NOTES AND MEMORANDA.

Discovery of Nuclei in Foraminifera.—In a previous communication[1] on *Quinquiloculina fusca* (Brady), forming a part of his valuable series of "Rhizopodstudien," Professor Eilhard Schulze already shadowed out his extremely interesting and unexpected discovery of a nucleus in Foraminifera, and in a recent memoir he confirms and gives the enlarged details of his remarkable discovery.[2] It is highly interesting that, simultaneously and independently, the same discovery has likewise been made by Professor Hertwig,[3] as to which see below.

Leaving aside the baseless views of the older authors, such as D'Orbigny and Ehrenberg, who ascribed to the sarcode body-mass of the Foraminifera a high organisation, it is well known that, from Dujardin (the first to correctly appreciate the real simplicity of their structure) onwards, through a long series of years, even to Haeckel in his 'Gastræa-Theorie,' this group of Rhizopoda has been regarded as destitute of nucleus—the body-mass apart from the shells, in fact, but a simple single cytode or an aggregate of cytodes. Hence the interest and value of the surprising announcement made by these independent observers.

That this important element in the organization should have so long eluded observation is, perhaps, not at all surprising when one considers the many difficulties in the way of its perception presented by the shell (perfect or broken), the numerous granules of varied nature, oily and pigment, food particles, &c., not to speak of the density and opacity of the sarcode-mass itself.

At the same time it is remarkable that the naturalist on board H.M.S. Challenger, who specially studied the pelagic Foraminifera, should have failed to detect it when devoting so much time and attention to these organisms.

It was only by the observation of very small species with a delicate pellucid shell, and by application of acids to the

[1] Schultze's 'Archiv für Mikr. Anatomie,' Bd. xi, p. 136.
[2] 'Archiv f. Mikr. Anat.,' Bd. xiii, p. 9, t. 2, 3.
[3] 'Jenaisch Zeitschrift,' Bd. x, p. 41.

larger, in order to deprive them of the calcareous coating, and by the use of staining media, that Professor Schulze succeeded in demonstrating the existence of the nucleus.

In *Entosolenia globosa* (Williamson) the author found a combination of several conditions which rendered it a favourable object for study in this regard. Its ovate or elongate calcareous shell, deeply invaginated at one end, is hyaline, delicate, and smooth, and perforated by extremely fine pores; the body-mass usually quite or almost quite fills the cavity of the shell, though it can become reduced to a ball lying at the "posterior" end of the shell, such seemingly due to diminished nutriment. In the finely granular sarcode body-mass there occur embedded numerous strongly refractive, probably oleaginous, partly colourless, partly brownish roundish corpuscles, amongst which, towards the posterior end, the author could see even in the living example a distinctly marked, clear, globular body. That this was a corpuscle and not a vacuole or a granuleless spot in the sarcode was concluded from the constancy of its position and occurrence, as well as from the sharpness of its contour. Upon application during observation of dilute acetic or pyroligneous acid, the pores in the shell became at first more evident, then the shell became dissolved almost completely, and the granules embedded in the sarcode became so nearly deprived of all colour, that this now finely granular body stood out distinctly, as against its pale environment, and with a sharply marked contour, as an indubitable *cell-nucleus*.

But in the many-chambered calcareous Foraminifera the demonstration of the nucleus was accompanied by greater difficulty.

For several reasons the author chose *Polystomella striato-punctata* as the starting-point of the research: first, the readiness with which he could obtain it in different conditions; secondly, its regular structure was in favour of its study; thirdly, its shell was of comparatively small mass, and less troublesome to eliminate by means of acids; and lastly, he wished to study this form owing to its having been so largely the subject of research by preceding observers.

The arrangement and figure of the successive "chambers," and the finger-like conjoining processes are best seen in the sarcode-mass after removal of the calcareous shell. Athough Max Schultze first experimented in this way he did not appear to have detected the mutual bridge-like connection of the body-segments belonging to each chamber, but, on

the other hand, considered them as isolated. Carpenter (1862) was the first to discover these. After some details as to the number and arrangement of these sarcode processes which the author found to be of a more hyaline and less granular character than the rest of the sarcode-body, as well as more prone to imbibe staining fluids, he proceeds to describe his experiments in order to test the presence of a nucleus.

He first directed his attention to the median primary chamber, distinguished from the rest by its globular figure, but in it he never found by any method a nucleus or any body at all like it; sometimes, indeed, a few clear round spots could be seen, but pressure constantly proved these were merely drops of probably oleaginous nature, and without any wall; sometimes they could not be seen. Almost giving up the research in despair, he was much surprised one day, on looking at a specimen whence the shell had been removed, to notice a spot in one of the segments about midway of a different aspect. He then stained the whole object in a decoction of logwood, and in order to render the preparation clear, then applied a dilute ammoniacal solution. To his great delight he now saw at the previously mentioned spot, lying in the now clear, blue, granular sarcode, a violet-coloured globular body (0·056 mm. in diameter) with a distinct, thick, outer membrane and clear contents, in which could be recognised several strongly refractive elliptic or roundish structures of different sizes (whether to be regarded as *nucleoli*, or as produced by the treatment, the author leaves undetermined)—in a word, *a nucleus of almost typical structure*. Treating a great number of Polystomella forms in the same way he always arrived at the same result though with some modification.

Ordinarily in each Polystomella but *one* such nucleus was to be found, and this, indeed, always in the middle of one of the middle segments; in rare cases he found *two* nuclei occurring in adjoining segments; only once did he see the two nuclei separated by a segment without any nucleus; and once he saw *three* nuclei in one animal—two in immediate sequence, and separated from the third by a segment. The nucleus in older examples occupies some one (or two) of the middle third of the whole number of chambers; but in very young forms (4-10 chambers) he found the nucleus far behind, say in the second chamber. Sometimes, indeed, the nucleus was not confined to a single chamber, but one part of it in one, the other in the adjoining chamber; in such cases the segments remained hanging together by a

narrow isthmus, occupying one of the mutual communications. One portion of such a nucleus might be sometimes very small as compared to the other, so much so as to be reduced to a mere tip. From this circumstance the author regards a passage of the nucleus from one chamber to another by means of the connecting canals as probable, and the disturbance of the normally globular figure to be due to the constriction caused by the narrowness of the passage.

The author found afterwards a similar nucleus in a Rotalina.

Whilst admitting that the foregoing observations do not as yet prove *all* Foraminifera to be nucleated, the author still would suggest the likelihood that they really are so. From the fact that the whole of the soft body of a many-chambered Polystomella or Rotalina normally has but a single nucleus, it follows that the whole animal has but the value of a *single cell*, and the old question as to whether the Polythalamia are to be regarded as single animals or colonies of animals is disposed of, as the Foraminifera at large (admitting possible exceptions) must be regarded as *unicellular animals*.

Subsequent to his study of the freshwater Monothalmia, Hertwig turned his attention to the marine Mono- and Polythalamia, with the view of investigating their probable relations to the former. Both groups naturally appeared to him to possess, on the whole, great affinity, whilst certain of the characters relied upon by most naturalists in recent times, as calling for their separation, seemed to him not to possess so broad a systematic importance, nor to be so comprehensive as was usually assumed, such as the characters of the pseudopodia, or the distinction between non-contractile vacuoles and contractile vesicles. He had, therefore, come to the conclusion that the only character left to systematically separate the freshwater Monothalamia from the Foraminifera was the absence of a nucleus in the latter and the presence of this important structure in the former. For if, as had been generally assumed, the Foraminifera represented only an undifferentiated, non-nucleated, test-bearing protoplasmic mass, then, in a histological point of view, they could only be regarded as *cytodes*, whilst the freshwater forms would possess the morphological value of one or more *cells;* the Foraminifera would, therefore, come more close to Haeckel's Monera.

Max Schultze was the only observer who had afforded us a knowledge of the structure of the soft body, the researches

of others being mainly confined to the shells. Although M. Schultze was of opinion that, on the whole, the Foraminifera were without nucleus, still in *Rotalia veneta* he described a clear body, which was rendered more distinct on the application of acetic acid, and which he was inclined to regard as a nucleus, though unable to study it more exactly. He observed something similar, too, in an example of *Textilaria picta*, in which he succeeded "in isolating from the two last chambers a nucleus-like structure." Also he had described nuclei in *Gromia oviformis*.

Under the circumstances of the doubt in which the question was involved Hertwig availed himself of the opportunity of a visit to Heligoland to submit living Foraminifera to a renewed study, and he also obtained from Professor Haeckel examples from the Mediterranean sea.

But he soon found out that no definite result was to be gained by the examination of only fresh specimens, so he applied himself to the use of reagents. He found that acetic acid, employed by Max Schultze, was of no service: it only coagulated the substance, and rendered the object too dark. Better results were obtained by the application of dilute solution of chromic acid; in it the protoplasm swelled and became partly extruded from the shell, remaining tolerably transparent; but then a coagulation set in, darkening the preparation, accompanied, indeed, by a bleaching-out of the brown pigment; but as regards the coagulation, the chromic acid was no improvement on the acetic acid. In most cases, however, it sufficed to render the nuclei evident; in others, again, he had to take refuge in staining; for this purpose he used Beale's carmine fluid. By its application he obtained favorable results in preparations which had lain for a day in chromic acid, which had then been washed out by frequent renewal of the water for several hours.

Young examples of Miliolæ offered the most conclusive results. The youngest were single chambered in the fresh state; in spite of the pellucid character of the shell, he could not discover a nucleus with any certainty; he could only distinguish a homogeneous, nucleus-like spot. This, however, coagulated on application of chromic acid into a sharply-contoured, minute circle (0·01 mm. in diameter), disclosing within a sharply-contoured little body (0·004 mm. in diameter). Still more distinctly did this structure come out with "Beale's fluid," the inner body assuming a ruby red. Whether this structure represents a nucleus differentiated into nucleolus and nuclear membrane, or (in the fresh state) a homogeneous nucleus, merely assuming, owing to

irregular coagulation of its substance, such an appearance, the author leaves undecided; but he thinks the latter improbable, and leans to the view that it coincides in structure with the nucleus of the freshwater Rhizopoda.

In the three-chambered Miliolæ, in one case, he saw only *one* nucleus; in two others two were present; in a four-chambered example he found seven, three appertaining to the first chamber, one to the second, and three to the third. They were all alike, except that one was smaller than the rest.

In large examples he was mostly unsuccessful in meeting with nuclei, probably due to the density with which the parenchyma became filled with foreign bodies.

The second Foraminifer in which the author succeeded in demonstrating the nucleus belonged to the Perforata—a little Rotalia, agreeing with M. Schultze's figure of *R. veneta*, the chambers arranged in a flat spiral, the shell-opening of considerable size, the protoplasm of brownish-granular and opaque (except in the last chambers), the pseudopodia richly granular, not very long, only slightly branched, and still more rarely inosculating. But rarely in the living organism could the author recognise the indication of the nucleus in the form of a clear spot of brownish granular protoplasm; he succeeded best in young single-chambered examples; in these he found a single round nucleus, and under chromic acid he could see the nucleolus. By the aid of carmine solution he found that, with the growth, an increase in number of the nuclei took place; thus, in a four-chambered Rotalia at one time four, at another three, nuclei; in a three-chambered, three nuclei; whilst in other cases, in many-chambered individuals, but one nucleus was recognisable. Still, it does not at all follow that more might not have been present.

On two occasions the author found in his collecting bottle little bodies, which he took, with the unassisted eye, for young Rotaliæ; under the microscope these proved to be clusters of thirty to forty young three-chambered individuals. In one case the connection of these was due to a common protoplasmic envelope, from which tufts of pseudopodia radiated in such abundance that they were perceptible to the unassisted eye. They thus resembled a colony of the freshwater *Microgromia socialis*. The shell was comparatively thick, and had a rough aspect, some ten to fifteen minute foramina disposed irregularly on the surface. The protoplasm filled the two first chambers completely, the third only partially, the pigment granules more copious in the first. The author could see nuclei herein only by the help of reagents. In most cases chromic acid sufficed, but the carmine fluid

answered still better. A nucleolus did not appear to exist, but rather the nucleus seemed to form a homogeneous mass; but its unequal coagulation might present a nucleolus-like aspect. There was only one nucleus, and that in the primary chamber.

We have manifestly here the starting-point of the reproductive process observed by Max Schultze and others. It consists essentially in this, that within the mother-shell, in a manner not yet more closely observed, daughter individuals are developed, surrounded by a proper shell. In Miliola and Nonionina the daughter individuals are one-chambered, in Rotalia—from the commencement—three-chambered. It is of interest to note that in both cases the young individuals are single-nucleated—are *unicellular*—regarding the nucleus as the centre of individuality of the cell.

From his experience, so remarkably confirmed by that of Professor Eilh. Schulze, then, the author feels justified in drawing the conclusion that all the Foraminifera—Imperforata and Perforata—are nucleated, thus removing the only ground of systematic separation of the Foraminifera from the Monothalamia, and both groups must be united in a single Class. The freshwater Monothalamia would, of course, most approach the single-chambered Gromia, Cornuspiræ, &c.

For such a Class the author urges the names "Polythalamia" and "Foraminifera," being applicable to only a part of the series, not to the whole, are manifestly each respectively unsuitable.

More appropriate would be Haeckel's term "Acytaria;" Haeckel, however, so designates the Foraminifera only, to the exclusion of the freshwater Monothalamia, which latter he includes under his Lepamœbæ. As regards its signification, the term might certainly be extended to embrace the Monothalamia; but Haeckel employed the name to distinguish Radiolaria without a central capsule from those furnished therewith. Now, there are whole series of sarcode organisms agreeing in this respect with Foraminifera which, so far as that is concerned, might be relegated to the Acytaria. The designation is, therefore, defective. For this reason the author proposes to call the class "Thalamophora," in allusion to their all-pervading characteristic.

As regards the subdivision of the Thalamophora, the author points out that two courses are open: either we might make the arrangement of the chambers the principle of division, or the finer structure of the chambers; in the one case we should subdivide them into Mono and Polythalamia, after Max Schultze; or, in the other case, into Imperforata or

Perforata, after Carpenter. The latter mode appears not only more generally recognised, but, from a scientific point of view, more to be commended. In Carpenter's system the freshwater Monothaliama would self-evidently have to be relegated to the Imperforata beside Gromida. The number of nuclei having evidently no relation to the number of chambers, it is, without doubt, that the latter can be regarded as but an external jointing of the shell, without any definite relation to the sarcode body.

In conclusion, the author recapitulates the characteristics of the

Thalamophora.

They are organisms whose soft body consists of undifferentiated sarcode, sending out changeable processes of the most different form—Pseudopodia—for the purpose of locomotion and capture of food. Cell-nucleus, single in young organisms, but may extraordinarily increase in the course of growth.

Fluid aggregations are almost constantly present in the interior of the body, either in the form of simple vacuoles or of contractile "vesicles."

All Thalamophora possess a skeleton, which is either purely chitinous or impregnated with lime, or covered with silicious particles. Its characteristic is the monaxial fundamental form; that is, a principal axis of the shell can be constantly recognised. . . . . This principal axis is in the simpler cases straight (Gromia, Nodosaria), in most cases spirally curved (Miliola, Rotalia, Polystomella, &c.); frequently this curvature takes place very irregularly, and gives rise to the appearance of a seemingly clustered arrangement of the sections of the shell, as if without rule (Acervulina).

By means of constrictions running vertically to the axis the shell may be subdivided into sections, one behind another (chambers), which may stand in mutual connection after very different modes (Polythalamatous forms).

According to the structure of the shell the author would divide the class into the two orders:

1. *Imperforata.*

No communications between the cavity of the shell and the outer world other than the constantly considerable shell-opening.

2. *Perforata.*

Numerous fine foramina perforate the shell-wall; hence the shell-opening proper is mostly minute and rudimentary.