Zoomorphology (1990) 109:295–301

Zoomorphology

© Springer-Verlag 1990

Formation and construction of asexual buds of the freshwater sponge *Radiospongilla cerebellata* (Porifera, Spongillidae)

Uwe Saller

Entwicklungsgeschichtliche Abteilung, Zoologisches Institut der Universität Bonn, Poppelsdorfer Schloß, D-5300 Bonn 1, Federal Republic of Germany

Received May 8, 1990

Summary. The buds of *Radiospongilla cerebellata* are formed asexually. Budding can be induced experimentally by injuring the sponge. The first sign of budding is a slight elevation of some surface areas, which proceed to rise rapidly so that they soon protrude conspicuously from the surface of the sponge. As a bud develops, the broad base joining it to the mother sponge narrows to a stalk, which finally breaks. The free buds drift in the water for 15–20 min and then settle, forming new sessile sponges. The buds, 1.5–2.5 mm in diameter, have an internal organization identical with that of the mother sponge. They are enclosed in a layer of pinacoderm perforated by dermal pores. Under the pinacorderm there is a shallow subdermal space, which is in communication with the incurrent canals leading to the choanocyte chambers. The water sucked into these chambers proceeds into the excurrent canal system and emerges from the sponge through the oscular tube. Spicules projecting radially from the bud bear apical tufts of microscleres. The skeletal spicules of the buds, like their choanocyte chambers, are smaller than those in the mother sponge. The chambers expand to their mature size by choanocyte mitosis. Buds and sponges are colored green by intracellular symbiotic algae of the genus *Chlorella.*

A. Introduction

Asexual reproduction is common in the Porifera, especially the freshwater sponges. It usually involves the formation of gemmules, clusters of cells within a hard shell that are able to withstand unfavorable environmental conditions. The development of the gemmules, the structure of the shell, and the eventual hatching of the new sponge have been intensively studied in recent years (see reviews by Simpson 1984 and Weissenfels 1989). Budding is another form of asexual reproduction, which has been considered unusual and has previously been observed only in a few marine sponges (see review by Simpson 1984). It has not been known in freshwater sponges.

The present paper is intended to clarify the process of budding and the cellular structure of the buds. The findings are compared with the morphological and functional aspects of budding in marine Porifera as they are currently understood.

B. Materials and methods

Radiospongilla cerebellata (Bowerbank, 1863) grows in the tropical greenhouse of the Botanical Garden of the University of Bonn, in a pool of water about 1 m deep that covers an area of about 10×20 m. The water temperature is kept at 28–29° C, and the vegetation consists mainly of tropical water lilies. The sponges were introduced unintentionally years ago, along with some imported plants, so that their precise geographic origin can no longer be ascertained. According to Penney and Racek (1968), the range of *R. cerebellata* includes tropical and subtropical regions of Africa, the Indopakistani region, Indonesia and China, and extends into the USSR and south-eastern Europe. Colonies of this sponge are colored an intense green; they grow best in light areas where they are not shaded by the large floating leaves of the water lilies. The growth form of *R. cerebellata* varies. Some are flat, while other colonies are cushion-shaped or branch like a tree. The last acquire their form primarily by growing over dead parts of plants.

Spicule preparations were obtained by soaking sponge pieces, buds and gemmules, overnight in chlorine bleach (sodium hypochorite). The disintegrated mass was then treated with 25% nitric acid. After repeated washing in distilled water, the isolated spicules were examined in the light and scanning electron microscope.

To observe bud development, the sponges were carefully separated from the substrate with a scalpel and transferred to polypropylene dishes, to which they attached after one or two days. The dishes were kept in a laboratory aquarium filled with water from the greenhouse pool, which was kept at 28° C (see Weissenfels and Langenbruch 1985).

The sponges produced many buds within the first few days after attachment to the dishes, as many as a dozen buds per sponge. Budding also occured, to a lesser extent (up to five buds per sponge), in uninjured sponges that remained in their "natural" habitat in the greenhouse. In the course of budding some material is lost from the mother sponge; the result is that its tissue becomes less dense, but there is no actual regression of the sponge. The fully formed buds were collected and placed in polypropylene

dishes, where they settled after 15–30 min. After 24 h the buds had transformed themselves completely into young sessile sponges. In the process, buds in close proximity fused with one another. The budding period lasted about one week, after which the sponges rapidly recovered. After growing in the aquarium for two to three months, most of the sponges began gemmulation. Even those that had developed from buds gemmulated. The young sponges that hatched from the gemmules were white at first, gradually acquiring a green coloration during their subsequent life in the aquarium.

Buds and sponges were fixed with 1% OsO_4 and 1% $K_2Cr_2O_7$ in 0.025 M sodium cacodylate buffer solution, pH 7.3, for light microscopy; 0.3% glutaraldehyde in the same buffer solution was used for electron microscopy. The subsequent preparation was as described repeatedly elsewhere (Langenbruch 1984; Saller and Weissenfels 1985). For scanning electron microscopy the sponge material was treated according to the method of Weissenfels (1982a, b).

C. Results

Anatomy of the sponge

R. cerebellata conforms to the typical organizational pattern of spongillids, as found for all the freshwater sponges so far investigated (review by Weissenfels 1989). The body of the sponge is clothed in a pinacoderm containing dermal pores. This outer covering, made rigid by radially arranged SiO_2 spicules, ends in a single-layered pinacocyte border around the base of the sponge. Below the pinacoderm is a shallow subdermal space continuous with the incurrent canal system. In the interior of the sponge, these canals lead to the flagellated chambers, about 45 µm in diameter. The apopyles of these chambers form passages to the excurrent canal system, through which the water current sucked in through the dermal pores emerges to the exterior of the sponge, by way of the atrium and egestion opening. Unicellular green algae (*Chlorella*) give the sponge its green color. The algal cells measure 1–4 µm and are found mainly in the ameboid cells of the sponge mesenchyme. An interesting feature of the mesenchyme, visible as light spots even under low magnification, are the aggregations of highly vacuolated cells (vacuolar cells) below the pinacoderm. These are described in detail in the context of bud structure.

R. cerebellata contains both macro- and microscleres (Fig. 1a). The former constitute the actual spicule skeleton. They are straight or slightly curved, smooth, pointed amphioxeas 290–380 µm in length (mean: 343 µm). The microscleres are amphistrongyles (Fig. 1a, b) about 75 µm long and 5 µm thick, with blunt ends and many thorny projections. They are found in the pinacoderm, where they remain within the cells that produced them (scleroblasts).

The gemmules are about 600 µm in diameter. The individual gemmules are held together by a stable spicule skeleton, which remains behind after gemmulation has been completed. The shell of the gemmule consists of a hard inner shell layer surrounded by a compartmented layer, the compartments of which are small and irregularly arranged. The micropyle is formed by a chimney-like pore tube. Microscleres are incorporated into the

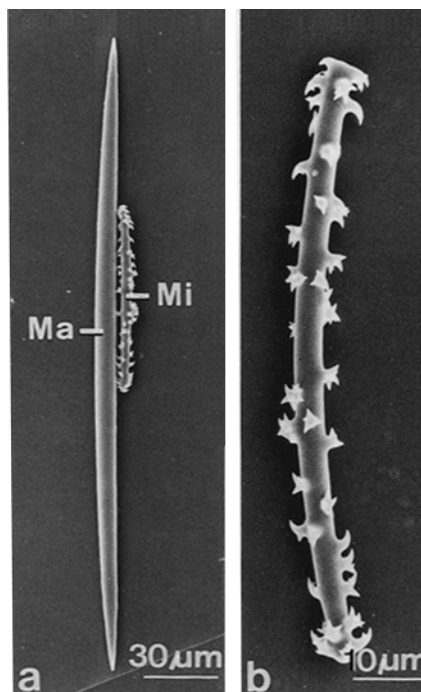

Fig. 1 a, b. Types of spicules. **a** Smooth macrosclere (*Ma*) and thorn-bearing microsclere (*Mi*). **b** Microsclere. The thorns are increasingly recurved toward the ends of the spicule. Scanning electron microscope (SEM)

compartmented layer and cover the surface of the gemmule shell in large numbers. The spicules of the gemmule are the same as those of the pinacoderm in both size and shape.

Formation of the buds

Many buds begin to form after the sponge has been removed from its habitat. The first buds have been completed after 24 h, and additional buds are produced on the following days. These sponges always form a limited number of buds in their original habitat as well.

The onset of budding is indicated by the appearance of slightly raised regions on the sponge surface. These elevations quickly become more pronounced, and soon they project well above the surface. Three young bud primordia (*B*) growing close together are shown in different stages of development in Fig. 2a–d. With reference to the middle bud in the figure, the process is as follows. In Fig. 2a the bud (*B*) is still joined to the mother sponge (*S*) by a broad, dense region of mesenchyme (*M*). This region is narrower and of lower contrast in Fig. 2b, and soon afterward (Fig. 2c) the connection to the mother sponge has been reduced to a thin strand of mesenchyme (*arrow*). In Fig. 2d the middle bud (*B*) has separated from the mother sponge, and in the region of the former connection only skeletal spicules (*Sp*) can be seen. At this time the middle bud possesses an egestion opening, turned toward the mother sponge, though it is too delicate to be visible in the picture. The young bud is capable of pumping water

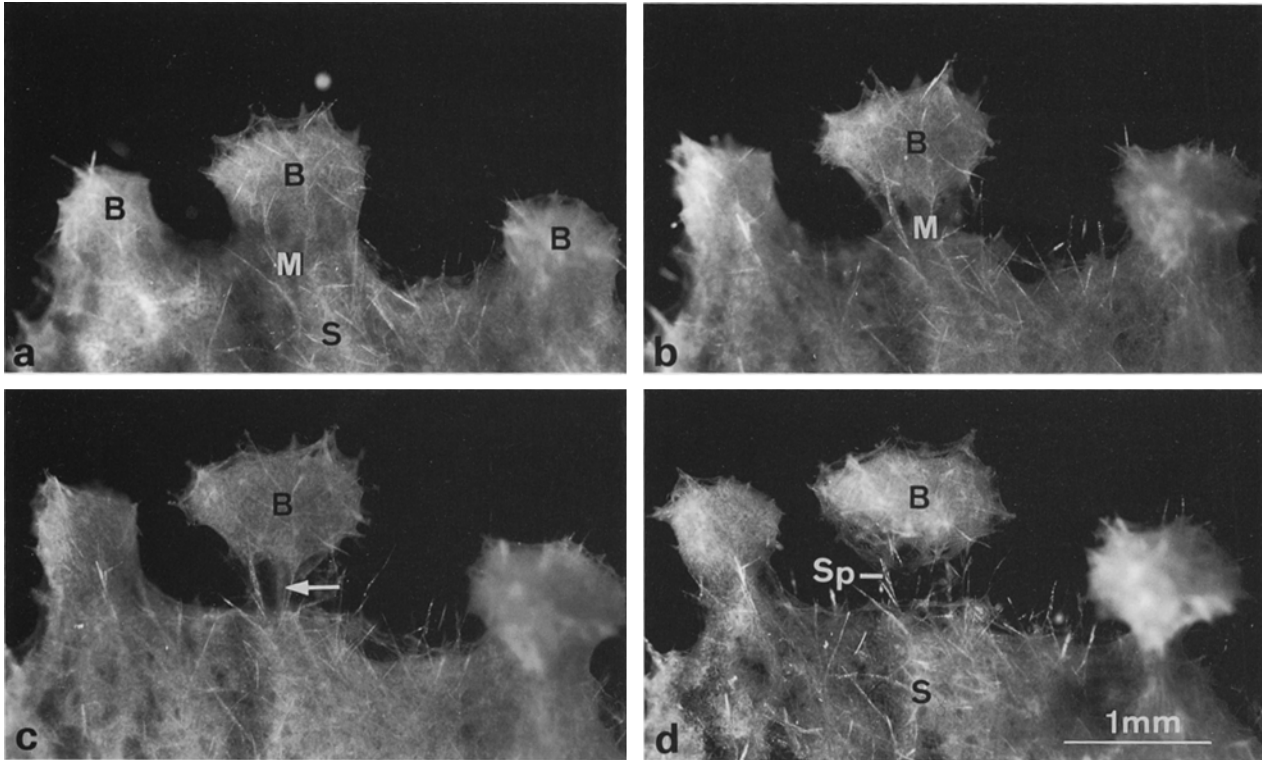

Fig. 2a–d. Formation of buds. **a** The young buds (*B*) project from the surface of the sponge (*S*). **b** The connection between the middle bud (*B*) and the mother sponge has been reduced to a stalk of mesenchyme (*M*), and in **c** it is little more than a strand (*arrow*). **d** Completed bud (*B*) with no connection to the mother sponge. Between **a** and **d** ten hours elapsed. *Sp* skeletal spicules. Stereomicroscope (StM)

during its entire development, as is evident in the continual inflow of particles through its dermal pores. Formation of the bud takes about 15 h altogether.

The crucial stages in bud development are summarized by the bright-field pictures of Fig. 3a–c, in which the mesenchyme appears in especially high contrast. The initially (Fig. 3a) broad, dense mesenchyme connection (*M*) between the young bud (*B*) and the mother sponge (*S*) becomes attentuated (Fig. 3b), at which time it contains a central excurrent canal (*EC*). Finally (Fig. 3c) the attachment is broken and the finished bud (*B*) lies on the mother sponge (*S*).

The buds are easily picked up and swept away by water currents. Their shape is spherical or oval to slightly flattened. They are 1.5–2.5 mm in diameter. The middle bud of Fig. 2a–d is shown again (*B*) after separation from the mother sponge, in Fig. 4a. It has a distinct oscular tube (*OT*). Radial spicules (*Sp*) protude from the surface of the bud, with tufts of microscleres (*SpT*) at their tips that function as barbed hooks. When two buds become hooked together by the spicule tufts, they subsequently fuse and grow into a single young sponge.

The buds very soon attach to the substrate; 15–20 min after they have separated from the mother sponge the buds have become scattered over the substrate and fixed to it. Figure 4b shows the bud (*B*) at the transition to the sessile stage. It first makes contact with the substrate by way of a long, projecting bundle of spicules. Figure 4c shows, at high magnification, such a bundle (*Sp*) projecting radially out from the bud surface with a tuft of microscleres at its tip (*SpT*). After they have become sessile, young sponges no longer possess these tufts.

Structure of the bud

The internal organization of the bud is the same as that of the sessile mother sponge having all the same cell types. The pinacoderm of the bud is 10–35 µm thick and is rich in collagen. In the pinacoderm (*PD*) of the oscular tube there are flagellated pinacocytes (*fPC*, Fig. 5a). All three of the endopinacocytes shown in the figure have a flagellum (*F*) in the region of the cell nucleus (*N*). Figure 5b shows a flagellated pinacocyte (*fPC*) as seen in the scanning electron microscope.

An especially striking feature is the aggregation of vacuolar cells (*VC*) shown in Fig. 6a. Such aggregations, found just below the pinacoderm (*PD*) of the bud, consist of a hundred or more cells with numerous vacuoles that appear more or less empty. In the electron micrograph of Fig. 6b some of the vacuoles (*V*) contain an indefinable material (*arrow*). Groups of cells of this kind are also present in the sessile sponges. Even under low magnification they are conspicuous as light structures in the otherwise green sponge tissue. In addition, the mesenchyme contains cells with high-contrast granules and a still darker subregion. Part of such a granulocyte (*GC*) is shown as an electron micrograph in Fig. 7.

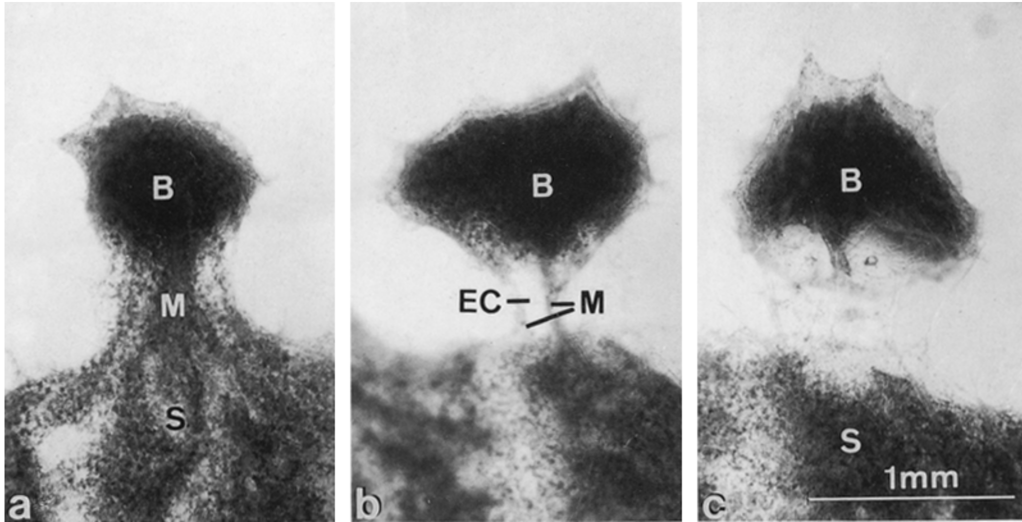

Fig. 3a–c. Bright-field micrograph of bud formation: **a** Young bud (*B*) connected to the mother sponge (*S*) by mesenchyme (*M*) over a broad area. **b** Almost completed but (*B*) with a thin mesenchyme bridge (*M*) and excurrent canal (*EC*). **c** Completed bud with no connection to the mother sponge (*S*). Between **a** and **c** seven hours elapsed. Standard light microscope (SLM)

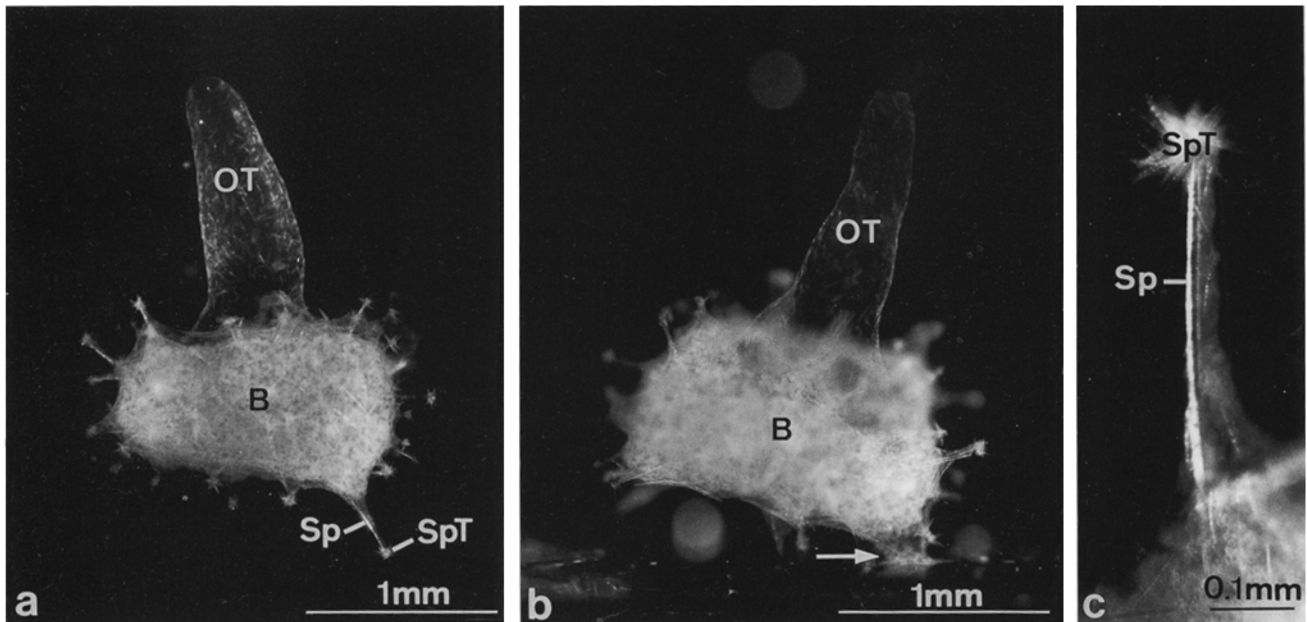

Fig. 4. **a** Free bud (*B*). *OT* oscular tube; *Sp* spicule bundle with apical tuft of spicules (*SpT*). **b** The bud (*B*) in the process of settling; a small region of the bud has become attached to the substrate (*arrow*). **c** Enlarged view of a spicule bundle (*Sp*) with apical spicule tuft (*SpT*). StM

The choanocyte chambers of the bud, with a diameter of about 31 μm, are distinctly smaller than those of the mother sponge. Many of the choanocytes are in mitosis. The scanning electron micrograph of Fig. 8 shows half of a choanocyte chamber (*ChC*) cut through the apopyle. Two flagella (*F*) of cone cells (*CC*) are clearly visible in the region of the apopyle.

Both buds and sponge are colored green by intracellular unicellular algae of the genus *Chlorella*. The algae are present in all cells of the sponge except for the vacuolar cells and the granulocytes. They are very abundant in the ameboid cells of the mesenchyme, and they are also numerous in pinacocytes and choanocytes. The algal cells are 1–4 μm in diameter. Figure 9a shows an archaeocyte (*AC*) containing many densely structured algae (*A*). Each individual alga is enclosed in a vacuole. The alga (*A*) in Fig. 9b was about to be passed on to another mesenchyme cell at the time of fixation; the part of the cytoplasm of the donor cell (*C1*) containing the algal cell is surrounded by a cytoplasmic process (*CP*) from the phagocytozing cell (*C2*).

The findings are summarized in a semidiagrammatic drawing of a median section through a bud, based on phase-contrast micrographs, in Fig. 10. The bud is com-

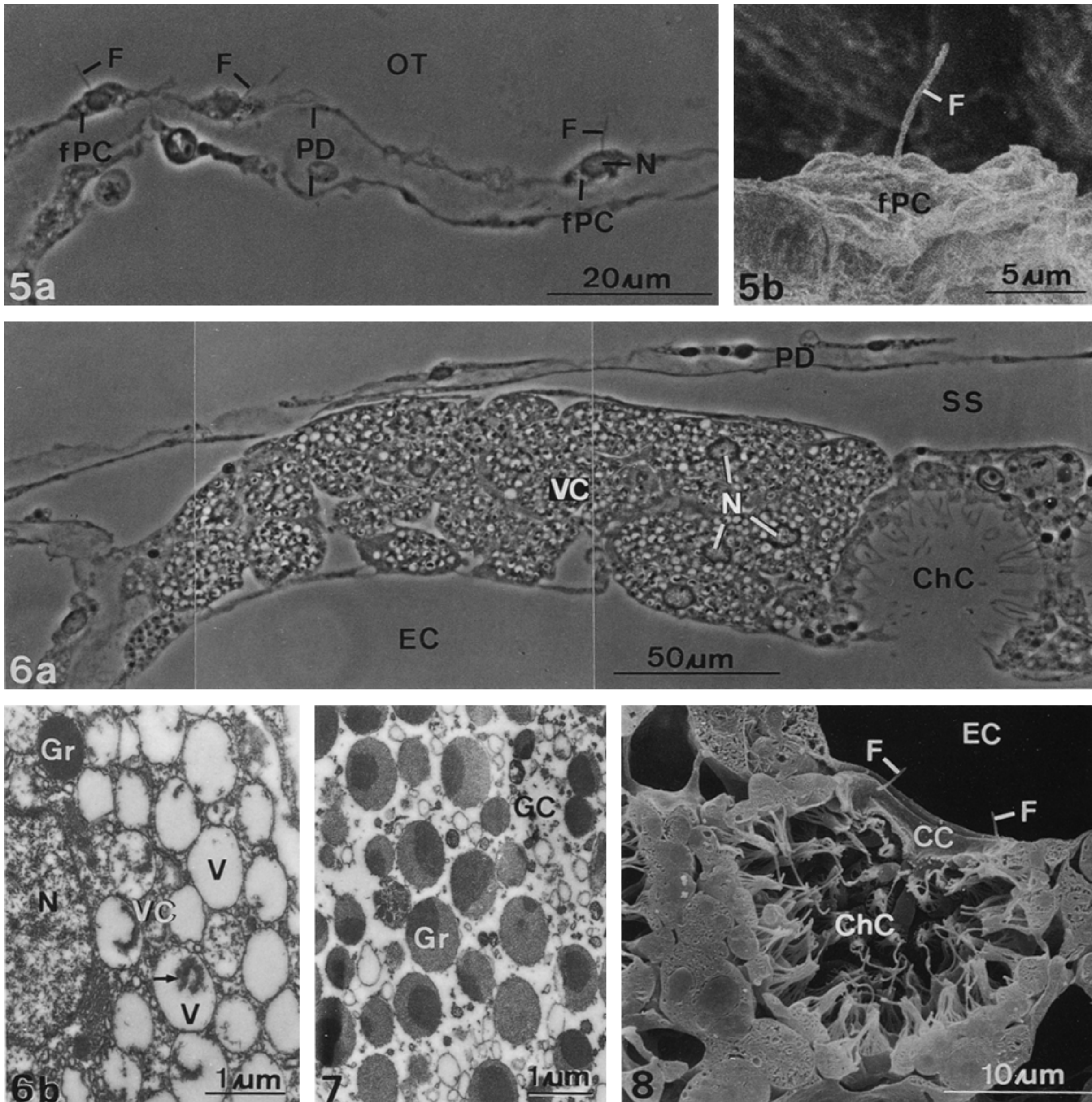

Fig. 5. **a** Pinacoderm (*PD*) in the region of the oscular tube (*OT*) with three flagellated pinacocytes (*fPC*). *F* flagellum; *N* nucleus. Phase-contrast microscope (PhM). **b** SEM picture of a flagellated pinacocyte (*fPC*). *F* flagellum

Fig. 6. **a** Aggregation of vacuolar cells (*VC*) just below the pinacoderm (*PD*). *N* nuclei of the vacuolar cells; *SS* subdermal space; *ChC* choanocyte chamber; *EC* excurrent canal. PhM **b** Electron micrograph of part of a vacuolar cell (*VC*). Some vacuoles (*V*) contain high-contrast material (*arrow*). *N* nucleus; *Gr* granule

Fig. 7. Part of a granulocyte (*GC*). The granules (*Gr*) contain amorphous material varying in contrast. Electron microscope (EM)

Fig. 8. SEM aspect of a choanocyte chamber (*ChC*); median section passing through the apopyle. The cone cells (*CC*) are clearly identifiable by their flagella (*F*). *EC* excurrent canal

pletely enclosed in a pinacoderm (*PD*) perforated by dermal pores (*DP*). Under the pinacoderm there is a shallow subdermal space (*SS*), which leads to the incurrent canals (*IC*). The latter conduct inflowing water to the choanocyte chambers (*ChC*) in the interior of the bud. By way of the excurrent canal system (*EC*) and the oscular tube (*OT*), the water flows back out of the bud.

The macroscleres that constitute the skeleton supporting the body mass have a length of 304 µm and are bound together by spongin, to form a three-dimensional framework. The spicules projecting radially out of the bud are connected to the spicule skeleton within the bud.

D. Discussion

Asexual reproduction of Porifera occurs by gemmulation or budding. Whereas the formation and subsequent

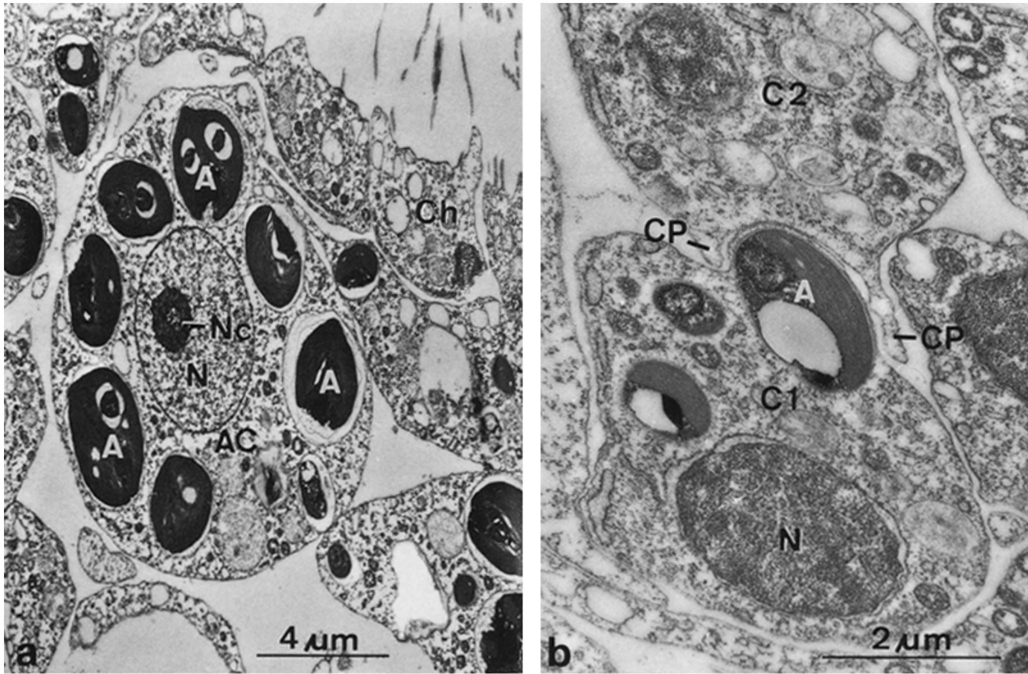

Fig. 9. a Archaeocyte (*AC*) with many intracellular algae (*A*). *N* nucleus; *Nc* nucleolus; *Ch* choanocyte. EM. **b** Transfer of alga. The cytoplasm of the donor cell (*C1*) that contains the algal cell (*A*) bulges out toward the recipient cell (*C2*). Processes (*CP*) of the recipient cell (*C2*) are closely apposed to this region. *N* nucleus. EM

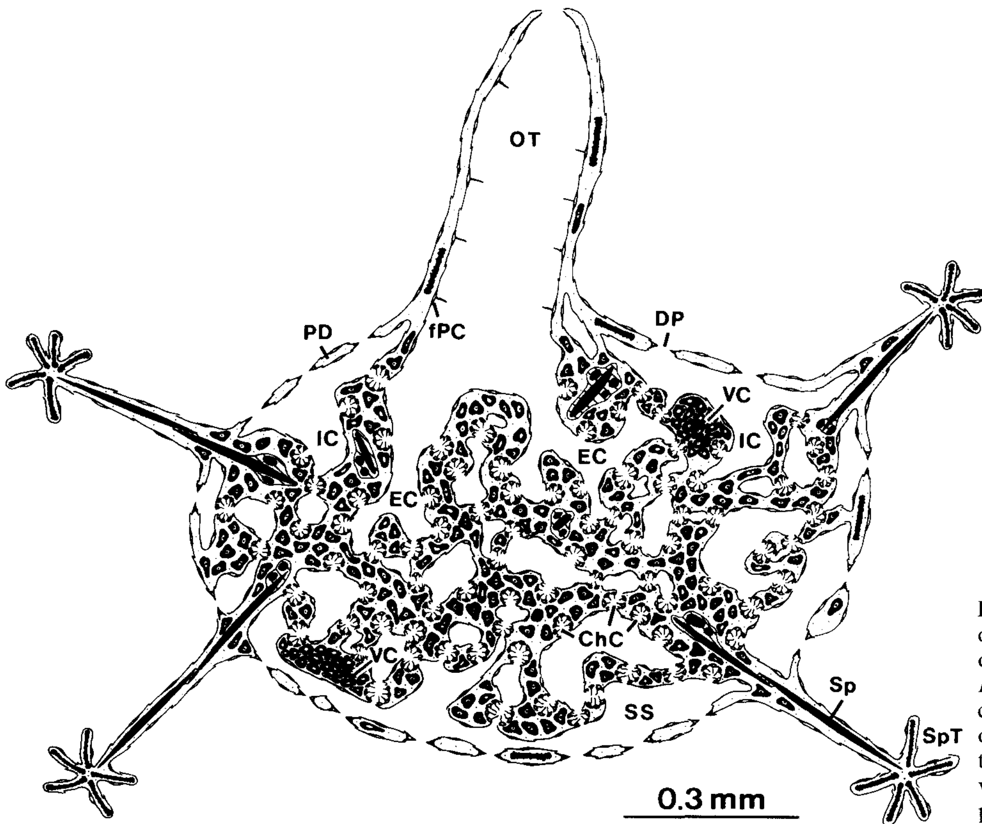

Fig. 10. Semidiagrammatic drawing of a bud. *PD* pinacoderm; *DP* dermal pore; *SS* subdermal space; *IC* incurrent canal; *ChC* flagellated chambers; *EC* excurrent canal; *OT* oscular tube; *Sp* spicules with spicule tuft (*SpT*); *VC* aggregation of vacuolar cells; *fPC* flagellated pinacocyte

hatching of gemmules are among the best-studied asexual reproductive process (reviews in Simpson 1984 and Weissenfels 1989), budding events have rarely been observed and descriptions are available only for a few marine sponges of the Demospongiae. In many cases the formation, the cellular construction, and the further development of buds are inadequately documented. Indeed, it had not been known that freshwater sponges form buds at all.

Of the few reports on budding in marine sponges, the most detailed refer to *Tethya lyncurium* (Pallas, 1766) (Connes 1967, 1968). Here budding could be induced experimentally by injuring the sponge. At first, when the buds of *T. lyncurium* are 1–2 mm in size, their cell

complement does not correspond to that of the mother sponge, for choanocytes and canals are absent. These elements do not develop until the bud has settled and attached to a new substrate.

The buds of *Axinella damicornis* Esper, 1794 (Boury-Esnault 1970), another representative of the Demospongiae, are also incomplete in that they lack choanocytes and spicules. On the other hand, the buds of *Mycale contarenii* (Martens, 1824) (De Vos 1965) contain all the types of cells present in the mother sponge. These buds are located in depressions in the mother sponge and are kept in place by spicules. The possibility that they arise from larvae that have not been completely discharged from the mother sponge, and continue to develop on the surface of the mother sponge, is under discussion.

While the buds of *R. cerebellata* are being formed, they continue to pump water. The position of the oscular tube of the finished bud results from a shift of the excurrent canal system; as the bud grows, the excurrent canal moves into the tissue that joins the bud to the mother sponge. After this link is broken, the oscular tube is formed from the part of the excurrent canal that remains with the separated bud, so that it necessarily occupies a position on the side toward the mother sponge. The significance of the vacuolar cells to the sponge and its buds is still unknown.

R. cerebellata is closely related systematically to two spongillids common in Central Europe, *Eunapius fragilis* (Leidy, 1851) and *Spongilla lacustris* (Linné, 1758). Both have monaxonal gemmule microscleres (gemmoscleres). With respects to the structure of the gemmule shell and the large number of thorny microscleres on the surface of the gemmules, *R. cerebellata* is the same as *E. fragilis*. On the other hand, *R. cerebellata* closely resembles *S. lacustris* in having thorny microscleres in the pinacoderm and its symbiosis with *Chlorella*.

According to Penney and Racek (1968), the thorny microscleres of *R. cerebellata* are immature gemmule microscleres (gemmoscleres). However, my investigation has shown no difference in size between the microscleres of the sponge and those of the buds or gemmules. Hence it seems more likely that the gemmoscleres are regular sponge spicules that have been put to use in constructing the gemmules.

The symbiosis of algae and sponge cells is facultative. The sponge is not dependent on the symbiont for its existence, since the young sponges that hatch from the gemmules contain no algae; the algae are acquired subsequently from the water. As in the symbiosis of *Chlorella* with *Spongilla lacustris* (see Saller 1989), the intracellular algae are transferred from one sponge cell to another.

Bud formation is an unusual mode of asexual reproduction in spongillids. Its chief characteristics are the brief duration of the budding process, the ease with which the buds drift away from the mother sponge, and the rapidity of their settling and attachment to the substrate. With respect to their internal organization, the buds are equivalent to spongillid larvae (Saller and Weissenfels 1985; Saller 1988).

Acknowledgements. I thank Professor Dr. N. Weissenfels, of the Zoological Institute in the University of Bonn, for supporting this project. The scanning electron microscopy was carried out in the Institute for Palaeontology, University of Bonn, by Mr. G. Oleschinski; his help is greatly appreciated.

References

Boury-Esnault N (1970) Un phénomène de bourgeonnement externe chez l'éponge *Axinella damicornis* (Esper.) Cah Biol Mar 11:491–496

Connes R (1967) Structure et développement des bourgeons chez l'éponge siliceuse *Tethya lyncurium* Lamarck. Arch Zool Exp Gén 108:157–195

Connes R (1968) Étude histologique, cytologique et expériementale de la régénération et de la reproduction asexuée chez *Tethya lyncurium* Lamarck (= *T. aurantium* Pallas) (Demosponges). Thèse Univ Montpellier, pp 1–193

De Vos L (1965) Le bourgeonnement externe de l'éponge *Mycale contarenii*. Bull Mus Nat Hist Natur (Paris) 37:548–555

Langenbruch PF (1984) Vergleichende rasterelektronenmikroskopische Darstellung der Gemmulaschalen von *Ephydatia fluviatilis*, *E. muelleri* und *Spongilla fragilis* (Porifera). Zoomorphology 104:79–85

Penney JT, Racek AA (1968) Comprehensive revision of a worldwide collection of freshwater sponges (Porifera: Spongillidae). Bull US Nat Mus 272:1–184

Saller U (1988) Oogenesis and larval development of *Ephydatia fluviatilis* (Porifera, Spongillidae). Zoomorphology 108:23–28

Saller U (1989) Microscopical aspects on symbiosis of *Spongilla lacustris* (Porifera, Spongillidae) and green algae. Zoomorphology 108:291–296

Saller U, Weissenfels N (1985) The development of *Spongilla lacustris* from the oocyte to the free larva (Porifera, Spongillidae). Zoomorphology 105:367–374

Simpson TL (1984) The Cell Biology of Sponges. Springer, Berlin Heidelberg New York

Weissenfels N (1982a) Rasterelektronenmikroskopische Histologie von spongiösem Material. Microsc Acta 85 (4):345–350

Weissenfels N (1982b) Bau und Funktion des Süßwasserschwamms *Ephydatia fluviatilis* L. (Porifera). IX. Rasterelektronenmikroskopische Histologie und Cytologie. Zoomorphology 100:75–87

Weissenfels N (1989) Biologie und Mikroskopische Anatomie der Süßwasserschwämme (Spongillidae) G. Fischer Verlag, Stuttgart

Weissenfels N, Langenbruch P-F (1985) Langzeitkulturen von Süßwasserschwämmen (Porifera, Spongillidae) unter Laborbedingungen. Zoomorphology 105:12–15