On the Occurrence of Pseudopodia in the Diatomaceous Genera, Melosira and Cyclotella.

By

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With Plate XLI.

THE diatoms on which these pseudopodia have been found do not belong, as might have been expected, to the motile, but to the non-motile forms, to the genera Melosira and Cyclotella. The Cyclotella is certainly C. Kützingiana. The Melosiras belong to one or two small species which have not yet been satisfactorily determined.

They were first met with in April, in the large pond in the gardens of the Royal Botanical Society of London, in the Regent's Park. This gathering consisted almost entirely of small isolated frustules of Melosira, with a few Cyclotellas and some Archerina Boltoni.[1] Later on filaments of frustules of Melosira became commoner, while Archerina increased enormously in number. Finally Cyclotellas replaced the Melosiras, and the Archerinas vanished.

I next met with pseudopodia on Cyclotellas at Stanstead, in Hertfordshire, whither I was directed by a friend.

Later I went to stay at Heytesbury, in Wiltshire, where I found the river Wiley and the brooks full of a Melosira in small isolated frustules, with long, delicate pseudopodia. Recently I have found a good set of Cyclotellas with pseudopodia in Kew Gardens, and others at Eastbourne. I infer that these

[1] See Professor Lankester's description of this organism, 'Quart. Journ. Micro. Sci.,' vol. xxiv, 1884.

small isolated Melosiras and Cyclotella Kützingiana have these pseudopodia normally. At Stanstead, Heytesbury, Kew, and Eastbourne there was no trace of Archerina. The reason for mentioning this is that it was suggested that the external protoplasm of Archerina migrated on to the diatoms.

The pseudopodia of the first gathering and of the Stanstead ones were easily seen for a part of their length with a $\frac{1}{6}$ object-glass, magnifying nearly 400 diameters; but in the case of the Heytesbury Melosiras and most of the Cyclotellas from the Botanical Gardens and Kew they are generally invisible, even when specially looked for. I think this helps to explain why they have not been found before.

To study the whole length of the pseudopodia I found it a good plan to dry the material on a cover-glass, which could then either be mounted dry or stained or roasted. All the figures on Pl. XLI are from specimens thus treated. Quite lately I have stained and mounted some of the Kew gathering without drying. The results I hope to give in a future paper. So far they have simply confirmed the main conclusions at which I had already arrived.

The principal points to notice in the structure of the pseudopodia are these: they are fairly stiff, and are non-retractile to ordinary observation.

The length varies in Cyclotella from two and a half to six times the width of the valves. In the Heytesbury Melosiras this reaches fully nine times the width. They are very permanent; a slide prepared in April by simply sealing the diatoms in the water in which they were found showed the pseudopodia apparently unchanged after five months. The dried slides show that the great majority of the pseudopodia are arranged fairly symmetrically round the margin of the valves. This is best seen in side view (figs. 6 and 7).

In slides of the Kew gathering prepared in the wet way a great many Cyclotellas show a series of small tooth-like projections of protoplasm round the margins of the two valves, arranged as regularly as the teeth of a circular saw. These projections are the thicker bases of the delicate pseudopodia. On a typical specimen I counted about forty-six of these projections, while a roasted cover of the same gathering gave about forty-six as the number of radiating ribs on the valve of the Cyclotella. Hence there would appear to be a close connection between the number of the pseudopodia and the structure of the diatom—a point of very great importance. Sometimes pseudopodia spring from the surface of the cingulum as well as from the valves.

The pseudopodia are generally fairly straight; occasionally they branch at some distance from the valve. This was especially the case in the earliest gathering (figs. 1 and 2), where they branched repeatedly.

On dried cover-glasses it is common to find two or three pseudopodia springing from a short thickened base. In water unstained these bases are extremely hard to see.

The number of the pseudopodia is, on the whole, strikingly regular. On dried covers seventeen to twenty is the ordinary number seen round a valve in side view (fig. 6); sometimes twice that number (fig. 7).

The pseudopodia vary a good deal in thickness in different gatherings. On dried unstained cover-glasses they often show short portions of their length more opaque and solid-looking than the rest, sometimes getting a beaded look. In this they agree with the pseudopodia of Archerina (figs. 5, 6).

Rarely one comes across quite thick pseudopodia. Fig. 5 represents a striking form, with a few very stout ones. It is noteworthy here that these four spring from the coarser markings on the valve. Occasionally one of the pseudopodia is thickened in the centre, as in fig. 6. Lastly, the pseudopodia of two diatoms seem to be able to fuse into each other, and increase greatly in width, so as to form a broad band connecting the two diatoms. Whole chains are thus formed, though two frustules only are more common (figs. 2 and 8).

Next as to the use of these pseudopodia, and the question why other diatoms do not have them. The chief point to be remembered is that these little Melosiras and Cyclotellas occur mainly as isolated frustules, and are without the power of locomotion. Under these circumstances the pseudopodia serve three purposes: 1. Protection. 2. Means of attachment. 3. Floats.

1. Protection.—The pseudopodia act in the ordinary way as defensive spines. I have often seen large predatory Infusoria knocking these about, and absolutely unable to touch them. The ordinary isolated diatoms can creep into mud or débris out of harm's way. To these the stiff pseudopodia would be quite useless.

2. Means of Attachment.—At Heytesbury I found the diatoms in running water, especially amongst filamentous weeds. Here the use of the pseudopodia is quite obvious.

3. Floats.—In the Botanical Gardens they are all over the still waters, and no doubt the large extra surface given by these pseudopodia helps to keep them floating. The remarkable pelagic diatoms Chætoceros also have long processes, but of a different kind: that is to say much coarser, and obviously forming part of the siliceous skeleton.

The next question is the substance of which these pseudopodia are made. I think the facts point conclusively to that substance being protoplasm. They are destroyed by nitric acid, while those of Chætoceros are not. All the finer parts are at once destroyed by roasting at the lowest red heat. The thick connecting bands and the thickened bases of the pseudopodia will stand a low red heat occasionally, while they, too, are entirely destroyed by a strong heat.

They stain readily with Kleinenberg's hæmatoxylin. With Schultze's solution they give no cellulose reaction, nor with iodine and sulphuric acid. Boracic carmine, which does not stain cellulose, stains the bases of the pseudopodia strongly, and just the same colour as the cell-membrane—the fine part slightly. It is quite probable that some kind of cuticle is secreted by the protoplasm in contact with the water, and that this gives to the bases and connecting bands their resisting power at a low red heat. The bases and cell-membrane always behave in nearly the same way to stains. Two other proteid stains, said not to stain cellulose, were tried. Picro-nigrosine stained the diatom bodies well, the bases fairly, and also the finer parts. Alcoholic safranin just stained the pseudopodia; but all alcoholic stains are apt to fail with them.

The evidence of the stains, therefore, joins with the other tests in pointing to the presence of protoplasm with or without a very fine cuticle of uncertain nature. The thick pseudopodia, with their variation in density (fig. 5), also point to a very fine cuticle with protoplasmic contents. There is no evidence in any of my slides of a layer of protoplasm normally present outside the diatom shell. The pseudopodia or their bases spring straight from the shell. Most probably there is a fine layer of protoplasm outside the shell, for Imhoff has shown that the internal protoplasm reaches the surface, but it is not thick enough to be visible in optical section.

In the Kew gathering I have met with two or three specimens snrrounded by a thick layer of what looks like a gelatinous substance, not granular. The pseudopodia, however, are independent of this, and are clearly traced through it up to the margin of the shell of the diatom.

I have also seen granular or fluffy substance round Cyclotellas, which might be protoplasm; but this is not at all common. I have seen it occasionally round other forms which have no pseudopodia.

Next we come to what has been said or suggested as to the meaning of the pseudopodia.

The first suggestion was that these were not diatoms at all, but the identification of a well-known species, Cyclotella Kützingiana, with pseudopodia has settled that point. Next it was suggested they might be extensions of the gelatinous layer which Professor H. L. Smith has shown to surround many diatoms.

But there is no evidence here of any kind of normal gelatinous envelope, and the stiffness of the pseudopodia and the permanence of the bases tell against this theory, which is further negatived by the fact already stated, that the pseudopodia penetrate the gelatinous layer when this is present.

A third hypothesis, based on the remarkable slides where the diatoms and Archerina are mixed in countless profusion, is that the pseudopodia are a vegetable growth covering everything in the field. But here the Heytesbury set are conclusive: on a single slide you may have some hundreds of diatoms of various kinds; about 200 may be the small Melosira, nearly every one of which will have pseudopodia, while nothing else in the field has any. Besides, botanists will, I think, agree that there is nothing plant-like in these forms. Another suggestion was that they are like the filaments of Polysiphonia. But these latter never branch and are not granular, and could not form connecting bands.

Another class of suggestions was that these pseudopodia do not belong to the diatoms, but to an investing animal like Vampyrella, which devours Gomphonema. But if they belong to an investing animal, where is the animal which invests? Vampyrella, any way, is visible both when wandering in search of diatoms and when investing. Besides, Vampyrella projects its pseudopodia from any part of the diatom, while here they are mainly confined to a definite tract. And Vampyrella and other predatory animals do not have pseudopodia so regularly symmetrical, nor so constant in number. I have seen what appeared to be Leidy's B i o m y x a v a g a n s, which is something like Vampyrella, investing diatoms. And here also the pseudopodia had no relation whatever to the structure of the diatom, while the animal itself was clearly visible outside of the diatom. But, strongest proof of all, Vampyrella devours the diatom and kills it in a couple of hours; while these diatoms lived for five weeks in one bottle, quite healthy all the time, and dead ones were not found.

I do not think that any theory based on Vampyrella or vampyrelloid animals (as suggested at the British Association) will explain the facts. If the pseudopodia are foreign to the diatom, it would have to be a case of symbiosis between the diatoms and unknown invisible animals. I know of no similar case.

All the phenomena seem to me to point to these pseudopodia being filamentous extensions of the cell protoplasm, probably strengthened by cuticular deposit.

The fact that they do not move or quickly retract under stimulus would not necessarily distinguish them altogether from true pseudopodia; at any rate, if the processes of Archerina are to be called by that name these must also, for it is impossible to separate them. It might, however, be an advantage to have some distinctive name for such stiff, slowly changing or unchanging pseudopodia.

It remains to treat briefly of the morphological resemblances between these diatoms and other forms. In doing this I wish simply to point out resemblances or differences, not to draw any large conclusions.

The first point that strikes one about these pseudopodia is their extreme unlikeness to any plant or part of a plant. Secondly, it is remarkable that when pseudopodia are discovered on diatoms these resemble a type which, so far as I know, is absolutely confined to the Heliozoa, forms with which the diatoms are already connected by the presence of a more or less siliceous test. All the Heliozoa are characterised by the relative stiffness of their pseudopodia; but in Archerina this relative stiffness becomes practically absolute, as in these diatoms. I do not know of any described form except Archerina which has these non-retractile pseudopodia.

But the similarity in type of the pseudopodia is not confined to rigidity; it extends to general form. As far as I know, every single detail of form in the diatoms can be matched amongst the Heliozoa with the exception of the repeated branching of those of Melosira. The resemblance of the diatom's processes to those of Archerina is still closer: nevertheless in 99 cases out of 100 it is quite easy to distinguish on a dry slide the pseudopodia of Archerina from those of the diatoms.

They are distinctly wider and taper regularly. But in the 100th case you get an Archerina colony the pseudopodia of which are absolutely undistinguishable from those of the diatoms. Their size and shape are the same as those of Cyclotella, and on a dry cover they show the thickened bases and the denser portions of the filament which I have described as characteristic of the diatoms.

If one bears in mind that the little Melosiras have very little silica, possibly none at all at times, and that both they and the Archerinas contain chlorophyll bodies, the resemblance becomes still more striking. It is also a striking fact that the connection of the diatoms by bands of protoplasm finds its counterpart amongst Heliozooid Protozoa, as in Monobia confluens. Another point of agreement is the fact that Archerina at times has very few but very large pseudopodia, just like the Cyclotella.

A fact about Archerina as yet unpublished is worth notice. In staining with Schultze's fluid and with iodine and sulphuric acid I obtained the clearest evidence that the cuticle of the chlorophyll bodies is made of cellulose. In this it is more plaut-like than the diatoms themselves, which do not give the cellulose reaction. Cellulose has been found in a number of animals, but still it is interesting that while the pseudopodia draw the diatoms nearer to the animals, this cellulose draws Archerina nearer to the plants. This fact is likely to be used as an argument in favour of Archerina being a case of symbiosis, and such a view may be extended to these diatoms. I will not discuss that view now. I do not think it is conclusive in the case of Archerina, much less in the case of the diatoms, where all the facts seem to point to the pseudopodia being integral portions of the diatoms.

EXPLANATION OF PLATE XLI,

Illustrating Mr. J. G. Grenfell's note "On the Occurrence of Pseudopodia in the Diatomaceous Genera, Melosira and Cyclotella."

FIG. 1.—Melosira of the first gathering in the Botanical Gardens, much branched. The second frustule shrivelled in drying. Stained.

FIG. 2.—Melosira of the same gathering. Stained.

FIG. 3.—Melosira filament from Botanical Gardens. Stained.

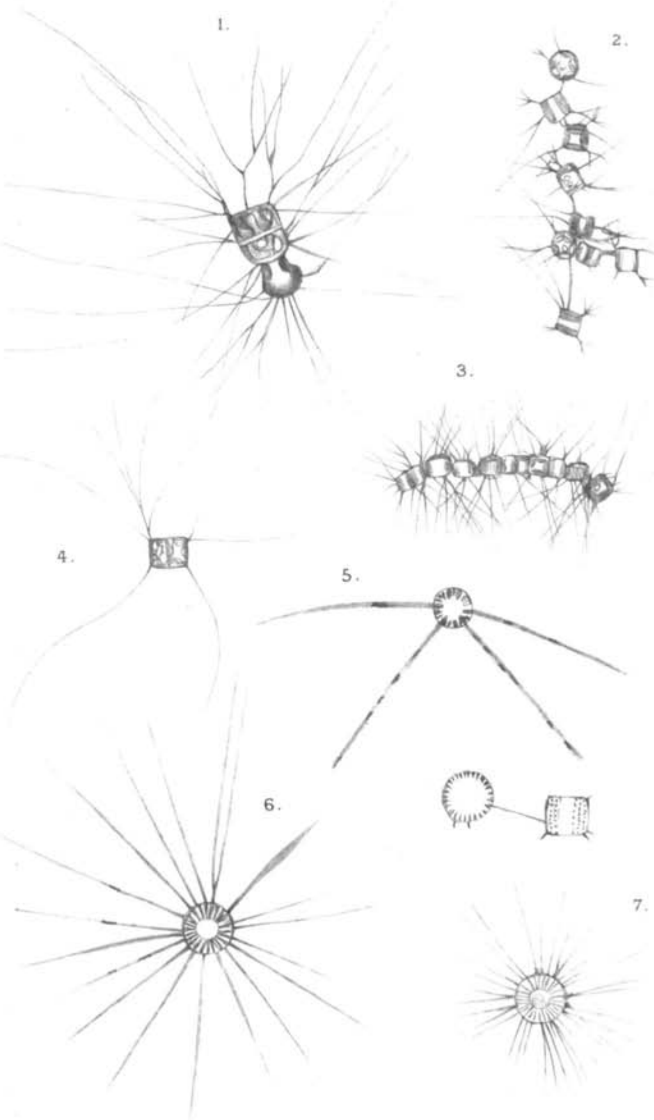
FIG. 4.—From Heytesbury, in Wiltshire. Unstained.

FIG. 5.—Cyclotella from Botanical Gardens. Four thick granular pseudopodia. Unstained.

FIG. 6.—Cyclotella Kützingiana from Botanical Gardens. Unstained.

FIG. 7.—Ditto, with more pseudopodia.

FIG. 8.—Two forms from Botanical Gardens, roasted at low red heat, showing projections and connecting band.



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