Development of *Carybdea brevipedalia* Kishinouye, 1891 (Cnidaria: Cubozoa: Carybdeida: Carybdeidae) collected from northern Japan

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**Abstract:** Envenomation by toxic box jellyfish species is known to be a serious problem to public health. In order to elucidate the problem, it becomes necessary to predict the occurrence of box jellyfishes, as well as understanding their ecology and life cycle. Mature medusae of *Carybdea brevipedalia* (Cubozoa: Carybdeida), which is a common species of box jellyfish in Japan, were collected from northern Japan to observe its early life history, including polyp formation. Fertilization occurred externally, and blastulae developed into planulae. Free swimming planulae settled and metamorphosed into tiny primary polyps with two forms, i.e. settled and creeper. Adult polyps formed cysts at temperatures below 15°C or when water replacement and/or feeding was stopped. Budding occurred in four-tentacled polyps, and the buds were released after commencement of budding. Complete metamorphosis of a whole polyp into a single medusa occurred at stable temperatures between 18 to 25°C (18, 20, 23, 25°C, respectively) or when temperatures were raised from 20 to 25°C. Newly released medusae had four tentacles. Our study demonstrated that polyps of *C. brevipedalia* survive and propagate over a wide range of water temperatures and that developmental features resemble closely those of some tripedaliid species, namely *Tripedalia cystophora* and *Copula sivickisi*, rather than *Carybdea marsupialis*. The morphological affinities of polyp in *C. brevipedalia*, *T. cystophora* and *C. sivickisi* support recent molecular results. However, further studies are needed to confirm the morphological contradiction between *C. brevipedalia* and *C. marsupialis* in the future.

**Key words:** box jellyfish, envenomation, life cycle, medusa, polyp

**Introduction**

The class Cubozoa, commonly called box jellyfish, has a wide distribution, with reports from tropical, sub-tropical and mild temperature localities in the Pacific, Atlantic and the Indian Ocean (Bentlage and Lewis 2012). Envenomations by cubozoans are well known as they can pose a serious threat to public health (Williamson et al. 1996). Several studies have been undertaken to investigate and understand the organisms including taxonomy (Gershwin 2005, 2006a, Bentlage and Lewis 2012), ecology (Lewis et al. 2008, Chiaverano et al. 2013, Kondo et al. 2018), envenomation (Williamson et al. 1980, 1996, Carrette and Seymour 2013), venom function and composition (Nagai et al. 2000, 2002, Nagai 2003, Underwood and Seymour 2007, Brinkman and Burnell 2009) and nematocysts (Yanagihara et al. 2002, Gershwin 2006b). At present, about 50 described cubozoan species are recognized, however, parts of the life cycle of only ten species are properly understood (Okada 1927, Werner et al. 1971, Studebaker 1972, Arneson and Cutress 1976, Yamaguchi and Hartwick 1980, Hartwick 1991, Toshino et al. 2013, 2015, Straehler-Pohl and Jarms 2011, Courtney et al. 2016, Toshino et al. 2016). Cubozoans have an asexual benthic polyp and a sexual planktonic medusa in their life cycles (Werner et al. 1971, Studebaker 1972, Arneson and Cutress 1976). A cubopolyp metamorphoses into a single medusa without residuum and this is unique within the phylum Cnidaria (Werner 1973). However, recent studies have reported that polyps of *Ca-
Development of Carybdea brevipedalia

Carybdea brevipedalia (Linnaeus, 1758), Morbakka virulenta (Kishinouye, 1910) and Carukia barnesi Southcott, 1967 exhibit a modified strobilation (Straehler-Pohl and Jarms 2005, Toshino et al. 2015, Courtney et al. 2016).

Carybdea brevipedalia Kishinouye, 1891 is the most common cubozoan species in Japan, with a maximum bell height and bell diameter of 44 mm and 33 mm, respectively, and extended tentacles of approximate length 50 cm (Uchida 1929). The common name for the species in Japan is "Andon-Kurage" because when swimming it resembles the Japanese traditional lamp "Andon". Carybdea brevipedalia was incorrectly identified as Carybdea rastonii by many biologists in the past (Uchida 1929, 1970, Kramp 1961). However, recent taxonomic investigations and molecular phylogenetic analyses suggest that C. rastonii from Japanese waters should be regarded as C. brevipedalia (Bentlage et al. 2010, Straehler-Pohl et al. 2017).

Medusae of C. brevipedalia have been reported from along the coastline of Japan during summer and fall (Kishinouye 1891, 1910, Uchida 1927, 1938, 1940, 1947, 1954, 1970, Yamazi 1958, Horita 1996, Kubota 1998). The medusae congregate in calm bays, coves and near beaches (Kishinouye 1910, Uchida 1929). The venomous sting of the medusae causes painful wounds to divers, fishermen and sea-bathers (Nagai et al. 2000). In order to investigate the causes of the blooms of C. brevipedalia, it is necessary to understand its life cycle and ecology. However, currently only the young medusae and embryological development of the life cycle of C. brevipedalia have been described (Uchida 1926, Okada 1927). The present study describes the development of C. brevipedalia from fertilized eggs to polyp formation to metamorphosis into medusae. These observations will be used to compare this species to other cubozoans to highlight potential relatedness.

Materials & Methods

Light traps were used because they are an effective method to collect cubomedusae, since box jellyfish exhibit positive phototaxis. In this study, seven mature medusae of Carybdea brevipedalia (Fig. 1) were collected using a ladle (170 mm in diameter) attached to the tip of a long rod (5 m in length); the individuals were attracted with an underwater fish-luring lamp (YF-500, Hapyson, Japan) at Onahama Port, Fukushima Prefecture, northern Japan (36°56'29.1"N, 140°54'.32.9"E; Fig. 2), between 18:30 and 20:30 on November 2, 2013 (water temperature 17.0°C, salinity 33). Medusae were transferred into a bucket with 20 L of freshwater at about 20°C, for transport. Spawning of box jellyfishes has been observed in dark conditions (Lewis and Long 2005, Toshino et al. 2013). In order to induce spawning, male and female medusae were kept in the bucket at about 20°C with dark and light (room lighting) treatments, respectively, in the laboratory at Kitasato University. Replacement of cultured water was halted for 24 hours to facilitate reproduction. Sex of the medusae was determined under an optical microscope (CX 21, Olympus, Japan) by observing the condition of the gonads. Fertilized eggs were collected by filtration of the culturing water, using a fine mesh (size 41 µm). A total of several thousand eggs were incubated in petri dishes (diameter 75 mm, height 45 mm) with filtered seawater (mesh size 0.22 µm) at about 20°C. The development of eggs was observed with a binocular microscope every day. Primary polyps developed from planulae were transferred into another petri dish (diameter 75 mm, height 45 mm). Artemia nauplii were chopped on a slide glass using a scalpel, and

Fig. 1. Carybdea brevipedalia Kishinouye, 1891, live, lateral view, in laboratory. Photo courtesy of Ryota Tamada (Nagasaki Penguin Aquarium). Scale bar = 1 cm.

Fig. 2. Map of the sampling site, Onahama port, Fukushima Prefecture, northern Japan.
fed directly to primary and secondary polyps using a fine needle every day. Water was completely replaced with filtered seawater (mesh size 1 \( \mu \)m) once a week, after a feeding event.

The adult polyps were exposed to a wide range of temperatures to determine the conditions favorable for metamorphosis. Temperatures were kept constant (10, 12, 15, 18, 20, 23, 25, 28, 30°C), raised (10\( \rightarrow \)15, 15\( \rightarrow \)20, 18\( \rightarrow \)20 and 18\( \rightarrow \)25°C) or lowered (30\( \rightarrow \)25, 25\( \rightarrow \)20, 20\( \rightarrow \)15°C) to approximate seasonal change in water temperature in the natural habitat (Table 1). During metamorphosis, the cultures were not fed nor was the water changed.

Newly detached medusae were kept in a disposable polypropylene cup (water volume 1000 mL) with filtered seawater (1 \( \mu \)m) at 23°C. Three to five individuals were reared per cup. Artemia nauplii were fed directly to the medusae on a daily basis. Culture water was replaced with fresh seawater approximately three hours after each feeding.

For nematocyst identification in the polyps (N=3), squashes prepared from fresh tissues were examined under an optical microscope (CX 21, Olympus, Japan). Nematocysts were identified according to Östman (2000), Gershwin (2006b), and Collins et al. (2011). For determination of the respective abundances of nematocyst types in polyps, a minimum of 200 nematocysts of all types were counted.

### Results

#### Fertilization and polyp formation

The transparent gonads of mature medusae became whitish to whitish-yellow in males and females. Fully mature ovaries contained mature eggs of ca. 0.10 mm in diameter (Fig. 3A, B), while testes were full of active sperm as denoted by the many fine parallel lines (see Gershwin 2005) (Fig. 3C, D). Fertilized eggs or planulae were not observed in gonads of the female medusae. In dark conditions, thousands of fertilized eggs were observed in the aquarium within 1 h, while spawning was not observed under light. Fertilized eggs were not found in the gonads of females, indicating that fertilization occurred externally. The eggs were demersal and were about 120 \( \mu \)m in diameter (Fig. 4A). When the eggs were sampled, most of them were at the two or four-cell stage (Fig. 4B, C). The eggs developed into the eight followed by the 16 (Fig. 4D) or 32-cell stage (Fig. 4E) within 2 h of initial cleavage. These eggs developed further into blastulae (Fig. 4F) within 2 h post 16-cell stage, and then developed into planulae within 8 h after the blastulae stage. Planulae (n=23) were 80 to 127 \( \mu \)m (mean: 107 \( \mu \)m) in diameter, 109 to 163 \( \mu \)m (mean: 141 \( \mu \)m) in length and had about 30 dark-reddish larval ocelli on their equatorial planes (Table 5, Fig. 4G, H). Free-swimming planulae settled within two days and metamorphosed into primary polyps within three days after settlement.

Primary polyps were either settled or actively detached to transform into a creeping phase (Fig. 5A, B). The shape of the settled polyps resembled a pouch (see Toshino et al. 2014) with a very short stalk and ovoid calyx around the mouth cone (Fig. 5A). Body length was 0.11 to 0.16 (mean: 0.15 mm) (n=6), the mouth disc diameter of the polyps was 0.05 to 0.09 (mean: 0.06 mm). The polyps in the creeping phase had a long, worm-shaped body (Fig. 5B). At a body length of 0.24 to 0.25 (mean: 0.25 mm), the mouth disc diameter of the polyps was 0.03 to 0.05 (mean: 0.05 mm). Both settled and creeping polyps had one to four tentacles and each tentacle bore two to three nematocysts (American football-shaped p-rhopaloids; see Toshino et al. 2014) in the tip of their tentacles (Fig. 6A).

The primary polyps developed into adult polyps within 80 days (Fig. 5C, D). The settled adult polyps were able to actively detach from the substrate, and creep in order to

### Table 1. Nominal temperature (°C) and duration of exposure of polyps (number) to the initial and transfer conditions.

| Initial temperature | Transfer temperature | No. of polyps |
|---------------------|----------------------|---------------|
| Constant            |                      |               |
| 10                  | 30                   |               |
| 12                  | 30                   |               |
| 15                  | 30                   |               |
| 18                  | 30                   |               |
| 20                  | 30                   |               |
| 23                  | 30                   |               |
| 25                  | 30                   |               |
| 28                  | 30                   |               |
| 30                  | 30                   |               |
| Raised              |                      |               |
| 10                  | 15                   | 30            |
| 15                  | 20                   | 30            |
| 18                  | 20                   | 30            |
| 18                  | 25                   | 30            |
| 30                  | 25                   | 30            |
| 25                  | 20                   | 30            |
| 20                  | 15                   | 30            |
| Lowered             |                      |               |

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Fig. 3. Gonad tissue from Carybdea brevipedalia. A: ovary; B: eggs; C: testis; D: sperm. Scale bar: A–C=100 \( \mu \)m; D=50 \( \mu \)m.
Development of Carybdea brevipedalia

change location (Fig. 5E). When the polyps began to creep, a peristomal cup was left behind (Table 3). The shape of the adult polyps was almost identical to the primary polyp stage, but they were larger (settled polyps: mean body length 0.50 mm, mean mouth disc diameter 0.25 mm; polyps in creeping phase: mean body length 1.30 mm, mean mouth disc diameter 0.14 mm) (Table 5, 6). Both settled and creeping polyps had three to eight tentacles and each tentacle bore five to eight American football-shaped p-rhopaloids and six to nineteen heterotrichous euryteles (Fig. 6B). The polyps (n=3) possessed three types of nemato-
cysts, viz. American football-shaped p-rhopaloids (two sizes) (Fig. 6C–F), heterotrichous euryteles (Fig. 6G, H) and spherical p-rhopaloids (Fig. 6I, J). Further developmental stages, i.e. budding and metamorphosis, only took place in settled polyps.

Asexual polyp reproduction

Asexual reproduction was observed when the 3-tentacled stage was reached (Table 2). Bud formation occurred on the middle part of the calyx. Seven days post initiation of bud formation, polyp tentacles and hypostome devel-
opened from a bud; on the next day a new polyp was released from the parent polyp. The shape of newly released buds resembled the parent polyps and the buds had three or four tentacles.

Cysts

At lower temperatures (15°C), culture water replacement and feeding were stopped, leading to deterioration of water quality or induced starvation. As a result, about 50% of the adult polyps formed resting stages (Fig. 7A). The polyps contracted into a ball-like tissue mass and were encapsulated by a soft layer, forming cysts. The cysts were yellowish, about 0.63 mm in diameter and attached to the substrate. After the temperature was raised above 20°C or the water was replaced causing an improvement of the water conditions, the nutritional condition of the cysts improved, and the polyps regenerated within two weeks, with excystation from the capsules (Fig. 7B).

Metamorphosis

Metamorphosis occurred at stable temperatures between 18 to 25°C (18, 20, 23, 25°C, respectively) or when temperatures were raised from 20 to 25°C. Metamorphosis from a single adult polyp into a medusa was observed (Table 3). The first evidence of medusa development was the formation of four temporary vertical furrows in the hypostome of the polyp (Phase 1). The bases of different numbers (one to three) of the polyp tentacles coalesced randomly.

![Fig. 6. Nematocysts of Carybdea brevipedalia. A: Tentacle tip of primary polyp; B: tentacle tip of adult polyp; C, D: American football-shaped p-rhopaloids (large). Intact (C), discharged (D); E, F: American football-shaped p-rhopaloids (small). Intact (E), discharged (F); G, H: heterotrichous euryteles. Intact (G), discharged (H); I, J: spherical p-rhopaloids. Intact (I), discharged (J); K, L: spherical isorhizas. Intact (K), discharged (L); M, N: ovoid isorhizas. Intact (M), discharged (N). Scale bars: A–F = 20 µm; G–N = 10 µm.](image-url)
Development of Carybdea brevipedalia

Development of Carybdea brevipedalia on the four corners of the peristomial edge. Two days after the onset of metamorphosis, the bases of the tentacles fused and thickened to bulbs and the free distal parts were absorbed (Phase 2). Pigmentation marks appeared on the inward-facing side of these tentacle base bulbs. One medusa tentacle formed between each of the tentacle base bulbs, for a total of four tentacles. Three days after the initiation of metamorphosis, the pigmented marks developed into two lens eyes and four ocelli, while statocysts emerged on the opposite side of the bulbs (Phase 3). A shallow horizontal groove appeared on the lateral sides of the polyp, dividing the calyx into a metamorphosing upper half part with plate-like medusoid tissue and a polypoid lower part with cylindrical cells. The orange color of the polyps changed into lemon yellow shades. Four days post initiation of metamorphosis, transformation of the polyp tentacles into four rhopalia and the hypostome into a manubrium was complete (Phase 4). The medusa opened the lips within the subumbrella. The entire calyx changed into medusoid tissue. Five days post initiation of metamorphosis, transformation of the polyp tentacles into four rhopalia and the hypostome into a manubrium was complete (Phase 5). The medusa began to pulsate and it detached from the substrate, before transforming into a single medusa without leaving any regenerative remnants, but leaving a thin periderm.

Medusa development

A total of 30 medusae were raised for 60 days. Newly detached medusae had a tetrameric, pyramid-like bell with a rounded top and were whitish to translucent in color (Table 3, 4, Fig. 8A–C). They (n=5) were 0.8 to 1.2 mm (mean: 1.0 mm) in umbrella height, 0.9 to 1.3 mm (mean: 1.1 mm) in umbrella width, and all had one pedalium and one tentacle per interradius. The exumbrella was sprinkled with large (0.06–0.14 mm) and small (0.02–0.03 mm) round nematocyst clusters, consisting mainly of American football-shaped p-rhopaloids (Fig. 6E, F) and spherical holotrichous isorhizas (Fig. 6K, L)-aligned along radial furrows (Fig. 8A). The manubrium had four lips and its length when extended was about 30% of umbrella height. One to two gastric filaments per corner were visible through the apex of the umbrella. The sensory niches were shallow and still roofless (Table 4). Tentacles appeared as a "string of

**Table 2.** The process of asexual reproduction (budding) in the polyps of Carybdea brevipedalia. Scale bar=0.1 mm.

| Day | 0 | 1 | 2 | 7 | 8 |
|-----|---|---|---|---|---|
| Oral view | 4-tentacled polyp (Just before budding) | Budding of asexual bud | Enlargement of bud | Development of tentacles and hypostome | Newly released settled polyp |
| Lateral view | Detachment of asexual bud |

![Fig. 7. Cyst of Carybdea brevipedalia. A: Cyst; B: polyp after excystment. All bars=2 mm.](image)
pearls” (Fig. 8D, see also Straehler-Pohl and Jarms 2011) with about twenty spherical shaped white nematocyst batteries consisting of American football-shaped p-rhopaloids (Fig. 6E, F) and ovoid isorhizas (Fig. 6M, N). When completely extended, the tentacles were up to 3 mm in length.

Five-day-old medusae were about 1.6 mm in height and about 1.6 mm in width (Table 4). The pedalium was elongated and thickened. The border between tentacle and pedalium was obscure. Fourteen-day-old medusae were about 2.1 mm in height and about 1.8 mm in width. A downwardly directed gelatinous roof formed above the sensory organ. The proximal base of the pedalium thickened. Twenty-day-old medusae were about 5.8 mm in bell height and about 5.2 mm in width. The border between tentacle and pedalium became clear. The sensory roof overhung the rhopalium. Sixty-day-old medusae were about 10.0 mm in height and about 8.0 mm in width. Leaf-shaped gonads appeared in the middle region of the interradial furrows. The pedalium developed to be scalpel-shaped. Heart-shaped rhopaliar niches were formed.

**Discussion**

The early life cycle of *Carybdea brevipedalia* was clarified from our laboratory rearing experiments (Table 5; Fig. 9). Fertilization of *Carybdea brevipedalia* occurred externally in dark conditions. In other cubozoans, external fertilization has been observed in *Alatina moseri* (Mayer, 1906) from Hawaii and Australia (Carrette et al. 2014), *Morbakka virulenta* (Kishinouye, 1910) (Toshino et al. 2013, 2015), *Carukia barnesi* Southcott, 1967 (Courtney et al. 2016), *Chironex fleckeri* Southcott, 1956 (Yamaguchi and Hartwick 1980) and *Chironex yamaguchii* Lewis and Bentlage, 2009 (Iwanaga et al. 2005), while, internal fertilization was observed in *Alatina* sp. from Puerto Rico (Arneson and Cutress 1976), *Carybdea marsupialis* (Linnaeus, 1758) from Puerto Rico (Studebaker 1972),

**Table 3.** The process of metamorphosis in the polyps of *Carybdea brevipedalia*. Scale bar=0.5 mm. MTe=medusa tentacle; MTi=medusoid tissue; Pe=Periderm; PT=polytentacle; Rh=Rhopalium; TBB=tentacle base bulb.

| Day | 0 | 1 | 2 | 3 | 4 | 5 | Periderm & young medusa |
|-----|---|---|---|---|---|---|------------------------|
| Oral view |
| Latera l view |
| Event | Fully developed poly (4-8 tentacles) | Tentacles coated at four corners of peristomial edge | Development of rhopalia by tentacle absorption | Development of eye spots and statocysts | Development of 4 medusa tentacles | Medusoid tissue appeared at upper calyx | Enlargement of medusa bell | Development of manubrium from hypostome | Elongation of medusa tentacles | Complete metamorphosis |
| | Appearance of nematocyst clusters on exumbrella | Pulsation and detachment of medusa | Residuum not remained |
Table 4. The process of young medusa development in *Carybdea brevipedalia*. 0, 5, 14, 20 days, live. 60 days, fixed. UD=umbrella diameter; UH=umbrella height; G=gonad; R=roof.

| Event/Stage                  | Cumulative interval (day) | Diameter (mm) | Length or height (mm) | No. of tentacles |
|------------------------------|---------------------------|---------------|-----------------------|-----------------|
| Fertilization (Fertilized eggs) | 0                         | 0.11–0.13     | —                     | —               |
|                              |                            | mean: 0.12    |                       |                 |
| Planulae                     | 1                          | 0.08–0.13     | 0.11–0.16             | —               |
|                              |                            | mean: 0.11    | mean: 0.14            |                 |
| Planulae settled             | 3                          | —             | —                     | —               |
| Primary polyps (settled)     | 6                          | 0.05–0.09     | 0.11–0.16             | 2–3             |
|                              |                            | mean: 0.06    | mean: 0.13            |                 |
| Primary polyps (creepers)    | 86                         | 0.03–0.05     | 0.24–0.25             | 2–3             |
|                              |                            | mean: 0.04    | mean: 0.25            |                 |
| Bud formation (Budding polyps) | 86                        | 0.11–0.18     | 0.23–0.40             | 3–4             |
|                              |                            | mean: 0.15    | mean: 0.32            |                 |
| Adult polyps (settled)       | 116                        | 0.19–0.35     | 0.40–0.66             | 4–8             |
|                              |                            | mean: 0.25    | mean: 0.50            |                 |
| Adult polyps (creepers)      | 123                        | 0.19–0.35     | 0.40–0.66             | 4–8             |
| Metamorphosis                | 128                        | 0.19–0.35     | 0.40–0.66             | 4–8             |
| Newly released medusae       |                            | 0.9–1.3       | 0.8–1.2               | 4               |
| 60-day-old medusae           | 188                        | 8.0           | 10.0                  | 4               |

Table 5. Chronology of developmental events in *Carybdea brevipedalia*.

| Event/Stage                  | Cumulative interval (day) | Diameter (mm) | Length or height (mm) | No. of tentacles |
|------------------------------|---------------------------|---------------|-----------------------|-----------------|
| Fertilization (Fertilized eggs) | 0                         | 0.11–0.13     | —                     | —               |
|                              |                            | mean: 0.12    |                       |                 |
| Planulae                     | 1                          | 0.08–0.13     | 0.11–0.16             | —               |
|                              |                            | mean: 0.11    | mean: 0.14            |                 |
| Planulae settled             | 3                          | —             | —                     | —               |
| Primary polyps (settled)     | 6                          | 0.05–0.09     | 0.11–0.16             | 2–3             |
|                              |                            | mean: 0.06    | mean: 0.13            |                 |
| Primary polyps (creepers)    | 86                         | 0.03–0.05     | 0.24–0.25             | 2–3             |
|                              |                            | mean: 0.04    | mean: 0.25            |                 |
| Bud formation (Budding polyps) | 86                        | 0.11–0.18     | 0.23–0.40             | 3–4             |
|                              |                            | mean: 0.15    | mean: 0.32            |                 |
| Adult polyps (settled)       | 116                        | 0.19–0.35     | 0.40–0.66             | 4–8             |
|                              |                            | mean: 0.25    | mean: 0.50            |                 |
| Adult polyps (creepers)      | 123                        | 0.19–0.35     | 0.40–0.66             | 4–8             |
| Metamorphosis                | 128                        | 0.19–0.35     | 0.40–0.66             | 4–8             |
| Newly released medusae       |                            | 0.9–1.3       | 0.8–1.2               | 4               |
| 60-day-old medusae           | 188                        | 8.0           | 10.0                  | 4               |
Tripedalia cystophora Conant, 1897 (Werner et al. 1971),
Tripedalia binata Moore, 1988 (Toshino et al. 2016) and
Copula sivickisi (Stiasny, 1926) (Toshino et al. 2014), dur-
ing similar night or dark conditions. Okada (1927) reported
that internal fertilization occurred in
C. brevipedalia from
Misaki, eastern Japan. However, fertilized eggs or planulae
were not observed in the gonads of the mature female me-
dusae.

Before spawning, the gonads of
C. brevipedalia changed
from transparent to whitish or whitish yellow in both sex-
es. Such alteration in gonad has been reported in other
cubozoans, such as A. moseri (Arneson and Cutress 1976,
Carrette et al. 2014), M. virulenta (Toshino et al. 2013), T.
binata (Toshino et al. 2016) and C. sivickisi (Toshino un-
published), and Scyphozoa, Nemopilema nomurai Kishi-
nouye, 1922 (Ohtsu et al. 2007). In the case of
N. nomurai,
gonadal maturation occurred by an increase in oocyte di-
ameter and matrix darkness (Ohtsu et al. 2007). Ohtsu et
al. (2007) observed that maturation and spawning in male
gonads were similar to those of females except that the
male spawning occurred within 30 min once exposed to
light and always preceded the female spawning. Therefore,
it was suggested that fertilization might take place in the
female gastrovascular cavity by sperm that came in from
the surrounding seawater. In the case of cubozoans, includ-
ing C. brevipedalia, it seems that the change in color of the
gonads is a sign of maturation, and fertilization occurred
continuously, as seen in
N. nomurai.

Polyps of
C. brevipedalia have never been found in the
wild. However, a wild population of
C. marsupialis
polyps from the Caribbean was collected growing on dead
Isogomon shells beneath mangrove roots (Studebaker 1972),
polyps of
C. fleckeri
were collected from stones (Hartwick 1991) and on the surface of dead coral for the species
Alatina morandini (Straheler-Pohl and Jarms, 2011) (Straheler-
Pohl and Toshino 2015). Medusae of
C. brevipedalia were
abundant near seagrass beds of
Zostera marina
Linnaeus, 1753. If the mature medusae spawned over soft bottom
substrate, embryos and polyps might be buried in the sedi-
ment. Under these conditions, the creeping polyps could
play an important role in successful recruitment on ade-
quate hard substrates, such as the leaves of
Z. marina,
stones and dead shells.

The polyps of
C. brevipedalia survived and propagated
over a wide range of water temperature conditions (be-
tween 10 to 30°C). Additionally, the polyps encysted below
15°C, when culturing water replacement and food were
stopped, and excysted when the temperature was raised
over 20°C. Water temperatures gradually decrease below
20°C from fall to winter, and drastic temperature and sa-
linity changes occur in rainy seasons in Japanese waters
(Japan Meteorological Agency 2017). The polyps of
C. brevipedalia might be encysted during winter in the wild
when temperature falls below 15°C, or when unfavorite en-
vironmental conditions such as low salinity and low food
concentrations occur. However, as the season changes and
the water temperature increases above 20°C or other fa-
vorable conditions act on the cysts, excystation may take
place.

Polyps of
C. brevipedalia can be distinguished from
other described cubozoan polyps by their shape and size
(Table 6). Polyps of other cubozoan species are flask-
shaped, as seen in
C. marsupialis
(Studebaker 1972, Straheler-Pohl and Jarms 2011) and
A. moseri
(Arnes-
on and Cutress 1976, Straheler-Pohl and Jarms 2011),
amphora-shaped in
A. morandini (Straheler-Pohl and

Fig. 9. Life cycle of Carybdea brevipedalia. a: egg and sperm; b: fertilized egg; c: planula; d: primary polyp (settled); e: primary polyp (creeping); f: primary polyp (budding); g: asexual bud; h: mature polyp (settled); i: mature polyp (creeping); j: cyst; k: mature polyp (budding); l–p: metamorphosis (phase 1–5); q: young medusa (just liberated); r: young medusa (1 month old); s: mature medusa.
Table 6. Comparative analysis of size (mm) and morphology of fully developed known cubozoan polyps.

| Cubozoan     | TBL   | MDD   | No. of tentacles | HL proportion | SL proportion | CL proportion | Nematocyst types | Bud type | Metamorphosis type | Culturing condition | References |
|--------------|-------|-------|------------------|----------------|----------------|---------------|------------------|----------|---------------------|---------------------|------------|
| Carybdea brevipedalia | 0.40–0.66 | 0.19–0.35 | 4–8 | 0.06–0.19 | 0.04–0.09 | 12% | 63% | HE, AFPR | settled | complete metamorphosis/ | a complete metamorphosis/ | stable (18–25°C) | TBL and 1/2 in MDD. |
| (from Puerto Rico) | mean: 0.50 | mean: 0.25 | mean: 6 | mean: 0.12 | mean: 0.07 | 23% | 14% | vertical | 3–4  | increase (from 20°C to 25°C) | b | |
| Carybdea marsupialis | 1.35–2.78 | 0.40–0.91 | 19–26 | 0.20–0.44 | 0.37–0.81 | 16% | 54% | ST | creeping | complete metamorphosis/ | c complete metamorphosis/ | strobilation stable (23–25°C) | |
| Tripedalia binata | 0.38–0.64 | 0.33–0.41 | 7–8 | 0.09–0.24 | 0.03–0.06 | 31% | 61% | HE, AFPR | settled | complete metamorphosis | stable (23–30°C) | |
| Tripedalia cystophora | 0.52–1.02 | 0.17–0.31 | 7–13 | 0.12–0.22 | 0.04–0.08 | 22% | 70% | HE | creeping | complete metamorphosis | stable (23–28°C) | |
| Copula sivickisi | 0.28–0.97 | 0.15–0.20 | 2–7 | 0.09–0.14 | 0.02–0.05 | 35% | 64% | AFPR | settled | complete metamorphosis | stable (23–30°C)/ | |
| Carkia barnesi | — | — | 4–24 | mean: 0.89 | mean: 11 | mean: 0.56 | mean: 0.33 | mean: 0.32 | vertical | swimming | strobilation | stable (28°C) | |
| Morbakka virulenta | 2.25–4.66 | 0.49–1.08 | 8–17 | 0.27–0.62 | 0.60–2.71 | 14% | 41% | HMT, SI | swimming | trirhopaloids | increase (from 15°C to 20°C) | g, h |
| Alatina morandini (identified as Carybdea morandini) | 0.83–1.80 | 0.34–0.74 | 9–18 | 0.16–0.36 | 0.22–0.54 | 21% | 48% | TR, SSPP | more than 30 | radial | complete metamorphosis | stable (23–25°C), day light | |
| Alatina moseri (identified as Alatina mordens or Alatina cf. moseri, respectively) | 1.43–1.63 | 0.41–0.46 | 11–19 | 0.19–0.22 | 0.21–0.41 | 14% | 60% | ST | creeping | complete metamorphosis | stable (25–29°C) | |
| Chironex fleckeri | 0.75 | 40–45 | — | — | — | — | — | vertical | 1 vertical | complete metamorphosis | stable (27–28°C) | |

Jarms 2011, Straeher-Pohl and Toshino 2015), and tulip-shaped in M. virulenta (Toshino et al. 2013, 2015) and C. barnesi (Courtney et al. 2016). Polyps of C. brevipedalia are pouch-shaped, similar to T. cystophora (Werner 1975, 1983), T. binata (Toshino et al. 2016) and C. sivickisi (Toshino et al. 2014) rather than C. marsupialis. When compared with C. marsupialis, the number of tentacles was smaller (6 vs 24 in average), while the tips of the tentacles contained multiple nematocysts (Table 6). Furthermore, the adult polyp of C. brevipedalia is smaller than that of C. marsupialis (1/4 in terms of TBL and 1/2 in MDD).

Metamorphosis in C. brevipedalia was complete and without any residuum as also occurs in the majority of known cubozoan life cycles, with C. marsupialis, M. virulenta and C. barnesi being exceptions (Straeher-Pohl and Jarms 2005, Toshino et al. 2015, Courtney et al. 2016). Carybdea marsupialis displayed two types of metamorphosis, complete and strobilation (Studebaker 1972, Straeher-Pohl and Jarms 2005). Straeher-Pohl and Jarms (2005) ob-
served that 45% of polyps transformed into a single medusa but left a residuum that regenerated into a small polyp. For C. brevipedalia, metamorphosis was induced at stable temperatures (18, 20, 23, 25°C) and when raised from 20 to 25°C. Metamorphosis was not observed at 15°C or when temperatures were lowered. In Japanese waters, young medusae of C. brevipedalia appear in July (Ueno 2003), when the water temperature gradually increases from approximately 20 to 25°C (Japan Meteorological Agency 2017).

Young medusae of C. brevipedalia resembled the tripedalid species, T. cystophora, T. binata and C. sivickisi rather than C. marsupialis. Carybdea brevipedalia and tripedalids, have four pearl string-like tentacles and aligned nematocyst clusters on their exumbrella (Strachler-Pohl and Jarms 2011, Toshino et al. 2014, 2016). All described cubozoans with the exception of C. marsupialis have four tentacles (Yamaguchi and Hartwick 1980, Hartwick 1991, Strachler-Pohl and Jarms 2011, Toshino et al. 2014, 2015, 2016, Courtney et al. 2016). Alatina moseri and species of the family Carukiidae viz. M. virulenta and C. barnesi, have regular nematocyst clusters pattern on the exumbrella and very short tentacles (Carrette et al. 2014, Toshino et al. 2015, i: Carrette et al. 2014. ML = manubrium length; UD = umbrella diameter; UH = umbrella height. Nematocyst types: AFPR = American football-shaped p-rhopaloids; AI = atrichous isorhizas; ME = microbasic euryteles; OI = ovoid holotrichous isorhizas; OHME = ovoid heterotrichous microbasic euryteles; OI = ovoid isorhizas; RHI = round holotrichous isorhizas; SHI = spherical holotrichous isorhizas; SE = sub-spherical euryteles; SOAI = small ovoid atrichous isorhizas.

Table 7. Comparative analysis of size (mm) and morphology of cubozoan carybdeid medusae with known life cycles.

| Species                  | UH  | UD  | ML proportion | No. of tentacles | Tentacle shape | Nematocyst types (tentacles) | Nematocyst types (exumbrella) | Nematocyst warts (exumbrella) | References |
|--------------------------|-----|-----|----------------|------------------|----------------|-----------------------------|-------------------------------|-------------------------------|-------------|
| Carybdea brevipedalia    | 0.8–1.2 to 2.0 | 0.9–1.3 | 0.43–0.54 | 4 | pearl | AFPR | AFPR small (0.06–0.14) and large (0.06–0.14), round, aligned along radial furrows |
| from Puerto Rico          | mean: 1.0 | mean: 1.1 | mean: 0.50 | 50% | string-like | OI | round, aligned along radial furrows |
| Tripedalia binata        | 1.1–1.5 to 2.0 | 1.2–1.4 | 0.23–0.41 | 4 | pearl | AFPR | very small (0.01–0.05 mm), round, aligned along radial furrows |
| mean: 1.2 | mean: 1.3 | mean: 0.32 | 30% | string-like | OI | medium large (0.06, 0.08 or 0.1 mm), round, aligned along radial furrows |
| Tripedalia cystophora    | 1.0–1.4 to 2.0 | 1.3–1.6 | 0.50–0.70 | 4 | pearl | OHME | very small (0.03–0.06 mm), round, aligned in two rows along radial furrows and two conspicuous nematocysts over sense organs |
| mean: 1.2 | mean: 1.5 | mean: 0.60 | 50% | string-like | OI | SE |
| Copula sivickisi         | 1.0–1.3 to 2.0 | 0.7–1.0 | 0.37–0.48 | 4 | pearl | OHME | very small (0.03–0.06 mm), round, aligned in two rows along radial furrows and two conspicuous nematocysts over sense organs |
| mean: 1.2 | mean: 0.9 | mean: 0.43 | 40% | string-like | OI | SO |
| Carybdea marsupialis     | 1.0–1.4 to 2.0 | 0.9–1.2 | 0.30–0.40 | 2 | pearl | OHME | large (0.14 mm × 0.06 mm), ovoid, ambilateral to the interradial furrows |
| from Puerto Rico          | mean: 1.2 | mean: 1.2 | mean: 0.36 | 30% | string-like | OI | SO |
| Carkia barnesi           | 0.3–0.8 to 2.0 | 0.5–0.8 | — | 4 | capitate | HMT, SI |
| Morbakka virulenta       | 2.3–2.8 to 2.0 | 1.7–2.7 | 0.41–0.91 | 4 | capitate | ME | innumerable, circular in shape, scattered in a dense, regular pattern over the whole exumbrella |
| mean: 2.5 | mean: 2.2 | mean: 0.77 | 30% | capitate | OI | OIME |
| Alatina morandinii       | 0.6–0.9 to 2.0 | 0.4–0.6 | 0.18–0.27 | 4 | filiform | AI | small (0.05–0.10 mm), innumerable, circular in shape, scattered in a dense, regular pattern over the whole exumbrella |
| (identified as C. morandinii) | mean: 0.7 | mean: 0.5 | mean: 0.21 | 30% | filiform | HI | very small (0.03–0.04 mm), circular in shape, irregularly scattered over whole exumbrella |
| Alatina moseri           | 1.2–1.6 to 2.0 | 1.1–1.5 | 0.24–0.30 | 4 | pearl | OHME | small (0.06 mm), innumerable, circular in shape, scattered in a dense, regular pattern over the whole exumbrella |
| (identified as A. mordens or A. cf. moseri, respectively) | mean: 1.4 | mean: 1.2 | mean: 0.27 | 20% | capitate | ME | i |

References: a: present study; b: Toshino et al. 2016; c: Strachler-Pohl and Jarms 2011; d: Toshino et al. 2014; e: Strachler-Pohl and Jarms 2005; f: Courtney et al. 2017; g: Toshino et al. 2015; h: Strachler-Pohl and Toshino 2015; i: Carrette et al. 2014. ML = manubrium length; UD = umbrella diameter; UH = umbrella height. Nematocyst types: AFPR = American football-shaped p-rhopaloids; AI = atrichous isorhizas; ME = microbasic euryteles; OI = ovoid holotrichous isorhizas; OHME = ovoid heterotrichous microbasic euryteles; OI = ovoid isorhizas; RHI = round holotrichous isorhizas; SHI = spherical holotrichous isorhizas; SE = sub-spherical euryteles; SOAI = small ovoid atrichous isorhizas.
(Bentlage et al. 2010). Though the genus Carybdea was recently revised by Straehler-Pohl et al. (2017), it remains necessary to clarify the life cycle of C. marsupialis from the Mediterranean and other carybdeid species, and compare their morphologies with C. brevipedalia in the future.

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