

Genetic Structure of the Jellyfish *Rhopilema esculentum* (Scyphozoa: Rhizostomatidae) in Korean Coastal Waters

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ABSTRACT

The edible jellyfish *Rhopilema esculentum* occurs in waters throughout northeastern Asia, including in Korea, China, and Japan. In Korean waters, *R. esculentum* has appeared in two regions (Gangwha and Muan). Based on the appearance of young medusae and coastal distribution records, these two regions may be key *R. esculentum* breeding sites. In the present study, we investigate and compare the genetic structure of *R. esculentum* in the two regions using mitochondrial sequences (16S ribosomal RNA and cytochrome c oxidase subunit I). The genetic diversity of the *R. esculentum* population at Gangwha exceeded that of the population at Muan. Despite considerable geographic separation (400 km) between the two regions (Gangwha and Muan), our haplotype network suggests that the Gangwha and Muan populations of *R. esculentum* are related. The simple and monotonous genetic structure of the Muan population shows that *R. esculentum* emergence is relatively recent. In contrast, the Gangwha population shows evolution. Moreover, jellyfish of the Gangwha population are genetically diverse and remain constant despite environmental fluctuations in the Han River. The Gangwha area is considered to be the old origin of *R. esculentum* in Korea.

Keywords: edible jellyfish, *Rhopilema esculentum*, mitochondrial gene, population genetics

INTRODUCTION

Since the 1970s, edible jellyfish have been commonly fished in South East Asia owing to the growth of the jellyfish market in North East Asia (Kitamura and Omori, 2010; You et al., 2016). Most edible jellyfish belong to the order Rhizostomeae (Omori and Nakano, 2001), of which two Rhizostomeae species, namely, *Rhopilema esculentum* and *Nemopilema nomurai* (Fig. 1), have been reported in Korean waters (Park and Chang, 2006; Ullah et al., 2015).

Rhopilema esculentum is smaller than *N. nomurai*, with an average bell diameter of 30–60 cm, whereas *N. nomurai* can reach diameters of up to 180 cm (Park and Chang, 2006; Dong et al., 2014; Ullah et al., 2015). *R. esculentum* is the most economically important edible jellyfish worldwide (You et al., 2007; Dong et al., 2009, 2014), and has been reported in the East China Sea, the western Yellow Sea (Bohai Sea and Shandong Province, China), and eastern Yellow Sea (Muan and Gangwha, Korea) (Dong et al., 2014; Ullah et al., 2015)

(Fig. 2).

Since 1980, the abundance of *R. esculentum* has increased after the artificial stock enhancement program in Chinese waters. However, overfishing since 1990 has resulted in jellyfish declines (Dong et al., 2014). The decline in *R. esculentum* fished in Chinese and Japanese waters and the preference for natural jellyfish has resulted in Chinese merchants purchasing *R. esculentum* from Korea since 2013.

R. esculentum typically inhabit low-salinity waters and are commonly found within 30 km of river mouths (Dong et al., 2009). Population structures with clear geographical separation and distinct and local breeding patterns have been observed in Chinese waters (Huang et al., 1985; Jiang et al., 2007). In Korea, similar coastal distribution patterns of *R. esculentum* were recorded (Fig. 2). Based on the separation in distribution, the *R. esculentum* population can be broadly divided into two regions: Gangwha (northern) and Muan (southern) in the Korean western coastal area (Fig. 2). Since 1980, jellyfish have been primarily fished in the northern Yel-

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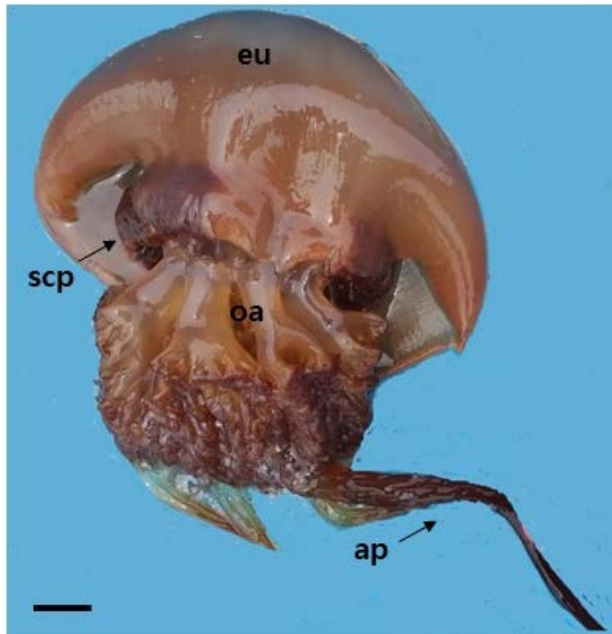


Fig. 1. Whole body (side view) of *Rhopilema esculentum* (ap, appendages; eu, exumbrella; oa, oral arm; scp, scapulet). Scale bar = 5 cm.

low Sea at Gangwha. However, since 2013, there has been a notable increase in jellyfish fishing in Muan in the southern Yellow Sea. In the present study, we determine two mitochondrial sequences (16S ribosomal RNA and cytochrome c oxidase subunit I [COI]) collected from *R. esculentum* in Gangwha and Muan to compare and understand the genetic structure of the two populations.

MATERIALS AND METHODS

Specimens, DNA extraction, and sequencing

R. esculentum jellyfish (Fig. 1) were sampled at Gangwha and Muan in 2015 ($n=41$ and $n=25$, respectively) and 2017 ($n=28$ in each region) (Fig. 2). Umbrella tissue samples of collected *R. esculentum* specimens were preserved in 99.9% ethanol before DNA extraction and nuclear DNA analyses. Genomic DNA (gDNA) was extracted from the preserved umbrella tissue of all 122 *R. esculentum* specimens. Before extraction, alcohol-preserved tissues were washed in distilled water to remove all traces of ethanol and to avoid contamination from the zooplankton contained in the stomach of the jellyfish. Total gDNA was extracted using the method outlined by Asahida et al. (1996). The partial sequence of the mitochondrial COI gene (534 bp) was amplified using the forward primer HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and reverse primer LCO 1490 (5'-GGT CAA

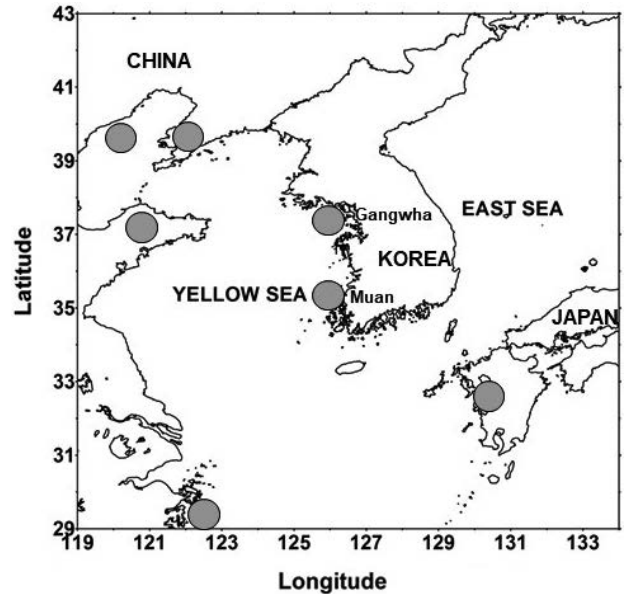


Fig. 2. Distribution map of *Rhopilema esculentum* in Korean and Chinese coastal waters.

CAA ATC ATA AAG ATA TTG G-3') (Folmer et al., 1994) on a thermocycler (Biometra, Göttingen, Germany) under the following cycling conditions: 30 cycles at 94°C for 8 min, 49°C for 2 min, 72°C for 2 min, 94°C for 4 min, 50°C for 2 min, and 72°C for 2 min; 33 cycles at 94°C for 45 s, 51°C for 45 s, and 72°C for 60 s; and a final extension at 72°C for 10 min; and then maintenance at 4°C. The partial sequence of the mitochondrial 16S rDNA (453 bp) was amplified using the forward primer Amt 16SIF (5'-AGG ATA YCA YAA TTC AAC AT-3') and reverse primer Amt 16SIR (5'-AAT RGC CGC GGT AWY TCT GA-3') under the following program: at 94°C for 5 min, 30 cycles (at 30 s, 47°C for 30 s, 72°C for 30 s); and a final extension at 72°C for 7 min; and then maintenance at 4°C. Amplified polymerase chain reaction (PCR) fragments were purified and sequenced on an automated DNA sequencer (ABI 3730XL; Applied Biosystems, Foster City, CA, USA). Sequences determined in this study were deposited in GenBank under the following accession numbers: OP602043–OP602056 and OR523266–OR523283 for mitochondrial COI and OR607940–OR607947 for 16S rRNA. *Rhopilema esculentum* were compared with Chinese, north-west Pacific Ocean, Russia) in GenBank data (HF536571, HF536572, HF536573, HF536574, HF930515; China EU373722, EU373723, EU373724, JQ353756, JQ353757, JX845347; Russia KF840718).

Population genetics analysis

The gene sequences of individuals collected from populations in Gangwha and Muan were analyzed and compared.

Sequences were aligned using ClustalX (Thompson et al., 1997) and the alignments were checked manually. Nucleotide frequencies for each haplotype were checked manually and estimated using MEGA 7 software (Kumar et al., 2016). Haplotype (Nei, 1973) and nucleotide (Nei and Tajima, 1981) diversities were estimated to determine the degree of genetic diversity between the two populations (Gangwha and Muan) using Arlequin 3.1 (Excoffier et al., 2005) and MEGA 7. A statistical parsimony network was constructed using TCS 1.21 (Clement et al., 2000).

RESULTS

Genetic diversity

The genetic distance, as assessed using mitochondrial 16S rRNA, within the phylum Cnidaria (Classes Anthozoa, Hydrozoa, and Scyphozoa) was 0.015–0.902 (Table 1). Within the class Scyphozoa, the genetic distance, determined using mitochondrial 16S rRNA (0.015–2.252) and COI mtDNA (0.059–0.332). However, the genetic distances of *R. esculentum* between the Gangwha and Muan populations were lower, in the range 0.000–0.008 (16S rRNA) and 0.000–0.025 (COI mtDNA).

Phylogenetic analyses for Scyphozoa based on mitochondrial 16S rRNA showed that *Rhopilema esculentum* populations in Korea were monophyletic, which coincided with *R. esculentum* in Bohai Sea (EU373726) (Fig. 3).

The genetic diversity of *R. esculentum* sampled from the Gangwha and Muan regions based on mitochondrial COI DNA and 16S rRNA is shown in Table 2 and Fig. 4. Based on both mitochondrial COI DNA and 16S rRNA, the genetic diversity of the Gangwha population is higher than that of the Muan population. *R. esculentum* genetic diversity in Muan based on COI DNA was markedly low, with only one haplotype identified among 19 individuals in 2015. Analyses of the COI sequences confirmed this result; genetic diversity was higher at Gangwha (0.9206 ± 0.0265) than at Muan (0.3148 ± 0.1024) in 2017. Genetic diversity of Muan in 2017 surpassed that of Muan in 2015.

Haplotype TCS network

Comparison of the haplotype network based on the mitochondrial COI DNA and 16S rRNA of *R. esculentum* showed high differentiation; minor haplotypes were derived from the major haplotypes (Fig. 5). Moreover, Gangwha and Muan populations showed genetic connectivity, with large genetic pools in Gangwha and a simple haplotype structure in Muan. Within the network analysis, a primary haplotype was identified, with several minor haplotypes branching out from this

Table 1. Pairwise distances within the phylum Cnidaria based on mitochondrial 16S rRNA

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 <i>Liriope tetraphylla</i> _AY512510															
2 <i>Craspedacusta sowerbyi</i> _EU29397	0.292														
3 <i>Aequorea aequorea</i> _AY512518	0.248	0.377													
4 <i>Physalia physalis</i> _AY935284	0.313	0.285	0.272												
5 <i>Rhopilema esculentum</i>_EU373726	0.374	0.432	0.390	0.357											
6 <i>Rhopilema esculentum</i> Korea	0.374	0.432	0.390	0.357	0.000										
7 <i>Nemopilema nomurai</i>_EU373727	0.358	0.433	0.342	0.374	0.067	0.067									
8 <i>Nemopilema nomurai</i> Korea	0.350	0.424	0.334	0.366	0.061	0.061	0.005								
9 <i>Aurelia limbata</i>_AF461403	0.383	0.506	0.384	0.446	0.210	0.210	0.189	0.182							
10 <i>Aurelia aurita</i>_AF461398	0.428	0.509	0.403	0.428	0.237	0.237	0.202	0.195	0.078						
11 <i>Aurelia aurita</i> Korea	0.428	0.509	0.403	0.437	0.252	0.252	0.215	0.208	0.089	0.015					
12 <i>Aurelia limbata</i>	0.428	0.488	0.403	0.418	0.230	0.230	0.215	0.208	0.067	0.030	0.036				
13 <i>Parombrosa polylobata</i>	0.358	0.460	0.375	0.411	0.243	0.243	0.236	0.229	0.213	0.214	0.221	0.207			
14 <i>Parazoanthus axinellae</i> _AY99593	0.822	0.902	0.758	0.745	0.763	0.763	0.755	0.740	0.662	0.635	0.635	0.648	0.755		
15 <i>Anthopleura kurogane</i> _EU190783	0.756	0.878	0.685	0.715	0.800	0.800	0.771	0.757	0.684	0.709	0.709	0.682	0.693	0.269	

Bold letters indicate Scyphozoan species and genetic distances between Scyphozoa.

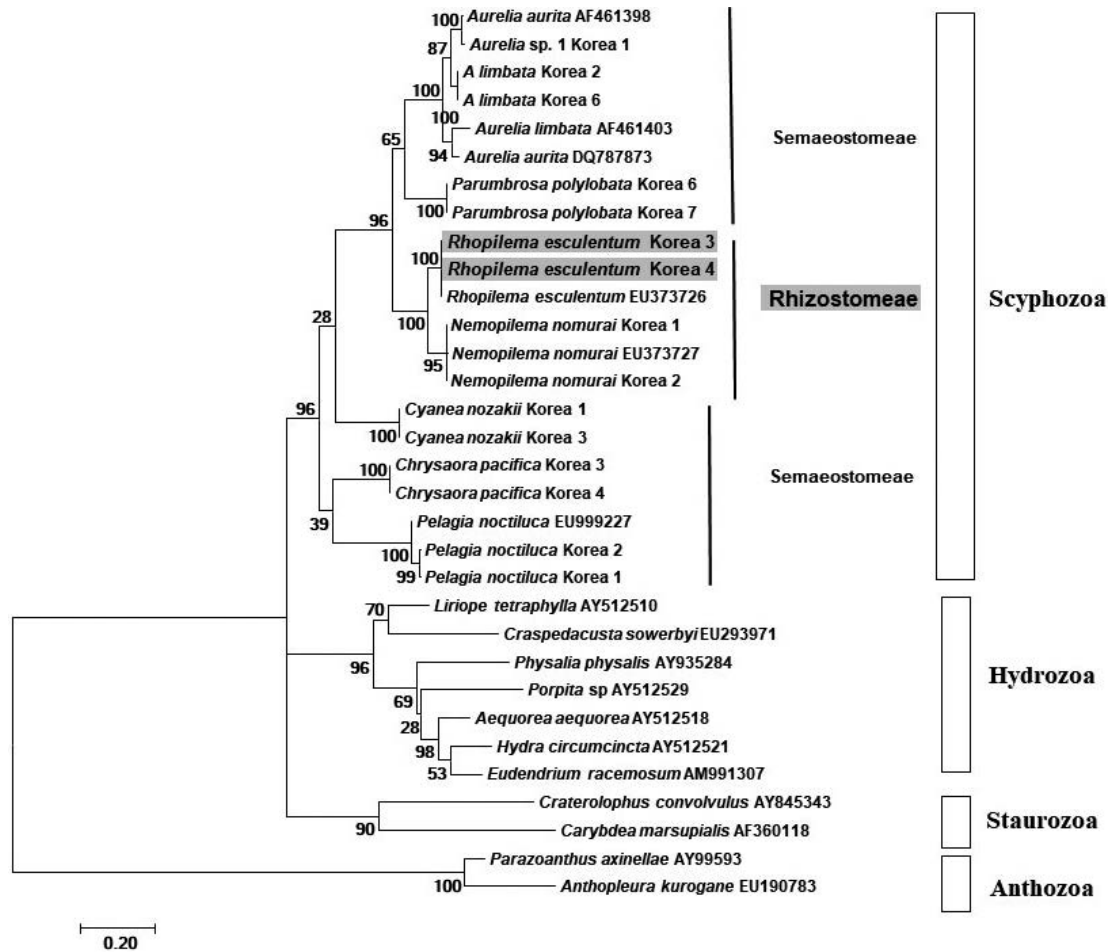


Fig. 3. Phylogenetic maximum likelihood (ML) tree constructed for the class Scyphozoa based on mitochondrial 16S rRNA.

Table 2. Genetic diversity of *Rhopilema esculentum* based on mitochondrial 16S rRNA and COI DNA (2015) and on COI DNA only (2017) in the Korean waters of Gangwha and Muan

Region	No. of sequences	No. of haplotypes	Genetic diversity	Gene
Gangwha_2015	41	7	0.3146±0.0940	Mitochondrial 16S rRNA
Muan_2015	25	2	0.1533±0.0915	Mitochondrial 16S rRNA
Gangwha_2015	35	18	0.9277±0.0239	Mitochondrial COI DNA
Muan_2015	19	1	0.0000±0.0000	Mitochondrial COI DNA
Gangwha_2017	28	13	0.9206±0.0265	Mitochondrial COI DNA
Muan_2017	28	3	0.3148±0.1024	Mitochondrial COI DNA

COI, cytochrome c oxidase subunit I.

central haplotype. Thus, it appears that the Gangwha population affected the Muan population through gene flow. The molecular phylogenetic tree among *R. esculentum* in the Yellow Sea (Korean and Chinese waters) and Pacific waters from the National Center of Biotechnology Information database (NCBI) confirmed that the genetic diversity of the Gangwha population was very high (Fig. 6).

DISCUSSION

Rhopilema esculentum inhabit coastal waters within 10–30 km from the shore, whereas *Nemopilema nomurai* (a giant jellyfish belonging to the Rhizostomatidae family) exhibit a much wider dispersion, ranging from the East China Sea to the Sea of Japan (East Sea), covering a distance of over 1,000

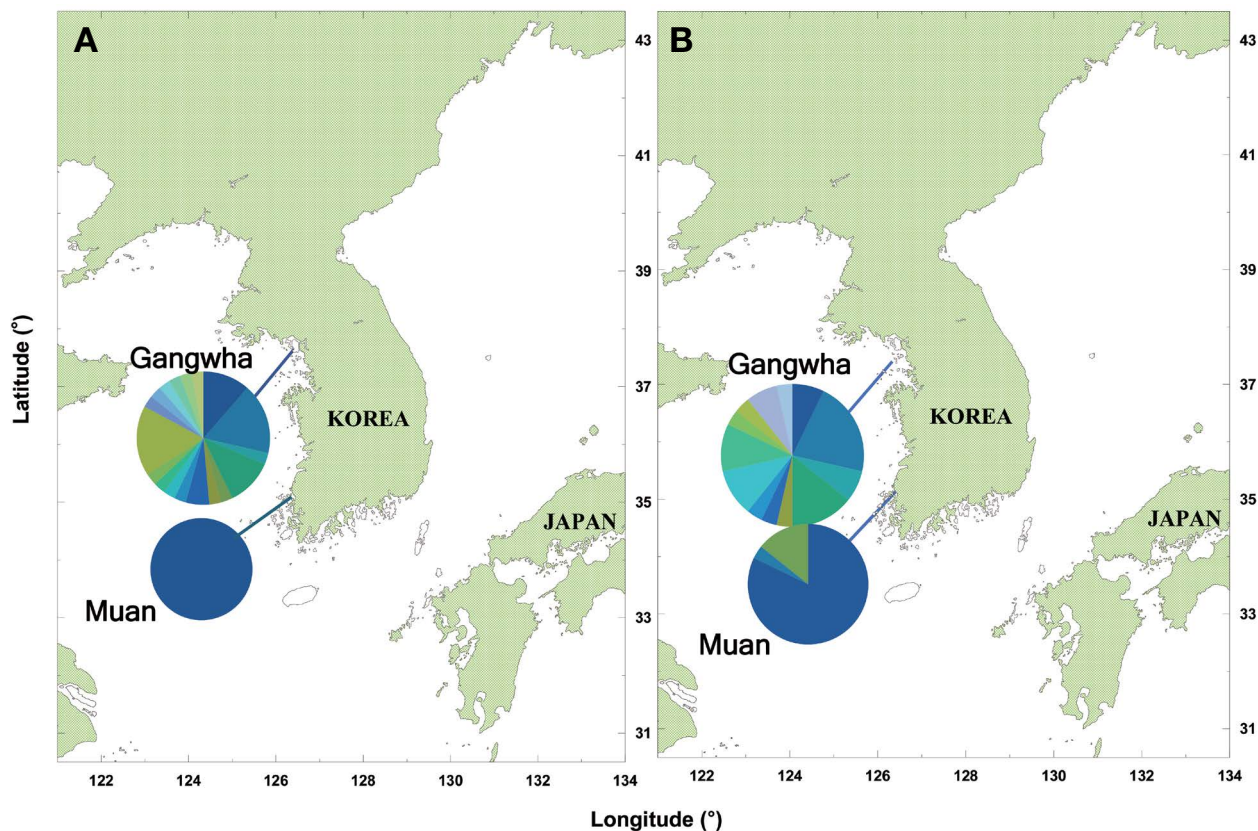


Fig. 4. Genetic diversity of *Rhopilema esculentum* based on mitochondrial cytochrome c oxidase subunit I DNA in Gangwha and Muan in 2015 (A) and 2017 (B) in Korean waters.

km (Gotho et al., 2017). In the present study, young medusae of *R. esculentum* with a bell diameter of ~2 cm were found in Gangwha and Muan, indicating that the two regions might be breeding sites for *R. esculentum*. However, the jellyfish were not found at the center of the eastern Yellow Sea.

In contrast, *R. esculentum* in Muan (southern Yellow Sea) appeared from May to August and from June to October in Gangwha (northern Yellow Sea) due to differences in water temperature and latitude (Fig. 7). Thus, the two regional groups (Gangwha and Muan) are geographically and temporally separated according to their geographic separation (400 km apart) and breeding time.

Our haplotype network suggests that the *R. esculentum* population of Gangwha affected that of Muan (see Fig. 5). Additionally, we demonstrate that populations in both areas are genetically linked despite their geographic separation based on mtDNA 16S rRNA and COI sequences. *R. esculentum* populations in Korean waters are considered to originate from the Gangwha area. Generally, the genetic diversity of populations in their site of origin tend to be higher than that of derived populations (Dawson et al., 2015).

In Korean waters, *R. esculentum* has been consistently

found in Gangwha over the last 40 years, whereas mass occurrence of the jellyfish in Muan have been recorded within the last 10 years. Capture records suggest that the *R. esculentum* population in Gangwha was established earlier than that in Muan. According to a fisherman’s testimony, *R. esculentum* appeared in Mokpo near Muan 30 years ago, but the habitat was damaged by coastal development. A population derived from Mokpo could have settled in Muan; however, it is difficult to assert that the Muan population originates from Gangwha or Mokpo when considering the ocean current pattern. Specifically, west Korean coastal current move north or south as the warm ocean currents expand (Hwang et al., 2014). In conclusion, the genetic diversity of *R. esculentum* in the Gangwha group is higher than that of the Muan group; the Gangwha group shows evolution over a long period (Table 2). Moreover, the genetic diversity of *R. esculentum* sampled in Muan in 2017 was higher than that of *R. esculentum* sampled in Muan in 2015. Taken together, our results suggest that the Muan group might have evolved recently.

As previously noted, Gangwha and Muan are ~400 km apart (from north to south) and consequently have different environmental characteristics. The water temperature is higher

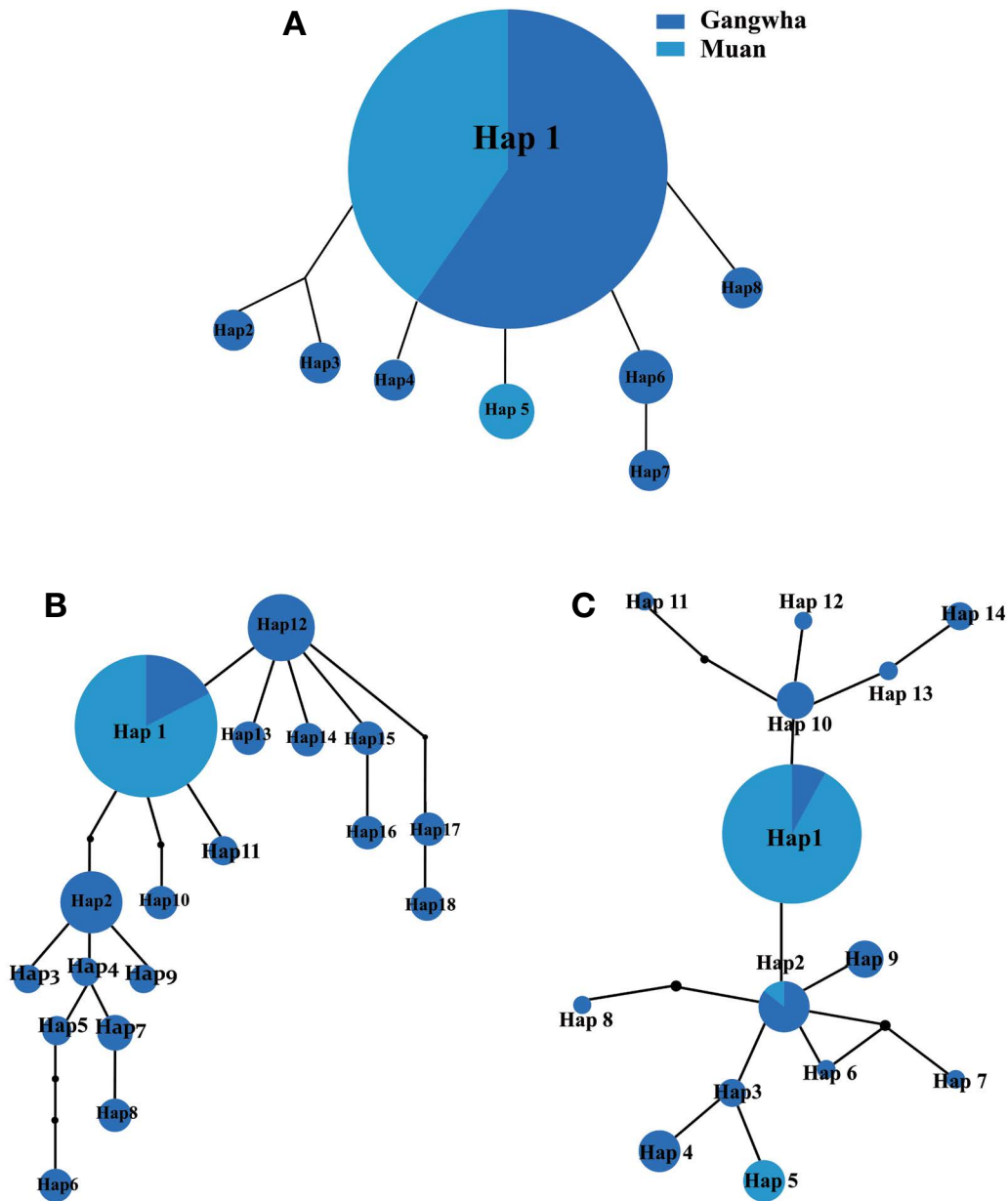


Fig. 5. Haplotype networks of *Rhopilema esculentum* in Gangwha and Muan: A, Mitochondrial 16S rRNA in 2015; B, cytochrome c oxidase subunit I (COI) mtDNA in 2015; C, COI mtDNA in 2017.

in Muan than that in Gangwha; constant salinity is conducive to jellyfish growth in Muan. Conversely, the Gangwha area is highly affected by the Han River, which has a wide range of salinity (16–26 PSU) (Fig. 7). Yearly, 300–500 tons of jellyfish are fished at Gangwha, in comparison to 100–2,000 tons fished at Muan. Despite considerable environmental fluctuations, the amount of jellyfish in Gangwha has remained constant and the genetic diversity of *R. esculentum* in the Gangwha region is high. Moreover, genetically diverse populations adapt to various environmental conditions relatively easily.

The elevated genetic diversity within the Gangwha population of jellyfish results in their consistent presence in regions experiencing considerable environmental fluctuations. Conversely, the Muan population appears in large numbers when environmental conditions are favorable, while its presence diminishes during less favorable environmental conditions.

To elucidate the genetic structure and dispersal pattern of *R. esculentum* in the Yellow Sea more comprehensively, future studies should incorporate and analyze more regional groups with sensitive molecular markers, including the *R. esculentum*

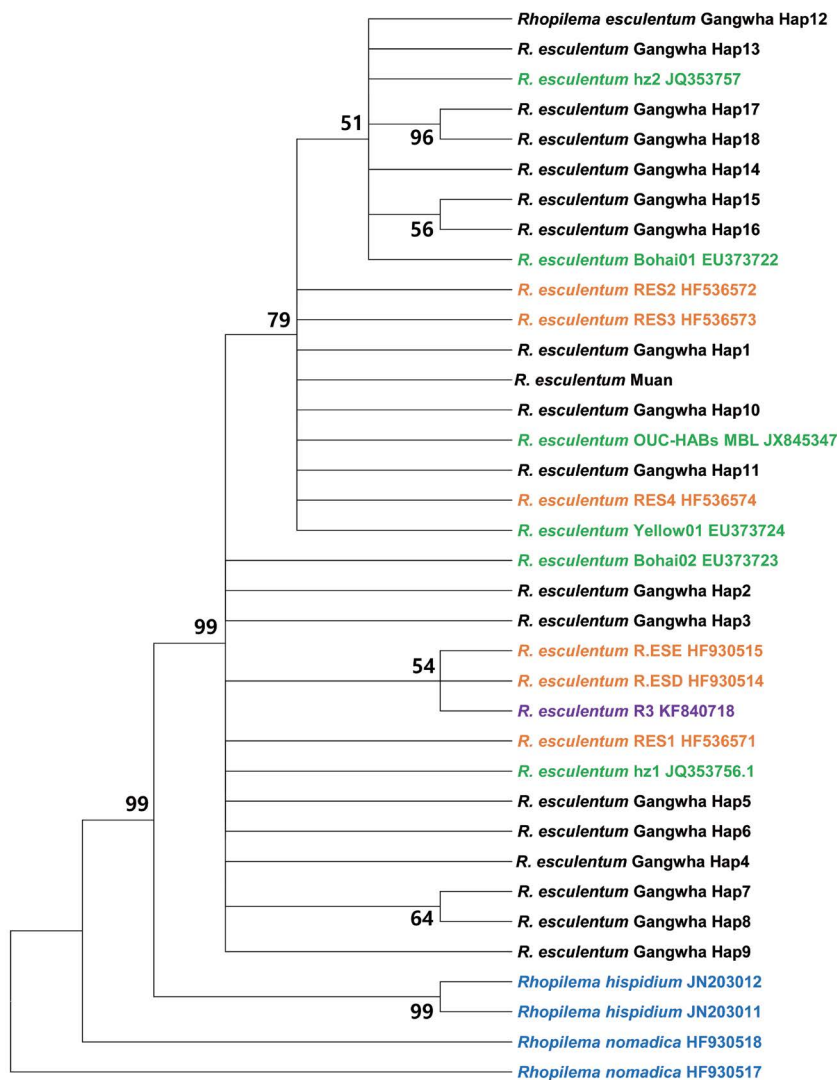


Fig. 6. Maximum parsimony tree of *Rhopilema esculentum* based on cytochrome c oxidase subunit I mtDNA in the northwest Pacific. Korean samples analyzed in the present study are indicated in black. Sequences obtained from the NCBI database are indicated in green (China), orange (northwest Pacific), and purple (Russia). *Rhopilema hispidum* and *Rhopilema nomadica* were analyzed as out-groups.

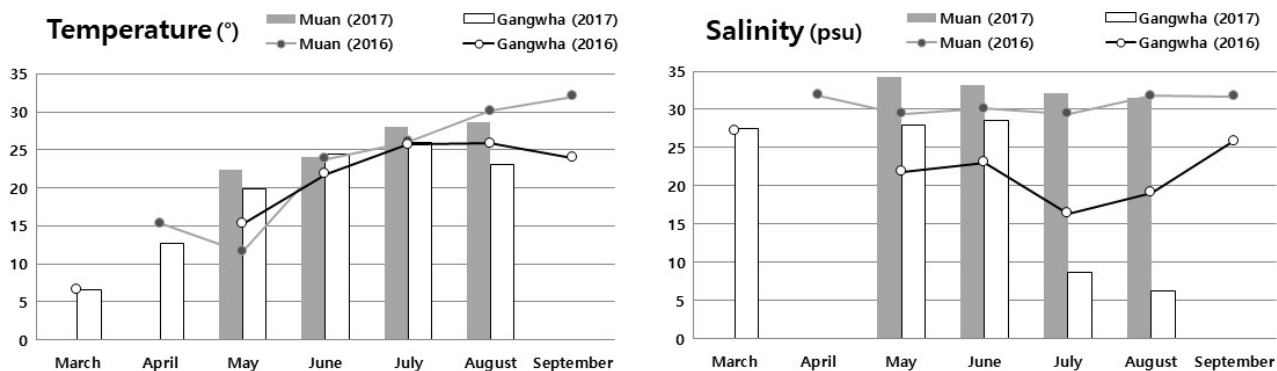


Fig. 7. Comparison of sea water temperature and salinity between Gangwha and Muan regions.

in Shandong, Bohai, Liaodong Bay, and the Yangtze River (Fig. 6).

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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