# The Oscula and Anatomy of Leucosolenia clathrus, O. S.

By

**E. A. Minchin, B.A.,**
Assistant to the Linacre Professor of Human and Comparative Anatomy, Oxford.

With Plate XXIX.

As is well known, Leucosolenia clathrus is a sponge in which the oscula have been hitherto supposed to be conspicuous by their absence. In Haeckel's "Kalkschwämme" will be found a full description of this sponge, together with an account of all observations upon it published previously to the publication of that monograph. The following is a summary of Haeckel's description; for further details I refer to the monograph itself.

Leucosolenia (Ascetta) clathrus was first described by Oscar Schmidt,[1] in 1863, as follows:—"Grantia ramosa, ramis 1 mmtro. latis, paulum compressis, varie et irregulariter implexis. Oscula in summitate ramusculorum brevium. Spicula triradiata, radiis obtusis. Color læte sulphureus." Later[2] Oscar Schmidt corrected his former description, and stated that though he formerly thought he had found oscula with a simple lens, he was unable to find them now with a "compositum." He further stated that he was unable to find a trace of a canal system in this sponge; the trabeculæ (Balken) composing it consisted of two very different layers, an outer colourless one containing the spicules, and an inner

[1] 'Spongien des adriatischen Meeres,' Suppl. I, p. 24
[2] Op. cit., Suppl. II, p. 8.

yellowish granular mass without spicules, filling up the whole space which in Leucosolenia botryoides constitutes the ramified cavity. Then Gray made this sponge the type of a new genus, Clathrina, which he characterised by simply translating the original Latin diagnosis of Schmidt given above.

Haeckel finds that Oscar Schmidt was in error in describing the sponge as solid, and as the result of his studies he divides his Ascetta clathrus into four varieties. (1) Ascetta labyrinthus, with a single layer of endoderm-cells, and no endogastric septa or partitions. (2) A. mæandrina, with the endoderm thickened to form a stratified epithelium of several cell layers, the uppermost bearing flagella. No endogastric septa or partitions. (3) A. clathrina, with the endoderm forming a stratified epithelium as in mæandrina, and the gastric cavity divided by partitions in which the embryos develop. (4) A. mirabilis, the colony consisting partly of A. labyrinthus and partly of A. clathrina. In A. labyrinthus alone did the spicules lie in a single layer; in the other three varieties they formed several layers. The plexus of the anastomosing tubes was much looser and with wider meshes in A. clathrina than in A. labyrinthus and mæandrina, and the tubes themselves averaged a larger diameter (1—2 mm., sometimes 3—5 mm.) in the two latter varieties than in the former (where they measured 0·5 to 1 mm., seldom more). In A. labyrinthus and mæandrina the tubes were much twisted and contorted, reminding one of the gyri of the mammalian brain. In all forms alike Haeckel found the colonies completely devoid of oscula, Auloplegma forms. He also at first thought he had seen oscula, but found he had mistaken artificial openings for such. Even the Olynthus forms were without openings, Clistolynthi. Since Haeckel's monograph I know of no work dealing with the question of the lipostomy and variations of this interesting and beautiful sponge. Metschnikoff[1] describes the histology

[1] "Spongiologische Studien II: Anatomisches über Ascetta," 'Zeitschr. f. wiss. Zool.,' xxxii, 1878-9, pp. 358—362.

with great accuracy, but says nothing of the oscula, though since he talks of "Tarrus forms" it might be inferred that he had seen them. Oscar Schmidt's work[1] on the development of this form contains no mention of oscula.

The curious phenomenon which is usually termed lipostomy has always been a great puzzle to me. How can a sponge exist without an osculum? The osculum is the central exhalent opening of the whole canal system, and to it converge all the currents which enter by the pores and flow along the canals. How can so important an opening be wanting? Haeckel attempts an answer to the question in the case of this sponge: "In the interior of the tubes the water goes in and out only by the pores."

Wishing to study the histology of this sponge, I was able, by the kindness of Sig. Lo Bianco, the well-known Conservator of the Naples Zoological Station, to go with the little steamer "Frank Balfour," of the Station, in order to collect and preserve Leucosolenia material fresh from the sea; and in the very first specimen of Leucosolenia clathrus which came on board I saw oscula of such a size that I was perfectly astounded. To be brief, I find that Leucosolenia clathrus in the fresh healthy condition not only has oscula, but in the full-sized specimens larger oscula than any other Leucosolenia known to me, whether from pictures or in the flesh.

The specimens of Leucosolenia clathrus may be for convenience divided into "large" and "small." By large specimens I mean the big, full-grown colonies, often 10 cm. in length and 3 or 4 cm. in height. By small specimens I mean the very young colonies of 5 mm. or less in extent. Fig. 1 represents two oscula of a large specimen seen in profile, natural size; one osculum (*a*) is widely open, the other (*b*) is partly contracted. Fig. 2 represents three more oscula from the same specimen (which had altogether ten oscula), seen

[1] "Das Larvenstadium von Ascetta primordialis und Ascetta clathrus," 'Arch. f. mikr. Anat.,' vol. xiv, 1877, pp. 249—263, Taf. xv, xvi.

from above. Fig. 4 represents the whole of a small colony with three oscula, magnified five times linear. It will be seen that the small colony forms a more or less flat plexus of narrow tubes, from which chimney-like oscular tubes arise of about 0·325 mm. in diameter. In fact, it grows in precisely the same manner as the specimens of Leucosolenia coriacea at Plymouth, in which I observed and described a sieve membrane over the oscular opening.

If now we study the edge of one of the larger oscula with a lens by transmitted light, we see that it has a margin of about half a millimeter in width where the wall is more transparent than in other parts of the oscular tube, and a very short distance above where this more transparent part begins an opaque line can be seen running round the whole osculum (fig. 2 *a*, *s*.).

This clearer margin indicates the line at which the collared endoderm stops short, so that the clearer margin is lined with ectoderm within and without, while the more opaque part of the oscula tube is lined by collar-cells on the inner side. The opaque line *s* is a muscular sphincter, by the contraction of which the osculum can be closed, and occurring alike in the larger and in the smaller oscula.

To proceed now to the study of sections and preparations. Fig. 6 *a*, *b*, *c*, *d*, represent four sections from a continuous series through one of the oscula of the sponge represented in fig. 4, after hardening in ½ per cent. osmic acid, soaking for an hour (on the slide) in picro-carmine, in order to counteract the blackening of the osmic acid, and finally staining with hæmatoxylin. *a*, *b*, and *c* are three sections about the middle of the series, *b* being the next section after *a*, and *c* the next but one after *b*; while *d*, in which the osculum is cut tangentially, is the thirteenth section after *a*. In all the sections the spicules have been carried by the edge of the razor towards the left, injuring the ectoderm a little.

Fig. 6 *a* shows well the general structure of the oscular wall. The collar-cells stop short suddenly at a point, and it can be seen plainly in figs. 6 *a* and *c* that they are continued directly by the flattened ectoderm-cells. In some sections (6 *b*) it looks as if there was an intermediate form of cell; but I am convinced that this is only due to ordinary collared cells being cut obliquely, so that only their bases are seen. A short distance above where the collar-cells stop, the sphincter is seen projecting like a ledge into the interior. Above it the ectoderm goes on for a considerable distance. The height of this oscular margin, formed only of two layers of ectoderm with some spicules and amœboid cells between, is really remarkable.

Fig. 7 *a* represents the sphincter of one of the larger oscula in section (the two sides of the oscula are of course not drawn at their natural distance apart, for then they would have to be separated by more than the length of the whole plate). 7 *b* is another section, rather thick, from the same series, showing the sphincter, which was a little crumpled, cut tangentially and obliquely. This osculum had been hardened with a saturated solution of corrosive sublimate in absolute alcohol, and the sections stained on the slide with borax carmine first, and then with hæmatoxylin, a method which I find exceedingly good for showing the ectoderm. Fig. 8 represents a transverse section of the sphincter of another osculum, prepared by the osmic-picro carmine-hæmatoxylin method.

The sphincter, as can be seen, projects as a ring-like ridge into the interior of the osculnm. We shall consider its minute structure presently. When this sphincter contracts it closes the osculum, which then, in the large colonies, has a very characteristic shape, which I do not know how to describe better than by comparing it to the human breast (fig. 3). In such a breast-shaped osculum the nipple is formed by the ectodermal margin of the osculum, and at the base of the nipple one finds the contracted sphincter. Fig. 9, *a*, *b*, and *c*, represent sections from a series through the osculum represented in fig. 3, hardened in abs. subl.,[1] and stained with borax carmine and hæmatoxylin.

[1] I use this as a convenient abbreviation for a saturated solution of corrosive sublimate in absolute alcohol.

To return now to this sphincter. It consists of two layers of fusiform ectoderm-cells arranged tangentially, between which one finds at intervals some of the large amœboid mesoderm-cells which occur throughout the sponge, but they are by no means common in the sphincter. The point I wish to emphasize is that the contractile muscular cells are the epithelial ectoderm-cells.

The simple two-layered nature of the sphincter is apparent from the transverse sections 6 *a*, *b*, *c*, 7 *a*, 8; but still more so from the tangential sections 6 *d*, 9 *a*, and *c*; less so in the thick section 7 *b*. Perhaps an even more convincing method of seeing it is to put a piece of the wall of a fresh living osculum into Ranvier's one third alcohol for twenty-four hours, and then to carefully pull the sphincter off with a needle and examine it laid out flat in glycerine, after previous staining with picro-carmine. In such a preparation one sees, by carefully focussing its surface, a layer of nuclei. If now the microscope be focussed deeper the layer of nuclei first seen vanishes, and a distinct layer of similar nuclei takes their place. There is absolutely no other cell layer but these two, unless one happens to find one of the scattered amœboid cells, which are by no means common.

These flat preparations offer the best means of studying the nature of the cells, and I find them differ on the opposite surfaces of the sphincter. On one surface they are spindle-shaped, elongated, and with distinct cell outlines. The spherical nucleus is surrounded by granules which form a fusiform figure, extending towards the two ends of the cell. Cells of this kind are shown in fig. 12. On the other surface the cells have similar nuclei, but no distinct cell outlines; the granules are sometimes arranged in a fusiform figure, sometimes not, but are much fewer relatively. Cells of this kind are shown in fig. 11. By focussing the preparation deeper from which fig. 12 was drawn, I could see cells similar to those represented in fig. 11; and similarly by focussing the preparation drawn in fig. 11, I could see cells like those in fig. 12. I have tried to represent this point more clearly in fig.

13, *a* and *b*. In the middle of each drawing is seen one of the amœboid wandering cells, one and the same cell both in 13 *a* and 13 *b*. Now 13 *a* is drawn with the microscope at the lower focus, and shows the fusiform cells; while 13 *b* is drawn at the upper focus, and shows the other kind of cells. 12 *a* represents one of the second kind of cells macerated out from the same preparation from which fig. 12 was drawn. I take it that the fusiform cells are more specially differentiated ectoderm-cells, while the other kind are more ordinary ectoderm-cells. In transverse sections of the sphincter the cells on one surface commonly appear more rounded and project higher than on the other (figs. 7 *a*, 8). I believe that the rounded projecting cells are the fusiform cells of the flat preparations, and the flat cells the others. It is difficult to be certain of this point; I infer it from the fact that in such sections the rounded cells are closer together than the flat ones. The two kinds of cell appear to occur indifferently on one or the other side of the sphincter. From the left side of fig. 7 *a*, it would appear as if both sides of the sphincter might, in places, be formed only of fusiform cells.

The nuclei of the cells composing the sphincter have a similar structure in both kinds of cells. They are spherical or slightly ovate, measuring in glycerine preparations about 6·5 $\mu$, in Canada balsam preparations (prepared in all points in the same manner as the glycerine ones) about 5·2 $\mu$. The nucleus rarely has one distinct nucleolus; more usually several small ones. In preparations hardened with Hermann's fluid, and stained by Flemming's method[1] with saffranin, gentian violet, and orange G., the structure of the nucleus is well shown, especially if the sections are cut very thin (4 or 5 $\mu$). Then the whole nucleus is seen to be filled with a fine network, which may be thickened at several nodal points, sometimes greatly at one, producing the appearance of a nucleolus. Without entering at present into further histo-

[1] Vide his paper in the 'Arch. f. mikr. Anat.,' vol. xxxvii (1891), "Ueber Theilung und Kernformen bei Leukocyten, und über deren Attractionssphären," p. 296.

logical details, I will state merely that these nuclei exactly resemble in size, structure, and appearance the nuclei of the remaining ectoderm, and differ in precisely these three points from the nuclei of the endoderm, still more so from the nuclei of the amœboid mesoderm-cells. The granules of the cells appear when carefully focussed as round black spots, but when the microscope is a little high or too low they appear as minute black rings round a central clear spot, and in sections often look not unlike fibrillæ cut transversely, which of course is not the case.

Thus, to recapitulate, this sphincter is composed of two layers of ectoderm, with a few scattered amœboid cells between, and the contractile cells are the ectodermal epithelium. Thus, in one of the simplest existing types of sponges, I have arrived at the same result as Topsent,[1] who in his work on the Clionidæ, finds that the ectodermal or endodermal "cellules de revêtement" are the contractile elements. The sphincter of the oscula of Leucosolenia clathrus is an especially favorable object in which to study this question, as the cells are so large compared with the minute cells of siliceous sponges, and the sphincter itself can be so easily prepared out or cut into sections. We have in this sphincter perhaps the most primitive type of muscle-cell in the animal kingdom; it can hardly be called a myo-epithelial cell, it is still a simple ordinary epithelial cell.

Various authors[2] have described muscle-cells lying in the mesoderm; and until Topsent wrote, I think I am right in saying that muscular cells in sponges were regarded as mesodermal. There is no reason why, in a highly differentiated sponge, muscle-cells originally forming part of an epithelium should not become more specialised and sink into the meso-

[1] "Contributions à l'étude des Clionides," 'Arch. de Zool. expér. et gén.,' tome v bis, Suppl. (1877—1890), p. 24, et seq.

[2] Vide Sollas's article "Sponges," 'British Encyclopædia;' "Monograph of the Tetractinellida," '"Challenger" Reports,' p. 42; von Lendenfeld, "Beitrag zur Kenntniss des Nerven- und Muskel-systems der Hornschwämme," 'S. B. k. pr. Akad. Wiss.,' Berlin, Nov. 12th, 1885.

derm. But the muscle-cells described by Topsent and in this paper make it, I think, to say the least, extremely probable that all muscular cells in sponges are of epithelial origin.

Dr. von Lendenfeld has published,[1] at divers times and in divers places, a classification of the Cœlenterata into Mesodermalia (sponges), in which the principal organs are derived from the mesoderm; and Epithelaria (other Cœlenterates), in which the principal organs are derived from the epithelia.

What are the principal organs of a sponge? I presume the ciliated chambers, skeleton, genital products, and the various kinds of muscle-cells, gland-cells, nerve-cells, &c. The skeleton certainly appears to be mesodermal, as far as we can judge, and perhaps also the genital cells. On the other hand, the ciliated chambers are almost certainly endodermal, and the muscle-cells of epithelial origin. There does not appear to be the slightest reason why the nerve-cells, so often described by von Lendenfeld, should (if they exist) be of mesodermal and not of ectodermal origin, as in other groups of animals; and the same may be said of their gland-cells. Thus it appears that the only principal organs of a sponge which can with any certainty be said to be of mesodermal origin are the connective-tissue system and the generative elements.

To return, however, to our oscula. We have in this sphincter a mechanism for closing the osculum, and in the sieve membrane over the oscula of Leucosolenia coriacea we have, I do not doubt, a structure which can be employed for a similar purpose, since Auloplegma forms of the latter sponge are so common. I look upon this as a good instance of two structures physiologically similar, but morphologically quite different. In my paper on the sieve membrane[2] I explained it as probably arising as a breaking through of the gastral cavity to the exterior in several places during the formation of the osculum, and hence as consisting of ecto-

[1] 'Monograph of the Horny Sponges' (London, 1889), p. 889; 'Proc. Zool. Soc.,' London, 1866, p. 566; 'Biol. Centralbl.,' ix, 1889, pp. 113—127, &c.

[2] "Note on a Sieve-like Membrane across the Oscula of a Species of Leucosolenia, &c.," 'Quart. Journ. Micr. Sci.' (n. s.), Part 2, January, 1892.

derm externally and endoderm internally. I see as yet no reason why I should depart from that opinion. On the other hand, it can hardly be doubted that the sphincter here described arises as a simple ingrowth of ectoderm, and consists of this layer only on both faces. In the young forms the sphincter shows only one or two cells on either face in transverse section (fig. 7, *a*, *b*, *c*), while in the older ones it consists of a great number lying side by side (figs. 7 *a*, 8, 11, 12, 13), so that it evidently grows with the osculum. I have not yet found an osculum devoid of a sphincter, but it is very probable that the young Olynthus would have none.[1]

In specimens of this sponge fresh from the sea the oscula were, as I have said, exceedingly conspicuous.[2] How is it these oscula have not been found before? I selected on my first collecting trip several large specimens of the sponge with widely open oscula, and put them into a separate vessel in sea water. What was my astonishment, however, when I got back to the Zoological Station, to find no trace of oscula in any of my specimens, not even an elevation to mark where they had been! The thin delicate walls of the sponge had completely collapsed, and the whole presented a shrivelled appearance, as different from the beautiful outlines and transparent yellow colour of the fresh living sponge as anything could be imagined. On a second occasion I selected another very fine specimen, and put it in a separate vessel, and brought it back with great care, changing the water several times on the way home. It was, however, of no avail; it arrived in the same shrivelled condition. The only indication that these sponges

[1] Since Haeckel observed only Clistolynthus forms it is possible that even the Olynthus has a sphincter. On the other hand, it would be quite possible for an Olynthus to contract itself completely without any special sphincter. Vide Metschnikoff's figure of a Clistolynthus of Ascetta blanca in longitudinal section, 'Zeitschr. f. wiss. Zool.,' xxxii, 1878–9, Taf. xxii, fig. 9.

[2] I cannot but express my astonishment that Haeckel did not see them, since he tells us in his monograph (p. 33) that he found this sponge growing in great quantities in a little bay (San Clemente) on the south side of the Spalmadori Cliffs on the coast of Lesina in 1871, and collected in a short time several hundred small and large colonies.

had ever had oscula was that the anastomosing tubes converged towards the points where the oscula had been.

These specimens, after being a few days in the aquarium, recovered slowly from their drooping condition, like a plant that has been transplanted. The tubes became rounded and of a healthy appearance, and sent out diverticula, which grew often to 10 or 12 mm. in length, and attached themselves to the side of the vessel. Such diverticula occur in the natural condition also (see fig. 2). From the places where oscula had been breast-shaped eminences raised themselves, which were normal closed oscula like fig. 3. Sometimes a small opening would appear in the "nipple," but only once did I observe in my aquarium that one of my specimens opened out a large normal osculum. Specimens with closed oscula like fig. 3 are of frequent occurrence in nature, and I have often observed them in specimens fished up fresh on the steamer. I went on three separate occasions to the only locality where this sponge occurs abundantly in the Gulf of Naples—a very sheltered grotto near Capo Miseno,—and the following short journal of observations may be of interest:

Oct. 2nd.—A fine bright day, the water smooth and clear. All the specimens had wide open oscula.

Oct. 8th.—The weather as before. All the specimens had open oscula, and on this occasion I preserved fresh from the sea the colony from which figs. 1 and 2 are taken. In one large specimen I observed a closed osculum.

Oct. 19th.—The sea was smooth, but the day was cloudy, and the water in the grotto was turbid, so that when I dived it was difficult to see the sponges clearly under water. There had been scirocco and bad weather previously. Every specimen examined had closed oscula.

Since this date the weather has been so bad and the sea so rough that the steamer has been unable to put out, and so my observations are extremely incomplete; but they give one at least the suspicion that the state of the sea and weather influence the sponge, and cause it to contract or open: and, indeed, one can hardly wonder that it should be so. Leucosolenia clathrus, in the widely expanded condition, is one of the most delicate organisms known to me, the least touch being sufficient to break or tear it. If it is even lifted out of the water the tubes and oscula collapse. In the whole Gulf of Naples it is only known to occur in profusion in one grotto. This is a kind of natural tunnel running through a rock peninsula, and putting a small bay in communication with the sea; but the tunnel runs through obliquely, and meets the shore-line at an angle which is acute towards the open sea: hence the waves can never break into it with much force, and it is exceedingly sheltered. But even here it might well be imagined that the sea would be too rough for this delicate animal. When the sponge is contracted, however, it is very much firmer and stronger, and can be handled with more safety. I noticed that the sponges brought home on Oct. 19th with closed oscula did not droop in the same manner as those brought home on Oct. 2nd and Oct. 8th, but remained healthy and firm. A specimen in a similar contracted condition would be much more able to withstand the force of the sea than one expanded.

It is not, however, only the oscula that can contract, but the tubes can also contract very greatly. In fig. 5 is represented, magnified four diameters, a small piece cut off a specimen which had been growing in my aquarium for a month, and which is still quite healthy. It has fairly attached itself on all sides, and is continuing to send out processes, many of which can be seen in the figure. This sponge, when it first came under my notice, was a specimen like that in figs. 1 and 2. After it recovered from the transplantation it several times completely expanded, and it was in this specimen that I saw the only completely open osculum I have ever seen in the aquarium. The manner in which the sponge had alternate periods of expansion and contraction was noteworthy. It would frequently be widely expanded one day and contracted the next. I could find no cause for these expansions and contractions; only about the last week in October the weather became very much colder, and a chilly tramontana blew for some days. The sponge contracted completely when the cold weather began, and has not expanded since; but it is still perfectly healthy, as shown both by the histology of sections from portions of it, and by its continuing to grow and send out processes.

If we compare fig. 5 with figs. 1 and 2, it is obvious that the tubes have shrunk to about one eighth of their former diameter. Imagine now a Leucosolenia tube, with its walls composed of ectoderm externally, jelly containing a single layer of spicules and a few cells, and most internally a continuous, closely packed lining of collared cells. If this tube contracts greatly what must be the result? There can be no longer room for the collar cells to form a single layer, and the spicules will also be closely packed, probably into several layers.

Figs. 15 *a* and *c* represent two sections from a series through some partly contracted tubes. The spicules now form at least two layers in the much-thickened mesoderm, and the collar cells are arranged in a stratified epithelium, of which the uppermost only bear flagella. In some places the endoderm is thrown into folds (fig. 15 *c*). In other words, we have before us Haeckel's variety Ascetta mœandrina.

Mr. Bidder, in his recent review of Dendy's 'Monograph of the Victorian Sponges,'[1] has written (p. 628), "In these Australian sponges (Calcarea Homocœla) there appears to occur none with a many-layered endoderm. This structure, observed by Haeckel, and since universally discredited, certainly appears in Ascetta clathrus." I must say that a many-layered endoderm as a normal feature of sponge anatomy is to me as inconceivable as that a sponge should be permanently without an osculum. In every preparation I have made of this sponge in the expanded condition I find a single-layered endoderm. On the other hand, if the sponge be sufficiently contracted, a many-layered endoderm does and must occur. One usually finds it in preparations made from

[1] 'Quart. Journ. Micr. Sci.,' vol. xxxii, part 4, October, 1891, pp. 625—632.

sponges living in the aquarium, and also in freshly preserved sponges which are contracted.

Fig. 14 represents a section taken at random from a series through the piece represented in fig. 5. Here the contraction has reached almost its limit. The spicules form in places as many as five layers (in the section figured the razor has displaced them a little, in a direction passing from the north to the south of the drawing), and the endodermic layer is now so thickened that the lumen of the tubes is reduced to series of narrow lacunæ. In some places the tube is even solid, as Oscar Schmidt described originally. It is evident from Oscar Schmidt's figure of the sponge that he had to do with a very contracted specimen. In almost every respect the sponge agrees with Haeckel's Ascetta clathrina, both in external form and in anatomy. It is true that the compartments (Fächer) are not separated from one another by "exoderm" (i. e. mesoderm), covered on both sides with endoderm; but if a specimen with folded endoderm, as in fig. 15 *b*, were to completely contract, that might be the case. It is true also that the compartments do not contain embryos, but that, I suppose, would depend on the time of year at which the sponge was observed.

Thus, to recapitulate: Haeckel's Ascetta labyrinthus is the ordinary expanded condition of this sponge, but with closed oscula, like the piece shown in fig. 3. His Ascetta mœandrina is the same a little contracted, as in fig. 15. Ascetta clathrina is the sponge in an extreme state of contraction, as in figs. 5 and 14. Finally, Ascetta mirabilis is this sponge partly expanded, partly contracted.

In the walls of the tubes also there are no elements to which the contraction could be due except the ectoderm-cells; and to the great power of contractility I attribute the fact that the ectoderm[1] in this sponge is, as Metschnikoff observed, so

[1] Mr. Bidder has recently described (loc. cit., p. 628) the ectoderm of this sponge as consisting of the mushroom-shaped cells described by Metschnikoff in the Olynthus (Clistolynthus!) form of Ascetta blanca. I do not wish at present to enter into histological details, which I hope to do in another

exceedingly distinct. I find the very greatest difference in this respect between Leucosolenia clathrus and L. coriacea occurring at Plymouth.

To sum up the results obtained:

Leucosolenia clathrus is not permanently lipostomous, but has very large and distinct oscula.

These oscula are provided with a sphincter by which they can be completely closed for a time, apparently as a protection against unfavorable external conditions.

Haeckel's four varieties of the sponge are only different states of contraction, and are no more zoological varieties than a polyp with contracted tentacles is a variety of a polyp with expanded tentacles.

The many-layered endoderm is also only a temporary condition, the mechanical result of the contraction of the whole sponge.

The contractile elements in all cases are the flattened ectodermal epithelium.

In conclusion, it is my pleasant duty to express my thanks to the staff of the Naples Zoological Station, and especially to my kind friend Sig. Cav. Lo Bianco, without whose help this work could never have been done.

NAPLES; November 10th, 1891.

## ADDENDUM.

WHILE the above was in the press, a work by von Lendenfeld has appeared, entitled "Die Spongien der Adria.—I. Die Kalkschwämme" ('Zeitschr. f. wiss. Zool.,' Bd. liii, Heft 2, pp. 185—321, Taf. viii—xv; and Heft. 3, pp. 361—433), containing a detailed account of Ascetta clathrus (pp.

paper, but as my figures might be thought to be erroneous I will only say that in freshly preserved material of the sponge the "Metschnikoff's cells" only occur sparingly, the predominant form of the ectoderm being flattened epithelium; and I have almost conclusive evidence to show that the "flask-shaped cells" are only the contracted condition of the flat cells.

210—217, Taf. viii, fig. 4; ix, figs. 27—37). The author divides the sponge into four forms, which he terms A, B, C, and D, rejecting Haeckel's varieties, "since these forms appear to arise one from the other in the course of the post-embryonal development." Form A consists of a mass of anastomosing tubes, 1—5 mm. in diameter, the walls of which have pores and contain numerous stellate connective-tissue cells, but no large granular elements; the endoderm forms a single layer. In Form B the tubes are only 0·3—1·5 mm. in diameter, and form a flat spread-out creeping network. Pores are rare, and the "zwischenschicht" (mesoglœa) contains, besides stellate cells, large granular, spherical or irregular cells; the endoderm is many-layered. Form C is similar externally to Form B, but has no pores in its walls; the same large granular cells occur as in B, and the endoderm is many-layered but more closely packed than in the latter form. Form D consists of a flat network of trabeculæ, 1·5—3 mm. thick, in which no pores were to be found; in the mesoderm large granular cells were not observed, and the endoderm fills up the interior of the tubes, leaving only irregular lacunes. All the forms agree in having no oscula visible to the naked eye, and are reticulate Auloplegmas. The author believes that the many-layered endoderm and the closing of the pores is connected with the ripening of the eggs (see p. 217). Dr. von Lendenfeld has made a considerable advance in rejecting Haeckel's varieties, but is nevertheless far from a correct explanation of the different forms, which are nothing more than different states of contraction of the sponge. Thus his fig. 30 (Taf. ix), representing a section of Form D, is in all essentials completely similar to my fig. 14, which is taken from a series of sections through the piece of sponge shown in fig. 5. This sponge, as above described, when first collected was like the specimens shown in figs. 1 and 2, and after completely contracting, frequently expanded again to this form. I have recently observed a similar contraction in another sponge, an Ascon of a beautiful orange-red colour, but with the spiculation of Ascetta primordialis, which when brought in by the fishermen was widely expanded, with large open oscula. In a few hours it contracted completely, the tubes shrinking to perhaps one tenth of their former diameter, and having no visible oscula. The following morning, being placed in a current of pure sea water, it again expanded to its former dimensions and opened its oscula; but the current being stopped, it slowly contracted again. In the evening I again placed it in the circulation, and the next morning it was expanded a third time, though not in all parts, the tubes furthest removed from the oscula being to a certain degree contracted. Some of its oscula opened completely, others were closed and breast-shaped, but at least visible; whereas, in its completely contracted state, it was impossible to see that the sponge had ever had oscula. On the strength of these so oft-repeated observations, I cannot but state my disbelief that any Ascon (or any sponge) is permanently lipostomous; and I have no doubt that where von Lendenfeld has described Auloplegma forms, e. g. in Ascetta spinosa (op. cit., p. 203), he has simply overlooked the oscula, as he has certainly done in Ascetta clathrus. The large granular cells in Forms B and C admit of a very simple explanation; they are simply closed pores, which the author has overlooked in Form D, where they are equally common. Other points of histology I hope to criticise in another place. I will only draw attention to the statement (p. 190), that in sponges the "skeleton forming, sexual, and muscular cells are formed in the mesoderm, and are not of epithelial origin" (compare also the account of the "zwischenschicht," pp. 398—405). After what I have already written, this statement requires no further comment.

NAPLES; 1st March, 1892.

# DESCRIPTION OF PLATE XXIX,

## Illustrating Mr. E. A. Minchin's paper on "The Oscula and Anatomy of Leucosolenia clathrus, O. S."

### PLATE XXIX.

All the sections of the sphincter have been drawn so that the inner (gastral) face of the sphincter looks towards the south, the outer face towards the north side of the plate.

The following letters are for all the figures.

*ect.* Ectoderm. *end.* Endoderm. *a. m.* Amœboid mesoderm-cell. *mes.* Jelly (mesoglœa) containing spicules. *s.* The muscular sphincter of the osculum.

FIG. 1.—Two oscula from a large colony, preserved fresh from the sea in 70 per cent. alcohol, in profile view. One of them (*a*) is widely open, the other (*b*) half closed. Natural size.

FIG. 2.—Three more oscula from the same colony viewed from above. Natural size.

FIG. 2 *a*.—The osculum of Fig. 2 viewed as a transparent object, magnified about three diameters. It is very slightly contracted.

FIG. 3.—A closed osculum. Natural size.

FIG. 4.—A small colony with three oscula, magnified five diameters.

FIG. 5.—A piece of a colony in a very retracted condition, the Clathrina clathrus of Oscar Schmidt, the Ascetta clathrina of Haeckel.

FIGS. 6 *a, b, c, d*.—Four sections from a series through one of the oscula of the colony represented in Fig. 4: 6 *a*, a section near the middle of the series; 6 *b*, the next section after 6 *a*; 6 *c*, the next section but one after 6 *b*; 6 *d*, the thirteenth section after 6 *a*. Magnified 350 times. Osmic half per cent., picro-carmine, hæmatoxylin.

FIGS. 7 *a* and *b*.—Two sections from a series through a large expanded osculum like those in Figs. 1 and 2: 7 *a*, a median section; 7 *b*, a thick tangential section. × 330. Abs. subl., borax carmine, hæmatoxylin.

FIG. 8.—A median (transverse) section of the sphincter of another large open osculum. Osmic half per cent., picro-carmine, hæmatoxylin. × 330.

FIGS. 9 *a, b, c*.—Sections from a series through the closed osculum in Fig. 3: 9 *a* and *c*, tangential section; 9 *c*, a detached muscle-cell. Abs. subl., borax carmine, hæmatoxylin. × 330. (In one place the section 9 *a* is slightly broken.)

FIG. 10.—Nuclei from a series of sections through the sphincter of an expanded large osculum. Hermann's fluid, safranin, gentian violet, orange G. Zeiss, compens. ocular 8, apochr. F.

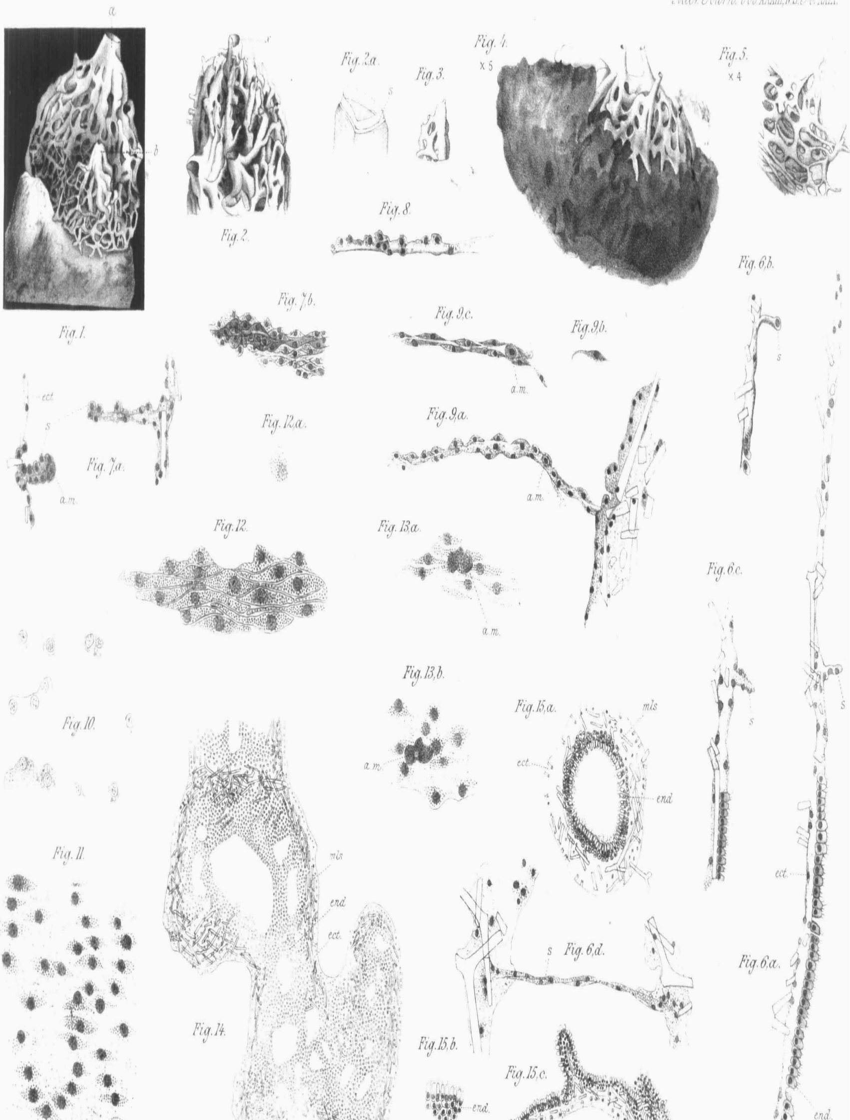
FIG. 11.—Surface view of a relaxed sphincter of the osculum of a large colony. One third alcohol, picro-carmine, glycerine preparation. × 500.

FIG. 12.—Surface view of another similar preparation. × 500. 12 *a*, an isolated cell from this preparation. The upper (north) limit of Fig. 12 represents the natural free edge of the sphincter.

FIGS. 13 *a* and *b*.—Two views of another preparation similar to that from which Figs. 11 and 12 are drawn: 13 *a*, drawn with the microscope at the lower focus; 13 *b*, with the upper focus. × 430.

FIG. 14.—Section from a series through the piece of sponge represented in Fig. 5. × 70. Abs. subl., borax carmine, hæmatoxylin.

FIGS. 15 *a*, *b*, *c*.—Sections from a series through a contracted sponge. 15 *b* is a portion of 15 *a* more highly magnified to show the many-layered endoderm. Abs. subl., borax carmine, hæmatoxylin. 15 *a* and *c* × 120.




E. A. Minchin del.

F. Huth, Lith^r Edin^r