

Article

Re-description of *Chrysaora pacifica* (Goette, 1886) (Cnidaria, Scyphozoa) from Korean Coastal Waters: Morphology and Molecular Comparisons

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Abstract : The nomenclature of the sea nettle jellyfish from Korea was initially described as *Dactylometra quinquecirrha* Agassiz, 1862. However its identity has been questioned on the basis of its local distribution and molecular data. Here, we examined morphology and DNA sequences of nuclear rDNA using specimens collected from southern Korean waters in August 2014. Based on morphological characteristics (bell size, umbrella pattern, number of tentacles and lappets) and distribution locality, we reassign the Korean *D. quinquecirrha* to *Chrysaora pacifica* (Goette, 1886), and provide a re-description accordingly. The molecular identity of *C. pacifica* was further confirmed by comparison of nuclear ribosomal DNA sequences.

Key words : sea nettle, *Chrysaora pacifica*, scyphomedusae, rDNA

1. Introduction

The genus *Chrysaora* Péron and Lesueur, 1810 contains more than 10 species. Due to morphological similarities among species, this genus has much of the confusion in their taxonomic descriptions. According to authors, numbers of nominal species in this genus were varied: for instances, 11 (Péron and Lesueur 1810), 6 (Eschscholtz 1829), 13 (Lesson 1843), 9 (Agassiz 1862), 10 (Haeckel 1880), 15 (Mayer 1910) and 11 (Kramp 1961). Most recently, Morandini and Marques (2010) re-described 15 species within the genus *Chrysaora* on the basis of morphology, geographical distribution, and phylogeny. These taxonomic ambiguities of jellyfish morphology can be solved partly by molecular approaches (e.g. Collins et al. 2006; Ki et al. 2008). These methods

led to differentiating cryptic jellyfish species, studying population genetics, and assessing the extent and impacts of invasions (Holland 2000; Wares et al. 2005).

In Korea, Park (2002) firstly described *Dactylometra quinquecirrha* Agassiz, 1862 using a specimen collected from Korean southern coasts. However identity of the species has been questioned (Bayha KM, Personal communication, Jul 26, 2007; Lee et al. 2013), because *D. quinquecirrha* is distributed only in the Gulf of Mexico, not other oceanic regions (Morandini and Marques 2010). Instead, *Chrysaora pacifica*, which is morphologically similar to *D. quinquecirrha*, distributes around Japanese coastal waters in North Pacific Ocean. In addition, phylogenetic comparison using molecular survey of Korean jellyfishes showed that it was closer to *Chrysaora melanaster* sensu lato than *Dactylometra*. Thus, we have presumed that *D. quinquecirrha* recorded in Korea by Park (2002) may be the other species belonging to *Chrysaora*,

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such as *C. melanaster* or *C. pacifica*. Although there are some differences in morphological characteristics and geographical distributions, *C. pacifica* has often been misidentified several times as *C. melanaster*.

In the present study, we re-describe morphology and DNA characters of already-known Korean *D. quinquecirrha* using specimens collected from Gyeongnam province, Korea, 2014. These results were compared with other species of the genera such as *Chrysaora* and *Dactylometra*, defining identity of Korean jellyfish.

2. Materials and Methods

Sample collection and morphological description

Jellyfish samples were collected at Namildae Beach (in Sacheon), Tongyeong, Jindong, and Jangmok in Korea during August 2014 (Fig. 1). Samples were obtained using hand nets or plastic buckets to avoid the damage of jellyfish. As morphological characters, we measured diameter of umbrella, and then immediately preserved whole samples with 5% formalin in seawater. To analyze DNA of nuclear ribosomal DNA (rDNA), oral arm and gonad tissues were preserved in 100% ethanol solution (Merk, Darmstadt, Germany).

Morphological characters were examined by using the preserved specimens collected from Namildae Beach (34°55.59N, 128°5.79E), Sacheon, Korea, on 12 August, 2014. We observed morphologies, including exumbrella, subumbrella, oral arms, marginal lappets, rhopalia, tentacles, and stomach using photos, and drew key characters. The number of tentacles and lappets were counted and expressed as the number per octant.

DNA extraction and molecular analysis

Prior to genomic DNA extraction, alcohol-preserved jellyfishes were washed in distilled water to remove all ethanol, and these procedures were repeated several times through overnight. Total genomic DNA was extracted using the CTAB (Cetyl Trimethylammonium Bromide) method described by Ausubel et al. (1989). Nuclear rDNA sequences spanning 18S rDNA to 28S rDNA gene were amplified by PCR using two Eukaryotic universal primers (a forward 18F01, 5'-TCG GCA GGT GAG TTG TTA CAC-3'; a reverse 28R1318, 5'-TGG TTG ATC CTG CCA GTA G-3'), according to Ki and Han (2005). PCR amplicons were then purified with QIAquick PCR purification Kit (Qiagen GmbH, Germany) and sequenced on a 3730XI DNA analyzer (Applied Biosystems, USA). Editing and contig assembly of the rDNA sequence fragments were carried out with Sequencher 4.7 (Gene Codes, MI, USA).

For molecular relationships, we constructed phylogenetic trees of separate 18S and 28S rDNA sequences belonging to Pelagiidae (Phylum Cnidaria) jellyfishes. In brief, the 18S rDNA sequences including an outgroup were aligned using Clustal W (Thompson et al. 1994). Various regions were further corrected manually, and unambiguous regions that could not be aligned were excluded from the analysis (i.e. 1,704 out of 1,830 alignment positions). Then, 18S Maximum-Likelihood (ML) trees were constructed using the General Time-Reversible (GTR) model in MEGA 6 (Tamura et al. 2013). Branch supports in the ML trees were obtained by bootstrap analysis using 1,000 replicates. Further a 28S phylogenetic tree was analyzed with a dataset (i.e. 1,014 out of 1,150 alignment positions) and

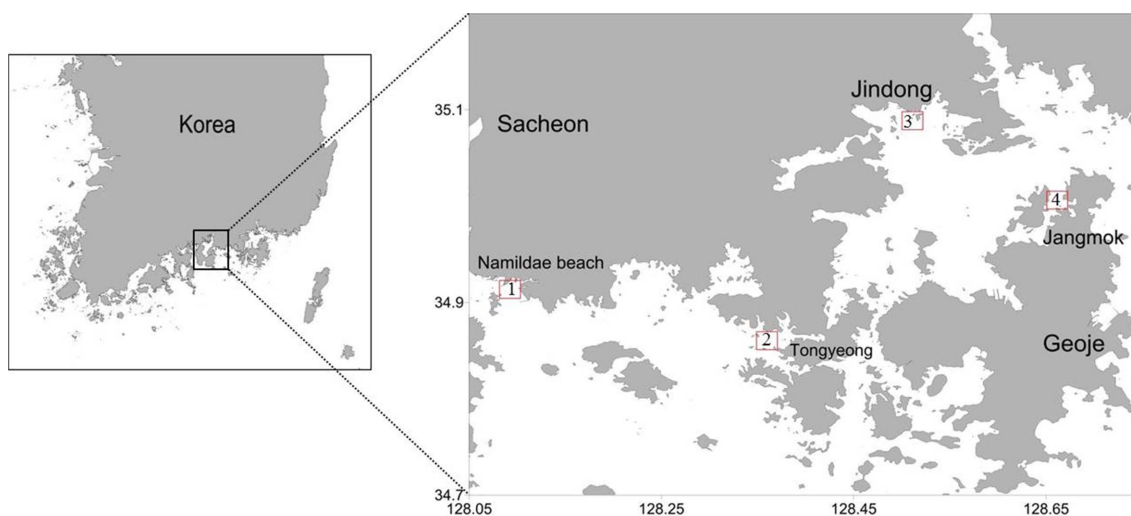


Fig. 1. A map of sampling sites of *Chrysaora pacifica* (1, Namildae beach; 2, Tongyeong; 3, Jindong; 4, Jangmok in Korea)

the same method used in 18S rDNA analysis.

3. Results and Discussion

Systematic account

Class Scyphozoa Goette, 1887
 Order Semaestomeae L. Agassiz, 1862
 Family Pelagiidae Gegenbaur, 1856
 Genus *Chrysaora* Péron and Lesueur, 1810

Chrysaora pacifica (Goette, 1886)

Dactylometra quinquecirrha var. *pacifica* Goette 1886: 834–835; Stiasny 1919: 81; Stiasny 1939: 183
Chrysaora pacifica: Kramp 1961: 327; Gershwin and Collins 2002: 128, 130, 133, Table 1, Fig. 2
Chrysaora melanaster: Calder 1972: 41; Arai 1997: 14, 134, 136, 175; Collins 2002: Table 1; Morandini, Silveira and Jarms 2004: 347, 348, 352, 353; Kawahara, Uye, Ohtsu and Iizumi 2006: 172; Kinoshita, Hiromi and Yamada 2006: 607, 611; Wares 2008: 81

Korean name

It has been named “Keoteun-wonyang-haepari” by Park (2002). “Keoteun” means a curtain and “wonyang” means offshore in Korean. This species belongs to Family Pelagiidae. Oral arms of this species are wrinkled and look like curtains. “Haepari” is Korean word indicating jellyfish.

Morphological description

Umbrella is almost hemispherical (Fig. 2A) and divided into eight sectors. The umbrella of young specimens is more slightly flattened than that of adults. The surface of exumbrella is finely granulated with 32 radial stripes.

Radial stripes start from midmost of exumbrella, some sample shows an indeterminate form at start point (sunburst type). Mesoglea is flexible and translucent and central portion is relatively thick, thinner at edge. Musculature is not distinguishable. Mouth is at the center of subumbrella, with 4 oral arms (Fig. 2B). Oral arms are approximately two-three times longer than diameter of umbrella. They look delicate, curtain-like. The margin of each oral arm is finely fringed and there are numerous nematocyst warts on the surface (Fig. 2C). Distal portion of oral arm is slightly spiraled (Fig. 2D). The marginal lappets are rounded (Fig. 3A, B) and there are six lappets per octant. Total number of lappets is 48 and there are numerous nematocyst warts on its external surface. The number of rhopalia is eight. Sense organs, rhopalia are located in deep clefts between lappets and contain a concretion without ocelli (Fig. 3C). Tentacle clefts vary in depth and five tentacles per octant are on the marginal lappets (Fig. 3D). Total number of tentacle is 40. The tentacle is extremely elastic and length of tentacles is two-three times longer than diameter of umbrella in live samples (Fig. 2A). Central stomach is circular and gastric filaments are in four interradial fields. Gonads are outlining gastric filaments and look like semicircular ring with greatly folded form.

Color

The color of the exumbrellar is whitish with thin brownish radiating stripes. Oral arm is milky white. Tentacles are brownish-orange color.

Size

The bell diameter of the collected specimens ranged between 15 and 21 cm.

Table 1. Comparison of *Chrysaora pacifica*, *C. melanaster* and *C. quinquecirrha*

	<i>C. pacifica</i>	<i>C. melanaster</i>	<i>C. quinquecirrha</i>
Tentacle number	40	24	40
Lappet number	48	32	48
Bell size (cm)	Up to 20	20–40	Up to 40
Umbrella pattern	Thirty-two brownish radiating stripes and finely granulated on exumbrella	Thirty-two brown rays on exumbrella and 16 black streaks on subumbrella	Background color whitish, pinkish or yellowish, sometimes with reddish to brownish specking and streaks
Distribution	North Pacific Ocean (Korea, Japan)	North Pacific Ocean (Kamchaka–Russia; Aleutian Islands, Bering Sea, Alaska, Oregon–USA)	Western North Atlantic, east coast of USA
Reference	Morandini & Marques 2010; this study	Gershwin & Collins 2002; Morandini & Marques 2010	Gershwin and Collins 2002; Morandini and Marques 2010

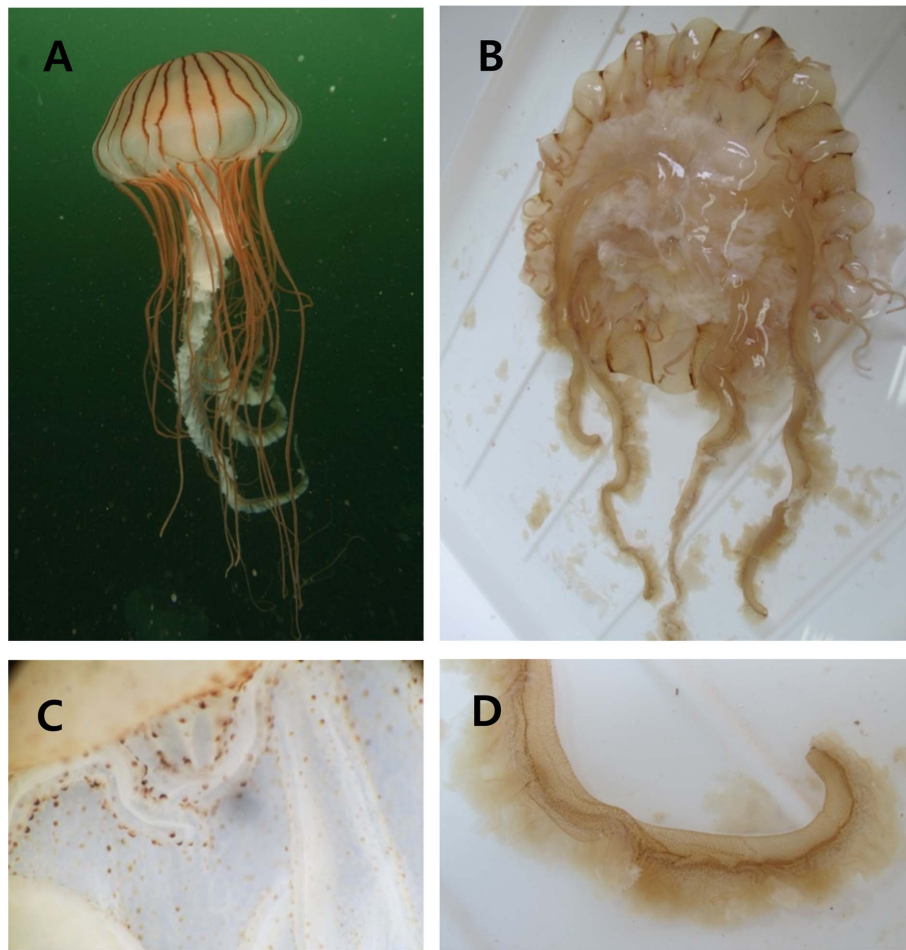


Fig. 2. *Chrysaora pacifica*. A, exumbrellar view of live sample; B, subumbrellar view of specimen; C, numerous nematocyst warts of oral arm; D, oral arm of specimen

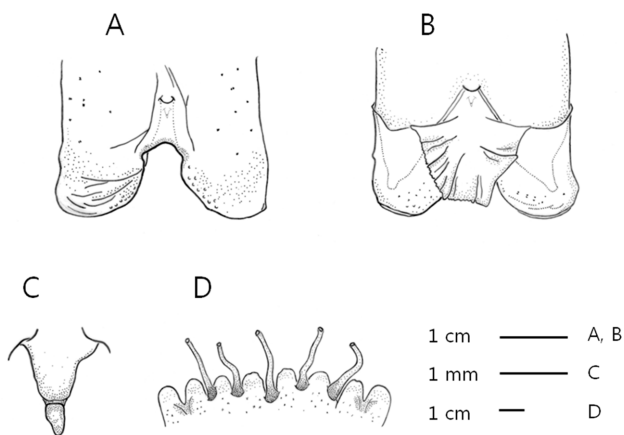


Fig. 3. *Chrysaora pacifica*. A, marginal lappet (exumbrellar view); B, marginal lappet (subumbrellar view); C, rhopalia; D, tentacles

Distribution

This species is distributed in North Pacific Ocean (most

Japanese coastal waters) (Morandini and Marques, 2010) and most coastal waters in Korea (mainly southern and eastern) and usually appeared from May to September.

Phylogeny of jellyfish, including the genus *Chrysaora*

The 18S and 28S rDNA sequences of *Chrysaora pacifica* determined here were deposited in GenBank (Accession Nos. KY249594 and KY249595). Pairwise genetic distances of 18S and 28S rDNA among Genus *Chrysaora* were calculated (Table 2), revealing that 18S rDNA sequences of the Korean *Chrysaora pacifica* were 100% similarity to that of *C. pacifica* available in GenBank (KY212123). However, it was different to those of *C. melanaster* (98.8%) and *C. quinquecirrha* (99.1%). In addition, its 28S sequence was 100% identical to that of *C. pacifica* (KY212123) and 94.7% to *C. melanaster*, respectively. Phylogenetic relationships of Pelagiidae jellyfishes, including *Chrysaora pacifica*, were inferred from their 18S, 28S rDNAs, respectively (Fig. 4).

Table 2. Pairwise genetic distances calculated for the regions of 18S (nearly complete) and 28S (partial sequence) among species of genus *Chrysaora*

	[2]	[3]	[4]	[5]	[6]	[7]	[8]
18S rDNA							
[1] <i>Chrysaora</i> sp. (KY249594)	100	98.8	93.4	98.9	98.8	99.1	99.2
[2] <i>C. pacifica</i> (KY212123)		98.8	93.4	98.9	98.8	99.1	99.2
[3] <i>C. melanaster</i> (AF358099)			94	99.8	99.6	98.5	98.5
[4] <i>C. helvola</i> (JX393280)				94	93.8	93.4	93.3
[5] <i>C. colorata</i> (AF358098)					99.7	98.6	98.6
[6] <i>C. fuscescens</i> (JX393279)						98.5	98.5
[7] <i>C. quinquecirrha</i> (JX393282)							99.6
[8] <i>C. lactea</i> (HM194810)							
28S rDNA							
[1] <i>Chrysaora</i> sp. (KY249595)	100	94.7	94.7	91.8			
[2] <i>C. pacifica</i> (KY212123)		94.7	94.7	91.8			
[3] <i>C. melanaster</i> (AY920780)			98.8	91.5			
[4] <i>C. fuscescens</i> (HM194868)				91.9			
[5] <i>C. lactea</i> (HM194863)							

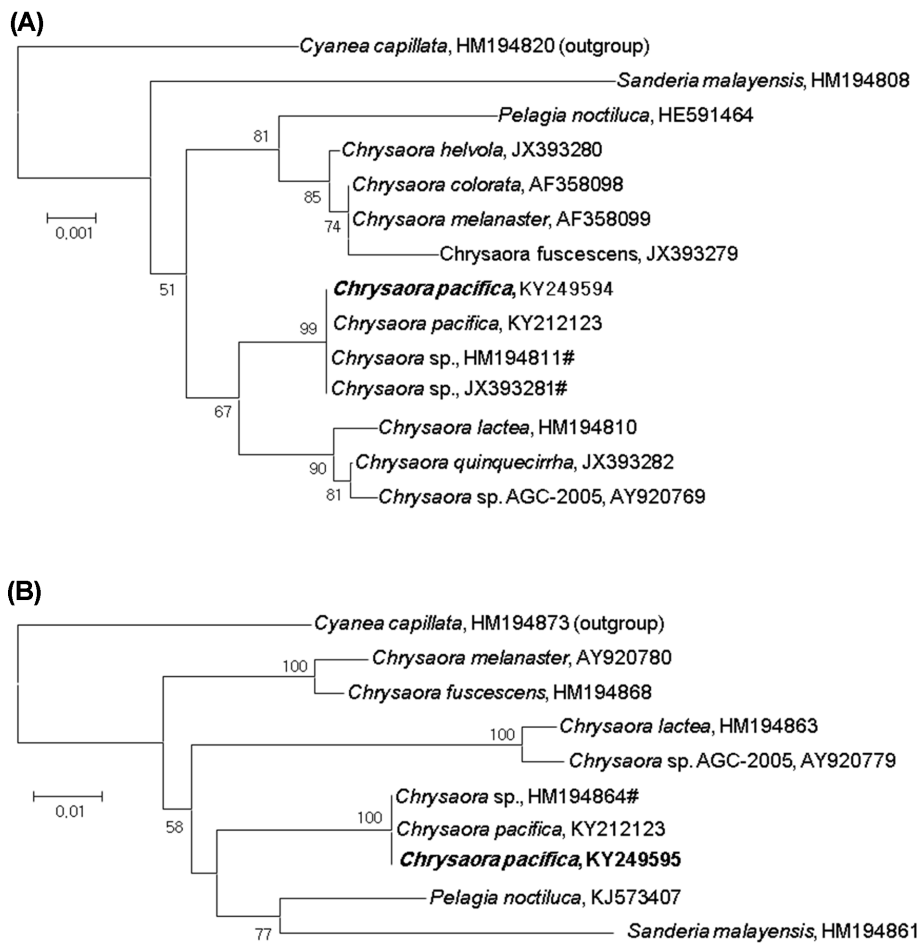


Fig. 4. Phylogenetic ML trees of Pelagiidae jellyfishes, including *Chrysaora pacifica*, using 18S rDNA (A) and 28S rDNA (B). Numerals above the branches refer to the percentage of the 1000 bootstrap replications supporting each node. # Represents tentatively assigned name, because its species did not be confirmed

Phylogenetic trees showed that our *C. pacifica* was clustered with other sequences of the same species, but it was segregated clearly to other species with high bootstrap supports. Molecular comparisons suggested that the Korean sea nettle was affiliated to *C. pacifica*, separating from close relatives *C. melanaster* and *C. quinquecirrha*.

4. Remarks and Conclusions

Chrysaora pacifica is common species in Korean coastal waters (mainly southern and eastern) every year from spring to summer. Because the sting of this species is serious, it is known as a dangerous jellyfish to swimmers and fishermen in Korea. Park (2002) recorded this species as *Dactylometra quinquecirrha*. However, the genus *Dactylometra* is not used taxonomically at present, thus *D. quinquecirrha* is accepted by *C. quinquecirrha* (Morandini and Marques 2010; WoRMS 2016). We think that there must have been some mistakes in Park's identification, because her morphological description of *D. quinquecirrha* was similar to those of *C. pacifica*. Umbrella of *C. quinquecirrha* has plain pattern and larger than that of *C. pacifica* which has stripe pattern. Moreover, *C. quinquecirrha* may be distributed only in eastern USA and Gulf of Mexico (Gershwin and Collins 2002).

C. pacifica is closely related to *C. melanaster*. However, there are some differences in morphology between these two species. The number of tentacles is different: *C. pacifica* has 40 tentacles, while *C. melanaster* has 24 tentacles. *C. pacifica* has up to 48 lappets, while *C. melanaster* has 32. *C. melanaster* can grow larger than 30 cm in bell diameter, whereas *C. pacifica* is much smaller (Morandini and Marques 2010). Moreover, *C. pacifica* is not a worldwide species, its distribution is restricted to the North Pacific Oceans (around Japan) and it thrives in sub-tropical temperatures. Toyokawa (2011) found wild polyps of this species in Sagami Bay, Japan. In contrast, Bering Sea nettle, *C. melanaster* is commonly found in sub-freezing waters (Kamchatka-Russia; Aleutian Islands, Bering Sea, Alaska, Oregon-USA, reviewed by Morandini and Marques 2010). In addition, the morphology of *C. pacifica* is very similar to *C. chinensis*, the only known morphological difference is a size of the holotrichous O-isoriza nematocysts. And *C. chinensis* is distributed only in a warmer area (western Pacific Ocean: China, Indonesia, Philippines, Sumatra) than *C. pacifica*.

To conclude, our results of morphological and molecular

analyses indicate that the sea nettle jellyfish collected in southern Korea was *Chrysaora pacifica*, not *Dactylometra quinquecirrha*.

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