

## Larval Development of Fertilized “Pseudo-Gynodioecious” Eggs Suggests a Sexual Pattern of Gynodioecy in *Galaxea fascicularis* (Scleractinia: Euphyllidae)

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**Shashank Keshavmurthy, Chia-Ming Hsu, Chao-Yang Kuo, Vianney Denis, Julia Ka-Lai Leung, Silvia Fontana, Hernyi Justin Hsieh, Wan-Sen Tsai, Wei-Cheng Su, and Chaolun Allen Chen (2012)** Larval development of fertilized “pseudo-gynodioecious” eggs suggests a sexual pattern of gynodioecy in *Galaxea fascicularis* (Scleractinia: Euphyllidae). *Zoological Studies* 51(2): 143-149. *Galaxea fascicularis* possesses a unique sexual pattern, namely “pseudo-gynodioecy”, among scleractinian corals. *Galaxea fascicularis* populations on the Great Barrier Reef, Australia are composed of female colonies that produce red eggs and hermaphroditic colonies that produce sperm and white eggs. However, white eggs of hermaphroditic colonies are incapable of being fertilized or undergoing embryogenesis. In this study, the reproductive ecology and fertilization of *G. fascicularis* were examined in Chinwan Inner Bay, Penghu, Taiwan in Apr.-June 2011 to determine the geographic variation of sexual patterns in *G. fascicularis*. Synchronous spawning of female and hermaphroditic colonies was observed between 17:30 and 20:00 (1 h after sunset) between 24-28 May 2011 (7-11 nights after the full moon in May), and at same times between 22-24 June 2011 (6-8 nights after the full moon in June). Red eggs were significantly larger than white eggs, although both types of eggs had a distinct nucleus, which was located at the edge of the eggs, suggesting that they were in the final stage of maturation and ready to release gametes. Crossing experiments showed that both white and red eggs could be fertilized *in vivo*, and they synchronously developed into swimming larvae, suggesting that instead of being pseudo-gynodioecious, the sexual pattern of *G. fascicularis* is gynodioecious. <http://zoolstud.sinica.edu.tw/Journals/51.2/143.pdf>

**Key words:** Gynodioecy, Pseudo-gynodioecy, *Galaxea fascicularis*, Reproductive mode, Synchronous spawning.

Sexual patterns and modes of development are the most important life-history traits in scleractinian corals, and have been one of the major research themes over the last 3 decades (reviewed in Richmond and Hunter 1990, Harrison

and Wallace 1990, Baird et al. 2009, Harrison 2011). Three sexual patterns (hermaphroditic, gonochronic, and mixed) and 2 modes of development (broadcast-spawned gametes and brooded larvae) were identified (Harrison 2011).

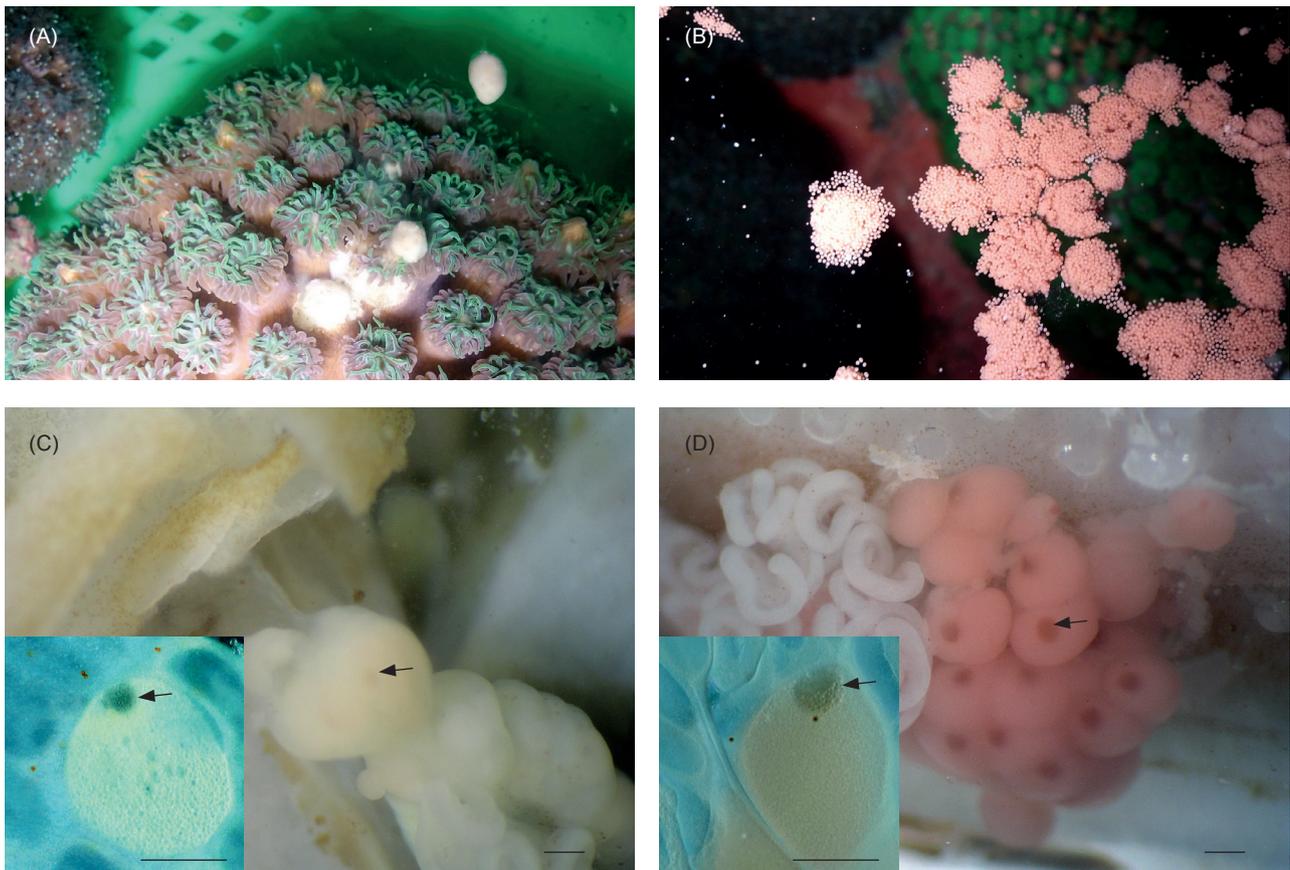
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Among the 444 species studied, 295 species are hermaphroditic and 109 are gonochronic (or dioecious). The remaining species are either mixed or have contrasting modes of reproduction. For the mode of development, 354 species spawn gametes into the water, and 60 species brood larvae (Harrison 2011).

*Galaxea* spp. were originally described as being simultaneous hermaphrodites (Harrison et al. 1984). However, subsequent research at the Great Barrier Reef (GBR), Australia demonstrated that *Galaxea* species have populations composed of female colonies that spawn pinkish-red eggs, and hermaphroditic colonies that produce sperm and lipid-filled white eggs (Harrison 1989). Hermaphroditic *G. fascicularis* colonies produce functional sperm that can fertilize spawned, pigmented eggs of female colonies (Fig. 1). However, white eggs contain unusually large lipid spheres, cannot undergo fertilization, and function to lift the sperm bundles up to the water surface where the buoyant pigmented eggs

accumulate, suggesting that these white eggs potentially enhance fertilization success (Harrison 1989). Harrison (2011) suggested that the pseudo-gynodioecious sexual pattern in at least some *Galaxea* species is therefore functionally gonochronic. However, this detailed observation was only made in the GBR, and the sample sizes of hermaphroditic and female colonies were relatively small ( $n = 2$  for each sex). Further studies outside the GBR with a larger sample size of colonies are necessary to confirm the pseudo-gynodioecious sexual pattern of *Galaxea* spp.

In this study, the reproductive ecology and fertilization of *G. fascicularis* were studied in detail at Chinwan Inner Bay (CIB), Penghu Is., Taiwan. *Galaxea fascicularis* is one of the dominant coral species of the scleractinian community at CIB (Hsieh 2008, Hsieh et al. 2011). This provided us with the opportunity to study the reproductive ecology and reexamine the sexual pattern of *G. fascicularis*.



**Fig. 1.** *Galaxea fascicularis* larval development. (A) White bundle containing white eggs and sperm; (B) red bundle full of red eggs only; (C) white eggs within a hermaphroditic polyp each with a clear nucleus (arrow), and (D) red eggs within a female polyp each with a clear nucleus (arrow). Scale bars = 200  $\mu\text{m}$ .

## MATERIALS AND METHODS

### Study site and sample collection

Coral spawning was observed at CIB (23°31'N, 119°33'E), Penghu Is., Taiwan in Apr.-June 2011. CIB is a semi-enclosed embayment where coral communities have developed on top of volcanic rocks, with 75 species of scleractinian corals described (Hsieh 2008, Hsieh et al. 2011). Over 50 colonies of *G. fascicularis* with a colony size of > 10 cm in diameter were collected, deposited in individual buckets, and moved to tanks with a continuous seawater flow and aeration system at the joint marine laboratory of the Biodiversity Research Center, Academia Sinica (BRCAS)-Penghu Marine Biological Research Center (PMBRC) at CIB. *Acropora muricata* was also collected for reference to compare developmental stages from fertilized eggs to elongated planular larvae (Miller and Ball 2002).

### Observation of spawning and crossing experiments

Observations of spawning behavior at CIB began on 12 Apr. 2011, 5 d before the full moon in Apr., based on previous observations (Chen et al. unpubl. data). Throughout the spawning period, seawater flow in the tanks was stopped daily, by turning the taps off after sunset (ca. 18:30 at CIB). If no spawning was observed on any particular day, seawater flow was restored after 22:30. The time of release of gamete bundles was recorded once polyps and tentacles were retracted, and colored bundles, either white or pinkish-red, were released to the surface of the buckets. On 24 May 2011, 10 colonies of *G. fascicularis* with white eggs and 10 colonies with red eggs were labeled for bundle collection. Gamete bundles released to the surface of the water in the buckets were separately scooped up using recycled plastic cups, and brought back to the laboratory for crossing experiments. Both white and red bundles were filtered through a plankton mesh with a 150- $\mu$ m-mesh size to separate eggs and sperm. Aliquots of eggs and sperm were collected for size measurements and density counts. Sperm density was diluted to  $10^5$ - $10^6$ /ml for the crossing experiment (Willis et al. 1997 2006). White and red eggs were mixed and fertilized with diluted sperm. Developmental stages were observed every hour and categorized based on stages described for *Acropora* by Miller and Ball

(2000) using the same terminology. A series of photographs was taken using an Olympus 5050 camera (Tokyo, Japan) attached to the eyepiece of an Olympus light microscope to obtain images of the developmental stages between white and red eggs until the swimming planular larval stage. *Galaxea fascicularis* white and red eggs inside the coral tissues were photographed under 40x magnification (objective lens 4x and eyepiece 10x) using an Olympus microscope (model SZ40) fitted with an Olympus C5050 digital camera. The gonads were placed in a Petri dish immersed in seawater without a cover. Images of white and red egg were photographed under 100x magnification (object 10x and eyepiece 10x) using an Olympus microscope (model CX31) fitted with an Olympus E510 digital camera. The same gonads were moved to a glass slide and gently put on the slide cover without any pressure. Time-series photos of *G. fascicularis* were taken under 40x magnification (objective lens 4x and eyepiece 10x) using an Olympus microscope (model SZ40) fitted with Olympus SP350 and C5050 digital cameras. The cameras were fitted directly to the eyepiece of the microscope to obtain the photos. Time-series photos of *Acropora muricata* were taken under 40x magnification with an Olympus C5050 camera. The egg size and scale shown in the photos were obtained by micro-ruler photo of a hemocytometer obtained at the respective magnifications.

## RESULTS

*Galaxea fascicularis* colonies at CIB, Penghu Is. were either female (pinkish-red eggs) or hermaphroditic (white eggs with sperm sacs) (Fig. 1A, B). No spawning was observed for *G. fascicularis* in Apr. 2011 (normal spawning period in Penghu begins from Apr.). However, on 24 May 2011, 7 nights after the full moon of May, synchronous spawning of *G. fascicularis* (> 30 colonies) was first observed at 19:30, 1 h after sunset at the Penghu Is. with a peak of gamete bundles released at around 20:00 (Fig. 1A, B). Continued release of bundles was observed the following 4 nights with a decrease in the number of colonies spawned on the 8th night after the full moon (Table 1). Another synchronous spawning event of over 30 colonies was observed on 22 June, 6 nights after the full moon of June (Table 1). Some colonies spawned multiple times either on different nights in May or continuously in June.

Dissecting gamete bundles suggested

that both white and red eggs of *G. fascicularis* were mature and had reached the same stage just before spawning. Some white eggs from hermaphroditic colonies possessed a clear nucleus close to the edge of the egg, as seen in red eggs (Fig. 1C, D). Spawning white eggs had a significantly ( $t$ -test = -72.1769,  $p < 0.01$ ) smaller mean diameter ( $290.20 \pm 2.60 \mu\text{m}$ ,  $n = 171$ ) than red eggs ( $438.58 \pm 3.13 \mu\text{m}$ ,  $n = 184$ ) (Fig. 2).

Fertilization experiments showed that both white and red eggs were mature, and embryo development was synchronous (Fig. 3). Two-cell cleavage was observed during the 1st hr after fertilization (Fig. 3A). The time of the initial development (cell-cleavage stage) cycle in *G. fascicularis* embryos was similar to that of *A. muricata* before reaching the prawn-chip stage (Fig. 3C-F). *Galaxea fascicularis* took 8 hr to reach the prawn-chip stage, while *A. muricata* needed at least 12 hr after fertilization (Miller and Ball 2000). Also, embryonic development from the donut to the pear stage in *G. fascicularis* was significantly faster than that of *A. muricata* (Fig. 3I-P). The swimming ability of planular larvae fertilized from white eggs did not differ from that of larvae from red eggs.

## DISCUSSION

Our study provides several lines of evidence, including final maturation, fertilization, and embryonic and larvae development, to demonstrate that the sexual pattern of the *G. fascicularis* population at CIB, Penghu Is., Taiwan is gynodioecious. This is the 1st record of the

**Table 1.** Month, date, days after the full moon, time of spawning (hours after sunset), and numbers of colonies spawned of *G. fascicularis* in Chinwan Inner Bay, Penghu, Taiwan in 2011

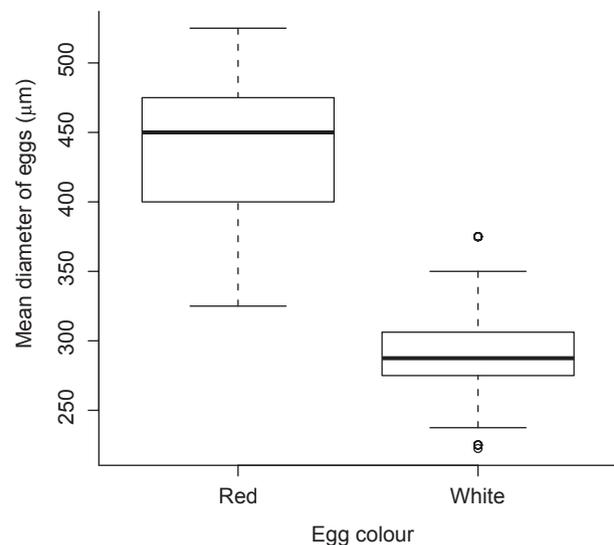
Month	Date	No. of days after a full moon	Time of spawning (h after sunset)	Number of colonies spawned from a total of $n = 50$
May	24	7	1	> 30
	25	8	1	> 30
	26	9	1	< 5
	27	10	1	< 5
	28	11	1	< 5
June	22	6	1	> 30
	23	7	1	-
	24	8	1	-

-, not spawning.

gynodioecious sexual pattern in scleractinian corals.

## Egg, embryonic, and larvae development in *Galaxea fascicularis*

According to a previous study (Harrison 1989) conducted at the GBR, Australia, the reproduction mode in *G. fascicularis* was reported to be pseudo-gynodioecious suggesting that white eggs produced by this species cannot be fertilized. This raises the question as to why white eggs of *G. fascicularis* at CIB, Penghu Is., Taiwan were fertile, but those in the GBR, Australia were not? Two possible scenarios are proposed to explain this difference. First, our observed results may have been due to geographic differentiation between *G. fascicularis* populations in the GBR, Australia and those at CIB, Penghu Is., Taiwan. In some scleractinian corals, sexual patterns and reproductive modes can vary in different geographic regions (reviewed in Harrison 2011). For example, histological studies on *Pocillopora damicornis* colonies in Japan indicated that brooded planulae develop from eggs, and may

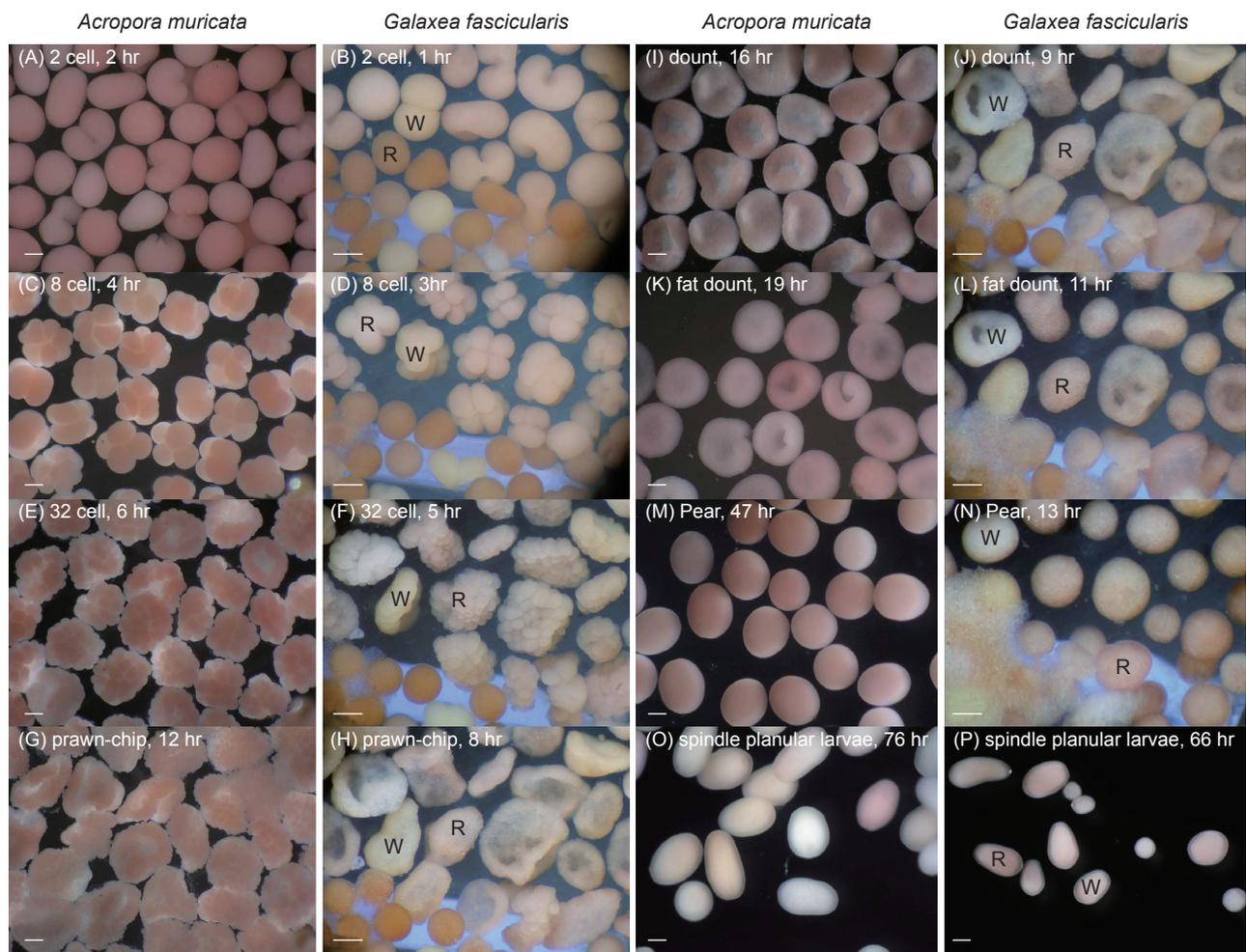


**Fig. 2.** Difference in egg sizes between female (red egg color) and hermaphroditic (white egg color) colonies of *Galaxea fascicularis*. The egg size data were plotted using software R to generate a box plot. The upper and lower hinge of the box indicate 75th and 25th percentile of the data set. The line in the middle of the box represents median for each data set of egg sizes indicating a skewed data set. Vertical dotted lines with whiskers at top and bottom represent maximum and minimum values. Circles in the figure are outliers with values outside the 25%-75% interval. The absence of circles for red eggs indicates that there were no outliers.

be produced sexually (Diah Permata et al. 2000). Different reproductive patterns occur in the eastern Pacific and Gulf of California populations of *P. damicornis*, which are characterized by the production of eggs and sperm and inferred spawning of mature gametes, but there is no evidence of brooding or planular production in those populations (Glynn et al. 1991, Colley et al. 2006, Chavez-Romo and Reyes-Bonilla 2007, Glynn and Colley 2009). It was suggested by Harrison (2011) that variations in reproductive characteristics and life-history traits recorded among populations in different regions indicate that these characteristics are unusually variable in this species. Alternatively, *P. damicornis* may be a species complex containing cryptic species with different reproductive patterns (Flot et al. 2008, Souter 2010). Determining whether *G. fascicularis*

with the fertilization capability of white eggs from CIB and the GBR is the same species with unusually variable life-history traits or there are cryptic species with different reproductive patterns requires further investigation using a molecular genetic analysis.

Another explanation is that results may have been due to the small sample size of *G. fascicularis* (2 female and 2 hermaphroditic) colonies utilized in the fertilization trials at the GBR (Harrison 1989). The percentage of mature white eggs in hermaphroditic colonies was relatively low compared to those large lipid bodies in gamete bundles released into the water column, thereby reducing the chance of obtaining fertile eggs for further observations of embryo development. In our study, large numbers of gamete bundles were collected from both female and hermaphroditic *G.*



**Fig. 3.** Embryo stages of *Galaxea fascicularis* and *Acropora muricata*. The time of each stage is indicated in hours after fertilization. (A, B) Two-cell stage; (C, D) 8-cell stage; (E, F) 32-cell stage; (G, H) prawn-chip stage; (I, J) donut stage; (K, L) fat-donut stage; (M, N) pear stage; (O, P): spindle planular larvae. R and W = Developmental stages form red and white fertilized eggs. Scale bar = 200  $\mu$ m.

*fascicularis* colonies, and fertilization took place with large quantities of gametes that increased the chances of observing serial embryo development of white eggs from hermaphroditic colonies. Further investigations of the percentage of fertile white eggs in gamete bundles of hermaphroditic colonies and of the survival, settlement, recruitment success, and growth of derived juvenile corals are needed to confirm the contribution of white eggs to *G. fascicularis* populations.

Overall, results from this study showed that embryonic development time is much shorter in *G. fascicularis* compared to that in *Acropora*. The length of embryonic development could affect dispersal and recruitment among different spawning corals (Nakamura and Sakai 2010). For example, among spawning pocilloporid corals, larvae that develop relatively more rapidly have higher recruitment at sites where adult coral cover is high. In contrast, recruitment is not related to adult coral cover in acroporid and poritid corals, the embryonic development times of which are relatively slow (Nakamura and Sakai 2010). The shorter embryonic development time might facilitate *G. fascicularis* settling locally faster, and helping it become the dominant species after a series of disturbances and disappearance of acroporid corals (*Acropora* and *Montipora*) after a cold shock event in 2008 at CIB (Hsieh et al. 2008 2011). The recruitment of acroporid corals may be slower because sources of larvae are from neighboring coral communities outside CIB.

### Sexual pattern of *Galaxea fascicularis* gynodioecy

Completion of embryonic and larval development of white eggs from hermaphroditic colonies suggests that the sexual pattern of *G. fascicularis* is gynodioecious, instead of pseudo-gynodioecious as proposed by Harrison (1989). Studies on plant reproductive systems have indicated that gynodioecy is a transitional step towards dioecy (gonochorism) from hermaphroditism (reviewed in Charlesworth 2006). This scenario might be applicable to the evolution of sexual pattern traits in *Galaxea*. *Galaxea* is the only coral genus that possesses a sexual pattern of gynodioecy, and phylogenetic studies have relocated *Galaxea* from the family Oculinidae to the Euphyllidae, where it forms a sister clade to the genus *Euphyllia* (Fukami et al. 2008, Dai and Horng 2010). The sexual patterns of 8 *Euphyllia* species can be divided into either dioecious species with spawned gametes

(e.g., *E. ancora*) or hermaphroditic species with brooded larvae (e.g., *E. glabrescens*) (Veron 2000). Gynodioecy in *Galaxea* might represent a transitional step of sexual pattern evolution in the family Euphyllidae. In addition, gynodioecy also suggests a unique inheritance mode of genetics in *Galaxea* compared to true hermaphroditic or dioecious species. Further work on ancestral reconstruction of life-history traits and genetic structuring of populations should provide insights into the evolutionary novelty of gynodioecy in *Galaxea* among scleractinian corals.

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