STORAGE CELLS AND SPERMATIC CYSTS IN THE CARIBBEAN CORALLINE SPONGE *GOREAUIELLA AURICULATA* (ASTROSCLERIDAE, AGELASIDA, DEMOSPONGIAE): A RELATIONSHIP ?

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E-mail: philippe.willenz@naturalsciences.beABSTRACT

Ultrastructural observations of the living tissue of the coralline sponge *Goreauiella auriculata* collected near Discovery Bay, Jamaica and in the Bahamas between 1984 and 2002 were conducted with transmission electron microscopy to pinpoint sexual reproductive stages. Two different structures situated at the base of the living tissue, between processes of the aragonitic skeleton were revealed. Firstly, masses of storage cells packed with various inclusions and resembling gemmular thesocytes were found in all specimens collected in Jamaica; they were scarce and small when male gametes were produced, but none were found in specimens from the Bahamas. Secondly, spermatic cysts containing primary spermatocytes occurred in the same region of the mesohyl on only one occasion. Although in many demosponges male gametes develop directly from choanocytes, we revive the view that the origin of these cells is different in different species. We suggest that storage cells can produce spermatic cysts in *G. auriculata.*

KEY WORDS

Coralline sponges, storage cells, spermatic cysts, reproduction, ultrastructure.

INTRODUCTION

Reproduction in coralline sponges has rarely been observed. Only *Astrosclera willeyana* and *Vaceletia crypta* are known to incubate parenchymella embryos (LISTER 1900; VACELET 1979; WÖRHEIDE, 1998). Among about 14 species of calcified demosponges described so far, 2 species concentrate masses of cells in basal cavities of their calcareous skeleton. Obvious similarities of these crypt cells in *Merlia normani* and *Acanthochaetetes wellsi* with thesocytes of the gemmules of a noncalcified demosponge, *Suberites domuncula* (CARRIÈRE *et al.,* 1974), led VACELET (1990) to ascribe to these masses of cells a similar storage role as gemmules, insuring an asexual reproduction to these species. Similar cells also occur in the Mediterranean calcisponge *Petrobiona massiliana* (VACELET, 1964). No evidence of oogenesis or spermatogenesis of these "living fossils" has ever been reported, and hence whether sexual reproduction actually occurs is still puzzling. In order to pinpoint reproduction stages in calcified demosponges, ultrastructural observations were repeatedly conducted on Caribbean species. This paper describes the cells of the deepest regions of the choanosome inserted between arborescent processes of the

aragonitic skeleton of *Goreauiella auriculata*, observed in light and transmission electron microscopy.

MATERIAL AND METHODS

Specimens of *Goreauiella auriculata* Hartman, 1969 were marked with plastic tags for further identification in a reef tunnel at a depth of 28 m at Pear Tree Bottom, 5 km east of Discovery Bay, Jamaica. Fragments were collected repeatedly in July 1984, February 1985, April 1986, April 1987, April 1997, August 1999 and May 2002. Samples were also taken in reef crevices at depths of 25 to 30 m on the fore-reef slope of the southern tip of Acklins Island (Jamaica Bay) and of Hogsty Reef, Bahamas in August 1985.

Observations in light microscopy and in transmission electron microscopy (TEM) were made on fragments removed with scalpel, with attached skeleton, from the periphery of sponges. Fragments were fixed *in situ* according to a modification of the "low osmium pre-fixative" technique of EISENMAN & ALFERT (1981) previously described (WILLENZ & HARTMAN, 1989). Sections were obtained with a diamond knife on a Leica Ultracut UCT ultramicrotome. Prior to sectioning, the siliceous spicules at the section plane were dissolved with 15 to 20 % hydrofluoric acid in distilled water for 5 min. Thin sections double-stained with uranyl acetate and lead citrate (REYNOLDS, 1963), were examined with a Zeiss EM 10 at 80 kV. Ground sections for light microscopy were prepared as formerly stated (WILLENZ & POMPONI, 1996). Semithin sections having a thickness of 1 µm were heat-stained with Methylene Blue.

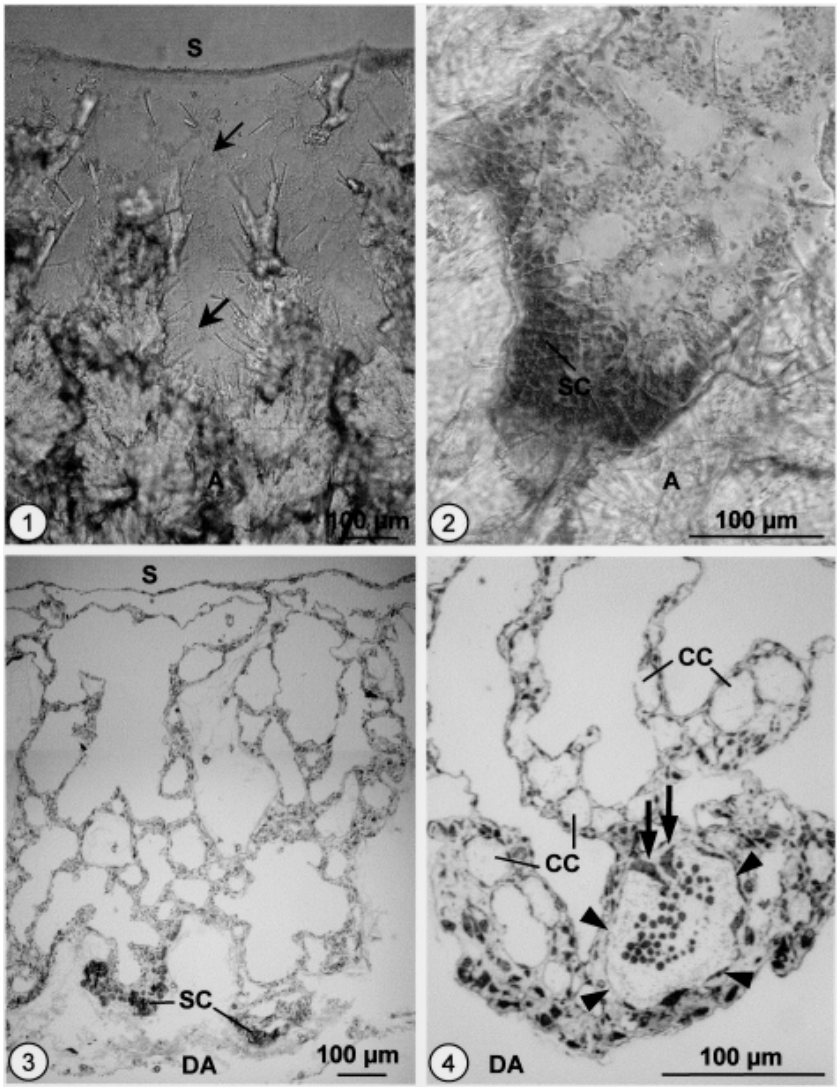
RESULTS

The living tissue of *Goreauiella auriculata* forms a thin veneer that extends between the arborescent processes of the aragonitic skeleton (HARTMAN, 1969). The basal skeleton confines short pseudocalicles (Fig. 1) at the lowest region of which two different types of cell masses were observed: storage cells and spermatic follicles.

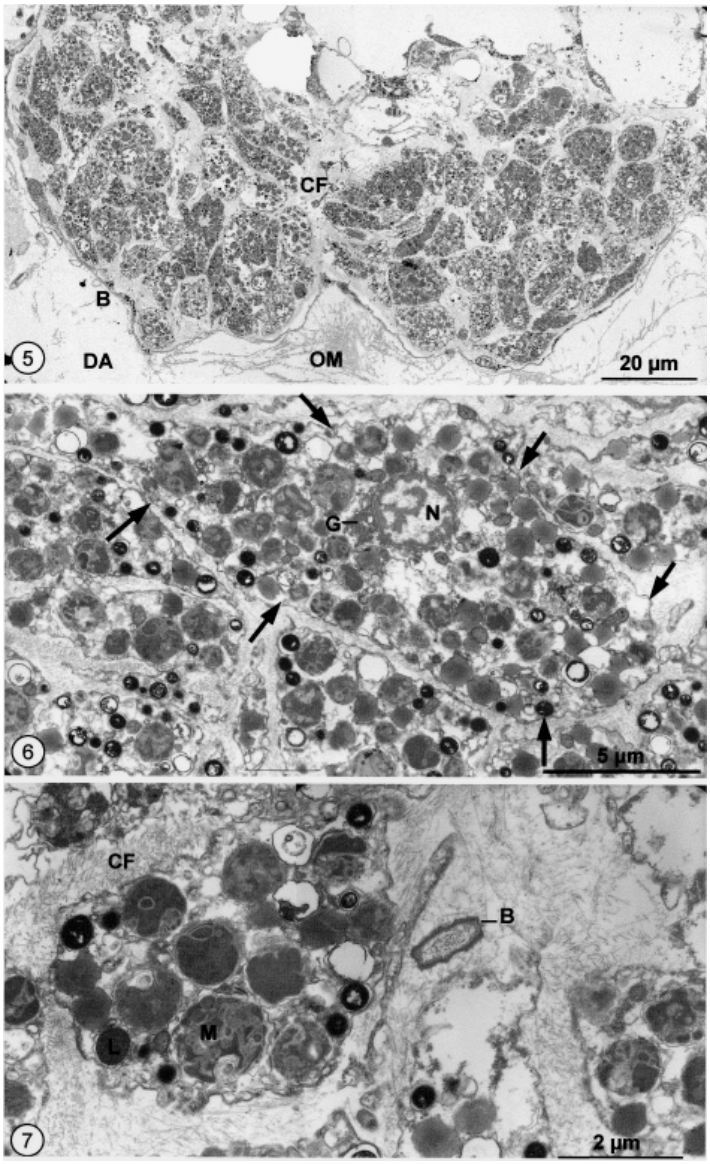
Storage cells

In all specimens collected at Pear Tree Bottom, from 1984 to 2002, except in those collected in April 1986, masses of cells were concentrated in each basal cavity of the skeleton (Figs 2, 3). The upper part of these cell masses was in continuity with the mesohyl, whereas the lower part was circumscribed by a single layer of basopinacocytes lining the aragonitic skeleton (Fig. 5).

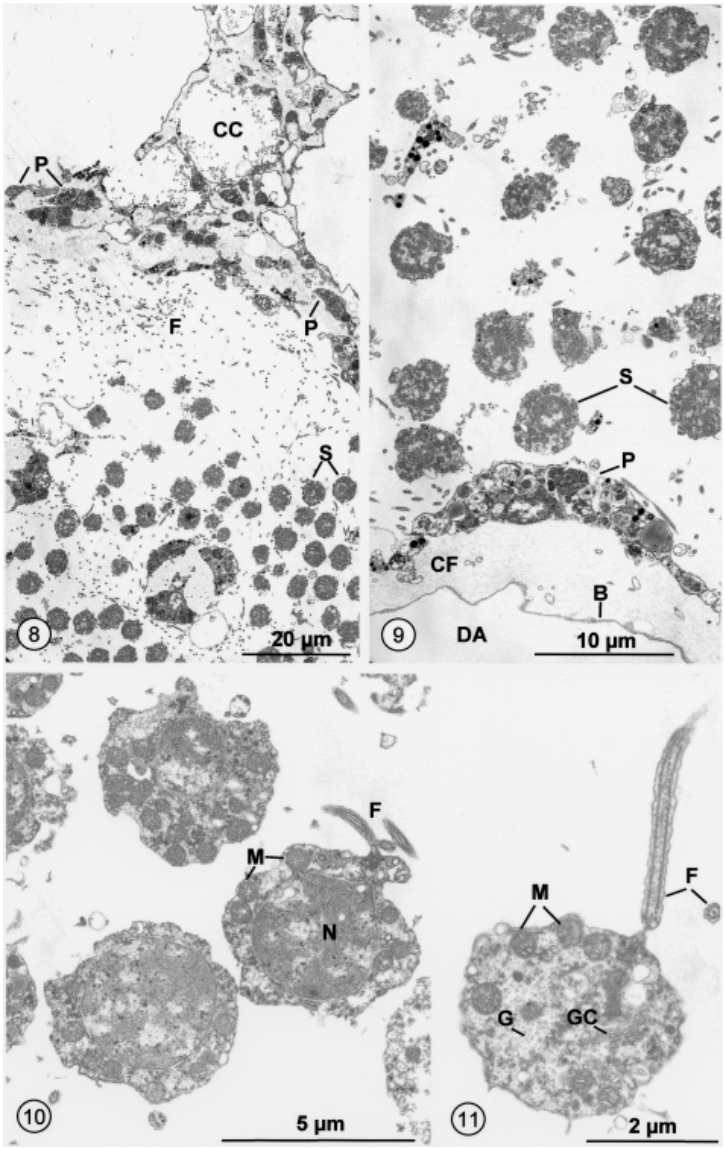
In TEM, all cells were alike, closely resembling gemmular thesocytes, with a cytoplasm densely packed with heterogeneous inclusions varying in size and density. These storage cells ($\bar{x}$ = 5.5 ± 1.6 µm x 11.0 ± 3.3 µm; n = 20) had an anucleolate nucleus ($\bar{x}$ = 3.2 ± 0.4 µm x 3.6 ± 0.5 µm; n = 20). A Golgi complex was present, but no mitochondria were observed (Fig. 6). A majority of inclusions contained stacked membranes that clearly resulted from phagocytosis ($\bar{x}$ = 1.0 ± 0.2 µm x 1.1 ± 0.2 µm; n = 20); relatively scarce smaller spherical inclusions ($\bar{x}$ = 0.5 ± 0.1 µm x 0.5 ± 0.1 µm) showed dense homogeneous lipidic material (Fig. 7). Dense bundles of smooth collagen fibrils separated storage cells from each other and contained occasional bacteria (Fig. 7).



Figs 1-4. *Goreauiella auriculata:* Organization of living tissue relative to basal skeleton, seen in light microscopy. **Fig. 1,** Arborescent processes of aragonitic skeleton with short pseudocalices harboring living tissue. Ground section perpendicular to surface seen under interference contrast microscopy. A = aragonite; S = sponge surface; arrows = living tissue. **Fig. 2,** Storage cells (SC) massed at bottom of pseudocalicle. Ground section: living tissue stained with acid fuschin. A = aragonite. **Fig. 3,** Semithin section of decalcified specimen. DA = space occupied by aragonite prior to decalcification; SC = storage cells. **Fig. 4,** Semithin section of decalcihed specimen. Arrowheads indicate pair of flattened cells pointing inward spermatic cyst, primary spermatocytes are gathered at center with flagella directed toward cyst periphery. CC = choanocyte chamber.



Figs 5-7. *Goreauiella auriculata*: Storage cells seen in TEM. **Fig. 5,** Mass of cells concentrated at base of living tissue, surrounded by collagen fibrils (CF) and separated from aragonitic skeleton by a layer of basopinacocytes (B). Thin section perpendicular to sponge surface. DA = space filled with aragonite prior to decalcification; OM = organic matrix. **Fig. 6,** Storage cells (surrounded by arrows) with cytoplasm packed with heterogenous granules varying in size and density. N = nucleus; G = Golgi complex. **Fig. 7,** Detail showing homogenous lipidic inclusions (L) and other inclusions filled with stacked membranes (M). CF = collagen fibrils; B = bacterium.



Figs 8-11. *Goreauiella auriculata*: Spermatic cyst seen in TEM. **Fig. 8,** Portion of cyst surrounded by unicellular layer of pinacocytes (P), close to choanocyte chamber (CC). F = flagella; S = primary spermatocytes. **Fig. 9,** Detail of spermatic cyst close to decalcified aragonitic skeleton (DA). Pinacocytes (P) enveloping cyst are surrounded with a layer of collagen fibers (CF); B = basopinacocytes; S = primary spermatocyte. **Fig. 10,** Primary spermatocyte with numerous mitochondria (M) and anucleolate nucleus (N); F = flagellum. **Fig. 11,** Detail of primary spermatocyte with flagellum (F) seen both in longitudinal and tansverse section. G = glycogene granules. M = mitochondria; G = Golgi complex.

Spermatic cysts

In only one occasion, in April 1986, two specimens collected at Pear Tree Bottom contained large spermatic cysts ($\bar{x}$ = 143 ± 63 µm x 69 ± 20 µm; n = 40) located in basal cavities of the skeleton (Fig. 4). In those sponges, masses of storage cells were scarce and small. Choanocyte chambers occurred throughout the mesohyl as in all specimens collected at other times ($\bar{x}$ = 32 ± 7 µm x 25 ± 5 µm; n = 40). In sections, each pseudocalicle contained only one or exceptionally two spermatic cysts limited by a monolayer of pinacocytes (Fig. 8). The lowest part of spermatic cysts was separated from basopinacocytes bordering the basal skeleton by a dense mat of collagen fibrils (Fig. 9). All cysts were at the same stage of maturity, containing synchronically developed flagellated cells ($\bar{x}$ = 4.7 ± 0.5 µm x 5.1 ± 0.4 µm; n = 20) identified as primary spermatocytes which were separated from each other by empty spaces. However, some spermatic cysts were considerably long, reaching 300 µm in length, partly segmented by pairs of flattened cells extending inward and parallel to each other, suggesting the amalgamation of several cysts (Fig. 4). The nucleus of primary spermatocytes was large relatively to the cell ($\bar{x}$ = 3.3 ± 0.2 µm x 3.5 ± 0.3 µm; n = 20) with abundant chromatin. The cytoplasm contained numerous mitochondria, a developed Golgi complex and many glycogen granules (Figs 10, 11). Only one of these two specimens was retrieved in May 2002, but no evidence of spermatogenesis could be detected.

DISCUSSION AND CONCLUSIONS

Storage cells, massed in the deepest regions of the living tissue of *Goreauiella auriculata*, sheltered between arborescences of the aragonitic skeleton showed conspicuous similarities with the cryptic cells previously described, enclosed in the calcareous skeleton of two demosponges: *Merlia normani* and *Acanthochaetetes wellsi*, and of a calcisponge: *Petrobiona massiliana* (VACELET, 1990). In addition, all storage cells of *G. auriculata*, were alike within single masses and no variation was observed along a gradient as reported for the three other species. These storage cells, unknown in other calcified sponges devoid of horizontally partitioned skeletons, were interpreted as dormant structures enabling the sponges to survive through deleterious periods, such as winter or trophic shortage (VACELET, 1990).

However, the skeleton of *G. auriculata* displays no particular structure to harbor storage cells like basal crypts with semiclosed septa as in *M. normani* or horizontal calcareous tabulae as in *A. wellsi* or even thin canals as in *P. massiliana*. Storage cells are thus unlikely to be protected by their situation in the sponge, in case of adverse environmental conditions. Moreover, summer vs winter temperature variations encountered by *G. auriculata* are relatively limited (30° C to 26° C), as recently measured through continuous monitoring in the Pear Tree Bottom cave where samples were collected (ROSENHEIM *et al.,* 2002). Therefore, another function might be ascribed to storage cells in *G. auriculata*.

Occurrence of spermatic cysts up to 8 times larger than choanocyte chambers in *G. auriculata* differs from today's widely accepted view that in demosponges, male gametes develop directly from choanocytes (SARÀ, 1974; SIMPSON, 1984; PAULUS & WEISSENFELS, 1986; GAINO *et al.,* 1986b; PAULUS, 1989; BARTHEL & DETMER, 1990; DE VOS *et al.,* 1991; KAYE & REISWIG, 1991). The establishment of a

choanocyte origin of spermatogonia in the case under discussion would have to involve the fusion of an ample number of choanocyte chambers in order to form the large cysts reported here, or that a high level of mitosis would have to be initiated in each chamber in order to develop the definitive cyst. Both explanations are unlikely to be adequate in *G. auriculata*, since the number and the shape of choanocyte chambers remained unchanged in breeding specimens. Density of mesohyl cells remained undisturbed as well. The precursory alternative explanation suggesting that mesohyl cells aggregate and form cysts (OKADA, 1928; FINCHER, 1940; LÉVI, 1956) could be considered in the case of *G. auriculata*, reviving the view that the origin of the spermatocytes differs in different species. This viewpoint was recently supported by PILATO (2000) in a review of the ontogenic origin of the germ cells in Porifera. In addition, in this instance, storage cells and spermatic cysts were found independently in the same area of the mesohyl, superceding each other. Therefore, we suggest that spermatic cysts could be generated from storage cells. However, despite the number of samplings over an 18 year period and because of the restricted seasons at which they were accomplished, no evident transition stage from storage cells to spermatic cysts could be observed. Also, the lack of storage cells in the Bahamian samples remains unexplained.

So far, this study of gametogenesis in *G. auriculata* has only demonstrated the occurrence of male elements; no events dealing with oogenesis have been observed. As reported for other species, such as *Oscarella lobularis*, only a few individuals of a sponge population can be found breeding (GAINO *et al.,* 1986 a,b). Consequently, further elucidation is required from samples collected more extensively before and during the reproductive period to determine whether there are separate male and female individuals or whether some individuals are hermaphroditic in *G. auriculata.*

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