 1995). *Aurelia* sp.1 was found to be distributed in major warm-temperate regions around the globe, including Australia, California, France, Japan and Korea (Dawson and Jacobs, 2001; Schroth et al., 2002; Dawson, 2005; Ki et al., 2008).

Jellyfish invasions are not easily distinguished due to species crypsis and morphological plasticity in new abiotic or trophic environments (Graham and Bayha, 2007). However, molecular genetics approaches are useful for distinguishing invading cryptic species with morphological plasticity. The cytochrome c oxidase subunit I (COI) gene is one of the most frequently used mitochondrial genes for genetic analysis because it is easily amplified using the polymerase chain reaction method and conserved primers (Folmer et al., 1994). Additionally, intra- and inter specific variations of the Medusozoan mtDNA COI gene make it an appropriate choice for use as a DNA barcode for species-level identification (Holland et al., 2004; Folino-Rorem et al., 2009; Ortman et al., 2010; Laakmann and Holst, 2014). For example, the *C. andromeda* invasion of the Hawaiian Islands was identified by examining the global molecular phylogeny of *Cassiopea* spp. based on the mtDNA COI gene (Holland et al., 2004). Furthermore, multiple cryptic species in the genus *Cordylophora* were revealed as invasive species based on molecular analysis of mtDNA COI, 16S rRNA and 28S rRNA sequences (Folino-Rorem et al., 2009).

In Chinese seas, the aggregation and blooms of *Aurelia* spp. have mainly been observed in harbors and inshore areas in temperate regions, including the Yellow Sea and Bohai Sea (Dong et al., 2010; Wan and Zhang, 2012; Dong et al., 2014; Wang and Sun, 2015). In a previous study, *Aurelia* spp. collected in the East Margin Sea (i.e., Japan and Korea) were identified to be a single species (*Aurelia* sp.1) (Dawson, 2005; Ki et al., 2008). However, the taxonomy and genetic connectivity of *Aurelia* spp. in Chinese coastal waters were not well resolved. In this study, we collected individuals of *Aurelia* spp. from six different sites close to the harbor in northern Chinese coastal waters. The aim of our study was to investigate the existence of cryptic species in the genus *Aurelia* in Chinese coastal waters. Finally, the phylogeographic patterns of this species were also revealed using the mtDNA COI gene.

2. Materials and methods

2.1. Sample collection

A total of 103 individuals of *Aurelia* spp. were collected in six geographic locations in the Bohai Sea and Yellow Sea during the local jellyfish blooming periods (between July and September) in 2013 and 2014 (Fig. 1; Table S1): (I) the Bohai Sea region (BH) including locations near Caofeidian (CFD) and Weifang (WF); (II) the Northern Yellow Sea region (NY) including locations near Yantai (YT) and Rongcheng (RC); and (III) the southern Yellow Sea region (SY) including locations near Qingdao (QD) and Rizhao (RZ). Medusae tissue extracted from the bell margin or gonads was preserved in 95% ethanol and then stored at −20 °C until DNA extraction.

2.2. DNA extraction, PCR amplification, sequencing and alignment

Total genomic DNA was extracted using the TIANamp Marine Animals DNA Kit (TIANGEN, Beijing, China). The mitochondrial COI fragments from *Aurelia* spp. were amplified using the universal primers LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) under the PCR conditions previously described (Folmer et al., 1994). The PCR reactions were carried out in a volume of 50 μL that consisted of 50–100 ng genomic DNA, 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 mM primers, and 2.5 U Taq DNA polymerase (TIANGEN, China). The temperature profile was defined as follows: 94 °C for 3 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 54.5 °C for 30 s and extension at 72 °C for 60 s; followed by a final extension at 72 °C for 5 min. The PCR products were analyzed by 1.0% agarose gel electrophoresis according to a standard method.

PCR-amplified DNA fragments were purified and sequenced with an ABI 3730 automatic DNA sequencer at Sangon Biotech Co., Ltd (Shanghai, China) using the same primers described above. All PCR products were sequenced in both directions to obtain accurate sequences.

The DNA sequence fragments were verified, edited and assembled with BioEdit 7.0 (Hall, 2005). The sequences were blasted in NCBI to confirm their identities. Additionally, related *Aurelia* spp. sequences were obtained from GenBank for phylogenetic analyses (Table 1). The total dataset consisted of 103 COI sequences from this study and 62 COI sequences obtained from GenBank. The alignments were conducted with MEGA 5.0 (Ballard and Melvin, 2010); the total length of the alignments was 576 bp. *A. aurita* (JX995329) was used as an outgroup for the phylogenetic analyses.

2.3. Data analyses

The nucleotide composition and variable sites were analyzed in MEGA 5.0. The genetic diversity indices of mtDNA (nucleotide diversity [π] and haplotype diversity [h]) were calculated using DnaSP 5.0 (Librado and Rozas, 2009).

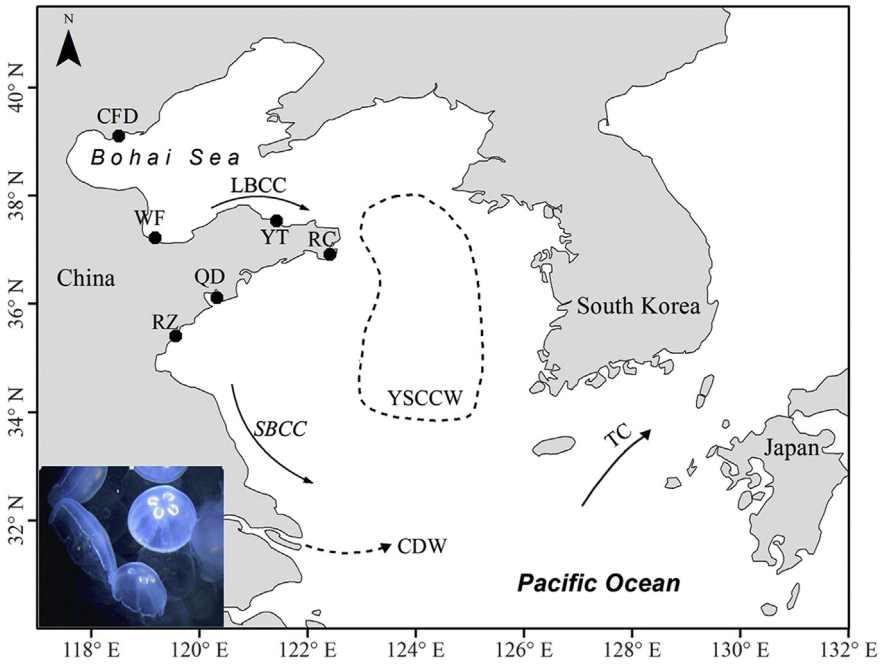

Fig. 1. Sampling sites of *Aurelia* spp. in Chinese coastal waters. Abbreviation IDs for geographic regions: CFD, Caofeidian; WF, Weifang; YT, Yantai; RC, Rongcheng; QD, Qingdao; RZ, Rizhao. Abbreviation IDs for currents: LBCC, Lubei Coastal Current; SBCC, Subei Coastal Current; CDW, Changjiang diluted water; YSCCW, Yellow Sea Cold Current Water; TC, Tsushima Current.

Phylogenetic analyses were conducted on two datasets (one consisted of all samples and 13 identified *Aurelia* species and the other consisted of all samples and *Aurelia* sp.1) using Bayesian methods. The best-fit model of evolution was selected by jmodeltest 2.1.4 (Darriba et al., 2012). The best suitable model under the Akaike information criterion (Akaike, 1992) was GTR + I (I = 0.614) for the first dataset and HKY for the second dataset. Only distinct haplotypes were used for phylogenetic analyses on both datasets using MrBayes 3.2 (Ronquist and Huelsenbeck, 2003). For both datasets, two parallel Markov chain Monte Carlo (MCMC) processes were run with four chains for 2,000,000 generations and sampled every 100 generations. The first 25% of trees were discarded as burn-in after checking the stationary using TRACER1.4.1 (Rambaut and Drummond, 2007). Phylogenetic relationships were visualized with FigTree 1.4.0 (Rivera et al., 2004). Relationships among haplotypes could be more intuitively and accurately visualized through networks. All populations of *Aurelia* sp.1 were used to construct networks with Network 4.6 (http://www.fluxus-engineering.com/) using the Median-joining method under default settings (Bandelt et al., 1999).

Genetic differentiation could be qualified by pairwise ϕ_{st} values. The TrN model was used with 10,000 permutations (Tamura and Nei, 1993). The relative proportion of variation within and between populations was obtained through the analysis of molecular variance (AMOVA). Both values were calculated by the program Arlequin 3.5 (Excoffier and Lischer, 2010). A spatial analysis of molecular variance (SAMOVA) was performed to further test for population structure (Dupanloup et al., 2002). Monmonier's maximum difference algorithm was then used to identify putative genetic barriers to gene flow across the oceanographic landscapes (Monmonier, 1973). The correlation between geographic and genetic distances was tested by a Mantel test with 1000 permutations using the Allele in Space 1.0 software (Miller, 2005).

The past demographic expansions were detected by the neutrality statistics Fu's Fs (Fu, 1997) and Ramos-Onsins and Rozas's R_2 (Ramos-Onsins and Rozas, 2002). Fu's Fs and R_2 have been suggested as the most powerful tests for detecting sudden population growth or contractions. All the neutrality statistics were calculated using DnaSP 5.0.

3. Results

3.1. Genetic variability

A total of 103 COI sequences revealed 19 haplotypes defined by 12 polymorphic sites, of which 8 were parsimony informative sites. The haplotype diversity (h) and nucleotide diversity (π) were calculated across geographic regions (Table 2). Overall nucleotide diversity in *Aurelia* sp.1 was $\pi = 0.0041$, while the corresponding haplotype diversity was $h = 0.66$. Across all samples, $\pi = 0.0034–0.0046$ and $h = 0.44–0.71$. The highest haplotype diversity was calculated in YT (0.713) and the lowest in RZ (0.436). The highest nucleotide diversity was calculated in RC (0.458) and the lowest in RZ (0.340).

Table 1
Related sequences from GenBank.

Species	Isolation locality	GenBank number
Aurelia aurita	Turkey: Bosporus	KC789082
Aurelia labiata	USA: Tomales Bay, California	AY903077
Aurelia limbata	Japan: Hokkaido	AY903189
Aurelia sp.1	USA: Marina del Rey, Los Angeles, California	AY903078
Aurelia sp.1	USA: Marina del Rey, Los Angeles, California	AY903079
Aurelia sp.1	USA: Marina del Rey, Los Angeles, California	AY903080
Aurelia sp.1	USA: Marina del Rey, Los Angeles, California	AY903081
Aurelia sp.1	USA: Long Beach, Los Angeles, California	AY903083
Aurelia sp.1	USA: Long Beach, Los Angeles, California	AY903084
Aurelia sp.1	USA: Long Beach, Los Angeles, California	AY903085
Aurelia sp.1	USA: Newport Beach, Los Angeles, California	AY903086
Aurelia sp.1	USA: Newport Beach, Los Angeles, California	AY903087
Aurelia sp.1	USA: Newport Beach, Los Angeles, California	AY903088
Aurelia sp.1	USA: Newport Beach, Los Angeles, California	AY903185
Aurelia sp.1	USA: San Diego, Los Angeles, California	AY903090
Aurelia sp.1	USA: San Diego, Los Angeles, California	AY903091
Aurelia sp.1	USA: San Diego, Los Angeles, California	AY903092
Aurelia sp.1	Australia: Mooloolaba Harbour, Queensland	AY903167
Aurelia sp.1	Australia: Mooloolaba Harbour, Queensland	AY903128
Aurelia sp.1	Australia: Greys Point, Port Hacking, New South Wales	AY903142
Aurelia sp.1	Australia: Coila Lake, New South Wales	AY903147
Aurelia sp.1	Australia: Coila Lake, New South Wales	AY903148
Aurelia sp.1	Australia: Coila Lake, New South Wales	AY903149
Aurelia sp.1	Australia: Coila Lake, New South Wales	AY903150
Aurelia sp.1	Australia: Lake Illawarra, New South Wales	AY903153
Aurelia sp.1	Australia: Lake Illawarra, New South Wales	AY903154
Aurelia sp.1	Australia: Tuggerah Lake, New South Wales	AY903155
Aurelia sp.1	Australia: Tuggerah Lake, New South Wales	AY903157
Aurelia sp.1	Australia: Tuggerah Lake, New South Wales	AY903158
Aurelia sp.1	Australia: Tuggerah Lake, New South Wales	AY903159
Aurelia sp.1	Australia: Tuggerah Lake, New South Wales	AY903160
Aurelia sp.1	Australia: Lake Macquarie, New South Wales	AY903161
Aurelia sp.1	Australia: Lake Macquarie, New South Wales	AY903162
Aurelia sp.1	Australia: Lake Macquarie, New South Wales	AY903163
Aurelia sp.1	Australia: Lake Macquarie, New South Wales	AY903164
Aurelia sp.1	Australia: Lake Macquarie, New South Wales	AY903165
Aurelia sp.1	Australia: Lake Macquarie, New South Wales	AY903166
Aurelia sp.1	Australia: Darling Harbour, New South Wales	AY903143
Aurelia sp.1	Australia: Millers Point, New South Wales	AY903130
Aurelia sp.1	Australia: Millers Point, New South Wales	AY903131
Aurelia sp.1	Australia: Port Jackson, New South Wales	AY903181
Aurelia sp.1	Australia: Port Jackson, New South Wales	AY903182
Aurelia sp.1	Australia: Port Jackson, New South Wales	AY903183
Aurelia sp.1	Australia: Huon Estuary, Tasmania	AY903151
Aurelia sp.1	Australia: Perth, Western Australia	AY903126
Aurelia sp.1	Australia: Perth, Western Australia	AY903127
Aurelia sp.1	Australia: Perth, Western Australia	AY903177
Aurelia sp.1	Australia: Perth, Western Australia	AY903178
Aurelia sp.1	Australia: Perth, Western Australia	AY903180
Aurelia sp.1	Japan: Miyazu Bay, Honshu	AY903168
Aurelia sp.1	Japan: Miyazu Bay, Honshu	AY903169
Aurelia sp.1	Japan: Miyazu Bay, Honshu	AY903170
Aurelia sp.1	Japan: Sakata Bay, Honshu	AY903186
Aurelia sp.1	Japan: Sakata Bay, Honshu	AY903187
Aurelia sp.1	Japan: Sakata Bay, Honshu	AY903188
Aurelia sp.1	Japan: Tokyo Bay, Honshu	AY903203
Aurelia sp.1	Japan: Tokyo Bay, Honshu	AY903204
Aurelia sp.1	Japan: Tokyo Bay, Honshu	AY903205
Aurelia sp.1	Japan: Tokyo Bay, Honshu	AY903206
Aurelia sp.1	Japan: Tokyo Bay, Honshu	AY903116
Aurelia sp.1	Japan: Uwa Bay, Inland Sea	AY903192
Aurelia sp.1	Japan: Ondo Strait, Inland Sea	AY903196
Aurelia sp.1	South Korea: coastal region of Incheon	EU010386
Aurelia sp.1	South Korea: Busan	EU366143
Aurelia sp.1	South Korea: Geoje-do	EU366144
Aurelia sp.2	Brazil: Cananeia, Sao Paulo	AY903121
Aurelia sp.3	Palau: Koror State	AY903096
Aurelia sp.4	Indonesia: Kakaban Island, Berau	AY903145
Aurelia sp.5	Croatia: Veliko Jezero, Mljet	AY903123
Aurelia sp.6	Palau: Ngell Channel	AY903099

Table 1 (*continued*)

Species	Isolation locality	GenBank number
Aurelia sp.7	Australia: Huon Estuary, Tasmania	AY903138
Aurelia sp.8	Croatia: Bay of Ston	AY903135
Aurelia sp.9	USA: Gulf of Mexico, Alabama	AY903172
Aurelia sp.10	USA: Kachemak Bay, Alaska	AY903067

3.2. Phylogenetic analysis

A total of 13 distinct clades including *A. limbata*, *Aurelia labiate*, *A. aurita* and ten other *Aurelia* spp. were detected in an unrooted Bayesian tree (Fig. 2). All the samples collected in Chinese coastal waters clustered together with the specimens collected from Japanese, Korean, American and Australian waters that were identified as *Aurelia* sp.1 (Dawson and Jacobs, 2001; Schroth et al., 2002; Dawson, 2005; Ki et al., 2008).

A phylogenetic tree was generated using Bayesian analysis for all haplotypes based on global *Aurelia* sp.1 mtCOI sequences (Fig. 3). The phylogenetic analysis revealed several well supported groups. Hap 35 and eleven additional haplotypes formed a strongly supported clade (posterior support = 100%), whereas Hap 39 and eight additional haplotypes formed a second well supported clade (posterior support = 93%). However, there was no geographical association of haplotypes in the two well supported groups.

TCA analysis of global *Aurelia* sp.1 generated an eight step statistical parsimony network connecting all 39 haplotypes (Fig. 4). In total, there were nine haplotypes (Hap 2, Hap 4, Hap 6, Hap 9, Hap 24, Hap 25, Hap 26, Hap 27, and Hap 30) that shared more than one geographical region (Fig. 4): Hap 25 and Hap 26 were shared by all six Chinese populations; Hap 4 was shared by the QD, American and Australian populations; Hap 9 was shared by the CFD, Australian and Japanese populations; Hap 2 was shared by the American and Australian populations; Hap 6 was shared by the Australian and Japanese populations; Hap 24 was shared by the RC and Japanese populations; Hap 27 was shared by the CFD and WF populations; and Hap 30 was shared by the QD and RC populations.

3.3. Population genetic differentiation

The genetic distance calculated using the Tamura-Nei model among the 39 haplotypes ranged from 0.002 to 0.016 with an average value of 0.008. Based on the sequence distances derived using the Tajima and Nei method, the AMOVA test showed that 92.82% of the genetic variation occurred within populations ($P < 0.05$), whereas 6.59% of the genetic variation occurred among populations within regions and 0.59% occurred among regions (Table 3).

Table 4 shows the pattern of genetic differentiation among populations observed by mean pairwise F_{ST}. These results indicated that the YT, RC and QD populations were significantly differentiated from the other two Chinese populations (WF and RZ) (F_{ST} range: 0.1698–0.2465, $P < 0.05$). However, the Mantel test showed that no significant correlation between genetic and geographic distances was found among Chinese populations ($r = 0.0053$, $p = 0.38$). The YT, RC and QD populations were also significantly differentiated from the Japanese population (F_{ST} range: 0.1585–0.1902, $P < 0.05$). However, no significant genetic differentiation was detected among CFD, RZ, WF and the Japanese populations. All Chinese and Japanese populations were significantly differentiated from the American and Australian populations (F_{ST} range: 0.2666–0.7328, $P < 0.01$). These results were further identified by SAMOVA analysis which recognized Chinese and Japanese populations as one group, American, Australian and Korean populations as the second group ($K = 2$, $F_{CT} = 0.4493$, $p = 0.011$). Fu's Fs test for the entire region was statistically significant negative (-5.352, $p < 0.01$). Meanwhile, a low value of the R_2 statistics (0.068) also indicated that the *Aurelia* sp.1 populations might have experienced population expansion.

4. Discussion

Recent genetic studies on moon jellyfish have reported high levels of intraspecific diversity, and at least 13 cryptic species have been revealed (Dawson and Jacobs, 2001; Schroth et al., 2002; Dawson, 2005; Ki et al., 2008). Among these species, *Aurelia* sp.1, *Aurelia* sp.4 and *Aurelia* sp.8 were thought to be introduced species (Dawson, 2005). In the present study, sequence analysis of 103 specimens from six localities revealed that a single cryptic species (*Aurelia* sp.1) was present in our collections. In previous studies, *Aurelia* sp.1 was also identified in Korea, Japan, California, Australia and the Mediterranean coast of France (Dawson and Jacobs, 2001; Dawson and Martin, 2001; Schroth et al., 2002; Dawson, 2005; Ki et al., 2008). The ocean model showed limited dispersion in the 1-year lifespan of medusa among Japanese, Australian and North American waters; therefore, the global distribution of *Aurelia* sp.1 in Australia and America is most likely due to anthropogenic translocation (Dawson, 2005). Moreover, the latitudinal range of *Aurelia* sp.1 distribution in these coastal regions was similar. Such a large geographic range that includes disjunct populations suggests that *Aurelia* sp. 1 may be an introduced species that is adapted to survive in warm-temperate seaports or adjunct seawaters (Dawson, 2005).

The genetic diversity of mtDNA COI sequences in *Aurelia* sp.1 in Chinese coastal waters (China: $h = 0.66$; $\pi = 0.0020$; $n = 103$) was lower than previously found in a Japanese population ($h = 0.87$; $\pi = 0.0063$; $n = 26$) (Dawson, 2005). Reduced genetic diversity was also reported in Australia (Australia: $h = 0.66$; $\pi = 0.0048$; $n = 37$) and California (California: $h = 0.53$;

Table 2
Genetic diversity of mitochondrial COI sequences in *Aurelia* sp.1 according to geographic region.

Geographic regions	Specimens (no.)	Haplotypes (no.)	Haplotype diversity $h \pm$ SE	Nucleotide diversity $\pi \pm$ SE (%)
CFD	26	7	0.692 ± 0.062	0.451 ± 0.030
WF	16	3	0.542 ± 0.098	0.377 ± 0.074
YT	17	7	0.713 ± 0.109	0.431 ± 0.079
RC	18	8	0.641 ± 0.130	0.458 ± 0.088
QD	15	4	0.543 ± 0.133	0.407 ± 0.094
RZ	11	2	0.436 ± 0.133	0.340 ± 0.104
Total	103	19	0.662 ± 0.034	0.446 ± 0.017

$\pi = 0.0020$; $n = 16$) compared to Japan. In this study, phylogenetic analysis based on global *Aurelia* sp.1 mtDNA COI haplotypes revealed that the Japanese haplotypes were distributed throughout the total tree (Fig. 3). These results suggested that *Aurelia* sp.1 might have dispersed globally from Japanese coastal waters.

A few studies have addressed the population genetic structure in scyphozoans, suggesting that both different reproductive strategies and dispersal ability may attribute to the population genetic structure (Stopar et al., 2010; Ramšak et al., 2012; Lee et al., 2013). In general, holopelagic scyphozoans with high dispersal potential showed genetic homogeny over large geographical distances (Stopar et al., 2010). In contrast, the increased genetic diversity observed for meroplanktonic scyphozoans may be closely linked to the benthic phase (Gibbons et al., 2010; Lee et al., 2013). For example, the holopelagic scyphozoan *Pelagia noctiluca* showed a lack of genetic structure among Mediterranean and East Atlantic populations (Stopar et al., 2010), while significant genetic structures distinguishing three populations in the meroplanktonic scyphozoan jellyfish *Rhizostoma octopus* were revealed in the Irish Sea and northeastern Atlantic (Lee et al., 2013). Similarly, phylogeographic analyses confirmed the separation of three *Aurelia* spp. in the Mediterranean Sea (Ramšak et al., 2012). However, no significant genetic differentiation was detected in *Rhizostoma pulmo* in the Mediterranean Sea. One possible explanation is that the dispersal ability (including dispersal with ocean currents and active swimming) might differ between *R. pulmo* and *Aurelia* spp. Horizontal directional swimming has been observed in different scyphozoans (Albert, 2011).

Complex life-history characteristics and habitat fragmentation may be important factors for these high levels of genetic differentiation in meroplanktonic scyphozoans. Schroth et al. (2002) indicated that two important life cycle traits (strobilation frequency and temperature for strobilation onset) might coincide with the genetic differentiation of *Aurelia* spp.

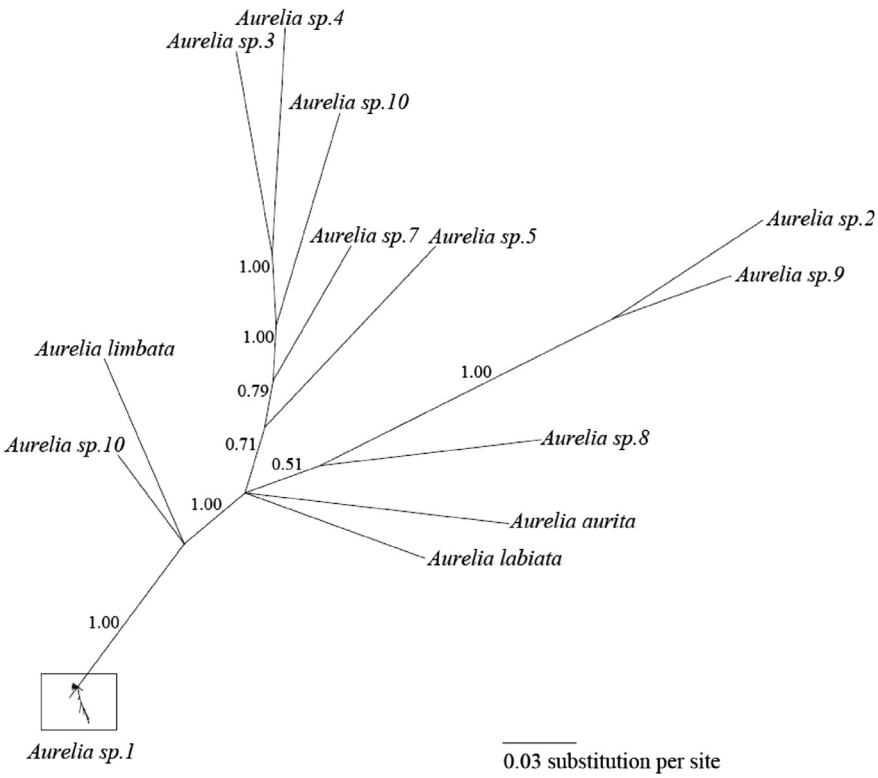

Fig. 2. Unrooted Bayesian trees showing the relationships of *Aurelia* sp.1.

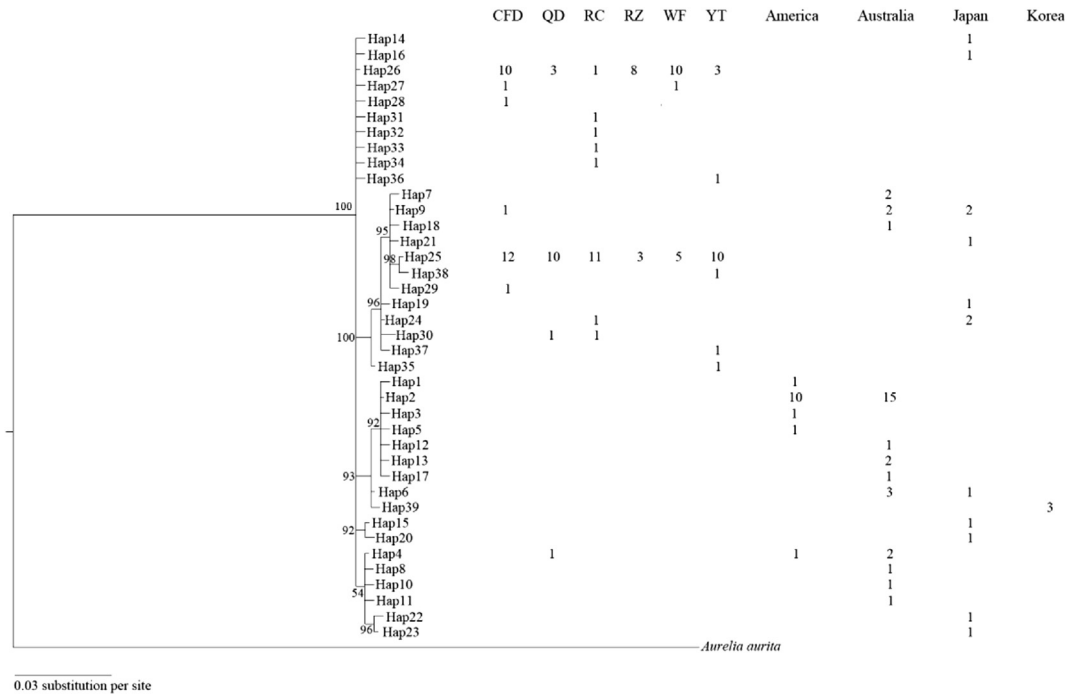

Fig. 3. Phylogenetic relationships within *Aurelia* sp.1 derived by Bayesian inference based on mtDNA sequences under the HKY model. Numbers at nodes indicate posterior probabilities. The distribution of haplotypes among populations is also presented.

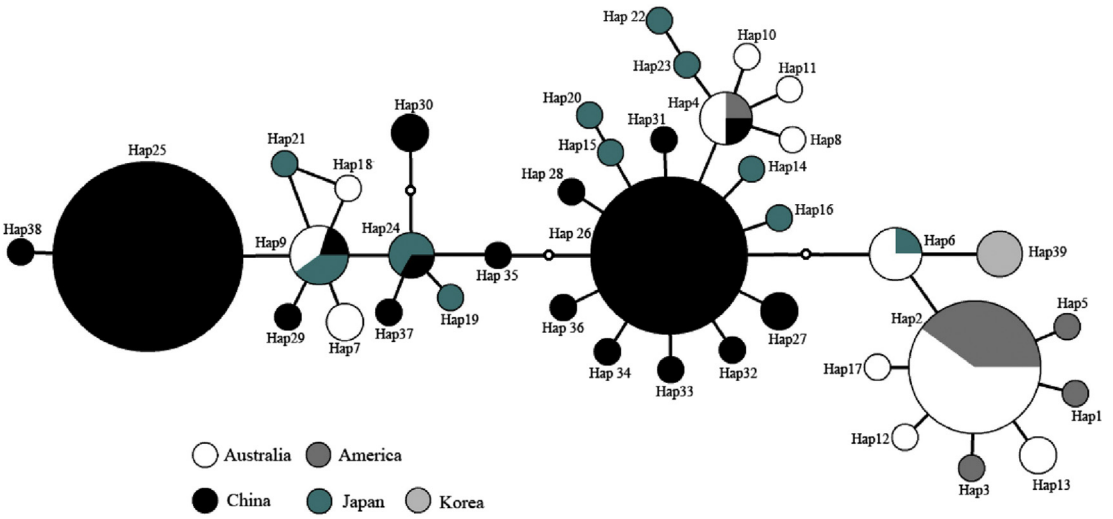

Fig. 4. Median-joining networks for all *Aurelia* sp.1 COI haplotypes. The color of the circle indicates the geographic region, and the size of the circle indicates the haplotype frequency. Each branch between any two shapes represents a single nucleotide substitution.

Table 3
Hierarchical analysis of molecular variance (AMOVA) of mtCOI haplotypes of *Aurelia* sp.1.

Source of variation	d.f.	Variance component	Percentage of variation	φ Statistic	*P* value
Among regions	2	0.00865	0.59	$\varphi_{CT} = 0.00592$	0.33376
Among populations within regions	3	0.0963	6.59	$\varphi_{SC} = 0.06626$	0.07337
Within populations	97	1.35711	92.82	$\varphi_{ST} = 0.07178^{*}$	0.02891
Total	102	1.46206	100		

Structure tested: Region 1 (Caofeidian and Weifang); Region 2 (Yantai and Rongcheng); Region 3 (Qingdao and Rizhao). Asterisks indicate significant level. * $p < 0.05$; ** $p < 0.01$.

Table 4
Fst analysis among geographical populations of *Aurelia* sp.1

Population	America	Australia	CFD	QD	RC	RZ	WF	YT	Japan
America									
Australia	0.0800								
CFD	0.6395**	0.3962**							
QD	0.7328**	0.4817**	0.0166						
RC	0.6982**	0.4647**	0.0080	−0.0561					
RZ	0.6547**	0.2944**	0.0545	0.2402*	0.1999*				
WF	0.6302**	0.3143**	0.0345	0.2026*	0.1698*	−0.0772			
YT	0.7325**	0.4883**	0.0218	−0.0551	−0.0439	0.2465*	0.2087*		
Japan	0.5430**	0.2666**	0.0753	0.1827*	0.1585*	0.0085	0.0217	0.1906*	

Fst values are calculated from genetic divergence data among haplotypes calculated with the method of Tajima and Nei (1984). Asterisks indicate significant level. * $p < 0.05$; ** $p < 0.01$. Probability *P* was calculated from 1000 replications.

populations. High pairwise F_{ST} values over short geographic distances (100 km) were observed between the QD and RZ populations. These results indicate that dispersal of *Aurelia* sp.1 between QD and RZ is limited. Similarly, two *Chlamys farreri* populations (RZ and QD) were also revealed to be genetically divergent (Zhan et al., 2009). Previous study suggest that habitat fragmentation formed by marine gyres and currents is much competent for the explanation of genetic differentiation for a fine geographical scale than isolation by distance (e.g., Launey et al., 2002; Zhan et al., 2009).

However, non-significant pairwise comparisons of the F_{ST} values were also found over both short geographic distances (i.e., YT, RC, and QD) and large geographic distances (i.e., RZ and Japan, WF and Japan, and CFD and RZ). Previous studies indicated that the absence of isolation by distance might be caused by a recent colonization event or by long-distance dispersal (Slatkin, 1993; Palumbi, 2003). The pelagic *Aurelia* spp. (ephyrae and adult medusa) occurred from April to October (Dong et al., 2012; Wan and Zhang, 2012). Therefore, the coastal currents in this region may play an important role in transporting planktonic medusa. In the summer, the Lubei coastal currents flow in this region (Su and Yuan, 2005) and may potentially enhance the dispersal of *Aurelia* sp.1 along coastal waters of the Jiaodong Peninsula (i.e., YT, RC, and QD). The pelagic medusa of *Aurelia* spp. are typically found in near-shore waters and shallow estuaries and rarely in deep waters, suggesting limited dispersal for this species (i.e., Dong et al., 2014). Therefore, long distance dispersal from Japan to coastal waters of the Bohai Sea and Yellow Sea might be limited. Thus, the identification of *Aurelia* sp.1 in RZ and WF that are genetically identical to those found in Japan is most likely due to anthropogenic translocation.

In conclusion, our results revealed that all *Aurelia* spp. samples collected in Chinese coastal waters were charactered as a single cryptic species (*Aurelia* sp.1), authough 13 cryptic species of *Aurelia* spp. have been revealed in the previous study. We also demonstrated a complex genetic population structure of *Aurelia* sp.1 in Chinese coastal waters. The phylogeographic patterns of *Aurelia* sp.1 in Chinese coastal waters were in relation to habitat fragmentation separated by marine gyres and currents, complex life-history characteristics of *Aurelia* sp.1, and possible anthropogenic introduction.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2015.02.018.

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