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Observations on the Ultrastructure of Flagellar Scales in the Genus *Synura* (Chrysophyceae)

David J. Hibberd

Culture Centre of Algae and Protozoa, Cambridge, England

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Summary. 1. Flagellar scales have been demonstrated for the first time in *Synura echinulata*, *S. sphagnicola*, *S. spinosa*, *S. uvella* and *Mallomonopsis ouradion* and on the smooth flagellum of *S. petersenii* in addition to those previously described for the long flagellum.

In *S. petersenii* the scales are annular, approximately 100 nm in diameter with a 50 nm hole and in *S. sphagnicola* linear, 200—300 nm × 60 nm (observations on both flagella); in *S. uvella* they are semicircular, 60—70 nm × 20 nm, and in *Mallomonopsis ouradion* oval, 100—120 nm × 60 nm (observations on long flagellum only). *Synura echinulata* is unique among the species studied in having a mixture of clavate scales, 200 nm × 60 nm, and annular scales 100 nm in diameter on the long flagellum, though only the annular scales have been found on the smooth flagellum. Annular scales have been detected with difficulty on the long flagellum only of *S. spinosa*.

2. The scales are not arranged in a regular way and their attachment to the flagellar membrane is tenuous, the annular scales being attached by their edges, the elongate scales by one end and the semicircular scales in *S. uvella* by their convex side.

3. The scales are accumulated, and almost certainly also formed, in a large temporary vesicle similar to the flagellar scale reservoir in *Pyramimonas* in the Prasinophyceae.

4. This type of flagellar scale is unlike that in any other class of algae and, within the Chrysophyceae, appears to be restricted to genera in the Synuraceae.

Application of electron microscopy to the study of algal flagella has revealed a wide range of appendages including spines, hairs of several types and scales (see Manton, 1965, for a review). Flagellar scales have so far only been found to be a general feature in the Prasinophyceae where there are usually nine rows of finely patterned overlapping unmineralised scales and an underlying layer of smaller scales on each flagellum (see Round, 1971, for literature); scales of a similar type to those forming the underlayer in the Prasinophyceae have recently been found in the Charophyta (Pickett-Heaps, 1968; Moestrup, 1970).

Motile cells in the Chrysophyceae characteristically possess one short, smooth, laterally-directed flagellum which bears a wedge-shaped swelling at its proximal end where it overlies the eyespot, and a long,

forwardly directed flagellum bearing stiff hairs ("Flimmer") laterally (Hibberd, 1970). Reports of other flagellar appendages in the Chrysophyceae are few, though two very different types of scale have been reported. On the one hand, *Sphaleromantis tetragona* Skuja is so far unique in having both the locomotory hairy flagellum and the smooth flagellum, in this case vestigeal, covered by the same two elaborate types of non-silicified scale which cover the body of the cell in this species (Manton and Harris, 1966), whereas, on the other hand, completely different, very small and apparently loosely attached annular scales have been detected by means of negative staining on the hairy flagellum of *Mallomonopsis paxillata* Bradley and *Mallomonas striata* Asmund by Bradley (1966a) and on that of *Synura petersenii* (Petersen) Korshikov by Bradley (1966a) and by Schnepf and Deichgräber (1969). The latter structures are of a very different type to the elaborate silicified body scales, several microns in size, which are characteristic for the genera *Mallomonas*, *Mallomonopsis* and *Synura* and which have been the subject of numerous electron microscope studies since their complexity was first revealed by Fott in 1955 (e.g. Fott and Ludvik, 1957; Harris and Bradley, 1960; Bradley, 1966b). The relatively late discovery and small number of observations on flagellar scales in these genera is therefore noteworthy, suggesting either tenuous attachment or extreme delicacy.

Material and Methods

Synura petersenii was obtained from three wild collections and as strains 960/1a, 960/1b and 960/1c from the Culture Centre of Algae and Protozoa, Cambridge; *Synura uvella* Korshikov was obtained from the latter source only, as strain 960/2. *Synura echinulata* Korshikov, *S. sphagnicola* (Korshikov) Korshikov, *S. spinosa* Korshikov and *Mallomonopsis ouradion* (Harris et Bradley) Harris, were obtained from wild collections only. Localities of wild collections are given in Table 1 as National Grid References.

Material for shadowcasting was fixed either on a coated grid by exposure to the vapour of 2% osmium tetroxide for periods ranging from 10 s to 2 min, by treatment of a cell suspension with a very small quantity of 2% osmium tetroxide or by fixation for up to 10 min in 2% osmium tetroxide in 0.1 M phosphate buffer, pH 7. In all cases, excess liquid was removed from the grid after allowing the cells to settle, and the material was then dried, washed in distilled water and dried again before shadowcasting with gold/palladium. Individual methods for each of the micrographs illustrated are not given since apparently identical methods produced inconsistent results and since the various species and different collections of the same species varied widely in their response to fixation.

Material for negative staining was fixed in osmium tetroxide vapour as above, after which 2% phosphotungstic acid was applied for 2 min, all but a thin film being removed with filter paper before drying. Material for sectioning was fixed in either 2% osmium tetroxide in 0.1 M phosphate buffer for 25 min (Figs. 4, 10, 11, 14 and 15) or in a 1:1 mixture of 2% osmium tetroxide with 4% glutaraldehyde in 0.1 M phosphate buffer for 15 min, followed by three 30 min changes of buffer

Table 1. Summary of data on flagellar scales in the Chrysophyceae

Species	Long flagellum		Short flagellum		Source
	Shape	Dimensions	Shape	Dimensions	
Synura echinulata	Clavate Annular	200 nm × 60 nm 100 nm diameter	Annular	100 nm diameter	Wild material; NGR SE263415
Synura petersenii	Annular		Annular	100 nm diameter	Wild collection A; NGR SE236419
	Annular	100 nm diameter	?	—	Wild collection B; NGR TQ226725
	Annular	hole c 50 nm	?	—	Wild collection C; NGR SU746648
	Annular		?	—	Cambridge 960/1a
	Annular		?	—	Cambridge 960/1b
	?	—	?	—	Cambridge 960/1c
	Annular(?)	—	—	—	Bradley (1966a); wild material
	Annular	c 0.1 μm diameter	—	—	Schnepf and Deichgräber (1968); Göttingen 960/3
Synura sphagnicola	Linear	200—300 × 60 nm	Linear	c 300 × 60 nm	Wild collection D; NGR SE123594
	Linear		Linear		Wild collection E; NGR SZ037853
Synura spinosa	Annular (rectangular?)	c 250 × 150 nm	?	—	Wild material; NGR SD384926
Synura uvella	Semicircular	60—70 nm × c 20 nm	?	—	Cambridge 960/2
Mallomonas striata	Annular	—	—	—	Bradley (1966a); wild material
Mallomonopsis ouradion	Oval	100—120 × 60 nm	?	—	Wild material; NGR TQ235718
Mallomonopsis paxillata	Annular	1350 Å diameter 500 Å hole	—	—	Bradley (1966a); wild material

and post-osmication for 2 h (Figs. 17 and 18). All material was then briefly rinsed in buffer before dehydration in an ethanol series and embedding in Epon. All fixation steps to dehydration in 95% ethanol were carried out at 4° C. Sections were cut with either a glass or diamond knife, mounted on formvar/carbon coated grids and stained with uranyl acetate and lead citrate. Observations were made on a Siemens Elmiskop I or an AEI EM6B in the Botany Department of Leeds University (micrographs prefixed DJH) and on an EM6B at the Natural History Museum, London (micrographs prefixed by a single letter).

Observations

Synura petersenii (Figs. 1—7)

In all five strains successfully examined the flagellar scales consist of annuli approximately 100 nm in diameter with a 50 nm hole (Figs. 3—5). They are thus of a similar size and shape to those described for *Mallomonopsis paxillata* by Bradley (1966a) and for *S. petersenii* by Schnepf and Deichgräber (1969). Sections show that the scales are attached to the flagellar axis only by their edges (Fig. 4) and this is taken to be their normal arrangement since it is unlikely that they are normally closely adpressed and only become loose during preparation. The conspicuous appearance of the scales in the original wild collection of this species can be seen in Figs. 1—3, and counts from preparations such as that in Fig. 2 give totals in the region of 800—1000 scales on the long (hairy) flagellum. Similar scales were also found on the short (smooth) flagellum of cells from this collection (Fig. 6) though they are encountered much less commonly and in much smaller numbers, indicating that they are far more readily shed. It is also probable that the short flagellum normally bears a smaller number of scales than the long one owing to its considerably narrower diameter (Fig. 1). In the remaining four strains, scales could be demonstrated on the long flagellum only. The scales on the long flagellum appear amorphous both in sections (Fig. 4) and in negatively stained preparations (Fig. 5).

Each of the six strains of *S. petersenii* examined behaved differently with respect to the ease by which the scales could be demonstrated using conventional shadowcasting techniques. One extreme is represented by the original wild material (Figs. 1—4 and 6) and the other by Cambridge Strain No. 960/1c in which it has not yet been possible to demonstrate the scales on either flagellum by means of shadowcast preparations. Even when scales are clearly present, preservation is variable and artifacts relatively frequent. Most commonly the scales on the long flagellum appear as lumps surrounded by a circular depression (Fig. 7), though they appear annular in sections of material from the same collection. Some or all of the scales on both flagella may also appear as solid discs without a central hole (Fig. 6). In this respect, it is note-

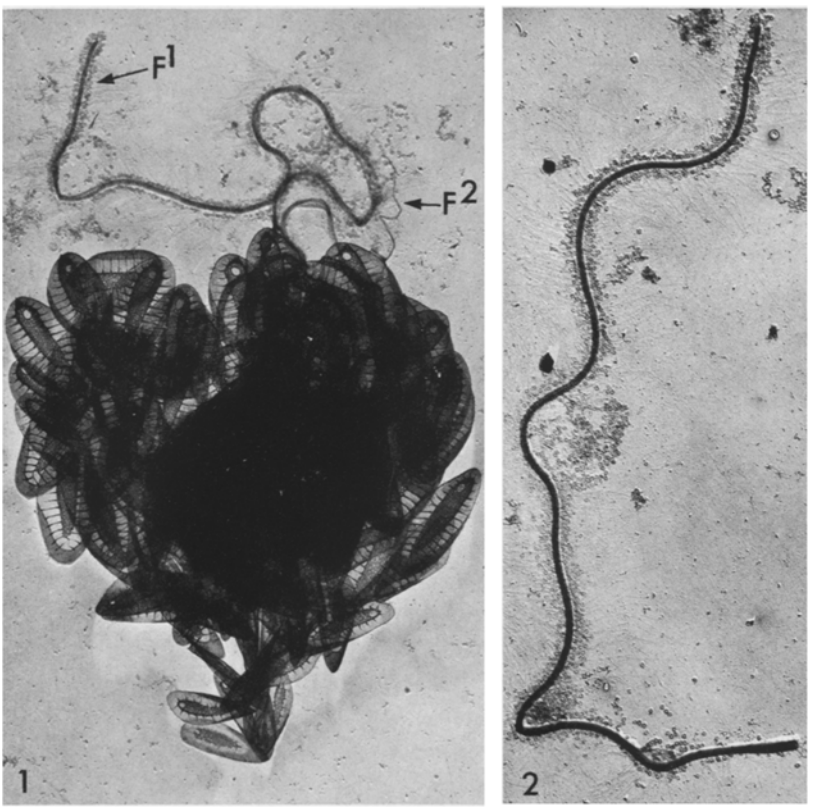


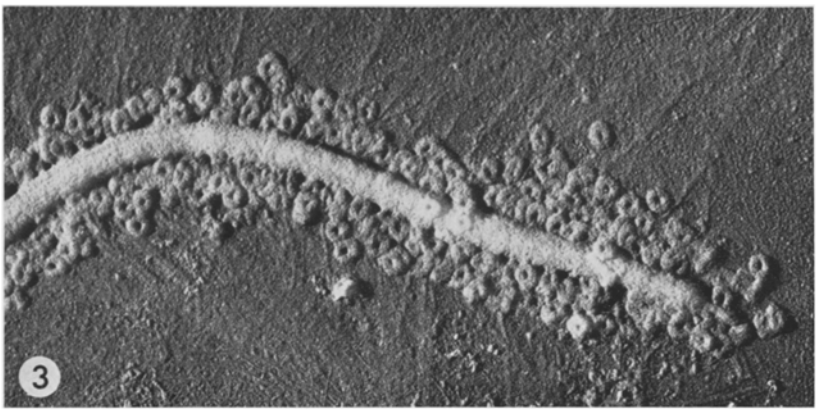
Fig. 1. Shadowcast preparation of a whole cell from a colony of *Synura petersenii* showing the covering of silica body scales and the two flagella, the one bearing hairs (F^1) densely covered with annular scales; F^2 smooth flagellum. Wild collection A. Micrograph DJH754 ×4000

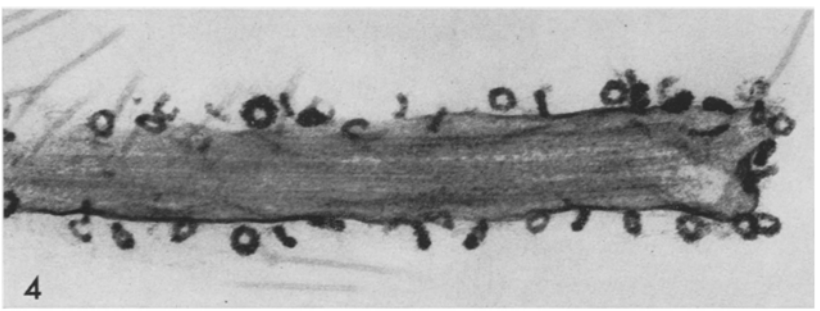
Fig. 2. A detached long flagellum of *Synura petersenii* with a dense covering of annular scales. Wild collection A. Micrograph DJH721 ×5000

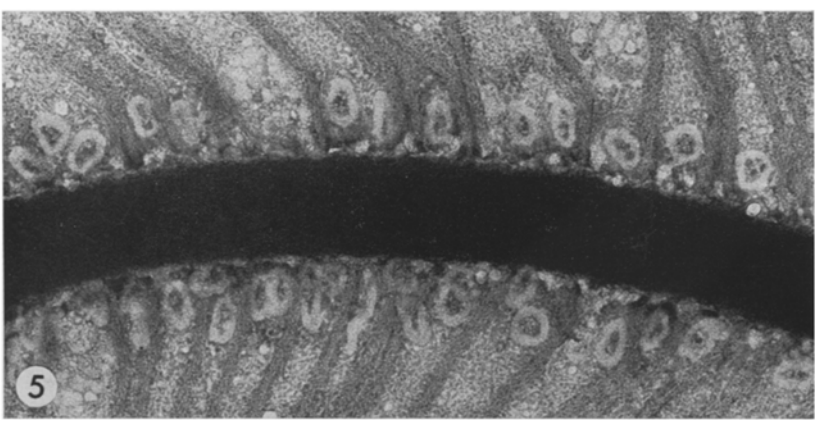
worthy that imperfect preservation of the scales is usually accompanied by excellent preservation of the flagellar hairs (Fig. 7) and *vice versa* (Fig. 3).

Synura echinulata (Figs. 8—10)

Synura echinulata is unique among the species studied in having a mixture of scale types on the long flagellum (Figs. 8 and 10). The majority are clavate, approximately 200 nm long × 60 nm maximum width and are attached to the flagellum by their narrow ends, the remainder being annular and of a similar diameter to those in *S. petersenii* (Figs. 8 and 10).







Figs. 3—5. Annular scales on the long flagellum of *Synura petersenii*

Fig. 3. Shadowcast preparation. Wild collection A. Micrograph DJH722, reversed print, ×20000

Fig. 4. Longitudinal section. Wild collection A. Micrograph K46 ×50000

Fig. 5. Negatively stained preparation. Cambridge strain No. 960/1b. Micrograph Q153 ×50000

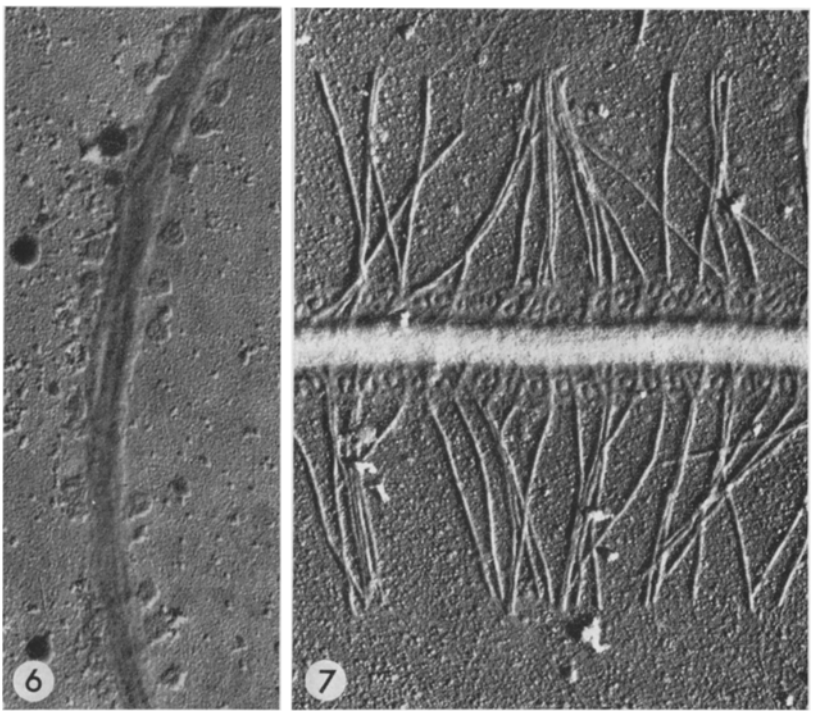


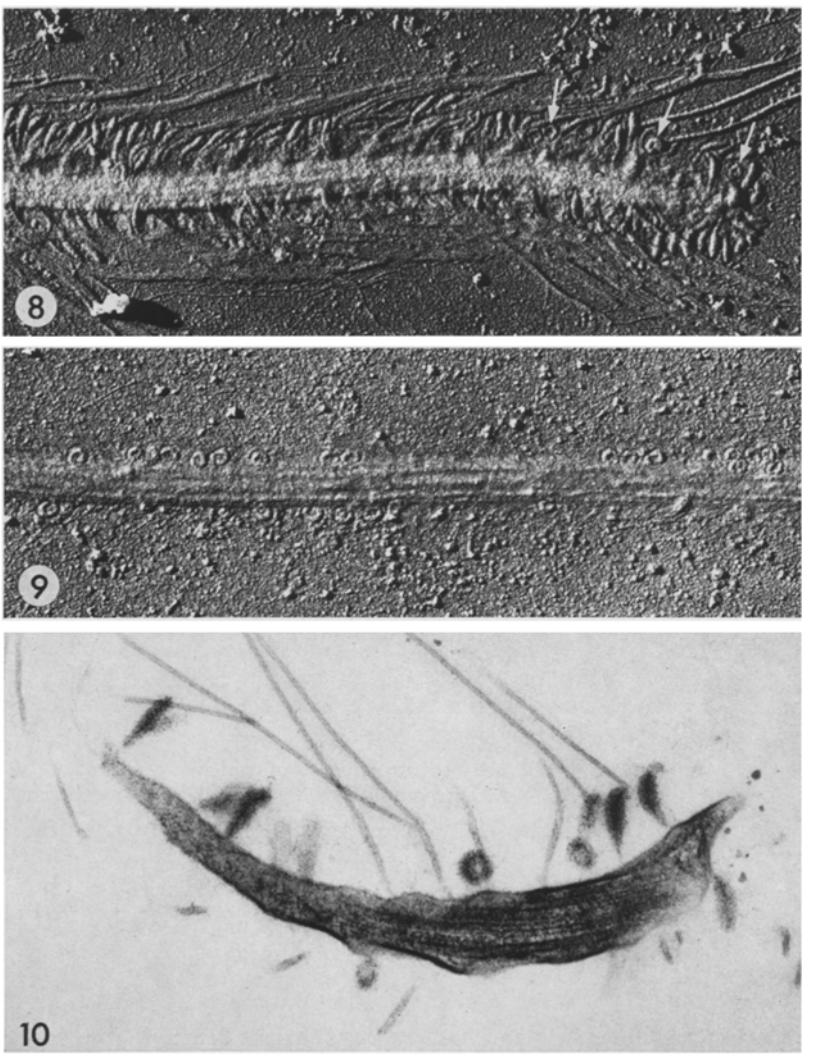
Fig. 6. Shadowcast preparation of a smooth flagellum of *Synura petersenii* showing the attached annular scales, the centres of most of which are occluded. Wild collection A. Micrograph DJH763 ×20000

Fig. 7. Shadowcast preparation of a long flagellum of *Synura petersenii* showing the well preserved flagellar hairs and the poorly preserved flagellar scales. Wild collection B. Micrograph J657, reversed print, ×20000

In section the inner region of both types of scale is denser than the outer part which often appears brush-like (Fig. 10). Only annular scales have so far been seen on the short flagellum (Fig. 9).

Synura sphagnicola (Figs. 11—13)

Synura sphagnicola has elongate flagellar scales which measure 200—300 nm × approximately 60 nm. They have been detected on both flagella (Figs. 12 and 13) and are attached by their ends (Fig. 11). They resemble the clavate scales on the long flagellum in *S. echinulata* in having a dense centre and more diffuse periphery (Fig. 11).

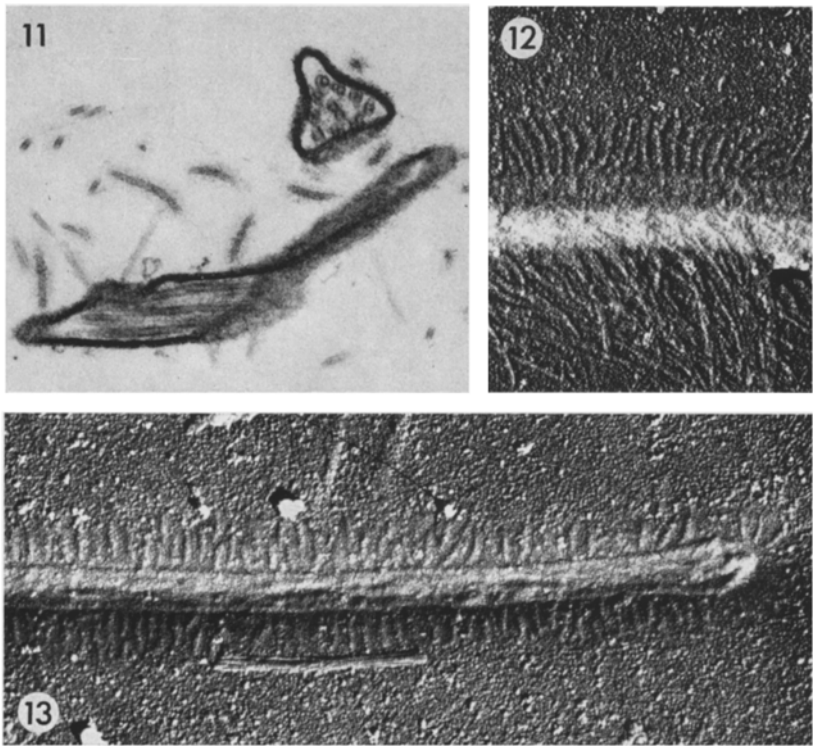


Figs. 8—10. Flagellar scales in *Synura echinulata*

Fig. 8. Shadowcast preparation of the long flagellum showing clavate and annular (arrowed) scales. Wild material. Micrograph DJH2323, reversed print, ×20000

Fig. 9. Shadowcast preparation of a smooth flagellum showing the annular scales. Wild material. Micrograph Q574, reversed print, ×20000

Fig. 10. Longitudinal section of the long flagellum showing the structure and attachment of the clavate and annular scales. Wild material. Micrograph J361 ×50000



Figs. 11—13. Flagellar scales in *Synura sphagnicola*

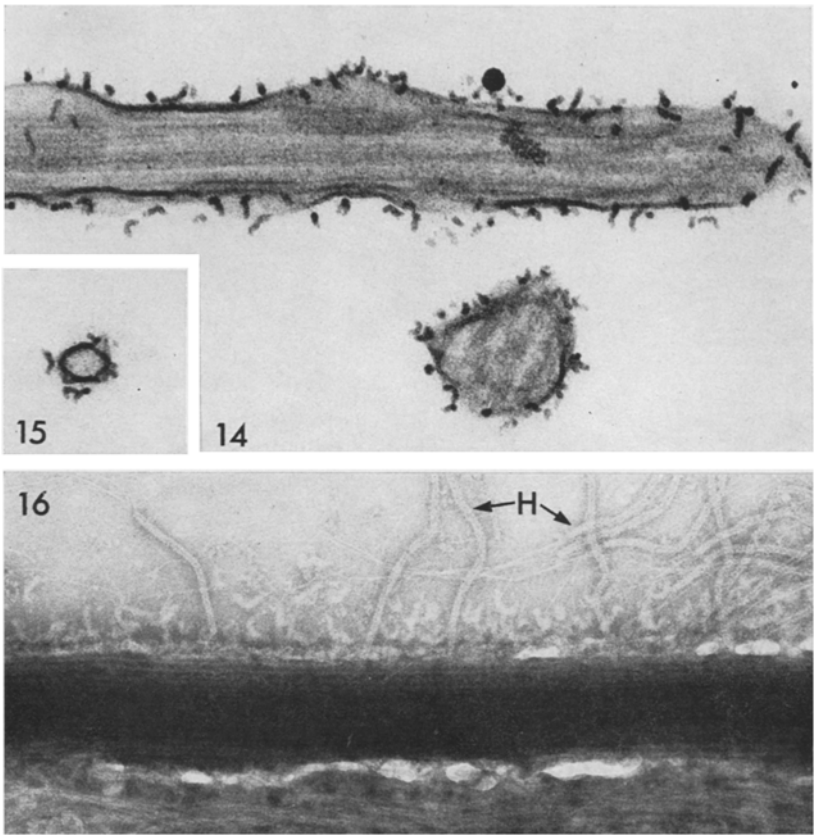
Fig. 11. Transverse and oblique sections of the long flagellum showing the linear scales with a dense central region and less dense periphery. Wild collection E. Micrograph R634 ×50000

Fig. 12. Shadowcast preparation of a long flagellum showing the linear scales. Wild collection D. Micrograph Q47, reversed print, ×20000

Fig. 13. Shadowcast preparation of a smooth flagellum showing the dense coating of linear scales. Wild collection E. Micrograph DJH2522, reversed print, ×20000

Synura uvella (Figs. 14—16)

Flagellar scales could not be detected in *Synura uvella* using shadowcast preparations though they are clearly revealed by sections and negative staining (Figs. 14—16). They are 60—70 nm long × approximately 20 nm thick and semicircular in shape. The point of attachment is not clear, though the great majority lie with their convex side



Figs. 14—16. Flagellar scales in *Synura uvella*

Fig. 14. Longitudinal and oblique sections of the long flagellum (flagellar hairs lost) showing the semicircular scales with their convex sides towards the flagellar axis. Cambridge strain No. 960/2. Micrograph R460 ×50000

Fig. 15. Transverse section of the distal part of a flagellum with semicircular scales. Cambridge strain 960/2. Micrograph R460 ×50000

Fig. 16. Negatively stained preparation of the long flagellum showing the semicircular scales; *H* flagellar hairs. Cambridge strain No. 960/2. Micrograph Q173 ×50000

towards the flagellar membrane. No definite observations have been made on the short flagellum, though Fig. 15 may represent a transverse section through the long narrow part of this flagellum.

Synura spinosa and *Mallomonopsis ouradion*

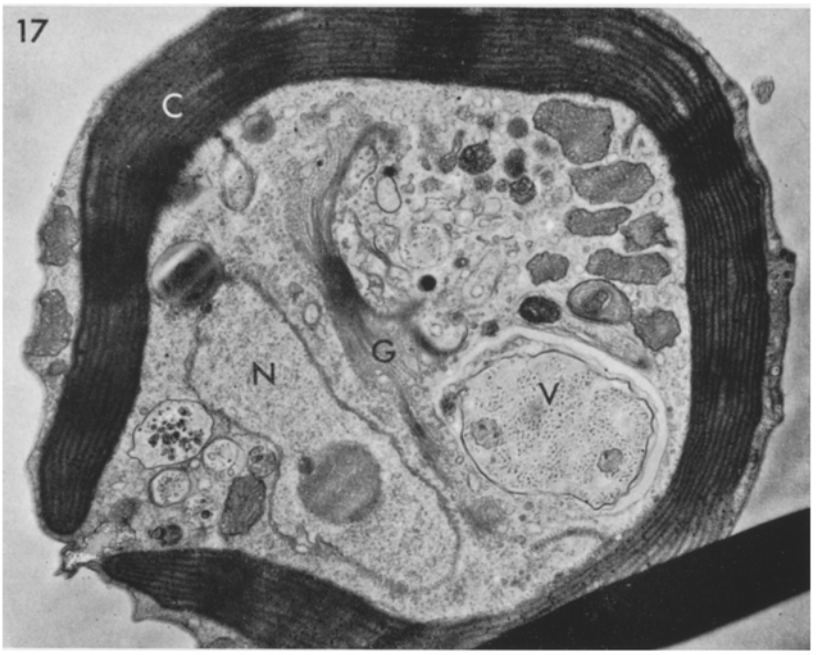
Flagellar scales have been detected only with difficulty in these species. In shadowcast preparations of *S. spinosa* they appear annular but almost rectangular in shape measuring approximately 200×150 nm. In *Mallomonopsis ouradion* the flagellar scales are not of the annular type reported for *Mallomonas striata* by Bradley (1966) but are oval, 100×60 nm and in section have a dense central core and less dense peripheral region as described for *S. sphagnicola*.

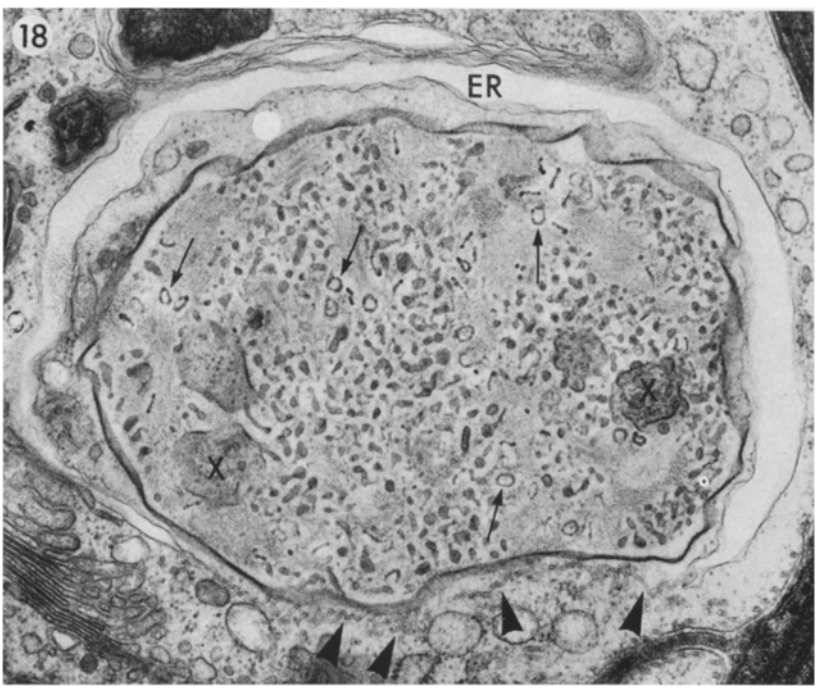
Observations on Scale Formation in *Synura petersenii*

The micrographs illustrated (Figs. 17 and 18) represent chance observations on a single specimen which have not so far been repeated, but they are included here because, if correctly interpreted, they illustrate new facts of great interest in the biology of the Chrysophyceae and provide interesting parallels with phyletically remote classes. The transverse section illustrated in Fig. 17 shows the position, relative to the nucleus (*N*) and Golgi body (*G*) of a large vesicle (*V*) containing (Fig. 18) annuli of similar size and appearance to the flagellar scales. This vesicle thus appears to be the site of accumulation, and probably production, of the flagellar scales; annular structures could not be detected in the cisternae of the adjacent Golgi body, though its unusual appearance in this cell possibly represents a division stage. In addition to annuli, the vesicle also contains fibrous material, small irregularly shaped globules and membrane bounded profiles (*X*); the latter contain ribosomes and small vesicles and are therefore probably sections through finger-like cytoplasmic invaginations, though connection with the vesicle membrane has not yet been seen. The vesicle is surrounded by a single dilated cisterna (Figs. 17 and 18) which is probably part of the ER system. Serial sections show that the cisterna may surround the vesicle more completely than in Fig. 18, though at all levels the edges taper finely and are linked by contorted tubular material 18 nm in diameter.

Discussion

Comparison with flagellar scales in the Prasinophyceae and Charophyceae demonstrates the unique nature of the present type in their relatively simple structure, irregular arrangement and tenuous attachment. The most important new observations from the point of view of general biological interest and comparison with other classes, however, are those on the proposed flagellar scale vesicle, since it has now been shown for two species of *Pyramimonas* in the green algal class Prasinophyceae, that whereas the body scales are produced in the Golgi





Figs. 17 and 18

body and liberated direct to the surface in vesicles, the flagellar scales are accumulated, and are almost certainly also formed, in a large flagellar scale reservoir enveloped by an ER cisterna and penetrated by cytoplasmic villi, and are liberated to the surface through a special duct (Manton, 1966, 1968). It is therefore of considerable interest to find a homologous structure in such a phyletically remote class as the Chrysophyceae. *Synura* also provides the first example of the operation of two distinct systems of scale production in one cell, neither of which is directly connected with the Golgi body, since the body scales have been shown to be formed by a unique process involving a special vesicle intimately associated with the chloroplast ER (Schnepf and Deichgräber, 1969). In contrast to the body scales, the flagellar scales are almost certainly unmineralised as demonstrated by their transparency to electrons, though further speculation as to their chemical nature is not yet possible.

The fact that flagellar scales have not been detected in most of the numerous studies of body scales in *Synura* and *Mallomonas*, and never on the smooth flagellum in species where they have been seen previously, is not difficult to explain considering the ease by which they are either shed or occluded, and their comparatively insignificant appearance beside the very much larger and more complex body scales. In addition, the use of a range of techniques on a variety of species and strains indicates that negative staining is greatly to be preferred to shadowcasting as a method of detecting the scales.

Flagellar scales of this type are possibly limited in occurrence to the Synuraceae, a family comprising genera morphologically specialised in a variety of ways but characterised by their silica body scales, since they have been found only in species of *Synura*, *Mallomonas* and *Mallomonopsis* among the increasing number of Chrysophyceae now studied in the electron microscope. They may therefore also be present in *Chrysosphaerella*, *Paraphysomonas* and other genera in this family, as well as in further species of *Synura*, *Mallomonas* and *Mallomonopsis*.

Fig. 17. Transverse section of the anterior end of a cell of *Synura petersenii* showing the position, relative to the nucleus (*N*) and Golgi body (*G*) of the large scale-containing vesicle (*V*). *C* chloroplast. Cambridge strain No. 960/1b. Micrograph 0838 ×7500

Fig. 18. High-power micrograph of the scale vesicle showing the annular scales (arrows), fibrous material, irregular globules and membrane bounded profiles (*X*). The vesicle is almost completely surrounded by a dilated ER cisterna (*ER*), the tapered edges of which are linked by contorted tubular material (arrowheads). Cambridge strain No. 960/1b. Micrograph 0841 ×25000

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Dr. D. J. Hibberd
The Culture Centre of Algae and Protozoa
36 Storey's Way
Cambridge CB3 ODT, England