

Development and polyp formation of the giant box jellyfish *Morbakka virulenta* (Kishinouye, 1910) (Cnidaria: Cubozoa) collected from the Seto Inland Sea, western Japan

SHO TOSHINO^{1,*}, HIROSHI MIYAKE¹, SUSUMU OHTSUKA², KAZUYA OKUIZUMI³, AYA ADACHI⁴,
YOSHIMI HAMATSU⁵, MAKOTO URATA⁶, KAZUMITSU NAKAGUCHI⁷ & SYUHEI YAMAGUCHI⁷

¹ Graduate School of Fisheries Sciences, Kitasato University, 1–15–1 Kitasato, Sagami-hara, Kanagawa 252–0373, Japan

² Setouchi Field Science Center, Graduate School of Biosphere Science, Hiroshima University, 5–8–1 Minato-machi, Takehara, Hiroshima 725–0024, Japan

³ Tsuruoka Kamo Aquarium, Tsuruoka, Yamagata 997–1206, Japan

⁴ Enoshima Aquarium, Katasekaigan, Fujisawa, Kanagawa 251–0035, Japan

⁵ Nagisa Aquarium, Suooshima, Oshima, Yamaguchi 742–2601, Japan

⁶ Marine Biological Laboratory, Graduate School of Science, Hiroshima University, 2445, Mukaishima-cho, Onomichi, Hiroshima 722–0073, Japan

⁷ Faculty of Applied Biological Science, Hiroshima University, 1–4–4 Kagamiyama, Higashi-Hiroshima 739–8528, Japan

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Abstract: The giant cubozoan *Morbakka virulenta* was collected from the central part of the Seto Inland Sea, western Japan in October 2009, in order to observe its development and polyp formation. Fertilization occurred externally. Demersal fertilized eggs were obtained during the incubation of mature females and males kept at a temperature of ca. 17 to 21°C. From the two-cell stage onwards, fertilized eggs developed into blastulae within 4 h. The development of the blastulae stopped for 21 days after forming blastocysts. This formation of blastocysts is up to now unique within the Cnidaria. The planulae, which developed inside the cysts and lacked larval ocelli, this being characteristic for the Cubozoa, finally metamorphosed into polyps bearing only a single tentacle. The 16-tentacled-stage in polyps was reached about three months after this metamorphosis. Budding occurred in eight-tentacled polyps and swimming polyps were released nine days after the commencement of budding. These unique developmental features of *M. virulenta* may shed additional light on the evolution of life history strategies in the Cnidaria.

Key words: life cycle, planulocyst, Scyphozoa, swimming polyp

Introduction

The Cubozoa so far comprise about 50 described species worldwide (Daly et al. 2007). However, a complete life cycle has been described only for *Tripedalia cystophora* Conant, 1897 (Werner et al. 1971, Werner 1973a, 1975, 1976a, 1983, Laska-Mehnert 1985), although parts of the life cycles are known for *Carybdea brevipedalia* (Kishinouye, 1891) (Okada 1927, as *Carybdea rastoni* Haacke, 1886), *Carybdea marsupialis* Linnaeus, 1758 (Studebaker 1972, Cutress & Studebaker 1973, Stangl et al. 2002, Straehler-Pohl & Jarms 2005), *Alatina alata* (Reynaud,

1830) (Arneson & Cutress 1976, as *Carybdea alata* Reynaud, 1830), *Chironex fleckeri* Southcott, 1956 (Yamaguchi & Hartwick 1980, Hartwick 1991a), *Copula sivickisi* (Stiasny, 1926) (Hartwick 1991b, Lewis & Long 2005, Lewis et al. 2008, as *Carybdea sivickisi* Stiasny, 1926), and *Carybdea morandinii* Straehler-Pohl & Jarms, 2011 (Straehler-Pohl & Jarms 2011).

Morbakka virulenta (Kishinouye, 1910) is one of the largest species in the class Cubozoa, with a maximum bell height of 250 mm, a maximum bell diameter of 200 mm and extended tentacle length of about 3 m. *Morbakka virulenta* has been identified as *Tamoya haplonema* by Uchida (1929, 1970). However, *Morbakka* lacks the vertical gastric phacellae that are a diagnostic character for *Tamoya* (Ger-shwin 2008, Collins et al. 2011). In addition, recent molec-

*Corresponding author: Sho Toshino; E-mail, mf09008y@st.kitasato-u.ac.jp

ular phylogenetic analyses and taxonomic investigations suggest that *T. haplonema* collected from Japan should be regarded as *M. virulenta* (Bentlage et al. 2010, Bentlage & Lewis 2012).

Morbakka virulenta has been reported from the coast of the Kii Peninsula and the Seto Inland Sea previously, as *Tamoya virulenta* or *Tamoya haplonema* (Kishinouye 1910, Kubota 1998). This species is well known by local fishermen and divers as a dangerous jellyfish due to its fiery sting. Although most cubozoan species occur in tropical waters (Studebaker 1972, Arneson & Cutress 1976, Yamaguchi & Hartwick 1980, Hartwick 1991a, b), *M. virulenta*

appears exclusively from fall to winter in Japanese waters (Kishinouye 1910, Uchida 1970). The life cycle of this species, or indeed any member of the genus *Morbakka*, has never been studied. The present paper describes its development and polyp formation.

Materials and Methods

Morbakka virulenta medusae (Fig. 1) were collected using an underwater fish-luring lamp (KU-5MB, KOTO Electric, Japan) equipped to the starboard side of the TRV Toyoshio-maru mooring at Uno Port, Okayama Prefecture,

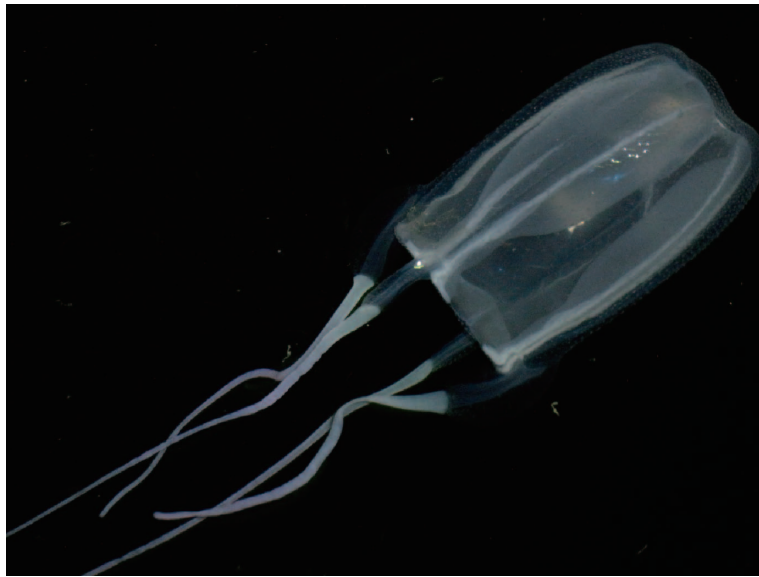


Fig. 1. *Morbakka virulenta* (Kishinouye, 1910), live, lateral view, in situ. Bell height of 200 mm.

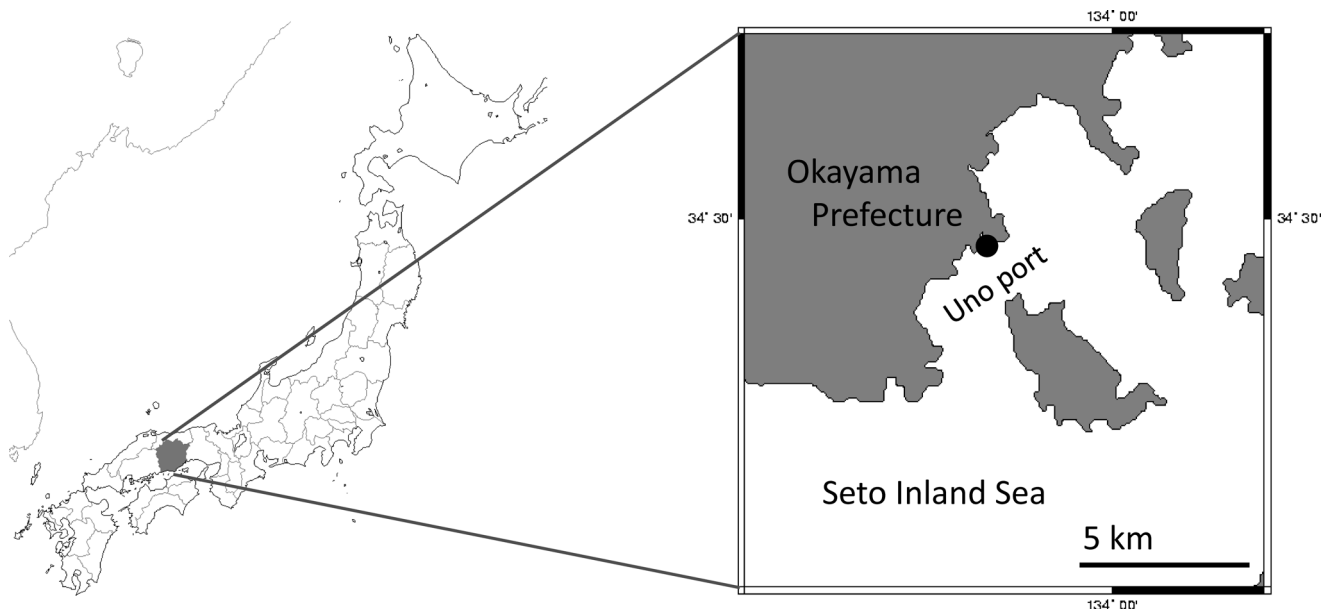


Fig. 2. Map of the sampling site, Uno port, Japan.

western Japan (give exact lat long; Fig. 2), on October 26, 2009. The medusae were taken by a dipnet (mesh size 2 mm, mouth diameter 25 cm). The bell height of medusae ranged from 100 to 250 mm. Male and female medusae were kept in an aquarium (length 60 cm, width 40 cm, height 60 cm, water volume 140 L) on the ship. The aquarium was set up in the onboard laboratory. Temperature in the aquarium was not controlled and varied within the range of room temperature (15 to 22°C). Sex of the medusae was determined under a microscope by observing the condition of the gonads (Fig. 3). All of water in the aquarium was changed with fresh seawater every morning. Fertilized eggs, which were identified under a binocular microscope, were collected by filtration of the aquarium water twice a day (morning and night), using a plankton net (mesh size 41 μm). A total of several thousand eggs were incubated in petri dishes (diameter 75 mm, height 45 mm) with filtered seawater (1 μm) at about 17°C in the onboard laboratory until the end of the cruise (October 30, 2009), and then incubation was continued at 21°C (temperature of the sampling site) in the laboratory at the Graduate School of Fisheries Sciences, Kitasato University. The development of eggs was observed with a binocular microscope every day.

Polyps developed from actively rotating planulae within the egg membrane were transferred into six-well culture plates (diameter 35 mm, height 20 mm, IWAKI, Japan) and kept at 21°C. Chopped *Artemia* nauplii were fed directly to the polyps using a fine needle, twice or thrice a week. All of rearing water was changed with filtered seawater (1 μm) about 3 h after feeding.

For nematocyst identification in the polyps, squashes prepared from fixed tissues were examined under an optical microscope. Nematocysts were identified according to Gershwin (2006). For determination of the respective abundance of nematocyst types in polyps, at least 200 nematocysts were counted.

Results

The gonads of mature medusae were whitish to whitish yellow in males and females. Fully mature testes were full of active sperm, while ovaries contained fully mature eggs of ca. 0.10 mm in diameter (Fig. 3a, b). Fertilized eggs were not found in the gonads of females, indicating that fertilization occurred externally.

On the night of collection, few eggs were observed in the aquarium. In the morning two days after collection, thousands of eggs were obtained. The eggs were demersal, about 0.10 mm in diameter (Fig. 4a). When eggs were sampled, most of the eggs were at the two-cell stage (Fig. 4b). The eggs developed to eight-cell stage or 16-cell stage (Fig. 4c) within 2 h after the two-cell stage. These eggs developed into blastulae (Fig. 4d) within 2 h after the 16-cell stage, and then developed into blastocysts within 30 h after the blastulae stage. The development of blastulae was stopped for 21 days after forming blastocysts. Within the egg membrane, blastulae finally metamorphosed into actively rotating planulae (Fig. 4e). Planulae were about 0.15 mm in diameter and had no band of larval ocelli, the lack of which is a characteristic of cubozoan planulae (Werner et al. 1971) (Fig. 4f).

Five days after the formation of planulae, planulae developed into polyps within the egg membrane. Since the basal discs of the polyps stayed attached to the egg membrane, the polyps seemed to hatch out of the eggs. The shape of the polyps resembled a tulip with a long stalk, and from the ovoid calyx only a single tentacle protruded (Fig. 5a). The calyx of the polyps was about 0.06 mm in mouth disc diameter and the polyps were about 0.90 mm in total body length. Polyps bore three nematocysts (spherical *p*-rhopaloids) at the tip of the tentacle (Fig. 6a). Thirteen days after transformation from planula into polyp stage, the polyps developed a second tentacle (Fig. 5b). The polyps developed four tentacles in three to four weeks (Fig. 5c), eight in about five weeks (Fig. 5d), and 16 in about three

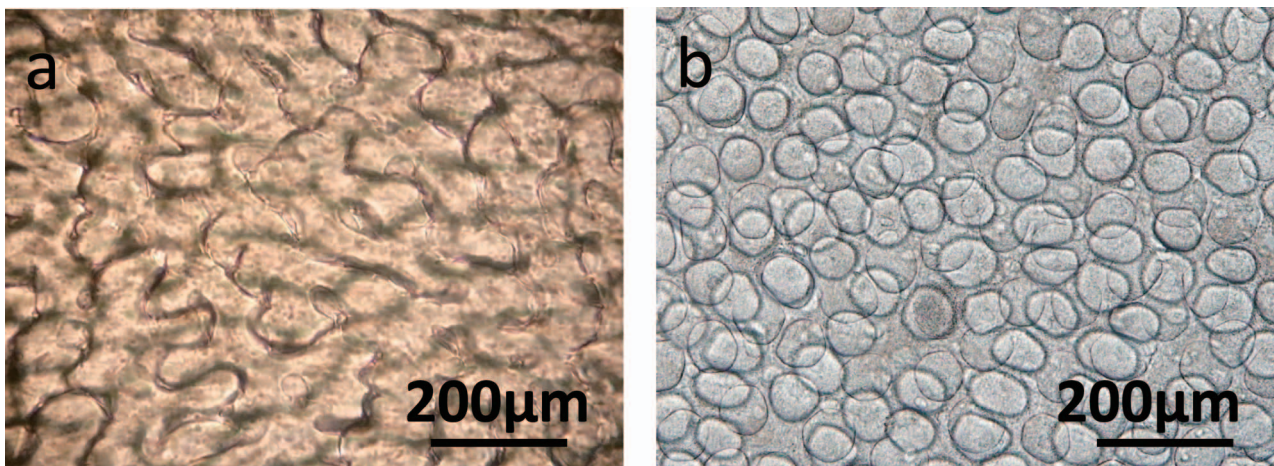


Fig. 3. Gonad tissue from *Morbakka virulenta*. a, testis; b, ovary.

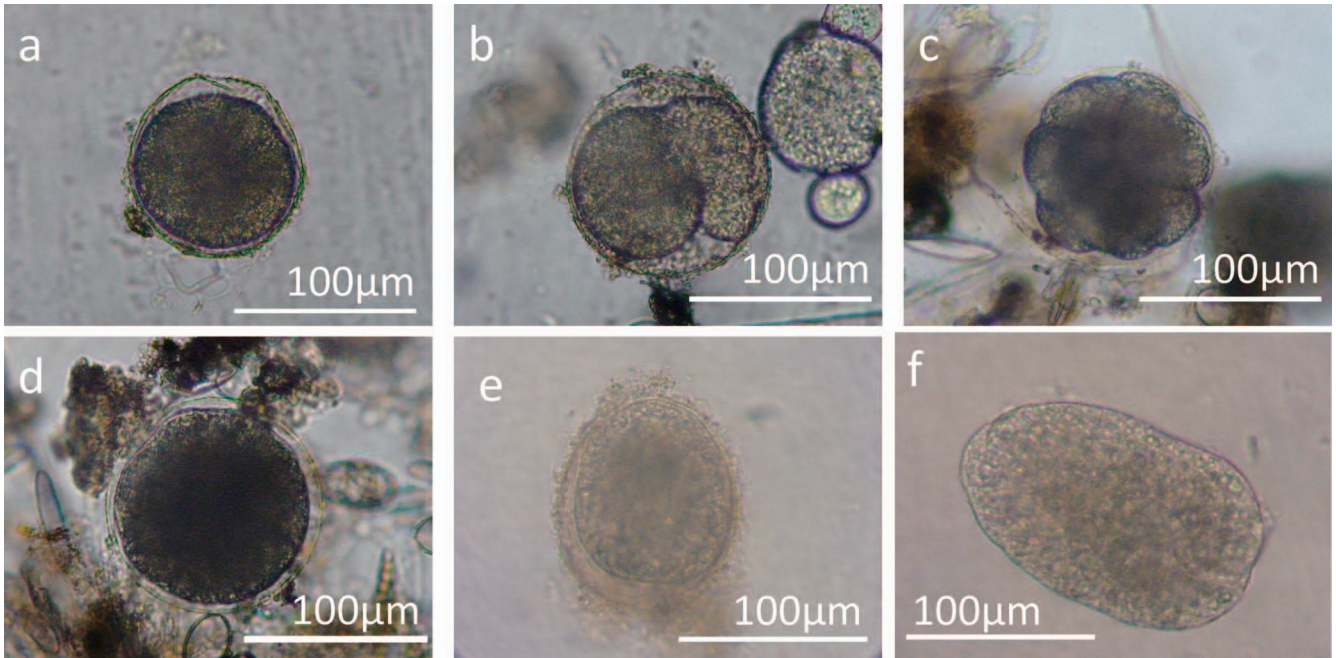


Fig. 4. Early embryogenesis of *Morbakka virulenta*. a, fertilized egg; b, 2 cell stage; c, 8 to 16 cell stage; d, blastula; e, planula rotating in egg membrane; f, planula.

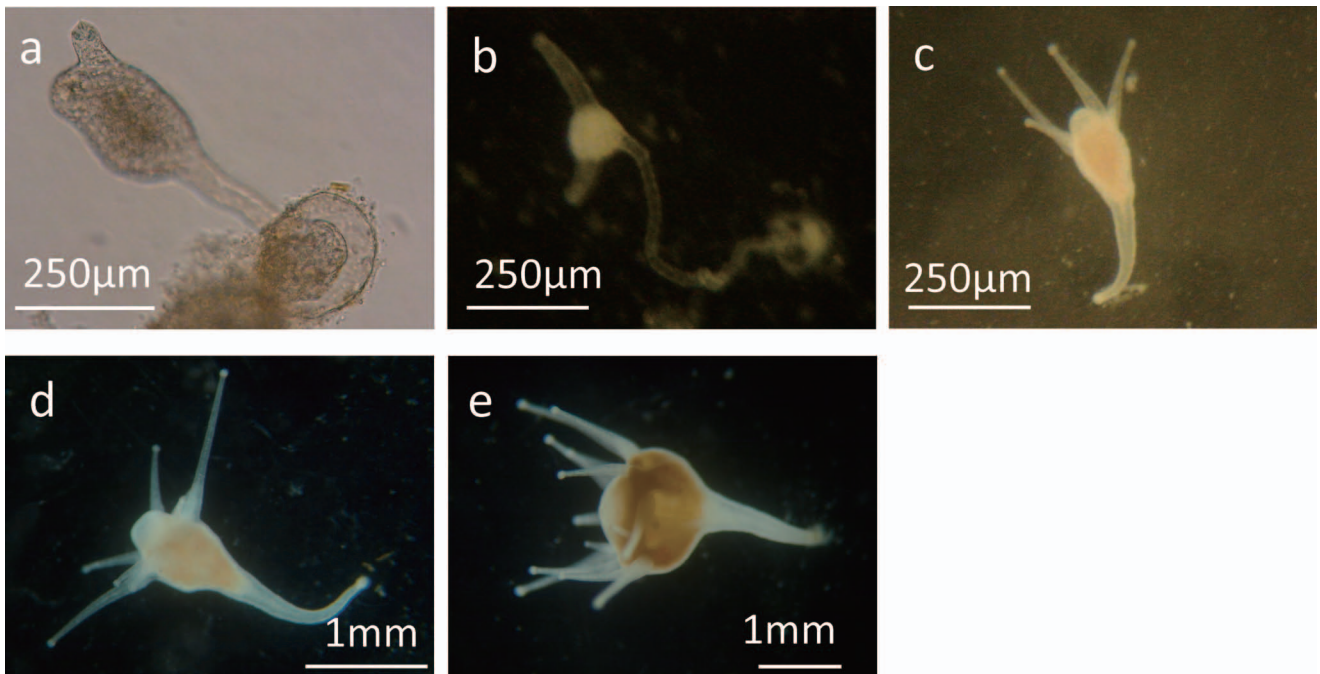


Fig. 5. Polyps of *Morbakka virulenta*. a, 1-tentacled polyp; b, 2-tentacled polyp; c, 4-tentacled polyp; d, 8-tentacled polyp; e, 16-tentacled polyp.

months (Fig. 5e). Fully-developed polyps were 1.08 mm in maximum mouth disc diameter and 4.66 mm in total body length and had up to 17 tentacles. The polyps had more than 30 nematocysts at the tips of each tentacle (Fig. 6b).

The polyps possessed two types of nematocysts, trirhopaloids and small spherical *p*-rhopaloids (Fig. 6c, d). The latter was more abundant than the former.

In eight-tentacled polyps, asexual reproduction was ob-

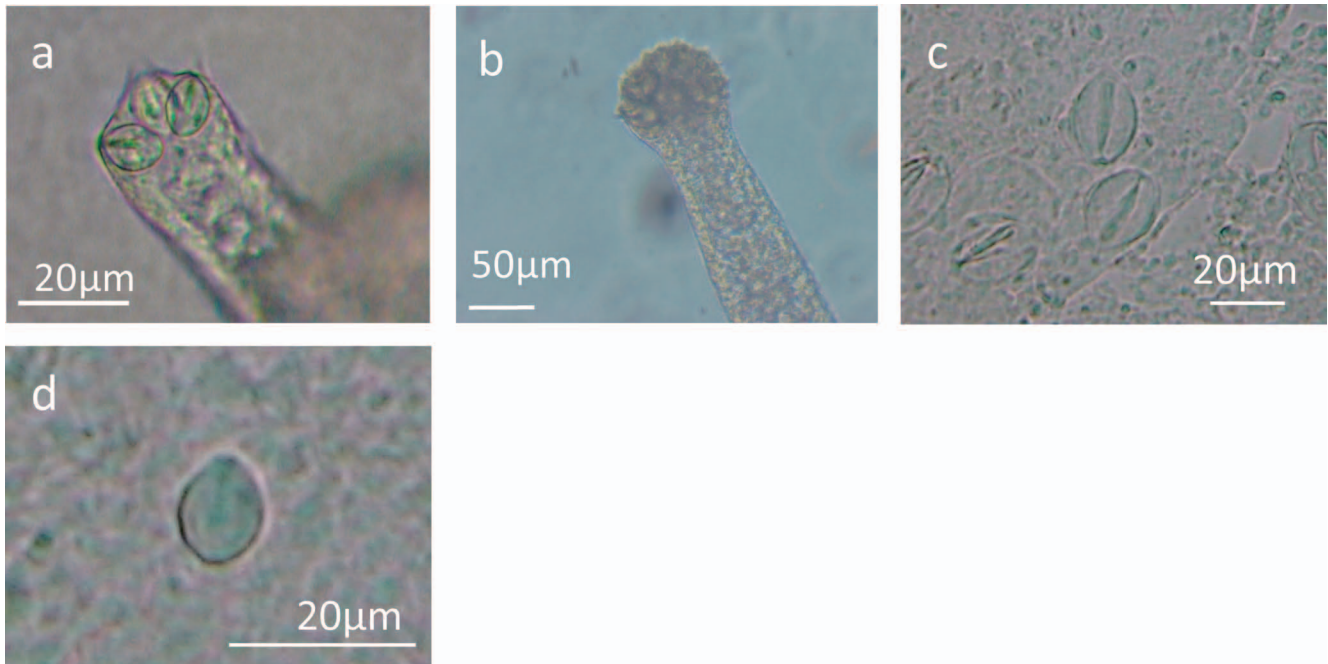


Fig. 6. Nematocysts of *Morbakka virulenta*. a, nematocyst of 1-tentacled polyp; b, nematocysts of 16-tentacled polyp; c, trirhopaloid; d, small spherical *p*-rhopaloid.

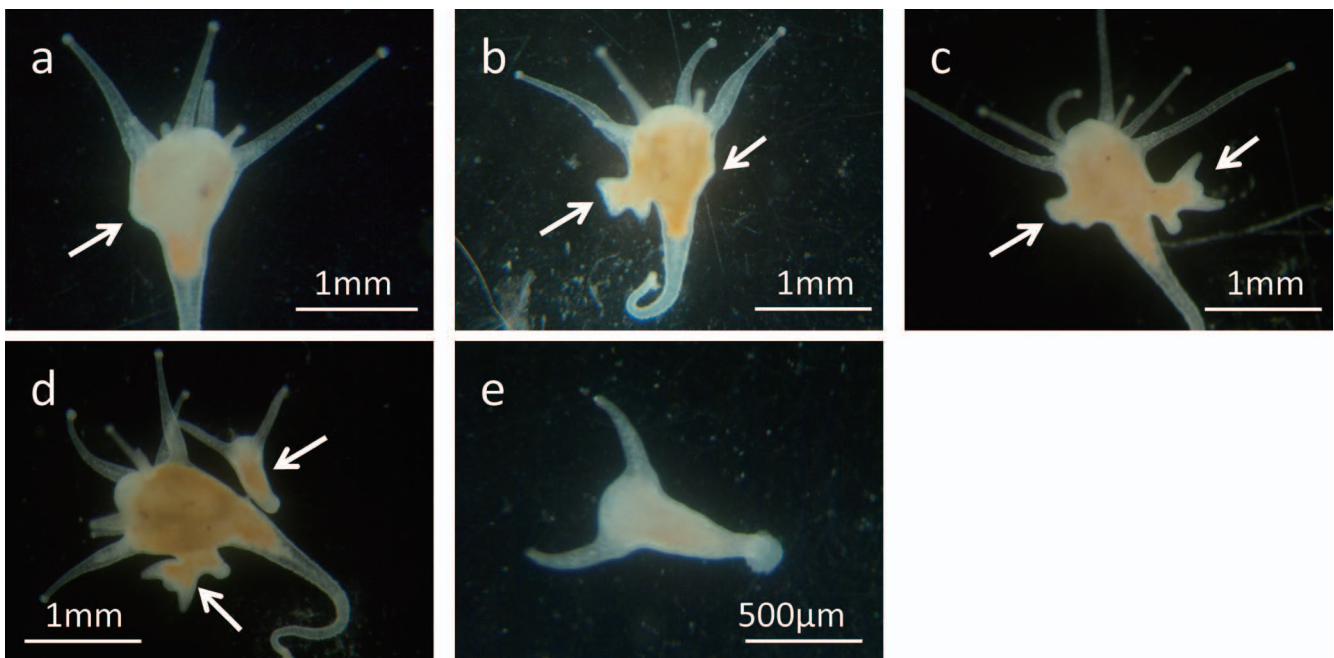


Fig. 7. The process of polyp budding. An arrow indicates a bud. a, just after budding; b, after 4 days; c, after 6 days; d, after 9 days, the right bud just released; e, swimming polyp.

served. The formation of buds occurred in the lower part of the calyx (Fig. 7a). Four days after the start of bud formation, two tentacles and the basal part were formed on the new bud (Fig. 7b, c). Nine days after the start of the

budding process, a new polyp was released from the original polyp (Fig. 7d). Swimming polyps were 0.7 mm in length and had two tentacles (Fig. 7e). They swam near the bottom or at the water surface. The swimming polyps set-

tled on the wall or near the edge of a petri-dish and transformed into adult polyps within two to three days.

Discussion

In *Morbakka virulenta* external fertilization occurred in the morning rather than at night as in the cubozoan *Alatina alata* (Arneson & Cutress 1976). Embryos of other cubozoans, such as *Alatina alata* and *Copula sivickisi*, metamorphose into primary polyps within eight or nine days after fertilization (Arneson & Cutress 1976, Hartwick 1991a, Lewis & Long 2005). In contrast, embryogenesis of *M. virulenta* stopped at the blastula stage for 21 days. This “blastocyst” seems to correspond to planulocysts which has been observed in the scyphozoan species *Cyanea capillata* and *C. lamarckii* and in the cubozoan species *Tripedalia cystophora* (Brewer 1976, Holst & Jarms 2007, Werner 1975).

Adult medusae of *M. virulenta* appear in autumn to winter (Kishinouye 1910), and they spawn fertilized eggs. Fertilized eggs, being negatively buoyant, sink to the bottom of the sea. If adult *M. virulenta* medusae spawned fertilized eggs over soft bottoms such as in Uno Port, fertilized eggs or embryos might be buried in the sediment in those areas. Blastocysts could play an important role in adaptation to low temperatures and unfavorable settlement conditions without solid substrate. Under the present conditions, embryos of *M. virulenta* formed blastocysts at low temperature (17°C) and blastocysts excyst at a higher temperature (21°C).

The planulae of *M. virulenta* lacked larval ocelli, which has been described as a character common to all cubozoan

planulae (Widersten 1968, Nordström et al. 2003). Polyps of *M. virulenta* were distinguished from hitherto described polyps of other cubozoans by shape and size (Table 1). Polyps of other cubozoan species were bag-shaped in *Tripedalia cystophora* (Werner et al. 1971, Laska-Mehnert 1985, Straehler-Pohl & Jarms 2011), flask-shaped in *Carybdea marsupialis* (Studebaker 1972, Straehler-Pohl & Jarms 2011) and *Alatina alata* (Arneson and Cutress 1976; Straehler-Pohl & Jarms 2011 as *Alatina moseri*) or amphora-shaped in *Carybdea morandinii* (Straehler-Pohl & Jarms 2011). Polyps of *M. virulenta* were characterized as being tulip-shaped. They are the largest of all known cubozoan polyps, and are as large as known scyphozoan scyphistomae (Morandini et al. 2004).

Polyps of *M. virulenta* have long stalks and bear at the base some traces of egg membrane. This hints to the possibility that primary polyps formed inside the egg membrane of blastocysts buried in soft sediments could excyst and use the egg membrane as a substrate in areas without hard substrate. The long stalk of the polyp may play a role in mitigating being buried by sediment.

The asexual buds of *M. virulenta* developed into swimming polyps. Morphs of swimming polyps are ovoid-shaped with two tentacles. In contrast, other cubozoan polyps formed from asexual buds are worm-like creeping polyps with a tiny hypostome cone surrounded by two to eight tentacles (Table 1). Creeping polyps moved on the substrate for a few days until attaching to the substrate (Werner 1975, Straehler-Pohl & Jarms 2011). The swimming polyps of *M. virulenta*, in contrast, swam near the bottom or the water surface, presumably being able to avoid non-optimal substrates such as mud and successfully

Table 1. Size (mm) and morphology of cubozoan polyps in previous and the present study. The proportion is the ratio of the mean length of that structure to the mean total body length.

	Total body length	Mouth disk diameter	Number of tentacles	Hypostome length proportion	Calyx length proportion	Stalk length proportion	Nematocyst types Number of nematocysts	Bud type Number of tentacles	References
<i>Morbakka virulenta</i>	2.25–4.66, mean: 3.50	0.49–1.08, mean: 0.65	8–17 mean: 13	0.27–0.62, mean: 0.48 14%	1.05–1.89, mean: 1.49 44%	0.60–2.71, mean: 1.52 41%	trirhopaloids, small spherical <i>p</i> -rhopaloids more than 30	swimming polyp 2	present study
<i>Carybdea morandinii</i>	0.83–1.80, mean: 1.28	0.34–0.74, mean: 0.51	9–18, mean: 13	0.16–0.36, mean: 0.26 21%	0.43–0.86, mean: 0.61 48%	0.22–0.54, mean: 0.39 31%	stenoteles 1	creeping polyp 4–6	Straehler–Pohl & Jarms 2011
<i>Carybdea marsupialis</i>	1.35–2.78, mean: 2.08	0.40–0.91, mean: 0.62	19–26, mean: 24	0.20–0.44, mean: 0.33 16%	0.72–1.50, mean: 1.12 54%	0.37–0.81, mean: 0.60 29%	stenoteles 1	creeping polyp 4–6	Straehler–Pohl & Jarms 2005
<i>Alatina mordens</i>	1.43–1.63, mean: 1.52	0.41–0.46, mean: 0.43	11–19, mean: 16	0.19–0.22, mean: 0.21 14%	0.87–0.94, mean: 0.91 60%	0.36–0.41, mean: 0.40 26%	stenoteles 1	creeping polyp 4–6	Straehler–Pohl & Jarms 2011
<i>Tripedalia cystophora</i>	0.52–1.02, mean: 0.83	0.17–0.31, mean: 0.26	7–13, mean: 9	0.12–0.22, mean: 0.18 22%	0.36–0.70, mean: 0.58 70%	0.04–0.08, mean: 0.07 8%	heterotrichous euryteles 20–40	creeping polyp 2–3	Straehler–Pohl & Jarms 2011
<i>Chironex fleckeri</i>	—	0.75	40–45	—	—	—	— 1	creeping polyp 4–8	Yamaguchi & Hartwick 1980

settle on adequate solid substrate when they reach it.

Polyps of *M. virulenta* can be distinguished from polyps of other cubozoan species by having a much longer stalk and a tulip-shaped body. This type of polyp is found in Scyphozoa (Gohar & Eisawy 1960, Sugiura 1963, Adler & Jarms 2009) and this character suggests that *M. virulenta* is a link species between Cubozoa and Scyphozoa. Indeed, the Cubozoa were been originally classified as a group within the Scyphozoa (Kramp 1961). Because cubozoan polyps differ in body structure from the scyphistomae of the Scyphozoa and because cubozoans display metamorphosis for medusa production instead of strobilation (Werner et al. 1971), the class Cubozoa was established to form an independent group from the Scyphozoa (Werner 1973b, 1975, 1976b). In addition, recent molecular analyses suggested that the monophyletic Cubozoa is a sister clade to the Scyphozoa (Collins 2002; Collins et al. 2006, Daly et al. 2007). The newly found similarities among life histories in Cubozoa and Scyphozoa that are discussed above are congruent with the hypothesis for the monophyletic clade Acraspeda containing both Cubozoa and Scyphozoa (Collins et al. 2006, Collins 2009). Furthermore, the data presented here provide new information to study the evolution of life history strategies in Cnidaria.

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