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Rare distribution of green fluorescent protein (GFP) in hydroids from Porto Cesareo, Lecce, Italy, with reference to biological meaning of this rarity

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Abstract. A reliable taxonomic character, distributional pattern of green fluorescent protein (GFP), that is contributable to species demarcation by observing living materials under the fluorescent microscope, has not been much done in hydroids in contrast to hydromedusae. We carried out such a fundamental study in diverse hydroids collected from Porto Cesareo, Lecce, Italy. In contrast to diversified distribution of GFP in the hydromedusae, GFP distribution in hydroids are unexpectedly very rare, and its biological meaning is deduced.

Key words: GFP, hydroid, hydrocaulus, campanulariid, developmental stage

Introduction

Distinct distribution pattern of green fluorescent protein (GFP) in small hydromedusae is contributable to species demarcation as is demonstrated in congeneric species of *Eugymnanthea* that is morphologically very similar due to parallel evolution (Kubota et al., 2008), whereas it may be convergently evolved among various taxonomic groups of small hydromedusae in higher classification levels as is demonstrated in Eirenidae (*Eugymnanthea vs Eutima*) or other families and in also much more higher levels (Kubota et al., 2010; Kubota, 2010). However, such a fundamental study has not been done in hydroids except for campanulariids that had been well-known to have obelin and clytin (Shimomura, 1985; Tsuji et al., 1995; Inouye and Sahara, 2007). Therefore, the present study is focused to carry out to detect GFP distribution and its contribution to taxonomy in hydroids with or without medusa stage. In this study, difference of fluorescent pattern according to developmental stages in metagenetic species is paid attention to analyze in order to deduce biological meaning of GFP distribution pattern in the life cycle.

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Materials and Methods

Distribution pattern of GFP was observed in fresh and living specimens collected from Porto Cesareo, Lecce, Italy in in middle period of September, 2010. Nine species of colonial hydroids belonging to Athecata (5 species: *Pennaria disticha, Coryne* sp., *Turritopsis* sp., *Eudendrium* sp., *Perigonemus* sp.) and Thecata (4 species: *Halecium* sp., *Dynamena disticha*, a hebellid, a campanulariid) of different taxonomic group (9 genera in 9 families in total) were examined. The number of colonies obtained were up to several in each species and every different colony was examined: hydranths and hydrocaulus of several zooids in each colony of every species were observed under fluorescent microscope (OPTIKA N-400LD) soon after collection and checked their GFP distribution on their body parts and photographed if present. In *Coryne* sp. a male gonophore could be examined, though other species are infertile colonies and found only one colony except *Pennaria disticha* and *Turritopsis* sp. In *Turritospis* sp. only one of several colonies bore only one medusa bud, but it soon degenerated and GFP of medusa bud could not be examined. In *Pennaria disticha* many released eumedusoids from several colonies and their unfertilized eggs spawned were also examined to clarify distributional difference among developmental stages.

Results

GFP was not found on hydranths, hydrocaulus and a gonophore of any hydroid species here examined such as *Pennaria disticha, Coryne* sp., *Turritopsis* sp., *Eudendrium* sp., *Perigonemus* sp., *Halecium* sp., *Dynamena disticha*, and a hebellid (totally 8 genera in 8 families). However, a few oblong shaped spots of GFP were detected on middle part of the hydrocaulus of a campanulariid hydroid, possibly *Clytia* sp. (Fig. 1, a, b). GFP was not detected in eumedusoids and eggs of *Pennaria disticha* as is the case of its hydroid stage, showing no GFP distribution in its entire life cycle.

Discussion

In contrast to wide distribution of GFP in small hydromedusae (Kubota et al., 2008, 2010; Kubota, 2010, 2011), it is very rare in hydroids as revealed in this study. It is possible that this difference greatly related to developmental stages, particularly between sexual and asexual one. Distribution pattern of GFP and its relative substances may be evolved independently even in sexual stage of medusa if present, then convergence occurred in various taxonomic groups in coupling to divergence observed in small hydromedusae (Kubota et al., 2010; Kubota, 2010). GFP distribution and bioluminescence will be also coupled to play its biological role, mainly sexual mating, judging from the fact that GFP and its relative substances are usually found in sexual stage of medusa (16 patterns are so far demarcated: Kubota, 2010, 2011) in contrast to presently clarified very rare distribution in asexual stage of hydroid except for campanulariids that had obelin and clytin as reported before (Shimomura, 1985; Tsuji et al., 1995; Inouye and Sahara, 2007). The biological role of these substances (Prasher et al., 1985, 1992; Shimomura, 2005) and luminous bacteria on the surface of the hydroids (Stabili et al., 2011) might defensive or warning purposes coupled to bioluminescence besides capture of prey known to a siphonophora species (Haddock et al., 2005; Haddock et al., 2010).

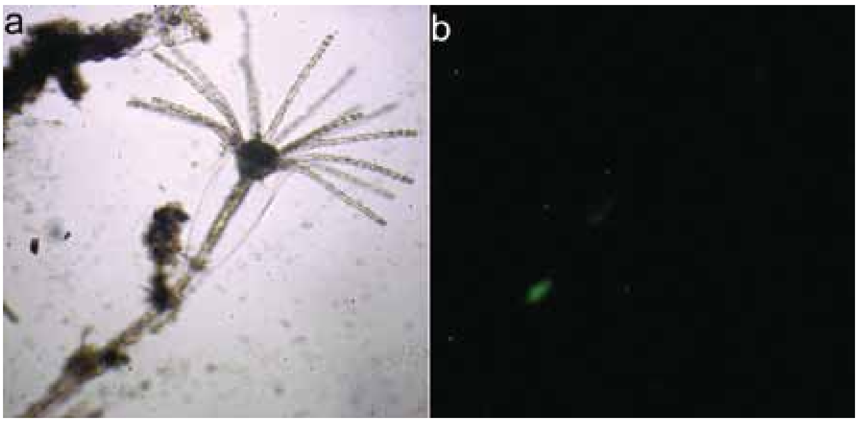

Fig. 1. Photomicrographs of a campanulariid hydroid, possibly *Clytia* sp. (a): a living material of a zooid of colony at transmitted light; (b): a portion of the same hydrocaulus at epifluorescence, showing GFP.

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