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# First Finding of a Bivalve-Inhabiting Hydrozoan (Cnidaria, Hydrozoa) from Korea

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## ABSTRACT

*Eutima japonica* Uchida, 1925, a bivalve-inhabiting hydrozoan was collected for the first time in Korea, associating with *Mytilus galloprovincialis*. The morphology of male medusae of this hydrozoan is clarified by culture and described as well as other developmental stages. As the present material from Korea is in good accord with that of the northern Japanese form of *E. japonica*, so the geographical distribution of the northern form of this species is widened, Japan, China and Korea. Green fluorescent protein distribution pattern of this medusa is also described and compared with that of the most related species *Eutima sapinhoa* Narchi and Hebling, 1975.

**Keywords:** culture, *Eutima japonica*, geographical distribution, green fluorescent protein, life cycle, medusan form, morphological variation, taxonomy

## INTRODUCTION

The solitary, bivalve-inhabiting hydroid supposedly evolved from the free-living, colonial *Eutima* without drastic modification of the medusa stage, and appeared as a specialized, distinct member in the Eirenidae (see Kubota, 2000 for details of the evolution of bivalve-inhabiting hydrozoans and the study history of them; Kubota and Collins, 2017). In Japanese waters, records of *Eutima* refer only to the bivalve-inhabiting species, *E. japonica* Uchida, 1925 (Uchida, 1925; Yamazi, 1958; Kubota, 1992, 2000), which is divided into the four forms that are parapatrically distributed, changing their morphology of the earliest matured medusae step by step (Kubota, 1997, 1999, 2000). The medusa of *E. japonica* was also reported in the central part of the north Pacific (Kramp, 1965), Cochin Backwater, India (Santhakumari and Vannucci, 1971) and in the Jiulong River esturary near Amoy China (Zhenzu and Jiachi, 1983), while never found in the east coasts of the Pacific, from where no bivalve-inhabiting hydrozoans have been recorded (Kubota, 2000), except for a recent appearance of polyp by Tsunami transport of the host *Mytilus galloprovincialis* that attached to small boat (Calder et al., 2014).

In Korea, 130 hydroid species, comprising 77 species or subspecies of the Thecata, 24 species of the Athecata and 29 species of the hydromedusae, are currently recorded in three monographical studies by the late Prof. Jung Hee Park (Park, 2010, 2011, 2012). However, notwithstanding her long-lasting efforts since her first paper published in 1979 (Rho and Park, 1979) and against our detailed survey at 30 sites along Korean coasts during the period between 1990 and 2006, no bivalve-inhabiting hydrozoans have so far been found (Kubota et al., 2006). Recently we conducted an additional survey in late April, 2018, and finally found a polyp colony associated with mussels of *Mytilus galloprovincials* from Yeosu for the first time in Korea.

To determine the exact taxonomic status of a bivalve-associating hydroid as well as its morphological and geographical form, the morphology of mature medusa after culture is prerequisite and indispensible (Kubota, 1997). Furthermore, green fluorescent protein (GFP) ditribution pattern of the medusa should be observed and compared with that



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of the most related species *Eutima sapinhoa* Narchi and Hebling, 1975 (Migotto et al., 2004). As a result of the studies, the mussel-inhabiting hydroid from Yeosu turned out to be *Eutima japonica* Uchida, 1925, which is a new record from Korea, and its form can be determined.

In this paper, we deal with the description of the male medusae of *E. japonica* as well as other developmental stages by culture, and with some brief comments on its morphological and geographical distinction according to the GFP ditribution pattern of the medusa. We also provide herein a measurements table showing morphological variation of well-developed medusae reared in the laboratory after release from a mussel-inhabiting hydroid polyp.

## MATERIALS AND METHODS

On April 30, 2018, 31 individuals of *Mytilus galloprovincialis*, that were cultivated in nearby mussel farms and sold in Yeosu seafood market, were examined alive soon after purchase. The water temperature in Yeosu was about 15°C on these days, according to the "Korea Real-Time Database for NEAR-GOOS" by Korea Hydrographic and Oceanographic Agency. Polyps were found inside only in one individual of these mussels, then polyps with medusa buds were picked out from the host at the Kujyukusima Aquarium, Nagasaki Prefecture, Japan, on May 1, the next day after purchase. On the other hand, no such polyps were found from 30 mussels purchased at Jagalchi seafood market at Busan, Korea on April 28, 2018.

Laboratory culture of the polyps with medusa buds was done individually and bringing them back to the laboratory in Shirahama, Wakayama, Japan. For each polyp, an 80-mL polystyrene vessel of 60 mm in diameter and 30 mm high was filled with filtered seawater (32 psu) from Shirahama, Wakayama Prefecture (supplied from the Seto Marine Bio-

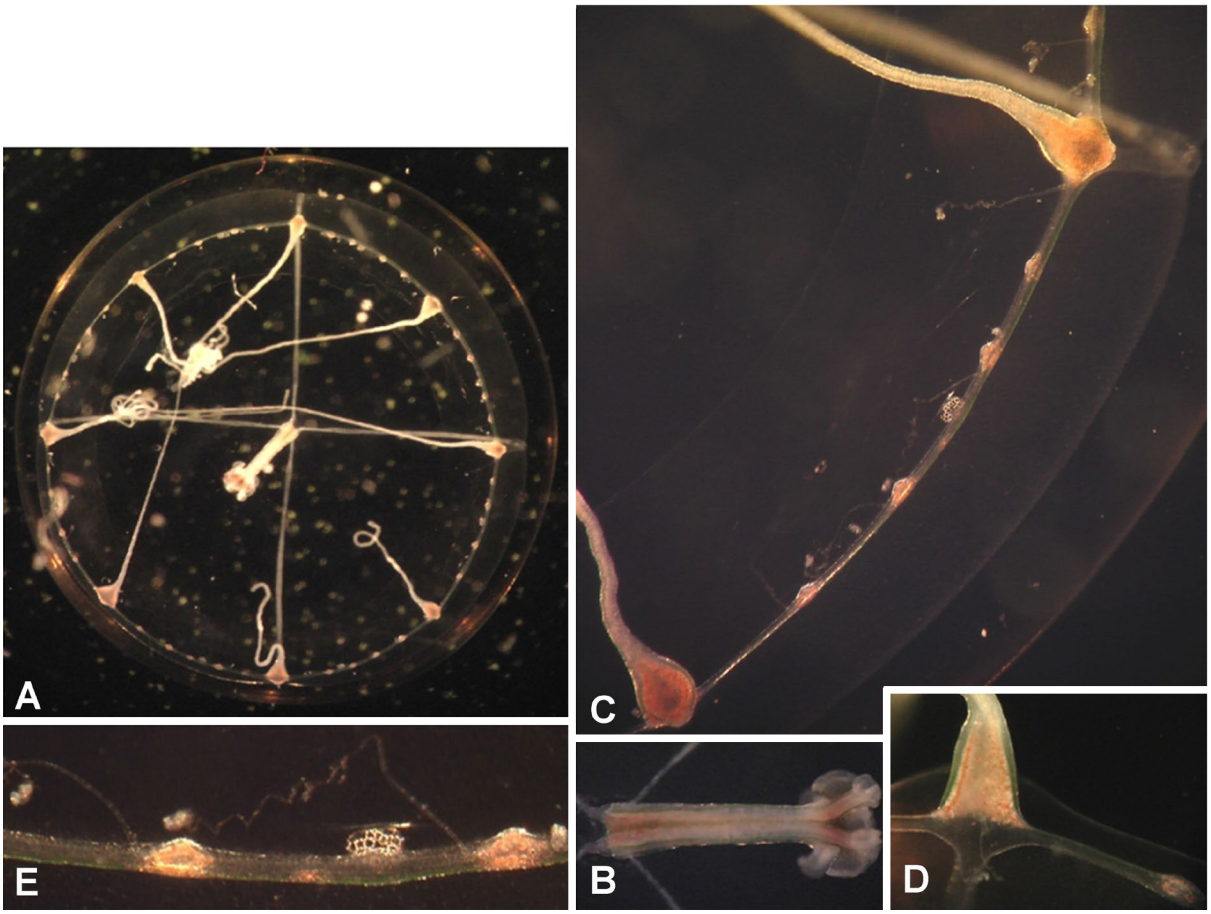


**Fig. 1.** A well-developed, but not yet fully matured, 1-month-old laboratory-reared medusa of *Eutima japonica* from Yeosu, Korea, 7.1 mm in diameter. A, Oral view; B, The manubrium with black line; C, Part of umbrellar margin, showing tentacular bulbs, marginal warts, and statocysts; D, A tentacular bulb with one abaxial cirrus; E, Part of umbrellar margin, showing marginal swellings with or without cirri and a statocyst containing many statoliths.

logical Laboratory, Kyoto University). In these vessels, material of every developmental stage was safely cultured and rarely died. Newly hatched *Artemia* nauplii were supplied in sufficient quantities to each medusa and polyp every day, then the seawater was changed daily. Water temperature was about 20−28°C (room temperature) during the culture. The last individual of the youngest medusa released from the polyp was obtained on May 19, 2018 in the laboratory in Shirahama, Wakayama Prefecture, Japan and cultured together with others released before.

For checking GFP patterns, a living individual was placed in a depression slide glass, and the GFP pattern was observed under OLYMPUS-SZX-RFL2 fluorescence microscope (Olympus, Tokyo, Japan) with blue light excitation, using the BP460-490 filter set, at Kinki University in Shirahama, Wakayama Prefecture.

## SYSTEMATIC ACCOUNTS

Order Leptothecata Cornelius, 1992
Family Eirenidae Haeckel, 1879
[1]*Genus *Eutima* McCrady, 1859

[2]***Eutima japonica* Uchida, 1925 (Figs. 1, 2)**

*Eutima japonica* Uchida, 1925: 93, fig. 17; Yamazi, 1958: 136; Kramp, 1961: 197; 1965: 85; 1968: 96, fig. 260; Kubota, 1983: 296, figs. 1−25, Pl. 10; 1985: 144, figs. 1−2; 1990: 104.

*Ostreohydra japonica* Yamada, 1950: 117, fig. 1.

*Eugymnanthea japonica*: Crowell, 1957: 162; Yamada, 1959: 30; Rees, 1967: 221.

*Eugymnanthea cirrhifera* Kakinuma, 1964: 51, figs. 1−4; Uchida, 1964: 103, fig. 4; Rees, 1967: 221.

*Eutima cirrhifera*: Kubota, 1978: 125, figs. 1−11; 1979: 225,

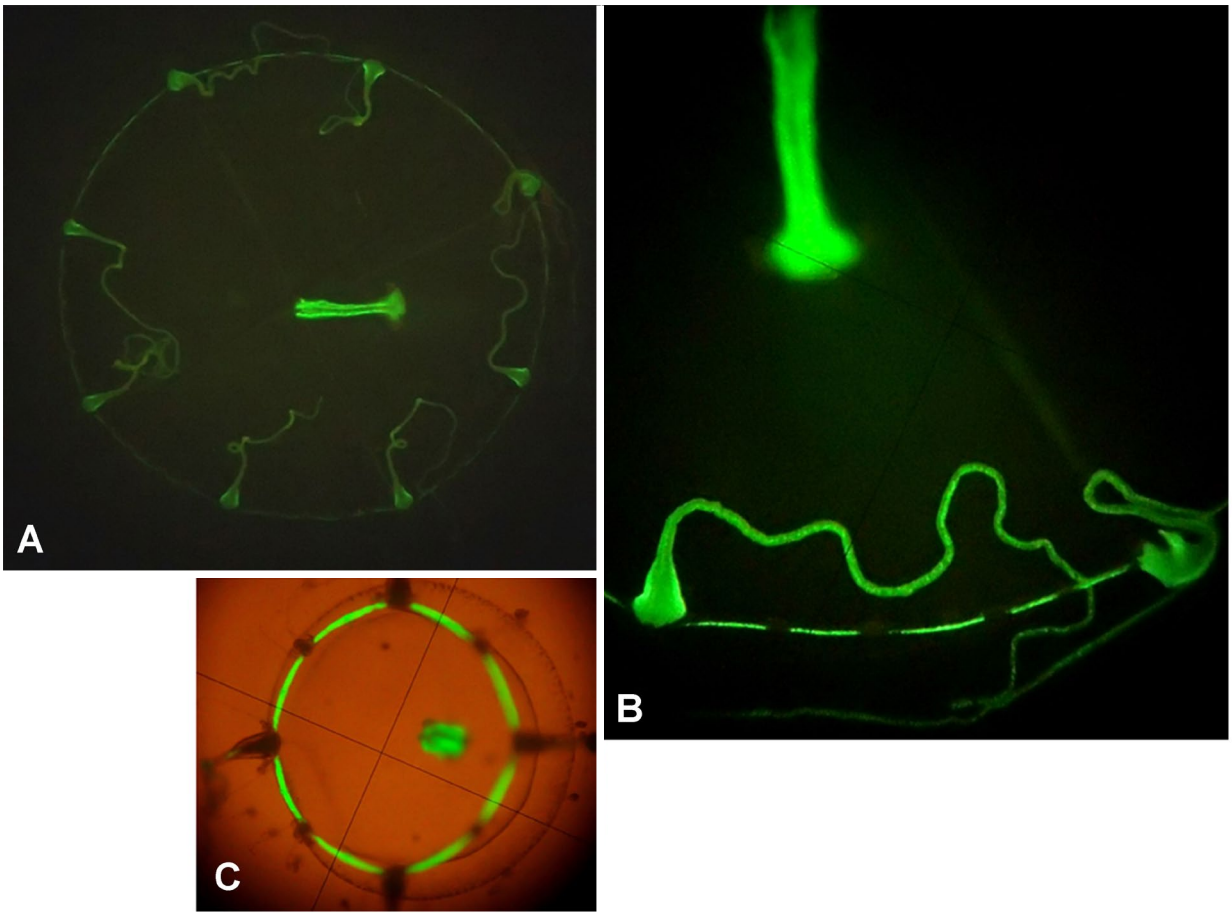


**Fig. 2.** Green fluorescent protein distribution of the laboratory-reared medusa in a well-developed, 1-month-old medusa (A, B) and that of a newly liberated medusa 1 day old (C) of *Eutima japonica* from Yeosu, Korea.

Korean name: [1]*조개살이히드라속, [2]*가랑잎조개살이히드라

**Table 1.** Measurements of laboratory-reared, well-developed medusa of *Eutima japonica* from Yeosu, Korea, about one month after release

| No. | Diameter (mm) | Height (mm) | Manubrium length (mm) | Peduncle length (mm) | Thickness of mesoglea at bell apex (mm) | No. of tentacles, statocysts, marginal warts (no./quardrant), and statoliths (no./statocyst) | No. of lateral cirri, max. no./tentacular bulb or marginal wart, and max. no./quardrant |
|---|---|---|---|---|---|---|---|
| 1 | 8.5 | 6.9 | 1.3 | 0.4 | 6.9 | 8, 8, 32 (4), 53 (6–8) | 21, 2, 9 |
| 2 | 8 | 6 | – | 0.5 | 3.1 | 8, 8, 34 (4–5), 50 (5–7) | 26, 2, 9 |
| 3 | 7.6 | 5.9 | 1.5 | 0.8 | 3.1 | 8, 8, 33 (3–5), 54 (6–8) | 30, 2, 10 |
| 4 | 7.5 | 5.6 | 1.6 | 0.8 | 3.5 | 8, 8, 32 (4), 57 (6–8) | 34, 3, 10 |
| 5 | 7.5 | 5.5 | 1.6 | 0.8 | 3.1 | 8, 8, 32 (4), 49 (5–7) | 23, 3, 8 |
| 6 | 7.1 | 6.3 | 1.3 | 0.6 | 3.4 | 8, 8, 32 (4), 54 (5–8) | 38, 2, 12 |
| 7 | 7 | 6.3 | – | 0.4 | 3.8 | 8, 8, 33 (4–5), 53 (6–9) | 27, 2, 8 |
| 8 | 6.9 | 6.3 | – | 0.9 | 3 | 8, 8, 34 (4-5), 67 (8–9) | 35, 2, 10 |
| 9 | 6.9 | 5.5 | 1.4 | 0.5 | 2.9 | 8, 8, 34 (4–5), 56 (6–8) | 29, 2, 11 |
| 10 | 6.9 | 6 | – | 0.6 | 3.1 | 8, 8, 32 (2–5), 51 (6–7) | 34, 2, 11 |
| 11 | 6.9 | 5.6 | 1.5 | 0.8 | 3.1 | 8, 8, 32 (4), 47 (5–7) | 28, 2, 9 |
| 12 | 6.9 | 5.6 | – | 0.5 | 3.1 | 8, 8, 34 (4–5), 62 (6–13) | 28, 2, 9 |
| 13 | 6.9 | 5.6 | 1.6 | 0.6 | 3.1 | 8, 8, 32 (4), 55 (6–9) | 21, 2, 8 |
| 14 | 6.9 | 5 | 1.4 | 0.6 | 3.1 | 8, 8, 34 (4), 54 (6–7) | 29, 2, 9 |
| 15 | 6.6 | 5 | 1.4 | 0.6 | 3 | 8, 8, 33 (4–5), 60 (6-9) | 32, 2, 10 |
| 16 | 6.4 | 5 | 1.3 | 0.6 | 3.1 | 8, 8, 34 (4–5), 55 (6–8) | 31, 2, 10 |
| 17 | 6.3 | 5 | 1.3 | 0.5 | 2.5 | 8, 8, 30 (3–4), 51 (5–7) | 26, 2, 9 |
| 18 | 5.6 | 5 | – | 0.3 | 3.1 | 8, 8, 34 (4–5), 56 (6–8) | 29, 2, 11 |

figs. 1–4.

*Eucheilota intermedia* Kubota, 1984: 454, figs. 1–3; 1985: 122, figs. 1–5, Pl. 1.

**Material examined originally.** Polyps, South Korea: Gyeongsangnam-do, Yeosu-si, Yeosu seafood market, 30 Apr 2018 (S. Kubota), from *Mytilus galloprovincialis*. After release from the polyps, 1-month-old, well-developed 18 medusae cultured in the laboratory, and among them 1.5-month-old 3 matured medusae.

**Description.**

**Polyp:** Among many zooids examined, a larger solitary polyp having 24 tentacles. Intertentacular web apparent. Medusa bud produced 1–4 in number per polyp, usually 1, on a lower part of hydrocaulus.

**Newly released medusa:** Medusae within one day after release from their polyps having 2–4 tentacles (N = 41): 10 medusae with 2 tentacles; 3 medusae with 3 tentacles, and 28 medusae with 4 tentacles. Eight statocysts present. Each statocyst containing 1 statolith. Four marginal warts on interradial part. Mouth simple and cruciform. Many cirri present besides tentacular bulbs and marginal warts. GFP pattern observed and compared with grown-up medusa below.

**Well-developed and mature medusae:** A well-developed medusa about one month old, 5.6–8.5 mm in bell diameter, having 4 immature, oblong gonads, 8 tentacles, 8 statocysts, and a well-deve1opped peduncle (Table 1, Fig. 1A). Manubrium not protruded from the umbrellar aperture (Fig. 1B). Each of 4 oral lips frilled only once (Fig. 1A, B). On umbrellar margin, usually 4 marginal warts in each octant (Fig. 1C); 30–34 marginal warts per medusa. A total of 21–55 cirri per medusa, maximally 3 cirri on one or both sides of tentacular bulbs, marginal warts, and abaxial side of tentacular bulbs (Fig. 1D, E). A total of 47–67 statoliths, 5–13 statoliths per statocyst (Fig. 1E). Black pigmented regions on manubrium and tentacular bulbs (Fig. 1B).

GFP on manubrium, tentacular bulbs, tentacles, and umbrellar margin (Fig. 2A, B). Note that GFP on tentacular bulbs, tentacles, and interradial marginal warts of 1-day-old medusa unclear (Fig. 2C).

Three medusae reared for 1.5 months, and their morphology not undergo further noticeable changes (Table 1), except for three characters: (1) manubrium protruded from umbrellar aperture; (2) every cirrus disappeared; (3) they became male mature medusae. Largest one 9.3 mm wide, 6.4 mm high, 3.6 mm in mesoglea thickness, with a peduncle of 3.6 mm long, 82 marginal warts (10–11 warts per octant), and 115 statoliths (13–17 statoliths per statocyst).

**Remarks.** Although just one colony could be examined in the present study, the morphology of every developmental stage of the present material from Korea is in good accord with those of the northern Japanese form of *Eutima japonica* from Japan, that have such characteristics as a well-developed peduncle but no cirri on umbrellar margin (Kubota, 1978, 1983). Therefore, the geographical distribution of this form of the present species is widened, Japan, China and Korea. GFP pattern of medusa of the present species is clearly different from that of *Eutima sapinhoa* (Kubota, 1997;

Migotto et al., 2004; Kubota et al., 2010), and the mesoglea of the latter species is thin. It is noticeable that in Tshushima Island that is very near to the southern coasts of Korea, only intermediate form of *E. japonica* is distributed among four forms, and *Eugymnanthea japonica* is sympatrically distributed (Kubota, 1991, 2012).

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