

Morphology of newly liberated medusae of *Turritopsis* spp. (Hydrozoa, Oceanidae) from Japan and abroad

Shin Kubota

Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University,
459 Shirahama, Nishimuro, Wakayama, 649-2211 Japan

Abstract. Morphology of the youngest medusae of an immortal jellyfish *Turritopsis* from Shirahama and Okinawa (Japan), Eilat (Israel) and Porto Cesareo (Italy) is compared to detect any difference among them. But no outstanding distinction is found among them that obtained by culture from their polyps kept for up to 2–6 years. No GFP is detected in all of them. Therefore, for the present, only molecular distinction might be possible for species demarcation.

Key words: culture, GFP, hydrozoan, immortal, morphology, *Turritopsis*, youngest medusa

Introduction

In southern Japan a small form of *Turritopsis* is distributed, while a large form that colored red is distributed in northern Japan (Kubota, 2005). A small form is, however divided two separate species by molecular analysis (Miglietta et al., 2007). In the present paper, in order to seek any morphological characteristics of these two molecular species, i.e. *Turritopsis* sp. from central Japan (assignable to a new species, probably endemic to Japan) and *T. dohrnii* (thought to be an introduced species from the Mediterranean), the youngest medusae from Japan and other foreign countries are examined in addition to GFP distribution analysis.

Materials and Methods

All the youngest medusae examined in the present study are obtained by culture of their polyps at Shirahama, Wakayama Prefecture, Japan by the same

culture methods. Four different materials such as one female colony collected from Okinawa Island, Japan in May, 2009 and it rejuvenated 12 or 13 times [as the world rejuvenation record now, but actually 14] (Kubota, 2011), one colony collected at Shirahama, Wakayama Prefecture, Japan in September, 2010 and rejuvenated twice, one colony from Eilat, Israel collected in December, 2013 (Fig. 1), and one colony collected in Porto Cesareo, Italy in June, 2010 (Kubota and Grravili, 2011), were maintained in steady water flow of a natural seawater (c. 32 psu) in a running system, fed with the newly hatched *Artemia* nauplii. All the polyps attached to 60 cc polystyrene vessel (60 mm in diameter; 20 mm high). Removing algae and other macroscopic organisms that grew on the bottom of the rearing vessels by frequent cleaning by a wooden stick with a pointed tip. As to Italian colony, although they grow very well, the first release of their medusae took 5 years after collection.

Fluorescence distribution pattern of medusa was observed under Nikon ECLIPSE 80i with blue light excitation as was observed before (Kubota, 2010).

*Corresponding author: kubota.shin.5e@kyoto-u.ac.jp

Results and Discussion

By the above-mentioned method *Turritopsis* polyp 4 colonies from 3 countries have been kept in laboratory for 2-6 years. During the period between on March 23 and April 10, 2015, from all these colonies the youngest medusae released nearly at the same time (such an event is the first occasion) and they could be examined all at once. Their umbrella sizes (always measured the diameter, sometimes height) are compared with one another.

As a result, no distinct size differences were detected among them (Table 1). They are usually higher than wide: in 21 Shirahama specimens 16 are

showed this condition, while only one is opposite and four ones are equal. Their umbrellar apex is usually flat, but sometimes a very small protrusion is found due to growth, e.g. in Shirahama specimens 20 are flat while 7 are pointed, in Okinawa specimens 14 are flat but 3 are pointed, in Israel specimens 18 are flat (Fig. 2) but 3 are pointed, and in Italy specimens 10 are flat but only 1 was pointed. All of these specimens observed (totally 122) have not exumbrellar nematocysts (34 medusae in Shirahama, 16 in Okinawa, 61 in Israel, and 11 in Italy). Almost all of the youngest medusae had 8 tentacles (34 medusae in Shirahama, 16 in Okinawa, 61 in Israel, 10 in Italy), but only a few medusae from Israel and Italy had

Table 1. Umbrella size (mean $\pm$ SD, range, n) of the youngest medusae of *Turritopsis* (1-day-old) released from their cultured polyps collected from 3 countries.

Country	Umbrella diameter (mm)	Umbrella height (mm)
Japan (Shirahama, Wakayama)*	0.57 $\pm$ 0.04, 0.50-0.72, 26	0.62 $\pm$ 0.02, 0.53-0.72, 21
Japan (Okinawa I., Okinawa)**	0.60 $\pm$ 0.02, 0.55-0.62, 16	-, 0.55-0.67, 3
Israel (Eilat)***	0.53 $\pm$ 0.04, 0.48-0.60, 21	Not measured
Italy (Porto Cesareo)**	0.56 $\pm$ 0.03, 0.50-0.60, 11	Not measured

* : *Turritopsis* sp. A

** : *T. dohrnii*

*** : ?*T.* sp. A/B/*dohrnii*

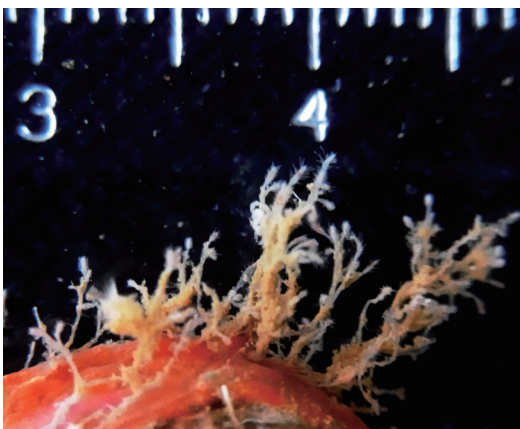


Fig. 1. A polyp colony collected at a wharf in Eilat, Israel on December 7, 2013.



Fig. 2. The youngest medusa released from the cultured polyp from Eilat, Israel.

one more very short tentacle. All of them had four yellow cramps on their interradal portions of the manubrium (Fig. 2). A few medusae from Shirahama had vacuolated portions above the manubrium, but the remaining all other many medusae from 3 countries had not such portions yet.

On the other hand, GFP marker, as is often used for the species-specific tool (Kubota et al., 2008), is not detected in all of the present materials as is partly observed before (Kubota, 2010). Therefore, molecular distinction might be, for the present, a sole marker (Miglietta et al., 2007).

As to mature medusae comparison, due to strong rainfall by an usual typhoon attacked to our region in middle of May, 2015, every cultured colony died (Kubota, 2015), therefore such comparison could not carry out.

Acknowledgements

The author wishes to offer sincere his thanks to Mr. Kazuhito Niina for his kind help to culture the materials.

References

Kubota, S. 2005. Distinction of two morphotypes of *Turritopsis nutricula* medusae (Cnidaria, Hydrozoa, Anthomedusae) in Japan, with reference to their different abilities to revert to the hydroid stage and their distinct geographical distributions. *Biogeography*, **7**: 41–50.

Kubota, S., Paguliara, P. and Gravili, C. 2008. Fluorescence distribution pattern allows to distinguish two species of *Eugymnanthea* (Leptomedusae: Eirenidae). *J.Mar. Biol. Assoc., UK*, **88**(8): 1743–1746.

Kubota, S. 2010. Various distribution patterns of green fluorescence in small hydromedusae. *Kuroshio Biosphere*, **6**: 11–14, 3 pls.

Kubota, S. 2011. Repeating rejuvenation in *Turritopsis*, an immortal hydrozoan (Cnidaria, Hydrozoa). *Biogeography*, **13**: 101–103.

Kubota, S. and Gravili, C. 2011. Rare distribution of green fluorescent protein (GFP) in hydroids from Porto Cesareo, Lecce, Italy, with reference to biological meaning of this rarity. *Biogeography*, **13**: 9–11.

Kubota, S. 2013. Difference of rejuvenation among three species of *Turritopsis* (Hydrozoa, Anthomedusae) from Japan. *Bull. biogeogr. Soc. Japan*, **68**: 139–142. (in Japanese with English abstract)

Miglietta, M. P., Piraino, S., Kubota, S. and Schuchert, P. 2007. Species in the genus : a molecular evaluation. *J. Zool. Syst. Evol. Res.* **45**(1): 11–19.

Kubota, S. 2015. *Turritopsis* (Hydrozoa, Anthomedusae) polyp cultured in the laboratory nearly annihilated after heavy rain caused by typhoon. *Annual Report, Seto Marine Biological Laboratory*, **28**: in press. (in Japanese)

(Received June 30, 2015; Accepted August 7, 2015)