Zoomorphology (1985) 105:367–374

Zoomorphology

The development of *Spongilla lacustris* from the oocyte to the free larva (Porifera, Spongillidae)

Uwe Saller and Norbert Weissenfels

Entwicklungsgeschichtliche Abteilung des Zoologischen Instituts der Universität Bonn, Poppelsdorfer Schloß, D-5300 Bonn 1, Federal Republic of Germany

Summary. During June and July oocytes appear in well-developed specimens of *Spongilla lacustris*. These differentiate from archeocytes, and during the first growth phase they reach a diameter of ca. 50 µm. At this time each oocyte is enclosed in a single-layered follicle epithelium, which is retained until emergence of the larva.

In the second phase the oocytes grow to about 220 µm by phagocytosis of trophocytes. When phagocytosis has come to an end, there is a distinct layering of the yolk material that has formed within the cytoplasm of the oocyte. Small yolk granules surround the centrally located nucleus, and peripheral to these is a layer of larger spheres of yolk.

Cleavage is totally equal to unequal. Some blastomeres are binucleate. In the 15-cell staged micro- and macromeres appear.

The embryo consists of uniform cells with high yolk content; at the periphery they are slightly flattened rather than spherical. In this stage of development the first scleroblasts appear.

Further development to the young larva is marked by the appearance of a cavity (the larval cavity) lined with pinacocytes. The cavity expands to occupy about half the volume of the larva at emergence, becoming hemispheric in shape. The cells at the periphery of the larva form a columnar, single-layered, multiseriate ciliated epithelium with teardrop-shaped nuclei.

The emerging larva breaks through its follicle and the wall of the excurrent canal system; occasionally larvae can be found in the canals. At this time the larva has developed a few flagellated chambers, which may already be integrated into the primordia of the excurrent canal system. The previously discernible scleroblasts have now formed isolated spicules, which may adhere to form spicule-spongin complexes.

A. Introduction

The Spongillidae are the only fresh water family in the otherwise marine phylum Porifera. Spongillids are also unusual in their sexual reproduction, for they do not undergo the inversion of layers that characterizes the marine sponges.

Offprint requests to: U. Saller

This paper describes the embryonic development of *Spongilla lacustris* (Linné 1758) (Spongillidae) from the young oocyte to the free larva. The spongillids are viviparous: that is, the development from egg to larva takes place within the tissue of the mother sponge. The parenchymula larva leaves the mother sponge by way of the excurrent canal system.

Histological study of the structure and development of the Spongillidae began in the middle of the nineteenth Century. The egg was discovered by Lieberkühn in 1856. Later Fiedler (1888) and Maas (1890) examined the embryonic development of spongillids, in particular *Ephydatia fluviatilis* and *Spongilla lacustris*. A new treatment of embryonic development in *Ephydatia fluviatilis* was published by Brien and Meewis in 1938. In view of the considerable methodological advances that have been made since then, we thought it appropriate to undertake a thorough reinvestigation.

B. Materials and methods

The specimens of *Spongilla lacustris* examined here were obtained directly from the field. To ensure that all stages of development were available for study, the sponges were collected at several different times from the River Sieg near Rosbach. There *Spongilla lacustris* grows in crusts 0.5–1 cm thick, on the undersurfaces of stones where it is protected from direct sunlight.

Small pieces of the sponge crusts were cut out under water and immediately transferred to fixative solution.

The pieces prepared on 16 June 1983 contained developmental stages from young egg cells to cleavage stages. The material from 25 July 1983 contained all developmental stages up to the fully developed larva, as well as gemmule primordia. The material fixed on 4 August 1983 contained only a few advanced cleavage stages plus larvae and gemmule primordia.

Number and size of the cells in the cleavage stages were determined in serial sections. Models were used to reconstruct the cleavage stages, providing information as to the positions of the blastomeres with respect to one another.

The preparation procedure was as follows.

1. Fixation: 1% OsO_4 and 1% $K_2Cr_2O_7$ in 0.025 M sodium cacodylate buffer solution, pH 7.3, 20° C, for 2 h in the dark.
2. Washing: 0.2 M (=6.9%) sucrose in 0.025 M sodium cacodylate buffer solution, pH 7.3, 20° C, 6 × 10 min.

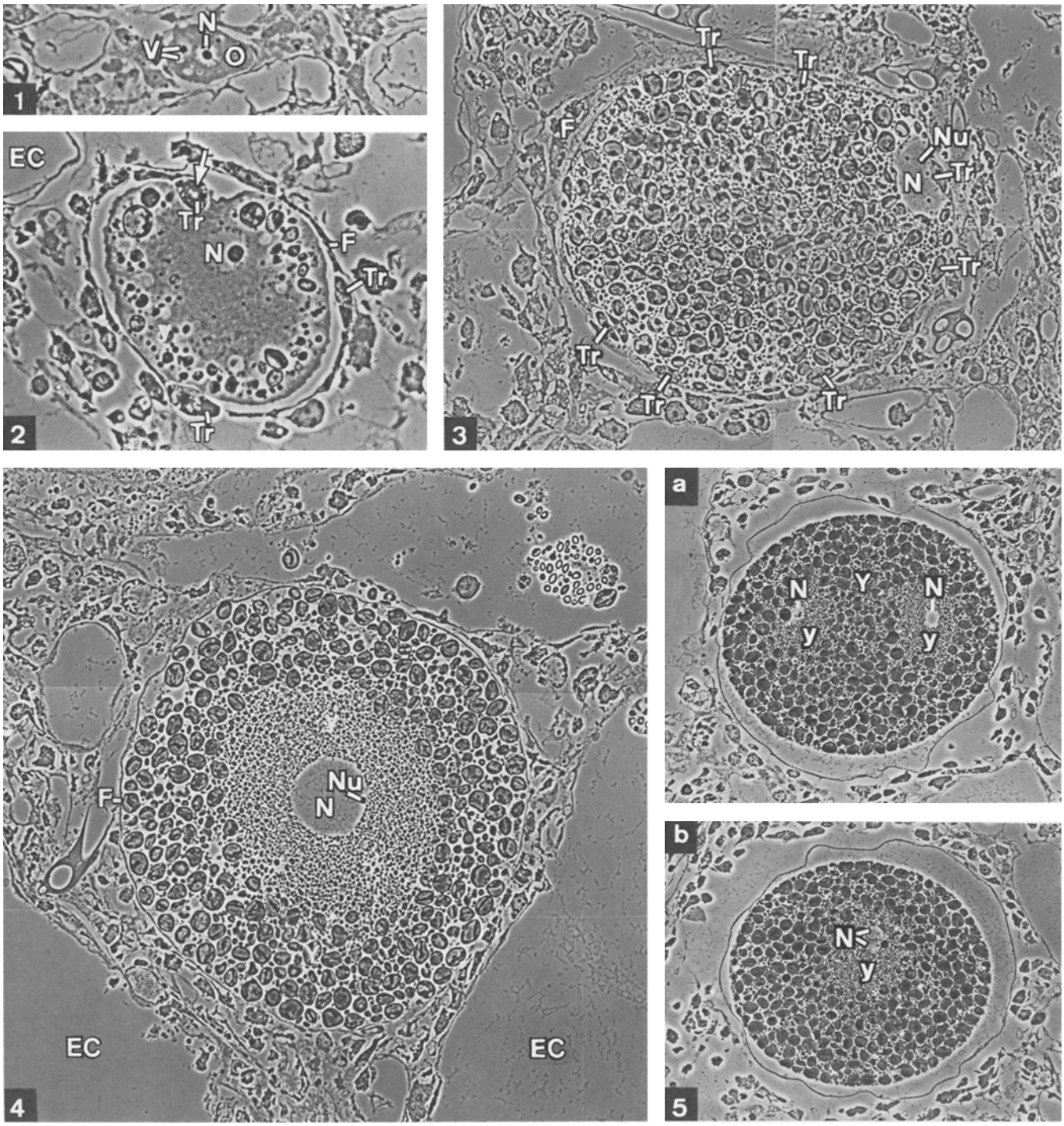


Fig. 1. Young oocyte (*O*) in the 1st growth phase. It contains a nucleus (*N*) with nucleolus and few vacuoles (*V*). ×490. This and all the following micrographs were made by phase-contrast microscopy of semithin sections

Fig. 2. Oocyte in the 2nd growth phase. It contains large spherical and small granular (*y*) yolk inclusions. Two trophocytes (*Tr*) lie within the follicle epithelium (*F*) and another in an indentation of the oocyte (←). *N* nucleus, *EC* excurrent canal. ×490

Fig. 3. Oocyte in the 2nd growth phase. The bean-shaped nucleus (*N*) with small nucleoli (*Nu*) is at the periphery of the yolk-filled egg cell. Many trophocytes (*Tr*) are within the follicle epithelium (*F*) and between it and the oocyte. *Nu* nucleolus. ×320

Fig. 4. Oocyte ready for cleavage, near excurrent canals (*EC*). The centrally situated, nucleolated nucleus (*N*) is surrounded by a zone of granular yolk (*y*). Outside this is the spherical yolk (*Y*). *Nu* nucleolus, *F* follicle epithelium. ×320

Fig. 5a, b. Two parallel sections of an oocyte ready for cleavage, which contains four nuclei (*N*). **a** Two nuclei (*N*) are separate from one another, each with a surrounding zone of granular yolk (*y*). **b** In the section 50 µm away in the series, two nuclei (*N*) are close together. *Y* spherical yolk. ×220

3. Desilication: 5% hydrofluoric acid in aqueous solution, 20° C, 60 min.
4. Rinsing: 0.2 M (=6.9%) sucrose in 0.025 M sodium cacodylate buffer solution, 3 × 10 min.
5. Dehydration: ascending ethanol series from 15% to absolute ethanol in 7 steps, 20° C, for a total of 3 h.
6. Postcontrasting: 1% phosphotungstic acid and 1% uranyl acetate in 70% ethanol of the dehydration series, 4° C, 2 h in darkness.
7. Embedding in mixtures of absolute ethanol and styrolmethacrylate in the proportions 3:1, 2:1, 1:1, 1:2, 1:3, for a total of 5 h until the final embedding in pure styrolmethacrylate.
8. Preparation of semithin sections with LKB-Ultrotome III.
9. Phase-contrast microscopy: Leitz-Dialux with Wild MPS 51/45 automatic photo attachment. Negative material: Ilford PAN F 18° DIN.

C. Results

Sexual reproduction of *Spongilla lacustris* in the Rhein/Sieg region takes place from the beginning of June to August. The developing stages are found in the middle and basal parts of the sponge. The oocytes are formed and develop over several weeks. A single sponge can contain various developmental stages at the same time.

The oocytes originate from archeocytes in the mesenchyme. The young oocytes can be distinguished from the regular archeocytes by their larger size.

1. Development of the oocyte

First growth phase. Single archeocytes, about 15 µm in diameter and containing a nucleolated nucleus, grow into young oocytes measuring about 50 µm.

Figure 1 shows the young oocyte (*O*) in its original ameboid form. Its mean diameter is 30 µm. The centrally located, nucleolated nucleus (*N*) is 10 µm in diameter. The homogeneous cytoplasm of the young oocyte contains light and dark vacuoles (*V*).

Second growth phase. By phagocytosis of nutrient-rich trophocytes, the oocyte grows from about 50 µm to 210–230 µm, enlarging its volume by 64–100 times. The trophocytes are generally located close to the oocytes in the mesenchyme. Their appearance there is closely correlated with the onset of sexual reproduction; if the mesenchyme contains only a few oocytes, or none at all, few or no trophocytes are present.

Figure 2 shows a 90-µm oocyte during phagocytosis (←) of a trophocyte (*Tr*). The cytoplasm of the oocyte contains much yolk in both spherical and granular (*y*) form. The nucleolated nucleus (*N*) occupies a slightly eccentric position within the yolk-free central cytoplasm. Two trophocytes (*Tr*) are within the follicle epithelium (*F*).

The oocyte in Fig. 3 measures 195 µm. The bean-shaped nucleus (*N*) is at the periphery of the oocyte and contains several nucleoli (*Nu*). The cytoplasm is filled with yolk material. The positions of some of the trophocytes (*Tr*) imply that at the time of fixation they were being, or were about to be, phagocytized by the oocyte.

Mature oocyte prior to cleavage. Just before cleavage begins, the oocyte is filled with trophocyte material. Its diameter is 210–230 µm.

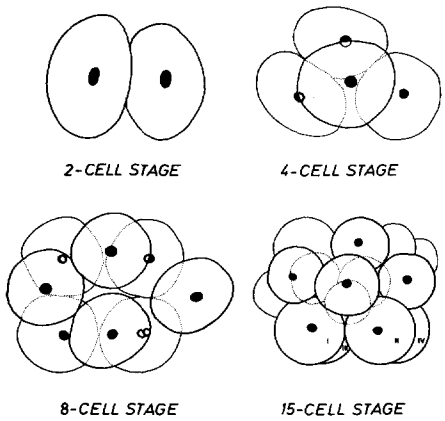


Fig. 6. Drawings to show the spatial arrangement of the blastomeres in four cleavage stages

Figure 4 shows a fully developed oocyte. It is enclosed in a follicle epithelium (*F*) and is situated in the mesenchyme near excurrent canals (*EC*). There is a distinct layering of the yolk material within the cytoplasm. The peripheral zone contains mainly 12-µm spheres of yolk (*Y*), with light inclusions embedded in a dense, homogeneous ground substance. The central region around the nucleus (*N*) is surrounded by a zone of smaller yolk granules (*y*), about 4 µm in diameter. The round, 40-µm nucleus contains a nucleolus (*Nu*) near the nuclear envelope; the interior of the nucleus is loosely structured.

In this stage of development the nature and distribution of the yolk material give no indication of polarity of the oocyte. The only differentiation in the yolk is the stratification by size, from inside to periphery. Hence there are no morphological criteria by which the animal and vegetal poles could be distinguished. In terms of the quantity of yolk present, this is a polylecithal egg.

Figure 5a, b shows two parallel sections from a mature oocyte, ready for cleavage. The distance between the two sections is 50 µm. Each section contains two 13-µm nuclei (*N*) containing nucleoli. In Fig. 5a the nuclei are far apart, and in Fig. 5b they are close together. All the nuclei are surrounded by granular yolk (*y*). This oocyte, then, contains four nuclei.

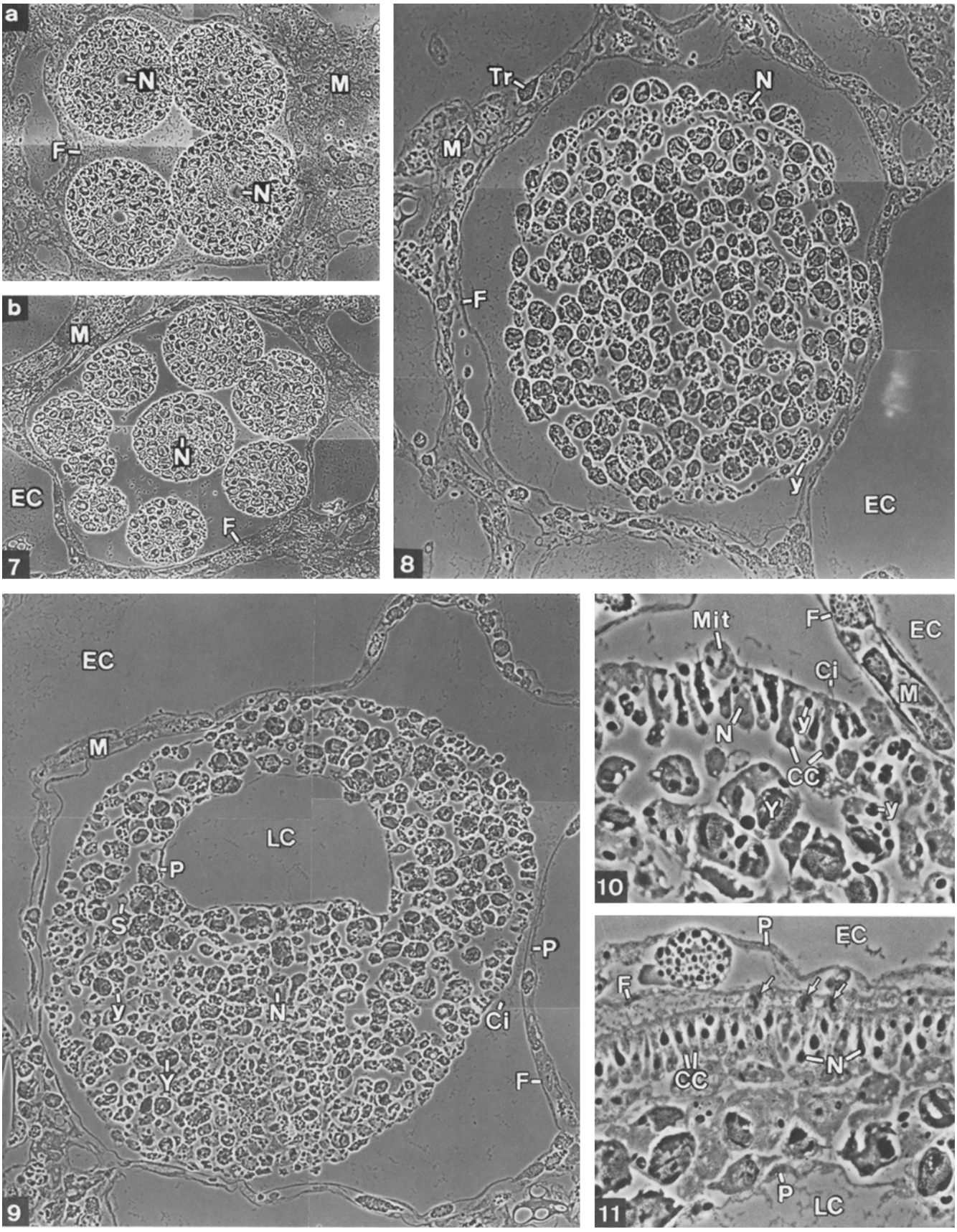
2. Cleavage

The spatial arrangement of the blastomeres in certain cleavage stages – the 2-, 4-, 8- and 15-cell stages – is illustrated in Fig. 6 by drawings based on reconstructions from serial sections. The two first blastomeres are closely apposed. In the 4-cell stage three cells lie in a plane with the fourth blastomere touching them. In the 8-cell stage four blastomeres are in one plane and the remainder in a parallel plane; one group of four is shifted with respect to the other. The 15-cell stage consists of four macromeres (I–IV) and smaller cleavage cells. No regular relationships in the blastomere positions can be discerned.

Figure 7 shows two sections of a 15-cell stage, through the 4 macromeres in (a) and through 9 of the 11 micromeres in (b).

3. Development of the larva

Stage I. The embryo shown in Fig. 8 consists of irregularly shaped cells about 18 µm in diameter, which contain many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yolk inclusions. The peripheral cells are slightly flattened and bulge outward. The diameter of the embryo is 220 μm; it comprises about 2,000 cells. The follicle epithelium (*F*) surrounds the embryo loosely. The layer of mesenchyme (*M*) outside it, 10–30 μm thick, contains trophocytes (*Tr*).

Stage II. A small eccentric cavity appears in the young larvae. It is bounded by flat pinacocytes. Some scleroblasts are beginning to form spicules.

Stage III. The surface of the young larva in Fig. 9 consists primarily of columnar cells. The cells in the interior of the larva are larger and contain more yolk than the more peripheral cells. The section passes through a scleroblast with a spicule (*S*). The larval cavity (*LC*) has expanded to a hemisphere. The mesenchyme (*M*) surrounding the young larva is reduced to a thin strip.

4. Development of the ciliated epithelium

Figure 10 shows part of the peripheral region of a young larva. The cells in the columnar epithelium are about 13 μm long. Some of them (*CC*) bear a cilium (*Ci*). The low-contrast nucleus (*N*), about 2 μm in diameter, is spherical to oval in shape. These epithelial cells have relatively few yolk inclusions (*y*). At the outer edge of the epithelium is a spherical cell in mitosis (*Mit*).

The ciliated cells (*CC*) of a somewhat older larva, shown in Fig. 11, form a tall, single-layered, multiseriate epithelium and are nearly yolk-free. The highly contrasting nuclei (*N*) are teardrop-shaped. Three round cells in mitosis project into the follicle space (←).

Stage IV. The larva in Fig. 12 is ready to emerge; its diameter is 320 μm. The large larval cavity (*LC*), lined with pinacocytes (*P*), takes up half the volume of the larva and appears sickle-shaped in section. The cells in the solid part of the larva contain more or less abundant yolk, and some of them have none. The tall, multiseriate ciliated epithelium (*C*) has an underlying layer of flattened cells. The larva is enclosed by a strip of mesenchyme only 2–5 μm thick.

Stage V. Having emerged from the follicle epithelium and the mesenchyme, the larva passes through the endopinacocyte wall into the excurrent canal system of the sponge (Fig. 13). The solid interior of the larva in this stage contains many flagellated chambers (*FC*) and scleroblasts with small spicules (*S*). The surface of the free larva is formed by a single-layered, multiseriate ciliated epithelium; in the region of the larval cavity the cells are much flattened.

The larval tissue (Fig. 14) also contains channels bounded by endopinacocytes (*P*). The presence of flagellated chambers (*FC*) in the typical arrangement, with apopyles (*A*), indicates that these channels are forerunners of the eventual water-conducting system (*EC*).

Comparison of Figs. 12 and 13 reveals a degree of reduction in the superficial epithelium of the larva after emergence. Some of the steps in this reduction are shown in the next series of pictures (Figs. 15–18).

When the larva is ready to emerge (Fig. 15), elongated columnar ciliated cells (*CC*) form a single-layered, multiseriate epithelium with highly contrasting, teardrop-shaped nuclei (*N*). Underlying the epithelium are mesenchymatic cells (*MC*), shown in Fig. 12 to be circularly arranged.

When the larva has freed itself from the mesenchyme and has reached the excurrent canal system of the mother sponge, the epithelial cells on the whole are less elongated (Figs. 16 and 17). As shown in Fig. 13, there is a gradient of progressive reduction from the solid part of the larva to the pole of the larval cavity. At the transitional stage shown in Fig. 16 the epithelial cells are shorter, and the underlying mesenchymatic cells (*MC*) contain many highly-contrasting vacuoles (*V*) about the size of the nucleus. The content of these vacuoles remains to be determined.

In Fig. 17 the epithelium is considerably altered in appearance. In addition to the original epithelial cells with teardrop-shaped to oval nuclei (cf. Fig. 15) there are now, at the same level, cells with large round nuclei like those of the mesenchymatic cells (*MC*) in the preceding figures. The cell on the left contains more or less contrasting vacuoles (*V*) about the size of the nucleus.

In Fig. 18, part of the peripheral zone of the solid region of a larva after emergence is shown. Here there is no longer any sign of a surface epithelium in the original sense (cf. Fig. 15).

The following finding is also significant in this respect. The material collected on 25 July 1983 contains not only all the stages of development in sexual reproduction, from young oocyte to larvae ready to emerge, but also gemmule primordia, in the same sponge. This observation is documented by Fig. 19, which shows the larva in the upper half of the picture and the incomplete gemmule (*G*) in the lower half. Because the development of gemmules in *Spongilla lacustris* is outside the scope of this paper, the reader is referred to the relevant literature (Rasmont 1955).

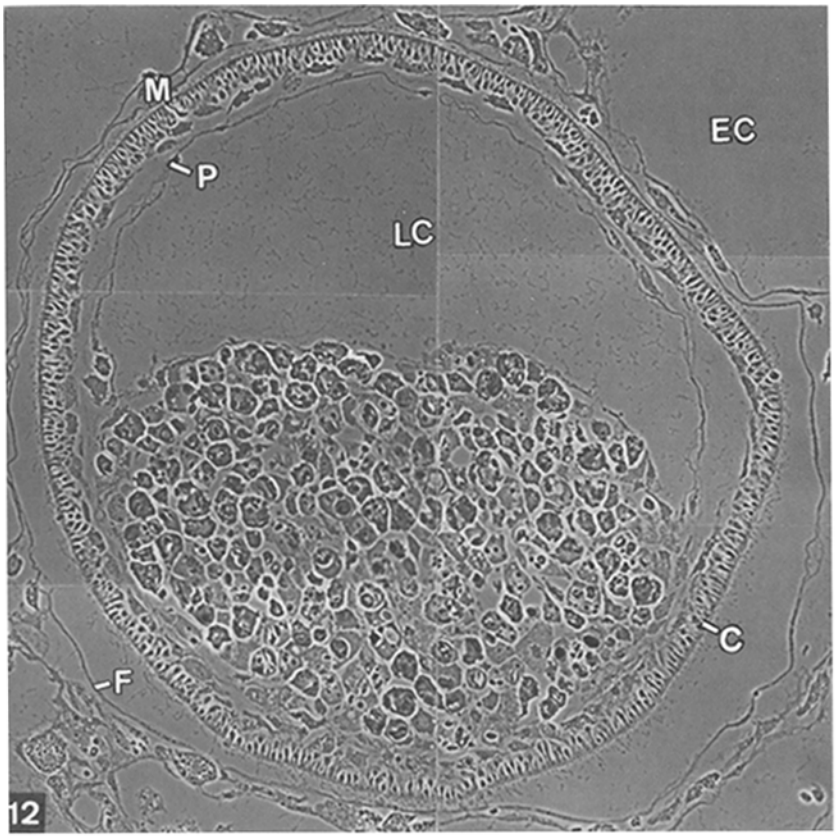
Fig. 7a, b. Two parallel sections through a 15-cell stage. **a** Four macromeres, **b** smaller blastomeres. *N* nucleus, *F* follicle epithelium, *M* mesenchyme, *EC* excurrent canal. ×210

Fig. 8. Embryo with yolk-filled cells. The outermost cells are somewhat flattened. *N* nucleus, *y* granular yolk, *M* mesenchyme, *Tr* trophocyte, *F* follicle epithelium, *EC* excurrent canal. ×320

Fig. 9. Young larva with hemispherical larval cavity (*LC*). *P* pinacocyte, *N* nucleus, *Y* spherical yolk, *y* granular yolk, *S* section of spicule, *Ci* cilia, *F* follicle epithelium, *EC* excurrent canal. ×320

Fig. 10. The marginal region of the young larva is formed by tall columnar cells. Some of them (*CC*) bear a cilium (*Ci*). One cell is in mitosis (*Mit*). *N* nucleus, *Y* spherical yolk, *y* granular yolk, *F* follicle epithelium, *M* mesenchyme, *EC* excurrent canal. ×920

Fig. 11. The single-layered, multiseriate ciliated epithelium of the somewhat older larva consists of elongated ciliated cells (*CC*) with teardrop-shaped nuclei (*N*). Three cells are in mitosis (←). *P* pinacocyte, *LC* larval cavity, *F* follicle epithelium, *EC* excurrent canal. ×920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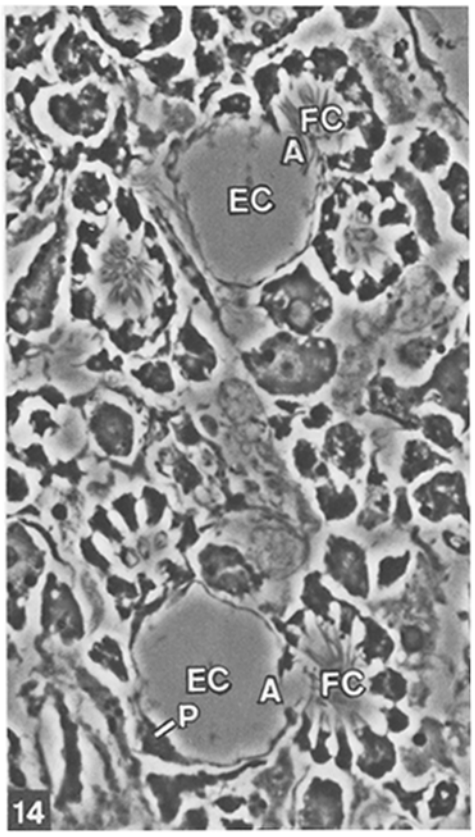
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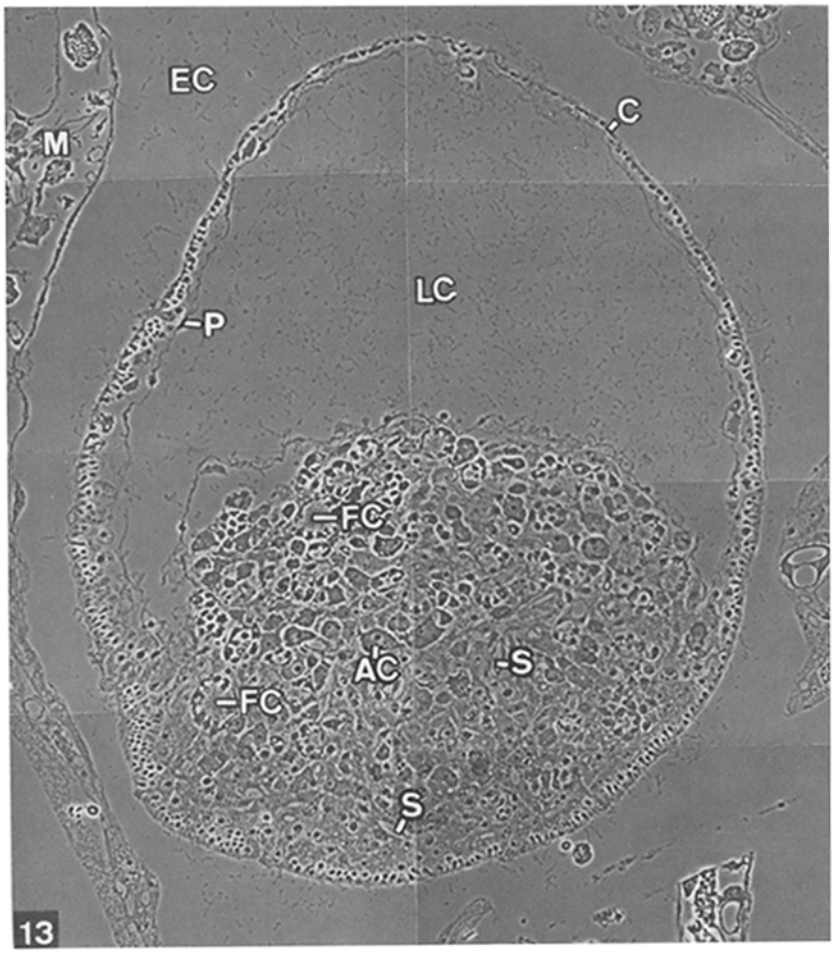
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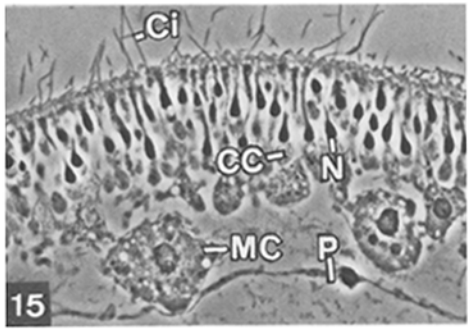
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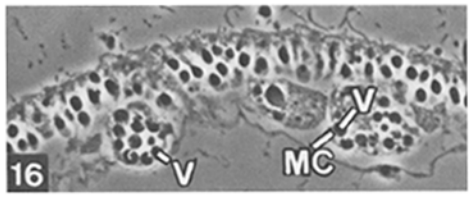
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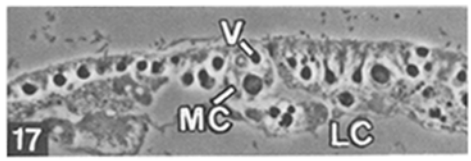
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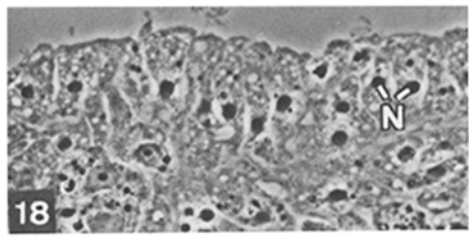
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D. Discussion

In the literature, the Spongillidae have been referred to as hermaphrodites (Leveaux 1941). According to Gilbert and Simpson (1976), *Spongilla lacustris* exhibits an alternating hermaphroditism, in which an individual is male or female one year and the other sex the next year. The sponges in this study were female in the summer of 1983, or else contained no sexual products.

According to Gilbert et al. (1975), at a given geographical latitude oogenesis is not dependent on season-specific factors.

The archeocytes of the mesenchyme are likely to be the originators of the oocytes, for they contain a nucleolated nucleus. Some archeocytes grow into young oocytes and are thereby differentiated from regular archeocytes. The subdivision of oocyte growth into a first and a second phase was adopted from the literature.

In the first growth phase the young oocyte at first retains its ameboid form, and in this respect resembles an archeocyte (Fig. 1). Then a squamous follicle epithelium develops from the surrounding mesenchymatic cells, and this epithelium remains intact until the larva leaves it.

The second growth phase is characterized by the phagocytosis of trophocytes (Figs. 2 and 3). Trophocytes are first seen in the vicinity of the oocytes; then they penetrate the follicle epithelium, are phagocytized by the growing oocyte, and finally are deposited as yolk material (Figs. 2 and 3).

Whether the peripheral position of the nucleus in second-phase oocytes is a criterion for the degree of egg maturation cannot be determined. So far no polar bodies have been observed, so that there is no indication of the time of meiosis. Meiosis and polar bodies of marine Porifera are thought to appear at the end of oocyte growth (Bergquist 1978).

The events associated with fertilization in the Spongillidae have not yet been described. In marine Porifera a carrier cell is thought to be responsible for transporting the sperm to the egg cell (Gallissian 1980).

The follicle cells have been referred to as collencytes (see, e.g., Brien and Meewis 1938). But because they are like the pinacocytes of the canal wall in every respect (Weissenfels 1980), we advocate this term.

The egg cell with 4 nuclei (Fig. 5a, b) is probably ready for the first two divisions, in which the 4 nuclei will be distributed among the 4 blastomeres. Because these 4 nuclei are equal in size, and because multinucleate blastomeres are occasionally also found in somewhat older cleavage stages, meiotic processes can be ruled out in this case. Multinucleate blastomeres have previously been observed in 2-cell and 8-cell stages. These cells are probably ahead of the other blastomeres in the cleavage process, which may lead to the occurrence of unusual numbers of blastomeres. In this respect the present data agree with the findings of Brien and Meewis (1938) in *Ephydatia fluviatilis*.

The thesocytes in the resting gemmule also have two nuclei (Brien and Meewis 1938; Höhr 1977; Langenbruch 1979), which in the germinating gemmule are distributed to two daughter cells by delayed cytokinesis (Höhr 1977).

Cleavage in the Porifera is considered to be radial as a rule. In *Spongilla lacustris* in the 8-cell stage, four blastomeres fit into the gaps between the remaining four (Fig. 6). Similar relationships, reminiscent of spiral cleavage, have been described for *Ephydatia fluviatilis* (Brien and Meewis 1938).

The subsequent cleavages produce first a morula and ultimately a solid embryo. Hence the larval cavity that appears later (Fig. 9) is not a blastocoel.

The cells in the peripheral layer are slightly flattened (Fig. 8); they divide to form the tall columnar cells of the ciliated epithelium. These cells are mitotically active (Figs. 10 and 11). It is unclear whether these cells leave the epithelium permanently in the direction of the follicle space. The ciliated epithelium of spongillid larvae is single-layered. Unlike the epithelium in marine Porifera, it is destroyed during larval metamorphosis by phagocytosis. This process begins while the free larva is still in the excurrent canal system of the mother sponge (*C* in Fig. 13), and there is every indication that the ciliated cells are gradually phagocytized by the underlying cells (Figs. 16 and 17). At least in the region that will become the attachment site, a new cell generation then forms the surface of the larva (Fig. 18). This process was also noted by Brien and Meewis (1938) in *Ephydatia fluviatilis*.

The first flagellated chambers appear when the larva has left its follicle. Their development in a young, growing sponge has been studied and described by Wierzejksi (1935), Brien and Meewis (1938), and Weissenfels (1981).

The spicules, which begin to form in the larva, are embedded into the skeleton of the young sponge. The joining of individual spicules by spongin, a scleroprotein of the collagen group, begins in the free larva. In the young sponge this primary skeleton is rapidly expanded by the incorporation of additional spicules (Weissenfels 1978).

Well-grown specimens of *Spongilla lacustris* in summer contain both larvae produced by sexual reproduction and asexually produced gemmules. There are parallels between the sexual and asexual reproductive processes in spongillids.

Fig. 12. Larva just before emergence, with large larval cavity (*LC*). *P* pinacocyte, *C* ciliated epithelium, *F* follicle epithelium, *M* mesenchyme, *EC* excurrent canal. × 320

Fig. 13. Larva in excurrent canal system (*EC*) of the sponge. *AC* archeocyte, *S* spicule, *FC* flagellated chamber, *LC* larval cavity, *P* pinacocyte, *C* ciliated epithelium, *M* mesenchyme. × 320

Fig. 14. Part of the solid cell complex of a free larva. Flagellated chambers (*FC*) are embedded in the walls of excurrent canals (*EC*). *A* apopyle, *P* pinacocyte. × 1,200

Figs. 15–18. Progressive reduction of the surface epithelium of free larvae. × 870. **15** Single-layered, multiseriate, tall columnar epithelium of ciliated cells (*CC*) with teardrop-shaped nuclei (*N*). *Ci* cilia, *P* pinacocyte, *MC* mesenchymatic cell. **16** The mesenchymatic cells (*MC*) below the ciliated epithelium contain highly contrasting vacuoles (*V*). **17** The epithelial tissue is greatly modified and now includes a few mesenchymatic cells (*MC*) with vacuoles (*V*). *LC* larval cavity. **18** The surface of this free larva consists of large columnar cells with round nuclei (*N*)

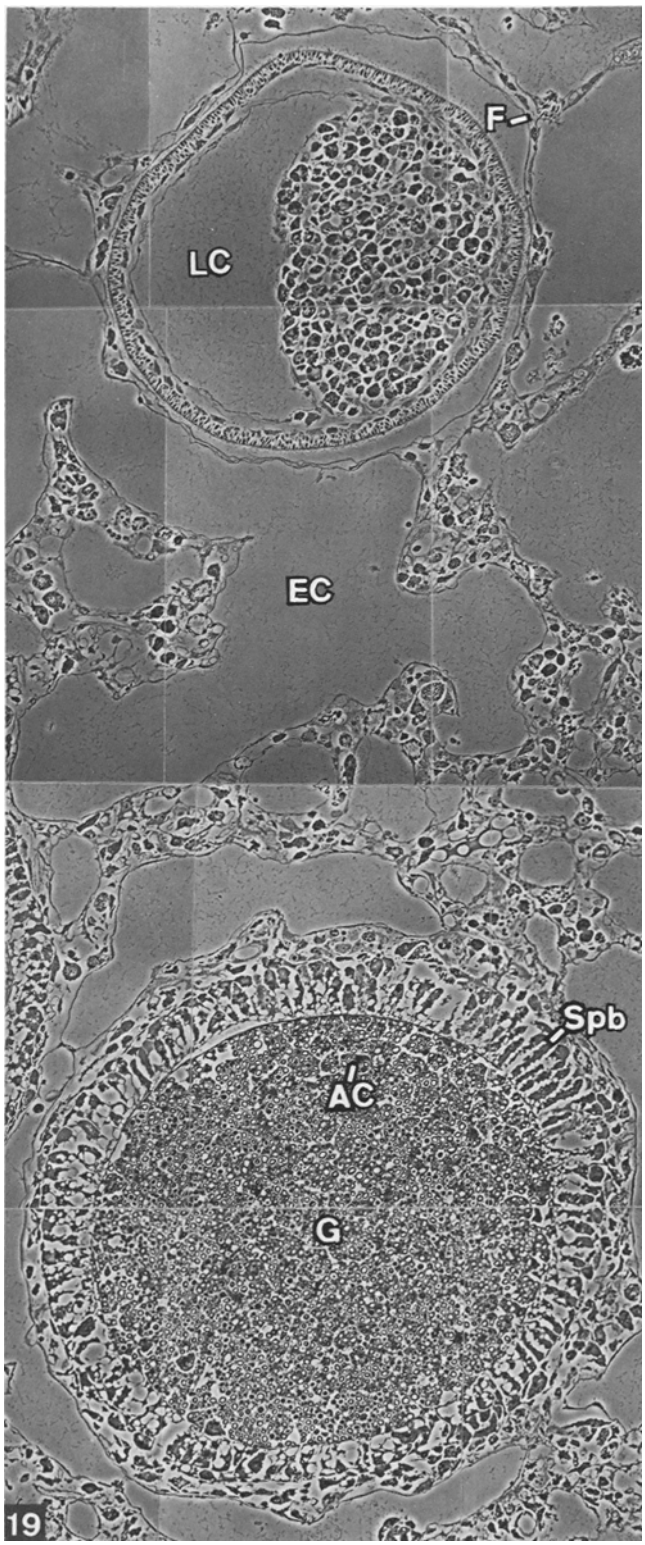

Fig. 19. Larva ready to emerge and young gemmule (*G*) in the same sponge. The latter consists of an aggregation of yolk-filled archeocytes (*AC*) which is surrounded by spongioblasts (*Spb*). *LC* larval cavity, *F* follicle epithelium, *EC* excurrent canal. ×180

Special nutritive cells (trophocytes) are involved in both. These cells appear only during the season of the year when reproduction occurs (Leveaux 1941). Whereas the lentiform yolk granules of the gemmule thesocytes are derived from trophocyte fragments (Langenbruch 1979), the growing egg cell phagocytizes the trophocytes in their entirety. The stages of yolk formation typical of gemmulation, as described by Langenbruch (1979) from the just phagocytized trophocyte particle to the yolk granule, proceed at a different rate from the corresponding events in oogenesis; in asexual reproduction yolk formation is rapid, and in oogenesis very slow.

According to Brien and Meewis (1938), the free larva attaches to a substrate by a region of its surface next to the larval cavity, and then metamorphoses. Before attachment, the larval ciliated epithelium in this part of the surface disappears (see above). The structural elements already present in the free larva develop further to produce a functional sponge.

Acknowledgement. This work was funded by the Deutsche Forschungsgemeinschaft. We thank Mrs. M. Geis, Mrs. U. Müller and Mrs. I. Nüssle for technical assistance.

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Received June 18, 1985