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Investigation of the budding process in *Tethya citrina* and *Tethya aurantium* (Porifera, Demospongiae)

Abstract The budding process has been studied in two congeneric Mediterranean species belonging to *Tethya* from different sampling sites: Marsala and Venice Lagoons (*Tethya citrina*); Marsala Lagoon and Porto Cesareo Basin (*Tethya aurantium*). Buds, connected to the adult by a spiculated stalk, differ between the two species in morphology and size, since those of *T. citrina* are small with elongated bodies, showing only a few spicules protruding from the apical region, whereas those of *T. aurantium* are round, larger, and show spicules radiating from the peripheral border. In *T. citrina*, cells with inclusions, varying in electron density and size, represent the main cell types of the buds. In *T. aurantium*, the cell component shows a major diversification, resulting from spherulous cells, grey cells, vacuolar cells and peculiar micro-vesicle cells. Neither canals nor choanocyte chambers were observed in the buds of the two species. In *T. citrina*, bud production is similar in both sampling sites. In *T. aurantium*, budding occurs more rarely in Porto Cesareo Basin, probably in relation with environmental factors, such as the covering of the cortex by sediment and micro-algae. Finally, in the buds of both species, the spicule size does not differ from that of the cortex of the adult sponges, further supporting the main involvement of the cortex in organizing the skeletal architecture of the buds.

Keywords Porifera · *Tethya* · Bud differentiation · Spicules · Ultrastructure

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Introduction

Poriferan asexual reproduction by fragmentation, gemmules and buds, together with the constitutively essential elements allowing fragments to reorganize and operate as independent organisms (Wulff 1991), represents a significant reproductive strategy for some species. These processes seem to occur to some extent in all or most sponges (Fell 1993), thereby maximizing the dispersal of these animals (Wulff 1995; Tsurumi and Reiswig 1997; Maldonado and Uriz 1999).

The structure of the gemmules along with the hatching of a new specimen has been intensively studied (reviews by Simpson 1984 and Weissenfels 1989). Less is known about the budding process, which typically occurs in marine poriferans (review by Simpson 1984; Boury-Esnault 1970; Chen et al. 1997; Corriero et al. 1998), and was first described in the freshwater species *Radiospongilla cerebellata* (Bowerbank 1863), by Saller (1990) after experimental induction by injuring the mother animal.

Buds consist of cellular masses that sprout out from the surface of the adults and are able to develop into new functional individuals. The buds of *Tethya* were first studied by Connes (1967, 1968) in a mixed population of *T. citrina* (Sarà and Melone 1965) and *T. aurantium* (Pallas 1766), where these species were confused in the entity *T. lyncurium* (Linné 1767). More recently, marked differences in the number of buds and in the lasting of this asexual process have been described in populations of these two species coexisting in the same environment (Corriero et al. 1996), without any interest in the process leading to bud differentiation.

Tethya citrina and *Tethya aurantium* conform to the typical organizational pattern of Tetractinomorpha. Among them, Tethydae are characterized by well-defined cortex and a skeleton consisting of strongyloxeas arranged in bundles, different types of both spherasters and micrasters. According to the exhaustive revision of Sarà (1987), the two species can

be distinguished on the basis of colour, surface structure and spiculation. In particular, *T. citrina* is light yellow in colour, has a thin cortical layer including oxyspherasters, and a single category of micrasters without distinction between cortical and choanosomal elements. *T. aurantium* is orange in colour, has a strong thick cortical layer including large spherasters, and two categories of micrasters (cortical and choanosomal elements).

In the present paper, we examine the various steps in the process of bud formation in *T. citrina* and *T. aurantium*, at the structural and ultrastructural levels, with the aim of verifying whether the budding process reflects a species-specific behaviour, and to what extent different environmental conditions can affect this asexual reproduction in congeneric species.

From the bulk of data, it has emerged that buds significantly differ in their cytological and skeletal organization, thereby proving that their differentiation is a species-specific process. Moreover, environmental conditions may locally enhance or depress this mechanism of asexual reproduction.

Material and methods

Animals and sampling sites

The study was carried out at sites where rich budding populations belonging to *T. citrina* (Sarà and Melone 1965) and *T. aurantium* (Pallas 1766) (Tetractinomorpha, Demospongia) have been previously observed (Corriero 1990; Corriero et al. 1996; Gaino et al. 2002). Budding specimens of *T. citrina* and *T. aurantium* were collected in October and November 2001. *T. citrina* was sampled in Venice (Adriatic Sea) and Marsala Lagoons (Tyrrhenian Sea, Northern Sicily). *T. aurantium* was sampled in the lagoons of Porto Cesareo Basin (Apulia, Ionian Sea) and Marsala. For each sampling site, five budding specimens were randomly selected and sponges were removed from the substrate within 2 weeks of the onset of the process.

Porto Cesareo Basin is a small, sheltered basin on the south-western coast of Apulia (40°15'N; 17°54'E) with a maximum depth of 2.5 m. A wide channel system allows considerable sea water inflow into the basin (Passeri 1974). Among 42 poriferan species present, a rich population of *T. aurantium* has been reported (Corriero 1990; Mercurio 2000). The species colonizes hard horizontally oriented substrates exposed to direct sunlight and it is covered by a continuous layer of sediment mixed with a dense population of filamentous microalgae. This layer, up to 5 mm thick, completely covers the sponge's surface hiding its typical orange colour and the cortical characters such as tubercles and lacunae. *Marsala* is a large lagoon along the north-western coast of Sicily (37°14'N; 12°40'E), widely communicating with the sea. The average depth is about 1 m. Forty-five species of Porifera have been recognized with rich pop-

ulations of *T. citrina* and *T. aurantium*, which both colonize the rhizomes of the phanerogam *Posidonia oceanica* (L.) Delile (Corriero et al. 1989). In both species, the external sponge surface is usually free of sediment and epibiontic organisms. The northern area of the *Venice Lagoon* (45°25'N; 12°26'E) is characterized by the presence of the Dese river which produces a marked haline gradient (Sconfietti 1989). At the mouth of the river, marine conditions prevail and the hard substrates are mainly colonized by different species of the Bivalvia, Bryozoa and Porifera. Among 20 poriferan species (Corriero et al. 2003), *T. citrina* is present with high density values, mainly colonizing vertical walls and small crevices between 3 and 5 m of depth (Corriero unpublished data). The external surface of the sponge appears free of sediment, or, at most, partially covered by fine sediment which fills the depression among the cortical tubercles. Epibiontic microalgae are lacking.

Methods

In order to investigate the skeletal elements, spicule preparations were obtained from fragments of animals as well as from whole buds dissected from adults. The material was treated by soaking it overnight in chloride bleach (sodium hypochlorite) and then it was dissolved in 60% nitric acid. After repeated washing in distilled water and in a graded series of ethylic alcohol, the isolated spicules were examined using an Olympus BH-2 light microscope. For each sampling site, buds were counted and separated into two size classes between 0 and 1.50 mm in diameter for *T. citrina*, and into five size classes between 0 and 3.75 mm in diameter for *T. aurantium*. Spherasters and oxyspherasters of five buds from each sampling site and from each bud size class were counted and measured. For comparative analysis, 50 spherasters and 50 oxyspherasters of both the choanosomal and cortical layers of each budding specimen were measured.

For histological observations, the budding region of the mother sponge and the outermost part of its choanosome were fixed in a solution of 10% formalin in filtered sea water, buffered with 0.1 N NaOH to a final pH of 7.6–7.8. Desilification of the samples was obtained by immersion in 5% hydrofluoric acid (HF) in filtered sea water for one and a half hours. Samples were then dehydrated in an ethanol series and embedded in paraffin. The 5–7 μm thick histological sections were stained with haematoxylin-eosin or toluidine, and observed under a Leica microscope.

For ultrastructural investigations (TEM, SEM), fixation was carried out for 2 h in 2.5% glutaraldehyde in a buffer of cacodylate (0.7 M) and filtered seawater to a final pH of 7.4. For transmission electron microscopy (TEM), selected material was post-fixed in 1% osmium tetroxide (1 h) in the same buffer and desilificated (30 min) in 5% HF. Afterwards, samples were dehydrated in a graded ethanol series and embedded in an

epon-araldite mixture. Ultra-thin sections obtained with an LKB ultratome were contrasted with uranyl acetate and lead citrate, and examined with a Philips EM 208.

Preparations for scanning electron microscopy (SEM) were carried out on the fixed material, then dehydrated in a graded ethanol series and critical point dried using a CO_2 Pabish CPD 750 apparatus, mounted on stubs with silver-conducting paint, and coated with gold palladium (20 nm) in a Balzers Union Evaporator. Specimens were observed under a Philips XL30 at an accelerating voltage of 18 kV.

Results

Buds sprouting out from the sponge's surface vary in size and extension since they are produced asynchronously, thereby appearing at different stages of differentiation. Their density and morphology differ according to the species.

Tethya citrina budding

The buds of *T. citrina* are small and emerge from the sponge's surface in a tight configuration (Fig. 1a). They typically appear in the form of filaments that tend to enlarge at their apex forming irregularly shaped and spiculated bodies. The buds mainly consist of a basal region, uplifted from the mother sponge, and of an apical region (Fig. 1b). Buds gradually grow through the swelling of their apical region, a process that makes the stalk of connection to the mother sponge more evident. The skeleton of the stalk consists of strongyloxeas arranged to form bundles (Fig. 1c), which irradiate from the mother sponge and gradually diverge in the apical region of the bud where they protrude outside. Micrasters are present in remarkable density around and within the spicule bundles (Fig. 1c) and become scattered in the apical region of the bud, just below its external surface.

Histological analysis reveals that spicule bundles emerging from the cortical layer of the mother sponge constitute the organizing centre that promotes bud differentiation (Fig. 2a). TEM images show that the cells of the buds are typically filled with inclusions varying in electron density and size. In the basal region of the stalk, spicule bundles prevail, and the number of cells surrounding the bundles is reduced (Fig. 2b). Moving slightly upward, the number of cells increases. Cells with inclusions have an elongated shape (Fig. 2c) and, together with the archeocytes, fill the matrix interposed among the spicules. Groups of spherulous cells occur on occasion, clearly distinct from the other cell types with inclusions because of their dimension and the marked electron-density of their spherules (Fig. 2d).

The apical region of the bud, delimited by a pinacodermal layer, shows lacunae separated by collagen matrix (Fig. 3a), which includes scattered cells, some of
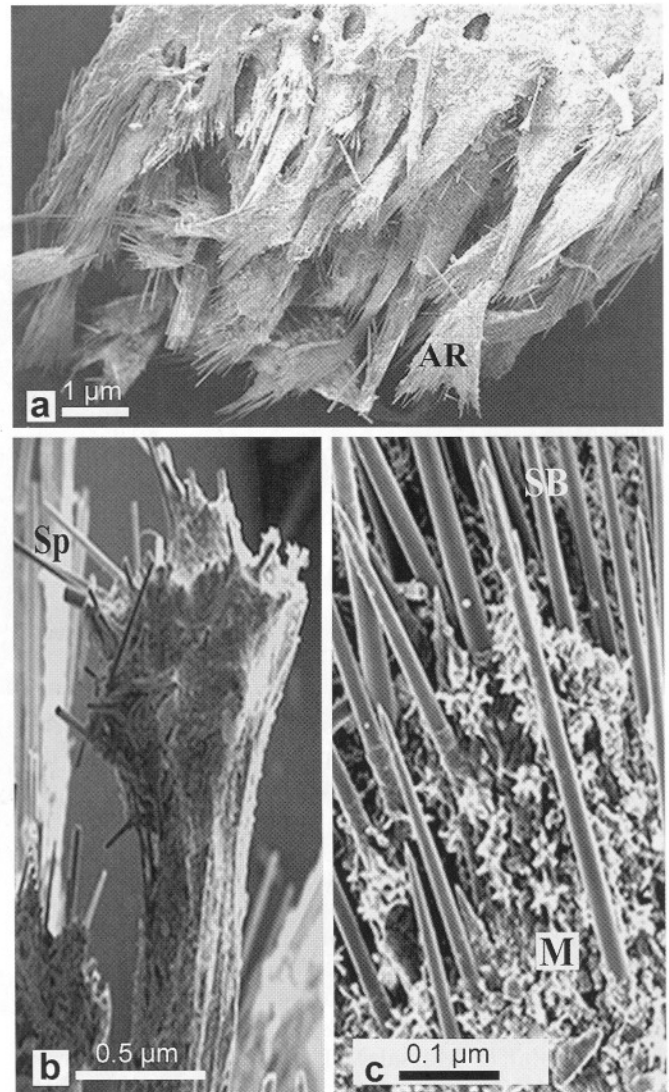

Fig. 1 Buds of *Tethya citrina* in scanning electron microscopy **a** Sprouting out of buds from the mother sponge surface. Each bud consists of a stalk (*S*) that enlarges in the apical region (*AR*). **b** Spicules (*Sp*) radiating from the apical region of the bud. **c** Micrasters (*M*) densely packed around the spicule bundles (*SB*)

which are characterized by a micro-vesicle cytoplasm (Fig. 3b). Moving towards the core of the bud, collagen fibrils become more abundant and are arranged to form bundles (Fig. 3c). The number of the cells with electron-dense inclusions progressively increases from the periphery of the bud towards its core. Here, these cells tend to be particularly elongated in shape, a morphology also shown by the archeocytes (Fig. 3d). Cells with inclusions have long thin cytoplasmic extensions that include granules arranged in sequence (Fig. 3e).

Cell density varies in the bud according to the abundance of spicules and collagen. In some areas the collagen matrix prevails (Fig. 3f), whereas in others the organization is very compact owing to the number of cells (Fig. 3g).

Fig. 2 a–d. *Tethya citrina*. Histological (**a**) and fine structural (**b–d**) organization of the buds. **a** Spicule bundles of the mother sponge (*arrows*) constituting the organizing centre of the bud (*B*) differentiation. **b** Prints left by the dissolved spicules (*S*) of the basal region of the bud, arranged in bundles and some cells (*arrows*) in between. **c** The upper region of the stalk showing cells with inclusions (*arrows*) and an archeocyte (*arrowheads*). **d** Spherulous cells showing large electron-dense spherules
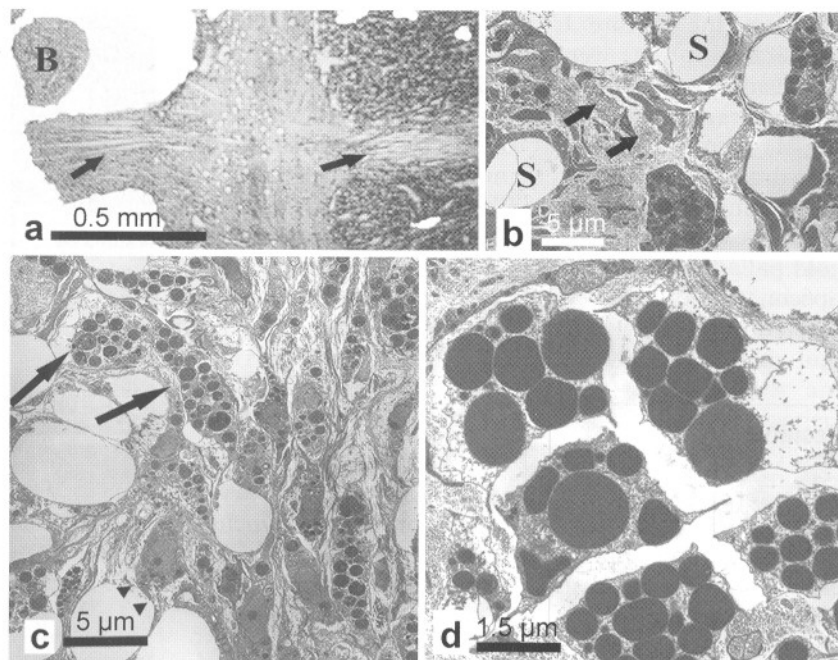
No choanocyte chambers or canals were observed either in the stalk or in the apical region of the bud that goes on to constitute the actual detached part.

Intraspecific comparative analysis

In *T. citrina* the budding process does not differ significantly between specimens living in the Venice and Marsala lagoons, as proved by the number of buds collected per site (475 buds in total in Venice; 305 buds in total in Marsala). The adults at Venice measured 21.4 ± 2.9 mm in mean diameter with mean volume of $4,472 \pm 1,724.4$ mm^3 and a total volume of $22,300$ mm^3, while their buds had a total volume of 988.2 mm^3 (each measuring 0.75 ± 0.2 mm in diameter with a volume of 2 ± 1.5 mm^3). The adults at Marsala measured 20.3 ± 2.6 mm in mean diameter with a mean volume of $4,180 \pm 1,143.1$ mm^3 and a total volume of $20,110$ mm^3, while their buds had a total volume of 763.1 (each measuring 0.85 ± 0.2 mm with a volume of 2.2 ± 1.7 mm^3). The volume of the buds at the latter site was 3.8% that of the adults, while it was 4.4 % of the adult's volume at Venice. Bud differentiation takes place fairly similarly in the two populations. In the two sampling sites, the mean values of the bud diameter (stalk excluded), measured within 2 weeks of their appearance, varied from 0.7 to 0.8 mm. The mean diameter of the oxyspherasters (about 60 µm) remains constant with the increase in the bud size (Table 1). The diameter of the oxyspherasters of the buds is very close to that shown by these spicules in the cortical layer of the mother sponge. In contrast, the choanosomal oxyspherasters of the adults are significantly smaller than those in the cortex of the adult and of those of the buds. Oxyspherasters are virtually absent in the smaller buds (Table 1)

Tethya aurantium budding

In *T. aurantium*, buds uplift from the mother sponge in the form of spiculated bodies (Fig. 4a) and remain attached to the adult surface by stalks. The onset of budding is marked by the differentiation of protuber-

Table 1 *Tethya citrina*: Spicule content and measurements in adults and buds

T. citrina		Mean numbers of oxyspherasters		Mean diameter of oxyspherasters (µm)	
		Marsala	Venice	Marsala	Venice
Choanosome	(Adult sponge)			47.9 ± 6.6	51.7 ± 8.3
Cortex	(Adult sponge)			57.2 ± 9.3	55.1 ± 7.7
Buds		0.6 ± 0.9	12.2 ± 20.1	59.0 ± 3.6	55.2 ± 5.1
Size classes of buds (mm)	0–0.75	0	6.8 ± 10	0	56.3 ± 4.1
	0.75–1.5	0.6 ± 0.9	5.4 ± 10	59 ± 3.6	54.5 ± 5.6

Fig. 3 *Tethya citrina.*
Transmission electron
microscopy (TEM) of the apical
region of the bud. **a** Pinacoderm
layer (*arrow*) bordering the bud.
Note the lacunae (*L*) among
scattered cells. **b** Below the
pinacodermal layer, a cell with
micro-vesicle cytoplasm is
evident. **c** Core of the bud
showing densely packed
collagen fibrils (*CF*) arranged to
form bundles. **d** Cells with
inclusions (*arrow*) and an
archeocyte (*double arrow*) filling
the core of the bud. Note the
elongated shape of both cell
types. **e** Granules fill the cell
cytoplasm and the thin
extensions (*arrow*).
f Collagenous matrix prevails in
some areas of the bud core.
g Cells with inclusions
accumulate in some areas of the
bud core giving rise to a more
compact organization
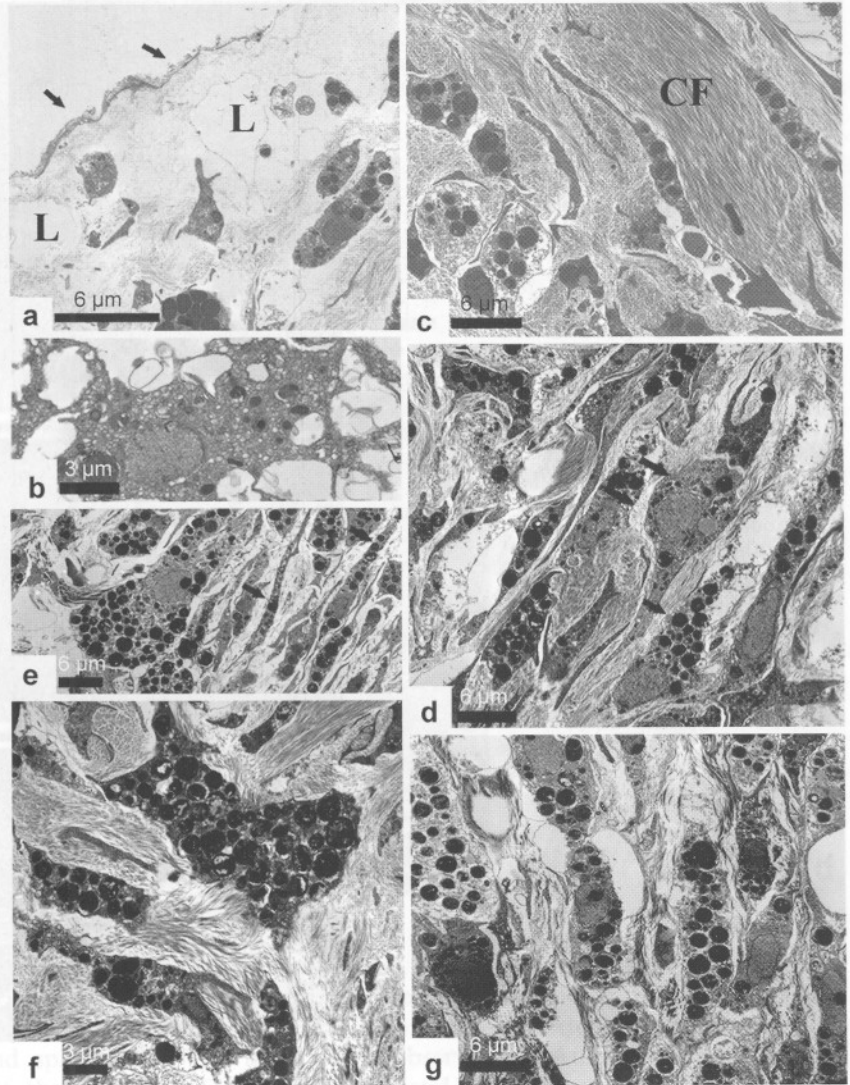
ances emerging slightly from the sponge (Fig. 4b). They gradually grow and become enriched with spicules radiating outside (Fig. 4a, c). In the buds experimentally detached from the adult, the stalk was fairly large (Fig. 4d). Strongyloxea spicules, unlike the rest of the bud, do not spread out from the surface (Fig. 4d).

Spicule bundles represent a remarkable component of the bud architecture. They can converge together or be arranged in parallel rows (Fig. 4e). Micrasters are uniformly distributed to support the pinacoderm layer (Fig. 4e).

Histological sections demonstrate that the onset of budding is triggered by the cortical layer of the mother sponge (Fig. 5a), which represents the organizing centre of the bud, providing it with collagen matrix and some cell types. The main cell category is represented by cells with inclusions, which are scattered in the mesohyl matrix and tend to concentrate along the outermost surface of the bud (Fig. 5b). These cells are also present in the choanosome (Fig. 5c) as well as in the cortex of the adult. In the buds, they are frequently observed gathered along the spicule bundles (Fig. 5d). Longitudinal sections confirm that the stalk mainly results from spicule bundles. These deepen into the choanosome of the mother sponge and contain collagen and a few scattered cells with inclusions (Fig. 5c).

Ultrastructure of buds in *T. aurantium*

TEM analyses allowed better definition of the bud organization. Collagen is abundant (Fig. 6a) while cells are scarce but represent various cell types. The superficial region of the bud is delimited by the pinacoderm below which cells with large inclusions, belonging to the spherulous cells, represent the most frequent type (Fig. 6a, b), coexisting with a few other smaller cells that vary in shape and in number of electron-dense granules. The emergence of the spicules interrupts the homoge-

Fig. 4 *Tethya aurantium.* Stereomicroscopy (**a**) and scanning electron microscopy (SEM) (**b–e**) of buds. **a** Sprouting out of the buds from the mother sponge surface. **b** The onset of the budding marked by the differentiation of protuberances (*P*) slightly emerging from the mother sponge surface. **c** Bud detached from the mother sponge showing the spicule bundles (*SB*) radiating outside. **d** Note the occurrence of a large stalk (*S*) in a bud experimentally detached from the mother sponge. The stalk does not show radiating spicules. **e** Spicule bundles (*SB*) arranged in parallel rows, sprout out from the bud surface whereas the numerous micrasters (*M*) are dispersed to support the pinacoderm layer
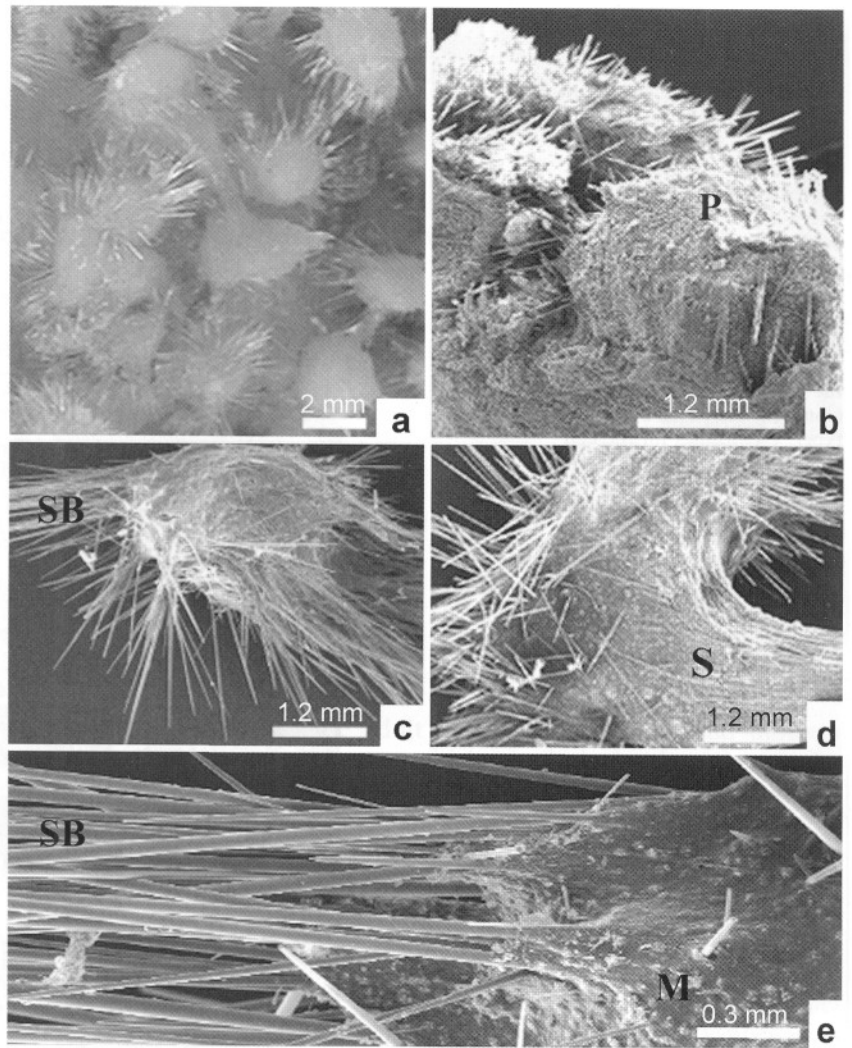
neous texture of the collagen (Fig. 6b). Sclerocytes are evident around the spicules (Fig. 6b). Moving inwards, the collagen matrix tends to be more consistent, even though the fibril bundles are more or less compact depending on the region of the bud examined, and spherulous cells assume a more rounded shape (Fig. 6c). Apart from the spherulous cells, other cell types dispersed in the collagenous matrix can be distinguished. They consist of (1) archeocytes, characterized by their phagosomes (Fig. 6d), (2) grey cells with glycogen rosette, few electron-dense inclusions (Fig. 7a) and often thin cytoplasmic extensions (Fig. 7b), (3) vacuolar cells characterized by large empty vacuoles disposed at the cell periphery (Fig. 7c) and showing irregular internal indentations (Fig. 7d), and finally (4) micro-vesicle cells morphologically resembling collencytes. The shape of the latter cells varies from fairly round (Fig. 7e) to elongate (Fig. 7f); they include electron-dense granules, the size of which corresponds that of the vesicles. The aquiferous system is missing, and only occasionally, some lacunae can be recognized in the apical region of that bud which will finally detach.

Intraspecific comparative analysis

In *T. aurantium*, budding appears more marked in the specimens from the Marsala Lagoon than in those of the Porto Cesareo Basin. At the former site, specimens were able to elaborate 295 buds with a total volume of 1,833 mm^3 (each measuring 2.1 ± 0.9 mm in mean diameter with a mean volume of 6.4 ± 4.7 mm^3), which constitutes about 5% of the adult's total volume of 392.4 mm^3 (each measuring 23.3 ± 3.5 in diameter with a mean volume of 6,990 ± 3,117.1 mm^3). At Porto Cesareo Basin, the specimens generated 67 buds with a total volume of 392.4 mm^3 (each measuring 1.9 ± 0.6 mm in diameter and a volume of 5.8 ± 3.7 mm^3) corresponding to 1.5% of the adult's total volume of 26,817 mm^3 (each measuring 19.4 ± 2.8 mm in diameter with a mean volume of 5,790 mm^3). The number of buds and volume ratio were thus lower at the latter site compared to Marsala. At both sites, the buds measured within 2 weeks after their appearance showed a wide range of size variation, encompassed between 0.9 and 3.6 mm. Spherasters are already present and numerous in the

Fig. 5 *Tethya aurantium.*
Histology of budding process.
Haematoxylin-eosin staining.
a Cortical layer of the mother
sponge showing the onset of the
differentiation of the buds (*B*).
b Outermost surface of the bud
showing the concentration of
the cells with inclusions (*arrow*),
which tend to disperse moving
towards the inner region of the
bud. **c** Cells with inclusions are
evident in the choanosomal
region (*ChR*) of the mother
sponge. Note that the stalk (*S*)
of the bud deepens into the
choanosomal region of the
mother sponge and contains
collagen and some cells with
inclusions (*arrow*). **d** Details of
the spicule bundles of the bud
showing cells with inclusions
(*arrow*) located along the
spicules
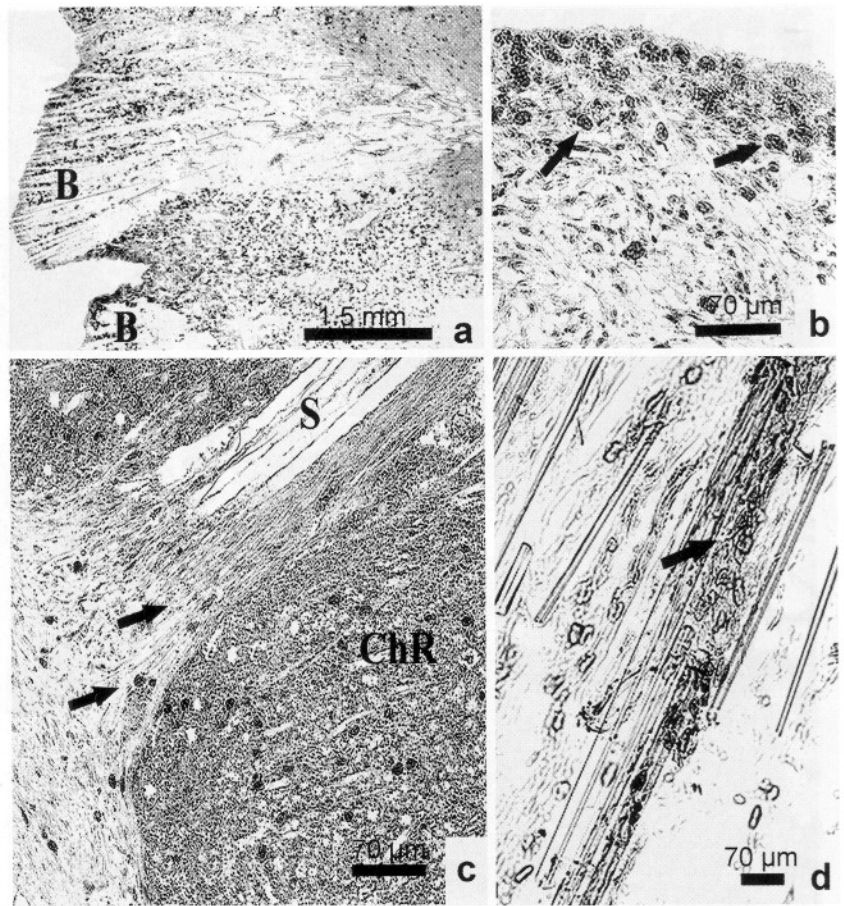
smallest buds (0.75–1.5 mm in diameter). At both sampling sites, these spicules measure about 65 μm and this value remains constant during the increase of bud size (Table 2). The mean diameter of the bud spherasters does not differ significantly from that of the cortical layer of the mother sponge. In contrast, the choanosomal spherasters of the adults are significantly smaller ($P < 0.01$, *t*-test) than those of the cortex of the adult and those of the buds. The number of spherasters per bud progressively increases with the bud growth at both sampling sites, reaching a mean value of about 2,000 elements in the largest buds (3–3.75 mm in diameter) (Table 2).

T. aurantium and for a large number of the tropical *Tethya* species (Bergquist and Kelly-Borges 1991; Sarà et al. 1993). Thin filaments extending outwards from the adult were observed in Australian specimens of *Tethya* sp. but distal buds were not apparent at the time of its finding (Fromont 1999). In addition, choanosomal buds are reported in the species descriptions of *T. bullae* Bergquist and Kelly-Borges 1991 and *T. levii* Sarà 1988, whereas *T. norvegica* Bowerbank 1872 develops into first growing buds, which in turn originate secondary buds (Merejkowski 1880). *T. robusta* (Bowerbank 1873) and *T. stolonifera* Bergquist and Kelly-Borges 1991 are able to elaborate stolons anchoring buds to the substratum (Bergquist and Kelly-Borges 1991; Sarà et al. 1993).

Discussion

Comparison with other *Tethya* species

Bud formation is a common means of asexual reproduction in *Tethya* species, since this phenomenon has been frequently observed in different species where it occurs with remarkable variations with regard to the origin, shape and size of the buds.

Production of cortical buds supported by spiculated stalks appears to be an extremely recurrent modality of budding for the Mediterranean *T. citrina* and

Comparison between *T. citrina* and *T. aurantium*

Structural and ultrastructural observations on the budding specimens of these species revealed that, even though the budding process is essentially borne by the cortical layer, the pathway leading to bud elaboration reflects the species-specific body structure (Sarà 1987). Indeed, in *T. citrina*, which has a thin cortex, the organizing centre of the bud is formed by the spicule bundles emerging from the surface. In *T. aurantium*, the cortex of
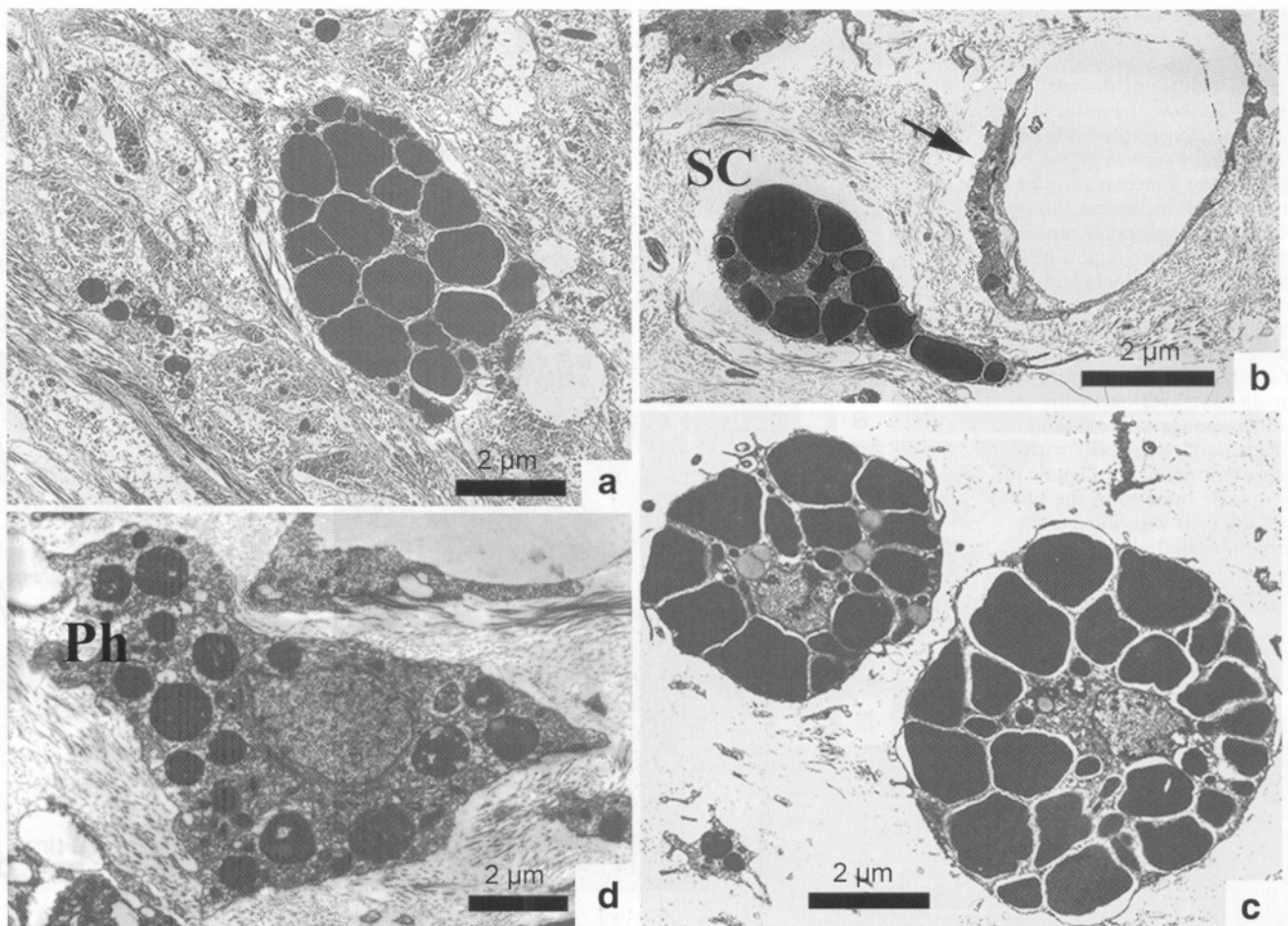
Fig. 6 Transmission electron microscopy (TEM) of various cell types constituting the bud of *Tethya aurantium*. **a** Abundant collagen matrix around spherulous cell. **b** Sclerocyte (*arrow*) bordering the hole left by a dissolved spicule. Note the spherulous cell (*SC*) with elongated shape. **c** Round-shaped spherulous cells. **d** Archeocyte with the typical phagosomes (*Ph*)

which is remarkably thick, bud differentiation takes place through progressive growth and bulging of this layer. With respect to *T. citrina*, the buds of *T. aurantium* are larger, thus suggesting a more rapid growth of the buds in this latter species that also leads to the differentiation of larger elements.

The occurrence of long protruding spicules, which radiate from the buds of *T. aurantium*, supports the notion that buds are able to float after their detachment and are at the mercy of the current. This feature allows dispersal and colonization of new habitats, even though, at present, we have no data about the distance they can spread or how this asexual process contributes to population biodiversity. However, the genetic uniformity of the population of *T. aurantium* in the Mediterranean basin (Sarà et al. 1989) has been used to state that the budding process has a remarkable impact on the recruitment of *T. aurantium* (Corriero et al. 1996).

Cell types are more diversified in *T. aurantium* than in *T. citrina* even though in both species the most recurrent elements are cells with inclusions whose electron density, size and distribution are markedly variable. Indeed, cells with inclusions represent one of the principle cell types

of asexual elements (Boury-Esnault 1970; Connes 1977; Chen et al. 1997; Ereskovskii 2003). The inclusion diversity we observed in *Tethya* probably reflects different functional phases more than different cell categories, apart from the typical spherulous cells and some others better defined on the basis of their fine morphology. Consequently, the differences observed in the buds of the two species with regard to both cell component and skeleton, seem to reflect a species-specific modality in the differentiation of these asexual elements.

Cells are numerous and densely packed in *T. citrina* whereas in *T. aurantium* they are more scattered, probably because of abundant collagen bundles. Cells commonly show cytoplasmic extensions, and tend to be arranged either in parallel rows or along the spicule bundles. These features suggest the ability of the cells to move from the mother sponge to colonize the newly formed buds. Migration of polypotent cells, and their subsequent differentiation into the definitive cells, is a typical feature occurring in the budding process that takes place as epimorphosis (Ereskovskii 2003).

Our data confirm previous observation (Bavestrello et al. 1992) that in *T. citrina* and in *T. aurantium*, the
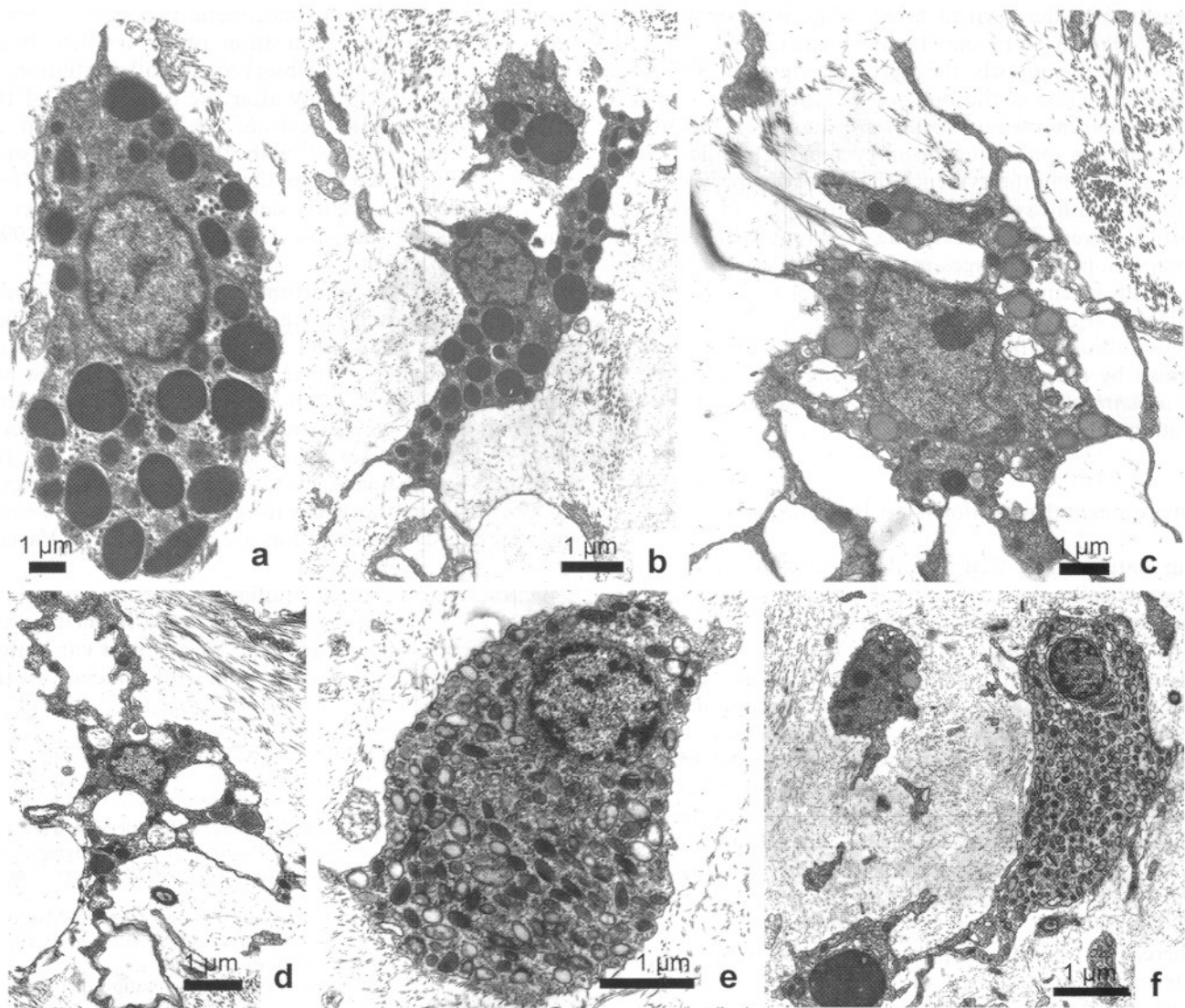
Fig. 7 *Tethya aurantium*. Transmission electron microscopy (TEM) of various cell types constituting the bud. **a** Grey cell. **b** Thin cytoplasmic extensions emerging from a grey cell. **c** Vacuolar cell with large and empty vacuoles. **d** Vacuolar cell with internally indented vacuoles. **e** Globular-shaped micro-vesicle cell. **f** Long-shaped micro-vesicle cell

Table 2 *Tethya aurantium:* Spicule content and measurements in adults and buds

T. aurantium		Mean number of spherasters		Mean diameter of spherasters (μm)	
		Marsala	Porto Cesareo	Marsala	Porto Cesareo
Choanosome	(Adult sponge)			37.5 ± 9.9	39.1 ± 11.7
Cortex	(Adult sponge)			66.3 ± 2.1	62.0 ± 0.7
Buds		$3,617.8 \pm 679.9$	$3,668 \pm 1,041.9$	66.3 ± 6.8	65.8 ± 9.3
	0.75–1.5	147.8 ± 191.3	165.6 ± 102.2	64.7 ± 11.6	65.2 ± 12.7
Size classes of buds (mm)	1.5–2.25	564.8 ± 326.7	501.4 ± 197.3	65.2 ± 10.3	65.6 ± 10.1
	2.25–3	922.2 ± 752.7	$1,222.2 \pm 426.2$	66.3 ± 7.2	65.5 ± 15.4
	3–3.75	$1,983 \pm 643.2$	$1,778.8 \pm 936.4$	67.8 ± 8.3	67.5 ± 10.3

mean diameter of both asters and oxyspherasters is significantly higher in the sponge cortex than in the choanosome. In addition, the size of spherasters and oxyspherasters in the buds of both species recorded within 2 weeks of the appearance of the bud on the surface of the mother sponge, does not differ from that

measured in the cortical layer, while it is significantly larger than those of the choanosomal region. This feature further supports the cortical origin of the buds. However, while in the buds of *T. citrina* the oxyspherasters occur in low numbers or are completely lacking, in *T. aurantium* spherasters occur even in the smallest buds, thus supporting the evidence that the two species follow different pathways with buds sprouting out from the cortical layer. These data are coherent with previous observations on changes in skeletal structure occurring in adult specimens of *T. citrina* and *T. aurantium* of different sizes (Bavestrello et al. 2000). According to these authors, while in *T. citrina* the growth is accompanied by the proliferation of strongyloxea bundles, in *T. aurantium* the enlargement is enhanced by the elaboration of new spherasters.

Environmental conditions and bud production

Our data suggest that the ability to differentiate buds is genetically determined and elicited by local environmental parameters. Among them, water temperature seems to play a pivotal role in the budding process. This is in keeping with the observations of Connes (1968) in the mixed deep-water population of *T. citrina* and *T. aurantium*. Likewise, the increase in the frequency of specimens with buds in the Mediterranean *Mycale contarenii* (Martens 1824) takes place concomitantly with the rapid decrease in water temperature (Corriero et al. 1998). In addition to the hydrological factors, our data lead us to hypothesize that the budding process can be enhanced or repressed by the different status of the sponge cortex. Indeed, whereas in budding specimens of both species the external surface is free of covering (Marsala) or, at most, moderately covered by a thin and discontinuous sediment layer (Venice), in Porto Cesareo, the specimens of *T. aurantium*, unable to elaborate buds, are covered by an up to 5 mm thick microalgal and sediment layer. According to Corriero (1990) this covering layer may protect the animal against light radiation, due to the schiaphylous habitus of the Mediterranean *Tethya* species (Corriero et al. 1989).

The provision of nutrients in buds

From the bulk of the cytological data, it has emerged that the organization of the buds of *T. citrina* and *T. aurantium* is typical of young sponges and that, apart from some very peculiar cell types, the remaining ones can be referred to those of the mother sponge, as reported by Connes (1968). Nevertheless, the lack of an aquiferous system in the buds hampers their water pumping, a mechanism that is typically involved in physiological functions in poriferans, among which the transportation of nutrients takes place. Cell inclusions could represent stored material useful to sustain the morphogenetic processes (Connes 1967) leading to the acquisition of a complete functionality. Connes (1967) observed the differentiation of the aquiferous system only after the detachment of the buds. In contrast, the buds of *M. contarenii* and *R. cerebellata* are equipped with the aquiferous system when still unreleased from the mother sponge, a feature that allows the newly detached elements to act as young functional animals (De Vos 1965; Saller 1990; Corriero et al. 1998).

The general organization and presence of fully developed canals and choanocyte chambers in the buds of *M. contarenii* has been considered to be in keeping with the possibility of their derivation from larvae not completely released from the mother sponge and undergoing their development on its surface (Simpson 1984). Likewise, a sexual origin was attributed to the armoured propagules, equipped with choanocyte chambers, from the excavating demosponge *Alectona* spp., previously considered as asexual products (Vacelet 1999).

On the other hand, an aquiferous system occurs in the buds of *R. cerebellata*, which are clearly formed asexually, thus proving that, in some cases, buds can differentiate an aquiferous system while still attached to the mother sponge.

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