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VARIOUS DISTRIBUTION PATTERNS OF GREEN FLUORESCENCE IN SMALL HYDROMEDUSAE

By

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Abstract

Twelve distributional patterns of fluorescent body parts, including complete non-fluorescence, were found in diverse taxonomic groups of small hydromedusae (≤21 mm in umbrellar diameter) collected in 2008-2010 in Tanabe Bay and a freshwater pond in Tanabe city, Wakayama Prefecture, Japan, and in Suma harbor, Kobe city, Hyogo Prefecture. Only three of the green fluorescence patterns have been described until now. Particular fluorescence patterns may have originated convergently more than once within this taxonomic group. In *Liriope tetraphylla* the distribution of fluorescence changes during development, and the eggs of *Clytia languida* also display fluorescence.

Introduction

Based on an examination of many medusae, Kubota *et al*. (2008, 2009) described interspecific differences in the green fluorescence distribution patterns of the medusae of three species of bivalve-inhabiting hydrozoan (*Eugymnanthea japonica*, *Eugymnanthea inquilina*, and *Eutima japonica*) and suggested that the exhibited fluorescence patterns were not congruent with the phylogeny of these species as inferred from other evidence. In order to corroborate this suggestion, many additional taxonomic groups of small hydromedusae have now been examined. New fluorescence patterns, including hitherto unreported combinations of fluorescent body parts, are reported here with photographs.

Materials and methods

By towing a small plankton net (mesh size 0.334 mm) vertically and/or horizontally, small hydromedusae were collected in Tanabe Bay, Wakayama Prefecture. For *O. dichotoma* (Plate 1E) and *Bougainvillia* sp. (Plate 1F), newly released medusae were obtained soon after collection of hydroids with medusa buds at the nearby coast of Shirahama town, Wakayama Prefecture. One species of freshwater medusa, *Craspedacusta sowerbyi*, was collected (mature females, 16-21 mm in umbrellar diameter) from a pond in Tanabe city, Wakayama Prefecture (cf. Kubota and Tanase 2006; Kubota 2007). All these specimens were collected from October, 2008, to January, 2010. In addition, three species, *Spirocodon saltator, Rathkea octopunctata*, and *Clytia languida* (eggs spawned in the laboratory were also examined in this species only) were found in plankton samples taken at Suma harbor, Kobe city, Hyogo Prefecture, Japan, by T.

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Yamada and his colleagues in January, 2010, by towing a plankton net (mesh size 0.315 mm).

Each individual live medusa was placed in a depression slideglass of suitable size and its fluorescence distribution pattern was observed under a fluorescence microscope (Nikon ECLIPSE 80i, Japan) with blue light excitation (using the B-2A filter set). Most of photographs are shown as fluorescence images superimposed on transmission images of the same individuals (Plates 1-3). Some medusae were examined after having been cut artificially with a pair of needles to confirm fluorescence of the manubrium (Plate 3C). For most species, as many individuals were examined as needed to check for individual variation, but for some species, only one individual was available for examination.

Results and Discussion

In the present study, as a rule, no individual variation was detected in species for which multiple individuals were examined. Up to now, three patterns, *viz.*, a subumbrellar fluorescence pattern, an umbrellar margin pattern, and a manubrium + tentacular bulbs + tentacles + umbrellar margin pattern, have been discriminated in small hydromedusae belonging to the family Eirenidae (Kubota *et al*. 2008; 2009), but in this study many new patterns were detected involving novel combinations of fluorescent body parts. All the distribution patterns of green fluorescence are listed below and are shown in Plates 1-3, and the species showing each pattern are named. Many specimens examined were immature and, therefore, fluorescent gonads are not described here. However, gonadal fluorescence was clearly displayed in some species, such as *Obelia* spp. (Plate 1D; Plate 2B; Plate 3A), *Clytia discoida* (Plate 2A), and *Clytia languida* (Plate 2C2), similarly to the earlier reported example of *Eugymnanthea inquilina* from Italy (Kubota *et al*. 2008).

(1) Subumbrellar pattern: *Eugymnanthea japonica* (Plate 1A, right; cf. Kubota *et al*. 2008); *Extopleura minerva* (Plate 1B; n=1); *Solmundella bitentaculata* (Plate 1C; n=1).
(2) Umbrellar margin pattern: *Eugymnanthea inquilina* from Italy (Kubota *et al*. 2008); *Obelia* sp. aff. *plana* (Plate 1D; n=1; cf. Kubota, 1981).
(3) Tentacular bulbs pattern: *Obelia dichotoma* (Plate 1E).
(4) Manubrium (excluding oral tentacles) pattern: *Bougainvillia* sp. (Plate 1F); eudoxid of unidentifiable species of siphonophore (Plate 1G; n=1).
(5) Manubrium + tentacular bulbs pattern: *Euphysomma brevia* (Plate 1H; n=1); *Clytia discoida* (Plate 2A; n=1); *Obelia* sp. aff. *dichotoma* (Plate 2B; n=1; cf. Kubota 1999); *Rathkea octopunctata*.
(6) Manubrium + tentacular bulbs + tentacles pattern: *Clytia languida* (Plate 2C1, 2C2).
(7) Manubrium + tentacular bulbs + tentacles + umbrellar margin pattern: *Eutima japonica* (cf. Kubota *et al*. 2009); *Obelia* sp. aff. *plana* (Plate 3A; n=1; cf. Kubota 1981).
(8) Manubrium + tentacles + radial canals + umbrellar margin pattern: *Spirocodon saltator* (Plate 3B).
(9) Subumbrella + manubrium pattern: *Proboscidactyla ornata* (Plate 3C).
(10) Tentacular tip pattern: *Liriope tetraphylla* (Plate 3D).
(11) Manubrium + tentacles + subumbrella + umbrellar margin pattern: *Liriope tetraphylla* (Plate 3E).
(12) Non-fluorescent: *Turritopsis* sp. (Plate 1A, left); *Craspedacusta sowerbyi*.

Various specimens of *Obelia* hydromedusae (probably referable to at least two species) displayed four different patterns of fluorescence as noted above. At present it is unclear whether this is an example of intraspecific polymorphism or, as was demonstrated in two species of *Eugymnanthea* (Kubota *et al*. 2008), a manifestation of species-specific fluorescence

patterns. The taxonomy of *Obelia* is not well-established. A clarification of the life-history of each species together with an account of both the hydroid stage's and the medusa's fluorescence pattern is needed (cf. Kubota 1981; 1999).

On the whole, the fluorescence patterns of the various hydromedusa species display convergence. One pattern may be found in representatives of two or more distinct higher taxa, and no single pattern is found corresponding to a unique taxonomic group. For example, among medusae that exhibited the subumbrellar pattern, *Eugymnanthea japonica* belongs to the order Leptomedusae, *Extopleura minerva* to the order Anthomedusae, and *Solmundella bitentaculata* to the order Narcomedusae (Kubota and Gravili 2009). *Euphysomma brevia* (Anthomedusae; Euphysidae) and both *Clytia discoida* and *Obelia* sp. cf. *dichotoma* (Leptomedusae: Campanulariidae) all display the manubrium + tentacular bulbs pattern. These findings support the conclusion of Kubota *et al*. (2009) that fluorescence patterns are not useful in reconstructing the phylogeny of hydromedusae. Such convergence as has been demonstrated here may be ascribed to ecological and/or physiological reasons that have not been resolved.

Two additional novel findings of the present study may be mentioned. First, fluorescence was detected in spawned eggs of two female medusae of *Clytia languida* (Plate 2C3), in contrast to the non- fluorescent eggs of two species of *Eugymnanthea* (Kubota *et al*. 2008). Second, in *Liriope tetraphylla* the distributional patterns of fluorescent body parts changed with development; differences are clearly evident between very young (Plate 3D) and nearly mature individuals (Plate 3E).

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Explanation of Plates

PLATE 1

Figures A-H: Various patterns of green fluorescence in small hydromedusae (1). A: *Eugymnanthea japonica* (right; mature female, 0.95 mm in diameter); A: *Turritopsis* sp. (left; 0.8 mm in diameter); B: *Extopleura minerva* (aboral view, 0.62 mm in diameter); C: *Solmundella bitentaculata* (aboral view, 0.63 mm in diameter); D: *Obelia* sp. aff. *plana* (0.80 mm in diameter); E: *Obelia dichotoma* (newly released from the hydroid, 0.40 mm in diameter); F: *Bougainvillia* sp. (side and oral views, newly released from the hydroid, 0.64 mm in diameter); G: eudoxid of unidentifiable siphonophore; H: *Euphysomma brevia* (mature female, 1.4 mm in diameter).

PLATE 2

Figures A-C: Various patterns of green fluorescence in small hydromedusae (2). A: *Clytia discoida* (mature male, 0.83 mm in diameter); B: *Obelia* sp. aff. *dichotoma* (0.75 mm in diameter); C: *Clytia languida* (mature female, 14 mm in diameter), C1, manubrium, C2, tantacular bulb and tentacle, C3, eggs (150-170 μ m in diameter).

PLATE 3

Figures A-E: Various patterns of green fluorescence in small hydromedusae (3). A: *Obelia* sp. aff. *plana* (mature female, 0.98 mm in diameter); B: *Spirocodon saltator* (young medusa, 4 mm in umbrellar height); C: *Proboscidactyla ornata* (originally 1.5 mm in umbrellar diameter); D: *Liriope tetraphylla* (young medusa, 0.85 mm in diameter); E: *Liriope tetraphylla* (nearly mature medusa, 16 mm in diameter, M, manubrium, S, subumbrella, T, marginal tentacles, U, umbrellar margin).

PLATE 1

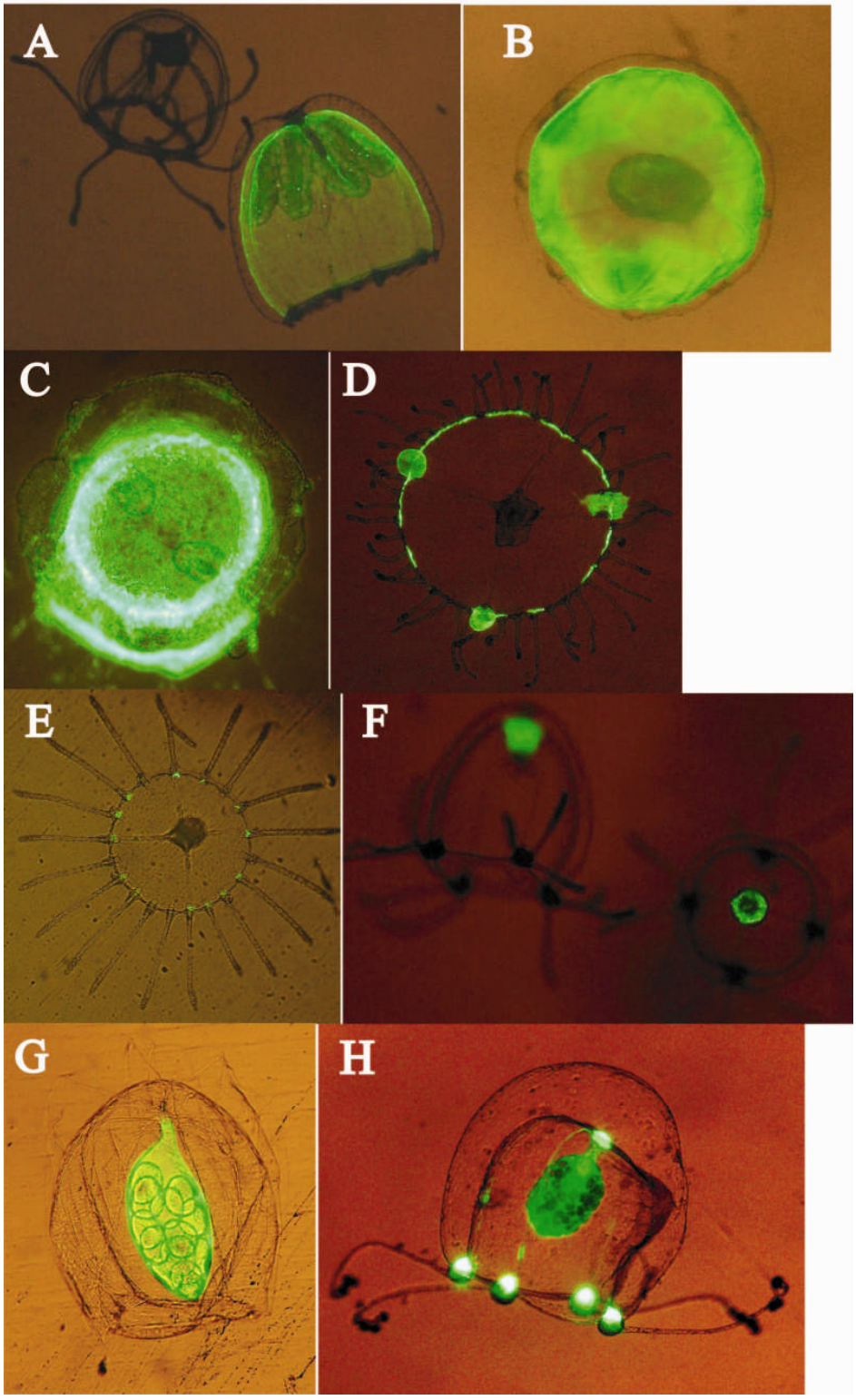

PLATE 2

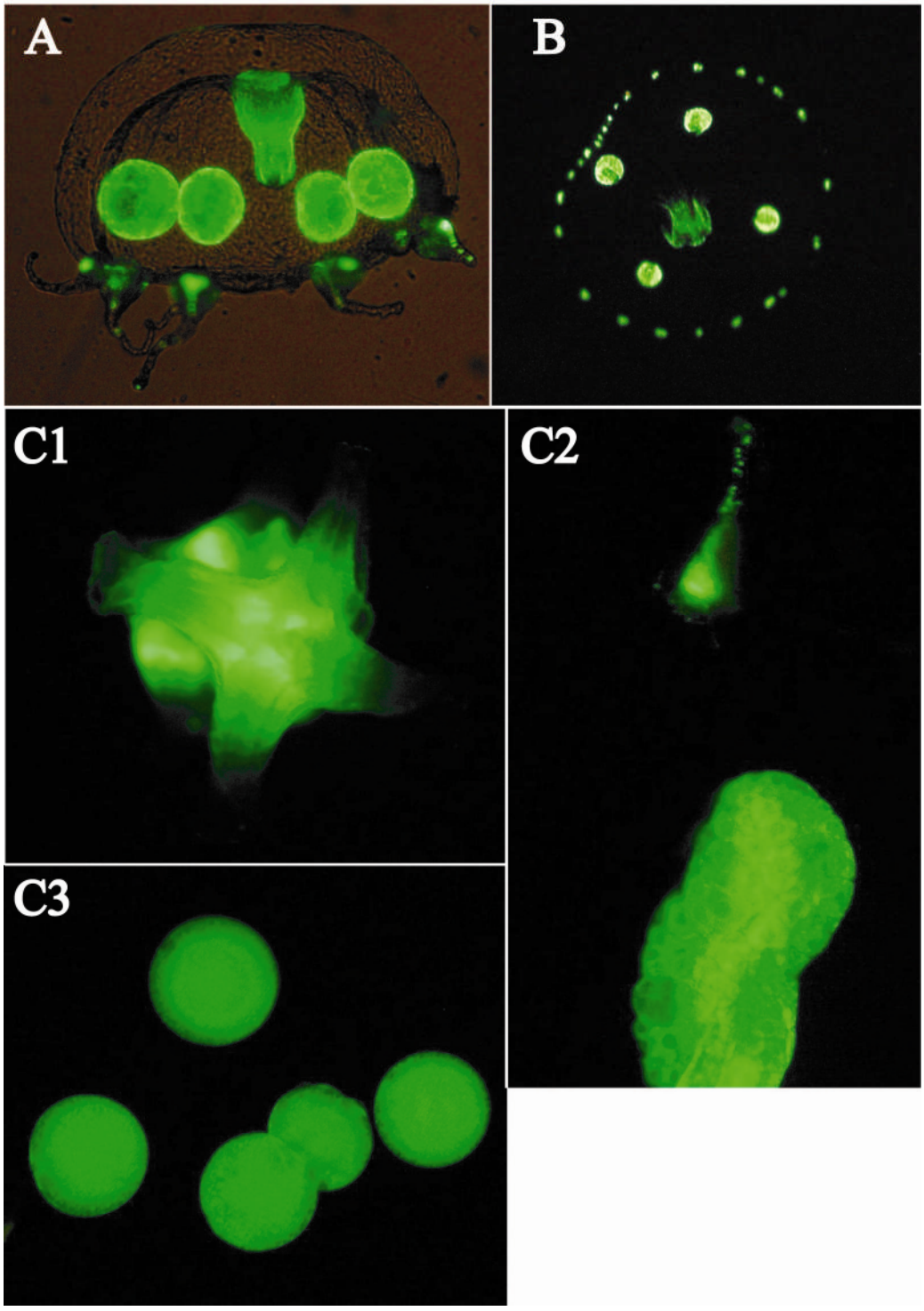

PLATE 3