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NANOPLANKTON FROM THE GALAPAGOS ISLANDS: TWO GENERA OF SPECTACULAR COCCOLITHOPHORIDS (*OPHIASTER* AND *CALCIOPAPPUS*), WITH SPECIAL EMPHASIS ON UNMINERALIZED PERIPLAST COMPONENTS

BY IRENE MANTON, F.R.S.,[1] AND K. OATES[2]
[1] *Physics/Administration Building, University of Leeds, Leeds LS2 9JT, U.K.*
[2] *Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster LA1 4YQ, U.K.*

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CONTENTS

On the basis of electron microscopy of dry whole mounts of wild material set up *in situ* mainly in the Galapagos Islands but with two introductory specimens from South Africa, the presence of unmineralized periplast components has been demonstrated in two genera of fully calcified coccolithophorids (*Ophiaster* and *Calciopappus*) and also in a broken cell, otherwise attributable to *Chrysochromulina* aff. *fragilis* Leadbeater. The

last possesses many small elliptical plate scales with characteristic surface markings, together with fewer but larger sheet scales, each membranous, flexible, and almost without patterning except at the edge which carries a narrow zone of sparse radial striations. Both types of scale recur in the two coccolithophorid genera, the small elliptical plates as an underlayer beneath the coccoliths and the peripherally streaked membranes individually attached to the proximal surfaces of coccoliths as an integral part of their structure. Though present, these are more difficult to detect in *Calciopappus* than in *Ophiaster* in which they have been clearly demonstrated in specimens from both South Africa and the Galapagos Islands. In addition, some types of *Ophiaster* have also been shown to possess completely patternless membranes, detectable only by their indirect effects, occupying the apparently vacant plate centres of coccoliths in special positions. Other aspects of coccolith substructure are discussed with special reference to recurring difficulties regarding speciation in the two genera. Revised generic descriptions are provided but specific descriptions are limited to *Ophiaster*. These include revision of the two existing taxa (especially necessary for '*O. formosus* Gran') and the erection of three additional new taxa (*O. reductus* sp.nov., *O. minimus* sp.nov. and *O. formosus* var. *inversus* var.nov.). The final discussion summarizes and comments on present knowledge of *Chrysochromulina fragilis* sens. lat. in relation to several genera of coccolithophorids including, but not limited to, *Ophiaster* and *Calciopappus*.

Historical introduction

Coccolithophorids are photosynthetic planktonic unicells with calcified periplasts. They are mainly but not exclusively marine, and occur at all temperatures, becoming the dominant group, numerically exceeding even diatoms, in tropical waters.

As is well known, the enormous amount of detailed information accumulated since the coccolithophorids were first recognized as a special group of flagellates (Murray & Blackman 1898; Lohmann 1902), has mainly involved the calcified components (coccoliths) which have recently (since Kamptner 1950, 1952; Deflandre & Fert 1952; Braarud *et al.* 1952*a*,*b*) been found to be exceptionally well suited for study by means of electron microscopy. In consequence, several hundred taxa can now be identified with reasonable certainty. An estimate of 200 was given by Black (1968) but many more can be named with the aid of compilations such as Deflandre & Fert (1954), Halldal & Markali (1955), McIntyre & Bé (1967), Borsetti & Cati (1970), Okada & McIntyre (1977), Heimdal & Gaarder (1980, 1981) and others. Concurrently, a fossil record extending at least as far back as the Jurassic period has been intensively studied and documented by geologists such as Black & Barnes (1961), Noel (1965), Deflandre & Deflandre-Rigaud (1967) and Black (1968) in a literature recurrently listed and updated by Loeblich & Tappan (1966 and later). Much of this literature is nevertheless incomplete and/or uncompletable for reasons that will become obvious.

The slow build-up of knowledge, throughout the 19th and early 20th century, following the initial detection of what we now know to have been fossil coccoliths in chalk (Ehrenberg 1836) can be taken as known since the historical facts have often been narrated (see especially: Noel 1965; Black 1965). Of more immediate importance in the present context are mid-20th-century observations based on cultures. Thus Parke & Adams (1960) were able to demonstrate unforseen complexities of life history in cultures established at Plymouth in which a recently described taxon, *Crystallolithus hyalinus* Gaarder & Markali, 1956, was shown to be a self-perpetuating motile phase in the life history of a longer-known non-motile coccolithophorid, *Coccolithus pelagicus* (Wallich) Schiller, 1930. These authors also showed that the motile cells of

the *Crystallolithus* phase carried two flagella and a third filamentous appendage essentially resembling the so-called haptonema in some uncalcified planktonic flagellates, notably *Chrysochromulina* spp. (Parke *et al.* 1955), thereby permitting the coccolithophorids as a whole (Kalkflagellaten as they had sometimes been called) to be allocated to a definitive taxonomic position with respect to other types of flagellates. They have since been generally regarded as calcified representatives of the class Haptophyceae Christensen, 1962, though recent changes in the International Rules of Botanical Nomenclature have caused this name to be replaced by that of Prymnesiophyceae Hibberd, 1976.

Cultured material from the same source (Plymouth) when sectioned (see, for example, Manton & Leedale 1963) provided unexpected new insight into coccolithophorid periplasts which had been found in some instances to contain uncalcified haptophycean-type scales in addition to coccoliths. The mode of origin and morphological nature of the latter were also greatly clarified (see especially: Manton & Leedale 1969; Manton & Peterfi 1969; Pienaar 1969, 1971) when sections permitted both coccoliths and scales to be traced with certainty to production sites in the cisternae of the Golgi system. In the unmineralized haptophycean genera *Prymnesium* and *Chrysochromulina* (see, for example: Manton & Parke 1962; Manton 1966*b*, 1967*a*, *b*), cognate facts were already known, as in other pigmented flagellates, both green (e.g. *Heteromastix* (Manton *et al.* 1965), *Mesostigma* (Manton & Ettl 1965), *Pyramimonas* (Manton 1966*c*)) and golden-brown (e.g. *Sphaleromantis* (Manton & Harris 1966)).

The coccoliths themselves, in three different taxa, notably *Coccolithus pelagicus*, *Hymenomonas* (= *Cricosphaera*) *carterae* Braarud and *Hymenomonas roseola* Stein, previously interpreted as calcified oval rings complete in themselves, were also shown to be attached to unmineralized plates in a manner recalling the scale rims of equivalent *Chrysochromulina* species. The unmineralized central region of such scales, still present in mature coccoliths whether outside or inside the subtending cell, were found to be unmistakable and fairly massive in these particular taxa when seen in sections (see especially Pienaar 1969), while, even without sections, a characteristic surface pattern could indicate the presence of unmineralized central material with equal certainty when seen in a shadowcast whole mount. Exceptionally such patterning may be sufficiently conspicuous to attract attention from the outset, in which case the facts will have been recorded at once in the type-description, as in the recently erected new genera *Wigwamma* and *Calciarcus* (Manton *et al.* 1977). More often, such patterning, though present, may be overlooked because of the extreme difference in optical contrast between calcified and uncalcified areas. Later, when emphasis changes and attention need no longer be concentrated solely on crystallites, the characteristic markings on other components can be recognized and interpreted. Thus the genus *Papposphaera* Tangen (Tangen 1972), amplified in this way by Manton & Oates (1975), Manton *et al.* (1976) and Thomsen (1981), exemplifies this trend. Deployment of newly devised methods for the more effective treatment *in situ* of the contents of freshly gathered water bottle samples, together with the routine use of shadowcasting, are currently providing equivalent additions (see, for example, Leadbeater & Morton 1973) to supposedly well known taxa such as *Syracosphaera pulchra* Lohmann and *Rhabdosphaera stylifera* Lohmann.

Experimental and observational work designed to explore the biochemistry, physiology and crystallography associated with coccoliths exists but does not immediately concern us. Nevertheless, all these methods of enquiry added together involve directly no more than a minute fraction of known coccolithophorids. This may not matter if all that is needed is a means of

compiling a catalogue of securely named specimens for stratigraphic or other practical purposes. Outside this context, however, a practice of treating partial structures as if complete would be hard to defend. With fossils, this may be unavoidable since the foreseeable likelihood that fragile components would almost always disappear early, in sediments ancient or modern, rules out certain desirable types of information from most fossil taxa. For this and other reasons, the importance of drawing on extant taxa as fully as possible, whenever material becomes available in a form and on a scale permitting much more difficult observations to be made than those required for mere recognition, becomes obvious.

In the present communication (and in others to follow), the selection of taxa for use in this way has been guided by two further considerations, beyond those of convenience and abundance of specimens. On the one hand, the spectacular morphology, individually distinctive but easily seen even with the light microscope, should make recognition less of a problem than might otherwise be the case. On the other hand, the positive advantages of treating selected genera together should increase the clarity with which conclusions based on mutual comparisons can be formulated.

Not unnaturally, the close attention to details required for elucidation of the more difficult types of specimen can also lead to new insight in unforeseen directions. For example, the availability of large collections of certain kinds can draw attention to an unsuspected degree of intraspecific variability which might and perhaps should affect the taxonomic treatment. Thus quantitative differences alone can be recognized as subordinate in value to qualitative differences, but even this proviso does not avoid a recurring dilemma when numbers are few, namely, that of distinguishing genuine specific criteria from accidental malformations or other local aberrations affecting a few exceptional individuals. A final decision may be impossible under such circumstances without more collecting and only the type and degree of morphological differences found can give guidance regarding the appropriate or necessary treatment to adopt as an interim measure in any one case. As we shall see, even one specimen may have to be used as the basis of a new species if its characters are different enough to prevent its inclusion within any others. Conversely, large populations separated from others in the same area by apparently slight differences may appear most conveniently treated as subspecies, while a few isolated specimens with single character-differences may be effectively placed on record if treated as no more than a new variety.

Examples of all these situations will be introduced below but since taxonomy, though necessary, has been intended as no more than a minor concern here, existing species and genera will remain untouched unless there is compelling new evidence to the contrary.

Material and methods

The sources of material are limited to two geographical areas, namely (1) South Africa (lat. 33° 56′ S; long. 18° 19′ E), visited by both authors in November 1972, based on the Botany Department, University of Cape Town, and (2) the Galapagos Islands (lat. 0° 56′ S; 91° 0′ W), visited in August 1977 by the senior author (I.M.) with other collaborators, notably Miss Joan Sutherland, A. D. Greenwood, Dr Margaret McCully and Mrs K. Greenwood, the last two operating as passengers on M.S. *Iguana* and the others based on the Charles Darwin Research Station on Santa Cruz Island.

Many samples from both areas were processed though only a few will be quoted here

(see table 1). The method of drawing water from a known depth by means of a hand-operated van Dorn bottle is standard, after which, within a few hours, the nanoplankton was concentrated by methods that changed slightly according to date and circumstances. In each case the first step was to pour some of the water (commonly 2 l at a time) through a fine nylon fabric (of 25 μm pore diameter) to remove debris and the larger organisms. After this, in South Africa (1972), the volume of water was reduced with the aid of a large centrifuge followed by a smaller, faster centrifuge, applied for not more than 7 min, to produce a pellet which, re-suspended in the last few millilitres of the mother liquor, could be used for making preparations.

TABLE 1. DETAILS OF WATER SAMPLES CITED IN FIGURE LEGENDS AS SOURCES OF SPECIMENS FROM CAPE TOWN (1972) AND THE GALAPAGOS ISLANDS (1977)

(For further information about the A samples see below.)

sample	locality	date	depth/m	temperature/°C
Cape VII	two miles NW of Houte Bay Neck	9 Nov.	20	9.25
Cape XI	between Robben Island and Cape Town	10 Nov.	10	10.25
Darwin 7	Academy Bay (near anchored ship)	12 Aug.	5	22
Darwin 8	Academy Bay (near anchored ship)	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 14	Academy Bay (further seaward)	13 Aug.	15	22
Darwin 15	Academy Bay (beyond M.S. *Iguana*)	13 Aug.	15	22
Darwin 18	Academy Bay (*en route* for Barrington)	16 Aug.	15	21.5
Darwin 21	Barrington Island (near NW point)	16 Aug.	15	18
Darwin 23	Plazas (between islands)	20 Aug.	8	18.5
A1	Bartolomé Island (Sullivans Cove)	15 Aug.	10 (on bottom)	22
A4	Isabella Island (Tagus Cove)	16 Aug.	surface	17
A8	James Island (Buccaneer Bay S)	17 Aug.	15	22

In the Galapagos Islands (1977), the large centrifuge was replaced by a Millipore filter (1.2 μm pore diameter), the final concentration being again achieved by means of a small bench centrifuge. On board ship, on the other hand, where centrifuging could not be attempted, the first-stage concentrate produced by filtration was bulk-fixed in 1% glutaraldehyde and brought to the Charles Darwin Research Station for finalizing. Samples so treated are designated A in table 1.

Dry whole mounts were set up in the usual way, either on glass slides for subsequent light microscopy or on copper grids coated with carbon-on-formvar support films for subsequent electron microscopy. Except for the A samples mentioned above, fixation was with osmium tetroxide, usually as vapour from a 2% aqueous solution, applied for 30 s to drops of the fresh concentrate after these had been placed in position on the slides or grids. They were then air-dried. No further fixation was needed for the A samples which were merely spun down and washed in de-ionized or distilled water with the aid of a bench centrifuge to remove the glutaraldehyde, after which drops of the fixed material could be used at once. As a final step, all types of preparation must be carefully rinsed with several changes of de-ionized water to remove salt crystals before being dried for a second time. They are then safe for transport or storage, without any further field treatment.

After returning to England, all grid preparations were shadowcast in the usual way with gold–palladium, after re-washing if necessary. Much later (1980), selected preparations, already studied by means of transmission electron microscopy, were rinsed in absolute alcohol and given a further coating with gold preparatory to scanning (for further details see Manton *et al.* 1981).

The transmission electron microscopy was carried out mainly by the senior author (I.M.) in several laboratories as opportunity offered. The African specimens (1972–3) were examined with an A.E.I. 801 microscope formerly in the Department of Biology in the University of Lancaster. The Galapagos specimens (1977–80) were surveyed in the first instance mainly on an A.E.I. EM6B microscope in the Cell Biology Unit in the University of Nottingham (by courtesy of Professor Cocking) supplemented by a similar microscope in the Department of Gynaecology, Leeds University, (by courtesy of Miss Ursula Lister) and, more recently, by a JEOL Temscan in the Lancaster Department. Finally, in 1980–81, this last instrument, used in the scanning mode by the junior author, became the source of the scanning electron micrographs illustrated on plates 1, 4 and 8.

The light microscopy was added last, by the senior author, in the first instance mainly as a means of verifying the correctness of calibration of electron microscopes which, used over a long period, can change their magnifications unpredictably. A Zeiss Photomicroscope 2 in the Cell Biology Unit at the Medical School, University of Liverpool, (by courtesy of Dr Stanley Walker), was used for this purpose. In the first instance it was set up for phase contrast and used with a $\times 40$ dry lens applied to the dry preparations without a coverslip. Photographs recorded on 35 mm film (Ilford Pan F) were subsequently adjusted to a final magnification of exactly $\times 1000$ by means of a Leitz Focomat enlarger belonging to the Royal Society but still available to the senior author in Leeds. Several of these calibration photographs have been reproduced beside micrographs of other kinds in the plates. Exceptionally, for special purposes, an oil immersion objective ($\times 63$) was also used. For this a selected cell was wetted with a drop of immersion fluid (in this case Objectol) into which the oil immersion objective was dipped, again without a coverslip. With the microscope set up for phase contrast, photographs taken in this way could be routinely enlarged to a final magnification of $\times 2500$ and several are reproduced here. This method is of course only applicable to a preparation on glass from which the immersion fluid can be removed at will by rinsing with amyl acetate.

In presenting the results we have been at pains to introduce technical information into the figure legends, thereby permitting every specimen to be traced back to a named water sample with the aid of table 1 and each micrograph to be attributed to a corresponding microscope. Such details, though apparently trivial, cannot be added later, and their uses are many, both as means of detecting errors and, foreseeably, for comparative purposes, more especially in an ecological context, in future publications dealing with different organisms from the same water sources.

Finally, the personal contributions made, as noted above, by each of the authors, towards either the initial collecting or the subsequent working out or both, should also be remembered though full responsibility for interpretation and especially for the formulation of taxonomic conclusions must rest squarely with the senior author.

Results. 1. *Ophiaster*

Introduction to Ophiaster

In a definitive study of the genus *Ophiaster* by Gaarder (1967) a diagnosis of the genus as then understood was presented in terms that can usefully be quoted (Gaarder 1967, p. 184).

> 'Cells spheroid to ovoid with two flagella (and a haptonema?); ordinary coccoliths normal-elliptical discs, irregularly arranged, touching each other closely; 4–6 coccoliths around flagellar area bearing centrally placed spines of about coccolith length; laterally compressed enlarged and transformed coccoliths linked by narrow ends into flexible arms, connected by looplike proximal ends to form a starlike structure attached near posterior cell pole. Two species.'

The two species referred to were *O. hydroideus* (Lohm.) Lohm. and *O. formosus* Gran, the first being accepted as the type species of the genus following an elaborate discussion of historical details. Both species have been recorded from parts of the Atlantic and Pacific oceans (Gaarder 1967; Hasle 1960), though *O. hydroideus* is generally thought to be the commoner (see also Okada & McIntyre 1977).

The material surveyed below cannot be included in *O. formosus* on existing definitions (but see below), though the genus is otherwise well represented in the Galapagos Islands. It will indeed be necessary to distinguish at least four distinct types, two (or three) common and two rare, some or all of which may eventually need to be distinguished as species. Before considering any of these it will nevertheless be helpful to introduce two exceptionally informative individual specimens from a different area (South Africa), one personally identified as *O. hydroideus* by Mrs Gaarder whom we wish to thank.

Observations on two South African specimens

The specimen of *O. hydroideus* illustrated in plate 1 and part of plate 2 is important for the clarity with which several critical details are displayed. The haptonema alone, seen loosely coiled near the flagellar bases in figure 1, at once removes the question mark placed against this organelle in the generic description.

Elsewhere the periplast is slightly incomplete, since a few body scales have been lost from the right hand side, while the posterior appendages have all become shortened by loss of an unknown number of terminal links. A few spined scales, of the type first detected by Gaarder (1967), are nevertheless still present near the anterior end of the cell and the tip of one of these can be seen entering the enlarged field reproduced for another purpose in figure 6*b*.

The crystallites in coccoliths of all morphological types are spaced apart to an unusual degree, thereby greatly facilitating descriptive analysis. The appendages in particular agree closely with the specific description given by Gaarder (1967, pp. 186–187). As may be seen in figures 3 and 4, each link is asymmetrical, the crystallites forming the edge on one side being smaller than those on the other. Here and there an edge crystallite on the convex side of the arm is replaced by a 'thorn', i.e. a shortly stalked flattened crystallite ending distally in a sharp point. The centre of each link, apart from an apparently vacant clear area located near one or both ends, is occupied by two groups of somewhat diagonally oriented, elongated crystallites, not described in detail by Gaarder. The special proximal link in each arm, on the other hand, is

exactly as described by Gaarder (1967), being longer, more asymmetrical, twisted and united to the base of the subtending cell by a characteristically widened end (figure 1).

The correctness of Mrs Gaarder's interpretation of the posterior appendages in this genus, as chains of strongly modified coccoliths, is endorsed by the more highly magnified views of ordinary coccoliths from the same cell, as illustrated in figures 6*b* and 7. Each coccolith consists of a calcified scale-rim bordering an oval, calcified, plate. The detailed substructure of the rims will be better understood later and it is enough to note here that two sorts of crystallite are involved, some larger and 'spade-shaped' and the others elongated tangentially and almost rodlike. The plate surfaces on the other hand are clearly traversed by a variable number of radially arranged bars (10–20 in these particular specimens), each attached by one end to the scale-rim and by the other to a central plaque with correspondingly indented edges. These plaques are commonly two or three in number, arranged parallel to the long axis of the plate or obliquely to it, though sometimes a plaque can replace a bar at one or both ends of the plate. Where indentations are omitted from the edge, radial bars are also absent, or may perhaps have fallen off. The degree of separation between adjacent bars can thus vary considerably from coccolith to coccolith, sometimes being so great as to leave large apparently vacant spaces as in figure 6*b*.

Evidence that such spaces, though unoccupied by crystallites are not empty can be obtained from figure 6*a*. This shows parts of two superposed coccoliths, one (right) lying with its distal face uppermost and the other (left) with its proximal face uppermost. Close scrutiny of both will reveal the presence of a system of short striations apparently attached to the rim peripherally and proceeding thence into an unoccupied area between crystallites in the right hand specimen and superposed on crystallites in the left hand specimen. These striations, in such a position, must represent peripheral patterning on an unmineralized membrane (or plate) underlying the bar crystallites, but, like them joined to the scale rim.

Other unmineralized periplast components are represented by the small elliptical scales visible here and there between and beneath the coccoliths (see especially figure 6*b*). They are sufficiently numerous to remove entirely the plausibility that two large plate scales with cruciform surface ridges (Gaarder 1967, pl. 3C) could be accepted as representing underlayer scales of an *Ophiaster* cell. We can now recognize these large scales as having come from a previously undescribed species of *Chrysochromulina* (Manton), in no way related to the *Ophiaster* against which they happen to have come to rest, doubtless as flotsam. The true underlayer scales of

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Description of plate 1

Ophiaster hydroideus. Shadowcast whole mount of a single cell collected in South Africa on 10 November 1972, sample Cape XI (table 1).

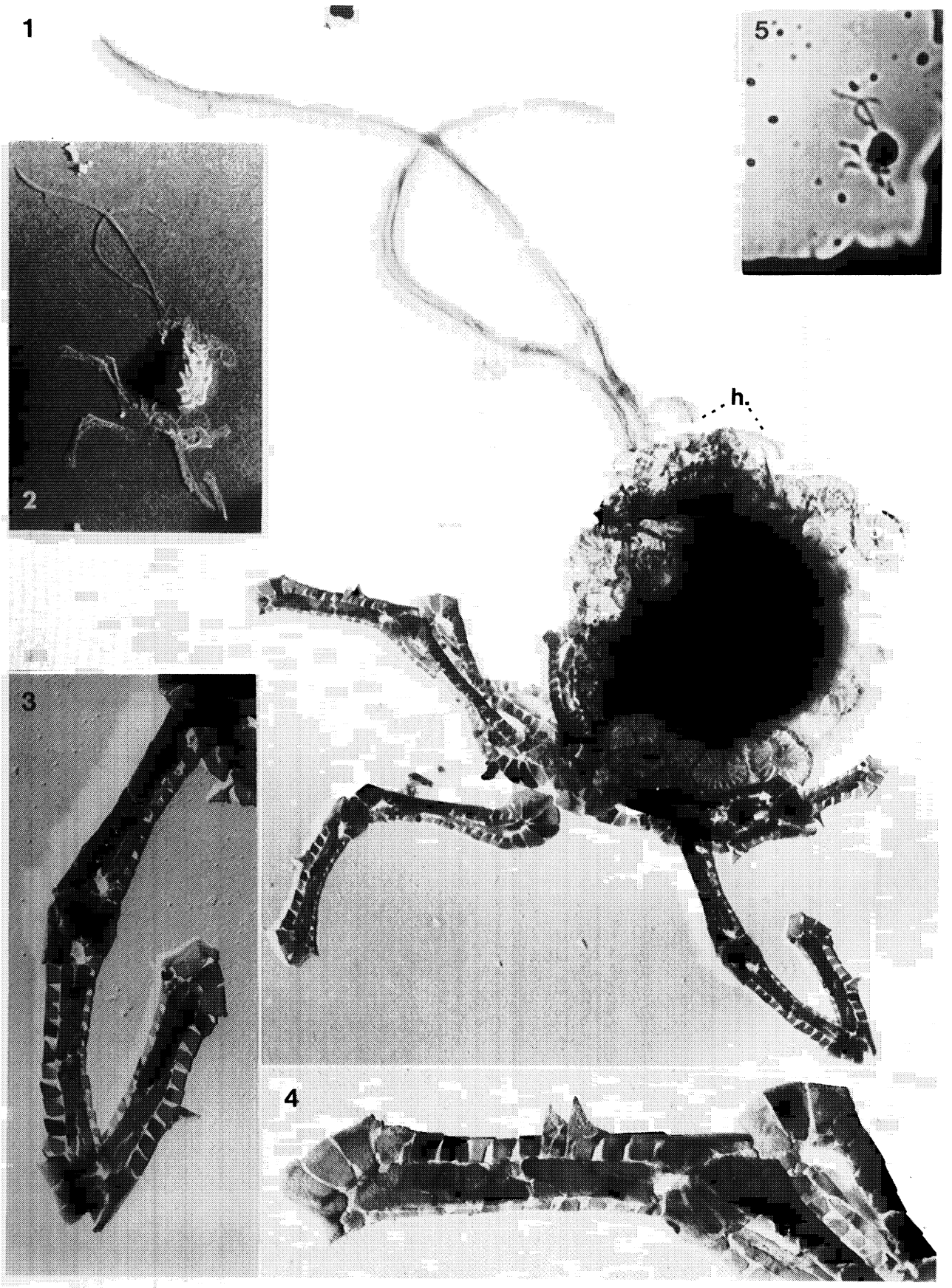
Figure 1. The cell as first found (1973). Transmission electron micrograph Y_L4692.26 (Lancaster £01); magn. ×10000.

Figure 2. The cell as seen with a scanning electron microscope (19£0). Micrograph $Y_0$8251.8 (Lancaster JEOL Temscan); magn. ×2000.

Figure 3. Part of an appendage arm from the cell of figure 1. Transmission electron micrograph Y_L4692.34 (Lancaster 801); magn. ×20000.

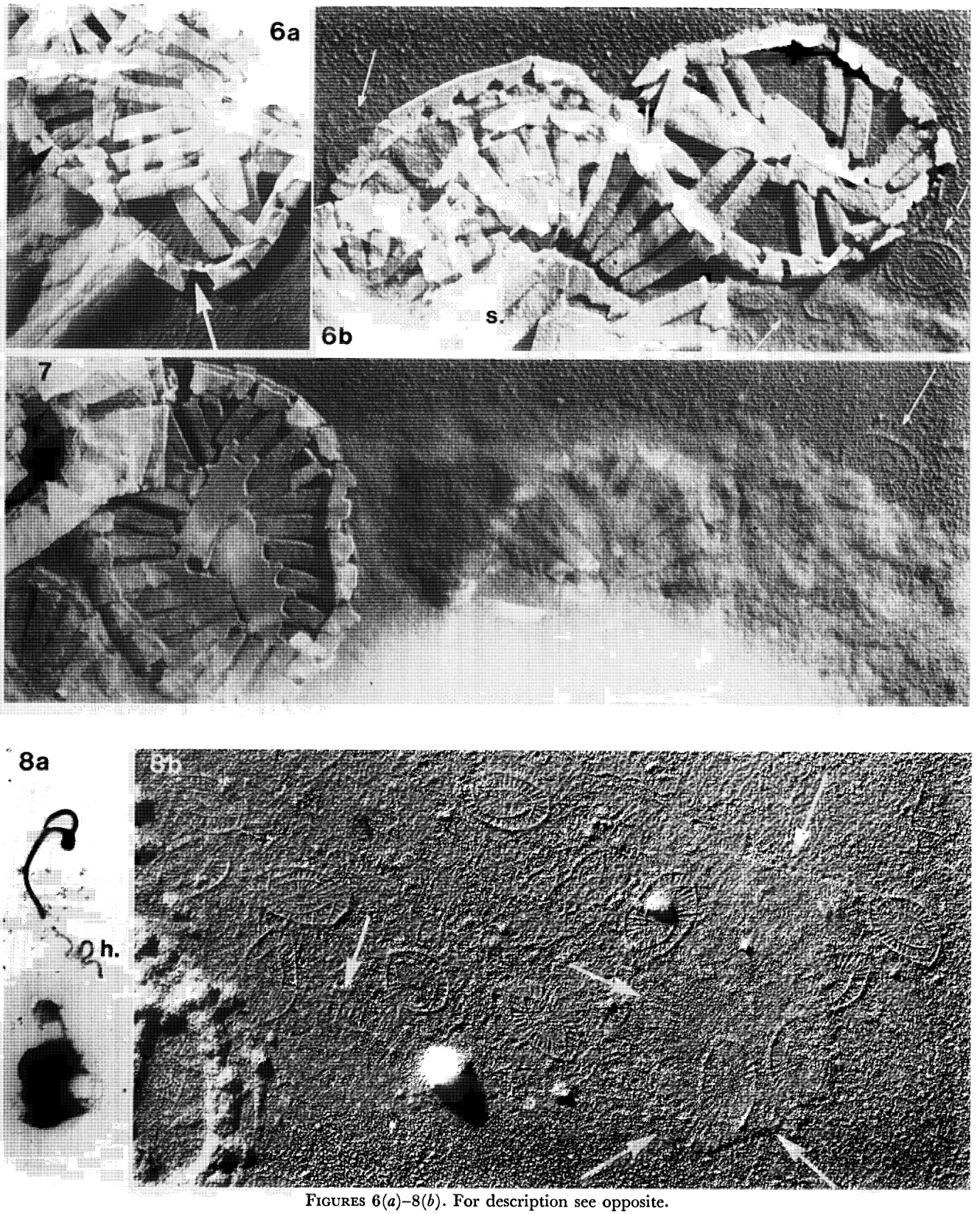
Figure 4. Another appendage link from the cell of figure 1 showing details of component crystallites. Micrograph Y_L4692.30 (Lancaster 801); magn. ×30000.

Figure 5. The cell of figures 1–4 after completion of the electron microscopy. Phase contrast light microscopy, film 174.30; magn. ×1000.



FIGURES 1–5. For description see opposite.

(*Facing p.* 442)



FIGURES 6(*a*)–8(*b*). For description see opposite.

Ophiaster are distinguished not only by their small size and elliptical shape but also by distinctive surface markings including a clearly delimited elliptical centre superimposed on radial ridges, as seen in figures 6*b* and 7. This observation has since been repeated many times with specimens of different origin from the Galapagos Islands (see below).

The second African specimen is illustrated in figures 8*a* and *b*. It is the last remnant of a severely disrupted cell in which only the short haptonema (figure 8*a*), still forming part of the torn-out protoplasmic appendages, indicates with certainty its haptophycean nature. The protoplast is otherwise uninformative but close scrutiny of the periplast fragments (figure 8*b*) will show many small elliptical scales corresponding in size and markings to those already seen in *Ophiaster*, together with some unobtrusive patches of patternless material (white arrows) delimited from the support film only by the presence of short peripheral striations. This specimen, if found in isolation, would have been identified as *Chrysochromulina* aff. *fragilis* Leadbeater. In the present context however it could represent the last stage of a disintegrating *Ophiaster* from which all the calcified parts have been lost leaving only an unmineralized underlayer. A decision in favour of one or other of these alternatives is, fortunately, not necessary since it is enough for our present purpose to have shown that unmineralized periplast components, morphologically similar to those encountered in *Ophiaster*, can be found independently. One additional detail should nevertheless be noted at once, namely the extraordinary lack of opacity of the sheet scales in figure 8*b*. Even when seen overlying the small elliptical plates the latter are scarcely concealed, the outlines remaining distinct, with even some of the surface markings detectable. This is only explicable on the assumption that these sheet scales are in fact extremely thin membranes and not substantial plates of the ordinary kind. The importance of this will be further discussed later (p. 455).

Observations on specimens from the Galapagos Islands

All the specimens assembled on plates 3 and 4 had been allocated at the outset to *O. hydroideus* sensu Gaarder since *O. formosus* seemed to be ruled out by total lack of agreement with certain, supposedly critical, parts of the description of that species, as already noted. However, after measurements had been made on a range of specimens, each carefully authenticated dimensionally by means of the light microscope as already explained (p. 440), discrepancies from the published descriptions of both taxa (Gaarder 1967) could be seen to have increased to a point at which either revisions had to be introduced or new hypotheses, including perhaps additional taxa, had to be erected. Recommendations on these and other matters, including those

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Description of plate 2

Figures 6*a* and *b*. Two parts of a single micrograph from the periplast of figure 1; reversed prints, showing peripheral striations (large arrows) indicating an unmineralized membrane in two places in figure 6*a* and with unmineralized underlayer scales near and beneath ordinary coccoliths (small arrows) in figure 6*b*. Electron micrograph Y_L4692.32 (Lancaster 801); magn. × 50000.

Figure 7. Another part of the surface of the specimen in figure 1, showing coccolith substructure (left) and an unmineralized scale (right). Micrograph Y_L4692.33 (Lancaster 801); magn. × 50000.

Figures 8*a* and *b*. Two views of a broken cell from South Africa collected 9 November 1972 in sample 'Cape VII' (table 1). (*a*) Flagella and a haptonema still present near the protoplast. (*b*) Many small elliptical scales and fragments of membrane with peripheral striations scattered in the field and identifiable as either *Chrysochromulina* aff. *fragilis* Leadbeater or a broken *Ophiaster* cell. (*a*) Micrographs Y_L5012.7 (Lancaster 801); magn. × *ca.* 5000. (*b*) Micrograph Y_L5012.8 (Lancaster 801); magn. × *ca.* 50000.

introduced by additional information assembled in plates 5 and 6, will be made. Before doing so however, it will be appropriate to consider basic facts relevant to understanding *C. hydroideus* sens. lat. in the Galapagos Islands, using plates 3 and 4 as originally intended.

(i) *O. hydroideus* (Lohm.) sens. lat.

Anyone who has successfully followed the descriptions of plates 1 and 2 will have no difficulty in understanding the gross morphology of whole cells and of coccoliths of the various categories, as exemplified in plates 3 and 4. The numerical variation in appendage arms is more or less as expected, five arms, six arms and seven arms being severally represented by figures 19, 9 and 10, among others. As usual, the commonest arm number is five but fewer than five, though sometimes seen, as in figures 13 and 22, almost certainly expresses accidental loss of arms by mechanical damage. Thus any appendage can become shortened by loss of an undefinable number of distal links, but complete arms can also break off, as indicated by figure 12. This figure illustrates a detached arm with no fewer than ten consecutive ordinary links still joined to the proximal link of an otherwise unknown cell. The other cell components need no further comment at this stage, the two flagella being clearly visible in many of the specimens illustrated, with the short haptonema also detectable in figure 24.

The substructure of individual coccoliths, though similar, is nevertheless not identical in every detail with equivalents illustrated from South Africa. Thus the crystallites in all the Galapagos specimens are more closely compacted together than those in figures 1–7. Further, the central plaques of the ordinary coccoliths are not normally indented at the edges, the

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Description of plate 3

Ophiaster hydroideus sens. lat. from the Galapagos Islands (1977), from dry whole mounts of wild material. Transmission electron micrographs taken with an A.E.I. EM 6B electron microscope in the University of Nottingham except where otherwise stated; other micrographs as specified in legends.

Figure 9. A cell with a six-armed posterior appendage and both flagella showing clearly; sample A1 (table 1). Oil immersion phase contrast light microscopy: exposure 196.42a; magn. × 2500. Inset: the same cell taken with a a dry lens, exposure 195.12; magn. × 1000.

Figure 10. A cell with a seven-armed posterior appendage; otherwise as figure 9. Photograph under oil immersion; exposure 196.26; magn. × 2500. Inset: the same cell taken with a dry lens; exposure 195.9; magn. × 1000.

Figure 11*a* and *b*. Two parts of posterior appendages of a cell from sample Darwin 14. Transmission electron micrograph Y_N 7935.18; (11*a*) magn. × 10000; (11*b*) magn. × 20000.

Figure 12. Detached arm of a posterior appendage showing ten ordinary links and a proximal link (right) still united. Light microscopy (phase contrast, oil immersion), exposure 197.20a; magn. × 2500.

Figures 13 and 14. Two cells from sample A1 with broad appendages and both flagella showing. Light microscopy (phase contrast, oil immersion), exposures 196.41a and 194.32; magn. × 2500.
Insets: the same cells with a dry lens; exposures 195.12 and 195.9; magn. × 1000.

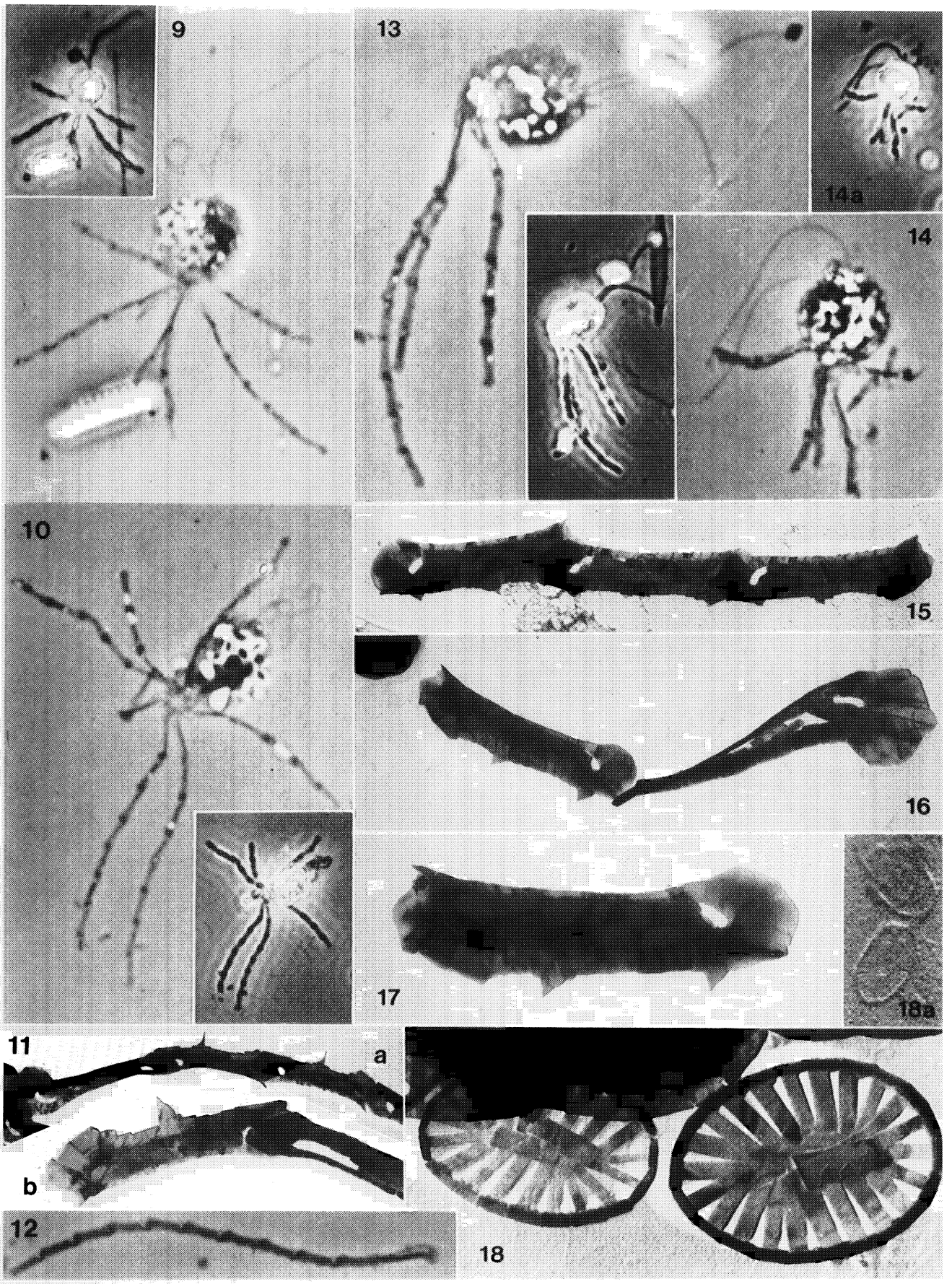
Figure 15. Chain of three consecutive ordinary appendage links (posterior ends directed left) from sample Darwin 11. Transmission electron micrograph Y_N 7918.1; magn. × 10000.

Figure 16. A proximal link (right) and an ordinary link from sample Darwin 13. Transmission electron micrograph Y_N 7904.14; magn. × 10000.

Figure 17. An ordinary link from sample Darwin 13, to show crystallites. Transmission electron micrograph Y_N 7976.30; magn. × 20000.

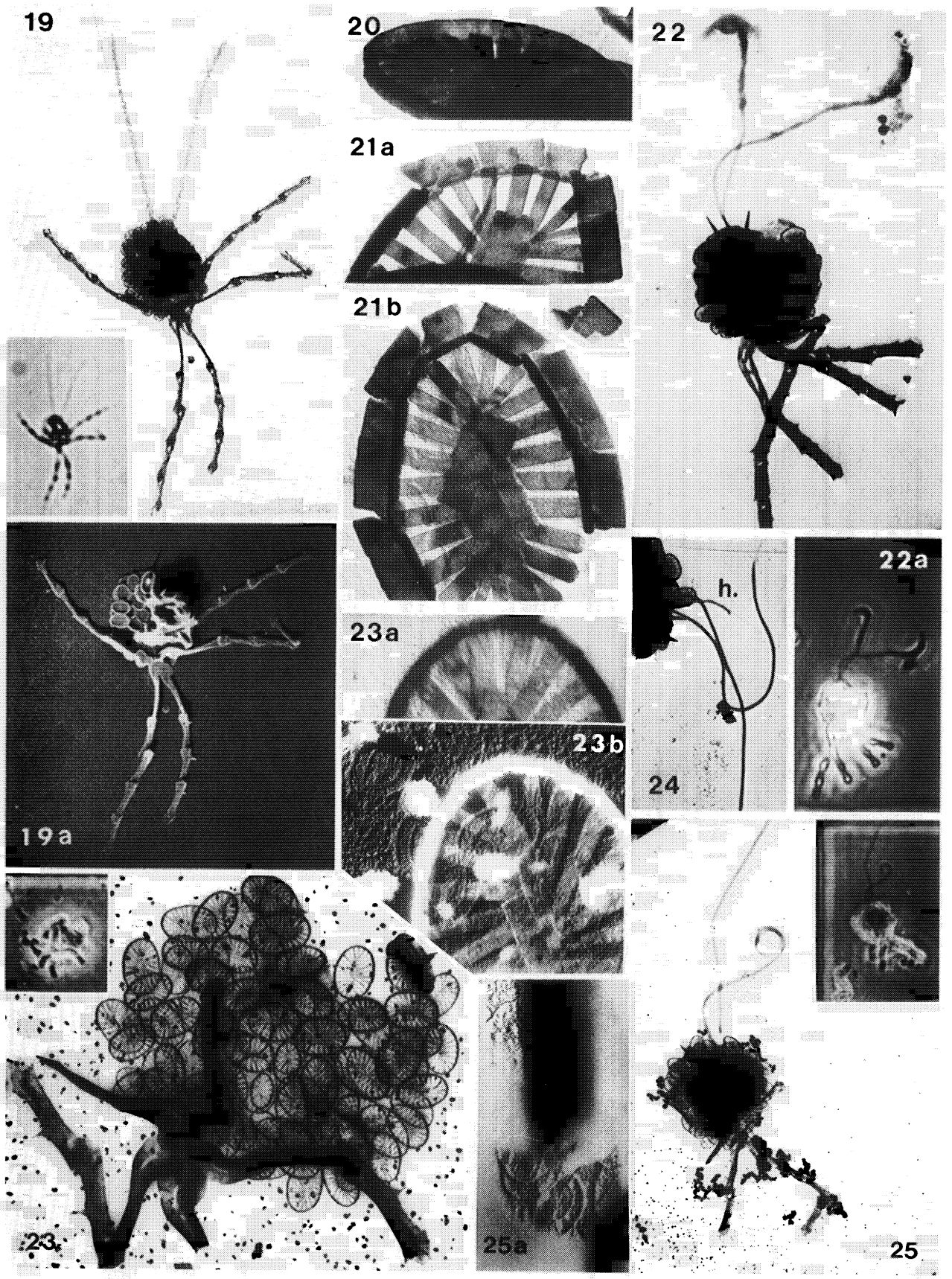
Figure 18. Ordinary coccoliths from a specimen from sample Darwin 14, showing central plaques, including tips of bar crystallites overriding a plaque surface (right), with faint peripheral striations visible over the bar crystallites near the rim at lower left. Transmission electron micrograph Y_N 7900.6; magn. × 40000.

Figure 18*a*. Another part of the field of figure 18, showing small unmineralized elliptical scales; magn. × 50000.



FIGURES 9–18. For description see opposite.

(*Facing p.* 444)



FIGURES 19–25. For description see opposite.

incoming ends of the corresponding bar crystallites tending to overlap onto a plaque surface (see especially figure 18, right), thereby partly concealing it from view. The plaques themselves tend to be more precisely rectangular than before and, though somewhat oblique, they do not seem to encroach on the bar crystallites at the ends of a coccolith as in figures 6*b* and 7. The rim crystallites, on the other hand, are more easily analysed in our present material, as may be seen in the three views provided: an edge view (figure 20), a top view (figure 21*a*) and a bottom view (figure 21*b*). When a rim is dismembered or spread out on the field, the larger 'spade-shaped' crystallites with undercut sides (figure 21*b* inset) are easily distinguishable from the tangentially elongated, more nearly rod-shaped crystallites, each carrying a thumblike process recessed into part of the lateral junctions between the others (see especially figure 21*a*).

The modified coccoliths of the posterior appendages show greater differences, not only from the African specimen but also relative to each other; hence some of the taxonomic problems to be discussed later. There is of course the same qualitative difference between proximal links (see, for example, figures 11, 16) and ordinary links, and the same type of asymmetry in the latter, with the edge crystallites along one of the long sides smaller than those on the other. The incidence of 'thorns' is more varied and they are sometimes greatly reduced if not absent (figure 15). The outlines of crystallites occupying a link centre are more difficult to see though

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Description of plate 4

O. hydroideus sens.lat. from Galapagos Islands (cont.); Nottingham EM 6B electron microscope except where otherwise stated.

Figure 19. Specimen from sample Darwin 14 with a five-armed posterior appendage and the two flagella showing clearly. Transmission electron micrograph Y_N 7945.1; magn. × 3000. Inset: light microscopy (phase contrast dry lens), exposure 166.3; magn. × 1000.

Figure 19*a*. The cell of figure 19 scanned and showing distortion (right) caused by protoplast shrinkage, but some other details, including a short spine, now clearly visible. Scanning electron micrograph (Lancaster Temscan), exposure Y_O 7984.45; magn. × 3000.

Figure 20. Edge view of a coccolith from the field of figures 18 and 18*a*. Transmission electron micrograph Y_N 7960.6; magn. × 40000.

Figures 21*a* and *b*. Parts of two coccoliths from a single field (sample Darwin 23), showing a distal face (*a*) and a proximal face (*b*) with two detached rim crystallites from the same coccolith at inset, top right; faint signs of appressed elliptical scales visible on the surface in (*b*). Transmission electron micrograph Y 7992.13 (Leeds EM 6B); magn. × 40000.

Figures 22 and 22*a*. Specimen from Darwin 14 with coarse appendages, perhaps numerically reduced by loss, and with two small spines flanking the flagella bases very clearly visible. (22) Transmission electron micrograph Y_N 7934.29; magn. × 3000. (22*a*) Light microscopy (phase contrast), exposure 191.2a; magn. × 1000.

Figure 23. Patch of coccoliths from the hind end of a broken cell from Darwin 21 with parts of a five-armed posterior appendage still attached. Transmission electron micrograph Y_N 7999K.3; magn. × 7500. Inset: light microscopy of the same cell, exposure 191.25; magn. × 1000.

Figures 23*a* and *b*. Parts of two coccoliths from the field of figure 23, magn. × 50000, to show unmineralized periplast components: (*a*) with peripheral striations; electron micrograph Y_N 7999K.7; (*b*) with unmineralized elliptical scales upon and beside the coccolith; transmission electron micrograph Y_N 7999K.9, reversed print.

Figure 24. Part of a specimen from sample Darwin 13, showing a short haptonema (h.) beside the two flagella. Micrograph Y_N 7976.14; magn. × 3000.

Figure 25. Specimen with slender appendages from sample Darwin 8, showing the two flagella, one broken at the base (see further figure 25*a*). Micrograph Y_N 7939.25; magn. × 3000. Inset: light microscopy of the same cell, exposure 191.6a; magn. × 1000.

Figure 25*a*. Unmineralized elliptical scales exposed near the broken base of the flagellum of figure 25. Micrograph Y_L 7999A.16 (Lancaster Temscan); magn. × 50000.

there is the usual clear area near one (but not both) ends of a link. Sometimes the central crystallites appear to be arranged in two rows (figure 17) though these are not then normally divisible into two groups by a median transverse separation, as in figures 3 and 4.

Dimensionally, the ordinary links of the posterior appendages seem to be relatively constant in length (3–4 μm), but the width is much more varied: our measurements of link width range from 0.3 to 0.75 (or 0.8) μm, the extremes being readily distinguishable with the light microscope as 'slender' and 'coarse' appendages respectively. Examples of the former are illustrated on the left of plates 3 and 4 and of the latter on the right of the same two plates.

Unmineralized components have already been introduced by these specimens since close scrutiny of figure 21*b* will show the faint outlines of appressed elliptical scales which have already contributed to the identification of the exposed face of this coccolith as proximal. Other examples will be found in figures 23*b* and 25*a*. The broken periplast illustrated as a whole in figure 23 contains many other examples of underlayer scales, while the ordinary coccoliths contribute excellent views of peripheral striations on their undersides (see especially figure 23*a*). There is thus no essential difference between such specimens and those from South Africa.

Up to this point there had seemed to be no serious objection to the allocation of all these specimens to *O. hydroideus* sensu Gaarder, but measurements of cell sizes exposed a major discrepancy. Whereas the cell diameters given by Gaarder (1967, p. 184) were 3.5–6.5 μm our own measurements on Galapagos specimens, including those illustrated and others, ranged from a little less than 0.6 μm to nearly 10 μm and were therefore substantially higher. This discrepancy was at first interpreted ecologically in terms of local gigantism induced by some (unspecifiable) environmental factors operating in the Galapagos Islands. Such an effect had indeed already been encountered on unimpeachable evidence in two collared flagellates, *Bicosta minor* in Manton *et al.* (1980) and *Polyfibula elatensis* in Manton & Bremer (1981). Further scrutiny of *Ophiaster* as a whole nevertheless indicated the need to reassess the taxonomic situation in the light of further evidence. This will therefore be attempted after the contents of the next two plates have been recorded and described.

(ii) *O.* aff. *hydroideus* = *O. reductus* sp.nov. (plate 5)

The specimens to be considered next, though less abundant than those already introduced, possess clear-cut morphological features of a qualitative kind, which should permit identification to be made with certainty, even on an incomplete specimen, provided of course that electron microscopy could be applied. Parts or all of six different individuals are assembled on plate 5, but the most complete is that shown in figure 26, with details of its periplast in figure 26*a*.

Though figure 26 can be designated 'type specimen' of this taxon, it is less than complete with respect to the posterior appendages. Some distal links from a different individual are therefore illustrated in figures 31*a* and *b*. Collectively these specimens recall the forms of *O. hydroideus* with 'slender' appendages though the cell size is rather small, *ca.* 3.5–4.5 μm diameter. 'Thorns' are also scarce on the appendages, being apparently restricted to near the ends of the ordinary links though they are undoubtedly easily broken since they appear to be exceptionally slender and brittle. The protoplasmic appendages on the other hand are fully normal, the flagella reaching a length of *ca.* 10 μm; both are present in figure 26 though one is folded and somewhat concealed by the body. The short haptonema is also clearly demonstrable (figures 26 and 27), coiled into not more than two gyres of a helix.

In spite of this general resemblance to *O. hydroideus* sens. lat. all the specimens on plate 5 and others are distinguished by two special attributes, one negative and the other positive. The negative attribute is the apparent absence of central plaques from the body coccoliths. The latter vary in length from *ca.* 1.0 to 1.2 μm and in width from 0.7 to 1.0 μm. Though these coccoliths are normally structured in other ways, central plaques are either absent (figures 26*a* and 30) or so much reduced as to be unrecognizable, hence the choice of '*reductus*' as the specific epithet. A more important diagnostic character is the positive possession of a small group of specialized coccoliths limited to the posterior end of the cell and almost or completely lacking calcification centrally. Examples are illustrated from four different specimens in figures 26–30. Their shape is narrowly elliptical *ca.* 0.7 μm long × *ca.* 0.25 μm wide, with mainly vacant centres but with fully calcified rims.

Unmineralized periplast components are demonstrable in several different ways. Thus the usual small elliptical underlayer scales can be seen emerging from beneath body coccoliths of the type specimen in figure 26*a*. Faint peripheral striations can also be detected here and there on the body coccoliths in the same field (e.g. at bottom) but they will probably be more clearly visible on a coccolith from another specimen selected for this purpose in figure 28. In this, as also in the body coccoliths of figures 27 and 27*c*, the presence of a membrane is further indicated by general opacity, as if the membrane itself had taken up some opaque substance.

No peripheral striations are ever detectable on the special posterior coccoliths though there is strong indirect evidence that their apparently vacant centres are not in fact empty. Two different kinds of indirect evidence can be found and both are to some extent illustrated in figure 27*c*. It is by no means unusual to find traces of calcification, sometimes expressed as single crystallites lying without visible means of support, within the area bounded by a fully calcified scale rim to which they are not directly attached. One such can be seen centrally in figure 27*c*. This condition strongly suggests the presence of an underlying supporting membrane not otherwise detectable and completely patternless. A similar conclusion is even more strongly indicated in figure 27*c* by the two clear spots located directly over the place where two of these special small scales are lying across one another with their central areas superposed. These clear spots cannot be explained away in terms of holes in the carbon-on-formvar support film which seems to be flawless. The only obvious alternative explanation involves perforations through one or both of the superposed scale centres which cannot therefore be empty but which must be bridged by a patternless membrane, in at least one and probably both of the superposed scales.

(iii) *O.* aff. *hydroideus* var. *inversus* var.nov. (plate 6, figures 32–34)

Two specimens, differing from all others so far encountered in the mode of attachment of the posterior appendages to the cell, are illustrated in figures 32 and 33. One carries a short haptonema beside its single remaining flagellum, suggesting that in life it would have been functionally normal. Both cells are large, that illustrated in figure 33 inset, measuring approximately 8 μm × 10 μm, and both might have been classed simply as *O. hydroideus* sens. lat. with coarse appendages, on account of their very large appendage links, had the unusual assembly remained unremarked. As it is we can see without difficulty that, in each of the three appendages still present among the two cells, the proximal link is upside down, the wide end being directed away from the subtending cell instead of towards it. This condition may not deserve taxonomic recognition at any level, since the two cells in question, having been found in one and the same water sample might represent no more than meaningless idiosyncrasies affecting a few abnormal

individuals in one area. However, the third specimen (figure 34), from a different water sample, is perhaps offering a clue in another direction. This specimen seems to be a detached proximal link from an otherwise unknown cell. It is more smoothly triangular and otherwise different in shape from all other proximal links illustrated hitherto. The orientation on the cell is of course unknown, but if, as its shape suggests, it could be shown to have been joined to the cell by the pointed end, this would greatly enhance the biological and taxonomic interest of the condition seen in the other two cells; hence the desirability of recording these facts in some way, if only as a means of introducing a new unsolved problem.

(iv) *O. minimus* sp.nov. (plate 6 figures 35, 35*a–c*)

The specimen occupying the remainder of plate 6 is so different from all others that no amended description of *O. hydroideus* or other known species could possibly be compiled that would include it without becoming so vague as to be useless. Though as yet represented by no more than a single individual, the distinctive characters are many and substantial.

(*a*) There is first the minute size. The protoplast is less than 3 μm across though the flagella reach 8 μm; a haptonema is unmistakably also present as is a six-armed posterior appendage.

(*b*) The ordinary coccoliths all over the cell are mostly calcified at their rims only. Exceptions are limited to the few spined scales at the anterior end which can show some irregular supporting crystallites at a spine base (figure 35*a*). These are nevertheless so few as to suggest that the main mechanical support for the spine itself is likely to come from an otherwise invisible membrane occupying the coccolith centre as described in another connection on page 447.

(*c*) The ordinary appendage links, though small (2–2.5 μm long × 0.25 μm wide) are

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Description of plate 5

Ophiaster reductus sp.nov. Transmission electron microscopy of shadowcast whole mounts from the Galapagos Islands taken with an EM 6B electron microscope in Nottingham except where otherwise stated.

Figure 26. The type specimen, collected in sample Darwin 13 (table 1) and showing the two flagella (one crumpled at right) and a short, coiled, haptonema (h.); the posterior appendages incomplete but the diagnostic small elliptical coccoliths with apparently vacant centres present near the posterior end of the cell. Micrograph Y_N7892.13; magn. × 10000. Inset: light microscopy of the same cell, exposure 174.13; magn. × 1000.

Figure 26*a*. Periplast details from the left side of the specimen of figure 26, showing elliptical unmineralized scales (arrow) emerging from beneath ordinary coccoliths, lacking central plaques but with traces of peripheral striations here and there. Micrograph Y_L7999A.9 (Lancaster Temscan); magn. × 30000.

Figure 27. Another specimen from the same water sample, showing the haptonema (h.) and posterior group of special coccoliths. Micrograph Y7987.8 (Leeds EM 6B); magn. × 10000.

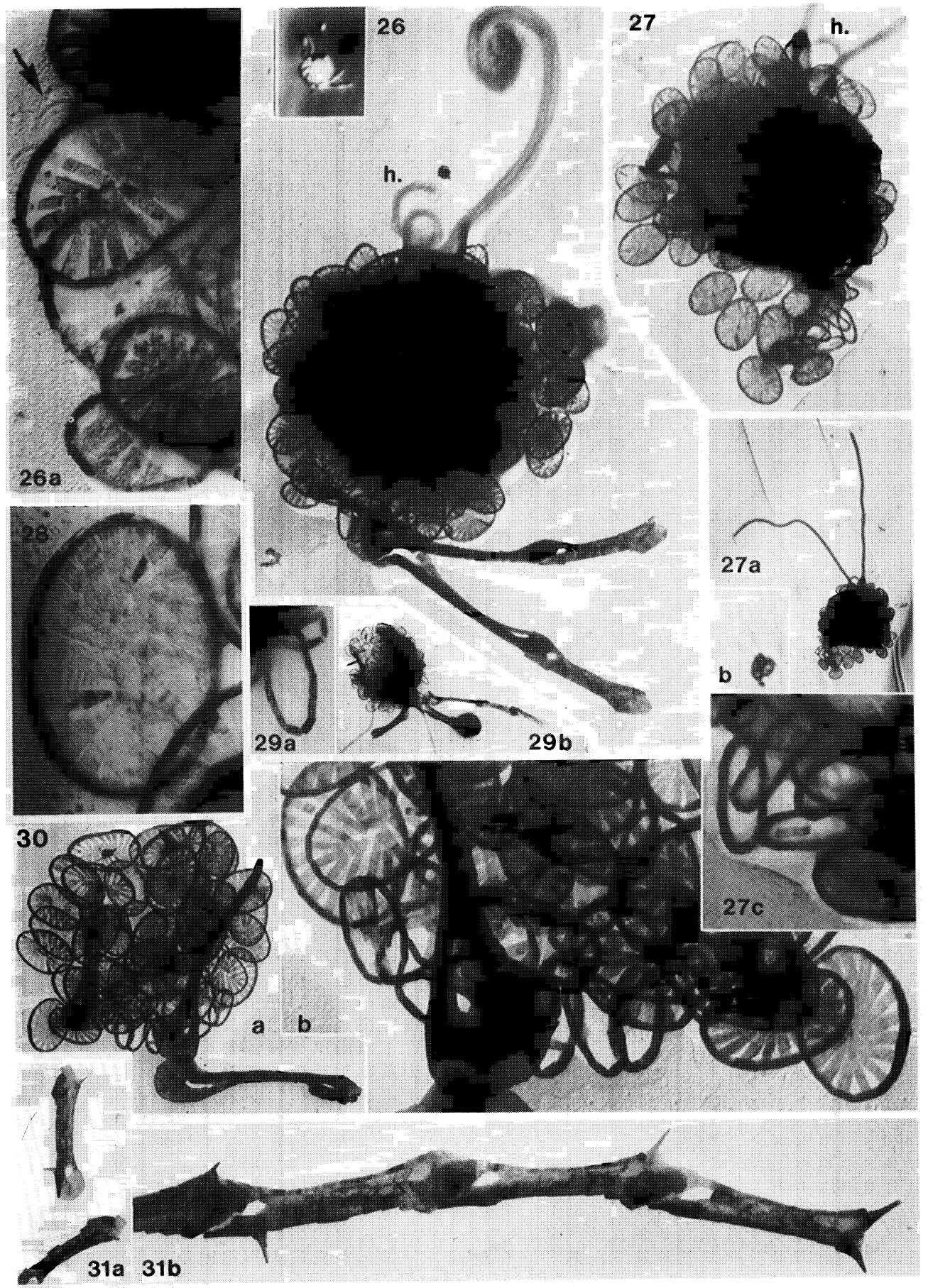
Figures 27*a–c*. Other views of the specimen of figure 27. (*a*) The whole cell; micrograph Y7987.7; magn. × 3000. (*b*) Light microscopy of the same cell, exposure 190.4; magn. × 1000. (*c*) Details of the special posterior coccoliths; micrograph Y7987.11; magn. × 30000.

Figure 28. Coccolith from Darwin 18, showing peripheral striations at left. Micrograph Y_L7999E.6 (Lancaster Temscan); magn. × 40000.

Figure 29*a* and *b*. Another specimen from the water sample Darwin 18. (*a*) A single posterior coccolith and (*b*) the cell with appendages and traces of flagella and with two spines at left. (*a*) Micrograph Y_L7999E.13 (Lancaster Temscan); magn. × 3000. (*b*) Micrograph Y_L7999E.8 (Lancaster Temscan); magn. × 10000.

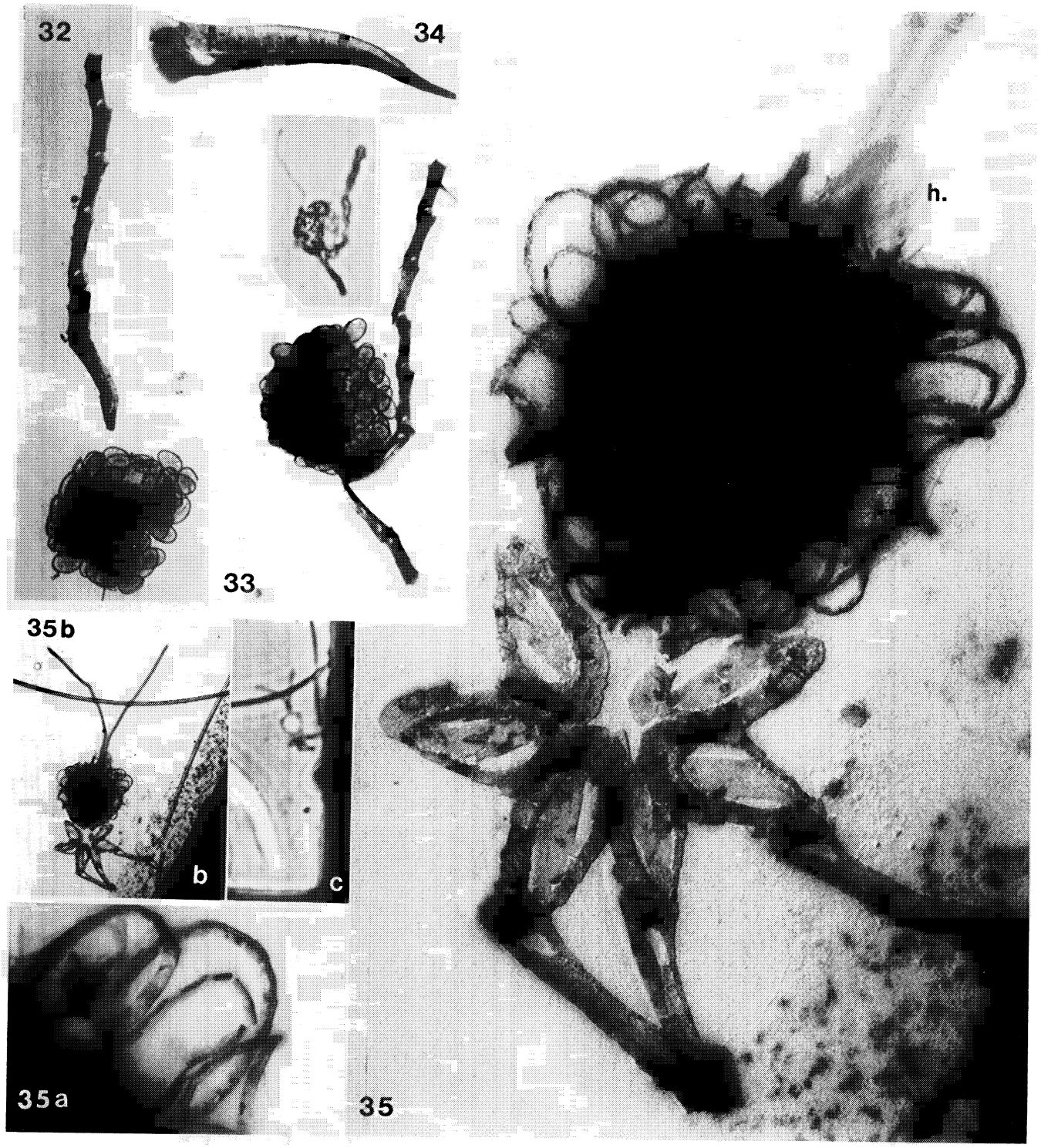
Figures 30*a* and *b*. Part of a broken periplast from Darwin 14, showing appendage links, the diagnostic small posterior coccoliths, and ordinary coccoliths lacking central plaques but some with a central spine. (*a*) Micrograph Y_N7935.8; magn. × 10000. (*b*) Micrograph Y_N7935.9; magn. × 30000.

Figures 31*a* and *b*. Terminal appendage links from another specimen (Darwin 15). Micrograph Y_N7963.2; (*a*) magn. × 10000; (*b*) magn. × 20000.



FIGURES 26–31. For description see opposite.

(*Facing p.* 448)



FIGURES 32–35(*c*). For description see opposite.

nevertheless longer (and not shorter) than the proximal links, a condition that has not been encountered before. They are otherwise unremarkable, being fully calcified and with the usual degree of asymmetry, including the customary uncalcified area near one end, but lateral 'thorns' seem to be absent.

(*d*) The proximal links are very different in shape from any of those seen hitherto. Each is more or less egg-shaped, 1.8 μm long × 1.0 μm wide, apparently untwisted, and with the wide central area crossed obliquely by a single plaque built up from several, laterally apposed, crystallites.

(*e*) Though traces of an unmineralized membrane can be made out with difficulty near the plaques of the proximal links, these are too faint to be likely to survive reproduction. The strongest evidence for the presence of membranes elsewhere is indirect as an expression of mechanical support to other structures (see item (*b*) above). Seen in this light, one of the special attributes of this species can plausibly be interpreted as absence of membrane patterning and not absence of membranes. The underlayer scales on the other hand, though not as yet seen, cannot be excluded on the basis of only a single cell and their presence or absence must remain problematical for the time being.

Discussion of Ophiaster including O. formosus Gran

The way is now clear for a more informed discussion of the status and indeed the reality of *O. formosus* Gran. As already noted this name had been discarded as inapplicable to our present material because of what appeared to be an insuperable conflict with supposedly essential parts of the formal description as given by Gaarder (1967). The most important structural difference between *O. formosus* and *O. hydroideus* sensu Gaarder had appeared to lie in the relative dimensions of the ordinary links of their respective posterior appendages, those of *O. formosus* being summarized in terms of a width/length ratio of 1/3 as opposed to 1/6 for this ratio in *O. hydroideus*. Since we had found no specimens approaching the extreme condition illustrated by Gaarder (1967, plate III, fig. E), nor any specimens exhibiting a width/length ratio of 1/3, this lack of accord seemed decisive. However, it must be recognized that both these descriptions were apocryphal in the sense that neither is based on a type specimen or a detailed type description; the validity of the terms used may therefore need to be re-examined.

Relevant measurements are summarized in table 2, those for the ordinary links in the posterior appendages being of special importance in the present context. For *O. formosus*, a range of

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Description of plate 6

Ophiaster spp. from Galapagos Islands (Nottingham EM 6B except where otherwise stated).

Figures 32 and 33. *O.* aff. *hydroideus* var. *inversus* var.nov. Two cells from the same water sample (A1), showing inverted position of the proximal link in each appendage arm. Electron micrographs (32) Y_N 7965.22 (Nottingham EM 6B) and (33) Y_L 7929F.4 (Lancaster Temscan); magn. × 3000. Inset: light microscopy 189.9; magn. × 1000.

Figure 34. A detached coccolith from an otherwise unknown cell from sample Darwin 21, perhaps a proximal link from another specimen of var. *inversus*. Micrograph Y_N 7904.9; magn. × 10000.

Figure 35. *O. minimus* sp.nov. The type and only specimen from sample A1. Micrograph Y_N 7974.1; magn. × 20000.

Figures 35*a–c*. Further details of figure 35. (*a*) Coccoliths, including one with a spine from field right of flagellar bases; micrograph Y_N 7996.20; magn. × *ca.* 30000. (*b*) The whole cell, showing flagellar length; micrograph Y_N 7973.29; magn. × 3000. (*c*) The same cell after completion of the electron microscopy; light microscopy, exposure 173.1; magn. × 1000.

lengths from 3.5 to 4.5 μm was given for this item although only a single round number (*ca.* 3 μm) was included for *O. hydroideus*. Actual measurements for link width were not supplied for either taxon but if we try to obtain these for *O. formosus* by applying the formula 1/3 to the other figures provided, we find an apparent range of link widths extending from 1.2 μm to 1.5 μm, all nearly twice as large as the actual sizes measured by us, which in no case exceeded a width of 0.8 μm. We must therefore again either discard *O. formosus* as irrelevant to our material or conclude that, unless actual measurements can be introduced to justify the formula 1/3, the imaginary figures for link width obtained with it could be unrealistic and misleading. The formula may therefore need to be discarded.

TABLE 2. TAXONOMICALLY SIGNIFICANT MEASUREMENTS ATTRIBUTED TO *O. HYDROIDEUS* AND NEAR RELATIVES

name	source	cell diameter μm	ordinary appendage links length/μm	ordinary appendage links breadth/μm	proximal link length/μm
O. formosus (Gran)	Gaarder 1967	4.5–10.5	3.5–4.5	—	4.5–6
O. hydroideus (Lohm.)	Gaarder 1967	3.5–6.6	*ca.* 3	—	4.5
O. hydroideus sens. lat.	this paper	*ca.* 5–10	*ca.* 3–4	0.3–0.8	4.5–6
O. reductus sp.nov.	this paper	3.5–4.5	*ca.* 2.5	0.25	4.5
O. minimus sp.nov.	this paper	3	*ca.* 2.5	0.25	1.8

Removal of the 1/3 formula from the description of *O. formosus* would at once bring this taxon back into contention and in so doing could remove the anomalous inference of gigantism from '*O. hydroideus*' in the Galapagos Islands. Our own measurements of cell diameters (*ca.* 5 μm–*ca.* 10 μm) remain high relative to those cited for *O. hydroideus* sensu Gaarder (3.5–6.5 μm), but they are completely covered by the equivalent figures (4.5–10.5 μm) cited for *O. formosus* (see table 2). Indeed, when it is remembered that the Gaarder figures must have been mainly if not entirely based on hydrated specimens whereas ours were all dry and therefore perhaps slightly shrunken, the difference between an upper limit of 10 μm and one of 10.5 μm can be seen as perhaps no more than procedural and not significant otherwise.

However, not all our specimens could or should be treated as *O. formosus* and a qualitative character, more meaningful when expressed verbally than it appeared to be when associated with a pseudo-mathematical formula, must also be introduced. This concerns the shapes of the posterior appendages which are severally termed 'bandlike' and 'cordlike' in the two descriptions. A glance at our own plates 3 and 4 will leave little doubt that these terms could be regarded as equivalent to our own 'coarse' and 'slender' appendages. In that case, the specimens in figures 13, 14 and 22 could be re-interpreted as *O. formosus*, those of figures 9, 10 and 19 remaining in *O. hydroideus* sens. strict. Such a rearrangement, though not enough to remove all outstanding anomalies, would nevertheless provide sufficient clarification to endorse fairly strongly the correctness of such a procedure.

The remaining anomaly, that *O. hydroideus* cells sensu Gaarder are still substantially smaller than our *lower* limit, is not difficult to explain with the aid of another plausible hypothesis. Reference to table 2 will at once show that the dimensions of our new taxon based on plate 5 and included here under the epithet '*reductus*' could exactly fill the place of the otherwise missing small cells of *O. hydroideus* sensu Gaarder. We cannot know whether any specimens with the special type of posterior coccoliths diagnostic of *O. reductus* were actually present among those measured by Gaarder, since her only references to small coccoliths (Gaarder 1967, pp. 186,

190) mean something quite different. Scrutiny of a much quoted published micrograph (Halldal & Markali 1955, pl. 13) leaves no doubt that the small coccoliths, in question there, are no more than a few reduced versions of ordinary coccoliths, as indeed can also be seen in our own figure 23. However, an undoubted example of an *Ophiaster* cell possessing the special type of modified coccoliths illustrated here in plate 5 has recently been recorded (H. A. Thomsen, personal communication) from the coast of Thailand, sampled in September 1981. This taxon is thus not distributionally limited to the Galapagos Islands and some other representatives of it could have been included in the Gaarder collections. If they were, the last dimensional anomaly between these records could be removed.

The conclusion therefore seems to be that provided certain misleading expressions are deleted from formal descriptions and we recognize three taxa and not merely two (for details see Appendix) all these difficulties can be resolved. Whether the taxa so recognized should be at the specific or at some lesser level is, however, a separate question which is still open to debate. Much will depend on whether even revised specific descriptions will permit borderline cases (if they exist) to be sorted out. If they will not, a different taxonomic and nomenclatural treatment from that advocated hitherto may have to be worked out.

One further topic should perhaps receive brief comment here, namely coccolith arrangement. The supposedly descriptive phrase 'irregularly arranged' in the formal generic diagnosis (p. 441 above) seems unlikely to have been based on direct or precise observation since we are also told that the ordinary coccoliths are 'so closely placed that in light microscope the cell cover of intact cells is not easily recognized as composed of separate coccoliths'. Further, we know that intact cells of about this size will inevitably be opaque when viewed by ordinary transmission electron microscopy in which the immediately facing 'cell cover' will therefore be invisible, as exemplified indeed in our own figures 1 and 26. Broken cells such as those illustrated here in figures 23 and 30 in which the coccoliths are not only violently disturbed but also superposed from opposite faces are inherently unsuitable for analysis in terms of initial coccolith arrangement. The same must be said of scanning electron microscopy if applied merely to an air dry specimen such as that in figure 2 since protoplast shrinkage in this case disturbs the overlying coccoliths so much as again to produce an unanalysable display. The only satisfactory way of ascertaining coccolith arrangement precisely, on small cells such as these, would be either to freeze or freeze dry them before scanning or to embed and section them after suitable fixation. None of these alternative procedures has as yet been carried out. The true situation is thus uncertain, difficult to detect, or obscure for some other reason, but that does not make it irregular.

We may suspect from our own evidence that the coccoliths of *Ophiaster* are sometimes at least arranged in a single layer and will then be expected to display hexagonal close-packing to the extent compatible with an even cover on a spherical surface. This is indeed strongly suggested by Mrs Gaarder's own diagram (Gaarder 1967, fig. 1 C). Nevertheless, pending the availability of precise information it is highly desirable that genuine uncertainty should not be glossed over in a formal description and that any statements made should not be such as to mislead a reader who may (legitimately) trust in the literal meaning of words. Further comment on this topic will be found in the discussion on the next genus.

Results. 2. *Calciopappus*

Like so much else, the pioneer work on *Calciopappus* was carried out in Norway, the type species, *C. caudatus*, having been described by Gaarder & Ramsfjell (1954*a*,*b*), followed by a second species, *C. rigidus* Heimdal, by Heimdal & Gaarder (1981). The genus as a whole is widespread, easily recognized and has been seen many times (cf. Okada & McIntyre 1977).

Specific recognition on the other hand is less easy and not many of our specimens can be allocated to species with confidence. Thus, two very different individuals from a single water sample in the Galapagos Islands are shown with the light microscope under oil immersion (amplifying a dry lens) at two focal levels in plate 7, with electron micrographs of others in plate 8. An internal spine, basis of the caudate condition in *C. caudatus*, is unmistakably present in figures 36*c* and 38 and (somewhat less clearly) in figures 42*a* and *b*, though not in any of the others. The cell body is nevertheless not always narrowly conical, as originally described for both species, since a cylindrical body, as in figure 37, is by no means uncommon, yet this shape has not as yet been recognized as present in either species. There is likewise no information on the taxonomic value (if any) of the considerable differences in spine thickness as well as in spine numbers, so conspicuous in plate 7. In contrast, the ring-shaped coccoliths subtending the bases of spines and shown in detail in figure 43 agree so closely with those illustrated for *C. rigidus* in Heimdal & Gaarder (1981) as to suggest that both species may have been present in our material though there is not as yet enough information of the right kind to sort them out.

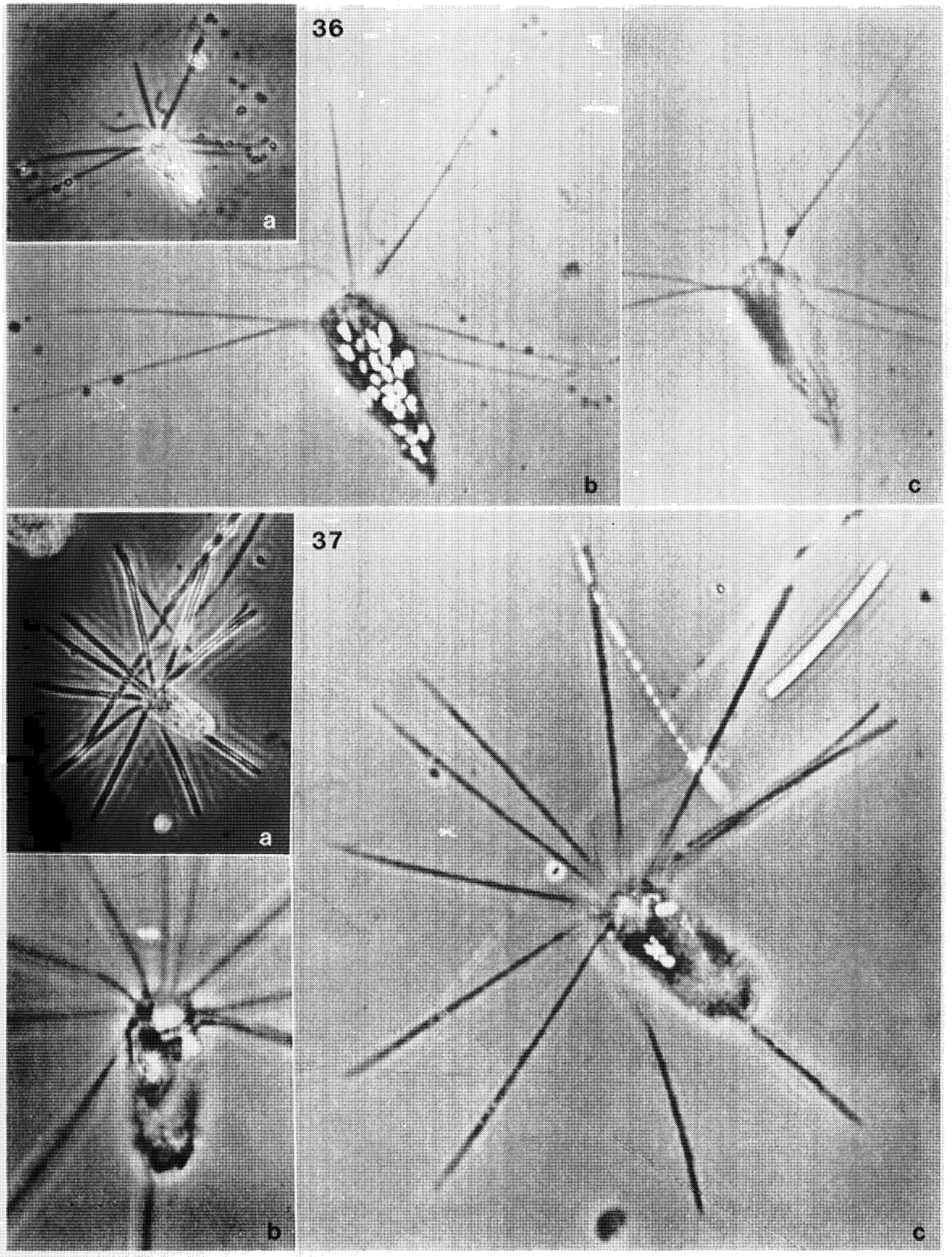
In spite of these difficulties and uncertainties, these specimens collectively contribute valid new information relevant to the genus as a whole. Thus the flagella, clearly visible in figure 36*a* and elsewhere, are unmistakably accompanied by a short haptonema in figure 40, thereby confirming that this organelle is indeed present though rarely exposed. Body shrinkage on drying, as illustrated best in figures 36*c* and 39, disturbs the periplast less than in the much smaller cells of *Ophiaster*, thereby leaving the coccoliths more easily detectable. Thus a dried cell on a glass slide, when immersed as already described, can retain air preferentially in or under the body coccoliths which then show up white (figure 36*b*); their longitudinal orientation and hexagonally close-packed arrangement can then be seen directly with the light microscope. Scanning electron microscopy, as in figure 42*a*, can in this case also reveal coccolith arrangement satisfactorily if allowance is made for minor local disturbances. Transmission electron microscopy on the other hand, in which top and bottom surfaces commonly appear superposed, can rarely be analysed.

Description of plate 7

Calciopappus Gaarder & Ramsfjell. Two specimens from the same water sample (A1 in table 1) dried on a glass slide and photographed with the light microscope under phase contrast.

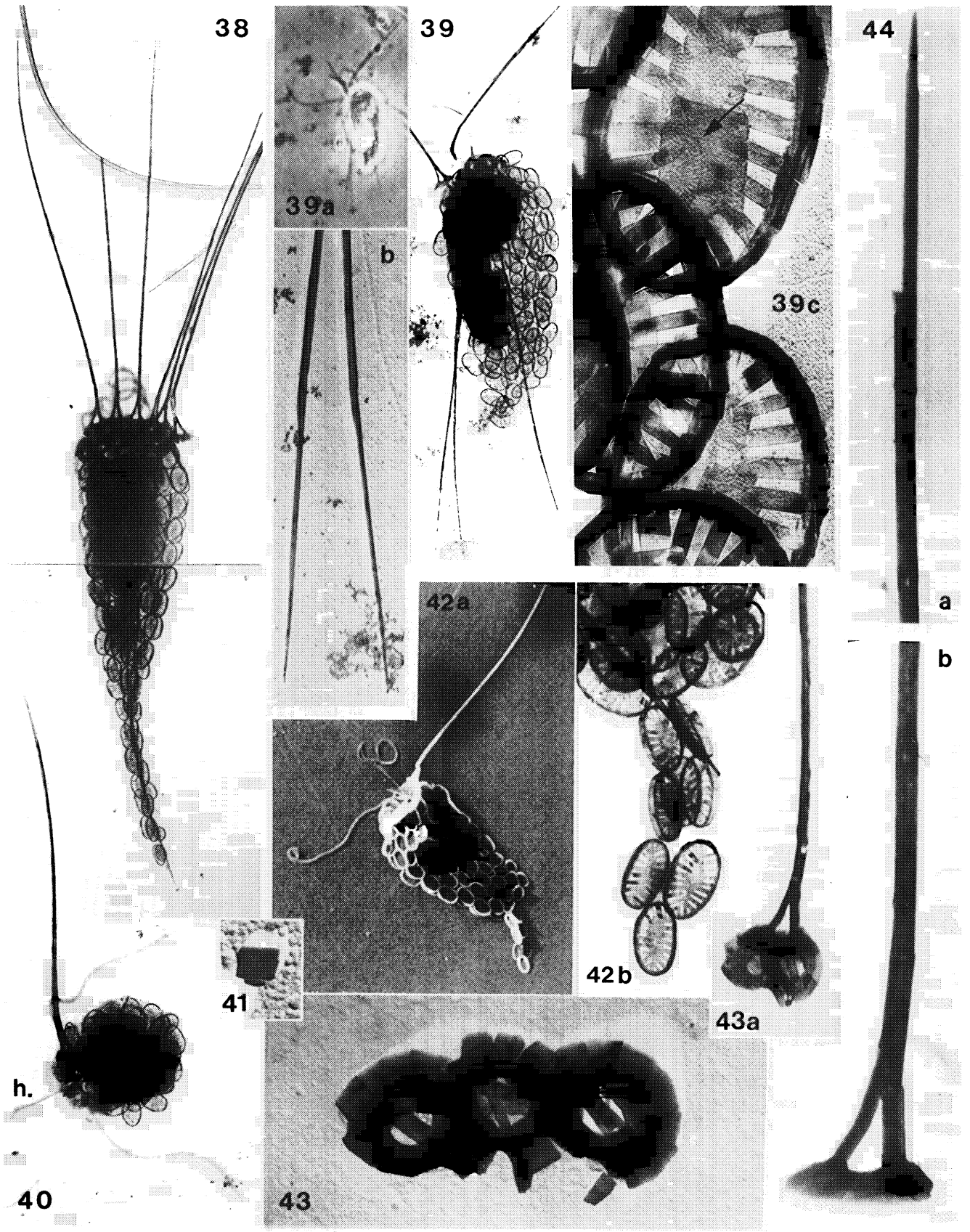
Figures 36*a–c*. A cell showing the two flagella and a crown of six spines. (*a*) Photograph taken with a dry lens. Exposure 195.27 (magn. × 1000). (*b*) The same cell newly immersed in Objectol and showing air trapped by the coccoliths which appear white. Photograph under oil immersion. Exposure 196.18 (magn. × 2500). (*c*) The same specimen after elimination of trapped air by a rinse in amyl acetate before re-immersion, coccoliths now visible in outline only but internal cell components more distinct, including the shrunken protoplast (left) and an internal spine indicative of *C. caudatus*. Exposure 198.38 (magn. × 2500).

Figures 37*a–c*. Another cell otherwise similar to the preceding but showing a cylindrical body and eleven thick spines, the apical depression visible in (*b*). (*a*) The cell among diatom appendages taken with a dry lens, exposure 193.30 (magn. × 1000). (*b*,*c*) Two different focal levels of the same specimen, immersed in Objectol (diatom appendages now transparent). Exposures 194.27 and 194.28 (magn. × 2500).



FIGURES 36–37. For description see opposite.

(*Facing p.* 452)



FIGURES 38–44(*b*). For description see opposite.

Apart from scale arrangement which will be further discussed below, the ordinary coccoliths (figures 42*b*, 39*c*) of *Calciopappus* sens. lat., though somewhat larger, resemble those of *Ophiaster* so closely as to be virtually indistinguishable in the absence of confirmation from the appendages. The central plaques tend to be broader and less oblique in *Calciopappus* but the basic construction of the plate as a whole is similar, including the presence of 'spade-shaped' crystallites (figure 41) in the rim. Even the appendages, superficially so different in morphology and position, are nevertheless in both genera built up from modified coccoliths of two different kinds. Each of the spines is a single coccolith in *Calciopappus*, modified in such a way that the scale centre has been suppressed while the two sides, except at the ends, are drawn out and pressed together, thereby providing the spine. Though much more longitudinally extended than the link coccoliths of *Ophiaster*, both are asymmetrical to an almost equal degree. At the base (figure 44*b*), a spine of *Calciopappus* is more massive on one side than on the other and if the two sides are traced to the extreme distal tip (figure 44*a*) the thicker side at the base is prolonged beyond the other distally to give the conspicuous bayonet-point, noted by Gaarder, with which the spine ends (see also figure 39*b*).

Modified coccoliths of a very different kind are illustrated in figure 43. The apical depression (figure 37*b*) is bordered by the ring-shaped coccoliths described in detail by Heimdal & Gaarder (1981), each oriented with its short point directed into the depression. Such modified coccoliths alternate with spines (figure 43*a*; see also Gaarder & Ramsfjell 1954, pl. IV*f*), and they alone mediate attachment between spines and the rest of the cell.

Unmineralized periplast components are more difficult to find than in *Ophiaster*, a difference that could be connected with the greater degree of coherence of the outer parts of the scale case in *Calciopappus*. This enforces a greater degree of spacial separation than in *Ophiaster*

Description of plate 8

Calciopappus Gaarder & Ramsfjell from the Galapagos Islands. Electron microscopy of dry whole mounts, all with an A.E.I. EM 6B microscope at Nottingham except where otherwise stated.

Figure 38. Specimen from the same water sample as those in figures 36 and 37, showing coccoliths and spines, including an internal spine directed backwards diagnostic of *C. caudatus*. Micrographs Y7965.28 and Y7965.29 (magn. × 3000).

Figure 39. A cell from Darwin 11, showing shrunken protoplast, coccoliths, spines and bases of the two flagella. Transmission electron micrograph Y_L7954.19 (Lancaster Temscan) (magn. × 3000).

Figures 39*a–c*. Details of figure 39. (*a*) The cell as seen with a dry lens (light microscopy). Exposure 182.2 (magn. × 1000). (*b*) Tips of two spines. Micrograph Y7954.16 (magn. × 10000). (*c*) Coccolith details and faint, superposed, unmineralized scales (arrow). Exposure Y7954.17 (magn. × 40000).

Figure 40. Specimen from sample Darwin 15 with one residual spine permitting generic (but not specific) identification but showing both flagella and a short haptonema (h.) clearly. Micrograph Y7963.45 (magn. × 3000).

Figure 41. A single 'spade-shaped' crystallite from the rim of an ordinary coccolith from another specimen fully authenticated by the presence of spines; sample Darwin 23. Micrograph Y7948.15 (magn. × 40000).

Figures 42*a* and *b*. A cell from sample Darwin 14. (*a*) Scanning electron micrograph showing coccoliths, a spine and the two flagella. Micrograph YO7984.36; magn. × 3000. (*b*) Transmission electron micrograph of the hind end, showing coccoliths and the tip of a posteriorly directed internal spine. Micrograph Y7944.24 (magn. × 10000).

Figure 43. Three ring-shaped coccoliths of the type attributed to *C. rigidus* Heimdal, from a specimen from sample A1. Transmission electron micrograph Y7922.23 (magn. × 20000).

Figure 43*a*. Base of a spine and two subtending ring coccoliths from another part of the field of figure 43. Micrograph Y7918.21 (magn. × 10000).

Figures 44*a* and *b*. Opposite ends of a single spine from another specimen (sample A4). Transmission electron micrographs Y7927.2 and Y7927.3 (magn. × 20000).

between the coccolith layer and the level at which underlayer scales may be located on the surface of a shrunken protoplast after drying. Nevertheless figure 39*c* contains two coccoliths showing unmineralized elliptical scales still pressed against their upturned proximal faces, which also exhibit here and there the peripheral striations on the plate bars near the junctions with the rim, essentially as in *Ophiaster*. This field (figure 39*c*) is part of the specimen of figure 39 and contamination with alien coccoliths or scales is thus excluded.

Finally, traces of reticulate membrane fragments can be seen crossing some of the supposedly empty spaces beside the central bars in the ring coccoliths illustrated in figure 43. These will be considered in another context elsewhere.

Discussion of Calciopappus

Apart from coccolith substructure and other morphological differences or resemblances between *Calciopappus* and *Ophiaster*, a functionally significant factor underlying some of the practical difficulties noted above with respect to speciation in *Calciopappus* can appropriately be considered here. This is spine length.

As is well known, haptophycean scales, whether calcified or not, are formed within the cytoplasm of the subtending cell. This is also true, though with differences of detail, for equivalent structures in other groups of planktonic unicells, including some now classed as animals such as the collared flagellates, one genus of which (*Bicosta*) has recently been discussed in this journal (Manton *et al.* 1980). It is therefore not surprising that many, and indeed most, of such organisms are limited in manufacturing their products by the length of the body. Large structures, if formed at all, as they often are, tend in consequence to be compound and built up from many, body length, units joined together in various ways. We have seen this in *Ophiaster*, in which the posterior appendages can reach a length several times that of the protoplast itself by virtue of their construction from chains of transformed, but still relatively short, coccoliths.

In *Calciopappus*, on the other hand, appendage length is achieved differently and much remains obscure about the process. If each spine is no more than a single modified coccolith, a mechanism is needed for production of spines so much longer than the protoplast in its normal form, as these appear to be. Moreover, cell replication and spine replication must necessarily be linked in some way though which process comes first in the cell cycle cannot be known without direct observation. Changes of cell shape, including perhaps cell length, must of course accompany cell division and such changes might provide temporary conditions in which exceptionally long internal structures could be laid down ready for eventual extrusion onto the cell surface. An explanation in such terms has recently been suggested in another connection with respect to the very long spines of *Bicosta spinifera* which, in arctic conditions, can exceed body length by a factor of 2:1 (Manton *et al.* 1980). A related problem, in *Calciopappus*, is the nature of the posterior projection in *C. caudatus*. This looks like a spine which for some reason is directed backwards and is incompletely extruded from the cytoplasm. This interpretation of its nature nevertheless raises the question as to whether a single spine in this condition can be a permanent feature of every interphase cell or whether it is in fact part of a process and therefore perhaps no more than a temporary, if regularly recurring, stage. These problems cannot be solved merely by indirect reasoning and until the cell cycle or salient parts of it have actually been observed in *Calciopappus* this genus will continue to present insuperable obstacles to clear formulation of many cognate topics, including the delimitation of specific boundaries.

A final comment is perhaps desirable on the choice of terminology acceptable for use in a formal diagnosis to define scale arrangement in this genus also. The phrase 'coaxial rings', sometimes used for this purpose in the past (Heimdal & Gaarder 1981; Gaarder & Ramsfjell 1954), if taken literally would be expected to mean 'scales arranged in a pile of circles centred on the long axis of the cell'. However, a beginner confronted with a specimen such as that of figure 42*a* might need to ask whether the curved and slightly tilted rows of coccoliths which seem to be crossing the exposed cell surface in a more or less transverse direction are really parts of circles or of some other configuration such as a helical system of some kind. If this distinction matters, as it might in some contexts, how can the facts be ascertained? The most obvious way would be to match up the curved lines exposed on the upper surface with exact equivalents on the concealed under surface, but such an exercise, in the absence of sections, would be virtually impossible and not unnaturally, an attempt has not yet been made. It must, therefore, be recognized that 'coaxial rings', though doubtless convenient for metaphorical use among experts, must not be taken literally. This is less than satisfactory in a supposedly exact scientific description and in our view the introduction of such a phrase into a formal taxonomic diagnosis, without qualification, should be strongly discouraged.

An emended generic diagnosis (but no specific diagnoses) will be found in the Appendix.

General discussion

There are several aspects of these findings with biological implications beyond the confines of the taxa so far discussed. First, the mere demonstrability of unmineralized periplast components in two additional genera beyond those listed in the Introduction significantly amplifies the evidence that such components are not trivial extras but integral parts of coccolith substructure, at least in some stages. This was not unexpected, but a second finding, namely the complete lack of surface patterning on some unmineralized components, which are then demonstrable only by means of secondary effects, introduces a new concept, which may have unforeseen implications. Thirdly, there is the question of thickness of unmineralized components, a topic that has not been considered in detail before but which may prove to be more important than might have been expected.

When seen in section, scales and unmineralized components of coccoliths can vary considerably in thickness. The most massive are the plate scales and coccolith bases of *Hymenomonas roseola* (see Manton & Peterfi 1969) and *Hymenomonas carterae* (see: Pienaar 1969; Manton & Leedale 1969). The thinnest occur in *Chrysochromulina fragilis* Leadbeater, 1972, represented in sections by a Danish variant illustrated in Manton & Leadbeater (1974) under the name of *C.* aff. *fragilis*. This differs both from the type specimens from Norway and also from the African equivalent illustrated here in plate 2, figure 8, mainly by possessing a wide band of spiralized peripheral striations on the sheet scales instead of a narrow band of short peripheral streaks as in *Ophiaster*, etc. Minor differences in the small elliptical scales occur but there is no disagreement about the pliability and extraordinarily thin texture of the large, peripherally striated, sheets. These, when seen in section (Manton & Leadbeater 1974, pl. IV, fig. 21), appear to be thinner than the plasmalemma and substantially thinner than the small elliptical scales. Even in the special case of *Ophiaster* and *Calciopappus*, the extreme transparency of the striated regions, when seen overlying crystallites exposing their proximal surface to view, is sufficient confirmation that the membrane here also is diaphanous to an extreme degree.

This fact could be relevant to some of the apparent anomalies presented by other coccoliths, such as those of *Emiliania* (*Coccolithus*) *huxleyi* (Lohm.) Hay & Mohler, when seen in sections. This taxon has been much studied because it is one of the few to have been long established in culture. It is also one of the few known, on the evidence of sections, to lack free scales other than coccoliths although alternative phases of life history, lacking coccoliths, can be covered with unmineralized scales as in species of *Chrysochromulina* (for literature see Paasche & Klaveness 1970). Recently, Klaveness (1972, 1976) has carefully investigated coccolith substructure and development, as traceable in this way, and has concluded that *Emiliania* coccoliths possess no equivalent to the massive, unmineralized central plate so conspicuous in sections of *Coccolithus pelagicus* and *Hymenomonas* spp. as listed above. However, the alternative possibility of a membranous *fragilis*-type sheet had scarcely then been thought of and the question might perhaps be asked as to whether the interpretation might have been different if guided by different expectations.

Other outstanding problems in need of further investigation, impossible at present without establishment of additional cultures of the right kind, include some major questions of life history. We have noted several times that *C. fragilis* as first described from Norway is not exactly like any of the several forms encountered subsequently, all of which, on no less than six different occasions, have been designated 'aff. *fragilis*' and not *fragilis*. Moreover, no two of these are exactly alike except those introduced in the present communication, which resemble each other but differ from the species itself as first described. One may therefore well ask whether *C. fragilis* is really a self perpetuating species of the ordinary kind or a stage of life history, or even a transitory individual condition, of some entirely different taxon, probably a coccolithophorid. Such a question cannot be answered by random sampling of the oceans but the range of diversity in details found on specimens actually caught in this way is enough to indicate that more than a single species is likely to be involved.

This forces attention on the minuter structural characters, if only as sources of phyletic guidance in certain cases. Such characters include not only the peripheral patterning on sheet scales but also the sizes, shapes and surface markings of underlayer scales accompanying them. When these are taken into account, substantial differences or resemblances, formerly unremarked, at once become conspicuous. Thus, in *Rhabdosphaera stylifera*, underlayer scales seem absent (Leadbeater & Morton 1973) as in *E. huxleyi*, while in *Syracosphaera pulchra* (Leadbeater & Morton 1973) they consist of large circular or square plates quite unlike anything otherwise attributable to *C. fragilis* itself. This difference at once counteracts the superficial resemblance conferred by the peripheral striations on their coccolith membranes. These genera therefore seem unlikely to be closely related, and this is of course endorsed by other aspects of their respective coccoliths. In contrast *Calciopappus* and *Ophiaster* have hitherto been separated by a considerable gap, as for example in the list of *Syracosphaeraceae* provided by Okada & McIntyre (1977) in which *Calciopappus* is genus 2 and *Ophiaster* genus 5 out of a total of six. Yet their underlayer scales as we have seen are of the same general type as are the other mineralized and unmineralized features of their ordinary coccoliths. These two lines of evidence thus concur and both together add appreciably to the sum of phyletically significant data previously available. We can now conclude with reasonable confidence that, in spite of the obvious differences in their appendages, *Calciopappus* and *Ophiaster* must in fact be more closely allied than could previously have been thought possible.

One final topic should perhaps at least be mentioned, namely ecology. The water samples

listed in table 1, themselves only a fraction of those processed, which, in the Galapagos Islands, totalled over 30, were very uneven in their yield of good cells, more than half of those illustrated here having come from only three samples. The richest sample (A1), from Bartolomé Island, contained all the taxa quoted for both genera except *Ophiaster reductus* but including all the more peculiar specimens of *Ophiaster* as illustrated on plates 3, 4 and 6. *O. reductus* itself was found in four different samples from Academy Bay, the richest part of that area being represented by Darwin 13 and Darwin 14 added together. Although it is known that the main inflow from outside the area in August comes from the south and might, therefore, be expected to affect Academy Bay earlier or more than Bartolomé Island, we are not yet in a position to distinguish recent or temporary immigrants from permanent residents. This also may nevertheless become more obvious in the near future, when a greater number of nanoplankton genera and species have been recorded and placed in their local context.

Conclusions

Thin, unmineralized, membranes, with or without peripheral striations, and resembling in size and texture the sheet scales of *Chrysochromulina fragilis* Leadbeater, are commoner than has previously been supposed, as integral parts of coccoliths of several different types. Such membranes have been positively demonstrated in four different genera of coccolithophorids, i.e. *Syracosphaera* and *Rhabdosphaera* (Leadbeater & Morton 1973), *Ophiaster* and *Calciopappus* (the present paper), with others expected to follow. Assessment of the functional significance of such membranes in the structure or development of these organisms cannot yet be made but, as phyletic markers, they increase substantially the available criteria on which relationships can be worked out. When taxonomic descriptions are amplified to include not only the structural details of coccoliths but also those of all unmineralized periplast components that may be present, including the morphology and surface patterning of unmineralized underlayer scales, the close phyletic relationship between, for example, *Ophiaster* and *Calciopappus* (but not *Rhabdosphaera* or *Syracosphaera*) becomes much more apparent. For these and other reasons, and in spite of, or indeed partly because of, their inconspicuousness on many intact cells and rapid disappearance from sediments, further attention to such components is highly desirable whenever opportunity offers.

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Appendix. Formal taxonomic diagnoses

Ophiaster Gran emend.

Cells spherical to ovoid, with two flagella and a short haptonema. Ordinary coccoliths normal elliptical discs probably arranged in a single layer and with a few (4–6 *fide* Gaarder) around the flagellar area bearing centrally placed spines each of about coccolith length; at the posterior pole a star-like appendage with flexible arms, 5–8 (or 5–11 *fide* Gaarder) in number, composed of elongated and transformed coccoliths linked together by their narrow ends and attached to the periplast by differently shaped proximal links. Small unmineralized elliptical scales present beneath the ordinary coccoliths together with unmineralized membranes individually attached to the under sides of the coccolith discs. Type species *O. hydroideus* (Lohm.) Lohm., and two or more other species. Distribution worldwide but mainly in fairly warm seas.

O. hydroideus (Lohm.) Lohm. emend.

Protoplast commonly 5–6 μm in diameter; flagella up to 15 μm long, the haptonema coiling into not more than about two gyres. Ordinary coccoliths 'incomplete caneoliths', commonly *ca.* 0.9 μm × 1.6 μm (or *ca.* 1 μm wide × 0.9–1.8 μm long *fide* Gaarder), the plate centres occupied by two or more plaques, often obliquely placed and with the rest of the coccolith surface crossed by rectangular crystalline bars, commonly up to 25 in number (10–30 *fide* Gaarder) distributed radially between the central plaques and the calcified rim, the latter composed of crystallites of two kinds, the larger spade-shaped and the smaller elongated tangentially but with a thumb-like extension recessed into part of each lateral junction between adjacent spade-shaped crystallites. Unmineralized scales present beneath the coccoliths, each elliptical, 0.2 × 0.4 μm, patterned with radial ridges visible on both surfaces and with an elliptical central area clearly delimited. An unmineralized, very transparent, membrane with short peripheral striations attached to the under side of each ordinary cocolith, both types of unmineralized components resembling equivalents in *Chrysochromulina* aff. *fragilis* Leadbeater. The calcified appendages cord-like, the ordinary links often constricted centrally and commonly *ca.* 2.4 μm long × 0.3 μm–0.5 μm wide, asymmetrical, with crystallites along one edge smaller than those on the other and with the link centres occupied by two groups of crystallites except for a space near one or both ends. Here and there small crystallites resembling 'thorns' projecting beyond the edge of a link in various places but mainly on the convex edge; the number of links variable and not normally exceeding 10 but easily detached, the distal link ending in a terminal 'thorn'. Proximal links longer than ordinary links, twisted and enlarged at one end, this end mediating attachment to the cell. Distribution probably worldwide.

O. formosus Gran sensu Gaarder emend.

Cells larger than *O. hydroideus* in the same area, protoplast commonly 8–10 μm in diameter, flagella up to 20 μm long but haptonema shorter than in *O. hydroideus*. Posterior appendages band-like, ordinary appendage links flat and not constricted centrally, 3.5–4.5 μm long (*fide* Gaarder) and up to 0.8 μm wide, the link centres occupied by two rows of crystallites closely pressed together except for a single clear area near the posterior end. Other details, including unmineralized components, as in *O. hydroideus*. Distribution probably worldwide.

O. formosus var. *inversus* var.nov. (L. = 'inverted')

As *O. formosus* but proximal links of posterior appendages more narrowly triangular and arranged upside down compared with the usual condition, the narrow end attached to the periplast instead of the wide end. Distribution: Galapagos Islands. Collected by Dr Margaret McCully in sample A1 from Bartolomé Island (Sullivans Cove) on 15th August 1977 on sea bed (10 m depth), sea temperature 22 °C. Type specimen: figure 33.

A var. formoso membris proximis appendicum posteriorum magis anguste triangulis, partibus angustioribus, non ut in illo partibus latioribus, periplasto adjunctis diversus.

Die 15 Augusti anni 1977 in exemplo 22 graduum Celsii 'A1' appellato 10 m sub aequore in fundo maris sinus Sullivans Cove insulae Galapagensis Bartolomé Island a M. McCully lectus, figura 33 typifica monstrata.

O. reductus sp.nov. (*L.* = reduced)

Protoplasts slightly smaller than *O. hydroideus* in the same area, commonly 3.5–4.5 μm in diameter with flagella up to 10 μm and a haptonema coiling into less than two gyres, the posterior appendages slender, otherwise normal but with few 'thorns' though these, when present, very slender and brittle. Ordinary coccoliths similar in size to those of *O. hydroideus* but lacking central plaques. In addition, a special group of small, narrowly elliptical coccoliths present at the posterior pole, six or seven in number, each *ca.* 0.3 μm × 0.7 μm with calcification almost limited to the rim but with a patternless membrane crossing the apparently unoccupied central area indicated indirectly by its mechanical effects. Other unmineralized components as in *O. hydroideus* and similarly resembling equivalents in *Chrysochromulina* aff. *fragilis* Leadbeater. Distribution: Galapagos Islands but recently detected off the coast of Thailand (H. A. Thomsen, personal communication) and therefore perhaps worldwide. Type specimen: figure 26, collected on 13 August 1977 in sample Darwin 13 from Academy Bay, Santa Cruz Island, at 10 m depth, sea temperature 22 °C.

Protoplasti paulo minores quam in O. hydroideo in eadem area collecto, plerumque 3.5–4.5 μm diam.; flagella ad 10 μm longa; haptonema in minus quam duos gyros convolutum; appendices posteriores tenues, praeterea forma usitata, spinis tamen paucis, debilibus, fragilibus. Coccolithi ordinarii ejusdem ut in O. hydroideo magnitudinis sed lamellis centralibus carentes. Praeter illos sex vel septem coccolithi parvi anguste elliptici gregem posticum formantes circiter 0.3 μm × 0.7 μm magni, praeter margines vix calcificati sed, indicante firmitate, membranis non visibilibus areas centrales occupantibus contenti. Ad elementa alia non calcificata O. hydroideo similis, ut ille Chrysochromulinam aff. fragili Leadbeater revocans.

Die 13 Augusti anni 1977 in exemplo aquae 22 graduum Celsii Darwin 13 appellato 10 m sub aequore in sinu Academy Bay insulae Galapagensis Santa Cruz lectus, figura 26 typifica monstrata, etiam (communicante H. A. Thomsen) ad oram Thailandicam lectus.

O. minimus sp.nov. (L. = 'very small')

Protoplast minute, *ca.* 3 μm in diameter, with flagella 8 μm long and a short but distinct haptonema. Coccoliths calcified mainly at their rims except for a few extra crystallites supporting the bases of spines located in the usual position near the anterior end of the cell. The posterior appendages fully calcified, the ordinary links *ca.* 2.5 μm long × *ca.* 0.25 μm wide but the proximal links very short, 1.8 μm long × 1.0 μm wide, broadly elliptical, blunt at each end, not obviously

twisted and with a single compound plaque crossing the widest part obliquely. Unmineralized membranes suggested by their mechanical effects but patternless.

Type, and only, specimen: figure 35 collected by Dr Margaret McCully in sample A1 from Bartolomé Island (Sullivans Cove) on 15 August 1977, on sea bed (10 m depth), sea temperature 22 °C.

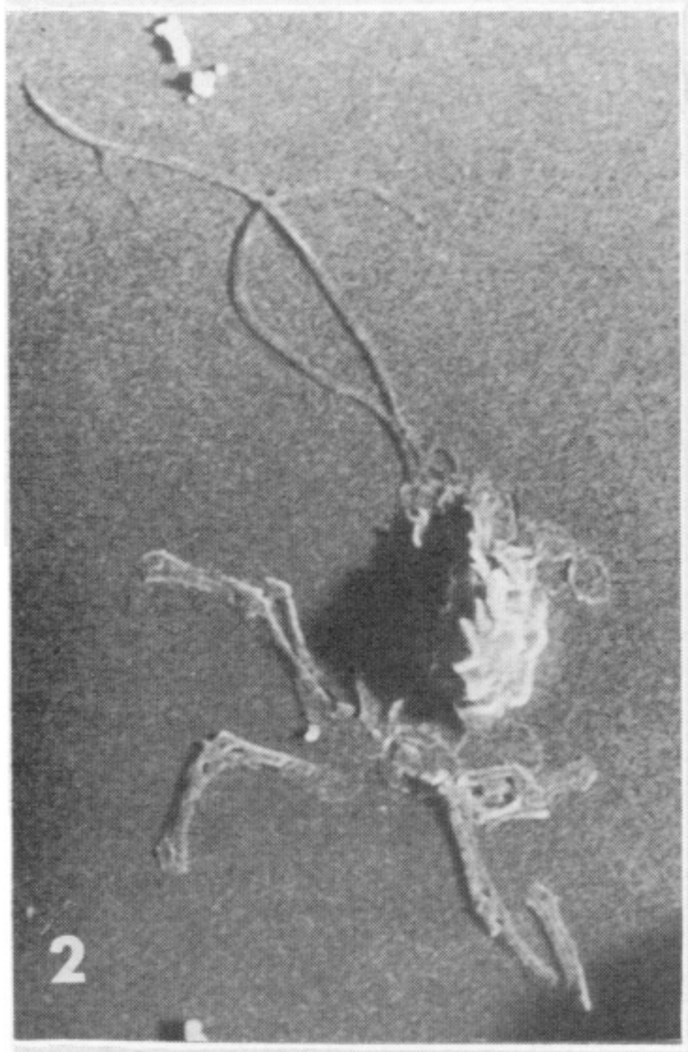
Protoplastus minutus, circiter 3 μm diam., flagellis 8 μm longis haptonemate brevi satis manifesto. Coccolithi imprimis margine calcificati, crystallitis supplementariis paucis spinas ut assolet prope apicem cellulae sitas basi suffulgentibus. Appendices posteriores omnino calcificatae, membris ordinariis circiter 2.5 μm longis, 0.25 μm latis, proximis 1.8 μm longis, 1.0 μm latis, late ellipticis, ad apices obtusis, non manifesto tortis, quoque lamella composita obliqua unica in medio trajecto. Membranae non visibiles indicante firmitate praesentes.

Cellula unica die 15 Augusti anni 1977 in exemplo 22 graduum Celsii A1 appellato 10 m sub aequore in fundo maris sinus Sullivans Cove insulae Galapagensis Bartolomé Island a M. McCully lecta, figura 35 typifica monstrata.

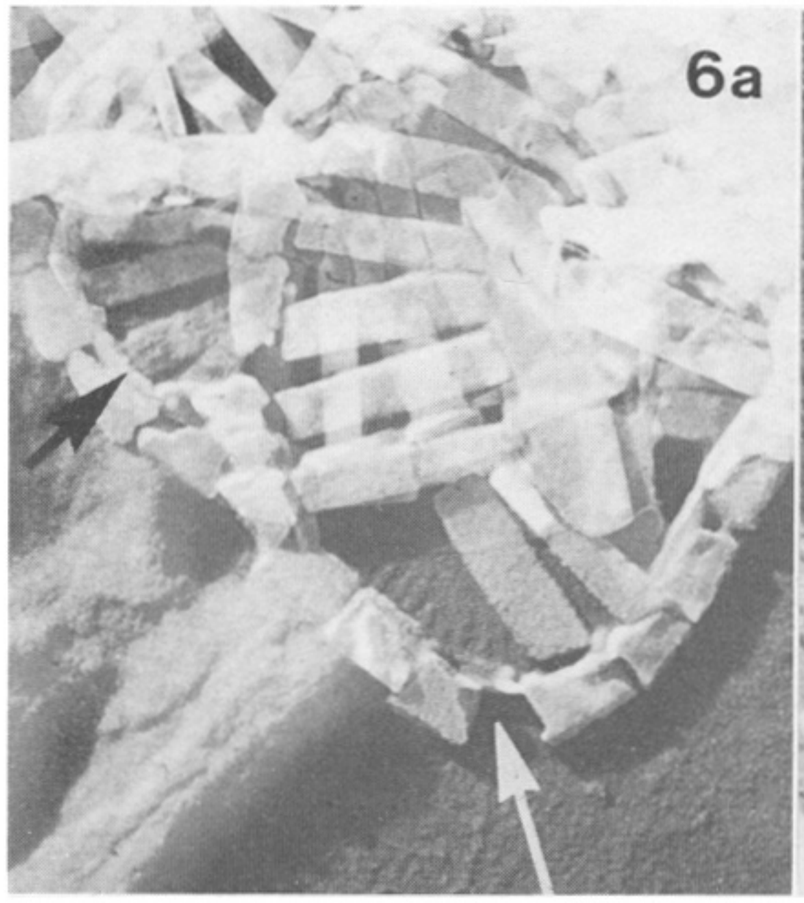
Calciopappus Gaarder & Ramsfjell emend.

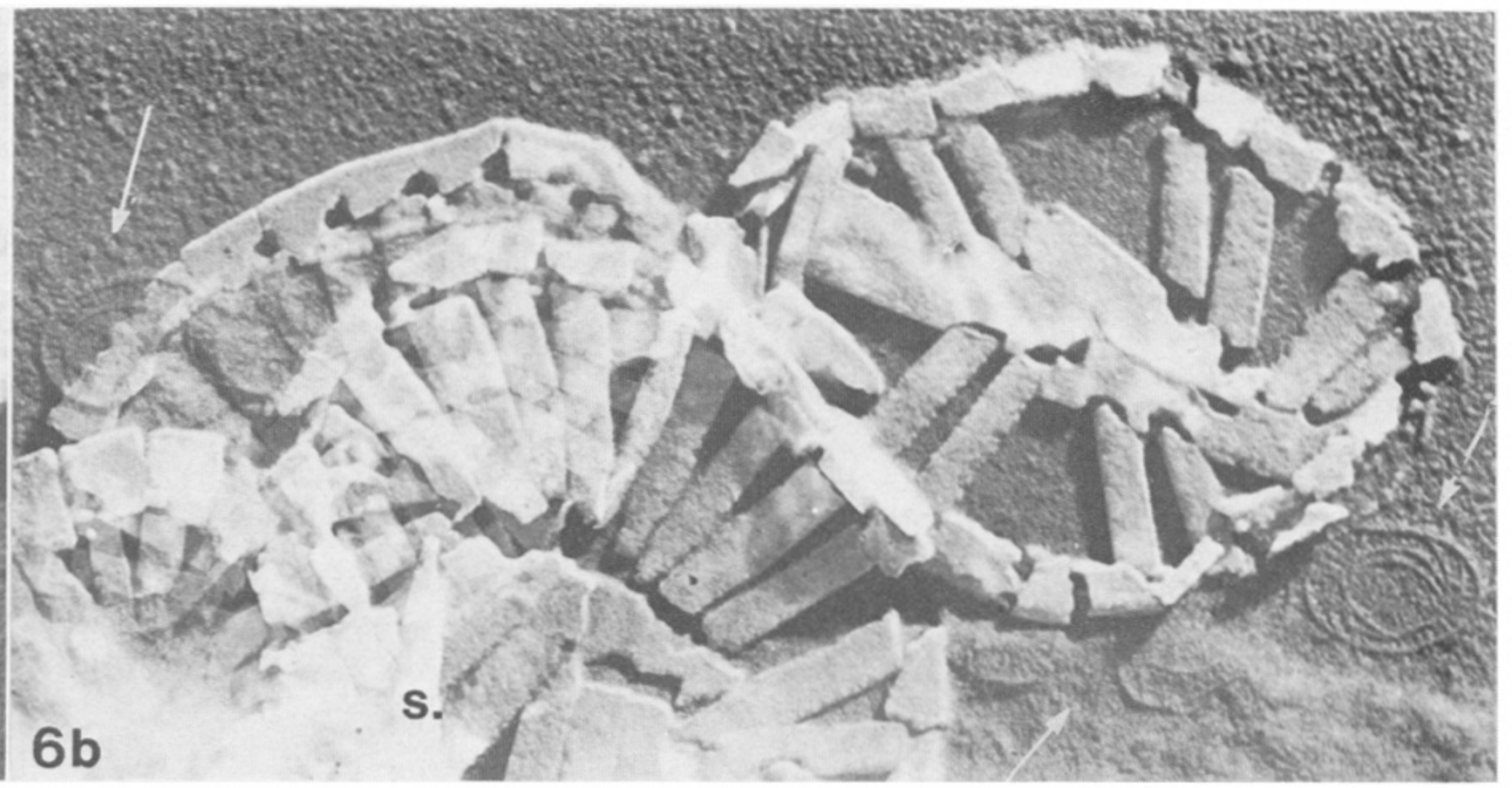
Cells cylindrical or narrowly conical with an apical depression and often with a posterior projection resembling an incompletely extruded, posteriorly directed, spine. Two equal flagella and a very short haptonema. Two chromatophores in the broadest part of the cell (*fide* Gaarder *et al.*). Ordinary coccoliths long-elliptical, nearly flat, narrow-rimmed (='incomplete caneoliths'), longitudinally oriented and arranged in hexagonal close-packing sometimes suggesting 'coaxial rings'. A whorl of five to eleven, or more, slender spines at the anterior end of the cell, each spine longer than the interphase protoplast and formed from a single transformed coccolith attached by a horseshoe-shaped basal end to a whorl of ring-shaped coccoliths distributed along the rim of the anterior depression. Unmineralized periplast components, including a peripherally striated membrane on the proximal face of each ordinary coccolith and small elliptical plate-scales underlying the ordinary coccoliths, closely resembling the equivalent in *Ophiaster*, but more difficult to see.

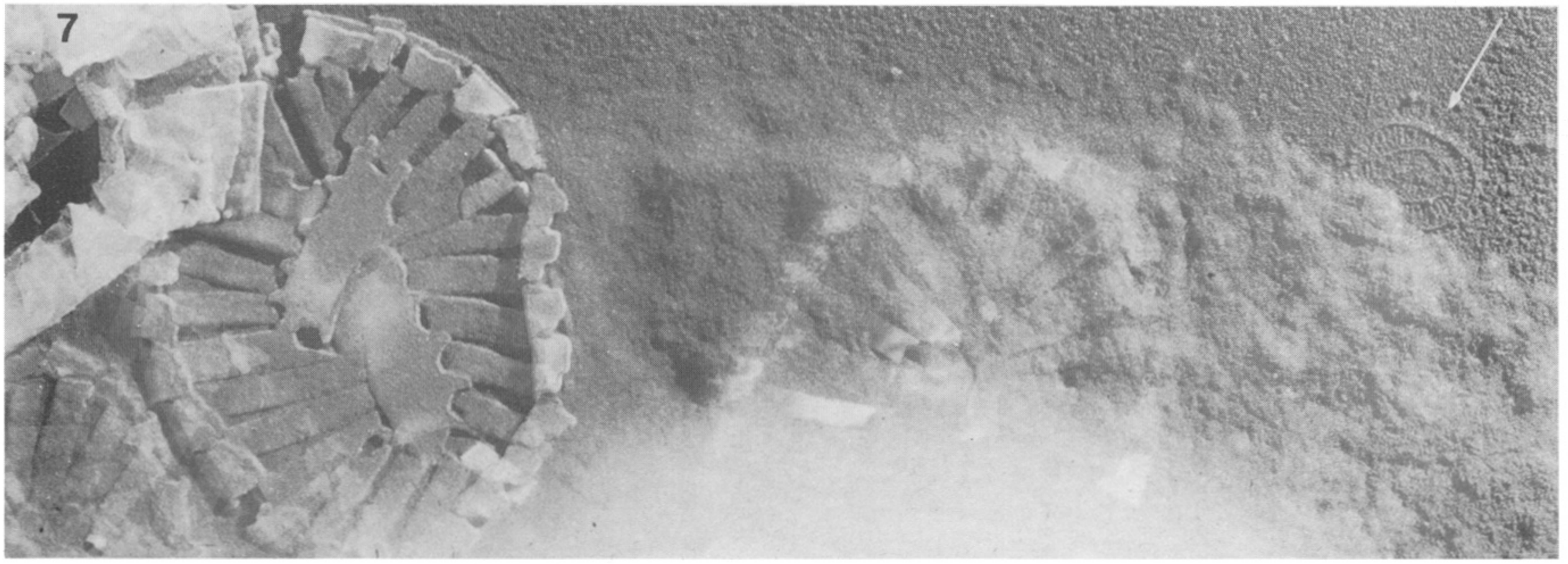
Distribution probably worldwide in coastal waters.

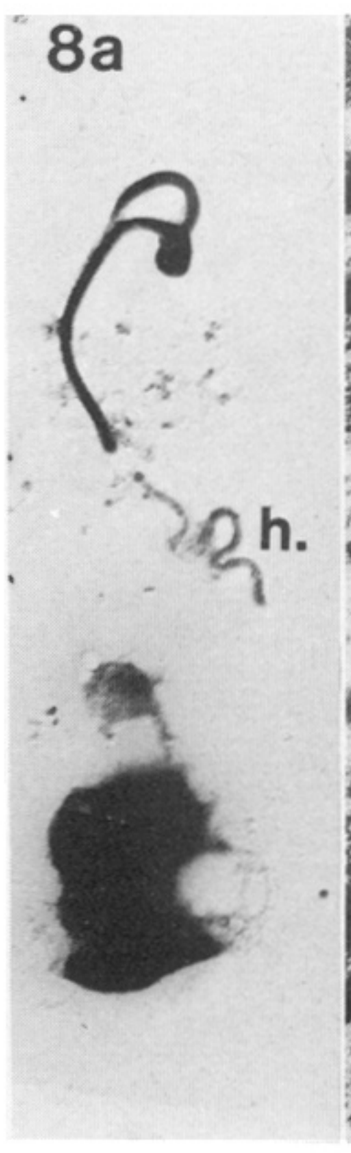


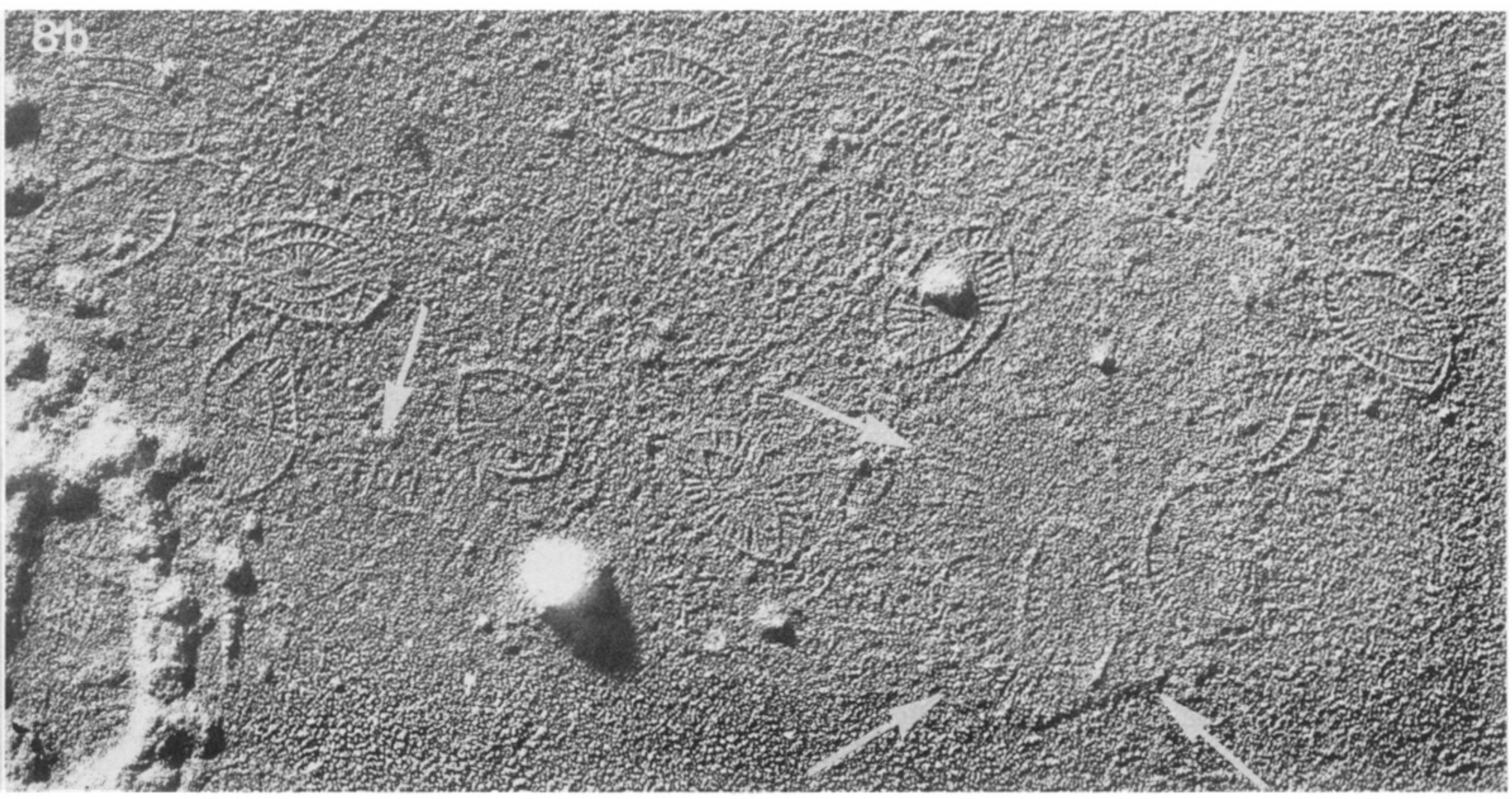
FIGURES 1–5. For description see opposite.



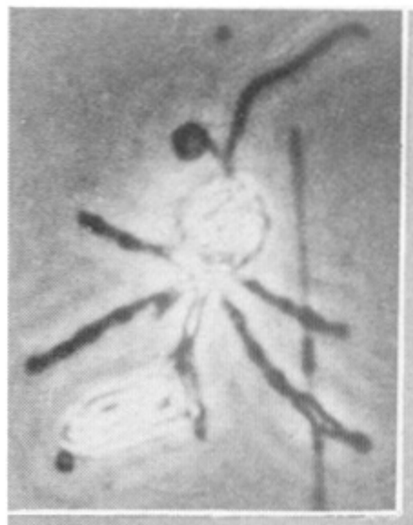


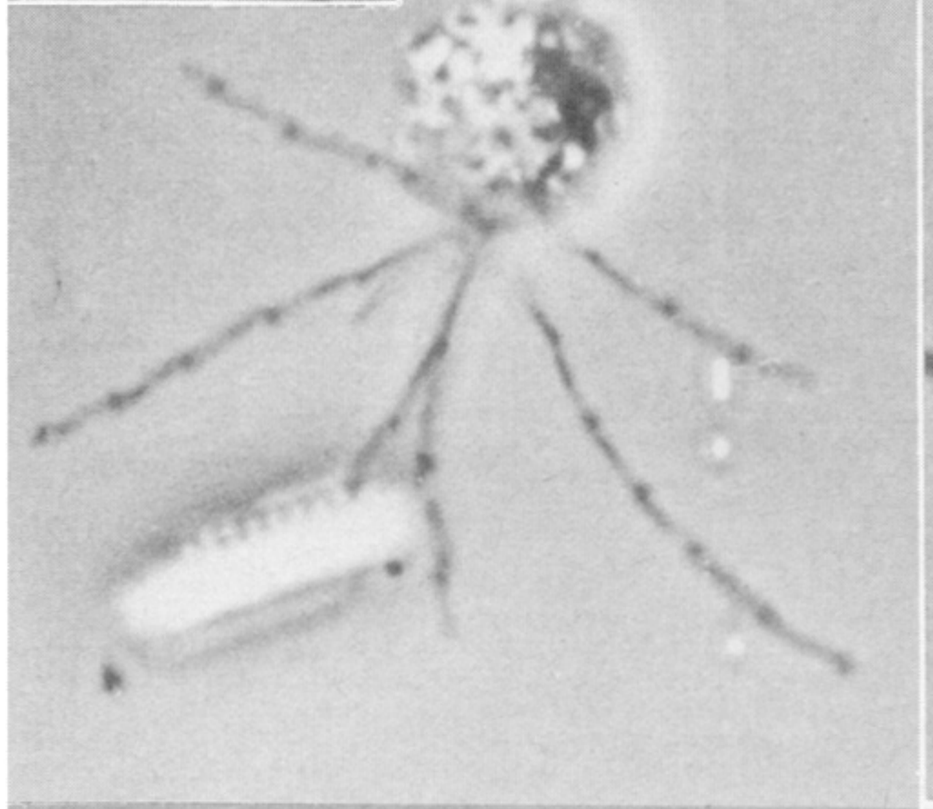


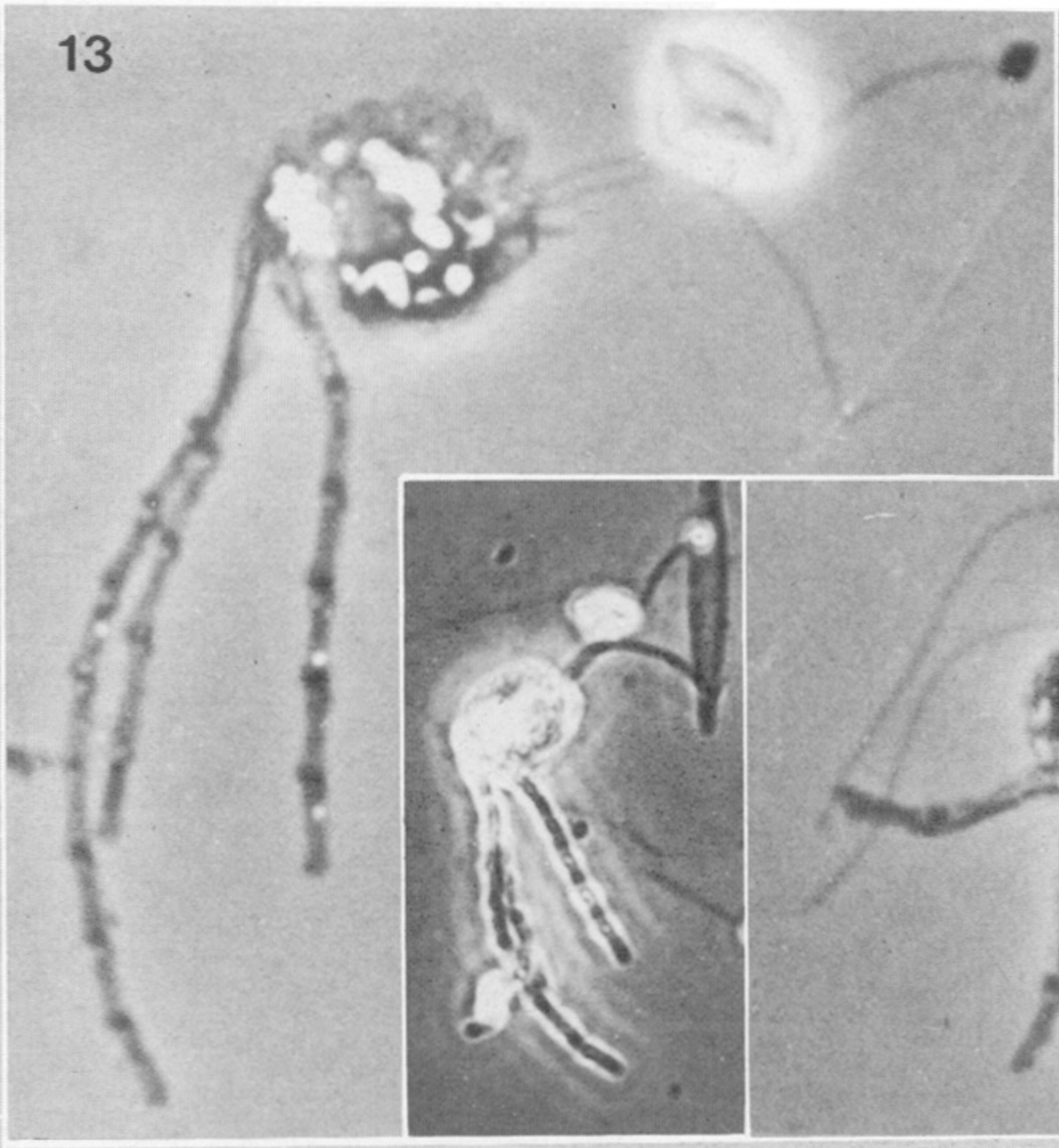


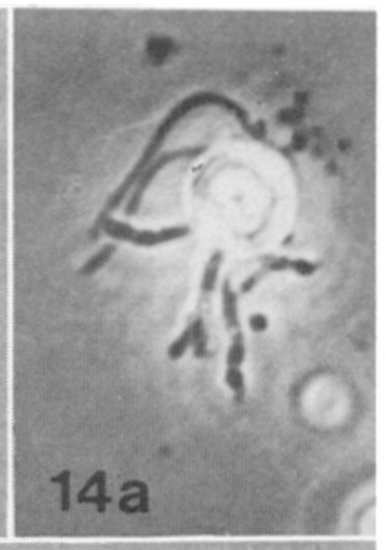


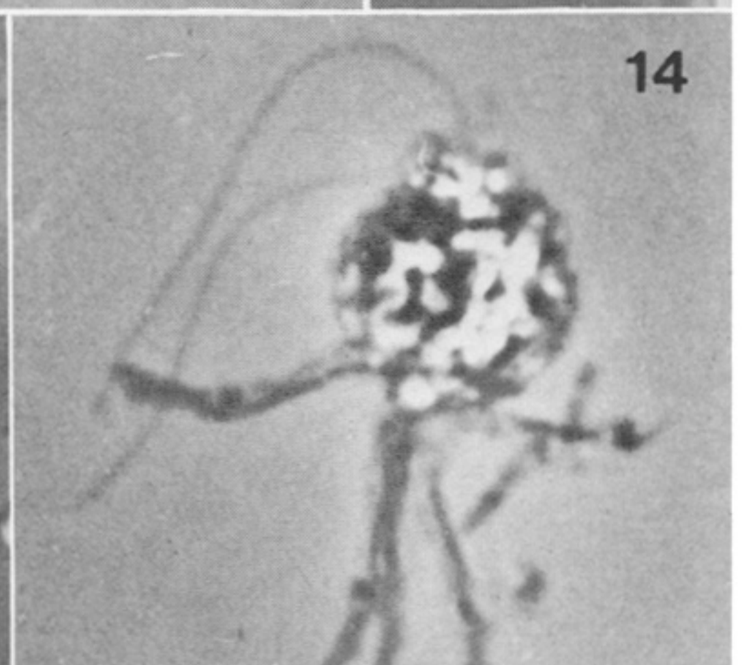
FIGURES 6(*a*)–8(*b*). For description see opposite.

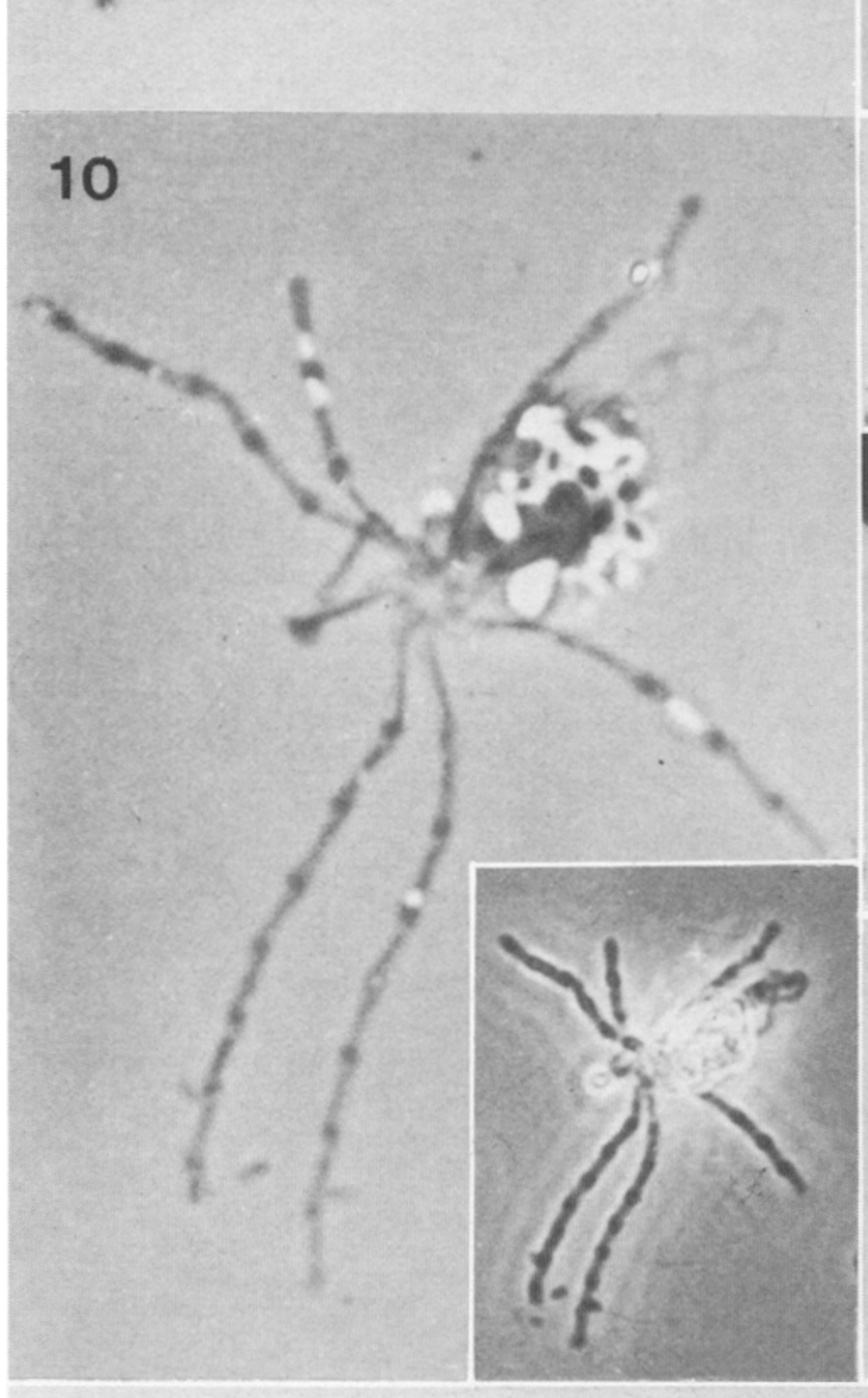


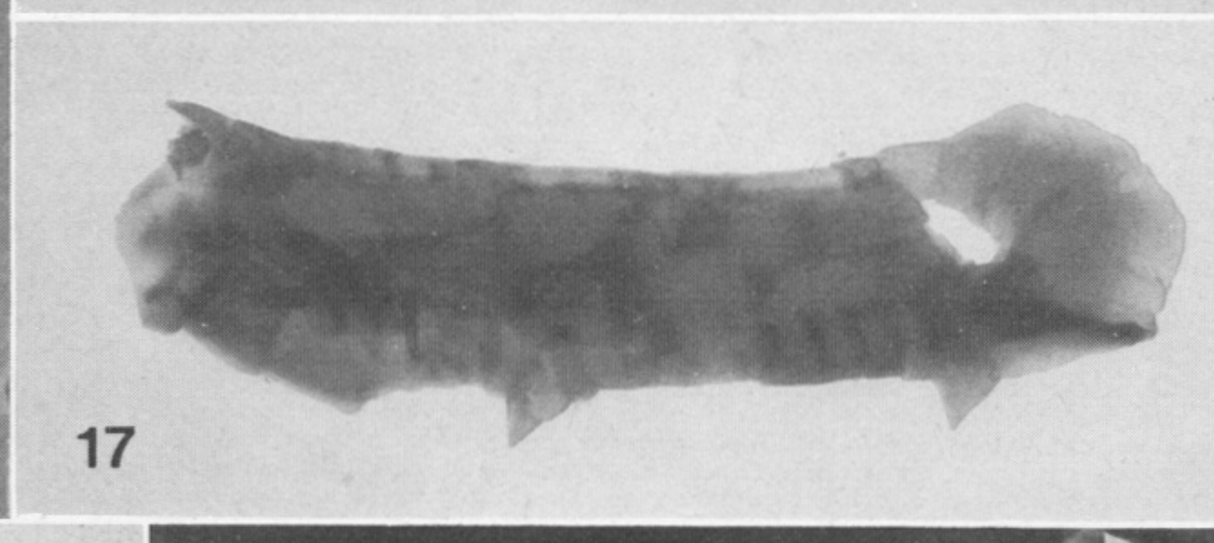


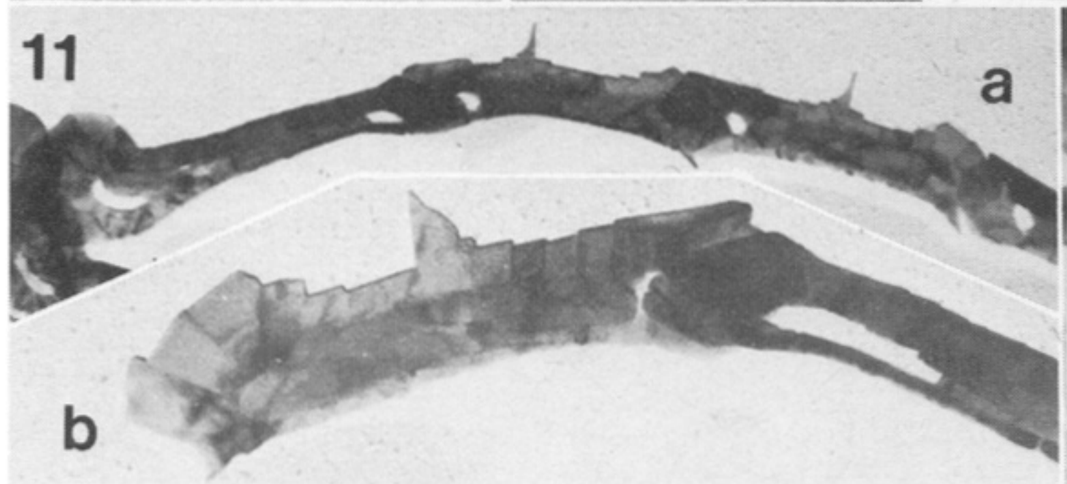


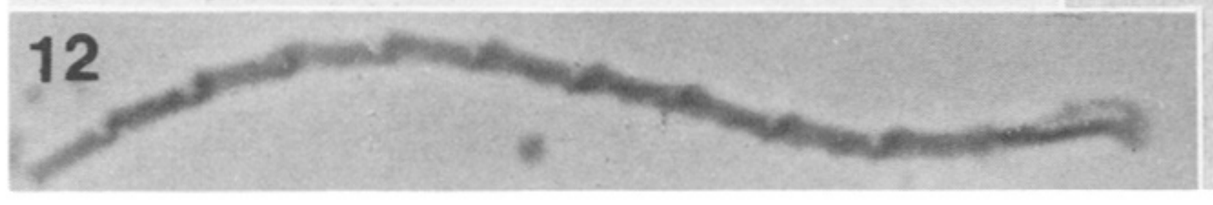




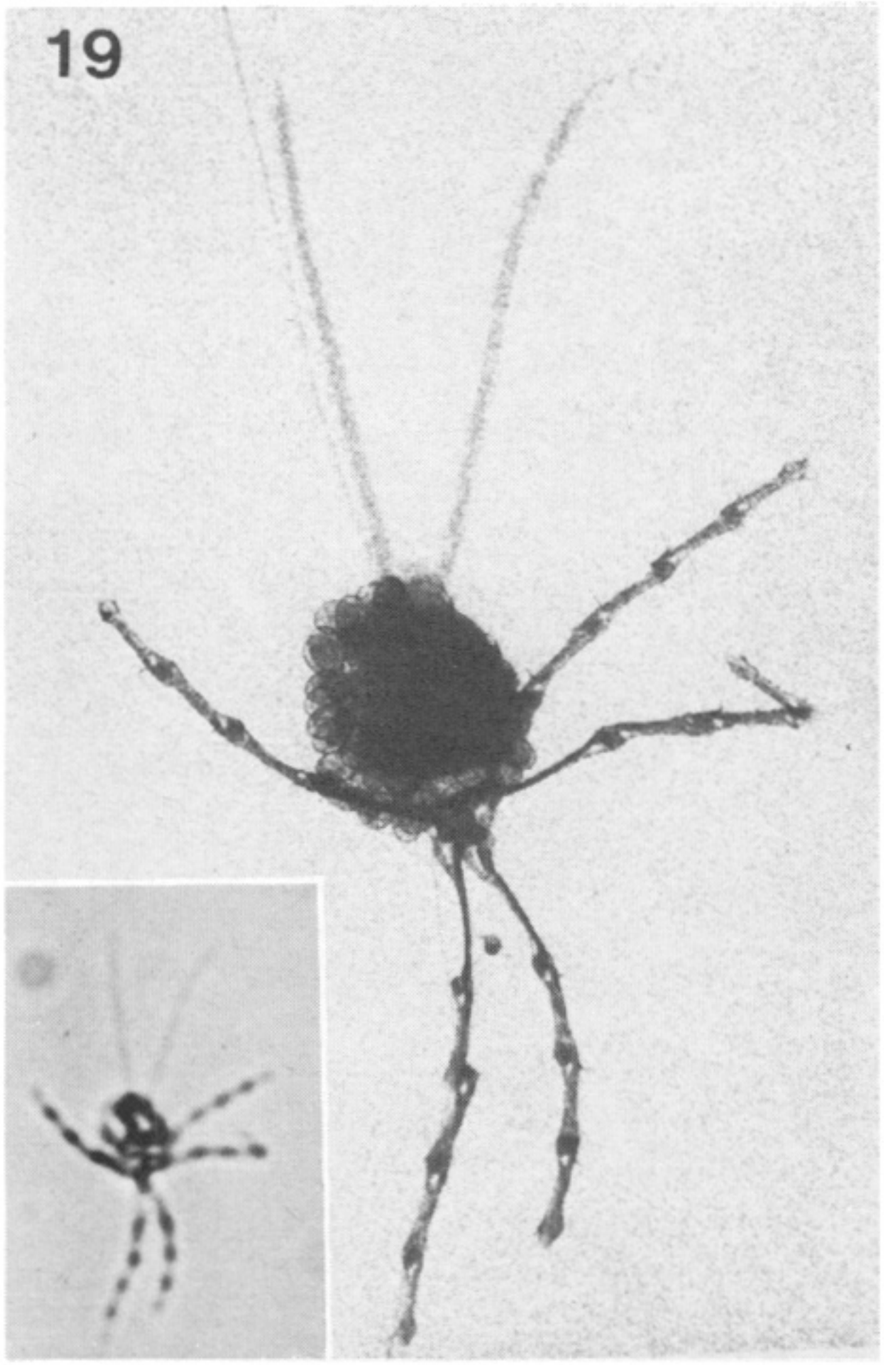


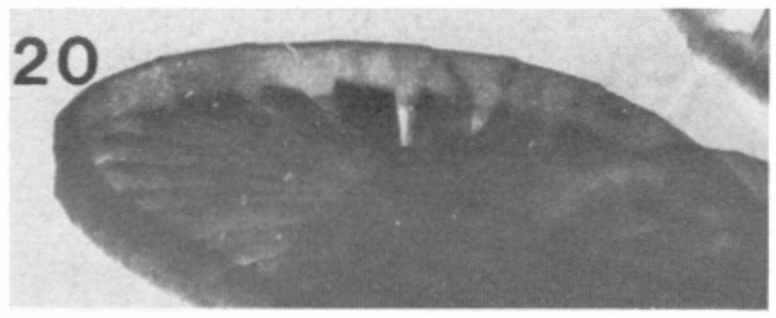


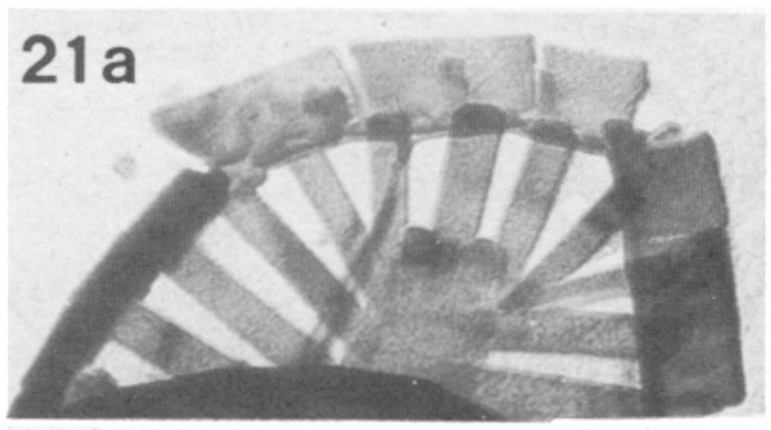


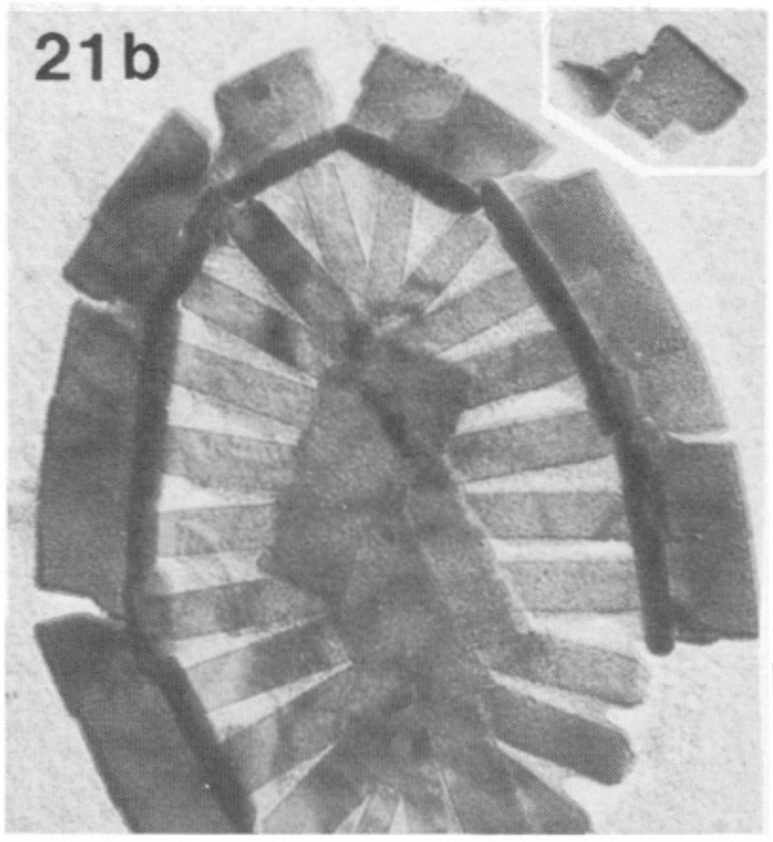


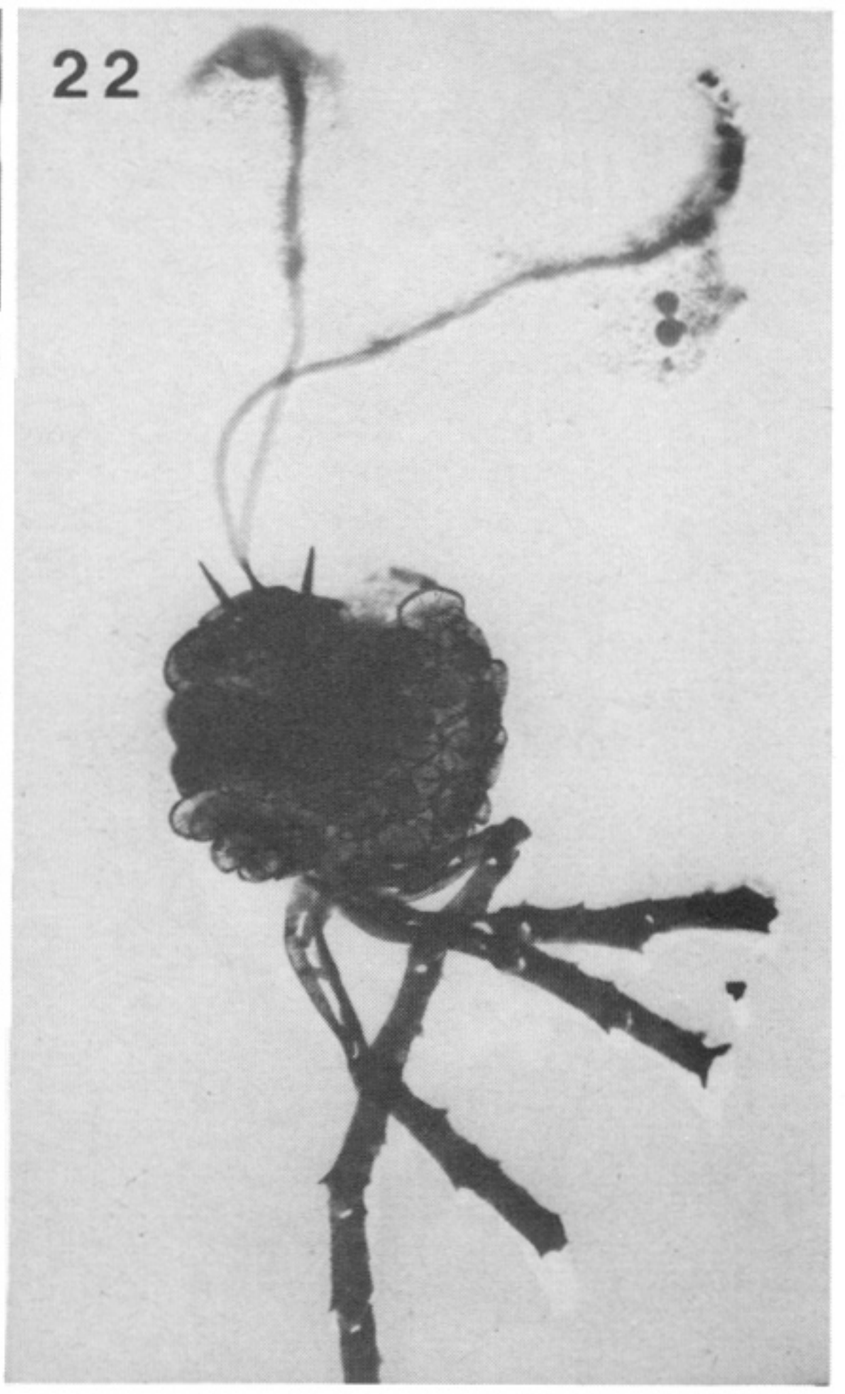
FIGURES 9–18. For description see opposite.

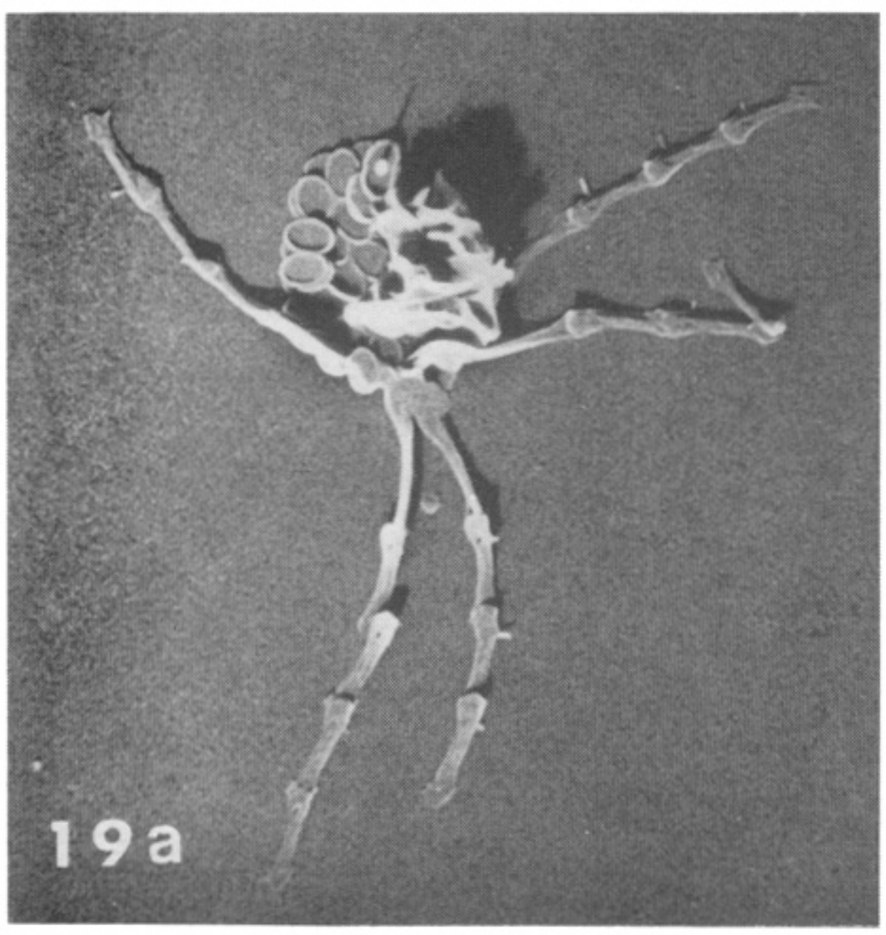


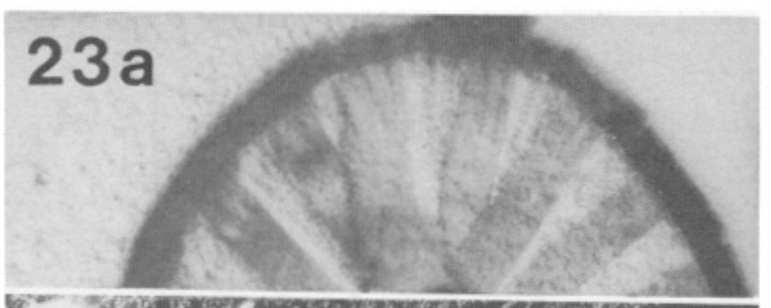


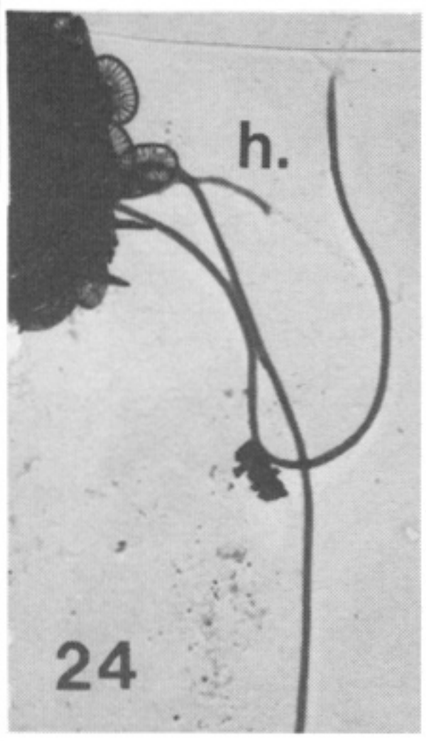


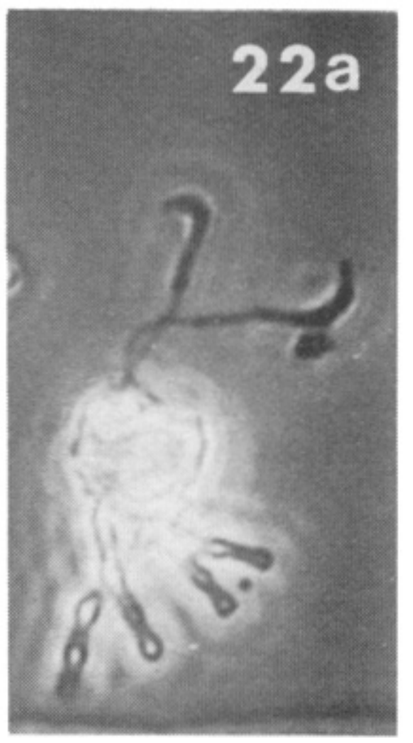


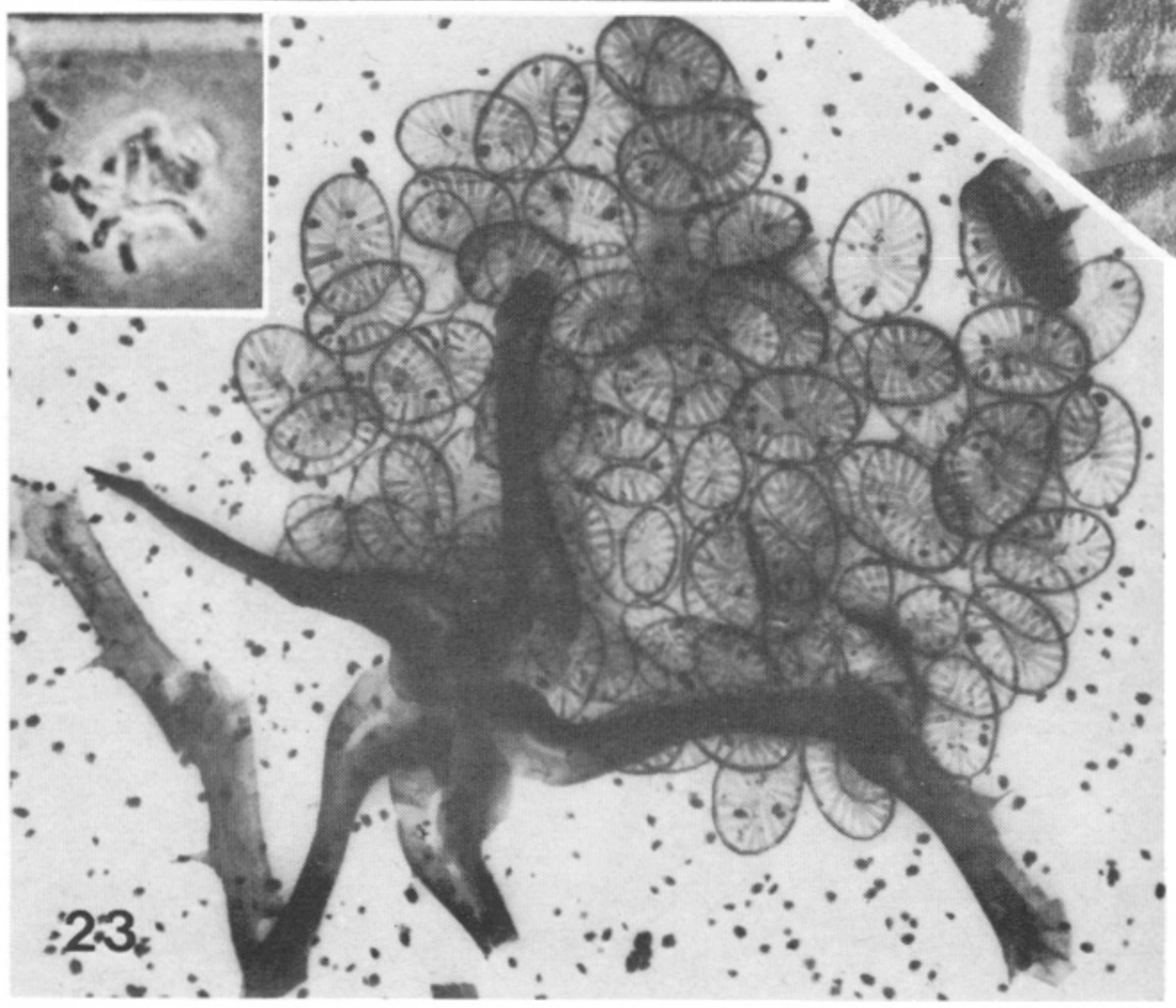


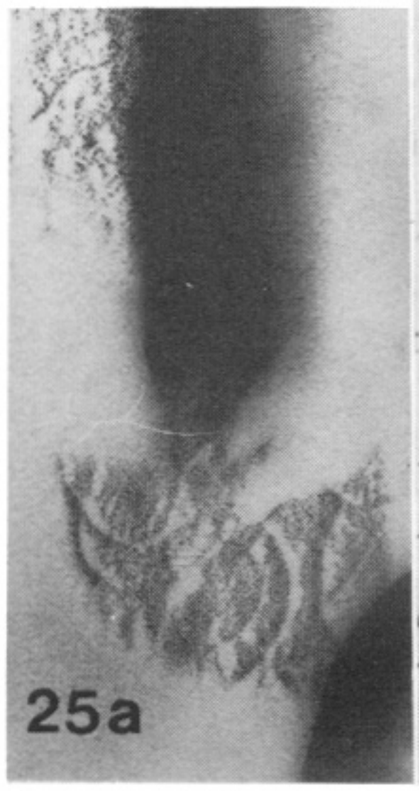


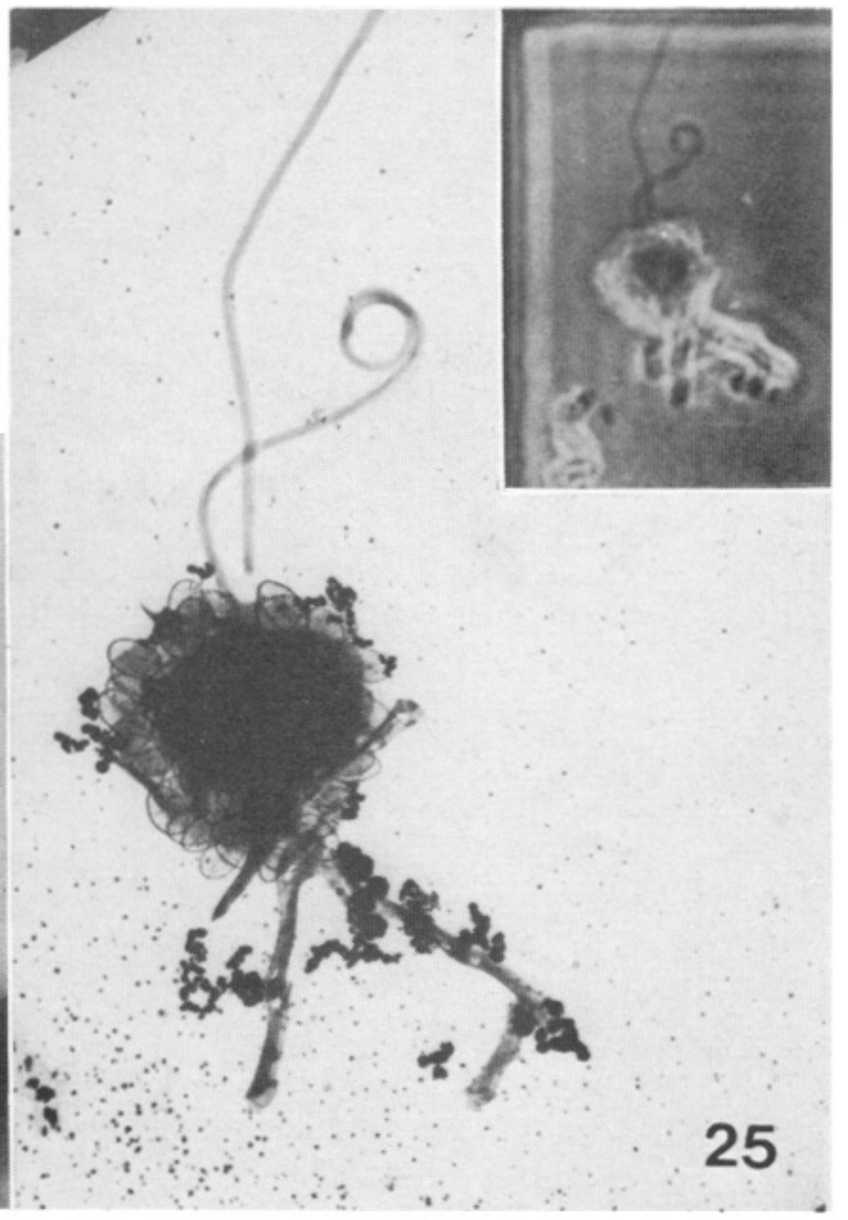




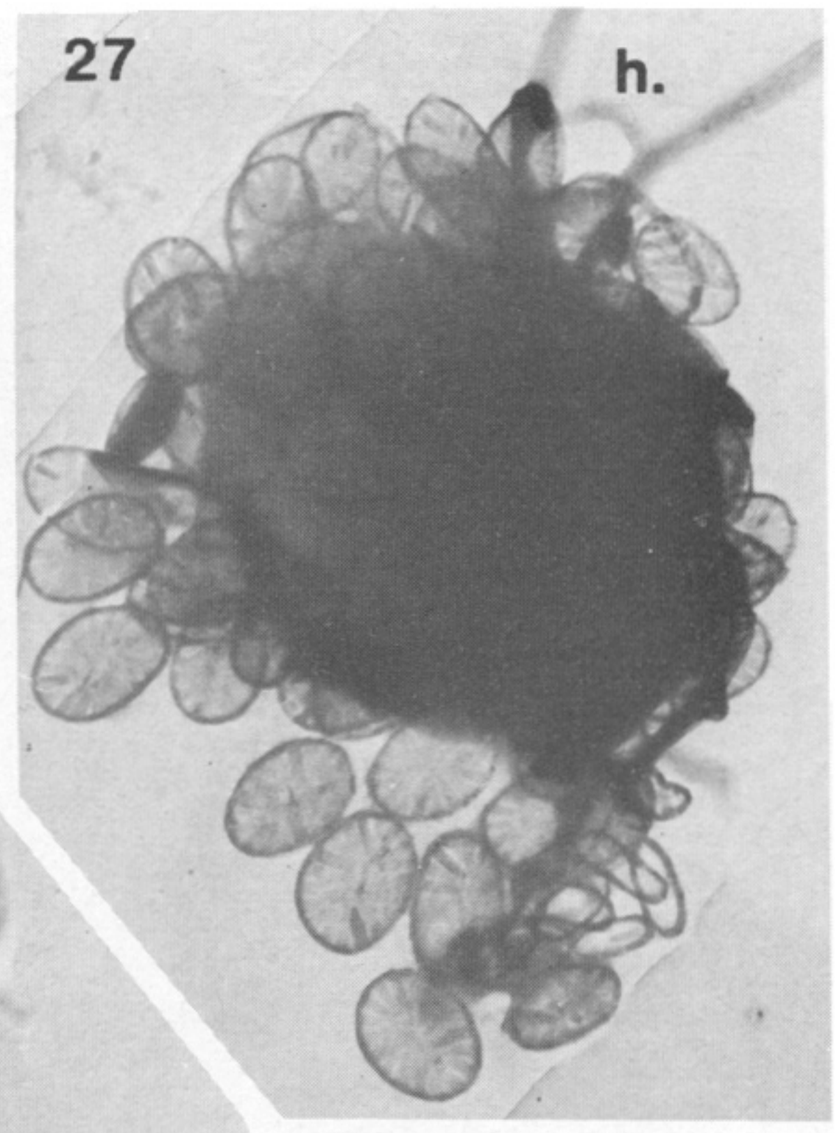


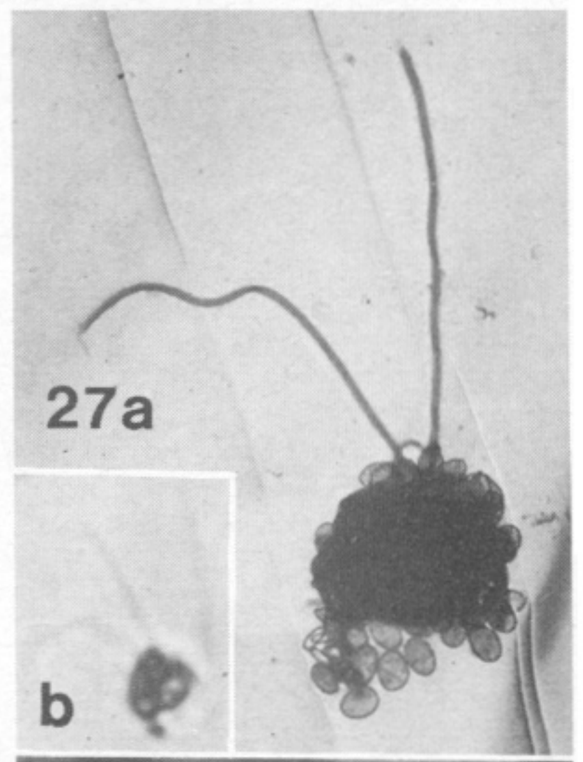


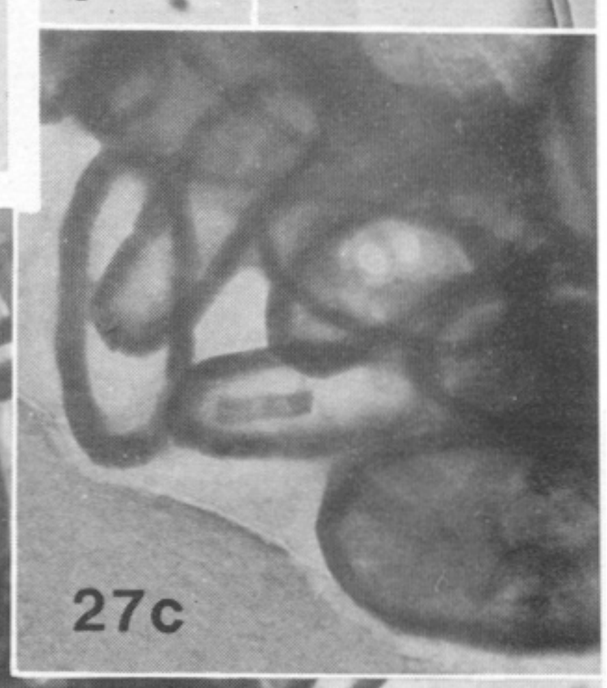


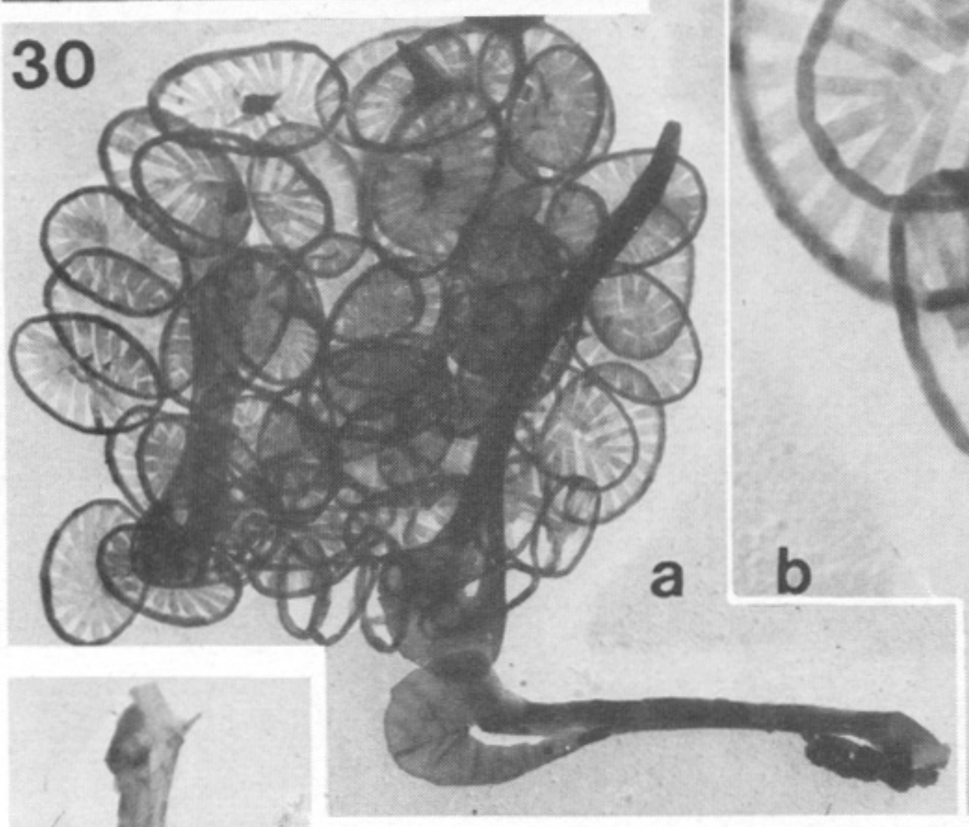


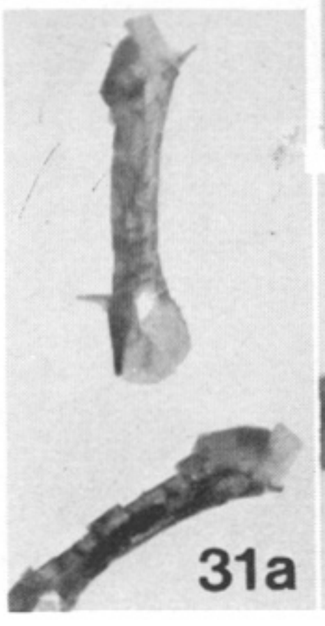
FIGURES 19–25. For description see opposite.

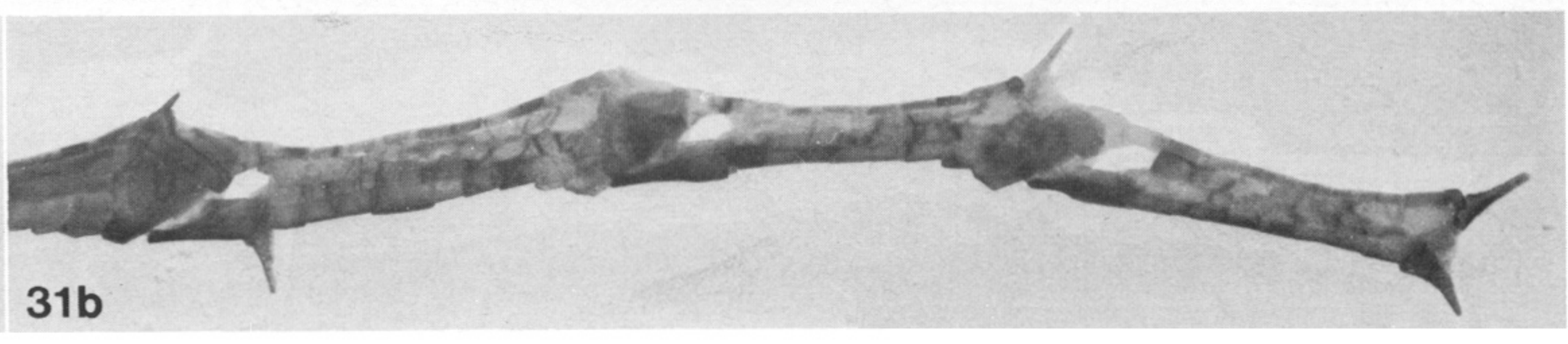




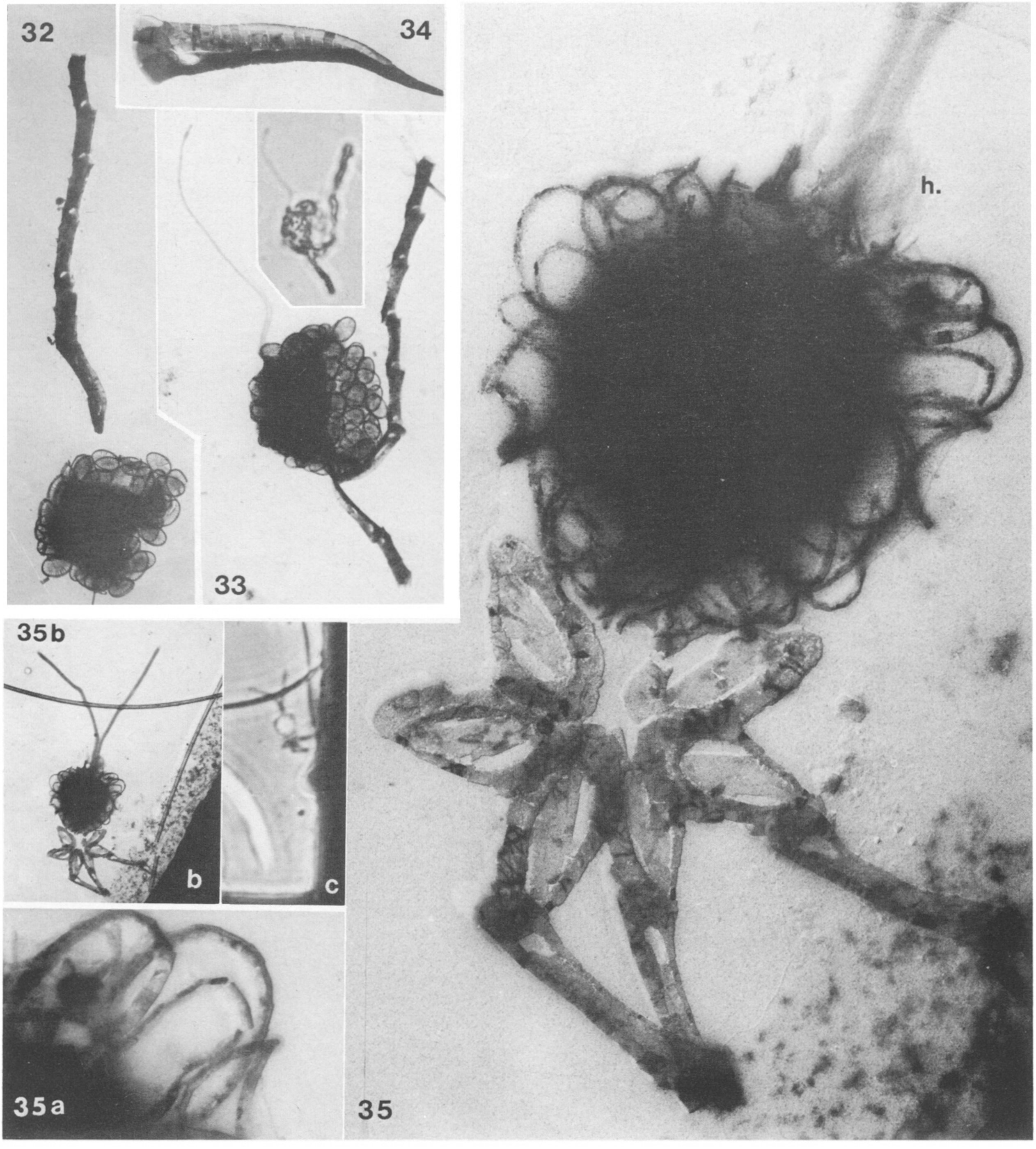




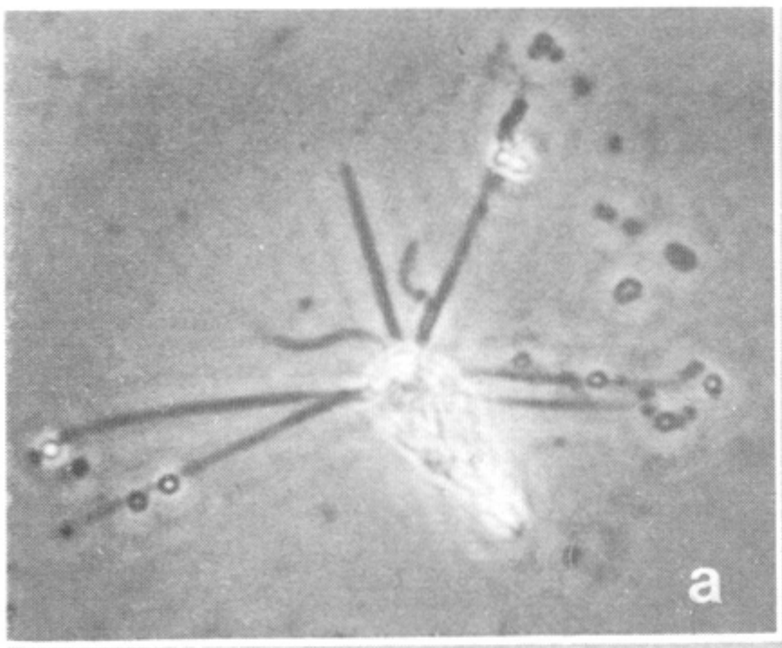




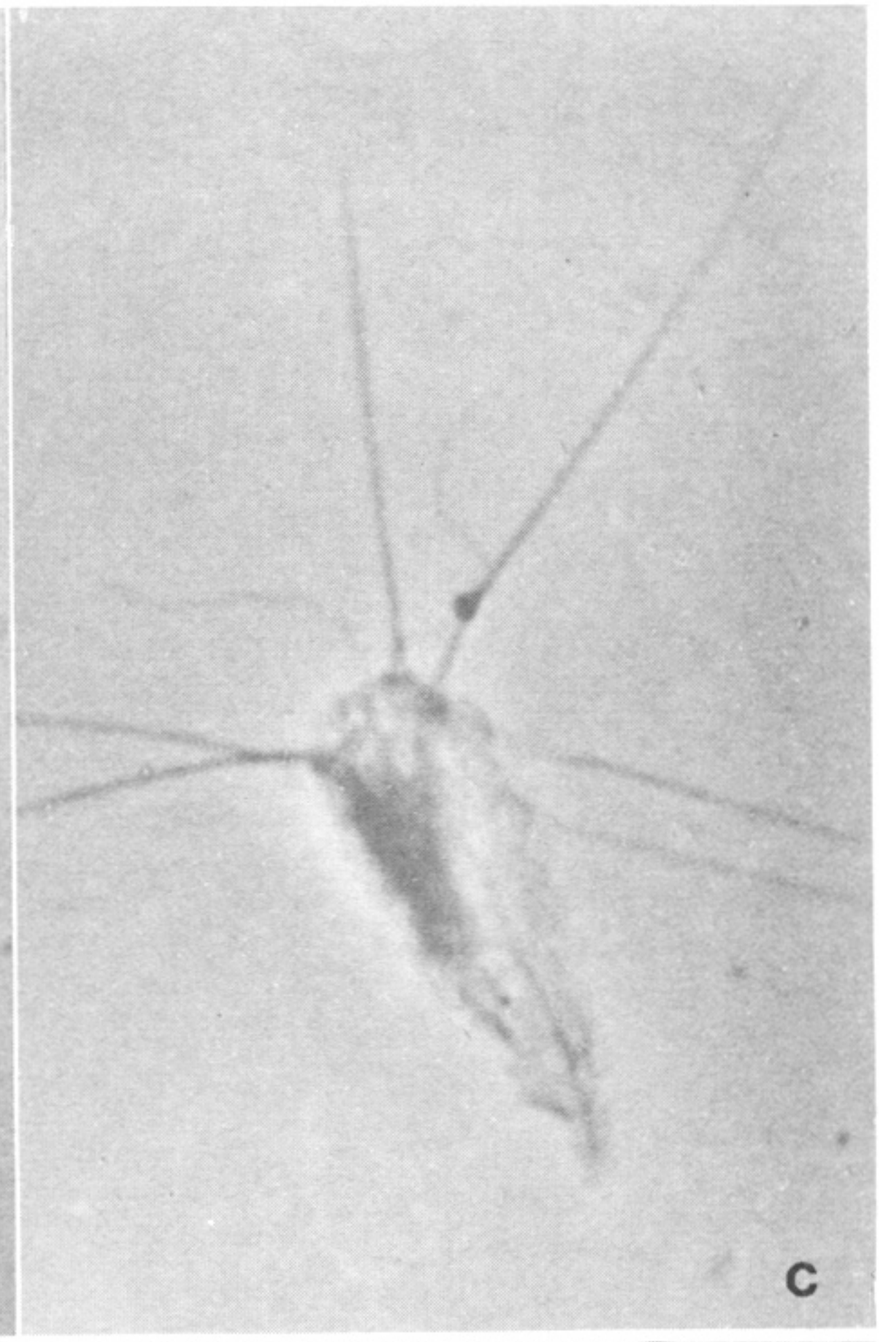
FIGURES 26–31. For description see opposite.

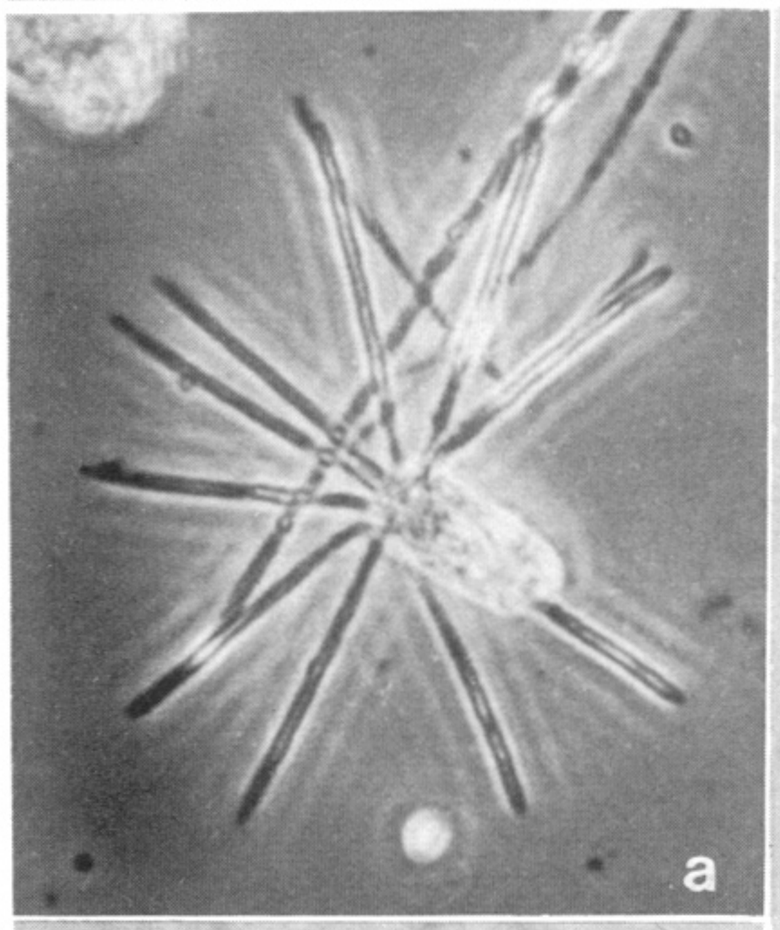


FIGURES 32–35(*c*). For description see opposite.

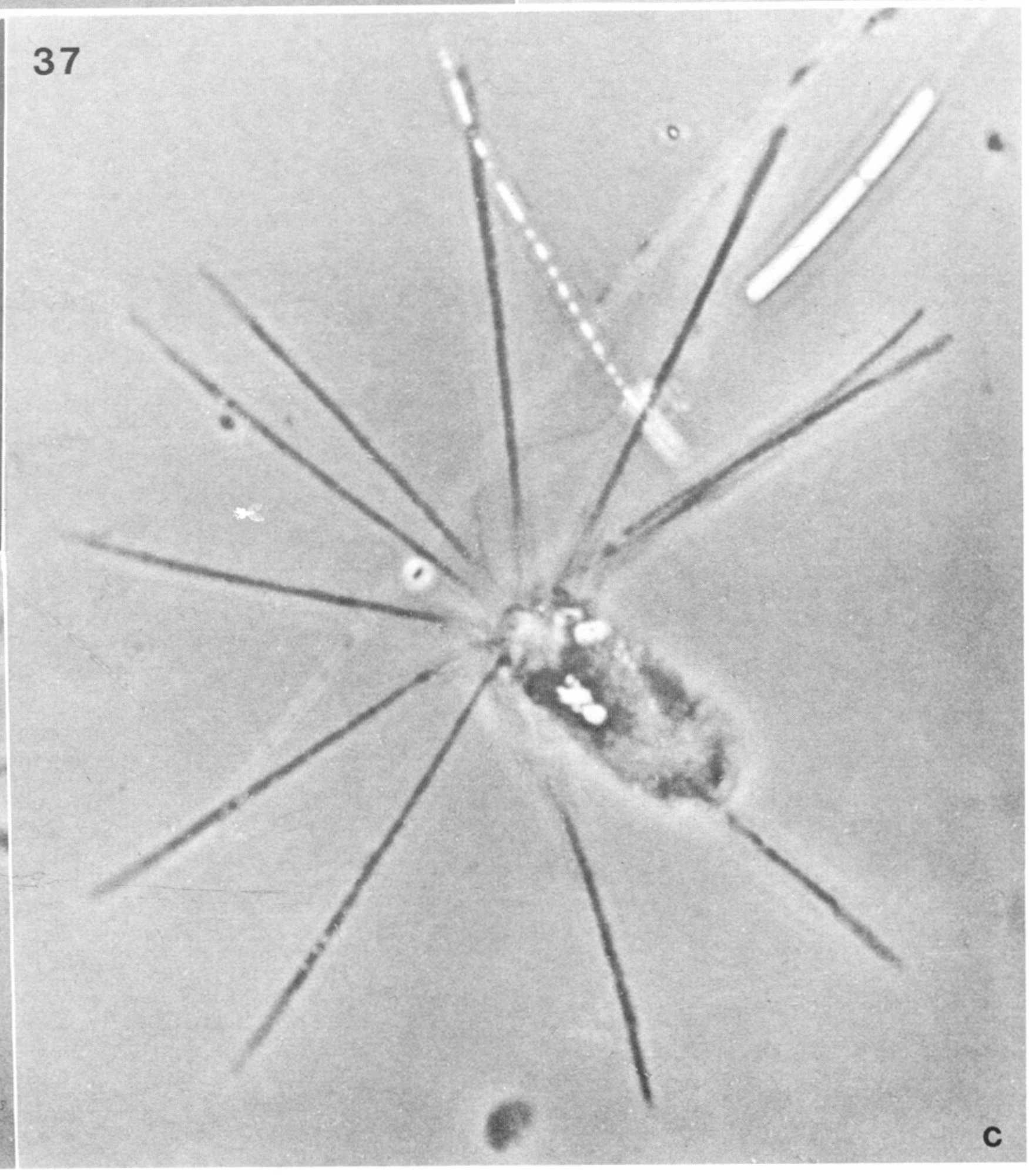


36

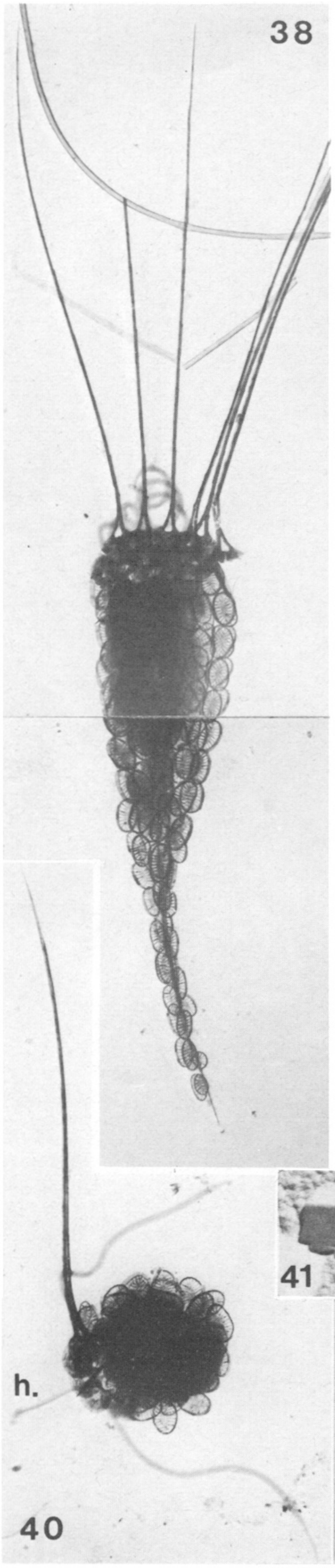


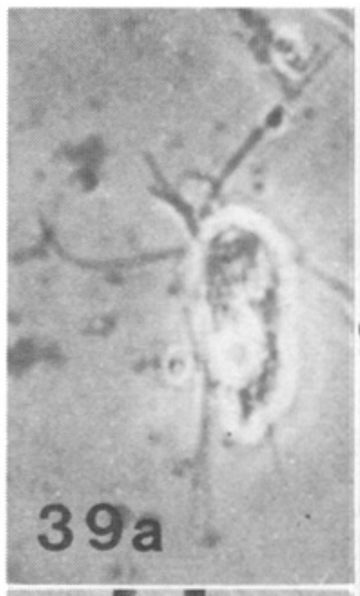


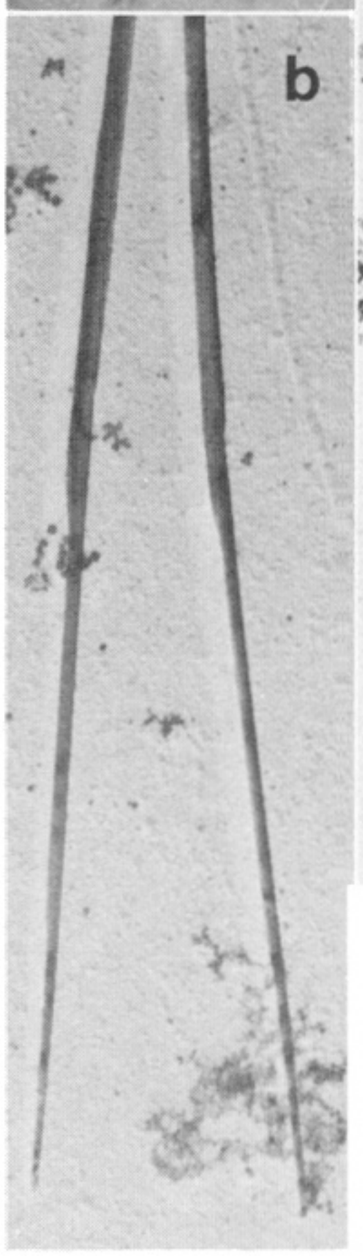
37

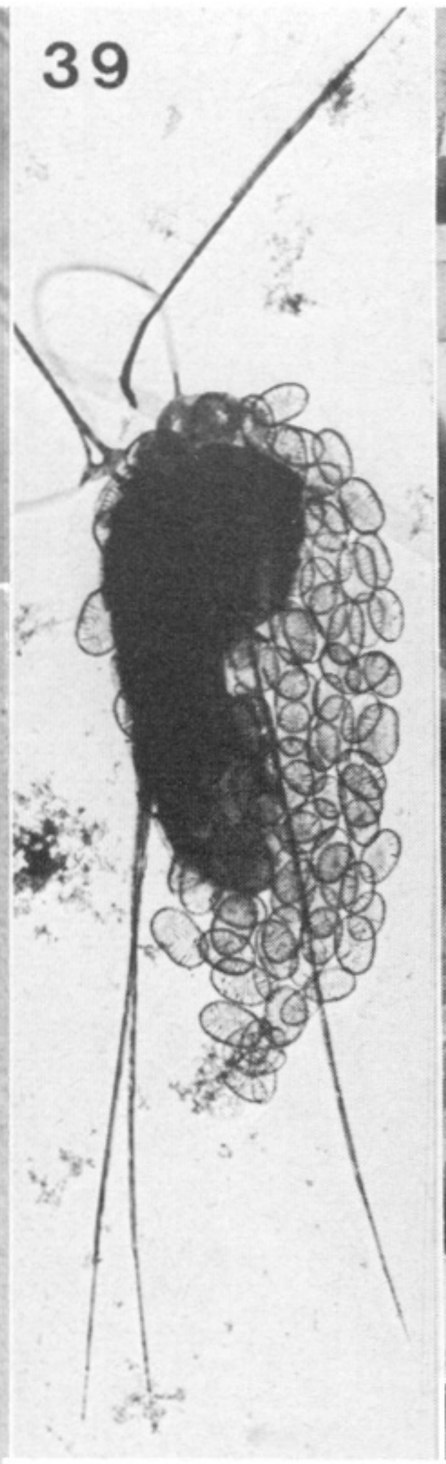


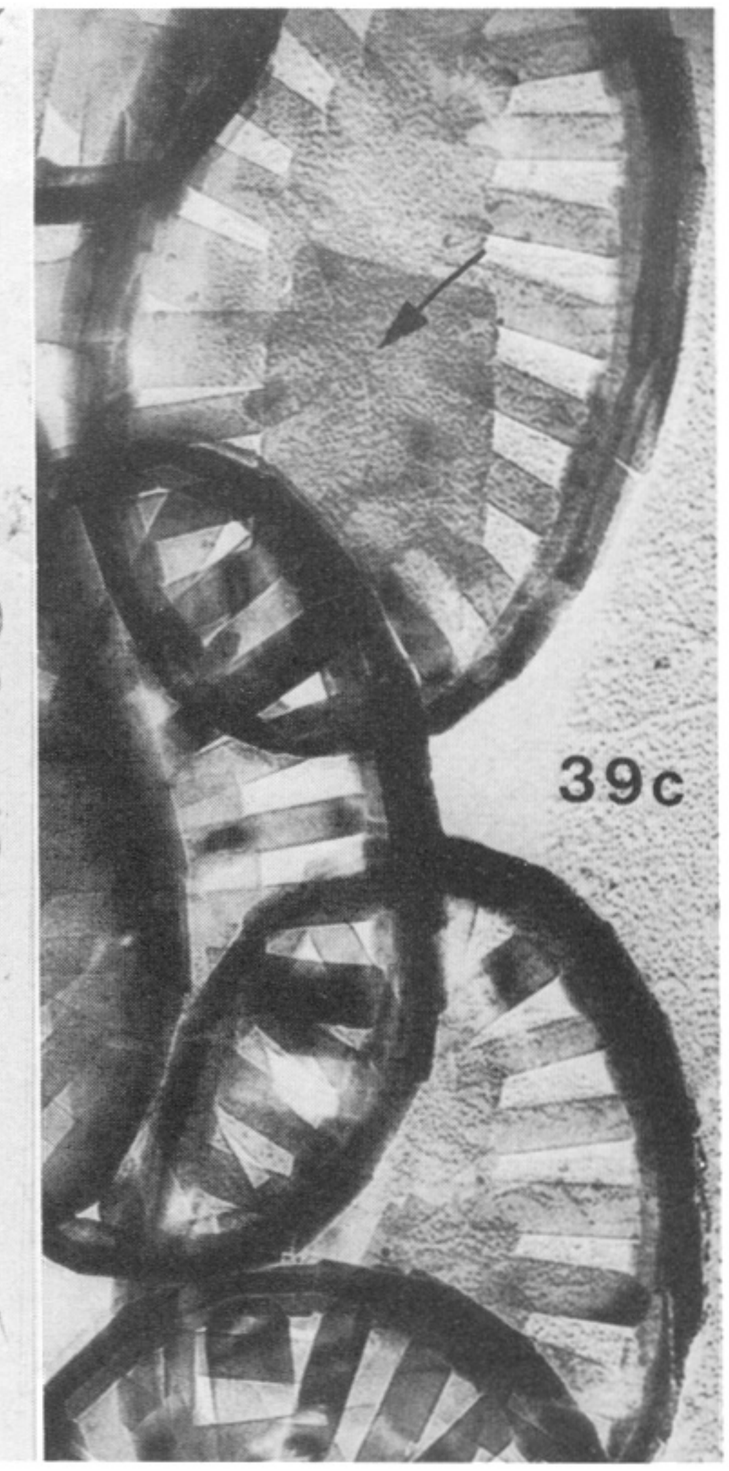
FIGURES 36–37. For description see opposite.

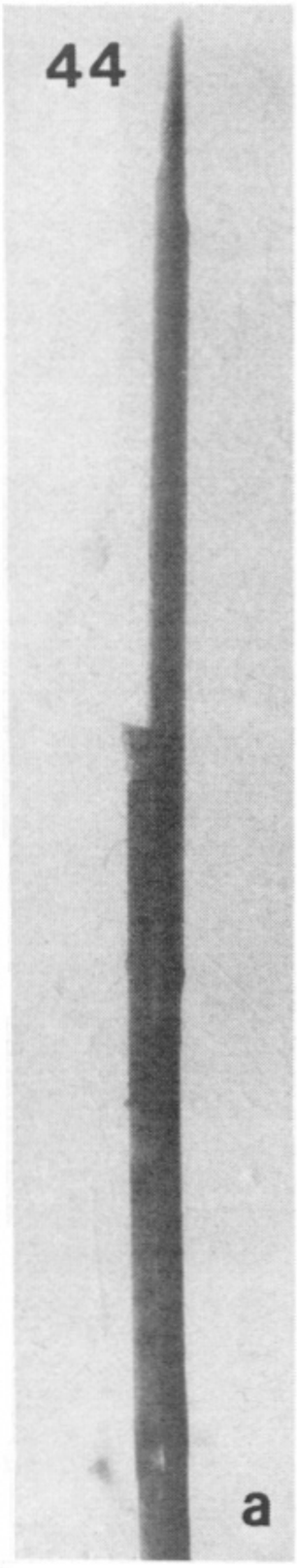


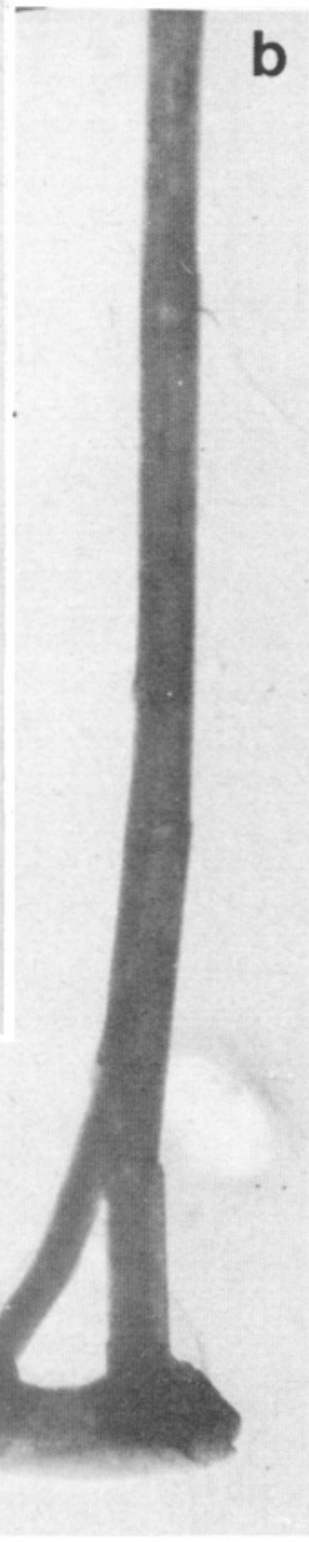


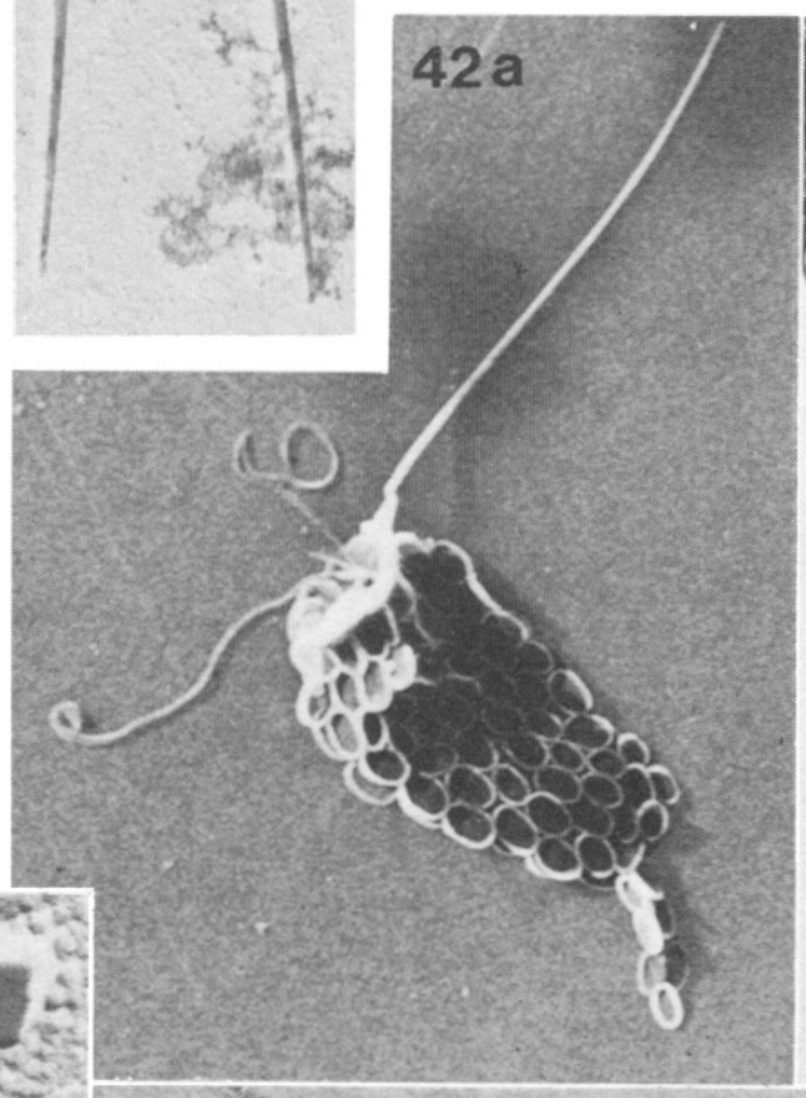


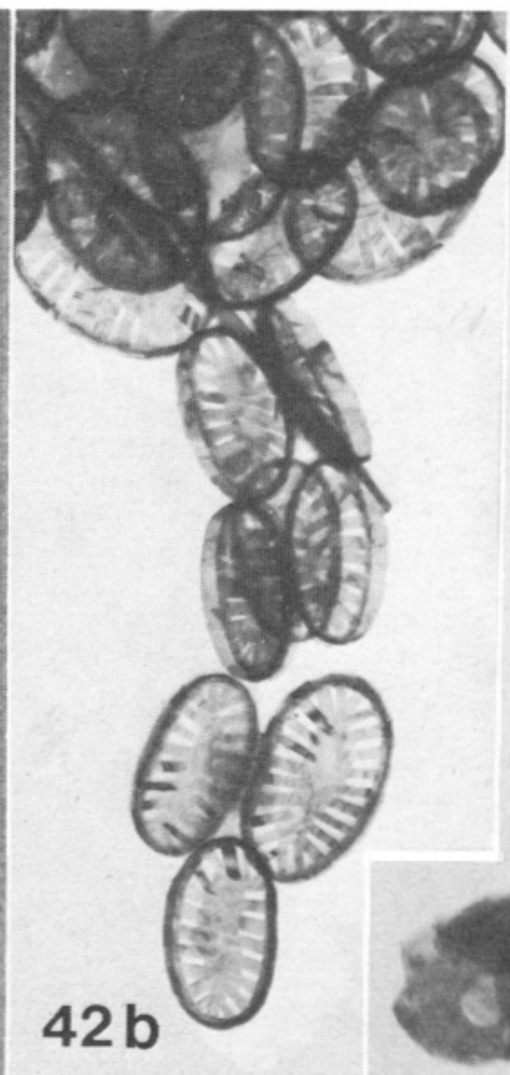


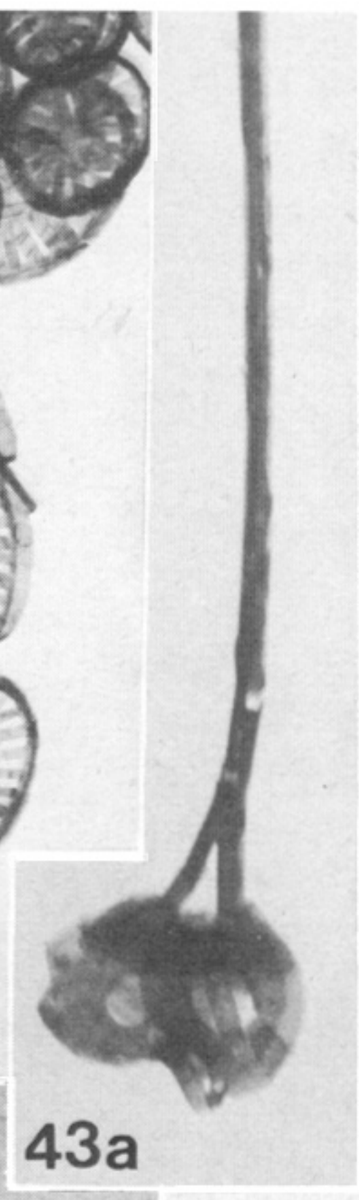


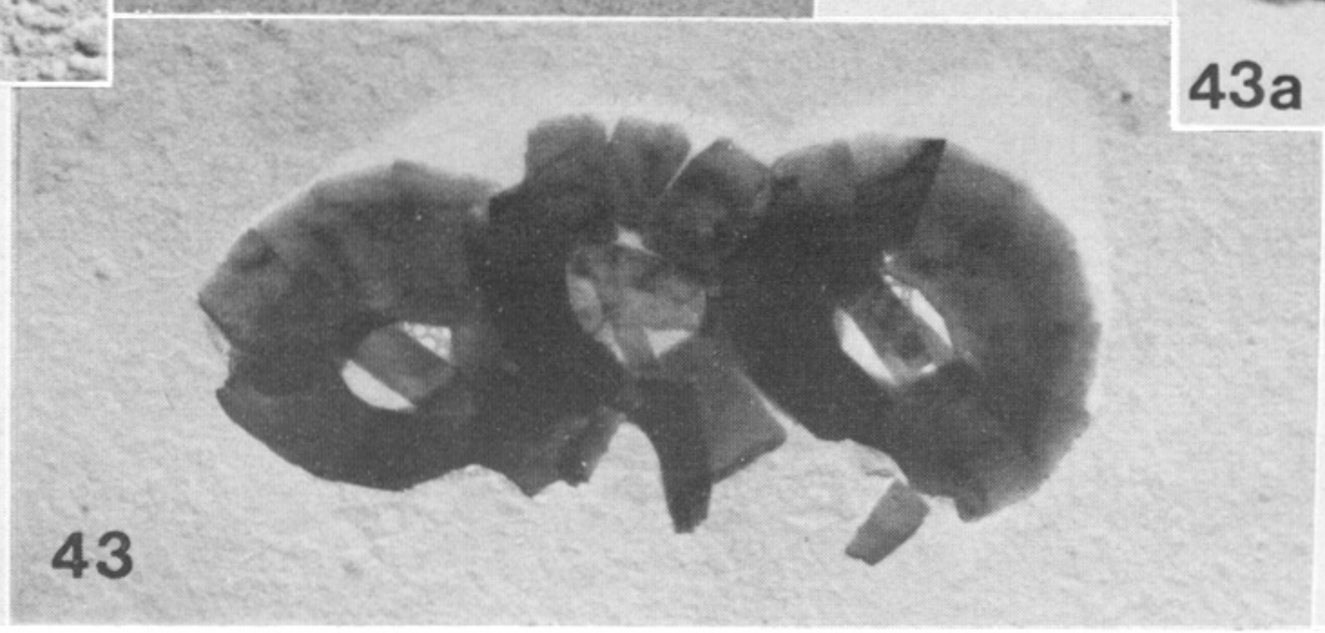












FIGURES 38–44(*b*). For description see opposite.