First Record of Box Jellyfish, *Carybdea brevipedalia* (Cnidaria: Cubozoa: Carybdeidae) from Korean Coastal Waters: Morphology and Molecular Descriptions

Jinho Chae¹, Won-Duk Yoon², ByeongHo Kim¹, Jang-Seu Ki³,*

¹Marine Environmental Research and Information Laboratory, Gunpo 15850, Korea
²Southeast Sea Fisheries Research Institute, National Institute of Fisheries Science, Tongyeong 53085, Korea
³Department of Life Science, Sangmyung University, Seoul 03016, Korea

ABSTRACT

We firstly described a box jellyfish, *Carybdea brevipedalia* collected from the southern coasts of Korea. It is morphologically characterized by gastric phacellae, a special digestive system of cubozoan jellyfish, and velarium, the thin muscle flap forming the opening of the subumbrellar cavity. The phacellae are linear-shaped, comprising numerous cirri branched from multiple roots. Each root also has multiple numbers of cirrus bundles. Basis of velarial cannels parts into two branches in each octant of velarium. Its geographic distributions were limited to Jeju-do and the middle-southern coasts where the organisms are seriously hazardous to bathers in summer. Numerous individuals and/or large-sized populations were observed specifically from beaches at Jeju-do, Namhae-do and a small fishing port of Namildae, while only a small amount of the individuals was observed in Gamak Bay, Jaran-Goseung Bay, and Geoje-do. We confirmed molecular identity of the Korean *C. brevipedalia* with comparison of nuclear ribosomal DNA sequences. Until now, *Carybdea brevipedalia* is the only cubozoan species reported to be distributed in Korean waters.

Keywords: Cubozoa, box jellyfish, Carybdeidae, *Carybdea brevipedalia*, new record, Korea

INTRODUCTION

Cubozoa, commonly called as box jellyfishes, is one of the smallest classes of medusozoan cnidarians, comprising approximately 50 species described (Bentlage et al., 2010; Bentlage and Lewis, 2012). The class involves two orders, Chirodropida and Carybdeida; in morphological comparisons, the carybdeid jellyfishes have only a single tentacle per pedædium in contrast to chirodropids.

Most species of box jellyfishes are venomous, which can cause medically significant stings (Gershwin et al., 2010). Chirodropid species such as *Chironex fleckeri* and *C. yamaguchii* are especially world’s most venomous marine creatures (Fenner and Williamson, 1996; Wiltshire et al., 2000), which can kill a healthy adult human within minutes. Envenomations by carybdeids are generally considered less of a threat, even though they may still be severely painful or lethal in some cases such as *Carukia barnesi* and *Malo kingi*, which cause irukanzi syndrome (Barnes, 1964; Fenner and Hadok, 2002; Gershwin et al., 2010; see review by Bentlage et al., 2010). High haemolytic activity was also reported in *Carybdea* species (Nagai et al., 2000; Bailey et al., 2005). Of them, *Carybdea brevipedalia* can cause severe pain and leave red marks where tentacles touch, which may be related to longer discharged nematocyst tubules (Kitatani et al., 2015).

Box jellyfish has received attention in Korea since 2013, when approximately 500 people were stung in Jeju-do and Namhae-do, according to official records from Jeju Fire Safety Headquarters and Namhae-Gun Health Center. Hundreds of victims of box jellyfish have been continuously recorded every year in Namhae-do, leading to necessity of study on the countermeasures. However, there are no records of any single species of cubozoan jellyfish in Korean waters, while we preliminarily identified the stinging species as *C. brevipedalia*. 
First Record of Box Jellyfish, Carybdea brevipedalia

Carybdea brevipedalia was firstly described in western Japan (Kishinouye, 1891) and has been known to be distributed broadly in the coastal waters through Japan (Uchida, 1929, 1938a, 1938b; Ueno et al., 1995). However, the species has never been recorded from waters of other countries, including Korea. In this study, we newly record the species from Korean waters with morphological description and molecular confirmation.

MATERIALS AND METHODS

Sample collection, morphological analysis and description
Through the present jellyfish surveys, we observed occurrences of tentatively-assigned Carybdea brevipedalia at Gimnyeong, Pyoseon, Hwasun, Yihoteu, and Hamdeok Beaches in Jeju-do, ENGang Bay (Wolpo-Dugok and Woncheon Beaches), Sangju and Songjeong Beaches in Namhae-do, Namildae in Samcheonpo, Goseong Bay, Jaran Bay, and Guyeong Beach in Geoje-do.

We used a high resolution camera (D810; Nikon, Tokyo, Japan) with close-up lenses (Micro-Nikkor 60 mm f2.8; Nikon and Makro-planar 100 mm f2.0; Carl Zeiss, Oberkochen, Germany, with a close-up tube, PK-13, Nikon) for recording morphology of live specimen as a whole without distortion or loss of any characters after fixing. Observations on small organs such as tentacle bands, nematocyst clusters, nematocyst-wart patterns, velarial cannels and rhophalia of both live and fixed samples were made under a stereo-microscope (SZX12; Olympus, Tokyo, Japan). For general terminology for taxonomy of carybdeid jellyfish, we followed Bentlage and Lewis (2012), Gershwin (2005), and/or Toshino et al. (2015).

DNA extraction and molecular analysis
Prior to genomic DNA extraction, alcohol-preserved jellyfishes were washed in distilled water to remove all ethanol, and repeated several times through overnight. Total genomic DNA was extracted using the cetyl trimethylammonium bromide method described by Ausubel et al. (1989). Nuclear ribosomal DNA (rDNA) sequences spanning 18S rDNA to 28S rDNA gene were amplified by PCR using two Eukaryotic universal primers (a forward 18F01, 5’-TCG CCA 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, Hilden, Germany) and sequenced on a 3730Xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). Editing and contig assembly of the rDNA sequence fragments were carried out with Sequencher 4.7 (Gene Codes, Ann Arbor, MI, USA).

For molecular relationships, we constructed phylogenetic trees of separate 18S-28S rDNA sequences belonging to Carybdeida. 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, an 18S maximum likelihood (ML) tree was constructed by 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 the same manner used in the 18S rDNA analysis.

**SYSTEMATIC ACCOUNTS**

Class Cubozoa Werner, 1973
Order Carybdeidae Gegenbaur, 1857
Family Carybdeidae Gegenbaur, 1857
Genus *Carybdea* Péron and Lesueur, 1810

*Carybdea brevipedalia* Kishinouye, 1891 (Figs. 2–4)

*Carybdea brevipedalia*: Kishinouye, 1891: 437–439, figs. 1–3; Bentlage et al., 2010: 493–501, figs. 1–3; Bentlage & Lewis, 2012: 2599–2607, fig. 3.

*Carybdea mora*: Kishinouye, 1910: 6, Pl. 1, figs. 4–9; Gershwin & Gibbons, 2009: 47–49, table 1.

‘Jagyeun’ and ‘Sangja’ mean ‘small’ and ‘a box’, respectively in Korean. The species is relatively small and has a squarish, box-like bell of typical characteristics of Cubozoa. ‘Haepari’ is Korean word indicating the jellyfish.

**Material examined.** Korea: 10 individuals, Jeju-do: Gimnyeong and Pyseon Beach, 13 Aug 2013, Yoon WD, Chae J; 13 individuals, Namhae-do, Wolpo-Dugok Beach, 10 Sep 2013, Chae J, Kim B; 24 individuals, Namildae, 10 Sep 2015, Chae J, Ullah MS.

**Morphological description.** Bell height 2.5–4.0 cm (mean = 2.9±0.4). Bell sharp cylindrical on the whole (Figs. 2A–C, 3G, H) with almost flat apex (Figs. 2C, D, 3A); becomes slightly narrower and round toward the end (Fig. 2B–D). Nematocyst warts round shaped; scattered through most of surface of bell (exumbrella) (Figs. 2A, C, D, 3A, D). Bell height of examined specimen of adults ranged from 2.6 to 4.1 cm (average 3.3±0.5, n=10), from 2.4 to 3.6 cm (average 2.9±0.4, n=13), and from 2.0 to 4.0 cm (average 2.8±0.4, n=24) at Jeju-do, Namhae-do, and Namildae, respectively.

Four gastric phacellae linear shaped; located in corner of stomach (Fig. 2B–D). Each phacella comprises multiple roots of cirrus bundles; each root has multiple numbers of cirri (Fig. 4A, B). Gonads leaf-shaped; attached on four corners of inner wall in subumbrellar cavity (Figs. 2B, 3D, E); covering almost full length of lateral inner bell (Fig. 3D). Manubrium four branched (Figs. 2C, D, 3A, 4C); length approximately one third (sometimes elongates to one half) of height of subumbrellar cavity when alive. Inner wing of pedalia scalpel-shaped (Figs. 2E, 3D); nematocyst warts scattered on outer pedalia with a row of larger ones near the outmost edge; no nematocyst warts on inner wing of pedalia (Figs. 2E, 3D).

Four rhopalia in rhopalia niche, the cavity opening to the environment, located one-fourth downward part of bell (Fig. 2C, F–I); each comprising one upper lens eye, one lower lens eye, one pair of pit eyes and one pair of slit eyes with a statocyst (Fig. 4D–F); rhopalia niche ostia, the exumbrellar openings of the rhopalia niche, heart-shaped (Fig. 4E, F).

One tentacle on each corner of bell (Fig. 2A, B). Tentacle banding pattern normal type (simple bands) with dense nematocysts (Fig. 2J–L); little space between bands near pedalia (Fig. 2J), while rest extremely elastic in live specimen (Fig. 2K). Velarial canals transparent in live samples (Fig. 3F–I), while dark-colored in fixed ones (Fig. 4G–L); two-branched in each octant of velarium, thin muscle flap forming the opening of the subumbrellar cavity (Fig. 4G–L).

**Distribution.** Pacific Ocean: Korea (South Sea), Japan (Hokkaido, Honshu, Shikoku and Kyushu).

**Remarks.** The species has been recorded only in Japan before the present study. Type specimen was recorded from Wagv village, Shima-zo, Mie prefecture, Japan. It is widely distributed in coastal area from Hokkaido to southern Kyushu, Japan.

Photomicrograph of nematocyst morphology of *Carybdea brevipedalia* occurred in Korean waters is shown in Pyo et al. (2016, fig. 1B), while the species is named as *C. mora*, the junior synonym now accepted as *C. brevipedalia* (see Gershwin and Gibbons, 2009; http://www.marinespecies.org). According to the drawings of Kishinouye (1910, Pl. I, figs. 4–9), it is suggested that *C. mora* was young medu-
Fig. 2. Morphological key characters of *Carybdea brevipedalia*. All photographs were gained from live individuals in situ (A-I) or under a stereo-microscope (J-L). A, Major taxonomic characteristics, lateral view; B, Dorsal view; C, Gastric phacellae, cubozoan digestive system in stomach, manubrium and location of rhopalium; D, Bundles of gastric cirri (gastric filaments) comprising gastric phacellae; E, Pedalia and pedalial canal with nematocyst clusters (nematocyst wart); F-I, Rhopalium from various angles; J, Tentacle near pedalium with numerous nematocysts; K, Middle part of tentacle; L, Tentacle bands with numerous nematocysts. ma, manubrium; ne, nematocyst; pc, pedalial canal; pe, pedalium; ph, phacellae; rh, rhopalium; te, tentacle; ve, velarium; vec, velarial canal. Scale bars: A, B = 1 cm, C-E = 0.5 cm, F-L = 1 mm.
Fig. 3. Key characters of *Carybdea brevipedalia*. All photographs were gained from live individuals in situ. A, Stomach and manubrium; B, Pedalia and upper tentacle; C, Middle of tentacle; D, Pedalium; E, Gonad from dorsal view; F-I, Velarial canal from various angles of alive individuals. ma, manubrium; new; nematocyst wart; pc, pedalial canal; pcb, pedalial canal band; pe, pedalium; ve, velarium; vec, velarial canals. Scale bars: A-I = 0.5 cm.
sa individuals of *C. brevipedalia* (Pitt and Lucas, 2014). In addition, *C. brevipedalia* has been most likely to be confused with *C. rastonii* (see Uchida, 1929, 1938a, 1938b; Kramp, 1961; Ueno et al., 1995), because morphological differences of these two species may not to be conspicuous. However, recent molecular phylogenetic analyses (see below) suggest that *C. rastonii* reported from Japan should be regarded as *C. brevipedalia* (Bentlage et al., 2010; Bentlage and Lewis, 2012; see also Toshino et al., 2015), while *C. rastonii* has been recorded from Hawaii and Australia (May-

Fig. 4. Key characters of *Carybdea brevipedalia*, individuals fixed with formalin. A, B, Gastic cirri (gastric filaments); C, Manubrium from ventral view; D–F, Rhopaliar from various angles of each different individuals, heart-shaped rhopaliar niche ostium and structure of rhopaliar comprised with eyes and statocyst; G–L, Velarial canal of various individuals. ma, manubrium; new; nematocyst wart; rn, rhopalia niche; rno, rhopalia niche ostium; rh, rhopalium; st, statocyst; vec, velarial canal. Scale bars: A, B, D–F = 1 mm, C = 0.2 cm, G–L = 0.5 cm.
Table 1. Pairwise genetic distances calculated for the regions of 18S (nearly complete) and 28S (partial sequence) among species of the genus *Carybdea*

|          | [1] | [2] | [3] | [4] | [5] |
|----------|-----|-----|-----|-----|-----|
| 18S rDNA |     |     |     |     |     |
| [1] *Carybdea arborifera* (GQ849091) |     |     |     |     |     |
| [2] *Carybdea branchi* (GQ849089) | 0.005 |     |     |     |     |
| [3] *Carybdea brevipedalia* (KY212121) | 0 | 0.005 |     |     |     |
| [4] *Carybdea cf. rastonii* (GQ849094) | 0.002 | 0.004 | 0.002 |     |     |
| [5] *Carybdea rastonii* (AF358108) | 0.002 | 0.004 | 0.002 | 0 |     |
| [6] *Carybdea xaymacana* (GQ849090) | 0.013 | 0.014 | 0.013 | 0.013 | 0.013 |
| 28S rDNA |     |     |     |     |     |
| [1] *Carybdea arborifera* (GQ849068) |     |     |     |     |     |
| [2] *Carybdea branchi* (GQ849066) | 0.070 |     |     |     |     |
| [3] *Carybdea brevipedalia* (KY212122) | 0.018 | 0.065 |     |     |     |
| [4] *Carybdea cf. rastonii* (GQ849071) | 0.035 | 0.063 | 0.021 |     |     |
| [5] *Carybdea rastonii* (AY920787) | 0.037 | 0.064 | 0.024 | 0.006 |     |
| [6] *Carybdea xaymacana* (GQ860995) | 0.112 | 0.100 | 0.109 | 0.108 | 0.109 |

Fig. 5. Phylogenetic maximum likelihood trees of Carybdeidae jellyfishes, including the genera *Alatina*, *Carukia*, *Carybdea*, *Copula*, *Malo*, *Morbakka*, *Tamoya*, and *Tripedalia*, using 18S rDNA (LogL = −4,422.64) (A) and 28S rDNA (LogL = −7,127.83) (B). Numerals above the branches refer to the percentage of the 1,000 bootstrap replications supporting each node. Bold represents our data.
er, 1906; Gershwin and Gibbons, 2009; Pitt and Lucas, 2014).

The 18S and 28S rDNA sequences of C. brevipedalia determined here were deposited in GenBank (accession Nos. KY212121 and KY212122). Pairwise genetic distances of 18S and 28S rDNA among genus Carybdea were calculated (Table 1), revealing that 18S rDNA sequences of the Korean C. brevipedalia were zero (100% similarity) to that of C. brevipedalia (GQ849093) and 0.002 (99.7% identity) to C. rastonii (AF358108). In addition, the 28S sequence of Korean C. brevipedalia was zero (100% sequence similarity) to those of C. brevipedalia (GQ849069, GQ849070), 0.018 (97.9% similarity) to C. arborifera (GQ849068), and 0.037 (96.5% similarity) to C. rastonii (AY920787), respectively. Phylogenetic relationships of Carybdea jellyfishes, including the genera Alatina, Carukia, Carybdea, Copula, Malo, Morbakka, Tamoya, and Tripedalia, were inferred from their 18S and 28S rDNAs, respectively (Fig. 5). Phylogenetic trees showed that the Korean C. brevipedalia is clustered with the same species, but clearly segregated to other species with high bootstrap supports. The 28S ML tree was more clearly separated each species than those of 18S rDNA. Carybdea brevipedalia was a sister relationship with C. arborifera.

This study firstly described morphological characteristics of a box jellyfish occurred in southern coasts of Korea, showed its distribution localities and confirmed its species identity by molecular analysis. In conclusion, Carybdea brevipedalia is the only species of cubozoan jellyfish found in Korean waters up to date.

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