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NOTE ON THE PLYMOUTH '*NITZSCHIA*' CULTURE

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(Plate I)

For nearly fifty years a small marine organism has been cultured at the Marine Biological Association's Laboratory at Plymouth, mainly to be used as a food supply in rearing marine larval forms.

The cultures were originated by the late Dr E. J. Allen in 1907. The first record of these cultures and the methods used to maintain them were published by Allen & Nelson (1910) in a paper describing methods for obtaining persistent cultures of eighteen species of plankton diatoms. One of these cultures flourished so successfully that subcultures have been distributed to many laboratories and institutions both in Europe and America. This organism was named *Nitzschia closterium* W. Sm. forma *minutissima*. The authors, however, did not describe the organism or produce an illustration, so the combination can be considered only as a *nomen nudum*.

In 1939 the care of the cultures passed to Dr Douglas P. Wilson who published the first detailed account of the organism together with illustrations (Wilson, 1946). Wilson found the organism to be pleomorphic and described ovoid, fusiform, triradiate, and cruciform forms. After discussing these forms in great detail Wilson concluded 'Both normals [fusiform] and triradiates produce ovals by division, and the ovals so produced can multiply to form further ovals, or can grow either two or three arms, generally two, to form normal or triradiate cells' (p. 268), and 'there is no doubt that as a general rule triradiates arise from normals only through the intermediary of oval cells' (p. 251).

As Allen & Nelson (1910) did not describe the organism there is no means of knowing whether the original cultures contained the varying forms or not.

It is certain that Allen & Nelson examined the organism under the microscope, and because of its small size must have used high-power objectives to do so. It is reasonable to conclude, therefore, that the original sample contained only fusiform specimens, or that these were strongly dominant, for had the original workers noticed the triradiate ones, it is almost certain that they would have commented upon them.

Wilson (1946) states that Dr Allen had seen occasional triradiate forms in the cultures, and that Mr Clifford Dobell, who examined some old exhausted cultures during the winter of 1910–11, noticed enormous numbers of triradiate

forms, and rightly concluded that they were present in the early cultures, and were not a product of long-continued artificial environment.

Recently considerable doubt has been expressed concerning the true taxonomic position of this organism and this paper describes efforts to clarify the problem. Hendey (1937) explained that *Nitzschia* Hassall (1845) is an absolute synonym of *Sigmatella* Kützing (1833), as both genera were based upon *Bacillaria sigmoidea* Nitzsch. The name *Nitzschia* was adopted until it was legally conserved, in the sense that most modern taxonomists have used it—that is, in the sense that W. Smith used it in his *Synopsis of British Diatomaceae* (1853)—taking *Nitzschia sigmoidea* W. Smith based on *Sigmatella Nitzschii* Kützing, which was *Bacillaria sigmoidea* Nitzsch, as the type of the genus.

Smith (1853) described *Nitzschia* as follows: '*Frustules* free, elongated compressed; valves linear, keeled, with one or more longitudinal lines of puncta; keel frequently eccentric.' This definition differs in no material respect from Hassall's, and, in explanatory notes that follow it, the importance of the keel, usually eccentric and punctate, is stressed as the dominant generic character.

Wilson (1946), in his description of the three phases of the organism, makes it quite clear that he was unable, with certainty, to see any of the characteristic markings associated with the genus *Nitzschia*. He states (p. 237): 'I have not been able to satisfy myself that I have seen any of the usual valve markings of the Nitzschioideae, the keel and canal raphe or the carinal dots.... Only in some gently incinerated specimens mounted in Sirax (a medium of high refractive index) were to be seen what might possibly be the keel and raphe with some slight suggestion of carinal dots, but it was impossible to be certain of the identity of the structures seen; they might very well have been artefacts.'

Commenting on the structure of the triradiate cell Wilson states (p. 237): 'It would be interesting to know how the keel and raphe, if present, are arranged, but it has not been possible to make them out.'

It is well known that some diatoms are very weakly siliceous and that definitive markings are difficult to observe, and, further, that some diatoms in culture have been induced to vacate their siliceous frustules and continue to live as naked bodies (Wiedling, 1941; Hendey, 1945, 1946), but these forms were derived from individuals possessing normal frustules. There can be no suggestion that the weakness of silicification and the consequent hyaline nature of the cell wall is attributable to culture conditions, or that a 'laboratory species' has, at some time, suddenly arisen due to conditions imperfectly known.

Wilson states (1946, p. 265), on the authority of Dr Mary Parke, also of the Plymouth Laboratory, that both the triradiate and fusiform phases were frequent in water samples from the Irish Sea off Port Erin.

It must be accepted, therefore, that there exists in nature a weakly siliceous organism having certain diatom characteristics, and that persistent cultures of it have been maintained over many years without producing any apparent change.

This polyphasic organism was ably described and illustrated by Wilson (1946) under the name of *Nitzschia closterium* (Ehrenberg) Wm. Smith forma *minutissima* Allen & Nelson. Wilson drew attention to the fact that a unicellular alga described by Bohlin (1897) as *Phaeodactylum tricornutum* agreed in all particulars with the triradiate of the Plymouth cultures. Bohlin had found this organism in Baltic rock pools; it had occurred with *Brachiomonas submarina* Bohlin and had multiplied rapidly in culture as the *Brachiomonas* died out.

Mr Michael Droop of the Marine Station, Millport, who is familiar with Bohlin's organism which he has himself collected in the Baltic, has intimated (in a private communication) that it commonly occurs there in rock pools on the skerries, and that he has found it within a few miles of Runmarö. Further, that in his experience, naturally occurring material always has been triradiate.

Mr Droop deposited a culture of the Baltic material at the Plymouth Laboratory, and comparisons made with it and the Plymouth specimens confirm beyond all possible doubt that both should be referred to Bohlin's species.

It should be noted that Allen & Nelson (1910) did not describe or illustrate their organism, but were content to list it as an undescribed variety of a well-known species. One of two explanations seem probable. First, that Allen & Nelson made a misidentification due to lack of information on the diatoms. The fusiform organism found by them in the original Plymouth material, whatever it was, was probably unknown to them. Its general appearance suggested a relationship with *Nitzschia closterium* Ehrenberg, but they satisfied themselves that it was not the type variety, and adopted forma *minutissima*, without describing it, merely as a matter of convenience. Secondly, that the original material cultured was predominantly a small *Nitzschia closterium* with a few specimens of *Phaeodactylum* which were, most likely, not noticed because of their small size. Subsequent subculturing at short intervals favoured the *Phaeodactylum* which replaced the *Nitzschia* very much in the same way as it replaced the *Brachiomonas* in Bohlin's cultures.

Dr D. P. Wilson (in private communication) favours the former view. He states that when Dr Allen spoke of the 'normals' in the cultures, he referred to those illustrated by Wilson (1946, p. 236, fig. 1, left-hand figures), which are fusiform specimens of *Phaeodactylum tricornutum* Bohlin, and that to the best of his knowledge and belief the original cultures had contained only 'normals', i.e. fusiform organisms. Dr Wilson also assures me that on some microscope mounts made by him in 1930 the triradiate form is present in small numbers but there are many more fusiform cells with them, and that no

true *Nitzschia* are there to be found. Whatever the cultures may have contained in the beginning, it is now clear that they have consisted only of *Phaeodactylum tricornutum* Bohlin in several phases for very many years.

The varying phases of the organism, ovoid, fusiform, triradiate, etc., and the ability to change one into the other, have been dealt with by Wilson & Lucas (1942) and Wilson (1946). It is not clear what factors determine the shape of the cells, but no doubt age of culture, availability of nutrients, and light intensity all play a part.

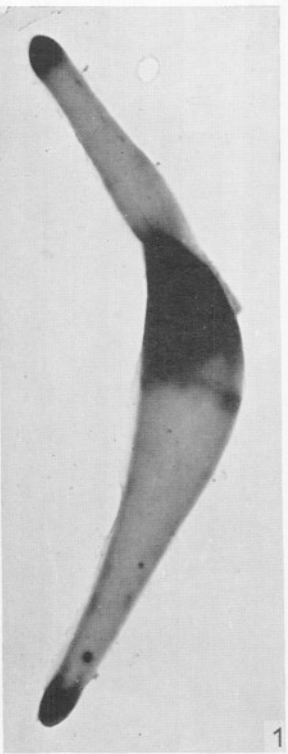
Bohlin (1897) described only triradiate specimens, Allen & Nelson (1910) indicated that only fusiform specimens were to be seen in their material. This does not exclude the possibility that some fusiform specimens were present in Bohlin's material, or that some triradiates were present in Allen & Nelson's, but only that they were not recorded. Droop intimated that his culture (on solid media) of the Plymouth organism has never given rise to triradiate forms, while a clone from the Baltic had been triradiate for most of the time since collection, but now, on solid media, contains only ovals which when subcultured into liquid media have given rise to fusiform cells but not to triradiates.

In an attempt to elucidate the problem, electron micrographs have been prepared. Pl. I, figs. 1 and 2 show the fusiform phase, and figs. 3 and 4 show the triradiate phase. The original prints of these were made at a magnification of × 10,000. The micrographs show the cell-wall to be completely hyaline and entirely devoid of any structures. The organism, as interpreted by the electron microscope, adds little to that already observed by the high-power optical microscope.

The true taxonomic position of this organism is still in some doubt. There appears to be evidence that the cells divide longitudinally as do the diatoms, but the weakly siliceous nature of the cell-wall precludes any positive observations to determine whether or not the cell is frustular. Wilson (1946, p. 237) sectioned the cells, but was unable to see any evidence that would suggest the presence of valves and girdles characteristic of diatoms.

Recently Dr Mary Parke has shown (unpublished) that leucosin is present in the cell of *Phaeodactylum tricornutum* from the Plymouth cultures. The presence of leucosin suggests that *Phaeodactylum* might be related to the Chrysophyceae, but too much importance must not be given to this suggestion without further research.

I wish to express my indebtedness to Mr F. W. Cuckow for the electron micrographs shown in Pl. I, and to Dr Mary Parke, Dr D. P. Wilson, and Mr Michael Droop for their kind assistance.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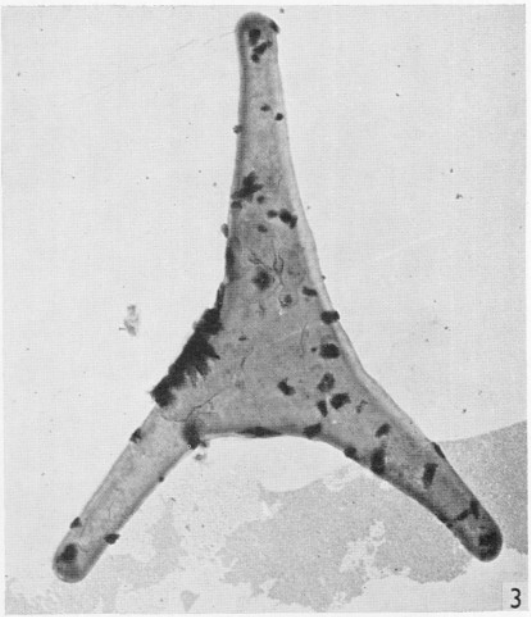
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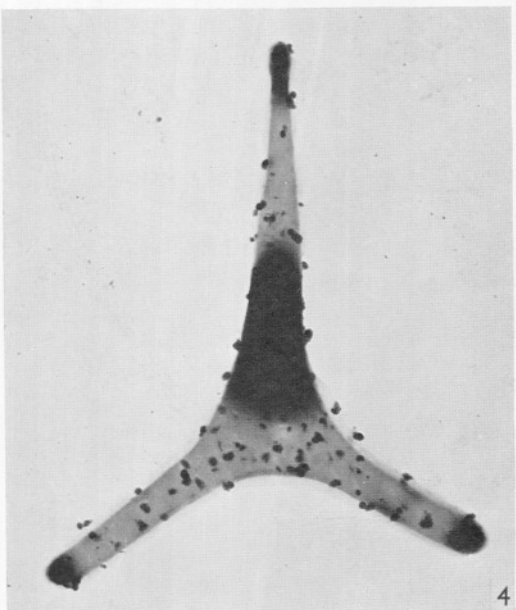
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EXPLANATION OF PLATE I

Phaeodactylum tricornutum Bohlin

Figs. 1 and 2. Cell in fusiform phase. × *c.* 6000.
Figs. 3 and 4. Cell in triradiate phase. × *c.* 6000.