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NANOPLANKTON FROM THE GALAPAGOS ISLANDS: *MICHAELSARSIA ELEGANS* GRAN AND *HALOPAPPUS ADRIATICUS* SCHILLER (COCCOLITHOPHORIDS) WITH SPECIAL REFERENCE TO COCCOLITHS AND THEIR UNMINERALIZED COMPONENTS

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Marine plankton flagellates attributable to *Michaelsarsia elegans* Gran (type species of its genus) and *Halopappus adriaticus* Schiller (sensu Gaarder) have been investigated by means of scanning and transmission electron microscopy supplementing light microscopy of dry whole mounts prepared *in situ* in the Galapagos Islands. Some external features, notably coccolith arrangement, have been re-interpreted, and information on others added or amplified. Some of the new details include the body coccoliths, which have been shown to be more complex than previously supposed,

the bar-crystallites in particular being compound in both taxa. In addition, unmineralized components are shown to be present in all types of coccolith. They include patternless membranes spread across the proximal faces of body coccoliths and occupying the apparently vacant centres of ring-shaped coccoliths, while a highly characteristic, fragile, reticulum is limited to the central areas of the elongated appendage links in both taxa. The impact of these findings on general biological concepts is discussed in a preliminary way, drawing on cognate data previously published for *Ophiaster* and *Calciopappus*. It is concluded that the presence of apical appendages (anterior or posterior) in each of these genera is an independently acquired adaptation to some as yet unknown environmental factor or factors, whereas coccolith substructure is phyletically more meaningful. This indicates that *Michaelsarsia*, to which *H. adriaticus* should be transferred, is more remote from the other two genera than has hitherto been supposed. Finally an attempt has been made, in the light of all the evidence, to assess for the first time the possible functional significance of the unmineralized coccolith components and some constructive suggestions have been tentatively formulated. The paper ends with a factual summary in the form of revised taxonomic diagnoses for *M. elegans*, *M. adriaticus* and the genus *Michaelsarsia*.

Introduction

This paper is the second half of a report originally planned to have dealt with four, supposedly related, genera. These are sometimes listed alphabetically (see Heimdal & Gaarder 1981) or, in the converse order, as *Ophiaster*, *Michaelsarsia*, *Halopappus*, *Calciopappus*, and all are well represented in the coastal waters of the Galapagos Islands. It had been expected from the existing literature that the new findings would be limited to unmineralized periplast components, which previous experience with South African material (see Manton & Oates 1983) had shown to be present, at least in *Ophiaster*. However, the need to unravel several other complexities within that genus, followed by the discovery that it could appropriately be paired with *Calciopappus* but scarcely at all with *Michaelsarsia* or *Halopappus*, led unavoidably to the need for subdivision of the report along the lines we now follow.

In the taxonomic literature based on light microscopy, notably Schiller (1930), several species are credited to each of two coccolithophorid genera: *Michaelsarsia* Gran and *Halopappus* Lohm. Electron microscopy, in contrast, though carried out by several authors including Lecal (1965), Borsetti & Cati (1970), Gaarder & Hasle (1971), Nishida (1979), Heimdal & Gaarder (1981) and perhaps others, appears to have involved no more than two species, one from each genus. Each of these species, namely *Michaelsarsia elegans* Gran and *Halopappus adriaticus* Schiller, seems to be widespread in warm, including tropical, seas though neither is said to be abundant. Specific recognition in both cases depended initially on comparisons with drawings made over 50 years ago with the light microscope but subsequent standardization has usually involved comparisons with published electron micrographs supplemented at need by personal consultation with authors. In the present instance, the naming of the material on which the following report is based, was supplied personally by B. R. Heimdal, K. R. Gaarder and others on the evidence of some, though not of course all, the micrographs reproduced here. This degree of taxonomic authentication has been exceptionally important in this particular context because it limits the terms of reference within which this part of the enquiry must necessarily be conducted.

A patternless membrane spread across a space previously interpreted as empty, is commonly undetectable by mere inspection of a whole mount. In contrast, a surface pattern not attributable to accompanying crystallites, is the most easily recognizable sign of the presence

of unmineralized material in a fully calcified coccolith. Examples are now known from several different genera: for a list, see Manton & Oates (1983). Exceptionally, but as yet only in a few specialized coccoliths in two minor species of *Ophiaster*, an intrinsically patternless membrane has been detectable as such by means of indirect effects or the fortuitous markings resulting from surface damage. This observation is highly relevant to *Michaelsarsia*, which displays the same phenomenon on a substantially greater scale.

In contrast, and more surprisingly, the crystalline parts of the ordinary body coccoliths of both *Michaelsarsia elegans* and *Halopappus adriaticus* differ from expectation based on the literature in several, highly significant, morphological features. This discovery focuses attention on aspects of these taxa that could (and should) have been detected by previous observers had closer attention been paid to their material. Collectively these observations now introduce a new situation permitting certain misconceptions of long standing to be recognized as such for the first time. They also permit, in conjunction with the first part of the enquiry (Manton & Oates 1983), some constructive comments to be put forward on a topic not previously approached directly at all, namely, the possible functional significance of unmineralized components of coccoliths in general.

Materials and methods

Both our species were collected on several occasions and in different water samples though most of those illustrated, as indicated in the legends, came from an exceptionally rich sample, termed 'A1'. This was collected at 16.30 h in clear weather on 15 August 1977 by Dr Margaret McCully (of Ottawa) and Mrs A. D. Greenwood, travelling together as passengers on M.S. *Iguana*, which had anchored near Bartolomé Island (Sullivan Bay), permitting their first sample to be drawn up in a van Dorn bottle from a depth of 10 m (on the bottom). Sea temperature was 22 °C. This sample was partly processed at once (see below) before being delivered, temporarily fixed in 1% glutaraldehyde, to the Charles Darwin Research Station for completion by the three shore-based members of the same party – the senior author (I.M.) assisted by A. D. Greenwood (of London) and Miss Joan Sutherland – by whom the other samples were collected. These, as listed in table 1, involved use of a dinghy with an outboard motor, operated from the Charles Darwin Research Station and therefore limited to various parts of Academy Bay. An exception was sample 'Darwin 21', collected by A. D. Greenwood and M. Zambriano (of Guayaquil), while travelling as passengers on a motor vessel chartered for another purpose by the Charles Darwin Research Station.

The practical details of making dry whole mounts of freshly gathered flagellates are now standard: for further information see Manton & Oates (1983). It is enough to say here that osmic fixation was usual, though the samples designated A in table 1 are those collected from M.S. *Iguana* and temporarily fixed in glutaraldehyde as noted above. All whole mounts made in the field, whether on glass slides or on coated electron microscope grids, were rinsed at once in de-ionized rain water to remove salt crystals before being stored, dry, until required for further processing in England. Grid-preparations were then routinely shadowcast with gold palladium in readiness for transmission electron microscopy. At a later stage, selected preparations (with a few notable exceptions) were given an additional coating with gold to prepare then for the use of a scanning electron microscope.

The three transmission electron microscopes used personally by the senior author (I.M.),

as specified in the legends, include an A.E.I. EM 6B microscope located in the Cell Biology Unit, University of Nottingham; a similar microscope in the Department of Gynaecology, Leeds University (figure 23 (inset only)) and a JEOL Temscan in the Lancaster department. The scanning electron microscopy, in contrast, did not involve the senior author directly but was carried out either on a JEOL T20 in the Portsmouth laboratory operated by G. Bremer or on the JEOL Temscan instrument listed above, used in the scanning mode by K. Oates.

TABLE 1. SOURCES OF ILLUSTRATED SPECIMENS OF *MICHAELSARSIA/HALOPAPPUS* FROM THE GALAPAGOS ISLANDS (1977)

sample	locality	date	depth m	sea temperature °C
Darwin 8	Academy Bay	12 Aug.	10	21
Darwin 11	Academy Bay (mid channel)	12 Aug.	15	21
Darwin 13	Academy Bay (near M.S. *Iguana*)	13 Aug.	10	22
Darwin 21	Barrington Island, off NW point	16 Aug	15	18
A1†	Bartolomé Island (Sullivan Bay)	15 Aug.	10 (on bottom)	22
A6†	Fernandina Island (Punta espinosa)	16 Aug.	surface	19
A8†	James Island (Buccaneer Bay)	17 Aug.	15	22

† These samples were collected by the boat party (Dr Margaret McCully of Ottawa and Mrs A. D. Greenwood of London) and partly processed on board M.S. *Iguana* before being delivered fixed in glutaraldehyde to the senior author for finalizing at the Charles Darwin Research Station (Academy Bay).

The light microscopy, carried out last by the senior author as a means of confirming the correctness of the magnifications cited, involved a Zeiss Photomicroscope set up for phase contrast in the Cytogenetics Unit at the Medical School, University of Liverpool. These observations were recorded on 35 mm film (Ilford Pan F) subsequently enlarged in a uniform manner on a Leitz Focomat enlarger belonging to the Royal Society but still available to the senior author in Leeds. Photographs taken with a dry lens (× 40) gave a final magnification of exactly × 1000, while those taken with an oil immersion objective (× 63) dipped into a drop of Objectol placed without a coverslip on a previously selected cell, gave a final magnification of × 2500. After this the Objectol could be removed without harm to the specimen by a rinse in amyl acetate. Use of immersion fluid in this way is of course only possible on a glass preparation, hence the limitation of light microscopy on grid preparations to the dry lens only.

In presenting the results we have followed our usual practice of recording in the legends the relevant technical details of water sample number (quoting table 1), exposure number and microscope used. These facts cannot be added later and they enhance the meaning of comparisons with earlier work, including Manton & Oates (1983), drawing on the same or similar material. While we have been at pains to make the legends self-explanatory as written, we have on this occasion made greater use than formerly of initial letters to summarize certain sorts of information. Thus the initial Y standing alone before an exposure number means an electron micrograph taken personally by the senior author fairly recently. YO or YB similarly placed means an electron micrograph taken by one or other of the co-authors. Finally the addition of an N or L subscript (Y_N or Y_L) means a micrograph taken recently by the senior author at either Nottingham or Lancaster. Where a specimen has been examined in both places, knowledge of this fact may be more important in assessing the reliability of calibrations, etc., than the mere listing of the microscope specifications, although these are also provided.

Observations

As is clear from the literature cited in the Introduction, the most conspicuous diagnostic feature of both *Michaelsarsia* and *Halopappus* is the crown of anterior appendages, each of which, when intact, is at least as long as the subtending cell body (see, for example, plate 5). At first sight these appendages recall those of *Calciopappus* Gaarder & Markali, though the construction is quite different. Whereas those in *Calciopappus* are spines, each consisting of a single, greatly modified coccolith (for some recent illustrations see Manton & Oates (1983), the equivalent in *Michaelsarsia* and its near relatives, though formerly described as 'jointed tubes' (Gran 1912; Murray & Hjort 1912) are now known to be chains of modified flat coccoliths: for a recent description see Heimdal & Gaarder (1981). Each chain-link consists of a narrow plate greatly elongated in the direction of its long axis and bordered by a fully calcified rim conspicuously outlining a relatively transparent slit-like centre. Adjacent links are united linearly, and in each of our two species there are characteristically three links per chain unless some have been lost by mechanical damage, which can occur all too easily as a preparation artefact. However, a loose link from the distal tip of a chain, even when separated from its place of origin, can be clearly recognized as such by its gentle taper and terminal 'bayonet-point' (figure 2, plate 1). Proximally, however, the whole 'parachute or pappus', a phrase used by Murray & Hjort (1912), is subtended by a whorl of differently modified coccoliths, each almost circular in outline, with a wide calcified rim surrounding an apparently empty circular centre.

The unmodified coccoliths covering the cell in both genera are elliptical plates, technically termed 'incomplete caneoliths' (Heimdal & Gaarder 1981). Each consists of a calcified rim, a thickened elliptical centre and a series of spoke-like bars bridging the gap between these two regions. In addition, a few, much smaller, coccoliths, rhomboid in shape and with the central thickening reduced to a short central projection, are limited to the depression immediately surrounding the flagellar bases, and for this reason are not always exposed.

Michaelsarsia elegans Gran

It will be convenient to begin with *Michaelsarsia elegans* (plates 1–4) even though it is less abundant than its fellow in the Galapagos Islands. The appendages are nevertheless inherently simpler though they are more easily lost. Such loss does not impede further study, in the light of existing knowledge, provided only that enough has been retained to ensure taxonomic recognition. This pre-condition is demonstrably met in each of the specimens (selected from others) illustrated in plate 1 and one, represented here by figure 4*b*, has also been personally identified by Mrs Gaarder whom we wish to thank.

The relative sizes and shapes of the more important types of modified and unmodified coccoliths can be ascertained in a general way by a glance at the fragments from a broken cell illustrated in figure 1, supplemented perhaps by figure 2. In each type of coccolith, the apparent emptiness of all regions not occupied by crystallites is as expected from the literature. When less damaged, there is generally no difficulty in recognizing the appendages as such, even with a dry lens (figures 3*a* and 4 *a*) though body coccoliths require better resolution and a higher magnification to become individually visible.

These requirements can of course be met in several ways. Thus a specimen dried on a glass slide (figure 3*a*), if immersed as explained above, can provide the three significantly different focal levels for a photographic record made with an oil immersion objective (figure 3*b–d*). Two

of these images show individual coccoliths on the top or bottom surface respectively (figure 3*b*, *d*) while the median optical section (figure 3*c*) displays not only the cell shape, including the apical depression, but also the greatly shrunken protoplast (left) thereby emphasizing the rigidity of the main periplast apart from the appendages.

An incidental detail that should perhaps be noted at once to avoid risk of misunderstanding is the reduced visibility amounting to virtual disappearance from figures 3*b–d* of the rod-like diatom appendage against which the cell had come to rest and which crosses the field of figure 3*a* so conspicuously when only a dry lens is used. This behaviour expresses no more than the difference in optical properties of a silicified versus a calcified object in the immersed and dry condition and it can indeed, under certain circumstances, be used as a substitute for a chemical test as between a diatom and coccolith.

All the other specimens illustrated on plate 1 had been mounted from the beginning on support films ready for electron microscopy. Oil immersion cannot therefore be applied to them though two relatively complete individuals were successfully examined by both transmission and scanning electron microscopy. One of these (figure 4*a–c*) had been alive when collected since the two flagella are still present (figure 4*b*) in spite of the loss of many appendage segments. The other cell (figure 5*a–b*), from a different water sample, may not have been alive when collected since disintegration has begun. The periplast of this is exposed somewhat end-on, hence the more circular outline compared with the acorn shape of the other cells.

The electron micrographs reproduced at low magnifications on plate 1 fail to suggest any significant departure from expectation based on the literature. However, higher magnifications reveal several unexpected new features. The first, and in some ways the most important of these, since a key to the general situation is thereby provided, concerns the ring-shaped coccoliths subtending the apical appendages. Here, the supposedly empty central areas can betray the

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Description of plates 1 and 2

Michaelsarsia elegans: light and electron microscopy from shadowcast whole mounts of cells dried on support films except where otherwise stated (figure 3*a–d*).

Figure 1. Field of detached coccoliths of the more important categories from a single broken cell in sample 'Darwin 8' (table 1). Transmission electron micrograph Y_N 7939.13 (EM 6 B, Nottingham), magn. ×*c.* 4000.

Figure 2. Two distal appendage-links from a broken cell in sample 'Darwin 11' (table 1) showing the terminal 'bayonet-point'. Transmission electron micrograph Y_N 7977.21 (EM 6B, Nottingham), magn. ×*c.* 8000.

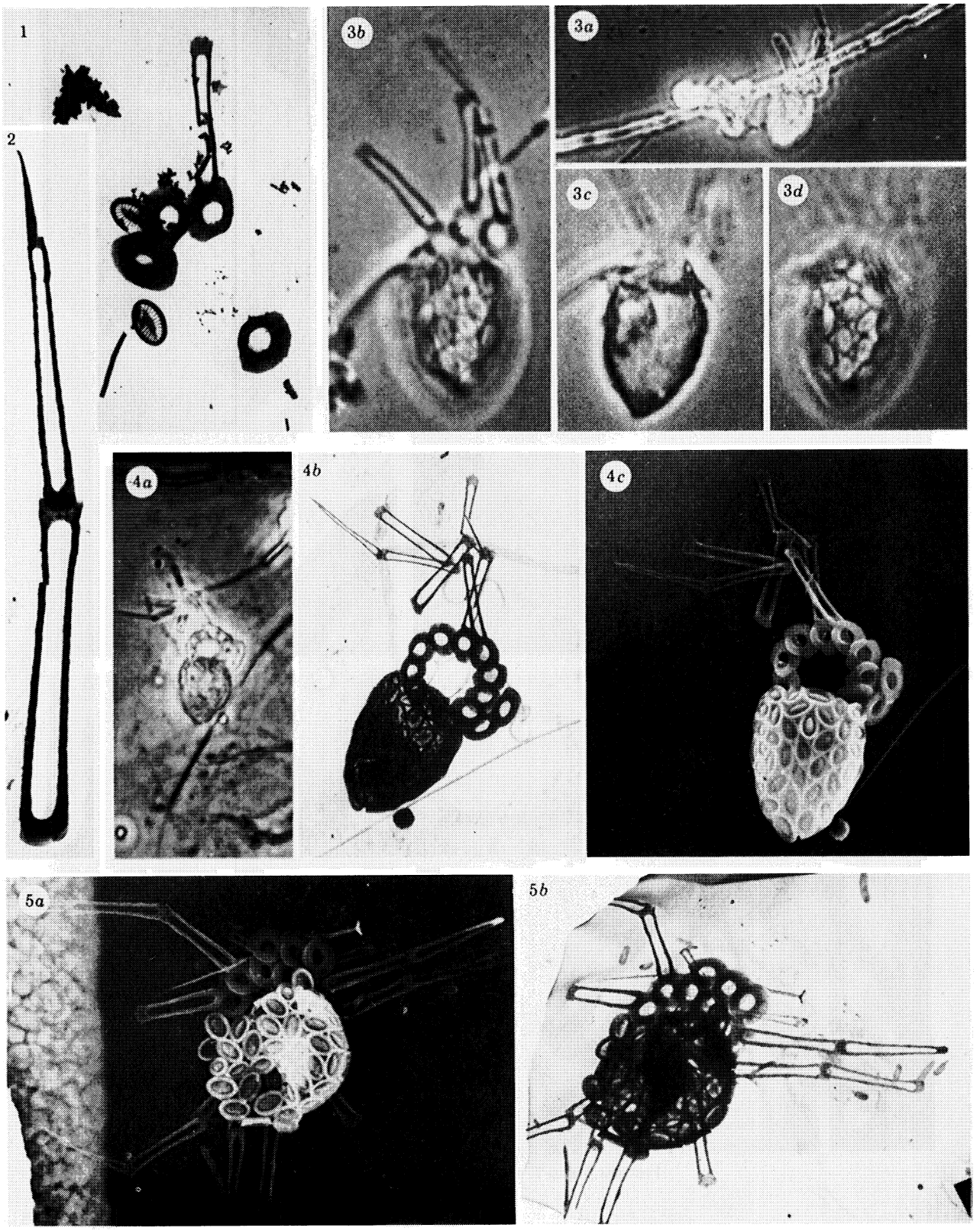
Figure 3. Light microscopy (phase contrast) of a cell from sample 'A1' (table 1), dried on a glass slide. (*a*) Photograph taken with a dry lens, exposure 241.1, magn. ×1000. (*b–d*) Three different focal levels photographed under oil immersion: exposures 192.29, 192.30, 192.31, magn. ×2500.

Figure 4. Another cell from sample 'A1' (for details see plate 2). (*a*) Light microscopy (dry lens), exposure 241.12, magn. ×1000. (*b*) Transmission electron micrograph Y_N 7933.13 (EM 6B, Nottingham), magn. ×2000. (*c*) Scanning electron micrograph YB 8229.5 (Portsmouth), magn. ×2000.

Figure 5. A cell from sample 'Darwin 21' (for details see plate 3). (*a*) Scanning electron micrograph YB 8227.5 (Portsmouth), magn. ×2000. (*b*) Transmission electron micrograph, taken last and showing the support film in the act of tearing. Micrograph Y_L 8232.8 (Temscan, Lancaster), magn. ×*c.* 2500.

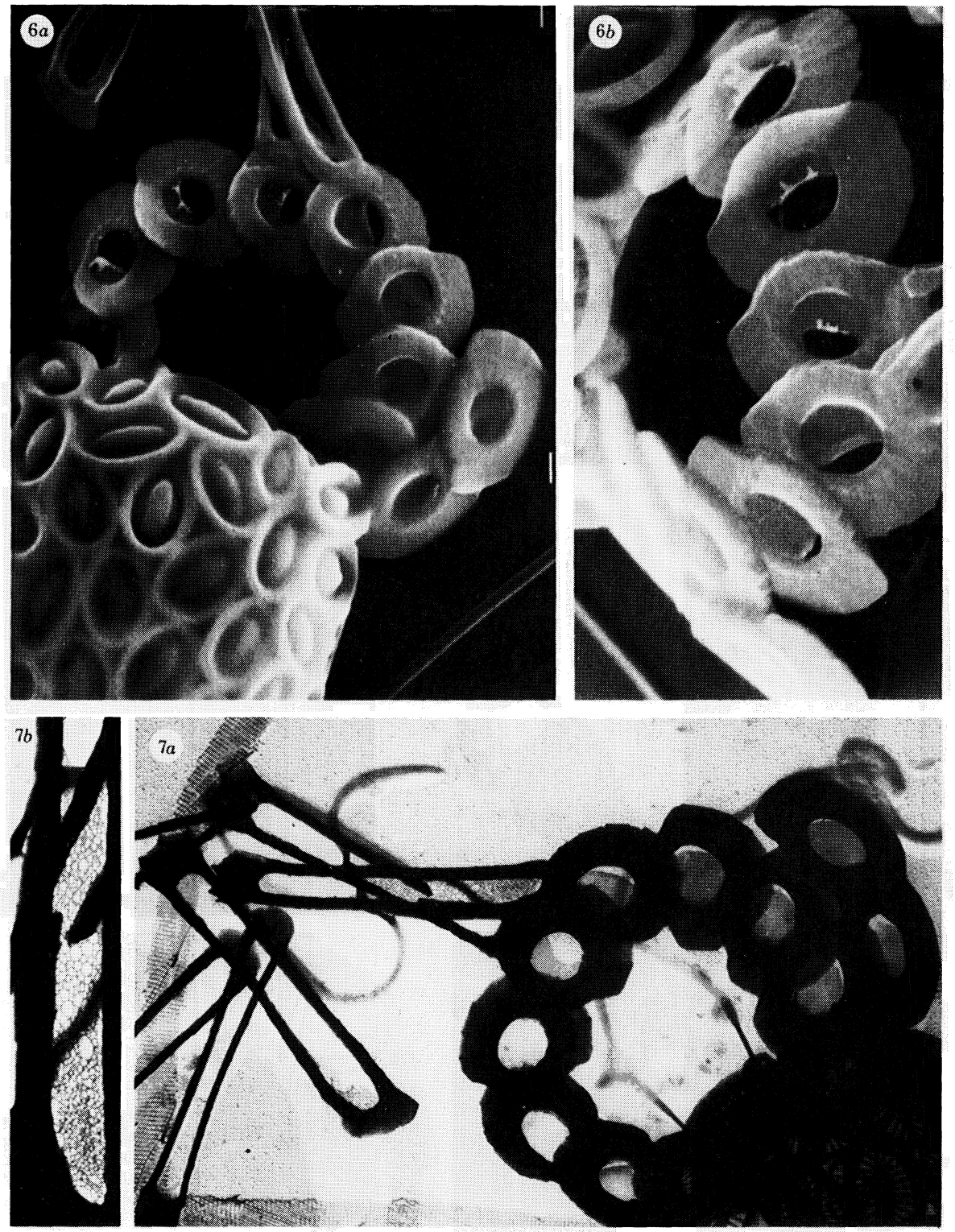
Figure 6. More highly magnified scanning electron micrographs of the anterior end of figure 4*b* showing splitting membranes in ring-coccolith centres with other details on ordinary coccoliths. (*a*) Exposure YB 8229.9 (Portsmouth), magn. ×7500. (*b*) The same specimen tilted (50°) and rotated through 80°; exposure YB 8230.10 (Portsmouth), magn. ×*c.* 15000.

Figure 7. The specimen of figures 6*a* and *b* showing enhanced contrast of unmineralized components conferred by the pretreatment involved in scanning. (*a*) The splitting membranes in ring centres now visible (compare shapes with those in figure 6*a*). Transmission electron micrograph Y_N 7933.14 (EM 6B, Nottingham), magn. ×7500. (*b*) Part of an appendage-link from the same field to show the unmineralized central mesh more clearly; magn. ×*c.* 18000.

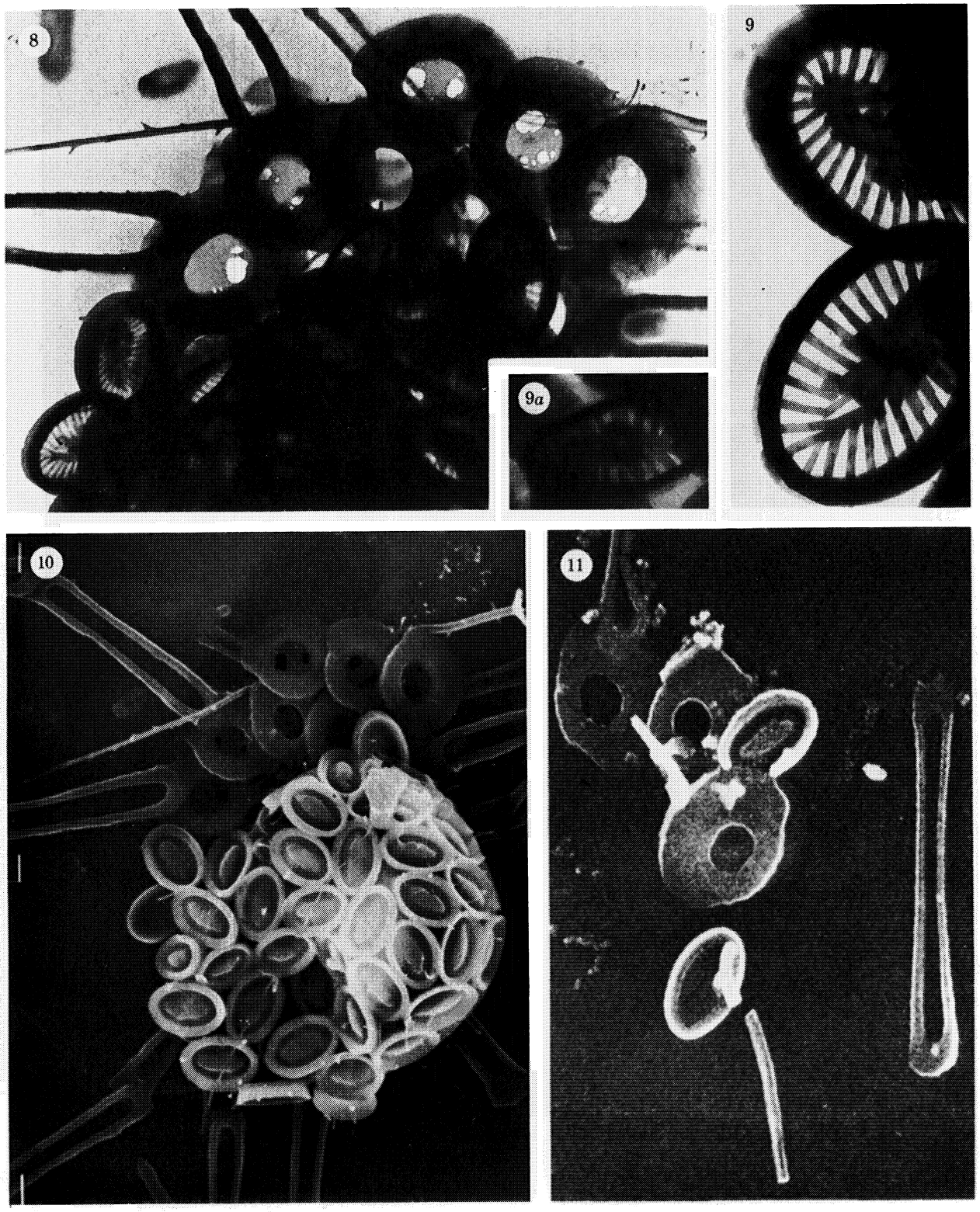


FIGURES 1–5. For description see opposite.

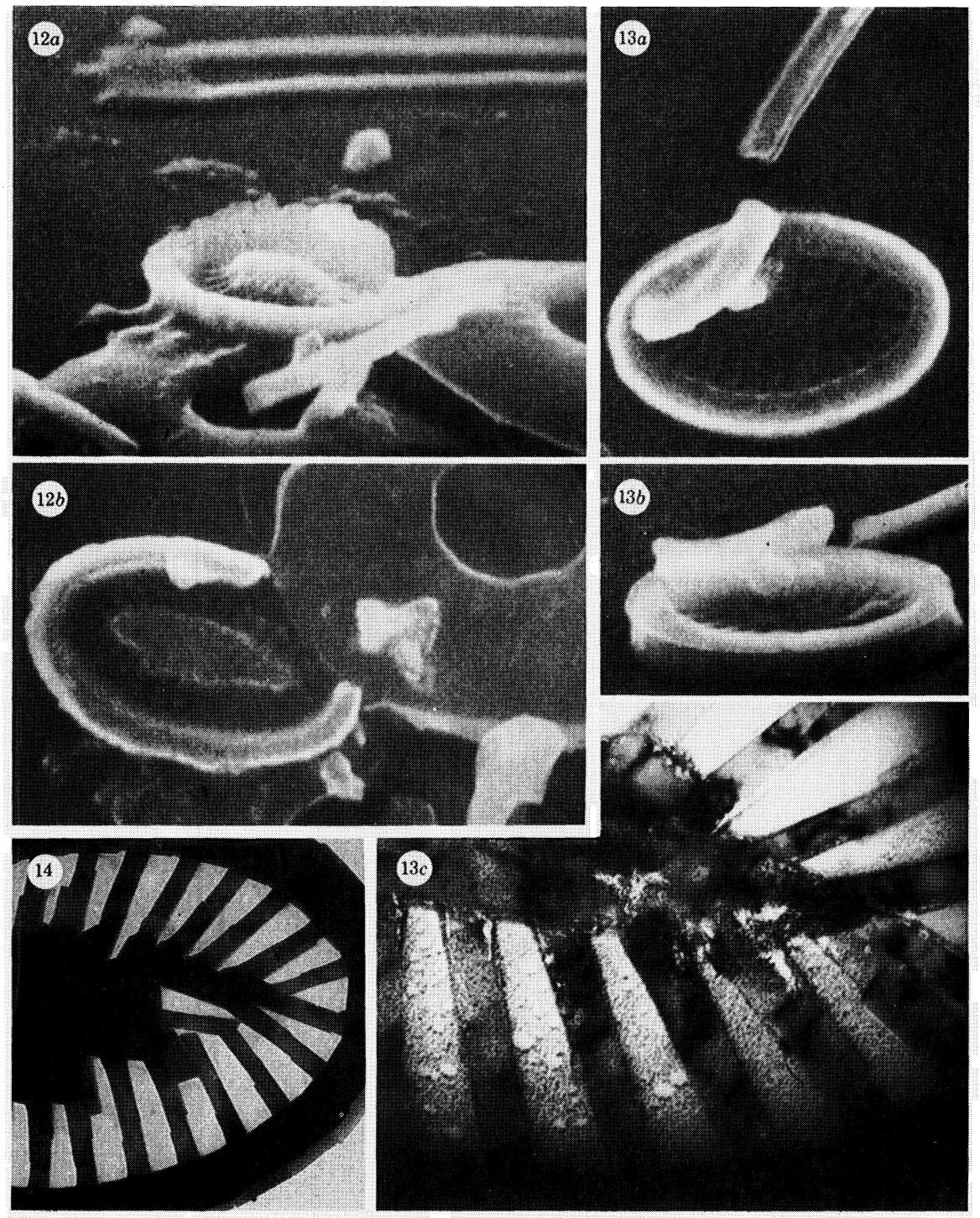
(*Facing p.* 188)



FIGURES 6 AND 7. For description see p. 188.



FIGURES 8–11. For description see p. 189.



FIGURES 12–14. For description see opposite.

presence of unmineralized material by exhibiting one or other of two alternative types of mechanical breakdown. These are illustrated respectively in plates 2 and 3. Plate 2, limited to more highly magnified views of parts of the cell of figure 4*a–c*, shows that when scanned (figure 6*a*, *b*) at these magnifications an arresting new observation is the split condition of centrally placed, unmineralized material in many, though not quite all, of the ring-shaped coccoliths. Transmission microscopy, when first applied at a comparable magnification, had given no noticeable sign of such structural damage but when the same specimen was re-examined after completion of scanning the differential opacity of unmineralized areas, conferred by the extra coating with gold, permitted easy recognition in a transmission electron micrograph (figure 7*a*), of split and unsplit membranous regions corresponding exactly in size, shape and position with those already seen in figure 6*a*.

Plate 3, based on the cell of figure 5*a*, *b*, provides a similar record of the second type of mechanical damage, namely puncturing in many places, presumably as a result of incipient chemical breakdown, in a cell that we already believe to have been dead before collection. In this case, although again undetected with the initial transmission microscopy, re-examination of the preparation after completion of scanning (and the addition of extra gold) showed the perforations to have become so conspicuous as to be even more clearly visible in figure 8 than when illustrated by scanning itself in figure 10. In both these micrographs, individual perforations can be separately identified and seen to correspond. The combined evidence for the presence of a membrane in each of these coccoliths is thus incontrovertible.

Evidence of an even more unexpected kind is obtainable from the appendages themselves. A delicate meshwork, broken away in many places but retained intact here and there, can be seen in several of the appendage links illustrated in figure 7*a*, one of which is shown more highly magnified in figure 7*b*. Fragments of such a meshwork are not uncommon in wholly detached appendages elsewhere and the undoubted emptiness of the slit-like centres of the two terminal

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Description of plates 3 and 4

Michaelsarsia elegans: further substructural details from the specimen illustrated on plate 1.

Figure 8. Part of the specimen of figure 5*a* taken after completion of scanning but before rupture of the support-film shown in 5*b*; the visibility of perforated membranes in ring-centres enhanced as a side effect of the pretreatment for scanning. Transmission electron micrograph Y_L 8232.4 (Temscan, Lancaster), magn. ×7500.

Figure 9. Body coccoliths from the field of figure 8 taken before scanning and therefore without enhanced contrast of unmineralized parts. Transmission electron micrograph Y_N 7907.6 (Nottingham), magn. ×20000 (for further details see figure 14). (*a*) A small rhomboidal coccolith with a tubular central excrescence. Transmission electron micrograph Y_N 7907.4 (EM 6B, Nottingham), magn. ×15000.

Figure 10. Another view of the specimen of figure 5*a*, showing the perforated membrane in ring-centres among other details, in a manner permitting comparisons with figure 8; an alien spine entering the field at top. Scanning electron micrograph YB 8227.7 (Portsmouth), magn. ×5500.

Figure 11. The specimen of figure 1 turned over and scanned, showing two body coccoliths exposing different surfaces (further details on plate 4). Micrograph YO 8300.19 (Temscan, Lancaster), magn. ×7500.

Figure 12. Two views of the same coccolith (upper) in the field of figure 11, both magn. ×20000. (*a*) Tilted 60°, scanning electron micrograph YO 8300.10. (*b*) Untilted. YO 8300.12.

Figure 13. Three views (Temscan, Lancaster) of one and the same coccolith (lowermost) from the field of figure 11. (*a*) Untilted; scanning electron micrograph YO 8300.14, magn. ×20000. (*b*)Tilted 60°; scanning electron micrograph YO 8300.6, magn. ×20000. (*c*) Transmission electron micrograph showing multiperforate membrane on the exposed proximal surface; exposure Y_L 8298.9 (Temscan, Lancaster), magn. ×100000.

Figure 14. Part of the lower coccolith in figure 9 without the enhanced contrast conferred by pretreatment for scanning; membrane perforations though present are faint (contrast with 13*c*); transmission electron micrograph Y_N 7907.6 (EM 6B, Nottingham), magn. ×40000.

links illustrated in figure 2 must therefore be artefactual. The exceptional interest of this particular unmineralized component may become more apparent later.

In contrast to the appendages, the body coccoliths are in some ways more difficult to analyse. This partly results from the stability of the periplast as a whole, which rarely permits single body coccoliths to be examined against a background of no more than the support film, an essential prerequisite to detection of several critical details. Two fortunately placed coccoliths are nevertheless present on the left of the specimen of figure 8 and these are shown again at a higher magnification in figure 9. These indicate clearly that the central thickening, detectable as no more than an elliptical streak on coccoliths seen with the light microscope, notably figure 3*b–d*, are in fact built up from relatively broad rectangular crystallites arranged in a mound since an undisturbed periplast scanned from without shows this region on each ordinary coccolith to be strongly convex (figure 6*a*). The radial 'spokes' on the other hand, though at first sight consisting of single rectangular crystallites are in fact compound, each being crossed more or less centrally by a somewhat zigzag joint where two crystallites, often slightly different in width, are united approximately end to end. The stepped outlines of several bars from part of the lower coccolith in figure 9, exposed in apparent silhouette at a higher magnification in figure 14, are perhaps sufficiently indicative of this general situation.

Figure 14 also contains evidence for the presence of a membrane, at least in the gaps between the radial bars, though the tiny perforations localized here are faint and may be difficult to see convincingly after mechanical reproduction. Fortunately supplementation from another specimen can be provided. Thus the field of scattered coccoliths already illustrated in figure 1 contains two superficially identical body coccoliths each showing the elliptical shape and central thickening to an almost equal degree. In contrast, when this preparation was turned over and scanned (figure 11), marked differences became evident. The central thickening is directly visible as a mound in one body coccolith but not in the other and the same difference is detectable between the two laterally projecting coccoliths when seen by scanning in figure 10. The explanation for these differences is at once revealed by tilting. When the field of figure 11 is tilted, it is immediately obvious that one coccolith (figure 12*a*, *b*) is lying with its distal face exposed, hence revealing the central mound directly, while the other (figure 13*a*, *b*) is lying upside down. The exposed under (proximal) surface can then be seen to be either flat or concave, an interpretation confirmed by other views of inverted coccoliths including at least one edge view detectable in other parts of the specimen of figure 10.

Since we know from this that the lower member of the pair of coccoliths illustrated earlier in figure 9 is inverted, it may not be coincidence that, without this knowledge, this coccolith had been selected for use in figure 14 rather than its fellow and the same preferential suitability for our present purpose is displayed by the inverted coccolith of figure 13*a*, *b*. As may be seen at a much higher magnification in figure 13*c* this specimen displays an almost colander-like perforated membrane not only between but also partly spreading over the surfaces of the bar-crystallites. This specimen thus completes the evidence for the presence of an intrinsically patternless very thin membrane spread across the proximal face of an ordinary body coccolith in this species.

Less can be said about the only remaining scale type, namely the small rhomboids. These are restricted to the apical depression and, being few in number, they are rarely exposed. An example from the field of figure 8 is nevertheless illustrated in figure 9*a* and this is noteworthy mainly because of the details of the central projection. This appears to be tubular, and not

a closed point as expected from the diagnosis given by Heimdal & Gaarder 1981. This somewhat trivial (in our opinion) departure from expectation will be further discussed later.

Finally, coccolith arrangement, as demonstrated best on plate 1 by means of light microscopy and scanning, seems to us very far indeed from 'coccoliths placed at random' (Heimdal & Gaarder 1981, p. 441). This matter will be further discussed below but it can be said at once that coccolith distribution seems to us to be not random but a clear example of hexagonal close-packing.

Halopappus adriaticus Schiller

Cells attributable to *Halopappus adriaticus* (plates 5–8) are substantially larger than those of *M. elegans* and since they are also more numerous there would have been no difficulty in multiplying examples of intact cells, complete with all their appendages, had this been desirable. As it is, plate 5 may sufficiently illustrate the range of body shapes – conical, elliptical or almost oblong – together with some of the variations in appendage numbers (eight in figure 16, nine in figure 15 and more in figure 18), characteristic of the Galapagos material as also elsewhere.

The calcified appendages are somewhat more slender than those of *M. elegans* though they are otherwise similar, except for one detail. The central slit in each appendage-link is less empty than in the other species. This feature, detectable in a general way even with the light microscope (plate 5), is of course more conspicuous when transmission electron microscopy is applied, as for example in figure 24*d*, plate 7. As will be shown, this is almost the only strictly qualitative character separating *H. adriaticus* from *M. elegans*.

A specific criterion sometimes treated as qualitative, though in our view mistakenly, is the arrangement of body coccoliths. This was discussed in detail with respect to *Calciopappus* in Manton & Oates (1983), p. 455, and the same considerations apply here. When seen in scanning electron micrographs such as those illustrated in plate 6, there is no difficulty in recognizing hexagonal close-packing as the basic arrangement of the body coccoliths. These also appear to be in almost transverse but slightly tilted curved rows to which the phrase 'coaxial rings' has sometimes been applied (Heimdal & Gaarder 1981). However, these rows, in this case arranged across a virtually cylindrical surface, cannot be rings. They seem necessarily to be parts of a spiral continuum of the kind inseparable from hexagonal close-packing in general. The equivalent appearance, suggesting an almost vertical alignment in *M. elegans* (figures 3*b–d* and 4*c*), is based on a quantitative difference involving several components such as cell size, the degree of curvature of the surface, and the individual dimensions of the coccoliths themselves. These are all quantitative rather than qualitative differences, the underlying coccolith arrangement (hexagonal close-packing) being essentially the same in both taxa.

Other quantitative, though less conspicuous, differences between these taxa are also present. Thus the periplast of *H. adriaticus* is less rigid than that of *M. elegans* and the coccoliths are in consequence more easily detached (as for example in figure 24 *c*, plate 7). Collapse of the periplast as a result of shrinkage of the protoplast on drying can then cause greater disturbance to body coccoliths especially when exposed on a cell mounted on a glass slide. Under these conditions (plate 5) the light microscope, used alone, could be highly misleading. Thus in figure 15, the white outlines of coccoliths produced by trapped air suggest a transverse orientation, while the equivalent view in figure 16 appears inextricably confused. Both these conditions nevertheless express no more than disturbance, thereby contrasting with the truer images obtainable by scanning (plate 6) of cells dried against the less rigid background of a

support film. That a comparable difficulty was not encountered with *M. elegans* was due to a minor difference of technical treatment. The immersed cell illustrated in figure 3*b–d* had been found last after the preparation had been rinsed in amyl acetate to remove air before being remounted in connection with a different object.

A qualitative character of doubtful value is supplied by the small rhomboidal coccoliths, which line the apical depression, as noted by Gaarder. Each carries a short, outwardly directed, central projection and two of these are visible in the expected position in figure 20*c*. Each projection is an open tube as expected (see also figure 23 inset) but because we failed to confirm that the equivalent in *M. elegans* is closed (cf. figure 9*a*), the dependability of this character for diagnostic use at any level seems to us more than doubtful.

In other respects the ordinary coccoliths of *H. adriaticus* are virtually identical structurally to those of *M. elegans*. The radial bars in particular are clearly compound (plates 7 and 8) and though individual crystallites may be narrower, notably those building up the central mound (figure 24*c*) the fact that they are also thinner and less opaque to electrons is important in only one practical context, namely that of distinguishing the two scale faces as such when only one is exposed. In *M. elegans* this could be done at once merely by means of scanning electron microscopy since in that species, as we have seen (plates 3 and 4), the central mound itself is only directly visible when a coccolith is scanned from the distal surface. In *H. adriaticus*, in contrast, the mound is equally visible from both surfaces no matter whether scanning or transmission electron microscopy is applied (compare, for example, figure 24*b*, *c*). Other criteria must therefore be used, as discussed further below.

The most important qualitative characters distinguishing *H. adriaticus* from other taxa are limited to the appendages. As already noted in connection with figure 24*d*, each appendage-link possesses a series of thin, almost rectangular, crystallites attached peripherally to the calcified rim and extending thence over part or all of the supposedly unoccupied centre. Somewhat similar additional crystallites border the circular central area in ring-shaped coccoliths (figures 22 and 23) also. These fringing crystallites, in both positions, are not only of interest in themselves, as illustrated in plates 7 and 8, but they also affect the ease or difficulty with which

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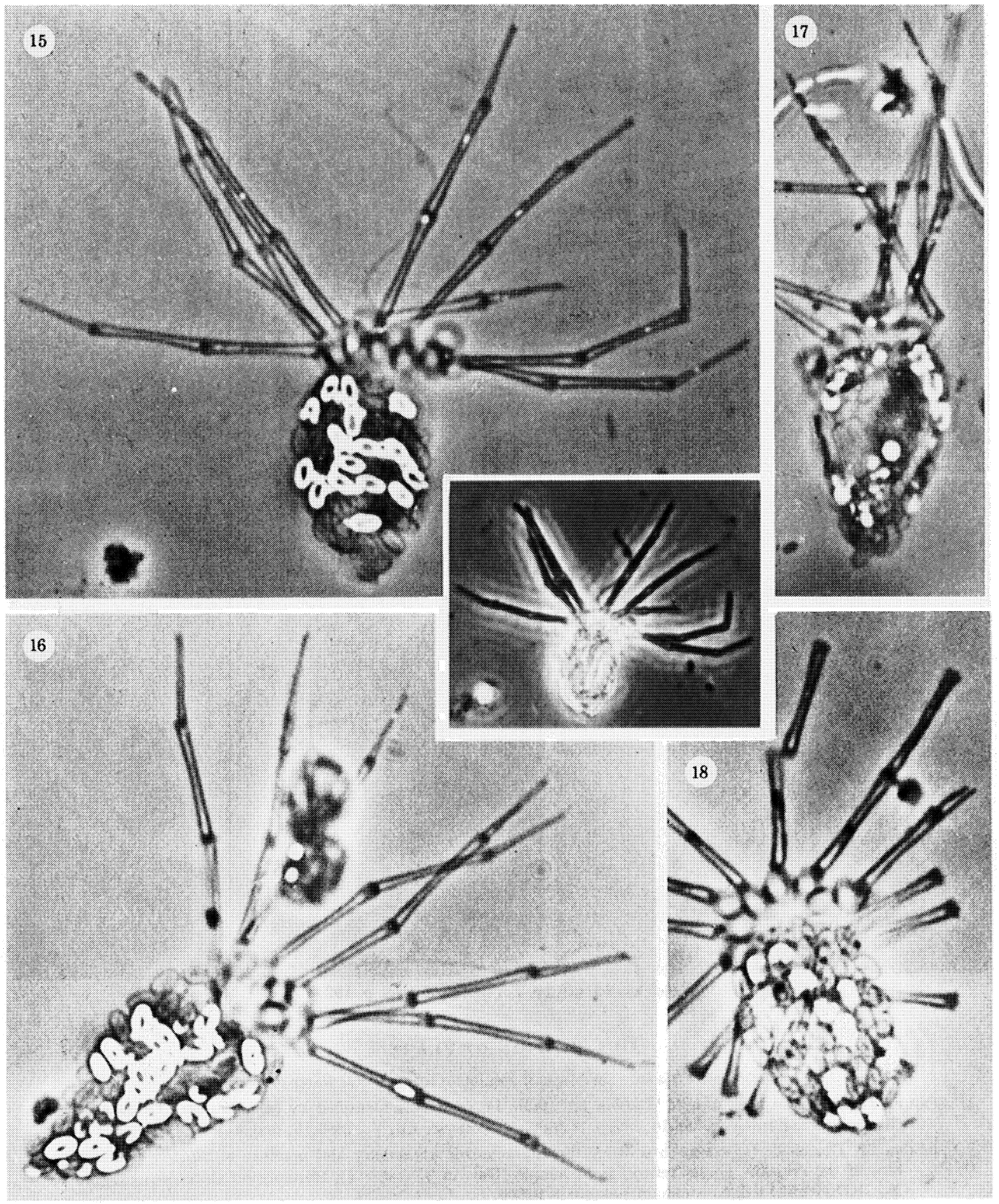
Description of plates 5 and 6

Halopappus adriaticus Schiller: six different cells from one water sample ('A1' in table 1) mounted dry on glass slides (plate 5) or on support films (plate 6).

Figures 15–18. Phase contrast light microscopy to show range of body shapes and appendage numbers, but see text for comments on coccolith arrangement. Exposures 196.16, 195.6A (inset), 192.18A, 196.15, 194.11. Inset taken dry with a dry lens, magn. ×1000; all others immersed and taken with an oil immersion lens, magn. ×2500.

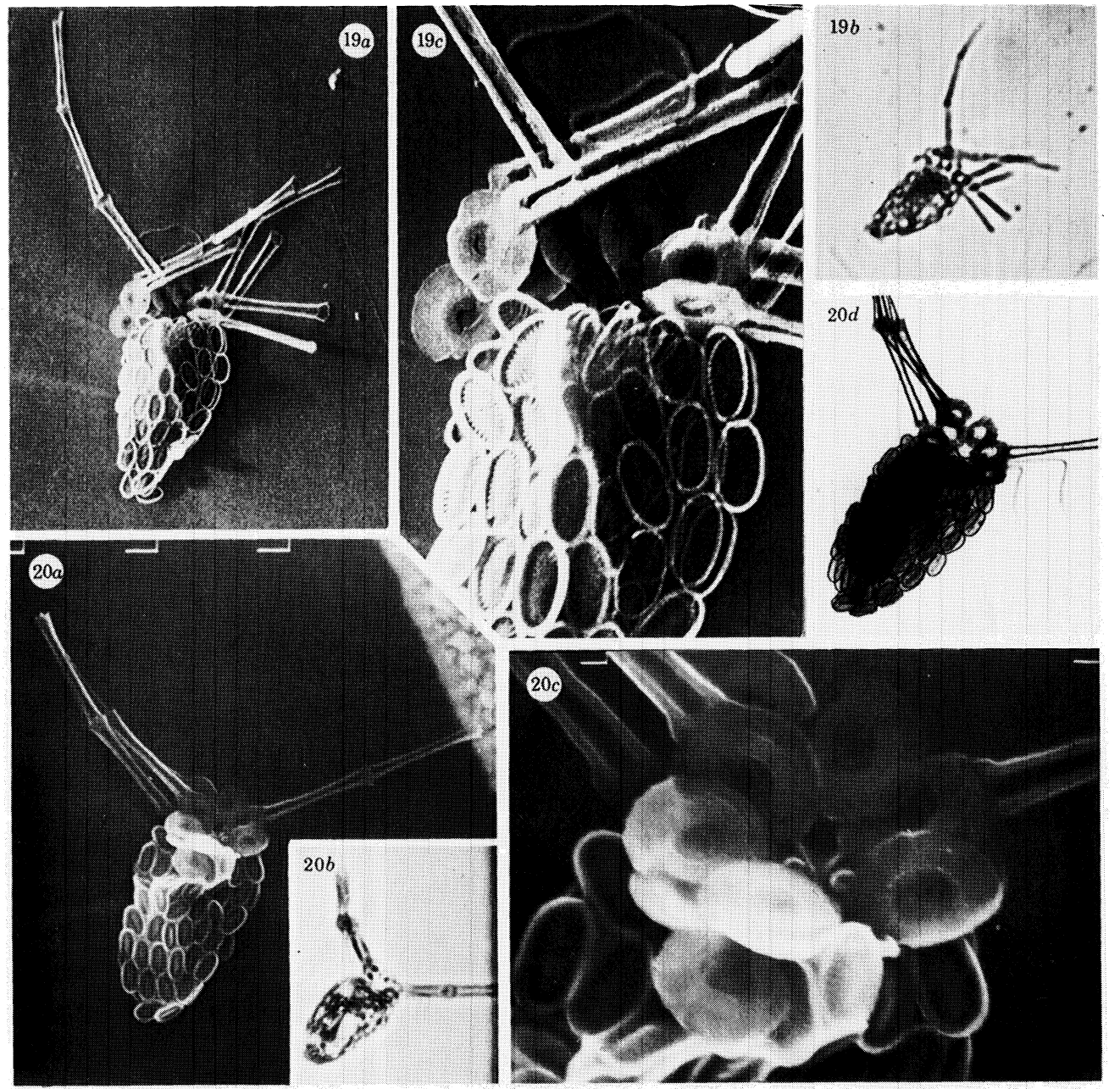
Figure 19. Cell with undisturbed coccolith covering but appendages mainly incomplete. (*a*) Scanning electron micrograph YO 7970.14 (Jeol Temscan, Lancaster); magn. ×2000. (*b*) Light microscopy (dry lens) to confirm cell size; exposure 166.7A, magn. ×1000. (*c*) Scanning electron micrograph YO 7970.15 (Temscan, Lancaster); magn. ×6000.

Figure 20. Cell with broken appendages and slightly disturbed periplast but still with two flagella and showing coccolith arrangement near the flagellar pole. (*a*) Scanning electron micrograph YB 8225.1 (Jeol T20, Portsmouth); magn. ×2000. (*b*) Light microscopy (dry lens) exposure 166.5, magn. ×1000. (*c*) Anterior end of the cell showing tips of cylindrical projections on two of the small coccoliths near the flagellar pole (see further figure 23 inset); scanning electron micrograph YB 8225.3 (T.20 Portsmouth); magn. ×7500. (*d*) Transmission electron micrograph of the cell to show the two flagella; exposure Y_L 7970.26 (Temscan, Lancaster); magn. ×2000.



FIGURES 15–18. For description see opposite.

(*Facing p.* 192)



FIGURES 19 AND 20. For description see p. 192.

DESCRIPTION OF PLATE 7

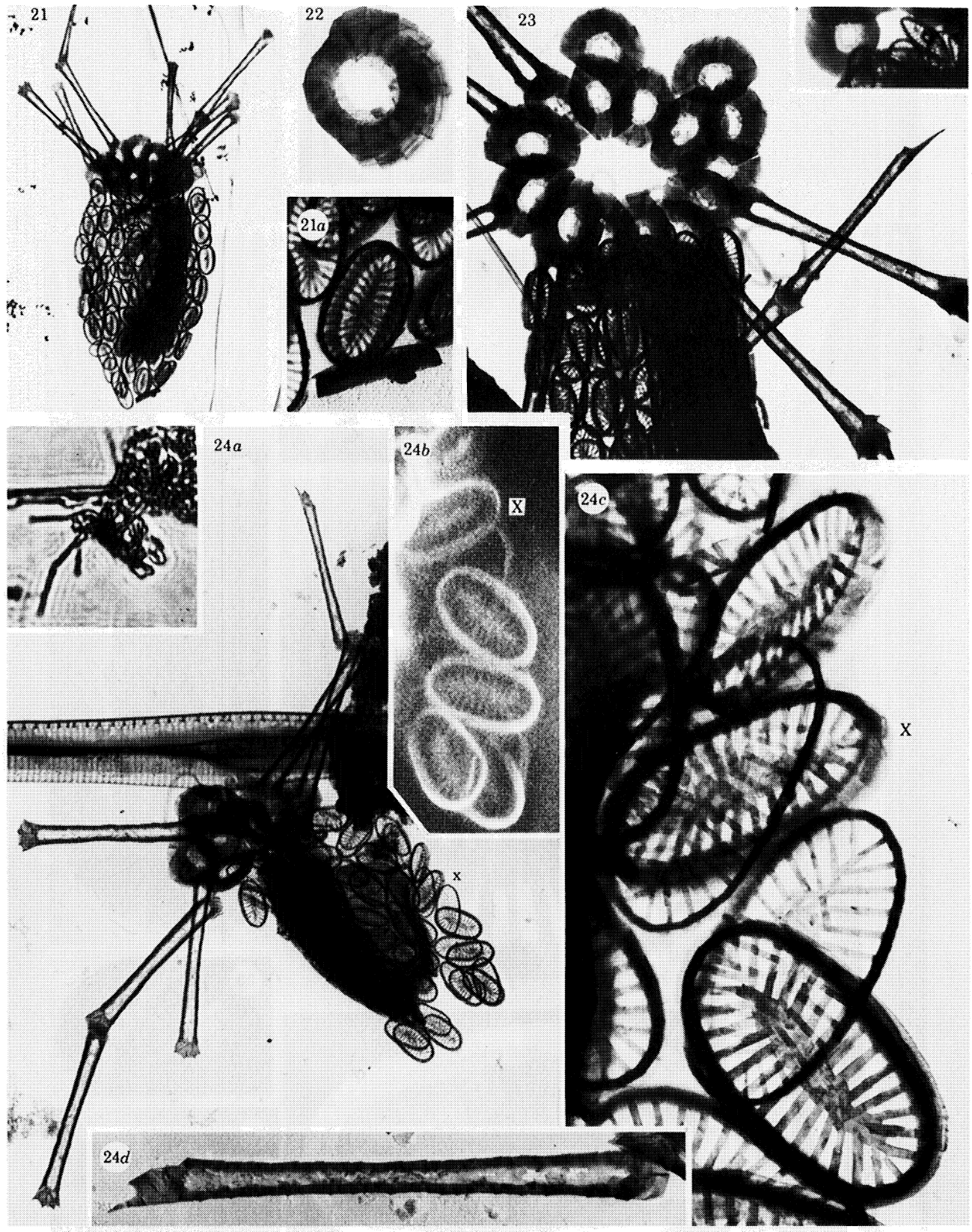
Halopappus adriaticus: shadow cast specimens from several water sources.

FIGURE 21. Specimen from sample 'Darwin 13' (table 1), personally identified by Mrs Gaarder. Transmission electron micrograph Y_N 7888.14; magn. ×3000. (*a*) Posterior tip; Y_N 7888.16; magn. ×10000.

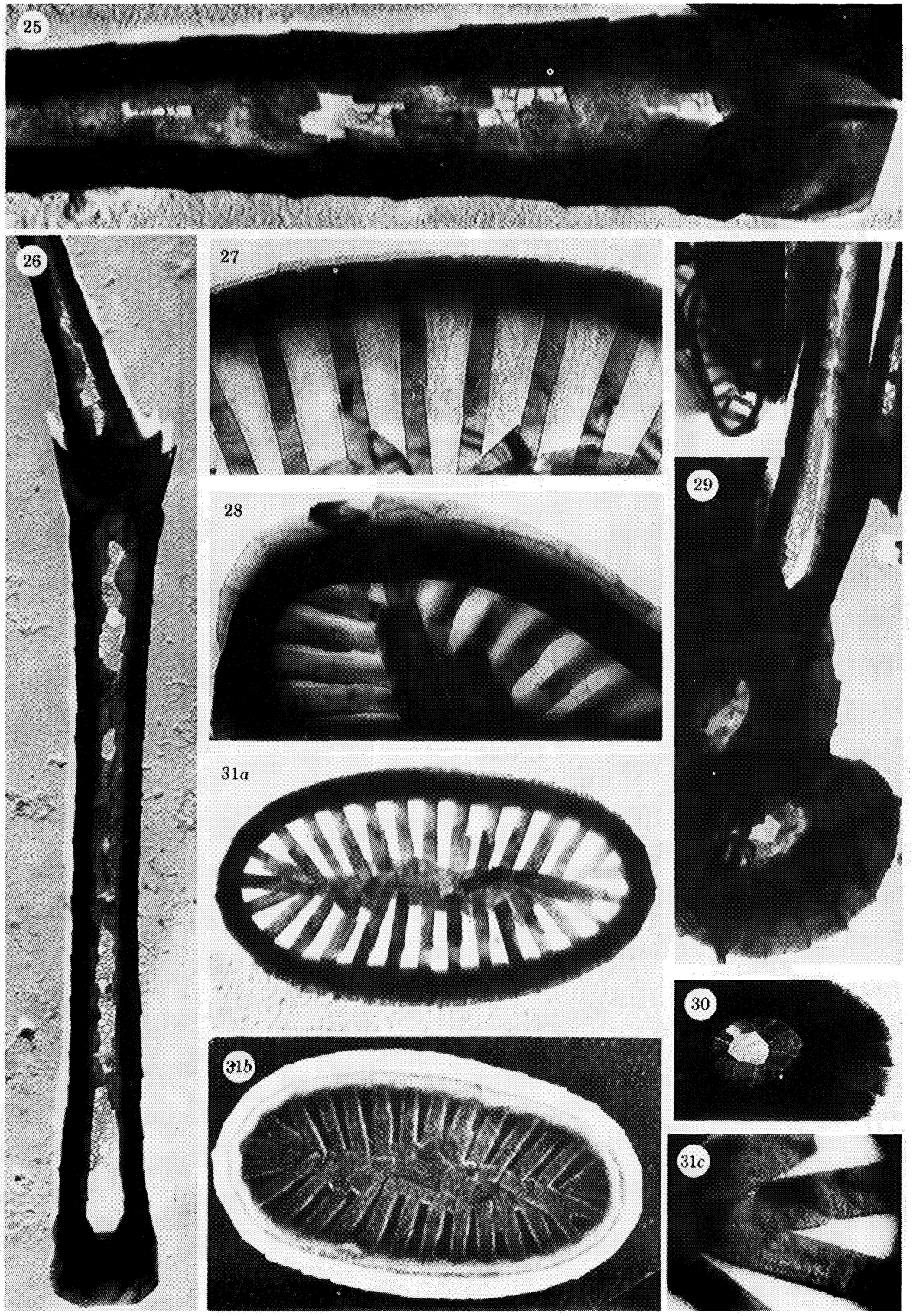
FIGURE 22. Detached ring-coccolith showing the characteristic asymmetry and presence of fringing crystallites around the optically empty centre; from sample 'Darwin 8' (table 1), transmission electron micrograph Y_N 7937.13 (EM 6B, Nottingham); magn. ×10000.

FIGURE 23. Anterior end of another specimen from sample 'Darwin 13' showing the complete assembly of ring-shaped coccoliths, some carrying tiny extraneous particles supported by an otherwise invisible central membrane. Transmission electron micrograph Y_N 7973.19; magn. ×5000 (inset, a similar specimen showing a rhomboidal small coccolith with tubular central excrescence, micrograph Y 7985.19 (EM 6B Leeds); magn. ×5000.

FIGURE 24. An exceptionally informative cell from sample 'Darwin 11' dried near a pennate diatom, see also figures 24*a–d*, 25 and 26. (*a*) Transmission electron micrograph Y_L 7955.31; magn. ×5000. Inset: light microscopy (dry lens) of the field, exposure 190.13, magn. ×1000. (*b*) Group of coccoliths from one side of the cell including the field marked X, scanning electron micrograph YO 8302.7 (Temscan, Lancaster); magn. ×7500. (*c*) Field of coccoliths near X in figure 24*b* showing substructural details of body coccoliths; transmission electron micrograph Y_L 7955.34; magn. ×20000. (*d*) Terminal segment of an appendage from top of field of figure 24 (see also figure 25); transmission electron micrograph Y_L 7955.31; magn. ×10000.



FIGURES 21–24. For description see opposite.



FIGURES 25–31. For description see opposite.

unmineralized components can be detected in coccoliths of the various categories. These will be considered in turn, with special reference to plate 8.

The presence of a very fragile membrane on the proximal face of body coccoliths is demonstrated in figures 27 and 28. The first (figure 27) is part of a coccolith newly fallen onto the support film from the distal tip of the specimen illustrated in figure 19*a*; it is therefore lying with its proximal face uppermost. Traces of an exceptionally delicate, somewhat shredded, membrane have been retained in many places. In a similar manner, figure 28 illustrates part of a body coccolith newly fallen from the top left hand side of the specimen in figure 24*a*. The proximal face is again exposed, with traces of a perforated membrane visible between the radial bars on the right hand side but replaced by a rugose deposit on parts of the bar surfaces on the left hand side where membrane disintegration is more complete. In a third coccolith shown in two different ways in figures 31*a*, *b*, similar rugosity present on parts of the exposed bar surfaces, shown more highly magnified in figure 31*c*, again represents the last remnants of a former membrane now disrupted. This specimen was subsequently tilted (micrographs not reproduced) as the only way of ascertaining that it, too, is lying with the proximal face exposed.

Demonstration of a similar membrane crossing the centres of ring-shaped coccoliths is impeded by the fringing crystallites already noted (figures 22 and 23). These not only reduce the unoccluded central areas by at least 50 % but they also cover (and obscure) the periphery, in which membrane splitting is most likely to occur. Fortunately intact membranes can still support small extraneous particles, some of which can be seen singly in several of the ring-coccoliths of figure 23. Perforations, on the other hand, though rare and inconspicuous, can sometimes be found, as, for example, in figure 30.

In contrast, the unmineralized reticulum present in the ordinary appendage-links is scarcely ever absent. It can be demonstrated without difficulty in most of the cells already used, as

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Description of plate 8

H. adriaticus: substructural details

Figure 25. Proximal end of the terminal appendage link (Darwin 11) in figure 24*d*, more highly magnified to show the reticular component among the fringe crystallites. Transmission electron micrograph Y_L 7955.36, magn. × 40000.

Figure 26. Middle link and base of a terminal link from sample A1 showing the reticular component between crystallites. Micrograph Y_N 7933.12; magn. × *c.* 20000.

Figure 27. Part of the ventral surface of a body coccolith from the tip of the cell in figure 19*a* (sample A1) showing traces of a shredded membrane between the crystalline bars. Transmission micrograph Y_L 7999G.12; magn. × 75000.

Figure 28. Part of the ventral surface of a coccolith from the left side of the cell of figure 24*a* (sample Darwin 11) showing traces of a perforated membrane between the crystalline bars at right and a rugose deposit from a more completely disintegrated membrane upon the crystalline bars at left. Micrograph Y_L 7999G.9; magn. × 60000.

Figure 29. Field containing a basal appendage link with reticulum, two ring-shaped coccoliths with fringing crystallites covering traces of splitting membranes and (inset at top) part of a small rhomboidal coccolith showing compound bars, from sample A1, exposure Y_N 7933.9; magn. × *c.* 15000.

Figure 30. Central part of a ring-coccolith to show perforated membrane surrounded by fringing crystallites. Sample A1, micrograph Y_L 7969.4; magn. × *c.* 15000.

Figure 31. A single detached coccolith exposing its ventral surface (ascertained by tilting) from sample A6. (*a*) Transmission electron micrograph showing the compound nature of the crystalline bars Y_N 7949.28; magn. × 30000. (*b*) Scanning electron micrograph of the same specimen YO 8303.51; magn. × 30000. (*c*) Part of the same specimen more highly magnified to show rugose deposit on the exposed surfaces of the crystalline bars. Transmission electron micrograph Y_L 8310.7; magn. × 100000.

for example in figure 25, which represents the proximal end of the actual link illustrated in figure 24*d*. Other, less highly magnified, micrographs, illustrated in figures 26 and 29, show the same component in parts of different specimens of each of the three links represented in the make-up of an appendage: minor breakage is detectable here and there but never complete destruction as is so often exemplified by equivalent material in *M. elegans*.

Other unmineralized components can be passed over more briefly. Thus the two equal flagella are clearly visible in figure 20*d* but we have again failed to find a haptonema although Heimdal & Gaarder (1981) claim (without illustration) to have seen one. Further information is thus necessary before the presence or absence of this organelle can be accepted as known. The same should perhaps also be said for underlayer scales, which we have also failed to find. These were sufficiently conspicuous in *Ophiaster* and *Calciopappus* to suggest that they ought to have appeared in our present, abundant, material, had they existed. Further evidence, preferably from sections, is nevertheless desirable before a negative conclusion can be finalized.

Discussion

These observations have greatly clarified previous concepts while also complementing and extending recent findings for *Ophiaster* and *Calciopappus* (Manton & Oates 1983) as noted in the Introduction. While *Ophiaster* itself has hitherto been the only coccolithophorid genus known to us in which wholly patternless membranes have been recorded, albeit only on a few specialized coccoliths, the prevalence of such in *M. elegans* and *H. adriaticus* is one of the more interesting facts to have emerged from the present study. However, without the fortunate chance of exceptionally clear surface damage, these membranes might have remained undetected for much longer.

In contrast it is not difficult to believe that most, or indeed all, of these and other observations recorded above as new, could have been made by earlier workers using the preparations they had, if circumstances had conspired to draw attention to critical details. Thus figure 35 in Gaarder & Heimdal (1981), depicting a scanning electron micrograph of *Michaelsarsia elegans*, suggests strongly that splitting membranes, closely resembling those illustrated in our own figure 6*a*, *b*, may have been present, unnoticed. Likewise their figure 34 contains reticulate material crossing part of the central space in an appendage link of the same species, in a manner strongly recalling our own figure 7*b*. In isolation, and when first seen, such material might plausibly have been discounted as no more than a fixation artefact, based on mucilage or equivalent, which, in life, would not have shown reticulation. Such an interpretation is, however, no longer possible now that we know that, though fragile, a similar reticulum in an identical position can be found in two different species and after at least two different fixatives (osmic vapour and glutaraldehyde) have been used. There is no information in Heimdal & Gaarder (1981) regarding what, if any, chemical preservative may have been applied to their specimens after collection by continuous centrifugation at sea (voyage of M.S. *Meteor*), but unless they had been stored dry, a somewhat unlikely contingency, there is not a remote possibility that any treatment could have duplicated our own. We are therefore left with the necessary inference that the reticulate component found in the link coccoliths of both species, but limited to this position, cannot be artefactual but must represent real structures.

In contrast, the previous incompleteness in the record of some of the crystallographic details from the ordinary body coccoliths, is at first more surprising and other explanations for the

omissions are needed. Thus the compound nature of the bar-crystallites is less easily detectable by scanning than by transmission electron microscopy (compare, for example, figure 31 *a*, *b*) and had both methods been routinely used together, instead of separately with emphasis on scanning as the newer method, the missing facts could scarcely have failed to come to light. Another negative factor is perhaps the complex terminology which, though doubtless needed for comparative purposes in the special context of recent or fossil sediments, is not self-explanatory. Thus the unqualified allocation of body coccoliths to a single category, 'incomplete caneoliths', in each of the four putative genera under discussion, could easily have been mistaken for a description so complete as to render further enquiry redundant. The best means (as we now know) of detecting a misapprehension regarding structural affinities may thus have been excluded in advance.

The sum of information now available, regarding the two species under investigation here, is scientifically interesting in more ways than one. Though several gaps have been filled that were not previously known to exist this, in itself, is the least significant consequence of the new findings. Of greater practical importance is the fact that we can now, for the first time, distinguish authoritatively between inconspicuous but phyletically meaningful details and spectacular resemblances brought about by convergence. The latter is basic to the hitherto unquestioned assumption that the mere presence of anterior appendages, no matter how different in themselves, could mean that *Halopappus* and *Calciopappus* must be phyletically related. On the contrary, as we now know (Manton & Oates 1983), the details of body coccoliths indicate that the true affinities of *Calciopappus* lie with *Ophiaster*, a genus wholly without anterior appendages. This conclusion is endorsed by our present findings in which the body coccoliths of *Michaelsarsia elegans* and *Halopappus adriaticus* resemble each other closely in exactly those details in which both differ from *Calciopappus* and *Ophiaster*. It must therefore be concluded that coccolith substructure is phyletically significant, no matter how inconspicuous the details may be, whereas the mere existence of appendages, whether anterior or posterior, can be parallel adaptations to a shared environment and therefore misleading phyletically.

The exact functional significance of any of the structures analysed cannot, of course, be specified in precise detail. Thus appendages, even if adaptive, must relate to some ecological factor powerful enough to elicit equivalent but structurally different adaptations in different genera though, without direct observations on the lifestyle of the organisms with respect to their habitats, analysis can go no further. The unmineralized components of coccoliths, on the other hand, are more elusive. Their prevalence is such (for further details see Manton & Oates (1983)) as to imply a functional role of some kind though we can as yet only guess at what this role might be. There are at least three possible scenarios. The first, a mechanical function in holding the crystalline parts of a mature coccolith together, is the least likely since the contradictory effect of the fringing crystallites in *H. adriaticus*, in sustaining the integrity of the unmineralized components, is clear evidence of the intrinsic fragility of the latter. A second possible scenario is developmental, since we already know (for example, Manton & Leedale (1969); Manton & Peterfi (1969); Pienaar (1971)) that coccolith development is completed inside the cell, within the cisternae of the Golgi system. This may therefore perhaps involve processes in which the permanently unmineralized components might play a constructive part. A third possibility, not necessarily the only one, might involve some regulatory function outside the cell such as control of coccolith arrangement. This alternative is the most likely, since it is indirectly supported by one further fact. Thus we know that the normal position of body coccoliths on

the cell surface in all these genera is with their long axes parallel to the long axis of the cell and with the membrane-covered lower (proximal) face directed towards the plasmalemma. Exceptions occur only in the appendages, which project outwards, never becoming directly involved with the cell surface except via the ring coccoliths. This raises the question (not as yet answered) as to whether the concurrent structural difference between membranes on the one hand and a reticulum on the other might be positively correlated with this difference in ultimate position. Further comparative studies on the intimate substructure of appendages of other types, probably attainable only by means of sections, might confirm or refute this suggestion.

Finally, it is necessary to graft the new information onto the old by revising the formal descriptions of the taxa under investigation. Considered simply as species, *M. elegans* and *H. adriaticus* are very distinct, though the diagnostic differences are few, being mainly based on cell size and the presence or absence of fringing crystallites in the modified coccoliths. The resemblances, on the other hand, are so much more numerous than those previously recognized that the taxonomic necessity, or indeed propriety, of retaining two genera for them can be questioned.

Taxonomy and nomenclature are of course inseparably linked and generic criteria must necessarily conform to those of component species or be adjusted accordingly. However, the ease of doing this is very different in these two cases, mainly for historical reasons. Thus *M. elegans* is the type species of its own genus but *H. adriaticus* is not (for further details see Heimdal & Gaarder 1981). In addition, the latter's original allocation to *Halopappus* had been made in part on a misapprehension (Schiller 1930), namely, that the periplast, though calcified, does not contain coccoliths. Since other species recorded only with the light microscope (Schiller 1930) are also potentially involved, there is no way at present in which a revised definition of *Halopappus* could be put forward acceptably. Fortunately this impasse can be avoided by a simple nomenclatural change to bring both species together into one, suitably amended, genus: *Michaelsarsia*. This is the least change needed to bring taxonomy into line with the new information without prejudice to other, less well known, species. If this were done a more informative order of citation of the genera used in this enquiry would be *Calciopappus*, *Ophiaster*, *Michaelsarsia* (*Halopappus*) and this we believe to be phyletically meaningful.

Conclusions

This study of *Michaelsarsia/Halopappus*, supplementing that already published on *Ophiaster/Calciopappus*, has shown that unmineralized components of coccoliths are not only more prevalent but are also more varied in character than had previously been supposed. Wholly patternless, thin, membranes, hitherto only seen to a limited extent within one genus (*Ophiaster*), are shown to be present on all types of body coccoliths in *Michaelsarsia/Halopappus*, including both plate-coccoliths and ring-shaped coccoliths, while a new type of fragile reticulum, replacing a membrane, has been demonstrated on the specialized coccoliths of the appendages in each of two taxa. This has suggested, for the first time on positive evidence, that there may be a functional involvement of unmineralized components with coccolith positioning outside the cell. Further comparative information is therefore now needed regarding equivalent details for other types of appendages, especially those of *Ophiaster* which, in the absence of sections, have not as yet been analysed from this point of view. Other substructural details of

body coccoliths have confirmed the relative remoteness of *Michaelsarsia/Halopappus* from the genera previously studied and in so doing have permitted, for the first time, a clear distinction to be drawn between conspicuous but convergent characters such as coccolith arrangement and the presence or absence of appendages, both previously over-valued, and inconspicuous but phyletically more meaningful characters, previously overlooked. These include underlayer scales, if present, together with substructural details of coccoliths and their unmineralized components. The undoubtedly adaptive nature of appendages, which must have evolved independently in a different form on at least three different occasions, requires further elucidation from observations on living cells to ascertain the nature of the environmental factors to which they relate. Finally, removal of several misconceptions permits a revised generic diagnosis of *Michaelsarsia* to be formulated, in terms appropriate to the new information regarding the type species. It is then recommended that this genus should also be used to include *M. adriaticus* (Schiller) comb.nov., which is similarly re-defined.

Revised taxonomic diagnoses

Michaelsarsia Gran emend.

Motile coccolithophorids with two equal flagella; a haptonema not yet fully authenticated (but see Heimdal & Gaarder 1981). Cells with a conspicuous crown of slender anterior appendages equal to the cell body in length and composed of chains of strongly modified, linearly attached, elongated coccoliths, with apparently vacant centres, each chain subtended by a ring-shaped modified coccolith at the base. Cell body commonly bluntly pointed posteriorly with an apical depression surrounding the flagellar bases, the body-shape otherwise ranging from isodiametric to long-conical, elliptical or oblong. Periplast a single layer of elliptical coccoliths arranged in hexagonal close-packing with their long axes mainly parallel to that of the cell, each coccolith an 'incomplete caneolith' consisting of a fully calcified double rim, and an elliptical plate carrying a central thickening. The latter composed of stacked rectangular crystallites, and the plate traversed by spoke-like bar-crystallites, each compound, with a central joint. The spaces between bars bridged by an unmineralized, patternless, membrane spread uniformly across the posterior (proximal) face of the coccolith. The apical depression lined by similar but smaller coccoliths, rhomboid in shape and with the central thickening reduced to a small outwardly directed excrescence. Each of the ring-shaped modified coccoliths subtending the appendage chains possessing a relatively wide calcified rim but with the apparently vacant centre bridged by an unmineralized, patternless membrane. The link-coccoliths themselves with an unmineralized, centrally located, fragile reticulum apparently replacing the membrane of the other coccoliths types.

Geographical distribution: probably world wide in coastal waters of warm including tropical regions. Type species: *M. elegans* with one other adequately authenticated species, and several insufficiently known additional taxa, as yet recorded only with the light microscope.

Note. The revised descriptions given below include some numerical statements from Heimdal & Gaarder (1981), which do not exactly coincide with our own: all such cases are indicated by the initial HG against the alternative figures in brackets.

M. elegans Gran emend.

Cells small, body isodiametric or nearly so, commonly *c.* 11 μm long (9.0–11 μm HG), appendages three-linked, up to 20 μm long (HG) and said (HG) to be 12–21 in number but often fewer. Body coccoliths relatively massive and firmly attached, commonly *c.* 1.8 × 2.6 μm (HG, length only, 1.7–2.5 μm) the central thickening strongly convex distally but not visible by scanning proximally, component crystallites broader than the bar-crystallites crossing the plate, the latter 20–28 in number, anterior rim often reflexed, posterior rim a narrowly projecting knife-edge. Small rhomboid coccoliths in the apical depression, with externally directed central projections usually conical (HG) but sometimes tubular. Ring-shaped coccoliths subtending the appendages commonly 12 but sometimes a few more, each *c.* 2.6 × 3.0 μm with the diameter of the unobstructed central area nearly half that of the whole coccolith.

Distribution: positively attested in Mediterranean Sea, Atlantic and Pacific Oceans, including Galapagos Islands, often in coastal waters from the surface to 20 m.

M. (*Halopappus*) *adriaticus* (Schiller) emend. comb.nov.

Cells larger and more elongated than in *M. elegans*, the body commonly *c.* 9 × 15 μm (HG, length only, 13.7–29.0 μm) with 8–13 (16–17 Schiller) appendages each up to 25 μm long, narrow and with link-centres partly occluded by thin intrusive crystallites attached to the inner edge of the rim and considerably increasing the stability of the unmineralized reticulum. Ring shaped coccoliths similar to those of *M. elegans* except for the presence of short, fringing, crystallites with consequent reduction of the unoccluded central area by 50% or more; other dimensions of ring coccoliths unchanged. Body coccoliths thinner, flatter and slightly narrower than those of *M. elegans* though essentially similar otherwise, dimensions commonly *c.* 1 × 2 μm (HG length only, 1.8–2.4 μm), the central mound composed of narrower rod-crystallites. Equivalent projections on small rhomboid coccoliths tubular and never solid as often in *M. elegans* (HG).

Distribution: as in *M. elegans* but usually more abundant.

The main acknowledgements for financial and other official assistance have already been recorded in the first part of this enquiry (see Manton & Oates (1983), p. 457) but more personal thanks must be given here to the following (among many others): Professor Cocking (of Nottingham) for use of the EM 6B electron microscope, as noted in the legends, by the senior author during many years and Dr S. Walker (of Liverpool) for much personal help with the light microscopy; Mrs Gaarder and colleagues (Norway) for the initial identifications; Dr Barry Leadbeater (of Birmingham) for helpful comments and advice during compilation of the manuscript and Barry Herbert of the Department of Fine Art, Leeds University, for personal help to the senior author in finalizing plate 4.

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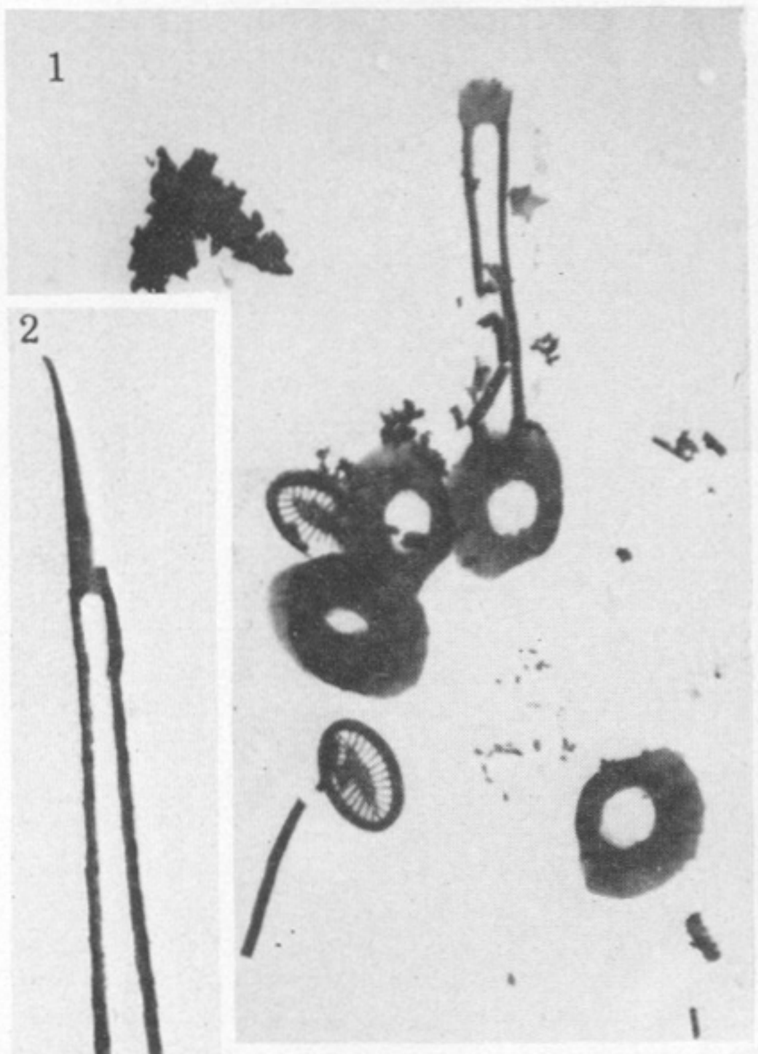
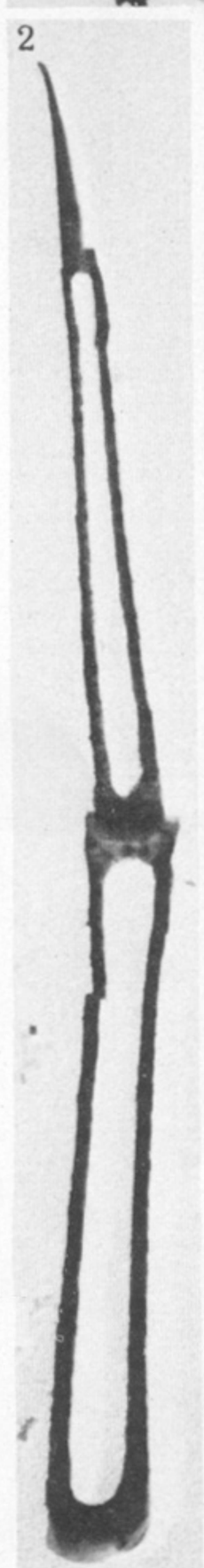
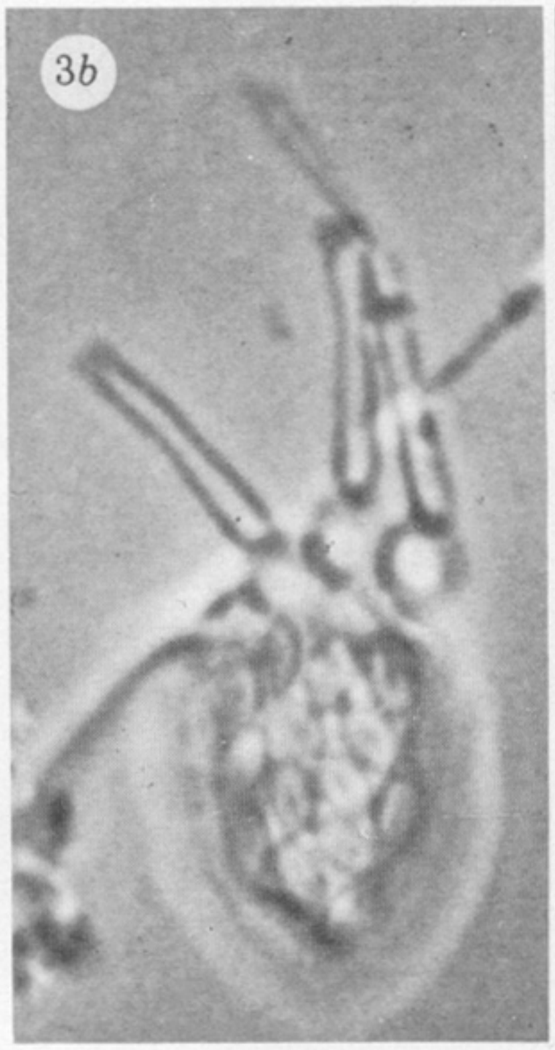
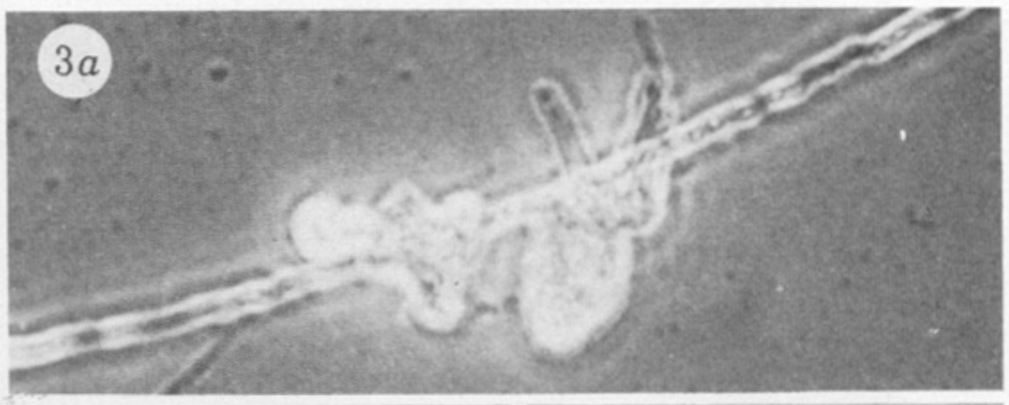
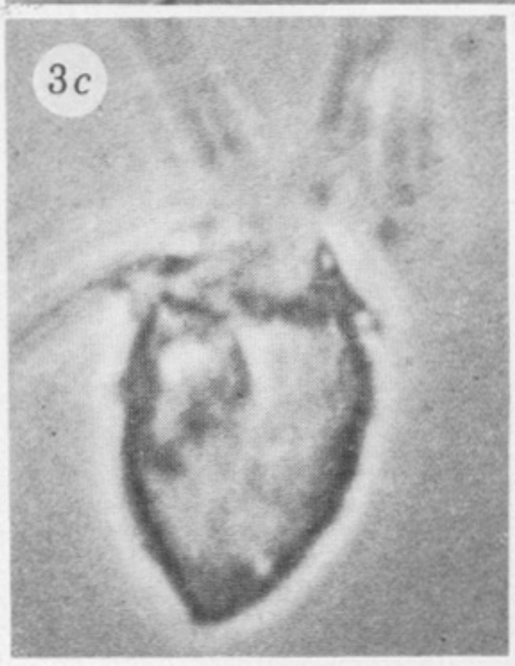
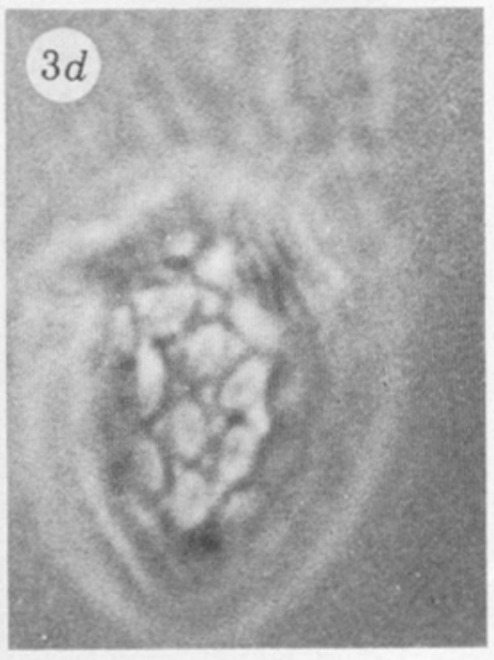


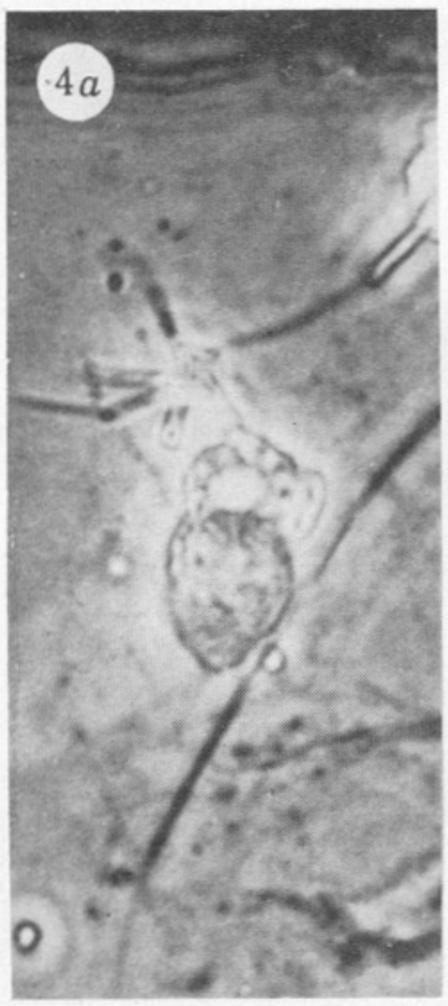


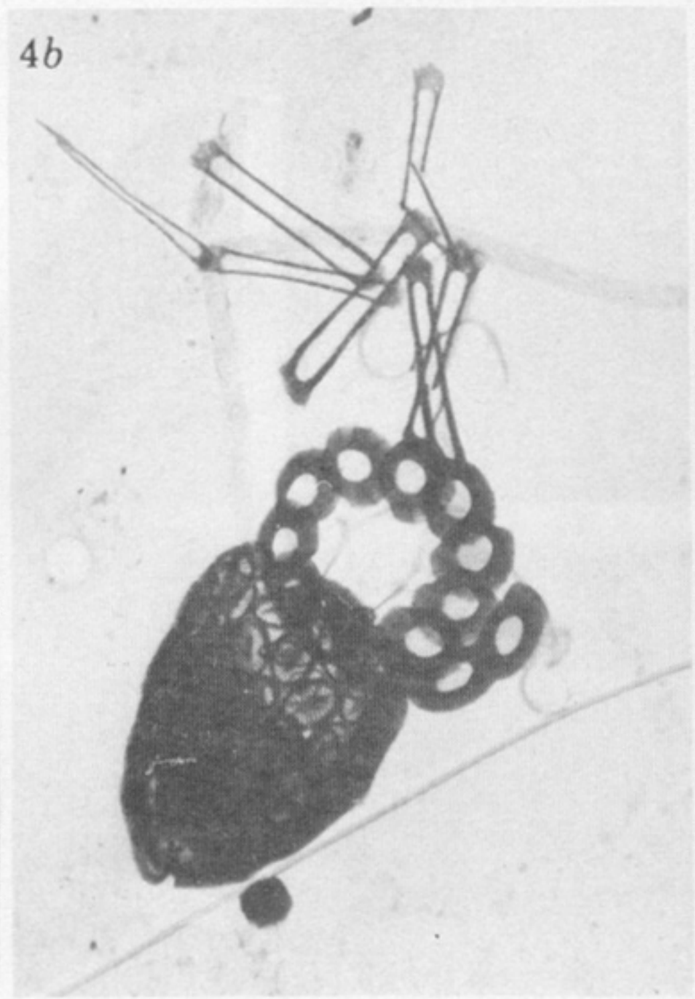


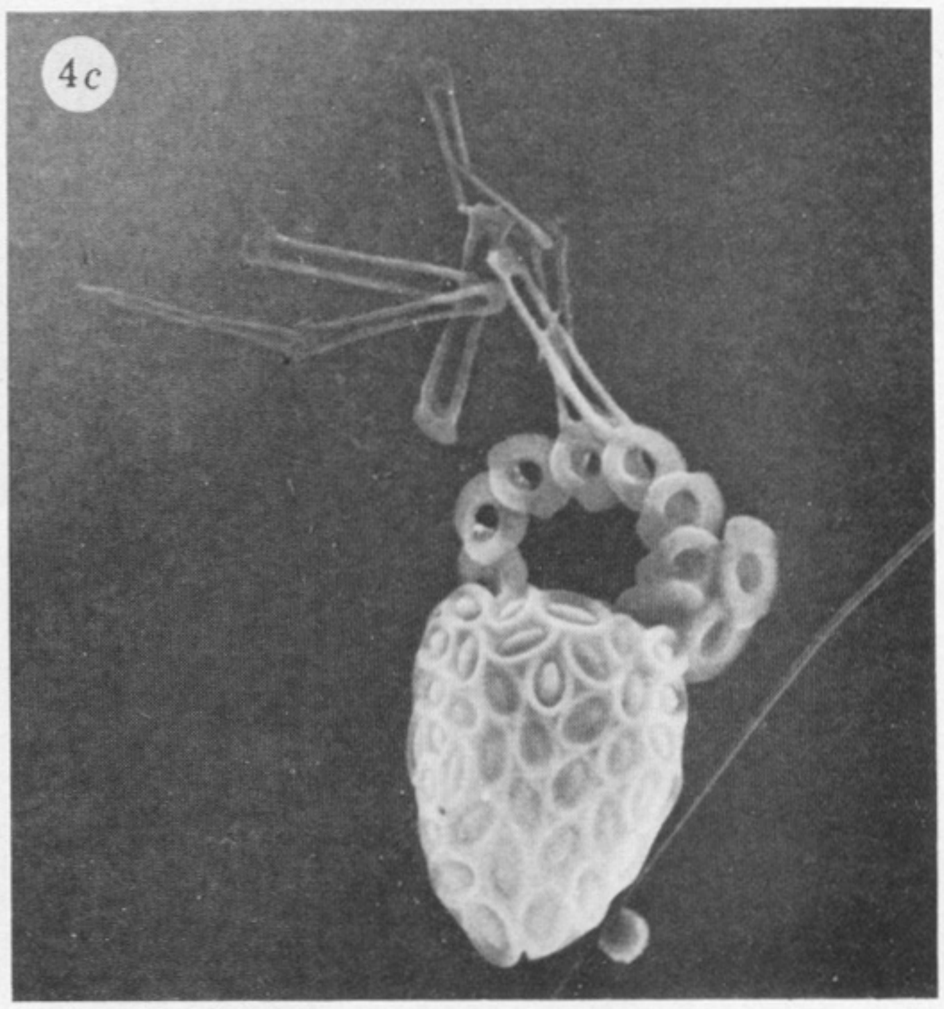


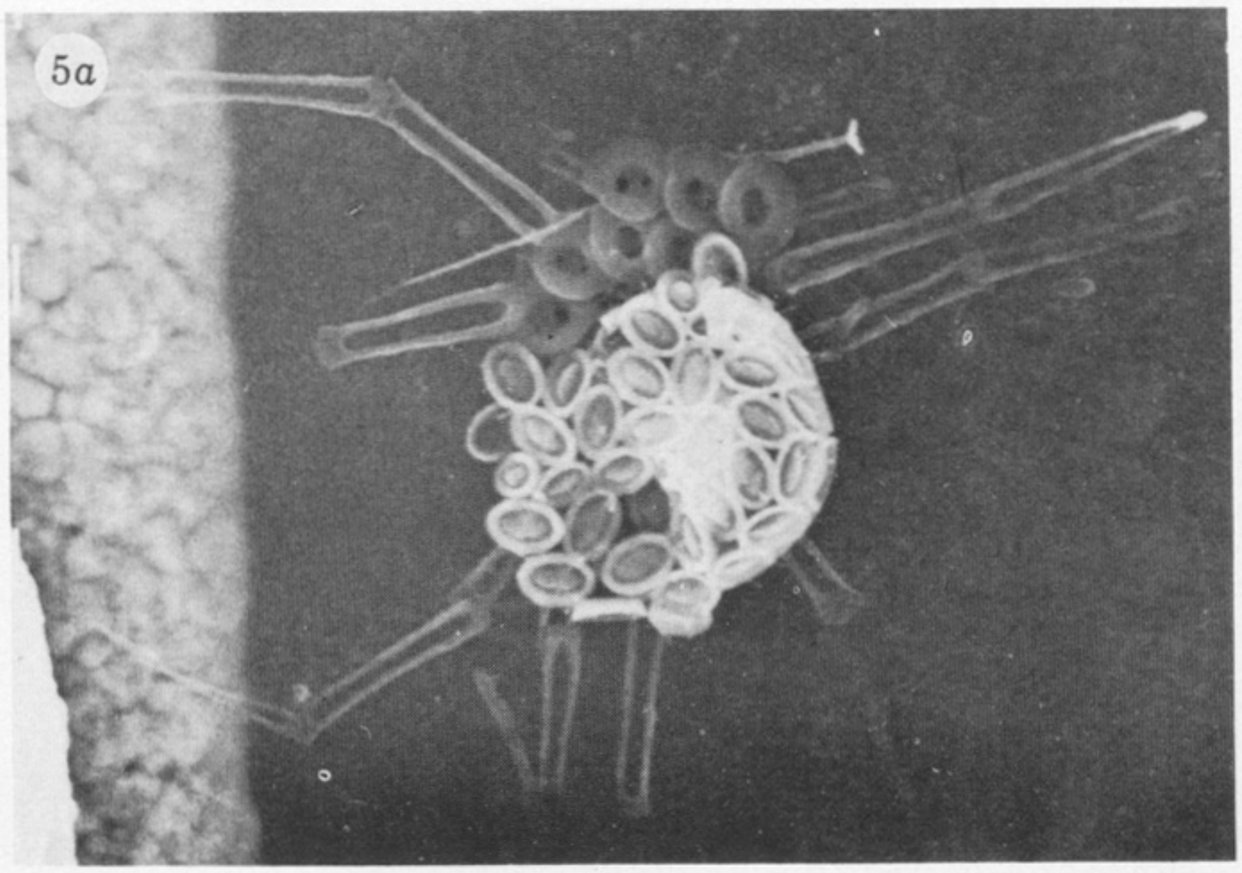


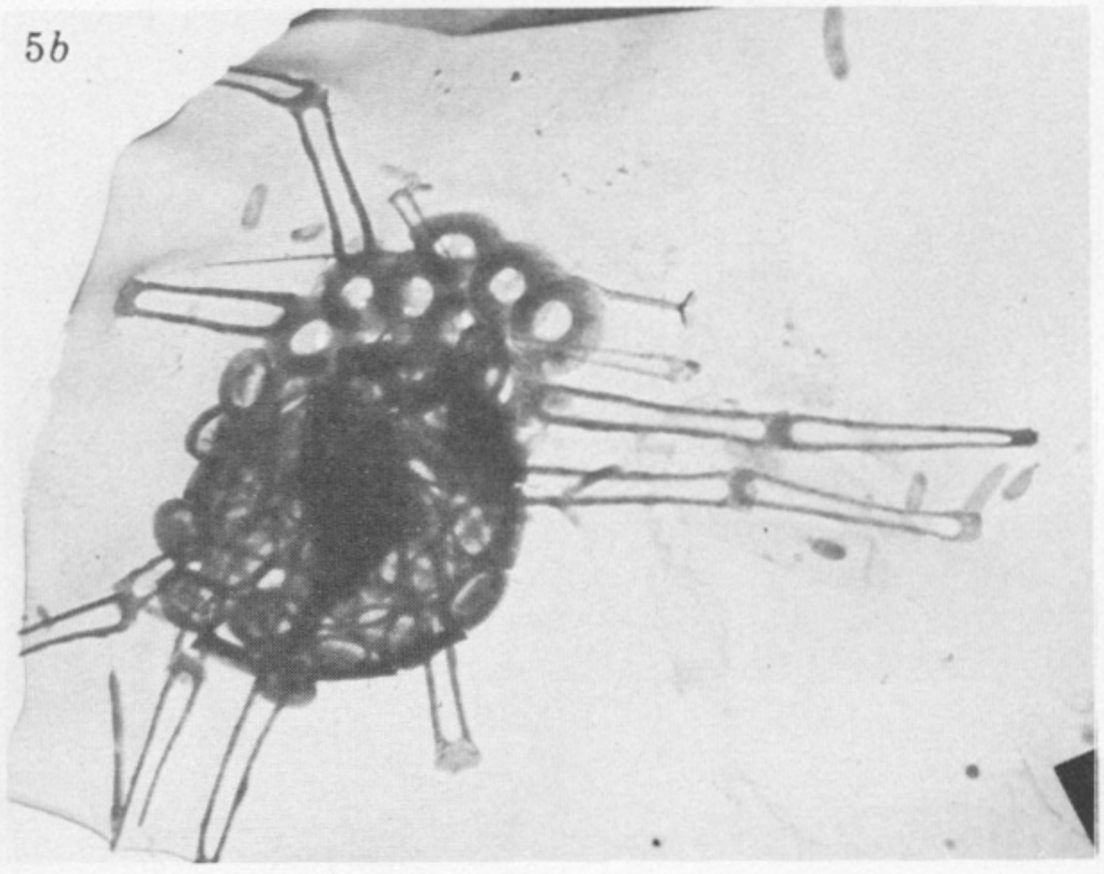




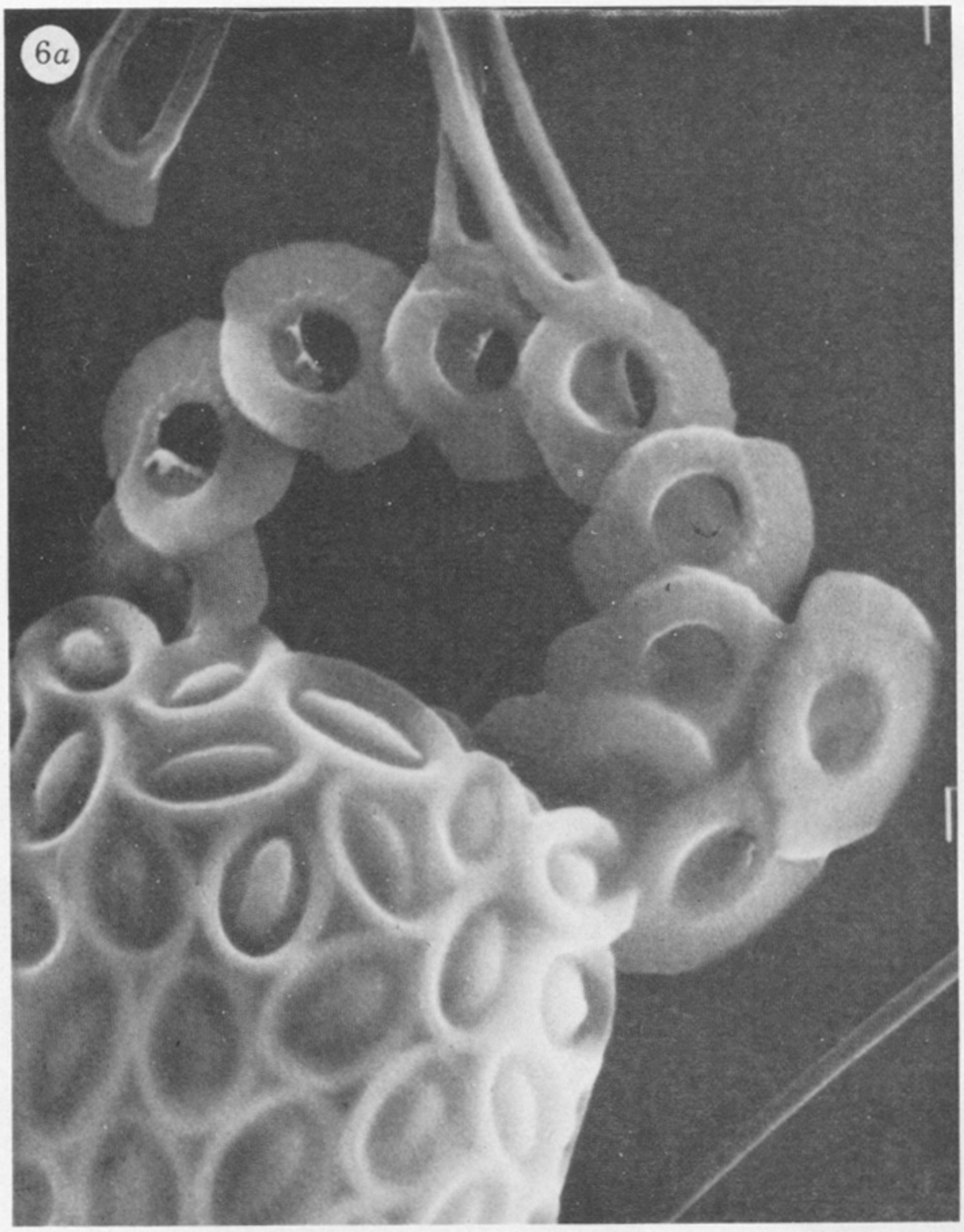


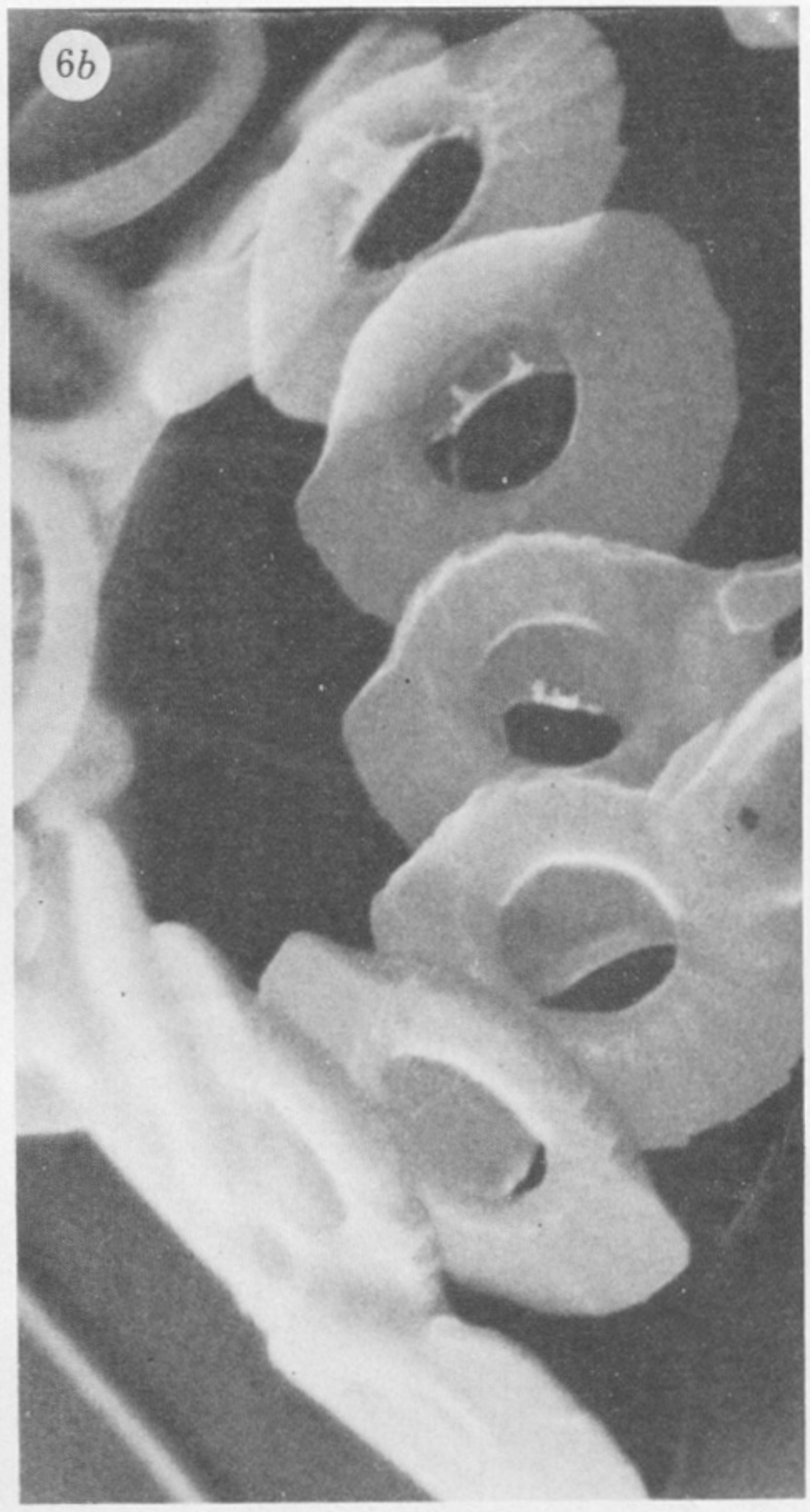


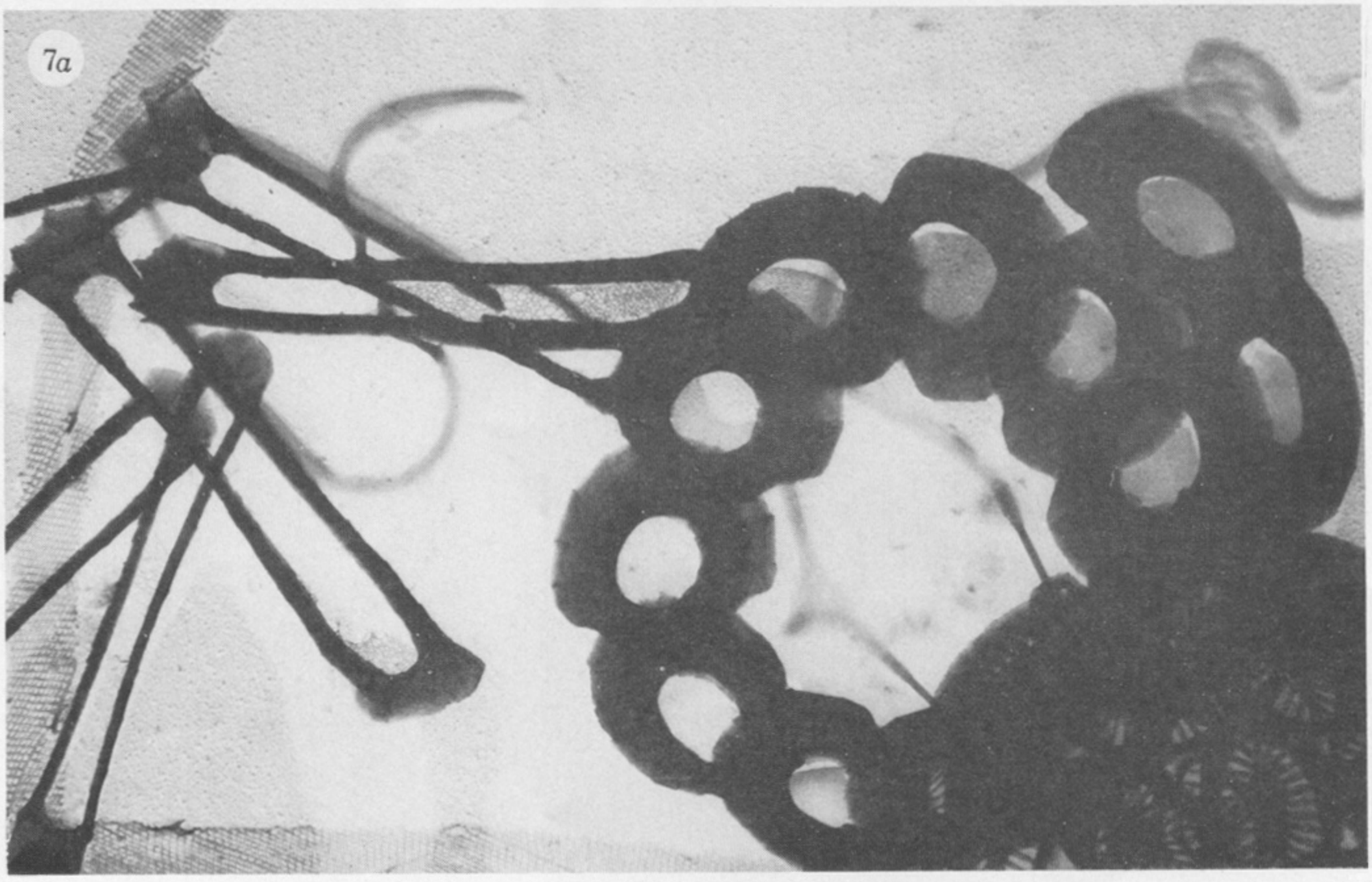




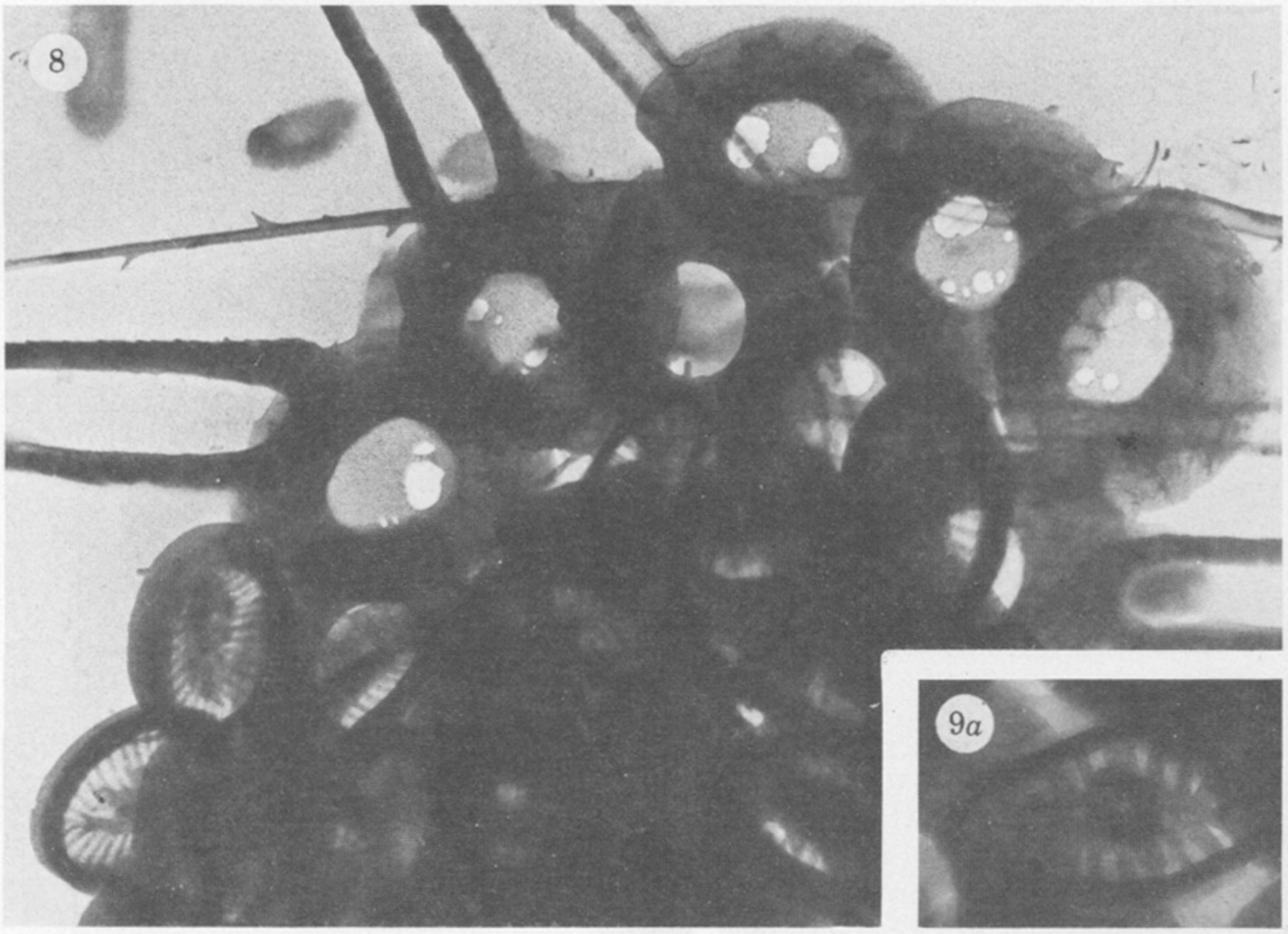
FIGURES 1–5. For description see opposite.

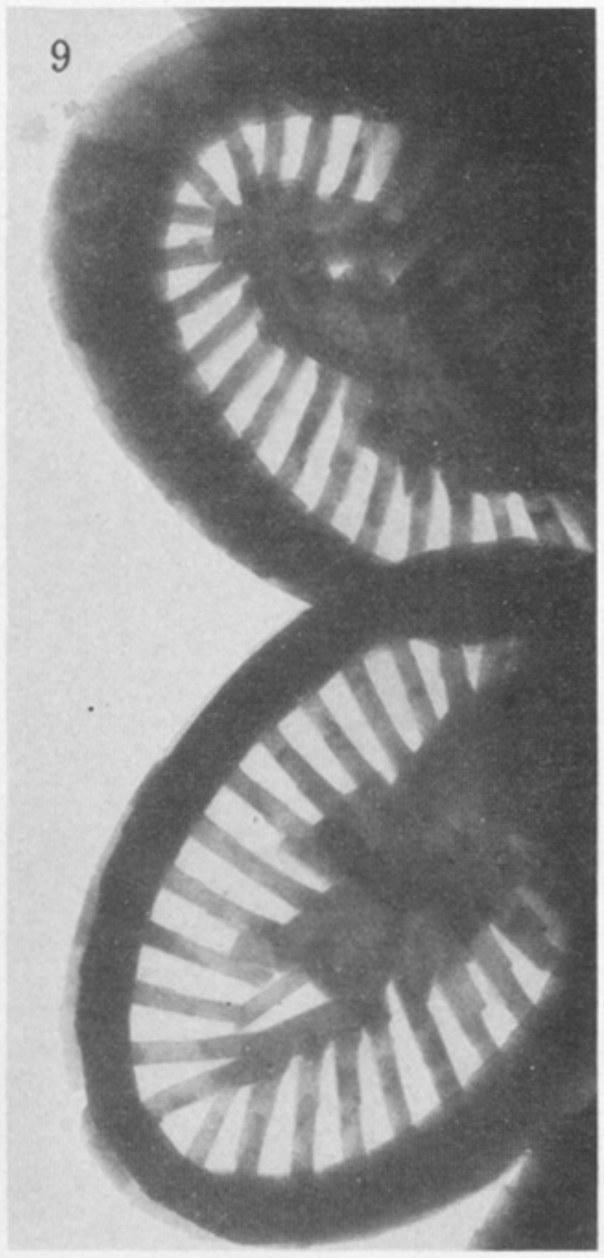


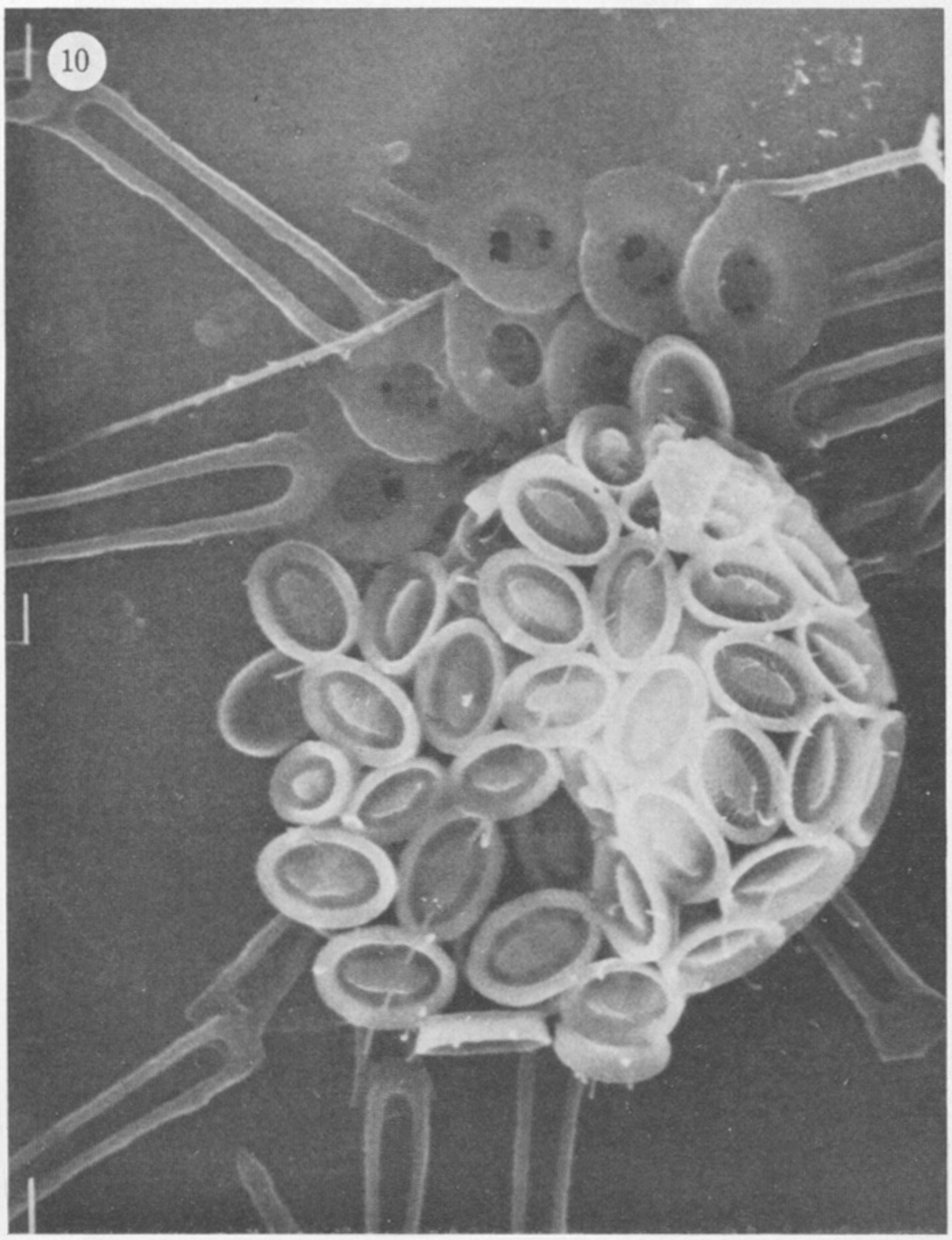


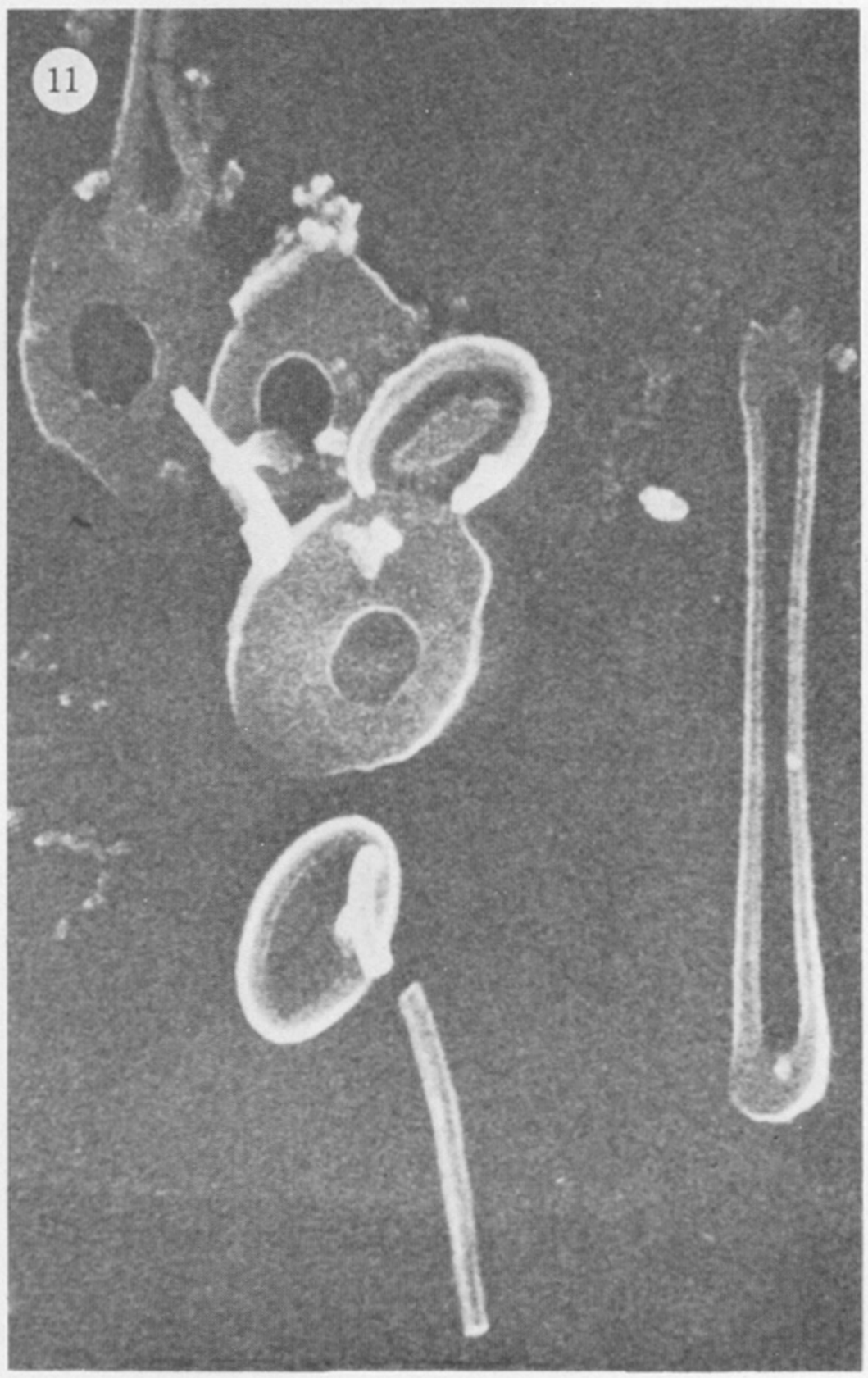


FIGURES 6 AND 7. For description see p. 188.

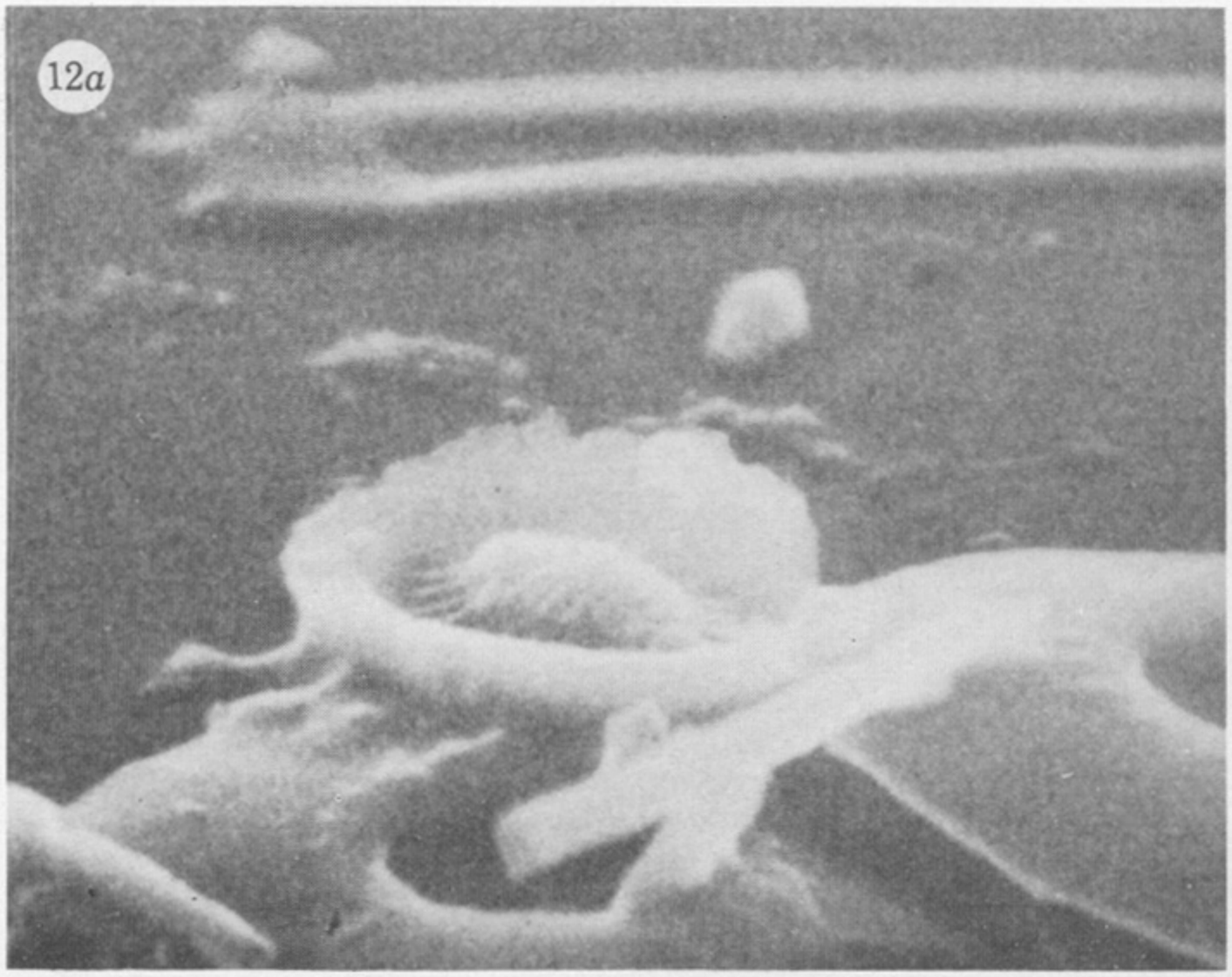


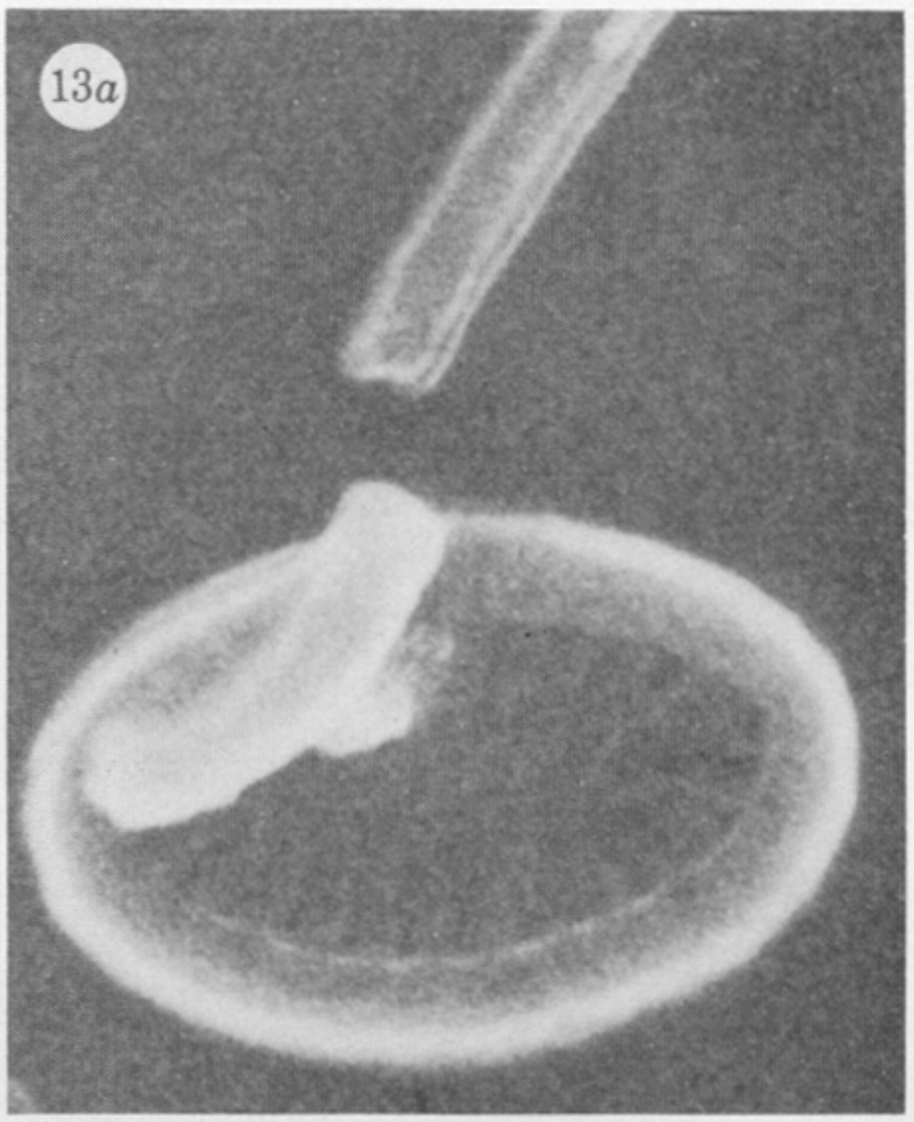


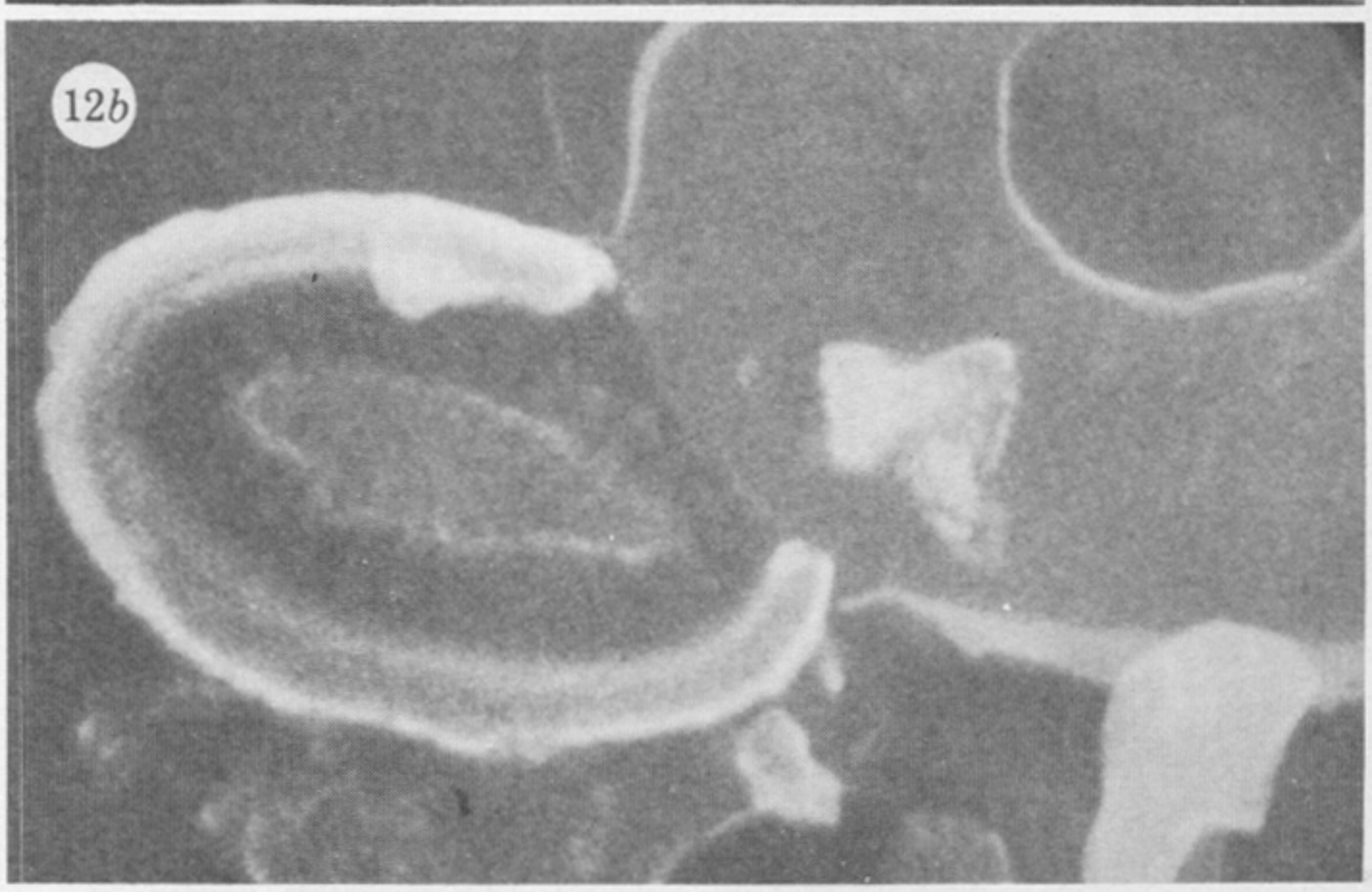


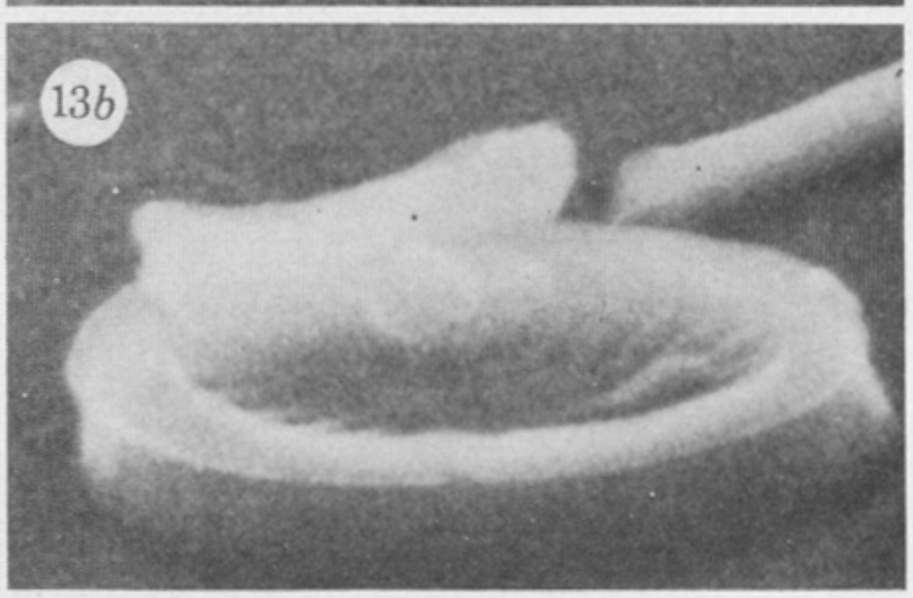


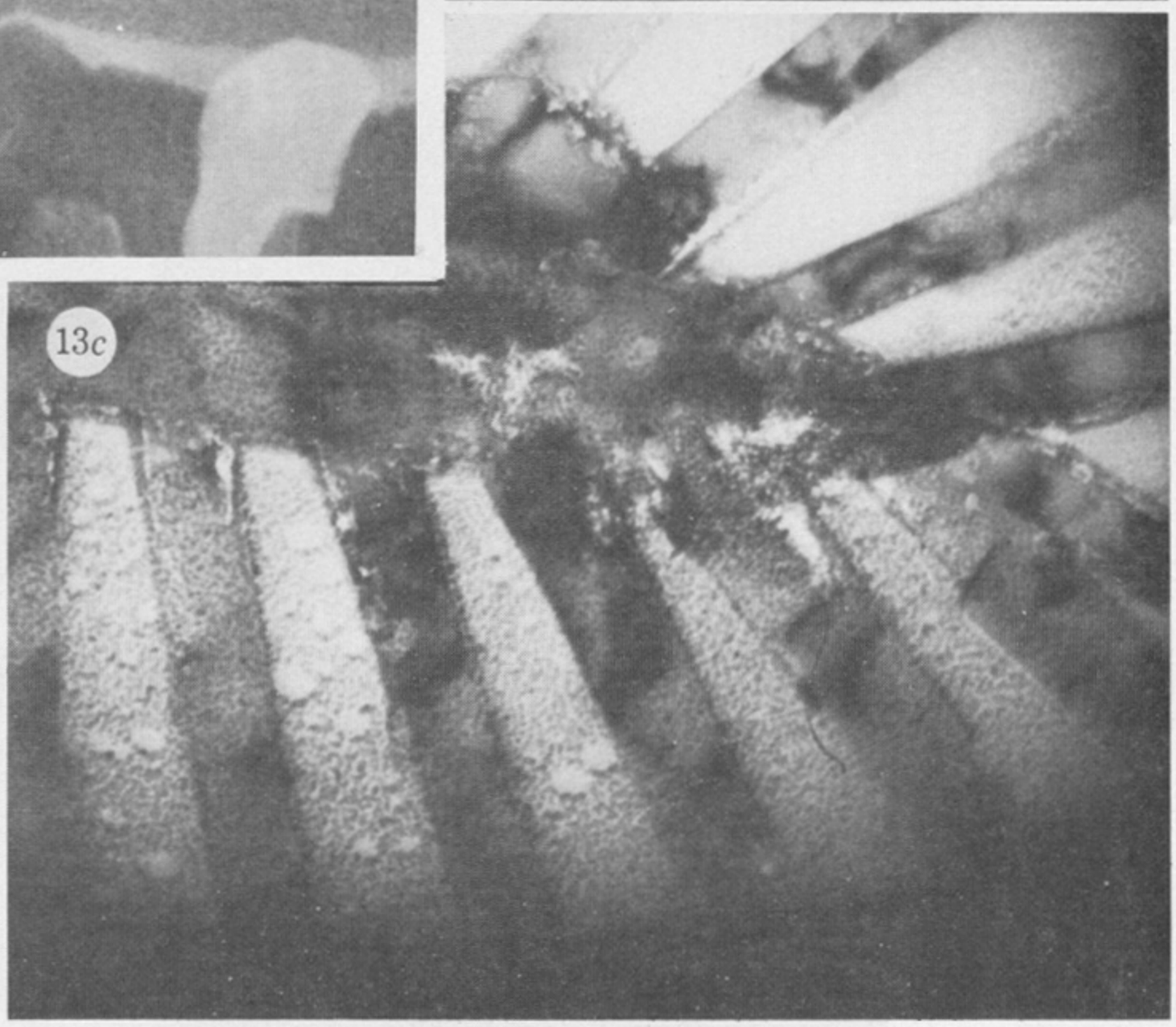
FIGURES 8–11. For description see p. 189.

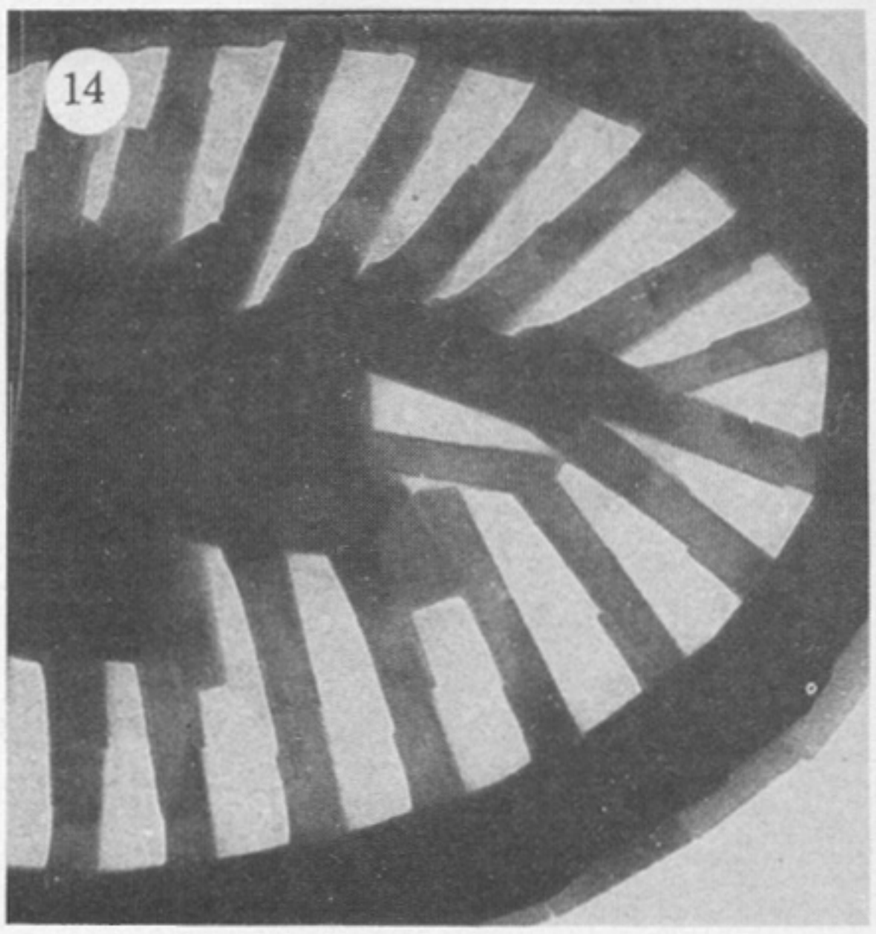




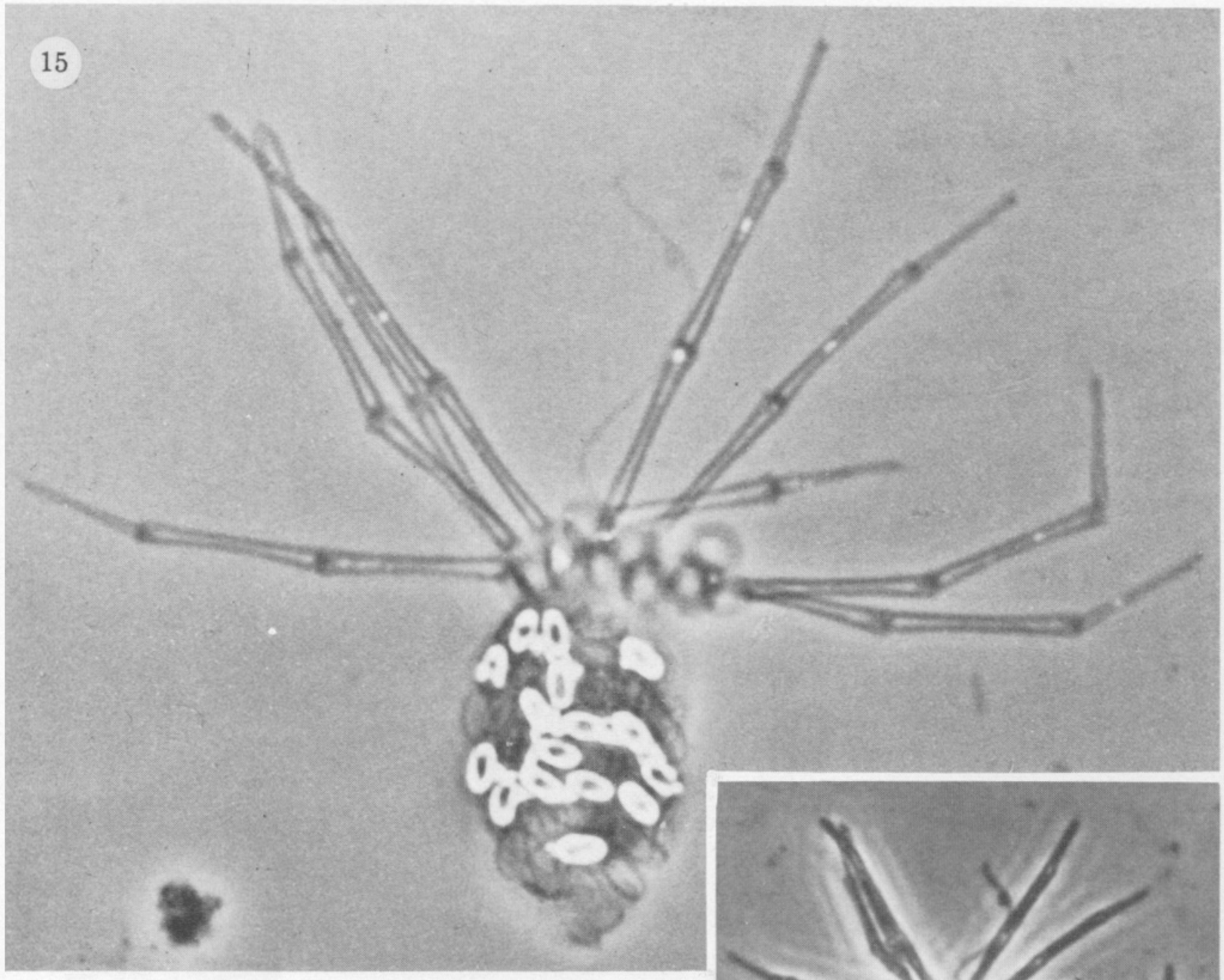


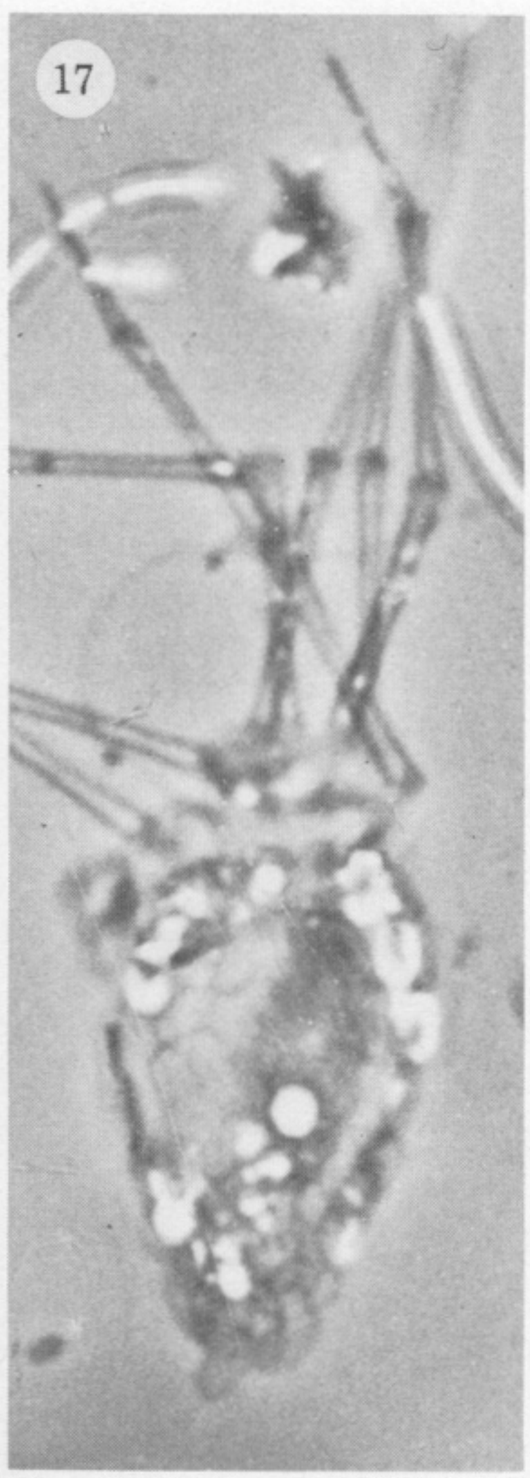


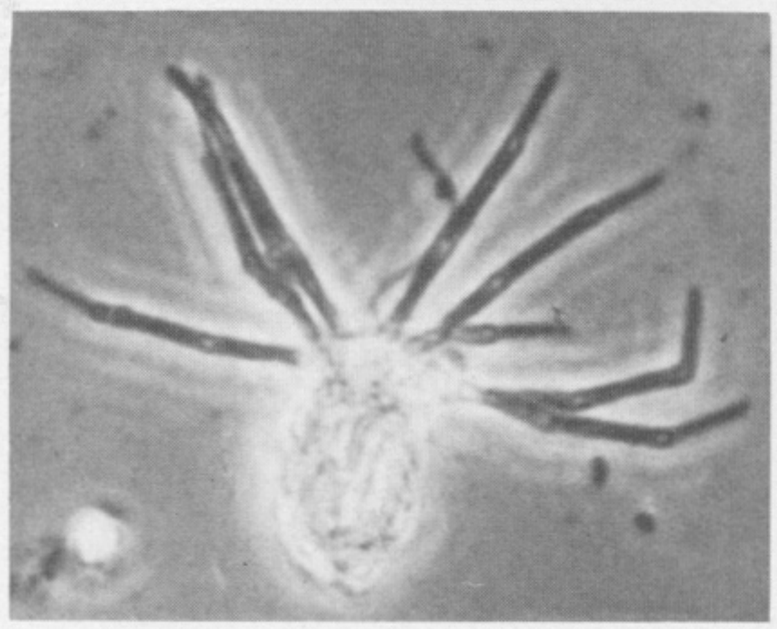


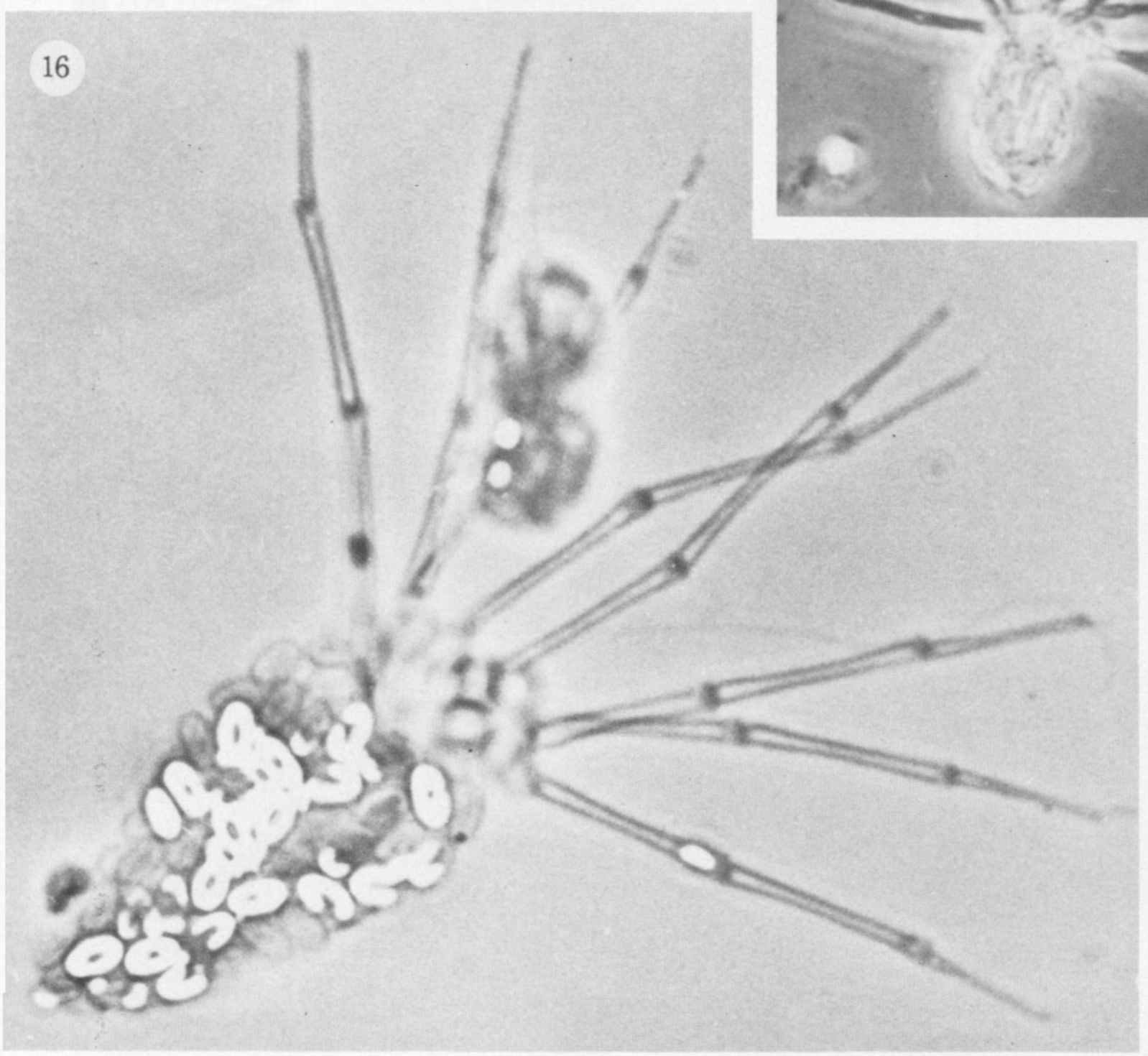


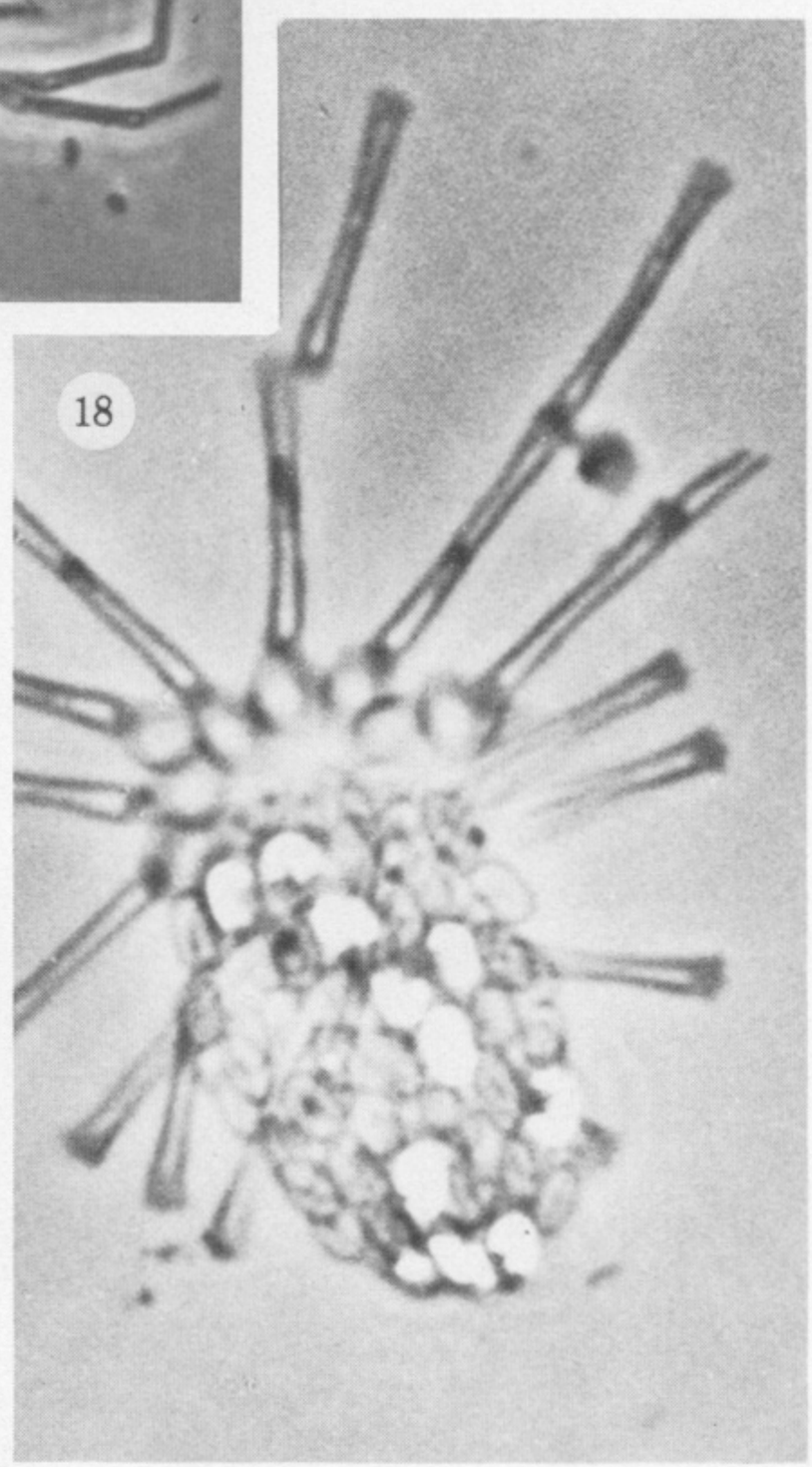
FIGURES 12–14. For description see opposite.



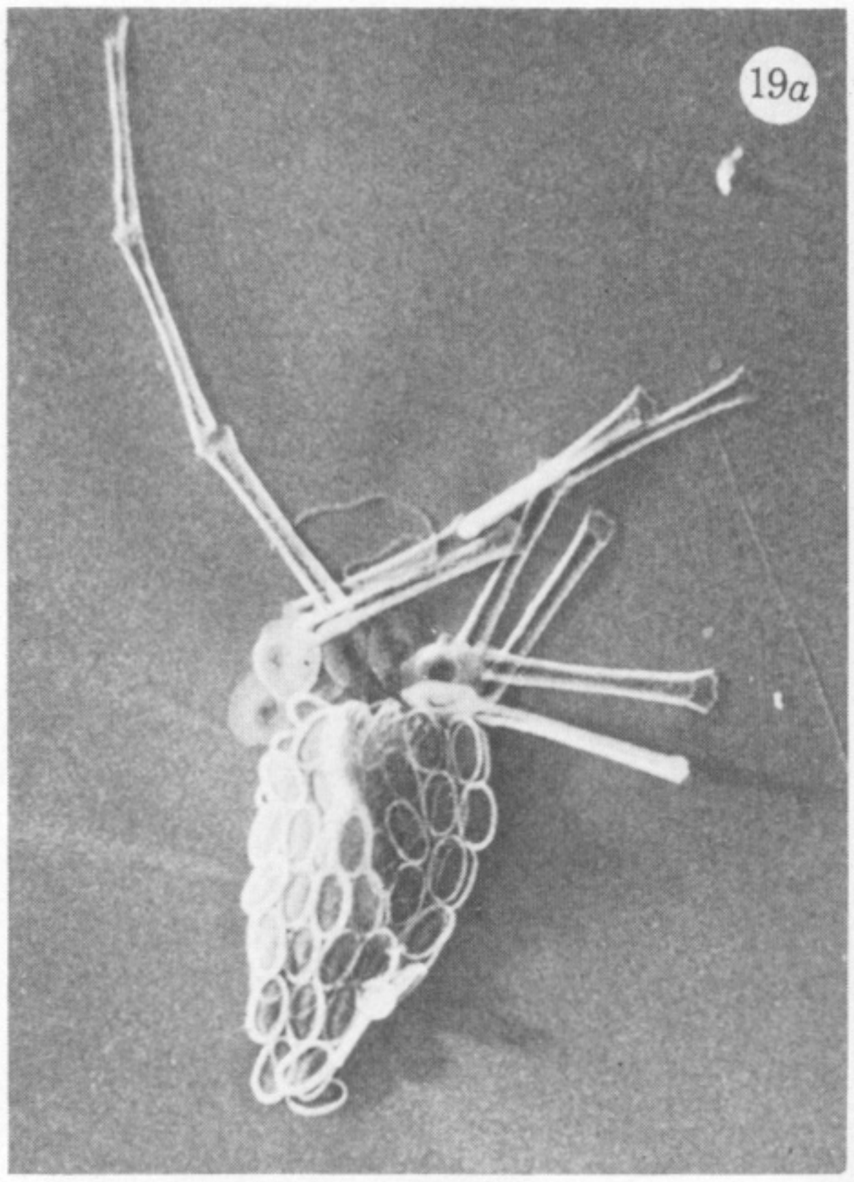


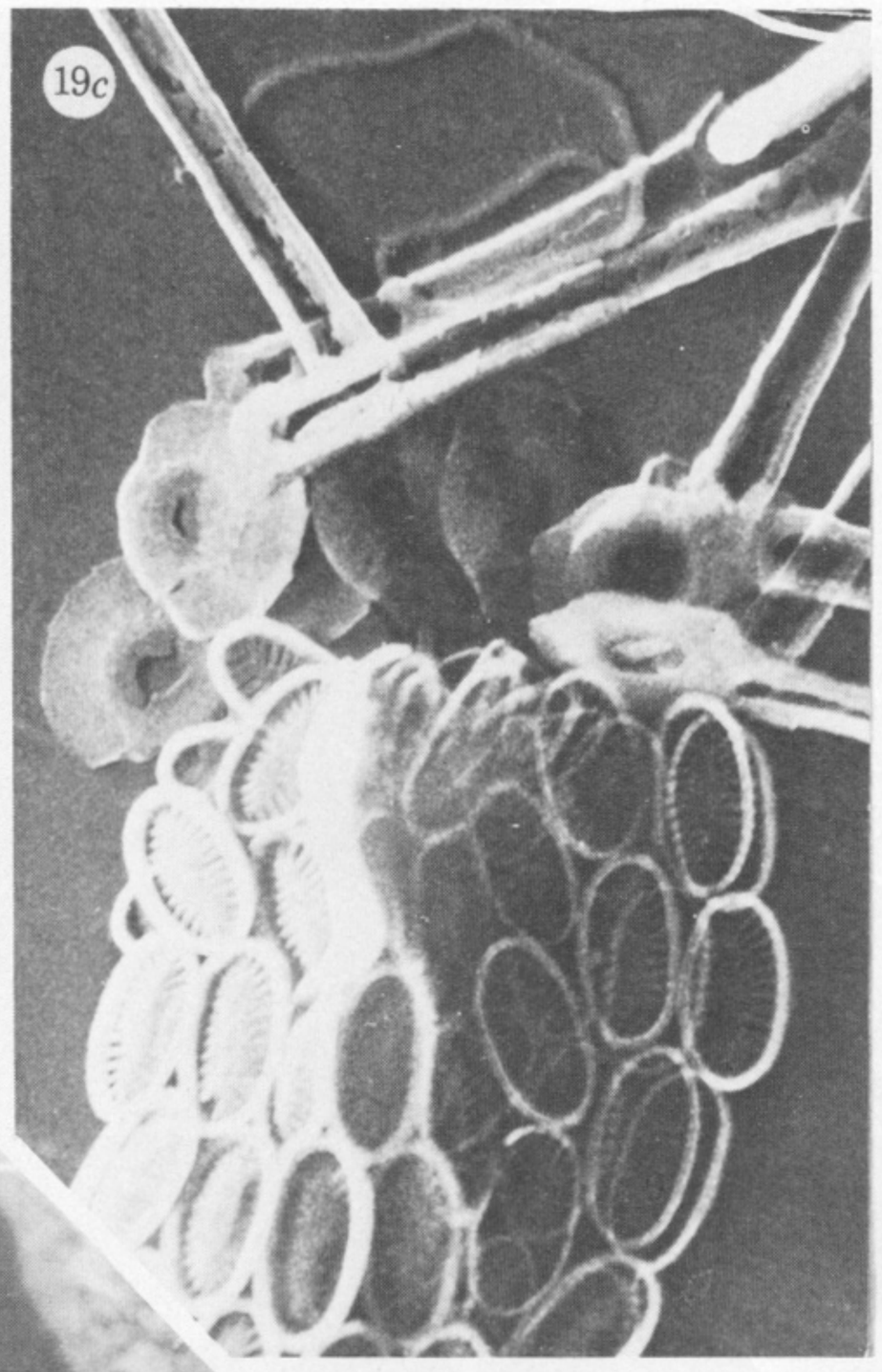


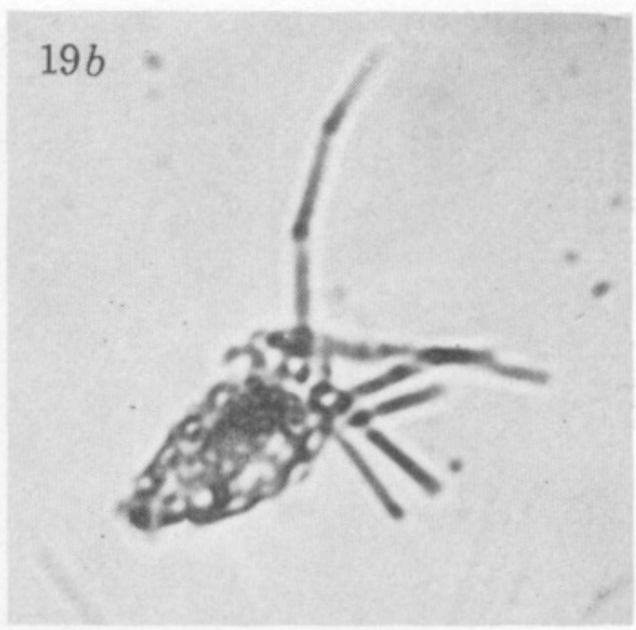


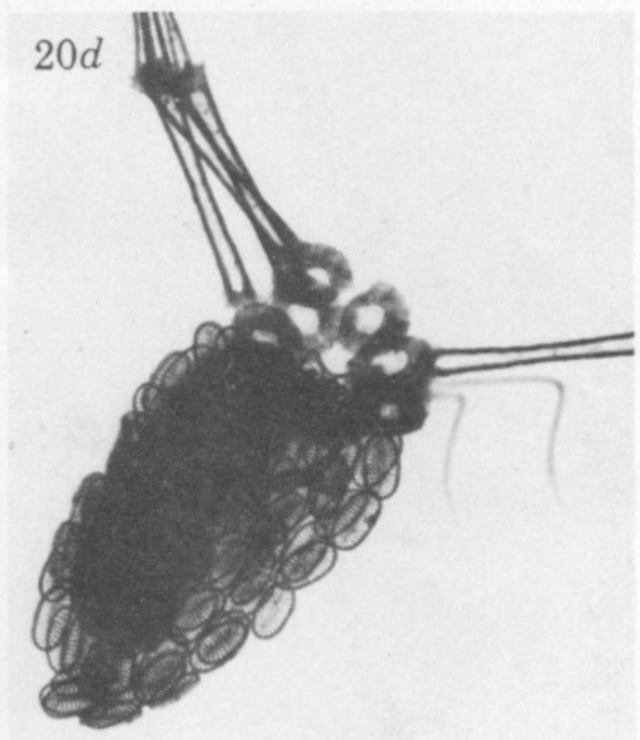


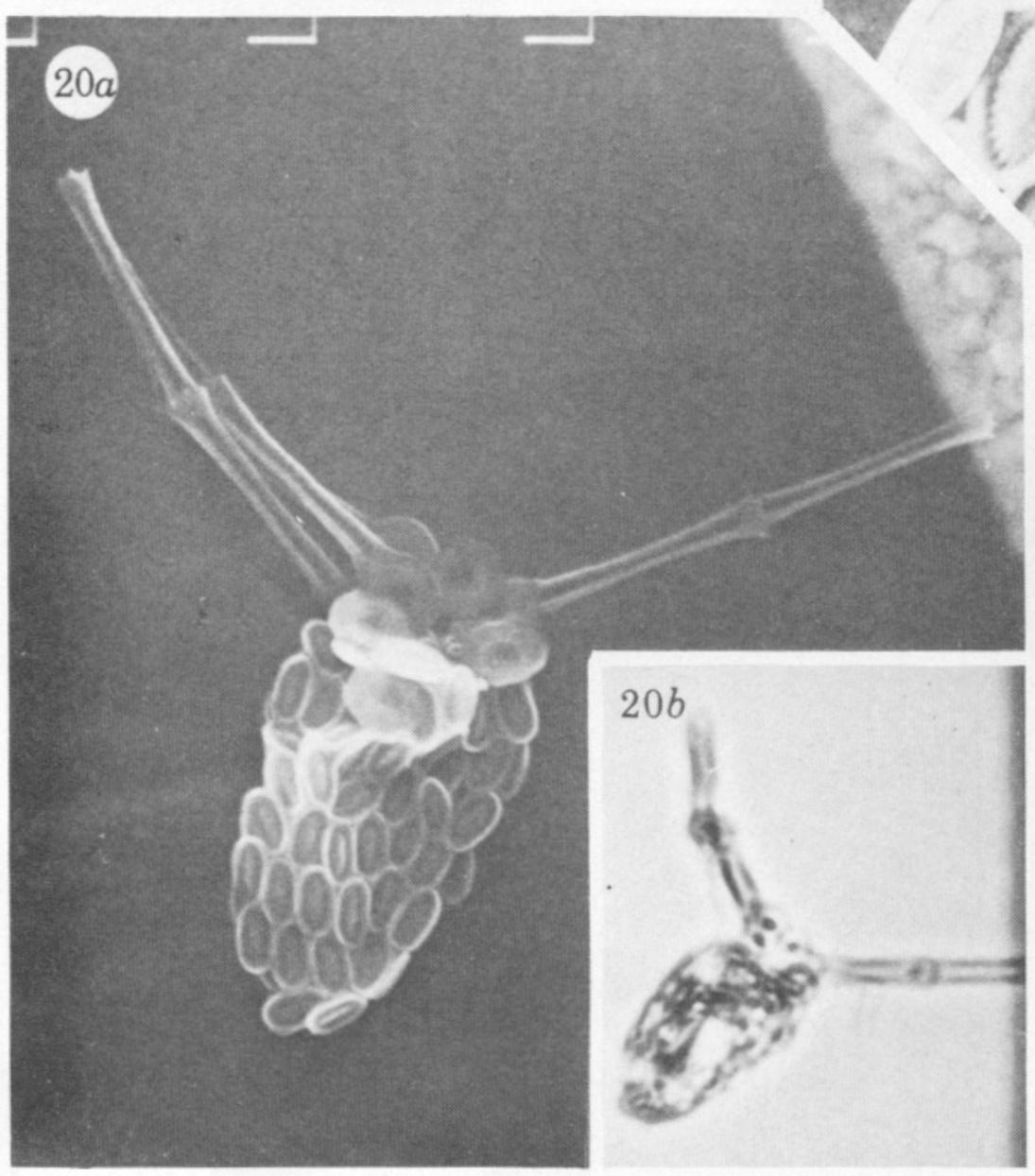
FIGURES 15–18. For description see opposite.

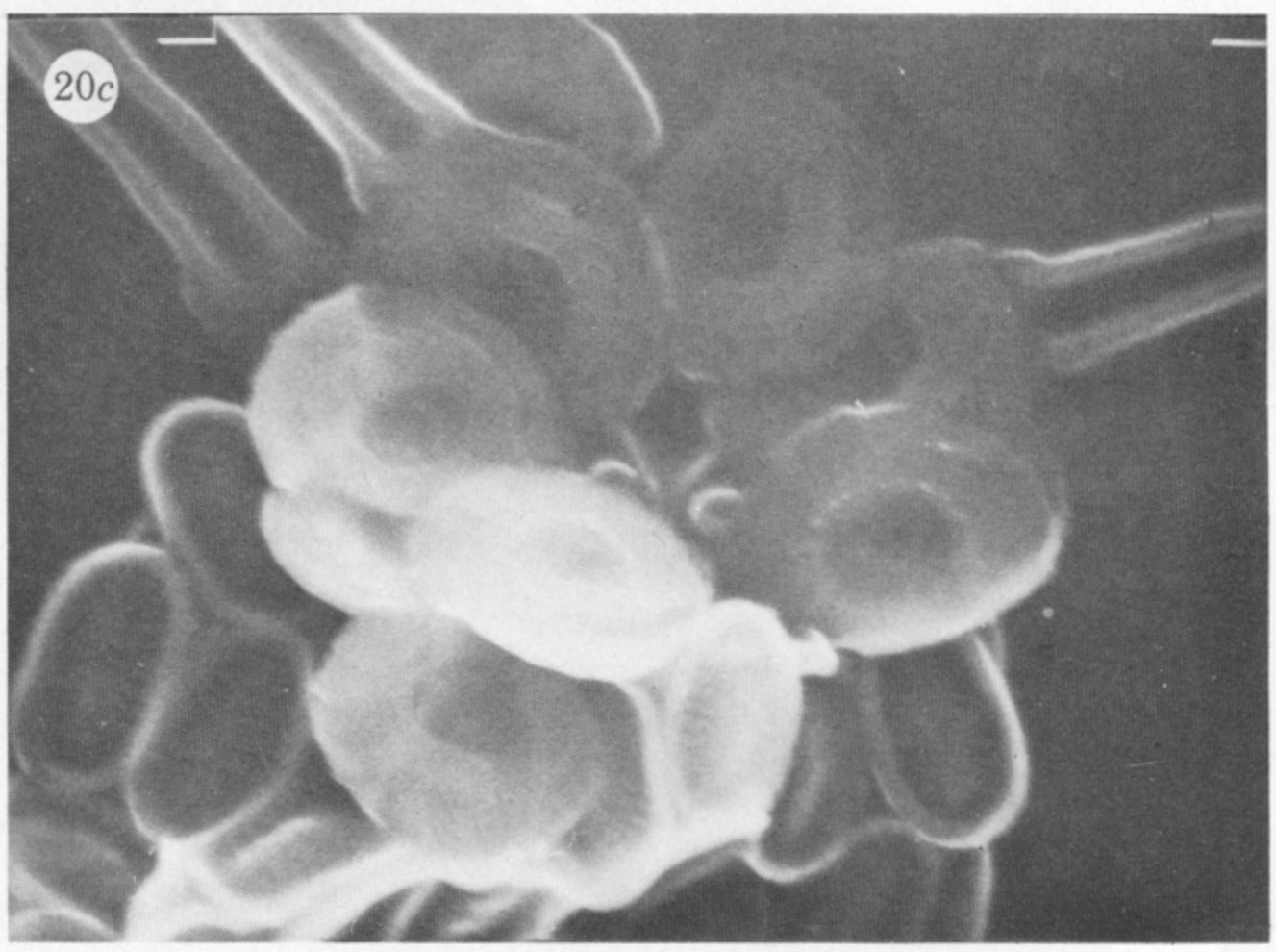












FIGURES 19 AND 20. For description see p. 192.

DESCRIPTION OF PLATE 7

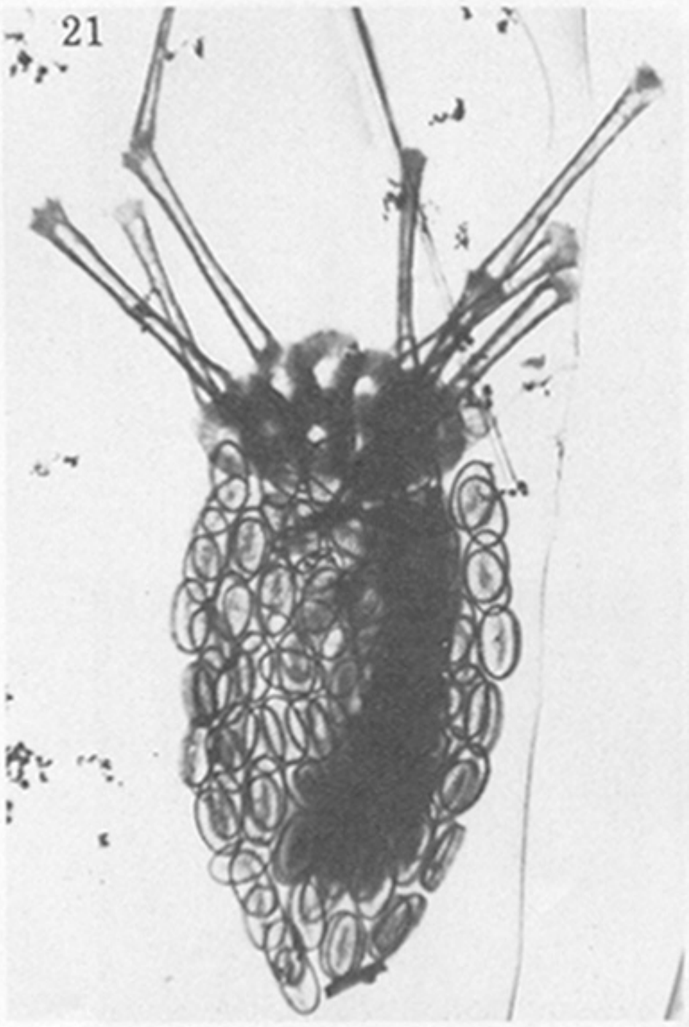
Halopappus adriaticus: shadow cast specimens from several water sources.

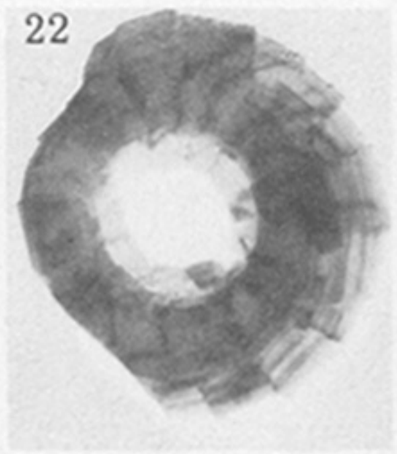
FIGURE 21. Specimen from sample 'Darwin 13' (table 1), personally identified by Mrs Gaarder. Transmission electron micrograph Y_N 7888.14; magn. ×3000. (*a*) Posterior tip; Y_N 7888.16; magn. ×10000.

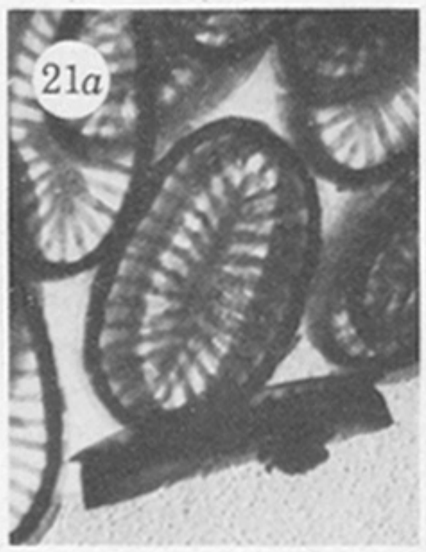
FIGURE 22. Detached ring-coccolith showing the characteristic asymmetry and presence of fringing crystallites around the optically empty centre; from sample 'Darwin 8' (table 1), transmission electron micrograph Y_N 7937.13 (EM 6B, Nottingham); magn. ×10000.

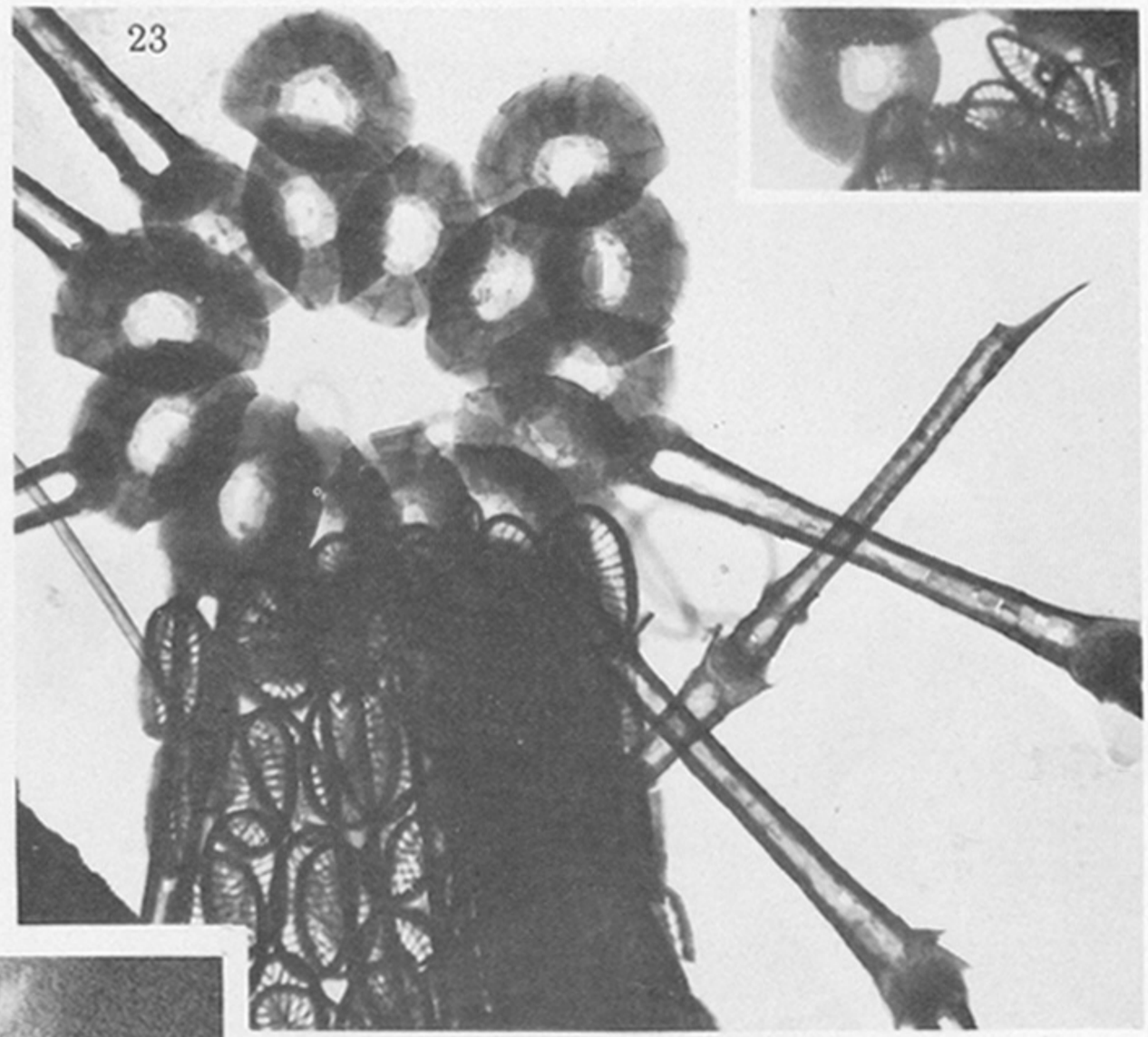
FIGURE 23. Anterior end of another specimen from sample 'Darwin 13' showing the complete assembly of ring-shaped coccoliths, some carrying tiny extraneous particles supported by an otherwise invisible central membrane. Transmission electron micrograph Y_N 7973.19; magn. ×5000 (inset, a similar specimen showing a rhomboidal small coccolith with tubular central excrescence, micrograph Y 7985.19 (EM 6B Leeds); magn. ×5000.

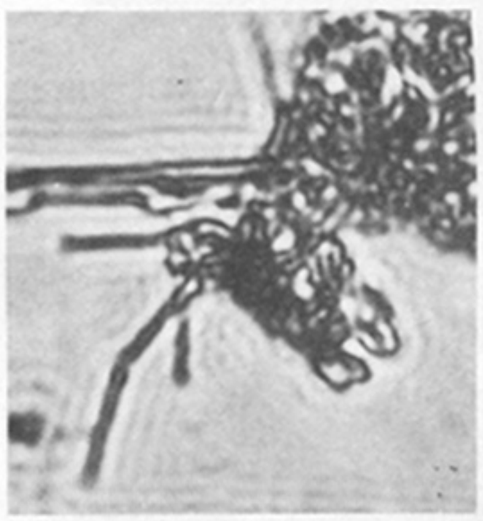
FIGURE 24. An exceptionally informative cell from sample 'Darwin 11' dried near a pennate diatom, see also figures 24*a–d*, 25 and 26. (*a*) Transmission electron micrograph Y_L 7955.31; magn. ×5000. Inset: light microscopy (dry lens) of the field, exposure 190.13, magn. ×1000. (*b*) Group of coccoliths from one side of the cell including the field marked X, scanning electron micrograph YO 8302.7 (Temscan, Lancaster); magn. ×7500. (*c*) Field of coccoliths near X in figure 24*b* showing substructural details of body coccoliths; transmission electron micrograph Y_L 7955.34; magn. ×20000. (*d*) Terminal segment of an appendage from top of field of figure 24 (see also figure 25); transmission electron micrograph Y_L 7955.31; magn. ×10000.



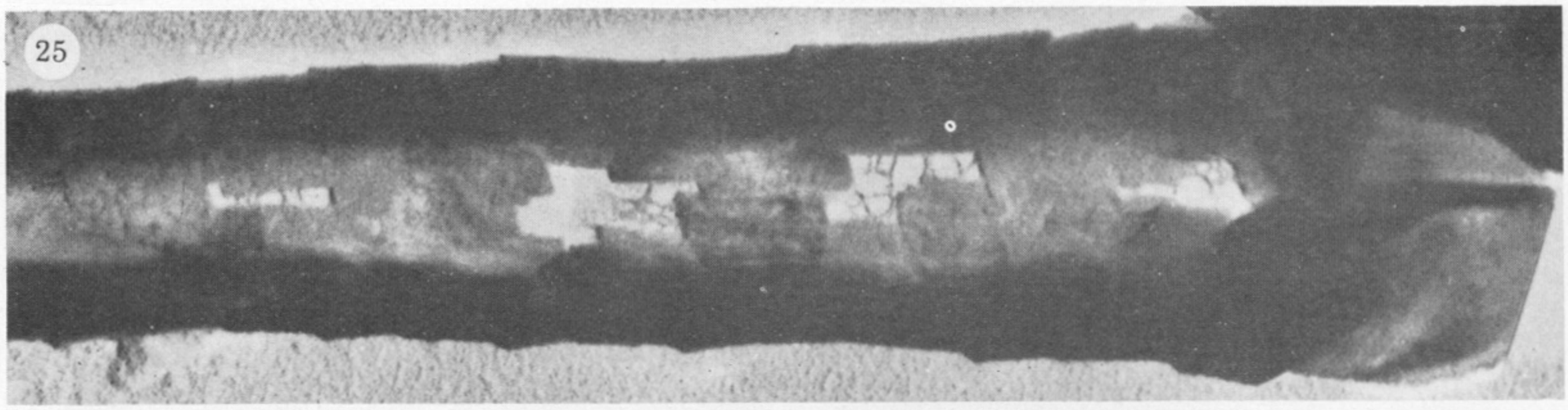


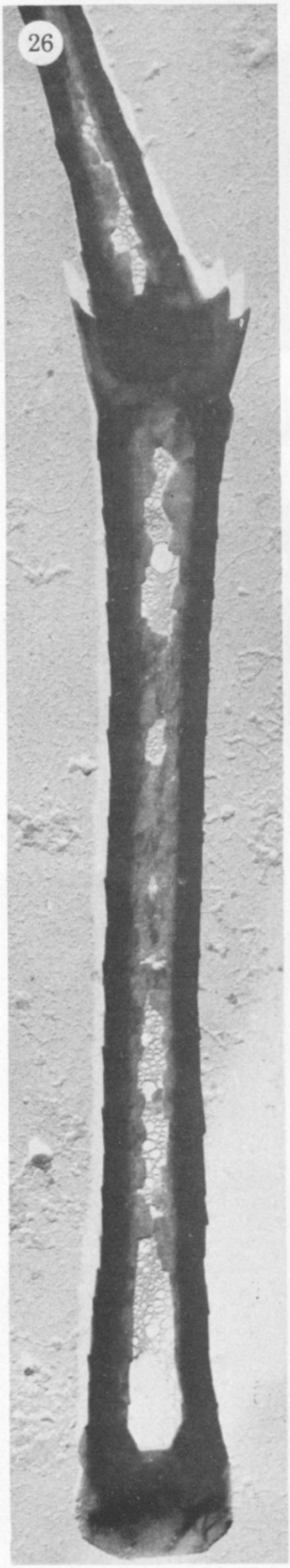


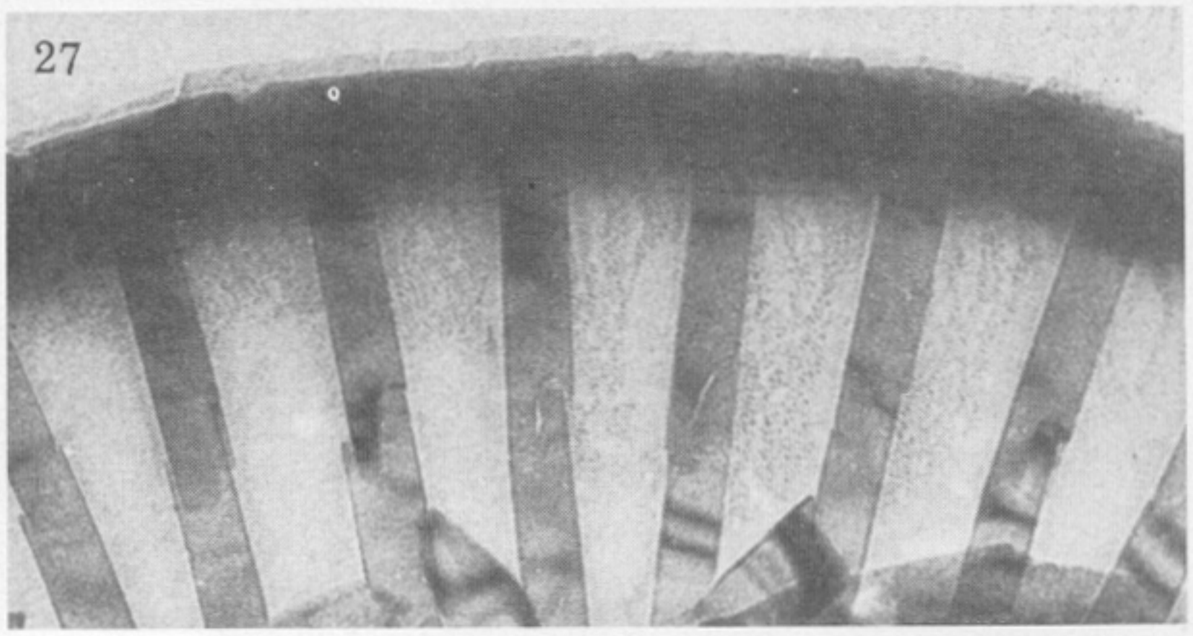


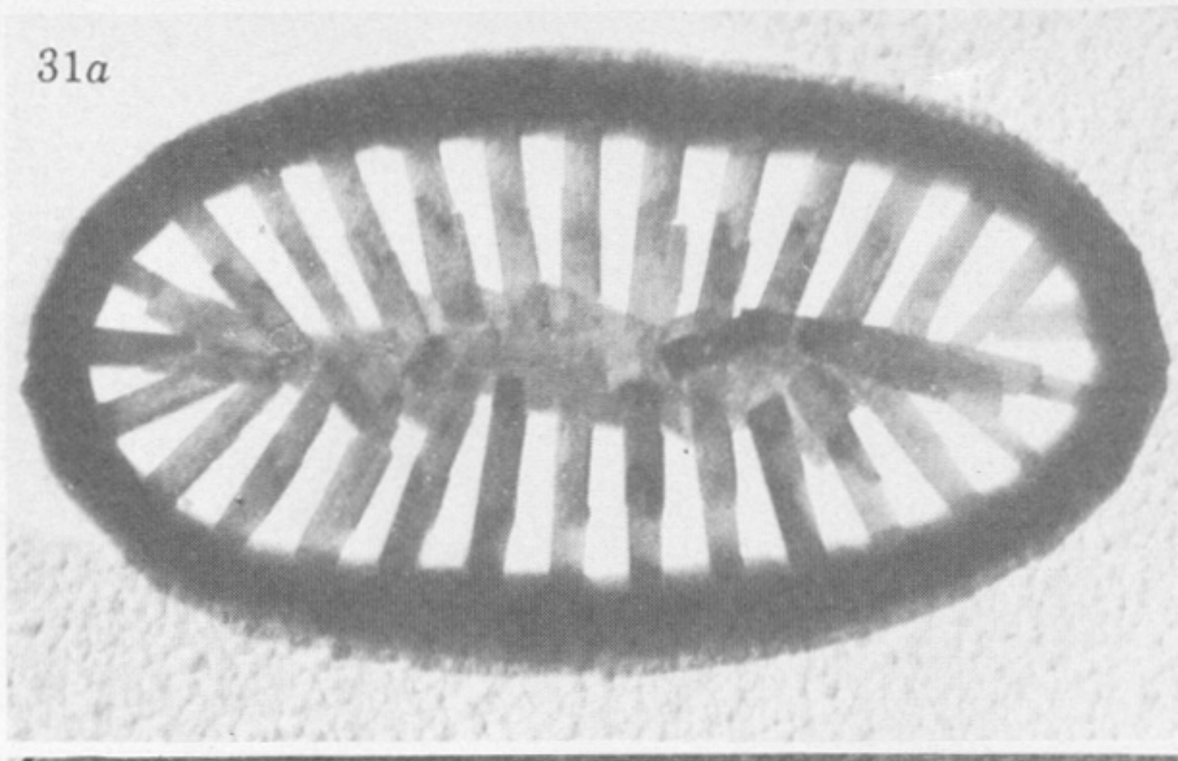


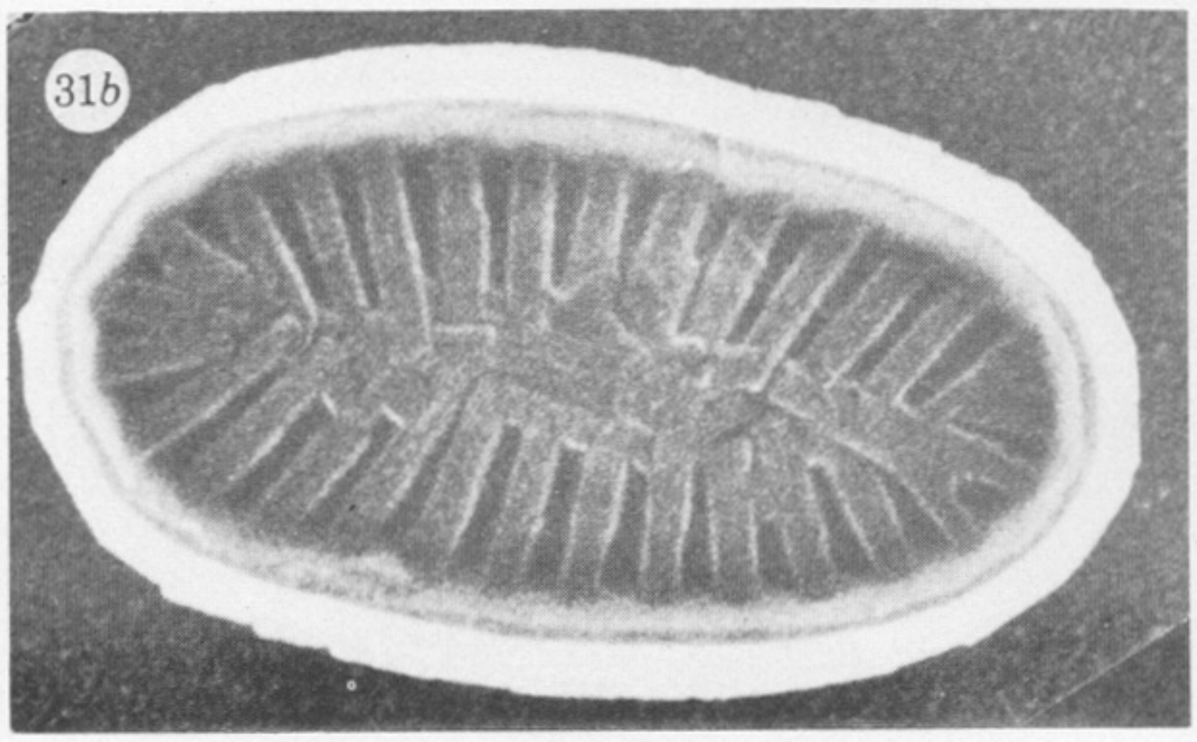
FIGURES 21–24. For description see opposite.

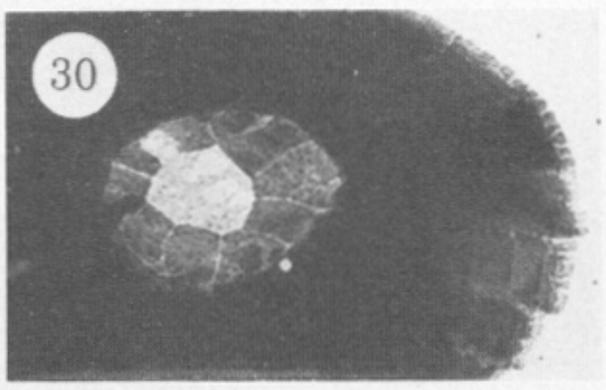


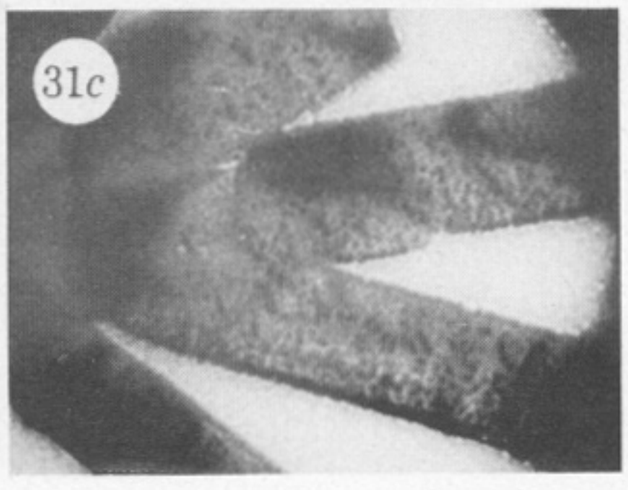












FIGURES 25–31. For description see opposite.