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Budding Cycle and Bud Morphology of the Globe-shaped Sponge *Cinachyra australiensis*

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Yung-Hui Chen, Chang-Po Chen and Kun-Hsiung Chang (1997) Budding cycle and morphology of the globe-shaped sponge *Cinachyra australiensis*. *Zoological Studies* **36**(3): 194-200. The asexual budding cycles of the globe-shaped sponge *Cinachyra australiensis* (Carter, 1886) were studied from 1987 to 1989 in the rocky intertidal area at Wanlitung, Hengchun Peninsula, southern Taiwan (22°00'N, 120°40'E). The small yellow buds are protruded by megascleres and are scattered over the entire surface of adults. The budding period generally started in late spring, peaked between late summer and early fall, and ended in early winter. Detached buds are oval in shape and negatively buoyant. Buds contain megascleres and microscleres similar to those of adults. At the ultrastructural level, 4 principal cell types can be identified within the buds: archaeocytes, special cells with numerous small cell inclusions, oval-shaped cells, and intermediate cells. The intermediate cells are characterized by intermediate numbers of cell inclusions and phagosomes as in archaeocytes and special cells. The oval-shaped cells are distributed more peripherally within buds than are the other 3 cell types.

Key words: Asexual reproduction, Bud, Porifera, Ultrastructure.

Sponges reproduce sexually and asexually. Asexual propagation includes fragmentation (e.g., *Iotrochota birotulata* in Wulff 1985), budding (e.g., *Tethya aurantium* in Burton 1948, *Axinella damicornis* in Boury-Esnault 1970) and gemmulation (e.g., *Haliclona loosanoffi* in Hartman 1958). Only a few studies have focussed on bud formation and their fates (Simpson 1984, Battershill and Bergquist 1985). Budding periods vary seasonally between sponge species. *Aaptos aaptos* buds from September to October (Ayling 1980), while *Tethya aurantium* produces buds from June to September (Burton 1948). Budding occurs throughout the year in *Tethya ingalli* (Bergquist et al. 1970), *Clathrina blanca* (Johnson 1978), and *Polymastia* sp. (Ayling 1980).

Bud formation also varies between sponge species dwelling in different habitats. Only intertidal individuals of *A. aaptos* produce buds, but *T. ingalli* produces buds regardless of depth (Bergquist et al. 1970). However, the number of buds of *T. aurantium* varies greatly between specimens collected from different locations (Burton 1948).

According to external morphological features, Bergquist (1978) has classified 3 types of buds: stalked surface bud, bud attached to a basal stolon, and armoured bud. The cell composition and tissue differentiation of buds differ between species. In *Mycale contarenii* the cell types of buds are similar to those of adults (De Vos 1965). The free-floating buds of *Alectona millari* have 3 layers which are composed of different cell types (Garrone 1974).

The globe-shaped sponge *Cinachyra australiensis* is distributed widely throughout Australia and the Eastern Indian region (De Laubenfels 1954), but information on its reproductive biology is not available. This sponge commonly occurs in the semi-open tidal pools along the rocky coast of

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southern Taiwan. In general, sponges are directly exposed to sunlight and are coated with a layer of sand grains through which megascleres protrude from the surface. However, sponges occurring inside caves or under rocky overhangs are free of sand grains (Chen 1988). The asexual budding cycle of the intertidal sponge *C. australiensis*, the morphology of its buds, and the ultrastructures of cell types occurring in the buds are reported.

MATERIALS AND METHODS

Field studies were carried out in the rocky intertidal area at Wanlitung, Hengchun Peninsula, southern Taiwan (22°00', 120°40'E). By wading or skin diving in the tidal pools, 30 to 50 individuals of the sponge *Cinachyra australiensis* (Carter, 1886) were randomly chosen monthly from September 1987 to December 1989 and examined for the appearance of buds. Seasonal changes of the budding cycle were investigated by calculating the budding percentage of total individuals examined each month. The diameters of individuals with buds were measured as well.

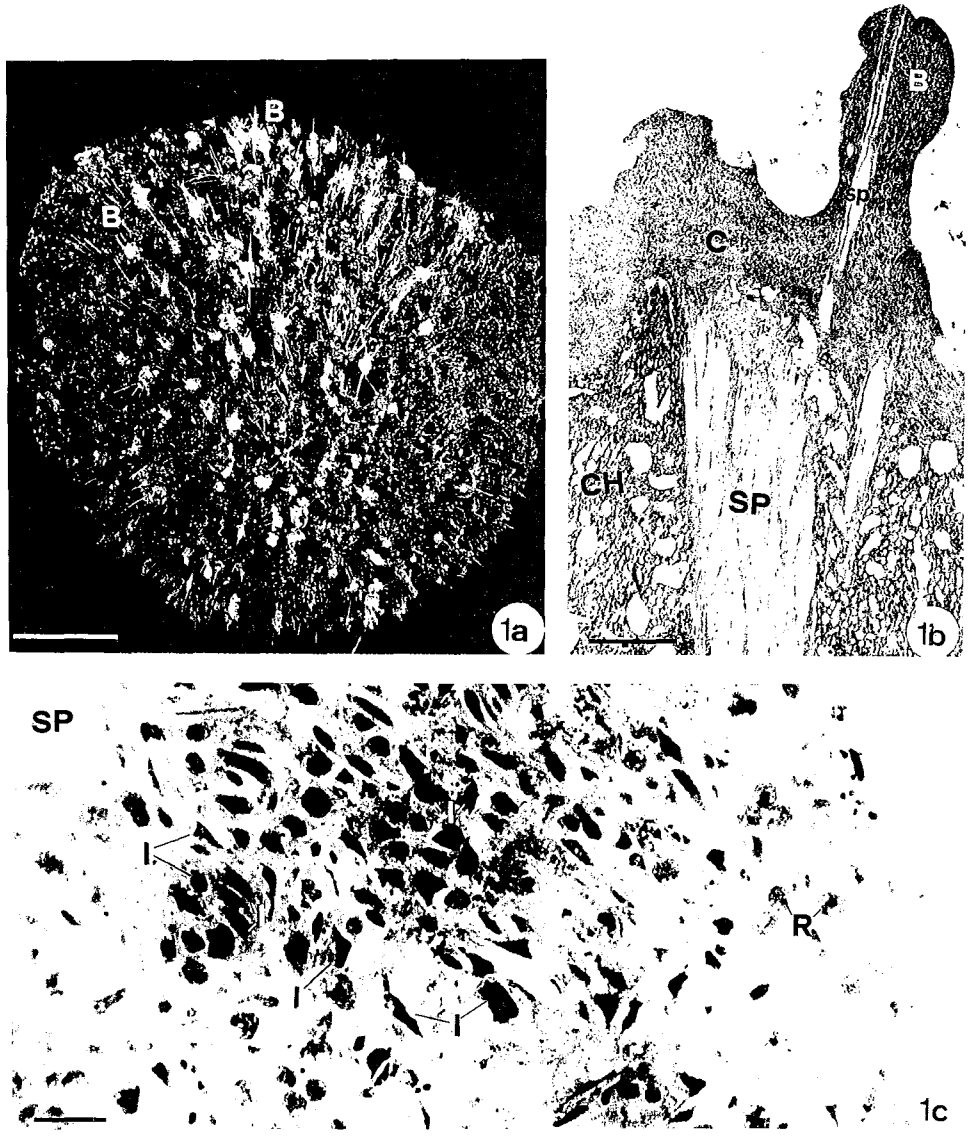

Fig. 1. (a) A budding individual of *Cinachyra australiensis*; scale bar = 0.5 cm. (b) Longitudinal section of bud formation; scale bar = 200 μm. (c) Thick section of bud showing cell distribution; scale bar = 100 μm.
B: Bud; C: Cortex; CH: Choanosome; SP: Empty space of spicule bundle dissolved by hydrofluoric acid; R: Oval-shaped cells; I: Irregular-shaped cells (archaeocytes, intermediate cells, and special cells).

The external morphology of each bud was examined under a light microscope. To study the spicules associated with the buds, several buds were boiled in 6 N nitric acid. The spicules were rinsed with distilled water and 95% alcohol 6 times. After being dried in an oven, the spicules were coated with gold and then observed under a scanning electron microscope (JOEL-JSM 35CF).

Detached buds and a portion of sponge tissue with protruding buds were fixed in Bouin's solution for 48 h, de-silicified in 5% hydrofluoric acid for 2 d, and then dehydrated through a series of alcohols, cleared in xylene and embedded in paraffin. Tissue sections of 5-8 μm were stained with Hematoxylin-eosin (Humason 1976).

Detached buds were prefixed in 5% glutaraldehyde and 4% paraformaldehyde in a buffer solution of 0.1 M sodium phosphate adjusted to proper osmolarity by adding sucrose at pH 7.2 for 30 min at 4 °C followed by post-fixing in 1% osmium tetroxide. The specimens were de-silicified in 5% hydrofluoric acid for 30 min, then dehydrated through an alcohols series and embedded in Spurr's resin. Sections about 1 μm in thickness were stained with toluidine blue. Under a light microscope, the numbers of cells with different shapes were counted along 5 transects vertical to the periphery to examine the distribution pattern of cells within the bud. Thin sections of 70 nm, double stained with uranyl acetate and lead citrate, were observed under a transmission electron microscope (JEOL-1OOS EM in 40 KV) to examine cell types and ultrastructures within the buds. Cells were classified according to their shapes, cell inclusions, phagosomes, and nuclei, using Simpson (1984) and Harrison and De Vos (1991) as references.

RESULTS

Buds were scattered over the entire surface of the globe-shaped sponge *Cinachyra australiensis* (Fig. 1a). They were attached to the cortex of adult tissue (Fig. 1b). Detached buds were negatively buoyant but could be resuspended by disturbance of the water.

The budding cycle varied seasonally and annually. In 1987, 63% of the individuals examined

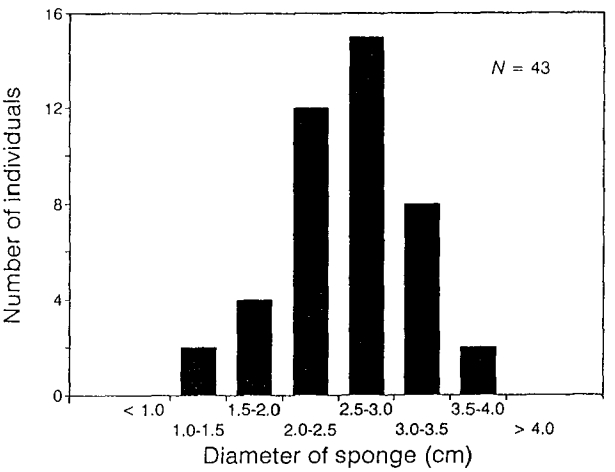

Fig. 3. Size distribution of budding individuals of *Cinachyra australiensis*.

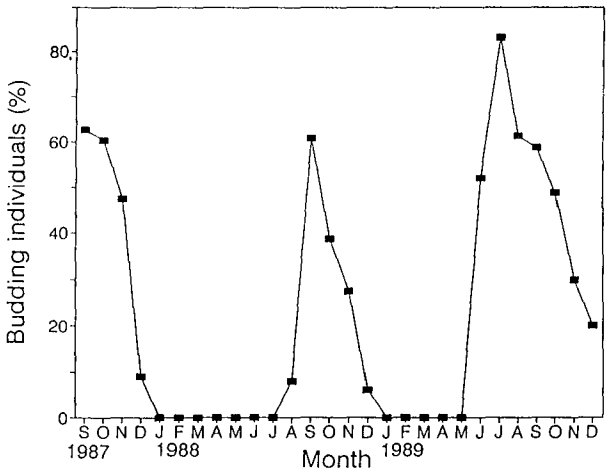

Fig. 2. Seasonal budding cycle of *Cinachyra australiensis* from September 1987 to December 1989 in rocky intertidal pools at Wanlitung, Hengchun Peninsula, southern Taiwan.

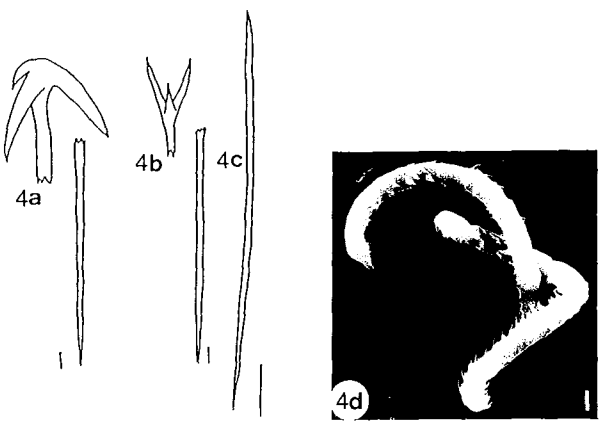

Fig. 4. Spicule types of a bud: (a) anatriaene (scale bar = 10 μm); (b) protriaene (scale bar = 10 μm); (c) oxea (scale bar = 500 μm); and (d) sigmaspires (scale bar = 4 μm).

produced buds in September, decreasing to 9% in December. In 1988, however, the cycle started in August, reached its peak of 63% in September, and then decreased to 6% in December. In 1989, it began in June, reached its maximum of 82% in July, and then dropped to 20% in December (Fig. 2).

The diameters of bud-producing sponges ranged from 1.3 to 4.0 cm with a mean of 2.6 cm (n = 43) (Fig. 3). The buds were yellow and were longitudinally penetrated by megascleres. They were round to oval in shape, ranging from 489 μm to 833 μm with a mean of 689 μm in length, and from 266 to 809 μm with a mean of 519 μm in

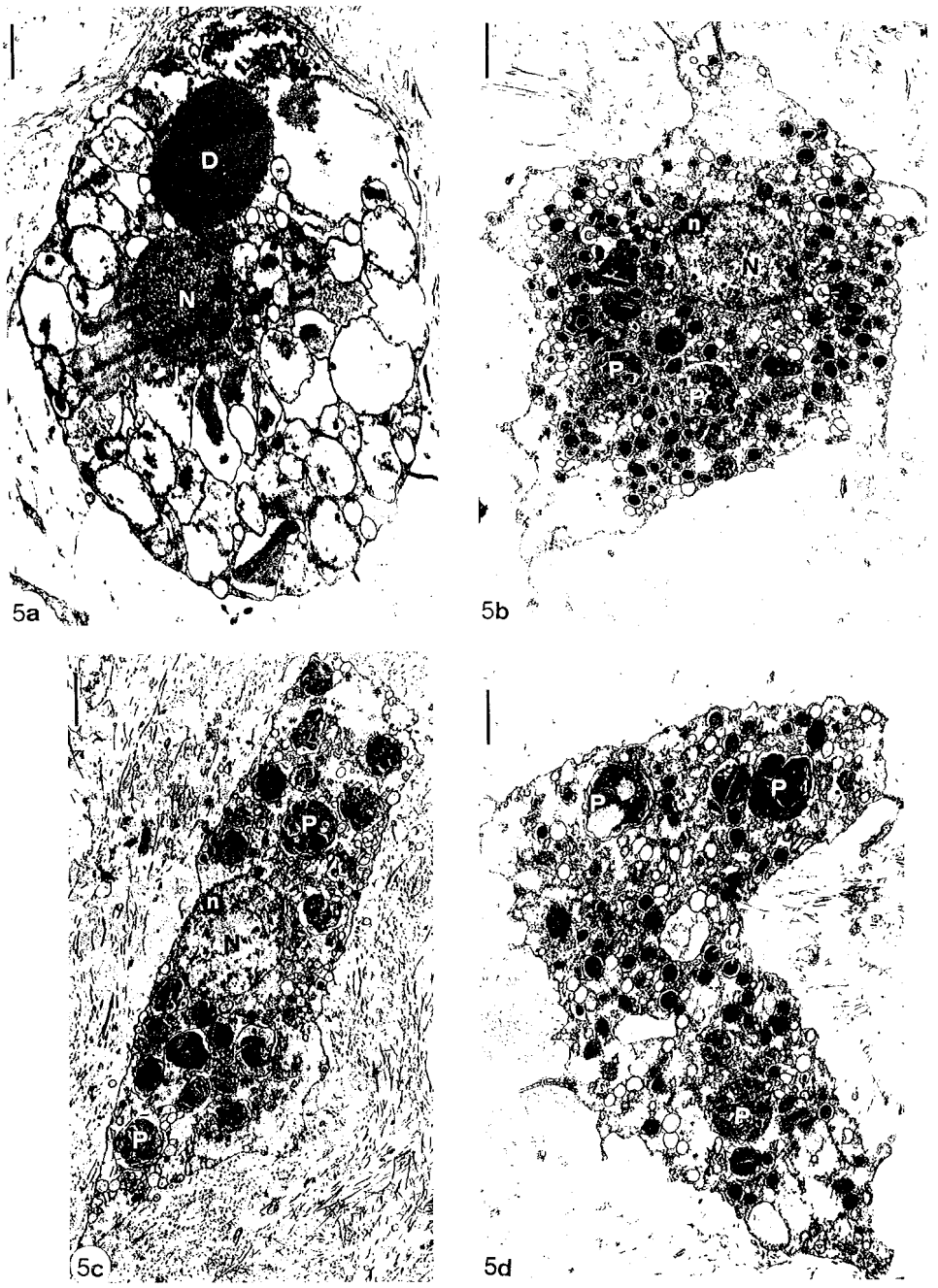

Fig. 5. Ultrastructures of the 4 principal cell types within a bud: (a) oval-shaped cell; (b) archaeocyte; (c) special cells; and (d) intermediate cell.
D: Dense cell inclusion; N: Nucleus; P: Phagosome; C: Small cell inclusion; n: Nucleolus; scale bar = 1.5 μm.

width (n = 37). Buds contained both megascleres: anatriaene, protriaene and oxea (Fig. 4a-c) and microscleres: sigmaspire (Fig. 4d). The oxea was the most common spicule type found within the buds examined.

Buds were composed of 4 principal cell types: oval-shaped cells, archaeocytes, special cells, and intermediate cells. Oval-shaped cells were uninucleate, ranging from 8.3 to 13.8 μm with a mean of 11.1 μm in diameter (n = 50). Numerous irregular cell inclusions containing amorphous material and a large rectangle-shaped, dense cell inclusion were found within oval-shaped cells (Fig. 5a). Special cells were nucleated and irregular in shape. They contained numerous small osmiophilic, membrane-bound inclusions and a few phagosomes; sometimes there were Golgi membranes (Fig. 5c). Both archaeocytes (Fig. 5b) and intermediate cells (Fig. 5d) were irregular in shape. Archaeocytes possessed many prominent phagosomes and a few smaller cell inclusions, whereas intermediate cells contained an intermediate number of both small cell inclusions and phagosomes (Table 1). Oval-shaped cells were distributed more peripherally than the other 3 irregular-shaped cell types (Figs. 1c, 6). Neither choanocyte nor water canal system was found within the buds. However, a detached bud of 3 mm in diameter found on the adult's surface was composed of 2 tissue layers similar to those of functional individuals. Buds attached themselves to the substrate after detachment from the adult sponge.

DISCUSSION

The present study reveals that the globe-shaped sponge *Cinachyra australiensis* produces buds seasonally: buds appeared in late spring, were most numerous in summer, and disappeared in early fall. Many environmental factors have been suggested to be related to reproductive activities of sponges such as photoperiod (Wilkinson and Vacelet 1979, Amano 1986), and water temperature (Bergquist and Sinclair 1973, Fell 1976, Benfey and Reiswig 1982). In our studies, insufficient environmental information was collected, therefore it is not possible to determine such seasonally variable relationships for the sponge *C. australiensis*. Further studies on this aspect are thus required.

The cells occurring in buds of *C. australiensis* are different in both ultrastructure and distribution. This suggests that each cell type might play a different functional role in the early development of a bud. Since there are neither choanocytes nor a water canal system within the newly detached buds, the energy needed for early development can not mainly be drawn from the surrounding water but more likely comes from the buds themselves. Among cell types occurring in buds of *C. australiensis*, special cells may be the major energy supplier since they contain more small cell inclu-

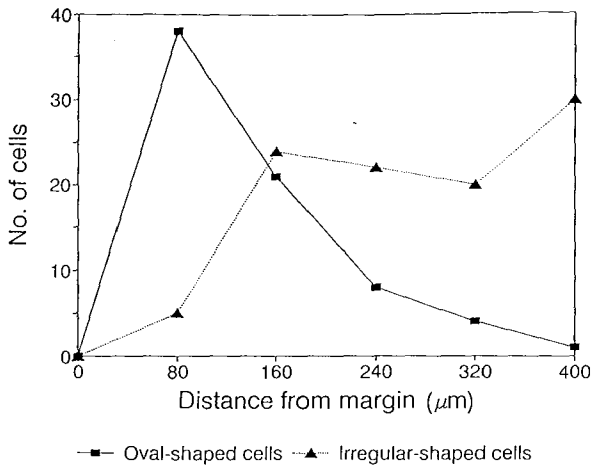

Fig. 6. Spatial distribution of oval-shaped cells and irregular-shaped cells, including archaeocytes, intermediate cells, and special cells, from the margins toward the center of a bud of *Cinachyra australiensis*.

Table 1. The range of number and size of phagosomes and of small cell inclusions in special cells, intermediate cells, and archaeocytes within buds of *Cinachyra australiensis*

	Small cell inclusions		Phagosomes		
Cell types	No.	Size (μm)	No.	Size (μm)	No. of cells
Special cells	97 – 128	0.16 – 0.56	2 – 4	0.2 – 0.8	n = 5
Intermediate cells	34 – 47	0.10 – 0.40	7 – 11	0.3 – 1.0	n = 5
Archaeocytes	2 – 13	0.10 – 0.30	9 – 21	0.3 – 1.4	n = 5

sions than the other cell types. Harrison and De Vos (1991) have suggested that in many cases small cell inclusions contain carbohydrates and might serve as a potential energy source. For example, gray cells, possessing numerous cell inclusions, were found to serve as an energy source in the external buds of *Tethya aurantium* (Connes 1968), in the regenerative blastema of *Hamigera hamigera* (Boury-Esnault 1976), and in the growth region of *Microciona prolifera* (Simpson 1963). Although the content of cell inclusions of special cells requires further biochemical analysis, their potential role in the early development of buds cannot be ignored. In the regions involved in morphogenetic processes, most of the gray cells are phagocytized by archaeocytes, suggesting that archeocytes can utilize such nutritional cells to differentiate into new cell types (Simpson 1984). It is likely that the archaeocytes occurring in buds of *C. australiensis* might interact with special cells to produce new cell types during early development. Intermediate cells with an intermediate number of phagosomes and cell inclusions could be "transitional" cell types during developing processes.

Oval-shaped cells of the globe-shaped sponge *C. australiensis* possess large cell inclusions similar to those of special cells. Simpson (1984) suggested that this type of special cell may be secretory cells. Even though this cell type has been found in buds of several sponge species (De Vos 1965, Connes 1968, Garrone 1974), its functional role remains undetermined. However, since oval-shaped cells are distributed more peripherally than other cell types in buds of *C. australiensis*, it is likely that they interact with archaeocytes to produce cells such as basopinacocyte which function to hold buds to the substrate.

Buds of the globe-shaped sponge *C. australiensis* are stalked surface buds. They are formed directly from protrusions of the adult surface and do not have the same protected wall as do armored buds that are produced in 2 genera, *Alectona* and *Thoosa* (see review in Bergquist 1978). This suggests that buds probably cannot withstand adverse environments after detachment. However, the detached buds of *C. australiensis* are negatively buoyant and are able to attach to the substrate after settlement. This ability enables buds to occupy a substrate quickly, which might reduce the risk of being transported to unfavorable environments. In addition, by the time buds have grown to a size of 3 mm in diameter, they already possess a structure similar to that of functional individuals, suggesting that buds develop rapidly after detachment. This characteristic could also reduce the mortality of buds because they can begin to take up nutrients directly from the environment. Buds of the intertidal sponge *Polymastia grannulos* can also develop rapidly to become functional after settlement in 3 d, which results in high recruitment rates (Battershill and Bergquist 1985). Both negative buoyancy and fast development may be essential for sufficient survival rates and recruitment in *C. australiensis*.

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澳洲球形海綿芽體生殖週期與芽體形態結構

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自 1987 至 1989 年間，我們在南臺灣恆春半島萬里桐岩岸潮間帶區，研究調查澳洲球形海綿 *Cinachyra australiensis* (Carter, 1886) 芽體生殖週期。澳洲球形海綿會產生無性生殖的黃色小芽體；每一個小芽體是由突起的大骨針，將其突出於海綿成體砂質表面。一般而言，澳洲球形海綿的芽體最早出現於晚春，最多見於晚夏初秋之際，入冬之後，則逐漸消失。芽體略呈卵圓狀，沉性，並未如芽球 (gemmule) 爲海綿纖維所包圍，但含有與成體相似的骨針種類。在電子顯微鏡的觀察下，芽體由四種主要細胞所組成：原始細胞 (archaeocyte)、含有許多細胞內涵物的特殊細胞 (special cell)、介於特殊細胞與原始細胞之間的中間型細胞 (intermediate cell) 和卵形細胞 (oval-shaped cell)。前三種細胞多分佈於芽體的中央，而卵形細胞則較分佈在外圍。

關鍵詞：無性生殖，芽體，海綿，顯微構造。

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