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The life cycle of a bivalve-inhabiting hydrozoan, *Eutima sapinhoa* (Cnidaria, Hydrozoa), from Florida, USA

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Abstract. The life cycle of a bivalve-inhabiting hydrozoan from Florida, USA, is clarified by laboratory-rearing for the first time. The morphology of the polyp (associated with *Crassostrea virginica*), newly released medusa, and mature medusa of this hydrozoan is described, and it is identified as *Eutima sapinhoa* Narchi and Hebling, 1975. The green fluorescent protein (GFP) pattern of the medusa is also described and compared with that of *Eutima japonica* Uchida, 1925.

Key words: life cycle, *Eutima*, culture, hydrozoan, medusa, GFP, geographical distribution

Introduction

Along the western shores of the Atlantic Ocean only one species of bivalve-inhabiting hydrozoan of clear taxonomic status has been described, *Eutima sapinhoa* Narchi and Hebling, 1975 (q.v.; see also Migotto *et al*., 2004). Two bivalve-inhabiting hydrozoans reported from Puerto Rico (Mattox and Crowell, 1951) and Florida, USA (Kubota and Larson, 1990), are still poorly known, and their mature medusae are uncertain despite well-advanced ecological studies (Samler, 2001; Winstead *et al*, 2004; Tolley *et al*, 2010). In the present study, the life cycle of the bivalve-inhabiting hydrozoan from Florida is clarified by laboratory-rearing for the first time, and its taxonomic status is determined. The polyp, newly released medusa, and mature medusa of this hydrozoan are described in detail; moreover, the green flourescent protein (GFP) pattern of this hydrozoan is also described and compared with that of *Eutima japonica* Uchida, 1925 from the Pacific Ocean.

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Materials and Methods

On November 18 and 19, 2011, 170 individuals of the American oyster *Crassosotrea virginica* (Gmelin) attached to many rocks were examined *in situ* at low tide. They were from five seashore sites near Tampa Bay, Florida, USA. Polyps found inside one oyster were picked out at the site: Bird Key, Sarasota, 27° 19.600′N, 82°33.755′W (32 psu; Table 1).

Laboratory culture of the polyps was done individually after bringing them back to Japan. For each polyp, a 60- or 80-ml polystyrene vessel of 60 mm in diameter and 15 or 30 mm high was filled with filtered seawater (32 psu) from Shirahama, Wakayama Prefecture. In these vessels, material of every developmental stage was reared. Newly hatched *Artemia* nauplii were supplied in sufficient quantities to each specimen every day and the medium was changed daily after feeding was over. Water temperature was maintained at 25℃ .

For checking GFP patterns, each living individual was placed in a depression slide and the GFP pattern was observed under an epi-flourescence microscope (Nikon ECLPSE 80i, Japan) with blue light excita-

tion (using the B-2A filter set). All GFP photographs are shown as fluorescence images superimposed on transmission images of the same individuals.

Description

Polyp

Among the many zooids examined, the largest was 1.24 mm long with 32 tentacles. Other smaller zooids had as many as 36 tentacles. Only one medusa bud, oblong in shape when well-developed but contractile and form-labile, was produced on the lower part of the hydrocaulus. The pedal disc was ovoid. One zooid that had reattached to the rearing vessel (Fig. 1) rotated clockwise and/or anti-clockwise for several days. No GFP was detected in the polyps, but well-developed medusa buds displayed a GFP pattern (Fig. 2).

Newly released medusa

Medusae within 0.5 day after release were 1.3-2.3 mm in diameter with four tentacles (N=6). There were eight statocysts on the umbrellar margin, each containing 3-6 statoliths for a total of 27-32, and 12-24 marginal warts (3-6 per quadrant) (Fig. 3). The mouth was simple and cruciform. No cirri were present. GFP was present exclusively on the umbrellar margin except for the tentacular bulbs, marginal

Table.1. Collection data of polyps from Florida, USA.

Locality	Date collected	No. of *C. virginica* examined	No. of *C. virginica* associated with polyps
Road crossing the entrance of Tampa Bay	Nov. 18, 2011	15	0
Brandenton Beach	Nov. 18, 2011	20	0
Bird Key, Sarasota	Nov. 18 & 19, 2011	81	1
Habourview Center	Nov. 19, 2011	39	0
Belleair Bluffs	Nov. 19, 2011	15	0

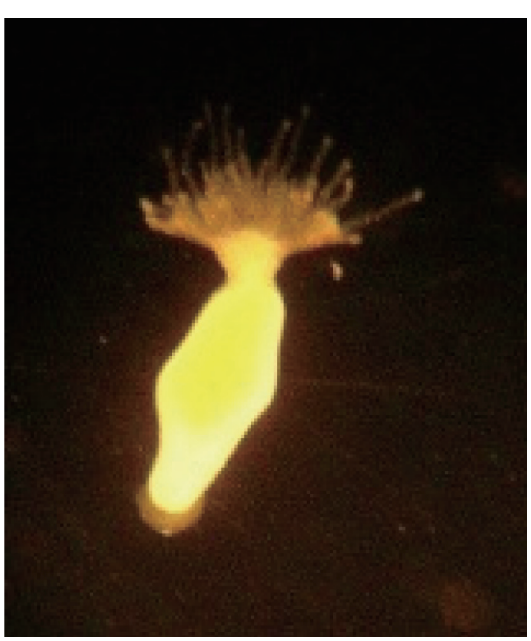

Fig. 1. Polyp of *Eutima sapinhoa* from Florida, USA, attached to a polystyrene rearing vessel.

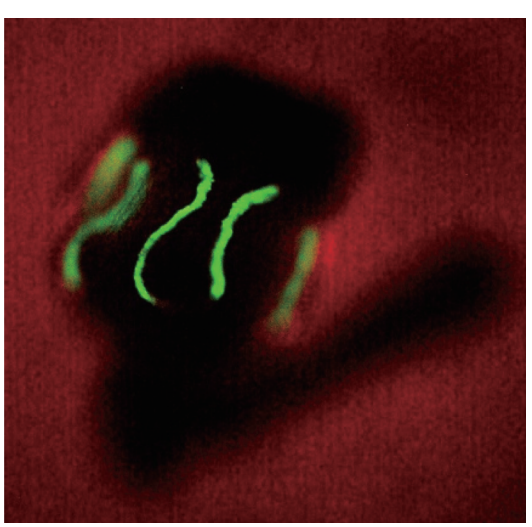

Fig. 2. GFP pattern of a well-developed medusa bud of *Eutima sapinhoa* from Florida, USA.

Fig. 3. Part of umbrellar margin of a newly liberated medusa of *Eutima sapinhoa* from Florida, USA.

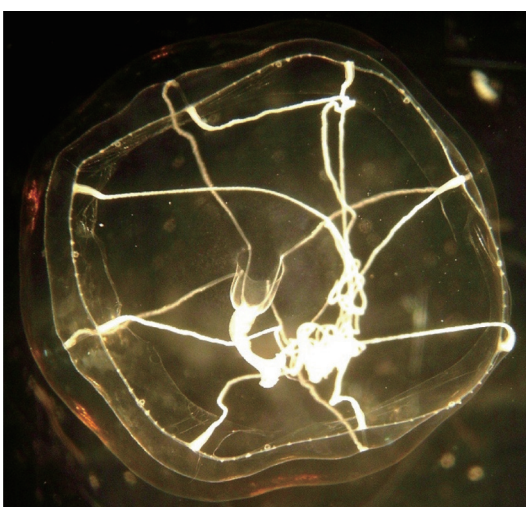

Fig. 5. Mature female medusa of *Eutima sapinhoa* from Florida, USA (9.6 mm in umbrellar diameter).

Fig. 6. Part of umbrellar margin and oral lips of a female medusa of *Eutima sapinhoa* from Florida, USA (same specimen as in Fig. 5).

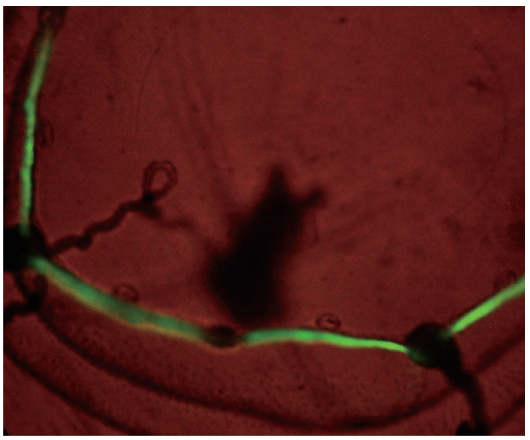

Fig. 4. GFP pattern of a newly liberated medusa of *Eutima sapinhoa* from Florida, USA (part of umbrellar margin; same specimen as in Fig. 3).

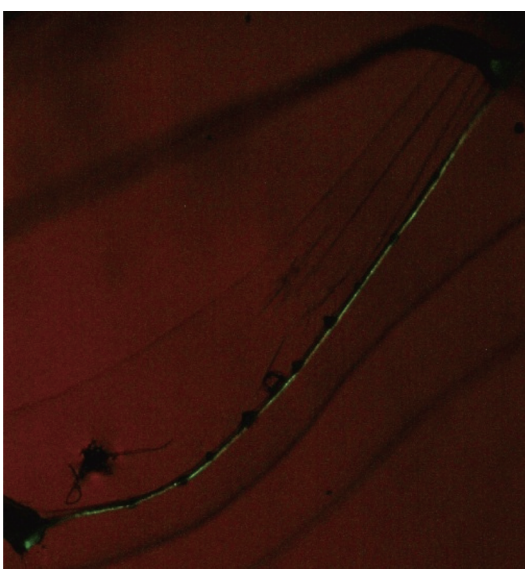

Fig. 7. GFP pattern of a mature medusa of *Eutima sapinhoa* from Florida, USA (part of umbrellar margin; same specimen as in Fig. 5).

Table.2. Morphology of laboratory-reared, mature female medusae from Florida, USA, 22-25 days after release.

No.	Diameter (mm)	Height (mm)	Manubrium (mm)	Peduncle length (mm)	Thickness of mesoglea at bell apex (mm)	Nos. of tentacles,statocysts, marginal warts & statoliths	No of statoliths per statocyst
1	9.6	6.4	1.8	3.6	3.6	8, 8, 77, 105	12-14
2	9.1	5.5	1.8	2.7	2.7	8, 8, 71, 85	9-12
3	8.6	4.6	1.4	3.6	2.3	8, 8, 75, 96	11-13
4	8.6	5.0	1.4	3.2	2.7	8, 8, 69, 90	9-13
5	8.6	5.5	1.4	2.7	2.7	8, 8, 69, 104	12-15
6	8.2	4.6	1.4	2.7	2.3	8, 8, 72, 94	9-14
7	8.0	5.0	1.4	2.7	2.6	8, 8, 71, 90	9-13

warts, and statocysts (Fig. 4).

Mature medusa

Every immature medusa became a mature female medusa by the 8th or 9th day after release. The eggs are produced in four gonads, each situated along nearly the whole length of one of the four radial canals. The earliest mature medusae (N=4) were 7.3-8.2 mm in bell diameter with eight tentacles, and with well-developed peduncle. The manubrium protruded from the umbrellar aperture. On the umbrellar margin four or five marginal warts were present in each octant. The oral lips were frilled.

Mature medusae 22-25 days after release were 8.0-9.6 mm across and 4.6-6.4 mm high with eight tentacles (N=7) (Fig. 5). They had a total of 85-105 statoliths, with each of the eight statocysts containing 9-15 of them. In each octant 9-14 marginal warts were present, for a total of 69-77 warts per medusa (Fig. 6). The thickness of the mesoglea at the umbrellar apex was 2.3-3.6 mm. The peduncle was distinct and long, measuring 2.7-3.6 mm in length (Table 2).

Only one medusa could be reared as long as 47 days, and its morphology did not undergo further change. It was 9.3 mm wide, 6.4 mm high, and 3.6 mm in mesoglea thickness, with a peduncle 3.6 mm long, 82 marginal warts (10-11 warts per octant), and 115 statoliths (13-17 statoliths per statocyst). No cirri were produced during the life span of this medusa.

The GFP pattern of the mature medusae was the same as that of the immature medusa (Fig. 7).

Remarks

Although just one female colony was examined in the present study, the morphology of every developmental stage of the present material from Florida is in good accord with that of specimens of *Eutima sapinhoa* from near São Paulo, Brazil, described by Migotto *et al*. (2004). The present material is thus identified as *E. sapinhoa*. This species appears to have a wide geographical distribution in the West Atlantic, from middle Brazil to the southern USA (Florida), and possibly also including Puerto Rico (Mattox and Crowell, 1951).

The GPF pattern of the medusa (including medusa buds) of the present species, reported here for the first time, is clearly different, and less complicated than that of *Eutima japonica* in the Pacific Ocean. GFP was found only along the umbrellar margin in the present species whereas in *E.japonica* it is found in other body parts as well, such as the tentacular bulbs, tentacles, marginal warts, and manubrium (cf. Kubota *et al*., 2010). The distinctive GFP patterns of these two related species thus prove to be a reliable taxonomic character allowing the species to be distinguished. This is also the case for another genus of bivalve-inhabiting hydroids *Eugymnanthea*, in which *Eugymnanthea inquilina* Palombi, 1935 from the Mediterranean Sea and *Eugymnanthea japonica* Kubota, 1979 from the Pacific Ocean differ in their GFP patterns (Kubota *et al*., 2008).

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