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ORIGINAL ARTICLE

Low genetic diversity and lack of genetic structure in the giant jellyfish *Nemopilema nomurai* in Chinese coastal waters

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ABSTRACT
The giant jellyfish *Nemopilema nomurai* is a scyphozoan species well-known in East Asian Marginal Seas for its damage to fisheries. The genetic diversity and population structure of *N. nomurai*, collected from five geographic regions in Chinese coastal seas, were examined based on mitochondrial cytochrome *c* oxidase subunit I (COI) gene and nuclear internal transcribed spacer one (ITS1) sequences. A total of 26 and five unique haplotypes were recovered from the COI and ITS1 genes, respectively. The overall genetic diversity of *N. nomurai* calculated by the COI and ITS1 sequences was low (haplotype diversity 0.727% and 0.108%, nucleotide diversity 0.212% and 0.039%). The median-joining network analysis revealed a star-like haplotype network. The hierarchical Analysis of Molecular Variance (AMOVA) of COI haplotypes showed that *N. nomurai* populations form a single population, with a low F_{ST} (0.0149, $p = 0.1036$). The dispersal ability, together with the biological characteristics, could be important factors for the lack of a geographically structured pattern in *N. nomurai* in Chinese coastal waters.

Introduction

Nemopilema nomurai Kishinouye, 1922 (Cnidaria: Scyphozoa: Rhizostomeae) is primarily distributed in the East Asian Marginal Seas around China, Korea and Japan (Omori & Kitamura 2004). Since the late 1990s, blooms of the large jellyfish *N. nomurai* have frequently occurred in the Bohai Sea, Yellow Sea and the northern area of the East China Sea (Cheng et al. 2004); such scenarios were also reported in Japanese and Korean waters (Kawahara et al. 2006; Uye 2014). The blooms of *N. nomurai* have caused serious damage to local fisheries in the East Asian Marginal Seas (Uye 2014). Moreover, the giant jellyfish *N. nomurai* is also responsible for most severe or fatal cases of jellyfish stings in Chinese seas (Dong et al. 2010).

In general, marine jellyfish species with good dispersal abilities should show little or no genetic structure over large ranges in the ocean (Stopar et al. 2010). However, species with metagenetic life histories may show increased genetic structure (Gibbons et al. 2010). For example, species of Semaeostomeae with both pelagic and benthic stages have been suggested

to show more geographically structured populations (Schroth et al. 2002; Dawson 2003). Population genetic analyses can yield valuable insights into biogeographic patterns by providing evidence of geographic dispersal barriers, directionality of migration or dispersal events, and the degree of gene flow among extant populations (Hardy et al. 2011). In recent years, some population genetic studies have been carried out on scyphozoans such as *Aurelia* sp., *Rhizostoma pulmo* (Macri, 1778) and *Pelagia noctiluca* (Forsskål, 1775), based on the mitochondrial COI gene and other genes (Ki et al. 2008; Stopar et al. 2010; Miller et al. 2012; Ramšak et al. 2012; Dong et al. 2015). For example, a recent genetic study using the mitochondrial COI control region and the nuclear ITS gene from the Atlantic scyphozoan *P. noctiluca* demonstrated significant population genetic structure between the South African and northern Atlantic populations (Miller et al. 2012). The global phylogeny of the genus *Aurelia* revealed 13 cryptic species, and most of them appear to be geographically restricted (Ki et al. 2008). The European scyphozoans *Aurelia* spp. and *R. pulmo* did not have concordant phylogeographic patterns (Ramšak et al. 2012). No significant genetic

structure was detected in *R. pulmo* across the Mediterranean Sea. In contrast, several proposed cryptic species of *Aurelia* spp. have been confirmed based on phylogeographic analyses in European seas (Ramšak et al. 2012).

The giant jellyfish *N. nomurai* has a metagenetic life history that involves a benthic asexual polyp phase and a pelagic sexual medusa stage (Arai 1997). Previous studies indicated that the ephyrae are released into the plankton from the benthic polyps during spring to early summer (Toyokawa et al. 2012; Sun et al. 2015). The ephyrae of *N. nomurai*, 1–2 mm in diameter, have been found in the southern Yellow Sea and northwestern East China Sea during May (Toyokawa et al. 2012; Sun et al. 2015). They then spend their planktonic life as medusae until they die in winter (Zhang et al. 2012; Sun et al. 2015). However, the genetic diversity and population genetic structure of the giant jellyfish *N. nomurai* have not been resolved. A recent genetic study revealed that populations of *Aurelia* sp. 1 were highly structured between most sampling sites in Chinese coastal waters (Dong et al. 2015). We hypothesize that similar genetic patterns occur in *N. nomurai* in the same area. Therefore, the giant jellyfish *N. nomurai* was sampled from five different localities, with the aim of this study being to reveal the genetic diversity and population genetic differentiation of *N. nomurai* in Chinese coastal waters, using mitochondrial cytochrome oxidase subunit one (COI) and nuclear ribosomal internal transcribed spacer one (ITS1) sequences.
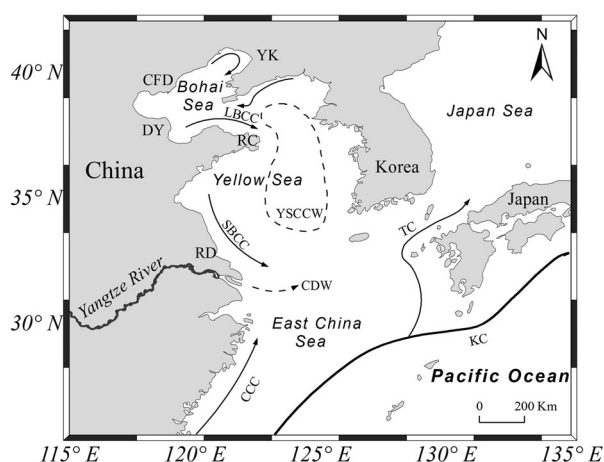

Figure 1. Sampling sites of *Nemopilema nomurai* in Chinese coastal waters. Abbreviation IDs for geographic regions: YK, Yingkou; CFD, Caofeidian; DY, Dongying; RC, Rongcheng; RD, Rudong. Abbreviation IDs for currents: LBCC, Lubei Coastal Current; SBCC, Subei Coastal Current; CCC, China Coastal Current; CDW, Changjiang diluted water; TC, Tsushima Current; KC, Kuroshio Current; YSCCW, Yellow Sea central cold water.

Materials and methods

Sample collection

The giant jellyfish *Nemopilema nomurai* mostly occurs north of 30°N (Dong et al. 2010; Sun et al. 2015). During local jellyfish blooming time in 2013, a total of 150 individuals of *N. nomurai* were collected from five localities along the coast of the Bohai Sea and Yellow Sea: (1) the Bohai Sea region including localities near Yingkou (YK), Caofeidian (CFD), and Dongying (DY); (2) the Yellow Sea region including localities near Rongcheng (RC) and Rudong (RD) (Figure 1). Individuals were collected using a hand net. Medusae tissue, extracted from the bell margin or gonads, was preserved in 95% ethanol and then stored at −20°C until DNA extraction.

DNA extraction, PCR amplification, sequencing and alignment

Total genomic DNA was extracted using the TIANamp Marine Animals DNA Kit (TIANGEN, China). The mitochondrial COI fragments were amplified using the universal primers LCO1490 and HCO2198, under the polymerase chain reaction (PCR) conditions previously described (Folmer et al. 1994). Internal transcribed spacer one (ITS1) regions were amplified using the primer pair ITS1 and ITS2 (White et al. 1990). 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). The temperature profile for the COI region 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 temperature profile for the ITS1 region was defined as follows: 95°C for 3 min; 35 cycles of denaturation at 95°C for 60 s, annealing at 48°C for 60 s and extension at 72°C for 90 s; followed by a final extension at 72°C for 1 min. The PCR products were examined on 1% agarose gels, stained with Genecolour™ (Biotium, USA), and photographed with transmitted illumination.

PCR-amplified DNA fragments were purified using the DNA Gel Extraction Kit (Sangon, China) 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. DNAMAN V6 (Lynnon BioSoft, Canada) was used to assemble and edit the contigs using the

Table I. Genetic diversity of mitochondrial COI and nuclear ITS1 sequences in *Nemopilema nomurai* in Chinese coastal waters.

Geographic region	COI				ITS1			
	No. of sequences	No. of haplotypes	Haplotype diversity h	Nucleotide diversity π (%)	No. of sequences	No. of haplotypes	Haplotype diversity h	Nucleotide diversity π (%)
CFD	29	11	0.727	0.182	19	1	0	0
DY	26	10	0.818	0.248	20	4	0.284	0.118
RC	22	8	0.654	0.157	24	1	0	0
RD	27	12	0.735	0.219	23	3	0.245	0.077
YK	28	9	0.669	0.238	22	1	0	0
Total	132	26	0.727	0.212	108	5	0.108	0.039

coelenterate mitochondrial code to verify the correctness of the COI sequence alignment at the amino acid level. The sequences were aligned with MEGA 5.0 (Tamura et al. 2011). The COI sequence of *Aurelia* sp. 1 (KP692786) was used as an outgroup.

Data analyses

The nucleotide composition and variable sites were analysed in MEGA 5.0. The genetic diversity indices for mtDNA and ITS (nucleotide diversity π and haplotype diversity h) were calculated using DnaSP 5.0 (Librado & Rozas 2009). Networks are useful tools to study intraspecific phylogenetic relationships (Bandelt et al. 1999). All COI haplotype sequences were included in network analyses. The networks were constructed using the program Network 4.6 (http://www.fluxus-engineering.com/) and the median-joining method (Bandelt et al. 1999) under default settings. Analysis of Molecular Variance (AMOVA) using the Tamura–Nei model (Tamura & Nei 1993) was conducted to measure the proportion of variation within and between the two regions (Bohai Sea and Yellow Sea) for the COI mtDNA haplotypes. Genetic differentiation was examined by means of pairwise Φst values, using 10,000 permutations to determine significance in Arlequin 3.5 (Excoffier & Lischer 2010).

Results

Genetic diversity

In total, the aligned sequence length of the mitochondrial COI sequences for 132 *Nemopilema nomurai* was 610 bp (GenBank Accession numbers: KU360807–KU360832; Table SI, supplementary material). The aligned sequence length of the nucleotide ITS1 sequences for 108 *N. nomurai* was 329 bp (KU363837–KU363841; Table SI). In total, 26 unique haplotypes were identified among the COI sequences that contained 28 polymorphic sites, of which 19 were parsimony informative. However, only five unique haplotypes were revealed by sequencing

the ITS1 region, defined by three segregating sites, of which two were parsimony-informative.

The haplotype diversity (h) and nucleotide diversity (π) across geographic regions are presented in Table 1. The haplotype diversity calculated from the COI and ITS1 sequences for the five sampling areas combined were 0.727 and 0.108, respectively. The sample from RC showed the lowest number of COI haplotypes (eight) and the lowest COI haplotype diversity (0.654), while the sample from DY possessed the highest COI haplotype diversity (0.818). From the 26 haplotypes defined by the COI sequences, only one haplotype (Hap 3) was found in all sampled areas, and 12 were restricted to one population: five in RD, two in YK, two in CFD, two in DY and one in RC. Haplotype 3 was the most common, accounting for over 51% of the haplotypes identified from all sampled areas (Figure 2). The nucleotide diversity calculated for the COI and ITS1 sequences from the five sampling areas combined were 0.212 and 0.038, respectively (Table I). The highest COI nucleotide diversity was calculated for DY (0.248) and the lowest COI nucleotide diversity for RC (0.157). Overall, the nucleotide diversities calculated from the COI sequences and the ITS1 region were much lower than in other scyphozoan medusae (Miller et al. 2012; Ramšak et al. 2012).

Population genetic structure

Because very few ITS1 region haplotypes were identified, the network analyses for *Nemopilema nomurai* was generated with all the mtCOI haplotype sequences. The median-joining network analysis revealed a star-like haplotype network (Figure 2). The most frequent haplotype (Hap 3), shared by all geographic regions, was inferred to be the ancestral haplotype, whereas the most derived ones are linked to this haplotype with a maximum branch length of two mutational steps.

Based on the sequence distances derived from the Tamura & Nei (1993) method, the AMOVA test showed that 98.51% of the genetic variation occurred within populations, whereas 0.79% occurred among regions
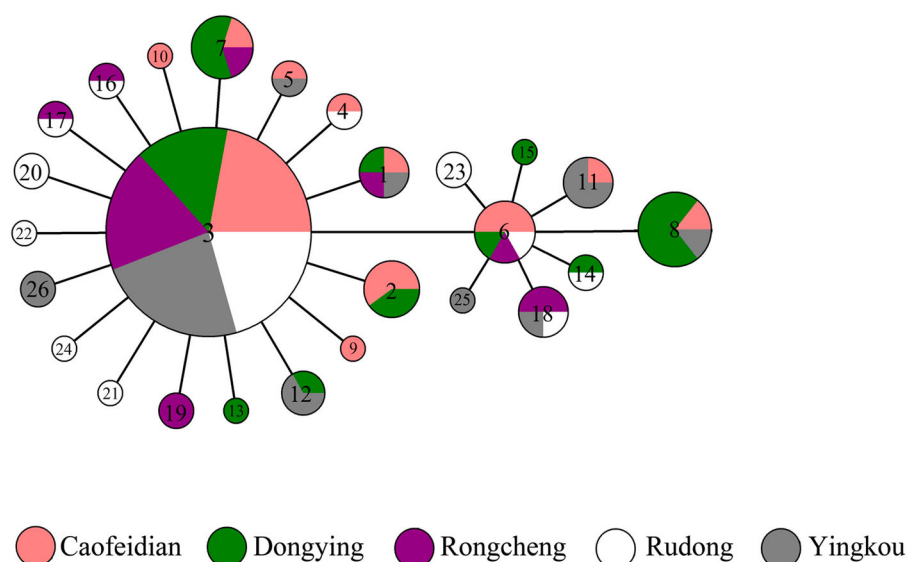
Figure 2. Median-joining network for all *Nemopilema nomurai* COI haplotypes. The colour of the circle indicates the geographic region, and the size of the circle indicates the haplotype frequency.

Table II. Hierarchical Analysis of Molecular Variance (AMOVA) of mitochondrial COI haplotypes of *Nemopilema nomurai*.

Source of variation	d.f.	Variance component	Percentage of variation	φ-statistics	P-value
Among regions	1	0.0058	0.79	$\varphi_{CT} = 0.0079$	0.2061
Among populations within regions	3	0.0051	0.70	$\varphi_{SC} = 0.0071$	0.2357
Within populations	127	0.7205	98.51	$\varphi_{ST} = 0.0149$	0.1036
Total	131	0.7314	100		

Structure tested: Region 1 (Bohai Sea: Yingkou, Caofeidian and Dongying); Region 2 (Yellow Sea: Rongcheng and Rudong).

Table III. Pairwise F_{ST} values in *Nemopilema nomurai* between geographic regions based on 10000 permutations.

	CFD	DY	RC	RD	YK
CFD	–	0.023	0	0.019	0
DY	0.021	–	0.038	−0.025	0.032
RC	0.008	0.026	–	0.033	0
RD	−0.011	0.019	−0.020	–	0.028
YK	−0.002	0.036	−0.006	−0.031	–

Below diagonal: pairwise values for COI; above diagonal: pairwise values for ITS1.

(Table II). Thus, the AMOVA revealed no genetic differentiation between the two regions (Bohai Sea and Yellow Sea) tested ($F_{CT} = 0.0079$; $P > 0.05$) and among populations in the total sample ($F_{ST} = 0.0149$; $P > 0.05$). Pairwise F_{ST} comparisons calculated from COI and ITS1 sequences showed no significant differentiation among all five localities along the coast of the Bohai and Yellow Seas (P-value from 0.061 to 0.593 for COI; P-value from 0.207 to 1.000 for ITS1) (Table III).

Discussion

Both oceanographic features and life-history characteristics influence the genetic structure of marine species, although the relative roles these factors play in shaping phylogeographic patterns remain controversial (Neethling et al. 2008). In addition to oceanographic currents, genetic differentiation may also be attributable to environmental heterogeneity that affects the survival or genetic drift of benthic polyps (Schroth et al. 2002; Dawson et al. 2015). Relatively few studies have been conducted to address the population genetics of blooming scyphozoan species (Dawson 2005; Stopar et al. 2010; Lee et al. 2013; Miller et al. 2012; Ramšak et al. 2012; Dawson et al. 2015; Dong et al. 2015; Glynn et al. 2016). Holoplanktic scyphozoan species seem to have little or no genetic structure over large stretches of ocean. For example, no strongly supported genetically or geographically distinct groups of *Pelagia noctiluca* were found across European seas (Stopar et al. 2010). A recent study using nuclear microsatellite markers and mitochondrial COI gene sequences revealed a high degree of connectivity in *P. noctiluca* across the northeast Atlantic Ocean and Mediterranean Sea (Glynn et al. 2016). However, significant population genetic structure was observed on a wider spatial scale between the South African and northern Atlantic *P. noctiluca* populations (Miller et al. 2012). Significant population differentiation or cryptic

species has been revealed for meroplanktonic scyphozoans in some studies (Dawson 2005; Lee et al. 2013; Ramšak et al. 2012). For example, two reciprocally monophyletic clades of *Catostylus mosaicus* (Quoy & Gaimard, 1824), which were geographically proximal to a provincial zoogeographic boundary near the Bass Strait, were distinguished based on mitochondrial COI (Dawson 2005). Similarly, significant genetic structure that distinguishes three populations of *Rhizostoma pulmo* (Macri, 1778) in the Irish Sea and northeastern Atlantic was also revealed, although this is not entirely consistent with prevailing physical oceanographic patterns (Lee et al. 2013). The phylogenetic study of scyphozoan *Aurelia* spp. showed significant geographically structured populations, and many cryptic species have been identified (Dawson 2003; Ki et al. 2008; Ramšak et al. 2012; Dong et al. 2015).

Nemopilema nomurai is endemic to the East Asian Margin waters around China, Korea and Japan (Omori & Kitamura 2004). The result of our analyses indicates that the scyphozoan *N. nomurai* is scattered in the Chinese coastal waters, including the Bohai Sea and Yellow Sea, with no significant geographical structure. The lack of geographically restricted groups indicated high levels of gene flow among *N. nomurai* populations in Chinese coastal waters. Their dispersal ability, together with their biological characteristics, could be important factors for the lack of a geographically structured pattern of *N. nomurai* in Chinese coastal waters. *Nemopilema nomurai* has a metagenetic life history (Kawahara et al. 2006). Previous studies indicated that *N. nomurai* has a long planktonic period during spring and winter (Toyokawa et al. 2012; Zhang et al. 2012; Sun et al. 2015). In the summer, the Lubei and Subei Coastal Currents flow in this region (Su and Yuan 2005; Figure 1) and may potentially enhance the dispersal of scyphozoans in Chinese coastal waters. Therefore, the hydrodynamic characteristics of Chinese coastal waters may contribute to the mixed populations. Meanwhile, the Changjiang diluted water (CDW) and the warm Tsushima Current (TC) operating in the region (Figure 1) may potentially enhance the dispersal of *N. nomurai* and keep populations well mixed. Moreover, active swimming activities have been observed in different scyphozoans that may enforce the dispersal of population (Albert 2011; Fossette et al. 2015).

Our present study was inconsistent with a previous study that revealed high genetic differentiation of *Aurelia* sp. 1 sampled in the same region (Dong et al. 2015). One possible explanation is that the dispersal abilities, including dispersal with ocean currents and active swimming, might differ between *N. nomurai* and *Aurelia* sp. 1.

Aurelia sp. 1 occurs mainly in near-shore waters and is rare in deep waters (Dong et al. 2014). However, *N. nomurai* is widely distributed in offshore waters of the Yellow Sea and Bohai Sea (Sun et al. 2015). These two regions differ greatly in ocean currents, which will influence the dispersal abilities of the two scyphozoan species. In addition, previous studies indicate that two important life cycle traits (strobilation frequency and temperature for strobilation onset) might coincide with the genetic differentiation of *Aurelia* spp. populations (Schroth et al. 2002; Gibbons et al. 2010). The benthic polyps of *Aurelia* spp. were observed in many locations, tolerating a wide range of temperatures (Miyake et al. 2002; Willcox et al. 2008; Purcell et al. 2009). However, the benthic polyps of *N. nomurai* have not yet been observed in the field in East Asian Marginal Seas. The Changjiang estuary offshore is indicated as one of the principal breeding places in Chinese waters (Sun et al. 2015). Therefore, the benthic polyps of *Aurelia* sp. 1 may survive in more diverse habits than those of *N. nomurai*.

Recently, landscape genetics and Lagrangian modelling of oceanographic dispersal have been used to explore patterns of connectivity in the scyphozoan jellyfish *Rhizostoma pulmo* in the Irish Sea and northeastern Atlantic (Lee et al. 2013). Tracking the origin of jellyfish blooms at a small or moderate geographical scale is useful. In the future, more detailed sampling of *N. nomurai* should be conducted in East Asian Margin waters. Moreover, multiple methods that couple population genetic and physical connectivity analyses will be useful in distinguishing whether the giant jellyfish *N. nomurai* has common origins in local demographic processes, originated from elsewhere, or is aggregated from a combination of sources.

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