

Article

**First Record of *Rhopilema esculentum* (Scyphozoa, Rhizostomae),
Edible Jellyfish in Korea**

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Abstract : A species of edible Scyphomedusae jellyfish has been used as food by the local people in Ganghwado, Korea since the 1990s. In order to identify this jellyfish, we collected specimens in Ganghwado during September 2013, and compared these specimens with original descriptions made by Kishinouye (1890). Mitochondrial cytochrome oxidase subunit 1 (CO1) sequences of these specimens were compared with those of *Rhopilema esculentum* retrieved from GenBank. Our specimens were also compared with jellyfish collected in China, previously confirmed as *R. esculentum*, based on morphological characters and DNA sequences. Using these two methods, the jellyfish specimens caught in Ganghwado were correctly identified as *R. esculentum*, a species new to Korean waters. This edible jellyfish has been named 'Gisusik-Yonghaepari' as Korean name.

Key words : CO1, edible jellyfish, *Rhopilema esculentum*, scyphomedusae

1. Introduction

Fifteen species of jellyfish have been used as food worldwide (Omori and Nakano 2001; Kitamura and Omori 2010), and their economic value in Asian countries is millions of dollars (Omori and Nakano 2001). The countries of China and Japan have the biggest markets of edible jellyfish. Korean people have also enjoyed jellyfish as a seafood for generations, and with the increasing unit cost of production, jellyfish imports from Indonesia and Thailand have increased and amounted to more than 8000 tons per year between 2006 and 2008 (The Korea Times 2009).

All edible jellyfish are from the order Rhizostomeae, including *Rhopilema esculentum* (Kishinouye 1891),

Rhopilema hispidum (Vanhöffen 1888), *Rhizostoma pulmo* (Macri 1778), and *Nemopilema nomurai* (Kishinouye 1922), of the class Scyphozoa. Among the 31 jellyfish species reportedly in Korea, only *N. nomurai* is edible. However, unidentified jellyfish have been observed from July to October, and have been used as food by the local people in Ganghwado and the Korean coastal area. The local people call this jellyfish, 'Gisusik-Yonghaepari'. Regarding the geographical distribution of edible jellyfish, such as those listed above (Omori and Nakano 2001; Kitamura and Omori 2010), *R. esculentum* is the most widespread species, having been reported from the Ariake Sea of Japan, as well as the BoHai Sea, the Yellow Sea, and the East China Sea (Hon et al. 1978; Dong et al. 2014; Kitamura and Omori 2010).

Rhopilema esculentum was first described (in Japanese language) as a new species by Kishinouye in 1890 and

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given its species name in a brief note by the same author (Kishinouye 1891). Kishinouye (1899) re-described the morphological characteristics of this Japanese jellyfish species in English.

In this study, we undertook morphological and DNA analysis of an edible jellyfish collected in Ganghwado, Korea and identified this species as *Rhopilema esculentum*. This is the first record of *R. esculentum* in Korean coastal waters.

2. Materials and Methods

Jellyfish specimens were collected with a set-net and set bag-net in Ganghwado, Korea from September to October 2013. Whole samples were preserved immediately in neutralized formalin. Partial samples, usually the gonads, were preserved in 70~80% ethanol for DNA analysis. Specimens from Liaoning Province, China that were previously identified as *R. esculentum* were also examined following the same DNA analysis protocol.

The morphological analysis consisted of observing whole specimens, including the exumbrella, subumbrella, oral arm, and scapulet, and a dissection of the gut, sensory organs, and gonads. The key morphological characteristics of the jellyfish are depicted in detail. For DNA analysis, genomic DNA was extracted from ethanol-preserved tissues of six jellyfish specimens using an MGTM Tissue Genomic DNA Extraction SV miniprep kit (Doctor Protein, Seoul, Korea).

The primers, FFDL and FRDL2, were used to amplify and sequence the short fragments of the mitochondrial cytochrome oxidase subunit 1 (COI) gene (Armani et al. 2013). The six sequences acquired were aligned with

sequences retrieved from GenBank (Table 2). We used the program MEGA ver. 5.1 to compute pairwise genetic distances and construct a Neighbor-Joining (NJ) tree based on the Kimura two-parameter model (Kimura 1980; Tamura et al. 2011). Node reliability was estimated by bootstrap analysis with 1,000 random replications.

3. Results and Discussion

Systematic account

Class Scyphozoa Götte, 1887

Order Rhizostomeae Cuvier, 1799

Family Rhizostomatidae Cuvier, 1799

Genus *Rhopilema* Haeckel, 1880

Rhopilema esculentum Kishinouye, 1890

Rhopilema esculentum Kishinouye, 1890, p.47, pl.2; AG Mayer, 1910, p.704, fig. 423; Kramp, 1961, p. 380;

Omori and Kitamura, 2004, 37-42, fig. 2-4.

Korean name

‘Gisusik-Yonghaepari’ is the Korean name used by the local people of Ganghwado, Korea, to refer to *Rhopilema esculentum*, which is appropriate given its scientific species name (*esculentum* is Latin for edible).

Material examined

Rhopilema esculentum: Ganghwado, Incheon. Korea, latitude; 37°.76'N, longitude; 126°.32'E (Fig. 1), collected by set bagnet on 25 Sep and 19 Oct 2013.

Morphological description

The umbrella of Korean specimens is round and bell shaped, hemispherical when expanded, and measures

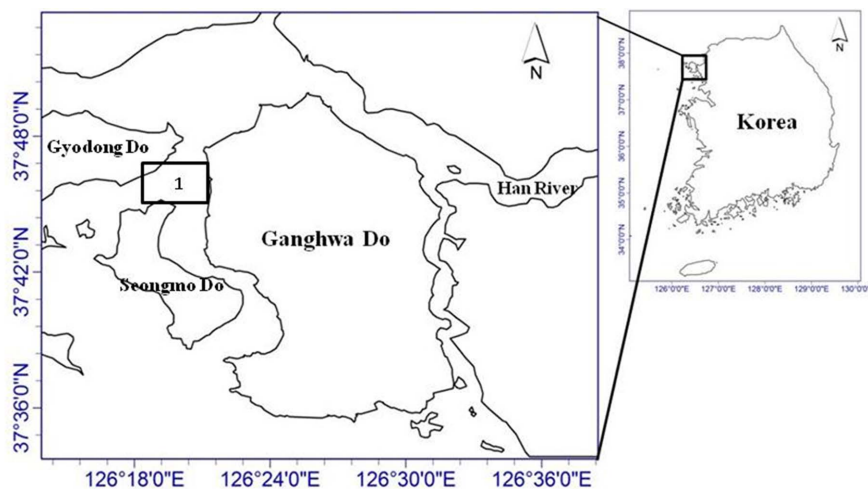


Fig. 1. Sampling area of edible jellyfish (Box 1, Ganghwado, Korea)

from 210 mm to 390 mm in diameter (Fig. 2A, C). The middle portion of the bell is thick, gradually thinning towards the bell margin. The bell margin is divided into eight parts, and each part contains 16~18 oval-shaped velar lappets.

The sensory organ (Fig. 2C) is located between two parts. The exumbrella surface is smooth, whereas the subumbrella surface is rather harsh (Fig. 2A, C). Circular and unitary muscles are well developed in the subumbrella.

Four arm disks (Fig. 2B, C (o.p)) are connected to the subumbrella; they are very thick and prismatic. Between every two arm disks, there is one large protuberance (Fig. 5C (pr)) situated at the subumbrella side on the interradial section. Each protuberance is very hard, coarse, and prickly. The Gastrovascular system is complex, and the circular canal is indistinct. There are four perradial canals,

four interradial, and eight adradial canals. The perradial and interradial canals extend to, and connect with, sensory organs at the bell margin; eight adradial canals are extended to the middle of the perradial and interradial canals, and then join with circular muscle. All 16 radial canals are inter-connected with one another by the circular muscle. Circular muscle is absent from the stomach area of the exumbrella side. Four strong, thick, and quadrate oral pillars are attached to the bell at the subumbrella side.

The stomach is under the four arm disks. Window of heart-shaped is located between the two arm disks. It has four subgenital cavities. Four oral arms are interconnected. A single peduncle-shaped tube connects to the oral arms with scapulets. The end of the tube is again divided into eight arm parts, onto which eight pairs of scapulets are situated.

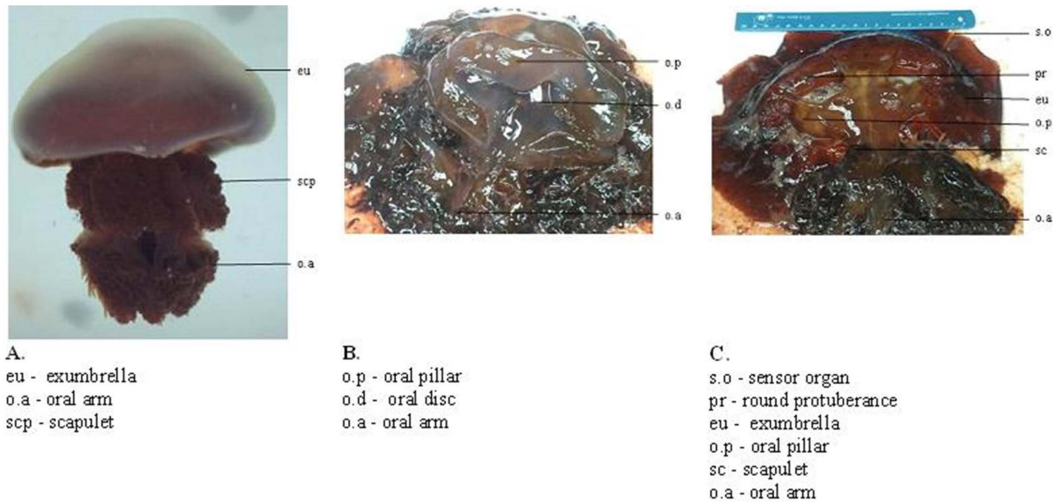


Fig. 2. (A) Jellyfish, (B) Without Bell, (C) Jellyfish (Subumbrella side)

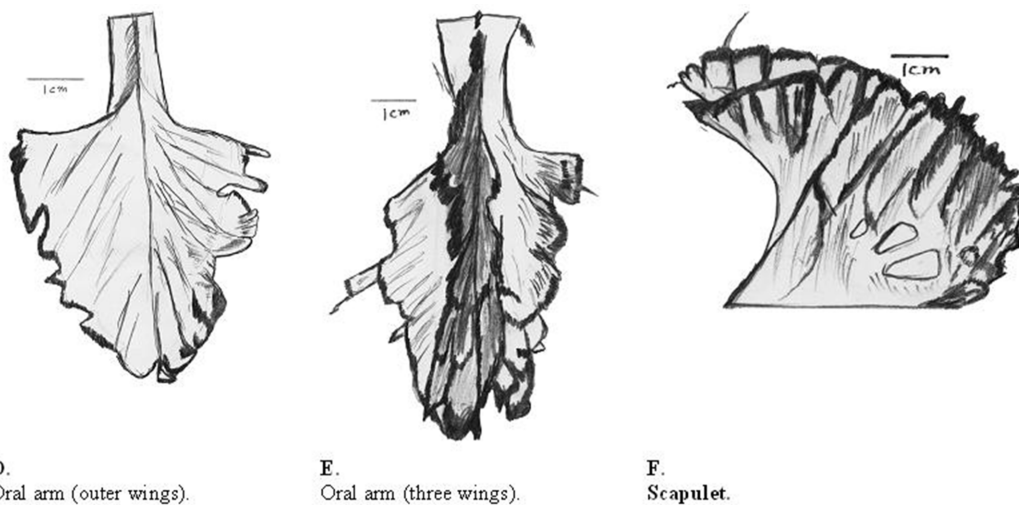


Fig. 3. Oral arm and Scapulet

The gonads are yellowish, or dark in color, and are attached to the lower part of the stomach wall with a complexly folded shape.

Arms

Four oral pillars are joined together and make a single rounded tube from where eight pairs of scapulets originate. The oral pillars divide into eight mouth arms, which are large, stout, triangular pyramids with a peduncle shape.

The scapulets (Fig. 3F) are concave, and the wings of each scapulet are divided into two parts at the distal end making a 'V' shape. Each oral arm has three wings (Fig. 2A, 3E) which occur at regular intervals. When viewed from the animal's central axis, two wings of each oral arm are outer and one side directed (Fig. 3D, E).

Each oral arm has a narrow arm canal, which is divided into several branches that are directed towards, and terminate at, the wing margin. The inner wing of each oral arm is frilled with numerous cirri (Fig. 3E).

Color

The color of the subumbrella is reddish brown or dark brown, and the exumbrella is colorless. The circular muscle, mouth arms, and scapulets are all reddish brown in color. Color variants of *R. esculentum* have been reported as deep blue and reddish brown (Hattori 1893; Kishinouye 1899), to light yellowish brown, golden, and even milky white (Hon et al. 1978).

Size

The bell diameter of the collected specimens averaged 33 cm and ranged between 21 cm and 39 cm.

Habitat

Water temperature was a minimum of 10°C in April and a maximum of 28°C in August, similar to the range reported by Lu et al. (1997), which was between 8°C and

34°C. Water salinity ranged from 7 to 28 Practical Salinity Units (PSU). Lower salinity generally occurs in July through August due to precipitation caused by Asian monsoons. *Rhopilema esculentum* has an optimal salinity range of 14 to 20 PSU, but can tolerate a range between 10 and 35 PSU as an adult and between 8 and 20 PSU as a juvenile (Chen et al. 1985; Lu et al. 1989). The pH of seawater varied from 7.4 to 7.9.

DNA sequence

DNA sequences of 5 specimens from Ganghwado, and one from China, were compared with *R. esculentum* and two unidentified Rhizostomatide jellyfish retrieved from GenBank (Table 1).

The partial COI gene sequences of *Rho. esculentum* were 624 bp in length (GenBank accession no.: xxxxxxxx-xxxxxxx). The interspecific pairwise distances among the species *R. esculentum*, *R. nomadica* (Galil et al. 1990), and *N. nomurai* were greater than 19%, whereas the intra-specific distances of *R. esculentum* ranged between 0 and 1.13% (Table 2).

The *R. esculentum* specimens analyzed in this study grouped as a single clade, separate from specimens in regions near the collection site (Fig. 4) Huang et al. (2008) reported that the intra-specific distances of select hydrozoan and scyphozoan species ranged between 2% and approximately 20%, which are larger distances than the ones we observed in *R. esculentum* (0~1.13%).

The average interspecific pairwise distances in Scyphozoa are 15.3% (Huang et al. 2008), but may be larger in certain genera, (e.g., 23.4% in *Cassiopea* (Holland et al. 2004) and 24.0% in *Aurelia* (Dawson and Jacobs 2001)), whereas the interspecific pairwise distances among *R. esculentum*, *R. nomadica*, and *N. nomurai* used in this study varied from 19.21 to 21.54%. Pairwise distances this large indicate that these species are taxonomically distinct (Mcfadden et al. 2011).

Table 1. Information of samples used in this study

Sample name	Species name	Geographical origin	Reference
Ganghwado 1~5	<i>Rhopilema esculentum</i>	Ganghwado, Incheon, Korea	This study (xxxxxxx-xx)
China 1	<i>Rhopilema esculentum</i>	China	This study (xxxxxxx)
China 2	<i>Rhopilema esculentum</i>	Bohai Sea, China	GenBank (EU373723)
China 3	<i>Rhopilema esculentum</i>	Jiaozhou Bay, China	GenBank (JQ353757)
Pacific Ocean 1	<i>Rhopilema esculentum</i>	Pacific, Northwest FAO 61	GenBank (HF536571)
Pacific Ocean 2	<i>Rhopilema esculentum</i>	Pacific, Northwest FAO 61	GenBank (HF536572)
<i>R. nomadica</i>	<i>Rhopilema nomadica</i>	Western Mediterranean FAO 37-3.2	GenBank (HF930517)
<i>N. nomurai</i>	<i>Nemopilema nomurai</i>	Pacific, Northwest FAO 61	GenBank (HF536563)

Table 2. Kimura 2-parameter pair wise distances (%) based on COI gene sequences

	1	2	3	4	5	6	7	8	9	10	11
1. Ganghwado 1 (this study)											
2. Ganghwado 2 (this study)	0.32										
3. Ganghwado 3 (this study)	1.13	0.81									
4. Ganghwado 4 (this study)	0.32	0.32	1.13								
5. Ganghwado 5 (this study)	0.32	0.32	1.13	0.32							
6. China 1 (this study)	1.13	1.13	0.97	1.13	1.13						
7. China 2 (EU373723)	0.16	0.16	0.97	0.16	0.16	0.97					
8. China 3 (JQ353757)	0.81	0.81	0.65	0.81	0.81	0.32	0.65				
9. Pacific Ocean 1 (HF536571)	0.16	0.16	0.97	0.16	0.16	0.97	0.00	0.65			
10. Pacific Ocean 2 (HF536572)	0.65	0.65	0.81	0.65	0.65	0.48	0.48	0.16	0.48		
11. <i>R. nomadica</i> (HF930517)	21.33	20.89	21.33	21.11	21.33	21.54	21.11	21.11	21.11	21.33	
12. <i>N. nomurai</i> (HF536563)	19.87	19.44	19.22	19.66	19.87	19.21	19.66	19.22	19.66	19.44	20.65

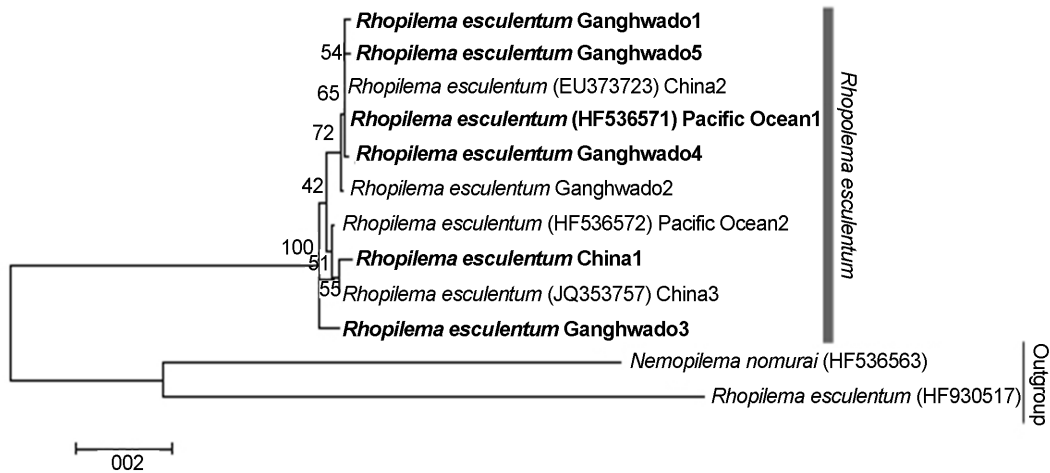


Fig. 4. Neighbor-joining (NJ) tree of *Rhopilema esculentum* based on partial COI gene sequences. Bolds are obtained in this study. *Nemopilema nomurai* and *R. nomadica* were used as out group. Number at branches indicates the bootstrap percentage of NJ tree

The morphological and molecular analyses performed in this study confirmed the occurrence of *R. esculentum* in Korean waters. Kishinouye’s descriptions of the key morphological characters helped to confirm our identification of this species (Kishinouye 1899).

Rhopilema esculentum displayed intraspecific distances 0~1.13% based on our COI gene sequence results. This gene has been adopted as a universal barcode for species identification (Meier et al. 2006).

4. Remarks and Conclusions

Park and Chang (2006) mentioned that *R. esculentum* and *N. nomurai* are very similar in shape and differ only

in color and body size; however, we noticed significant morphological differences among *R. esculentum*, *R. hispidum*, *N. nomurai*, and *Rhizostoma pulmo* (Table 3). Based on our morphological and molecular analyses, the edible jellyfish collected from Ganghwado was identified as *Rhopilema esculentum* and has been given the Korean name, ‘Gisusik-Yonghaepari’.

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Table 3. The morphological differences between *Rhopilema esculentum*, *Rhopilema hispidum*, *Nemopilema nomurai* and *Rhizostoma pulmo*

	<i>Rhopilema esculentum</i>	<i>Rhopilema hispidum</i>	<i>Nemopilema nomurai</i>	<i>Rhizostoma pulmo</i>
Marginal lappets	Marginal lappets rounded and 16 to 18 at each octant. (Kishinouye 1890, 1899; Mayer 1910; Kramp 1961). Small one has 7 to 11 (Omori and Kitamura 2004).	8 velar lappets in each octant (Vanhöffen 1888; Kramp 1961; Omori and Kitamura 2004).	Marginal lappets 8 in each octant and each lappet divided two round sub-lappets (Omori and Kitamura 2004).	8~12 evenly rounded small velar lappets per octant (Mayer 1910; Kramp 1961).
Exumbrella	Exumbrella smooth (Kishinouye 1890, 1891, 1899; Mayer 1910; Kramp 1961; Omori and Kitamura 2004).	Warts present on exumbrella (Omori and Kitamura 2004).	Covered by colorless granular warts (Omori and Kitamura 2004)	Exumbrella surface finely granular and covered with small netting warts (Mayer 1910; Kramp 1961).
Mouth arms	Mouth arms are associated with each other about one-fourth of their length (Omori and Kitamura 2004). Mouth arms without terminal appendages (Kitamura and Omori 2010).	Mouth arms are associated with each other about half of their length (Omori and Kitamura 2004). Mouth arms with club-shaped appendages (Kitamura and Omori 2010).	Mouth arms are J-shaped in lateral view (Omori and Kitamura 2004). Mouth arms with whip-shaped appendages (Kitamura and Omori 2010).	The bell diameter is about equal to the total length of the mouth arms including terminal clubs (Mayer 1910; Kramp 1961). Mouth arms with large club-shaped appendages (Mayer 1910; Kramp 1961; Kitamura and Omori 2010).

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