XXII.—NOTES ON FRESHWATER SPONGES.

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I.—THE BUDS OF *Spongilla Proliferens*, MIHI.

The buds that form so characteristic a feature of this species arise as thickenings of the strands of cells accompanying the primary spicule fibres of the skeleton, which project outwards from the surface of the sponge. These thickenings originate beneath the surface and contain, at the earliest stage at which I have as yet examined them, all the elements of the adult organism (*i.e.*, flesh spicules, ciliated chambers, efferent and afferent canals, parenchyma cells of various sorts) except skeleton fibres, gemmules, and a dermal membrane. A section at this period closely resembles one of an adult sponge, except that the structure is more compact, the parenchyma being relatively bulky and the canals of small diameter.

As the bud grows it makes its way up the fibre, pushing the dermal membrane, which expands with its growth, before it. The skeleton fibre does not, however, continue to grow in the bud, in which a number of finer fibres make their appearance, radiating from a point approximately at the centre of the mass. As the bud projects more and more from the surface of the sponge the dermal membrane contracts at its base, so as finally to separate it from its parent. No aperture is left when this occurs, the membrane closing up the gap completely. The newly liberated bud already possesses numerous minute pores, but as yet no osculum; its shape exhibits considerable variation, but the end that was farthest from the parent sponge before liberation is always more or less rounded, while the other end is flat. The size also varies considerably. Some of the buds float, others sink. Those that float do so either owing to their shape, which depends on the degree of development they have reached before liberation, or to the fact that a bubble of gas is produced in their interior. The latter phenomenon only occurs when the sun is shining on the sponge at the moment they are set free, and is due to the action of the chlorophyl of the green bodies so abundant in certain of the parenchyma cells of this species. If the liberation of the bud is delayed rather longer than usual, numbers of flesh spicules are produced towards the ends of the primary skeleton fibres and spread out in one plane so as to have a fanlike outline; in such buds the form is more flattened and the distal end less rounded than in others, and the superficial area is relatively great, so that they float more readily. Those buds that sink, usually fall in such a way that their proximal, flattened end comes in contact with the bottom or some suspended object, to which it adheres. Sometimes, however, owing to irregularity of outline in the distal end, the proximal end is uppermost. In this case it is the distal end that adheres. Whichever end is uppermost, it is in the uppermost end, or as it may now be called, the upper surface, that the osculum is formed. Water is drawn into the young sponge through the pores and, finding no outlet, accumulates under the dermal membrane, the subdermal space being at this stage even larger than it is in the adult sponge. Immediately after adhesion the young sponge flattens itself out. This process compresses the water in the subdermal space and apparently collects a large part of it at one point, which is usually situated near the centre of the upper surface. A transparent conical projection, formed of the dermal membrane, arises at this point, and at the tip of the cone a white spot appears. What is the exact cause of this spot I have not yet been able to ascertain, but it marks the point at which the imprisoned water breaks through the expanded membrane, thus forming the first osculum. Before the aperture is formed, it is already possible to distinguish, on the surface of the parenchyma, numerous channels radiating from the point at which the osculum will be formed to the periphery of the young sponge. These channels as a rule persist in the adult organism and result from the fact that the inhalent apertures are situated at the periphery, being absent from both the proximal and the distal ends of the bud. In the case of floating buds the course of development is the same, except that the osculum, as in the case of development from the gemmule in other species (see Zykoff, *Biol. Centralb.*, xii, p. 713, 1892), is usually formed before adhesion takes place.

The life of the individual is very short in *S. proliferens*, never lasting for more than a few weeks. So far as I have observed, sexual reproduction does not take place, but gemmules are produced in large numbers at the same time as the buds, often when the sponge is less than 100 sq. mm. in superficial area. A continuous succession of generations takes place throughout the year.

The above observations have been made chiefly on specimens in my aquarium in the Museum, but they have been corroborated, as far as possible, by a periodical inspection of others living in natural conditions in a pond.

Buds of a somewhat different nature are sometimes produced by *S. carteri* Bwk., and appear to be identical with the reproductive bodies described in *S. lacustris* by Laurent (*C. r.*, Sé. Ac. Sc. Paris, xi, p. 478, 1841) many years ago. With these I hope to deal on another occasion.

II.—GEMMULES OF *Trochospongilla phillottiana*, MIHI.

In my original account of this species (*Journ. Asiat. Soc. Bengal*, 1907, p. 22) I stated that the covering of the gemmule was thin. An examination of numerous specimens has shown me that this statement was incorrect as regards the majority of examples, in which the coating of granular substance is thick but unevenly distributed. Viewed from the external surface, the gemmules appear to be covered with little pits. These coincide with the position of the gemmule spicules and are in fact funnel-shaped passages leading from the external surface of the gemmule to the outer rotula of each spicule. So characteristic and so constant does this feature appear to be that I am inclined to think that in the type of the species the gemmules were not fully developed. In my description of these gemmules "cylindrical" is a *lapsus calami* for spherical.

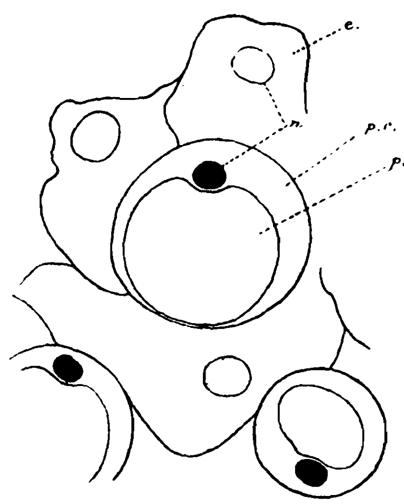
III.—EMBRYOS OF *Ephydatia blembingia*, EVANS.

Dr. Richard Evans in his original description of this species (*Quart. Journ. Micr. Sci.*, 1901, p. 71) notices certain minute basket-shaped bodies lying in cavities in the sponge, and is inclined to regard them as examples of a parasitic species, although their spicules only differ from those of the adult *E. blembingia* in their small size. Dr. F. Harmer, of Cambridge, has recently sent me one of Dr. Evans's specimens, and I have been able, thanks to its excellent state of preservation, to examine these bodies. They appear to me to be embryos in different stages of development. The smallest consist of rounded masses of cells, which in some cases can be seen to be of two sorts, a number of smaller ones and several larger ones. The compressed form of the larger examples is probably due, as Dr. Evans himself suggests, to shrinkage in preservation. In their later stages the bodies lie in well-defined cavities in the sponge, each body being surrounded by a delicate membrane secreted by a layer of flattened cells that apparently belong to the parent sponge. The body itself consists of an external layer of columnar cells and of an internal mass containing a large cavity. The cavity is situated towards the narrower end of the body and is enclosed at this end by a thin layer of cells that mostly have a starlike outline. The main bulk of the mass is below the cavity and consists of cells of several kinds, amongst which may be distinguished spiculiferous cells bearing spicules that are smaller, as yet, than those of the adult sponge. In short, an optical section of the body, apart from the membrane in which it is enclosed, closely resembles the actual section of an embryo of *Spongilla lacustris* figured by Evans in fig. 9, plate xxxvi, vol. xlii of the *Quarterly Journal of Microscopical Science*, 1899. In his account of *Ephydatia blembingia* he says that he was unable to make out the exact structure of these bodies, because none of his sections passed through them. If, however, a small piece of the sponge is teased up, stained with hæmatoxylin, cleared and mounted, it is not difficult to see the structures I have alluded to, although this method of observation does not permit of a minute examination of the cell anatomy. I have found closely similar embryos in *Spongilla carteri* both in summer and in spring, and also in an indeterminate sponge (probably an *Ephydatia*) taken by Mr. H. C. Robinson and myself at Biserat in the Siamese Malay States in autumn. I am indebted to Dr. Evans for the opportunity of re-examining, in a critical manner, the latter specimen, which I had handed over to him before I took up the study of the freshwater sponges myself.

IV.—THE NATURE OF THE PORES IN *Spongilla*.

The exact nature and origin of the external apertures of the inhalent canals in the Demospongia has been much disputed. Several authors claim to have established the fact that these apertures are intracellular and that the cells which contain them are porocytes homologous with those of the Calcarea. This view has been opposed by Minchin and others on theoretical grounds. An examination of fresh and well preserved specimens of the species of *Spongilla* occurring in Calcutta has convinced me that the structure of the pores is variable even within the limits of this genus. Two types can in fact be distinguished in the species examined, while from the descriptions of other species it seems probable that they also exemplify one or other of these types. Before describing the different forms of pores it will be as well to state the methods of investigation adopted. I find that in a tropical climate the best preservative for the dermal membrane is absolute or nearly absolute alcohol. The cutting of serial sections is not a satisfactory method of investigating this part of the sponge under any conditions, and in a climate such as that of Calcutta is almost impossible. If the dermal membrane does not adhere closely to the parenchyma, a piece of it may be detached with a pair of needles, floated off, stained—I find Ehrlich's acid hæmatoxylin an excellent stain—and mounted for examination. In many species, however, it is difficult to remove a large enough piece of the membrane in this way, and in such cases I find the best method is to shave the surface of the hardened sponge with a sharp razor. A portion of the parenchyma usually adheres to the membrane thus removed, but this does not very much matter, as sufficient clear spaces remain for the purposes of examination.

The first type of pore is found in those species (e.g., *S. carteri*) in which the subdermal space is small and the pores correspond in position more or less exactly with the distal extremities of the canals. Such species have comparatively large pores and as a rule there is no projecting collar round the osculum. The pores are simply gaps in the membrane, being surrounded by cells which do not differ from the other epithelial cells of the membrane except that they are often slightly attenuated in a horizontal plane.



Outline of a small portion of the external surface of the dermal membrane of *S. proliferens*, showing flattened epithelial cells (*e. c.*) and pores (*p.*), × about 1290. The membrane was taken from the edge of the sponge and stained with hæmatoxylin. The outline was drawn with a camera lucida and slightly enlarged. *n.* = nuclei ; *p c.* = pore cell.

In the majority of *Spongillæ* that occur in Calcutta, however, the pores can only be detected with the aid of a fairly high power of the microscope and open not directly into the termination of the afferent canals but into the subdermal space, their exact arrangement differing in different species. In such forms the subdermal space is often very large. Sponges which have this form of pore differ widely in other respects; those with which I am best acquainted are *S. crassissima* and *S. proliferens*. In such forms the pore is as a rule surrounded by a single cell. The actual hole is almost circular ; the cell that surrounds it has a minutely granular cytoplasm and a small nucleus that stains very deeply. For the greater part of its circumference the cell is attenuated to a mere filament; at the point at which the nucleus is situated it swells out considerably in both planes, while it is most attenuated at the opposite extremity. In all cases, so far as my observations go, the cell completely surrounds the pore, if only one cell is present, without a sign of secondary fusion at any point; but the relative proportions of the cell and the pore differ considerably and in some cases the latter is nearly in the centre of the former. Without further information it would be impossible to escape from the conclusion that the pore was intracellular ; but even were this the case, it would not be necessary to assume that the porocyte was homologous with that of the Calcarea. Indeed, there is one important difference, *viz.*, the pore-surrounding cell in *Spongilla* is not contractile, and cannot close the pore. In some cases, moreover—in *S. crassissima* at any rate—the pore is not surrounded by one such cell, but by two. In such cases each cell is bent into a semi-circle, having a crescentic outline, and the two adhere together round the pore by their extremities. The nucleus and cytoplasm of such cells do not differ from those of cells that apparently contain an intracellular pore. I am inclined to believe, therefore, that even in the latter instance the pore is not really intracellular, but is surrounded by an originally crescent-shaped cell, the two ends of which have fused together. I have not been able to detect any trace of true porocytes in connection with the ciliated chambers in any species examined

V.—THE SYSTEMATIC POSITION OF *Ephydatia meyeni*, CARTER, AND *E. indica*, MIHI.

As these are the only species of the genus that I have been able to find in Calcutta, my conclusions as to their specific and generic identity, after examining a large number of specimens, may be useful to others studying the group. *E. meyeni* was described in 1849 by Carter, who in 1881 stated that he believed it to be a variety of the widely distributed *E. fluviatilis*. Weltner (*Archiv. f. Naturg.*, lxvi (1), p. 124, 1895), however, assigned it to *E. mülleri* (Liebk.). During the present season I have found a form that agrees closely with Carter's descriptions, growing in the Museum tank in Calcutta. Its gemmule spicules have long shafts with scattered spines, but their rotulæ are very irregularly serrated; as a rule the spicules surround the gemmules in two rows. The skeleton spicules are smooth and sharp, and although the skeleton is rather fragile, it is hard, and spongin webs can be detected at its nodes. An important character was necessarily passed over by Weltner, who had only examined a dry specimen of this form, *viz.*, the presence of large numbers of vesicular cells in the parenchyma. These agree closely with Weltner's figure (*Archiv. f. Naturg.*, lix (1), pl. viii, fig. 14) of a cell of this kind from the parenchyma of *E. fluviatilis*, and as their presence is recognized to be diagnostic of *E. fluviatilis*, I believe that Carter was right in regarding *E. meyeni* as belonging to this species; it should therefore be called *E. fluviatilis* var. *meyeni*. Very possibly *E. robusta* (Potts) from North America is identical with this form.

E. indica, described by myself in 1907 (*Journ. Asiat. Soc. Bengal*, 1907, p. 20) is an interesting form as being to some extent intermediate between the genera *Ephydatia* and *Spongilla*. Even in the best developed specimens the rotulæ of the gemmule spicules are small and inconspicuous, consisting merely of a ring of spines but little differentiated from those that occur on the shafts. The spicules are arranged, however, in the upright position common in gemmules of *Ephydatia*, and the whole appearance not only of the gemmules but of the sponge itself resembles that of other species of this genus. Numerous specimens were obtained by Mr. R. Kirkpatrick and myself in a tank on the Calcutta maidan in May last. On examination these specimens proved to differ in several points from the types of the species. In the first place, the skeleton spicules were sharply pointed and distinctly inflated at the ends and sometimes in the middle, closely resembling those of a form found by Hanitsch (*Irish Naturalist*, 1895, p. 128, pl. iv) in Ireland and provisionally referred by him to *E. crateriformis*, Leidy. The gemmule spicules, moreover, were not or barely birotulate; the majority of them ending in a sharp, stout, vertical spine surrounded by a ring of transverse spines barely to be distinguished from those on the shaft. The spicules were, however, placed upright in the coat of the gemmule, and although many of the latter were still immature, some of them appeared to be fully formed. Large numbers of similar spicules occurred scattered in the parenchyma, and I also found a few free spicules of an irregularly massive outline. In July I obtained fresh specimens from the same tank and submitted them to a careful examination. In these examples the majority of the gemmules were fully formed, their spicules being distinctly birotulate and agreeing with those of the type of the species. The skeleton spicules were no longer pointed and expanded at the ends, although their outlines were still rather irregular, but closely resembled those of the type. Numerous free birotulates were found in the parenchyma. From this I conclude that the specimens found in May were immature, and that their peculiarities were due to their immaturity. *E. indica* is, as I suggested in my original description, closely allied to *E. crateriformis*, but the aperture of the gemmule is situated on a distinct prominence and is not markedly crateriform.