Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Calciosoleniaceae (Coccolithophorids) from the Galapagos Islands: Unmineralized Components and Coccolith Morphology in Anoplosolenia and Calciosolenia, with a Comparative Analysis of Equivalents in the Unmineralized Genus Navisolenia (Haptophyceae = Prymnesiophyceae)

Irene Manton and K. Oates

Phil. Trans. R. Soc. Lond. B 1985 **309**, 461-477
doi: 10.1098/rstb.1985.0093

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1985 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B **309**, 461–477 (1985) [461]
Printed in Great Britain

CALCIOSOLENIACEAE (COCCOLITHOPHORIDS) FROM THE GALAPAGOS ISLANDS: UNMINERALIZED COMPONENTS AND COCCOLITH MORPHOLOGY IN *ANOPLOSOLENIA* AND *CALCIOSOLENIA*, WITH A COMPARATIVE ANALYSIS OF EQUIVALENTS IN THE UNMINERALIZED GENUS *NAVISOLENIA* (HAPTOPHYCEAE = PRYMNESIOPHYCEAE)

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.*

(*Received* 10 *April* 1984)

[Plates 1–4]

CONTENTS

PAGE

Introduction 462

A note on left–right reversal in transmission electron microscopy 463

Materials and methods 464

Results 466

1. *Anoplosolenia brasiliensis* (Lohm.) Defl. 466
2. *Calciosolenia* aff. *murrayi* Gran 469
3. Comparison with *Navisolenia aprilei* Lecal ex Leadbeater and Morton 471

Discussion 473

Conclusions 476

References 476

Coccolith morphology in *Anoplosolenia brasiliensis* (Lohm.) Defl. and *Calciosolenia* aff. *murrayi* Gran from the Galapagos Islands has been investigated three-dimensionally mainly by means of scanning electron microscopy used with a tilting stage to supplement transmission electron microscopy and light microscopy. The rhomboid coccoliths in both genera are shown to be concave proximally with a central groove and convex distally with a central ridge. An unmineralized membrane with characteristic peripheral striations is demonstrated on the proximal face of the

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

rhomboids in both genera, with less complete evidence suggesting a second, patternless, membrane on the distal face of the coccoliths in *Calciosolenia*. Other newly described details are illustrated, and the existence of left–right reversal in some of the observations recorded in the standard literature is noted. When this is corrected, the uniform orientation of coccoliths in position on the cell surface in both genera is discussed in a preliminary way. Finally, comparisons are made with the wholly unmineralized cells of *Navisolenia aprilei* Lecal, ex Leadbeater and Morton, recently described in the literature, which resemble *Calciosolenia* so closely in salient features of cell shape, scale shape, scale arrangement and aspects of surface patterning, that a phyletic connection seems unavoidable. Some possible taxonomic consequences of these findings are discussed in a preliminary way in relation to the known antiquity and specialized condition of all members of Calciosoleniaceae though the need for further information on all of them is stressed.

Introduction

Marine plankton flagellates covered with rhombic coccoliths are so unusual that the isolated position of *Calciosolenia* and its near relative *Anoplosolenia* has long been recognized. Thus the geologist, M. Black, writing in 1968 on taxonomic problems in the study of coccoliths, summarizes the situation, under the heading Calciosoleniaceae Kamptner, in terms that are still valid:

> 'Most coccolithophorids have a spherical, or pear-shaped body. The Calciosoleniaceae differ in being cylindrical or fusiform, and their coccoliths instead of being circular or elliptical, take the form of a narrow parallelogram. There are probably four of five living species, and although the family characters are unmistakable, their systematics at generic and specific levels are not easy. Fossil representatives are never common, but have been found at intervals in the geological column down to the Cretaceous, the earliest British occurrence being at the base of the Cenomanian.... The interesting feature of this record is that the earliest specimens differ so little from living material: the family characters with their eccentricities are fully developed at the first appearance, there is no clue at all about relationships to other coccolith taxa, and no suggestion of any evolutionary change during the long interval from the middle Cretaceous to the present day.' (Black 1968, p. 803).

The reference to 'four or five living species' is likely to have been based on a well-known handbook (Grassé 1952, plate I, figure 356) in which *Anoplosolenia* Defl. is introduced as a new genus, illustrated with drawings made with the light microscope and published a decade earlier, as a variety of *Calciosolenia*, by Kamptner 1941. *Calciosolenia* itself, discovered by Gran in 1911 (see Murray & Hjort 1912) is represented in Grassé by a different drawing, quoting Schlauder 1945, placed beside two others attributed to a third genus *Acanthosolenia* Bernard 1939 about which nothing more seems to be known.

The advent of electron microscopy into the study of these organisms (for example, Halldal & Markali 1955; Lecal 1965; Gaarder & Hasle 1971; Borsetti & Cati 1972; Kling 1975; Nishida 1979 and perhaps others) has done little to clarify the situation at species level, in part because the range of specific epithets carried over from preceding light microscopy has introduced a confused synonomy not as yet fully resolved. Perhaps in consequence, several experienced authors have explicitly noted their choice of the simplest external characters as the sole means of taxonomic recognition, thereby precluding more than generic identification.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Thus Okada & McIntyre (1977, p. 19) state with respect to *Calciosolenia murrayi* Gran (type species of this genus) 'all coccolithophores with a cylindrical body and scapholith-type coccoliths were included in this species throughout our study'. Similarly Heimdal & Gaarder (1981, p. 40) state with respect to *Anoplosolenia brasiliensis* (Lohman) Deflandre 'all spindle-shaped coccolith cases with scapholith-type coccoliths and tapering at both ends into long horns were included in this species throughout our study'. The word 'scapholith' in both these statements had been defined by Halldal & Markali (1955) as meaning 'boat-shaped', a definition scarcely adequate, even in this context, as we shall see. However, the mere fact that these statements were made suggests that other characters and therefore perhaps additional taxa may have been present but disregarded in the collections available to these authors.

In the account which follows, our own procedure has been essentially that listed above by Okada & McIntyre (1977) and Heimdal & Gaarder (1981), respectively. We can accept their interpretations that the names used are the valid ones to designate the type species of each of the two genera, though whether these should be interpreted as monotypic or compound is a matter that cannot usefully be considered until more accurate information can be provided than that known hitherto. The first object of the enquiry will therefore be to amplify, and if necessary correct, the factual records for each of the two taxa undoubtedly representing the Calciosoleniaceae in the Galapagos Islands, to the extent permitted by our material. Inherent problems of speciation should thereby become clarified though not necessarily immediately resolved.

A further consequence of the enquiry, unforeseen at the outset but perhaps of greater scientific interest, is that improved descriptive understanding of the coccoliths themselves becomes highly relevant to phyletic interpretation of the wholly unmineralized flagellate *Navisolenia* Lecal. The results will therefore include a more informed critique of hypotheses already to be found in the literature on this unusual but undoubtedly related organism than could previously have been compiled.

Note on left–right reversal in transmission electron microscopy

Unintended left–right reversal is a hazard of exceptional relevance to the present enquiry. For this reason some preliminary explanations may perhaps be helpful, together with a recommended code of practice appropriate to the presentation of results.

Every photographer knows that an ordinary photographic print is obtainable, by contact or enlargement, from a negative so arranged that the emulsion side faces the light-sensitive surface of the paper during printing. This ensures that negative and print will be mirror images of each other and that the latter will correctly record the orientation of the object photographed. In transmission electron microscopy, on the other hand, it is less commonly realized that a printed micrograph, which may or may not conform to the orientation of the object itself, will always be left–right reversed with respect to the image on the fluorescent screen in the microscope, if normal photographic processing is adhered to. This is because the position of the camera in relation to the microscope column is such that the electron beam falls directly onto the light-sensitive emulsion (following removal of the fluorescent screen or its avoidance in some other way), thereby producing a negative which is oriented in exactly the same direction as the screen image. Printing by normal photographic procedures will automatically reverse this.

On many, and indeed most, occasions such an effect can be ignored in transmission electron microscopy since the relative positions of left and right are rarely important. Exceptionally,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

however, when accurate information concerning direction is needed, it is not difficult in a modern microscope (though impossible in some early ones) to change the screen image at will by manipulating the specimen. If a grid is turned over and replaced in the microscope, a mirror image of it will be formed compared with that in the previous position. A selection can then be made, in the light of other evidence, permitting operational procedures to be standardized in an appropriate manner to avoid errors.

With scanning electron microscopy, the circumstances are different. Both the image to be recorded and the camera are located outside the microscope column, permitting normal photographic procedures to be followed without risk of distortion. Specimen positioning must nevertheless be rigorously controlled since a grid, if inserted into the microscope with the wrong surface uppermost, will yield only a blank image of the back of the support film when surface-scanned.

This limitation can lead to an acute dilemma under the special circumstances in which a single microscope, such as a Temscan, is used concurrently for both sorts of microscopy. It is then found that the obligatory position for successful scanning is upside-down for convenient transmission microscopy. Identical printing procedures, if applied to both negatives, will produce mutually conflicting prints, one a mirror image of the other. This apparent contradiction can of course be resolved by printing one negative normally and the other through the back. Image reversal as between negative and print will be prevented in the latter but an unwise choice as to which micrograph to select for each process can all too easily lead to production of two mutually consistent prints both of which are directionally false.

At this point, some empirical guidance is perhaps helpful, of the kind obtainable by use of a familiar man-made object such as a coin. This, when surface-scanned followed by normal photography, as illustrated on the front cover of *Proc. R. Micro. Soc* (1983, part 4), is unmistakably the right way round. We can therefore accept as authoritative the proposition that results obtained by surface-scanning, when fully processed in the normal photographic manner, will be correctly oriented with respect to the object, whereas those from transmission microscopy may not be. This information can then be used as a guide to appropriate treatment of micrographs intended for publication while also giving a rational explanation as to why some historically important transmission micrographs, published in the past by careful observers, may in retrospect be found to have been interpreted back to front.

Materials and methods

Specimens of both taxa were collected in August 1977 from coastal waters in the Galapagos Islands (latitude 0° 56′ S; longitude 90° 20′ W) mainly by means of a van Dorn bottle drawing water from known depths ranging from the surface to 19 m and with other details as listed in table 1. All samples designated 'Darwin' were fully processed at the Charles Darwin Research Station on Santa Cruz Island, either at once or after standing in the shade for no more than a few hours. In contrast, those designated 'A' were part-processed at sea by means of a Millipore filter but without a centrifuge, by two collaborators (Dr Margaret McCully of Ottawa and Mrs A. D. Greenwood of London) travelling together as passengers on M.S. *Iguana*. These part-processed ('A') samples were temporarily fixed in glutaraldehyde before being delivered to the Charles Darwin Station for finalizing by the shore party consisting of the senior author, A. D. Greenwood and Miss Joan Sutherland of Dundee, formerly of Ottawa.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

All the technical details required for converting the contents of water-bottle samples into dry whole mounts have been used before and described many times (see, for example, Manton *et al.* 1980; Manton & Oates 1983). It is therefore enough to say here that brief osmic fixation was applied to the centrifugate from a known volume of water, except for the 'A' samples which were merely spun down with a centrifuge, and rinsed to remove the glutaraldehyde, after which the centrifugates of both kinds were deposited drop by drop onto carbon-coated grids or glass slides and allowed to dry. A careful rinse in de-ionized water to remove salt crystals ended the field treatment.

After returning to England, all grid preparations were shadowcast with gold–palladium prior to transmission electron microscopy, after which, selected preparations received a further coating with gold, prior to scanning. The electron microscopes used by the senior author included an A.E.I. EM6B in the Cell Biology Unit at the University of Nottingham (courtesy of Professor E. Cocking, F.R.S.) supplemented by a Jeol Temscan in the Lancaster department. The latter instrument, used in the scanning mode by the junior author (K.O.) provided all the scanning electron micrographs except one. Figure 2*c* had been obtained earlier on a Jeol T20 scanning electron microscope at Portsmouth during previous joint work with G. Bremer on another genus and it will be introduced onto plate 1 (by courtesy of G. Bremer) because of the convenience of the graduated scale, included automatically in the field scanned, and therefore usefully confirming the correctness of calibration of this micrograph and others.

TABLE 1. SOURCES OF SPECIMENS OF *ANOPLOSOLENIA* AND *CALCIOSOLENIA* IN THE GALAPAGOS ISLANDS (1977)

sample	locality	date	depth/m	temp./°C	*Calciosolenia*	*Anoplosolenia*
Darwin 8	Academy Bay	12 Aug.	10	21	—	×
Darwin 11	Academy Bay	12 Aug.	15	21	×	×
Darwin 13	Academy Bay	13 Aug.	10	22	×	×
Darwin 14	Academy Bay	13 Aug.	15	22	×	—
Darwin 16	Baltra near ferry	15 Aug.	surface	23	—	×
Darwin 21	Barrington Island	16 Aug.	15	18.5	—	×
Darwin 23	Plazas (between islands)	20 Aug.	8 (on bottom)	18.5	—	×
A1	Bartolomé Island	15 Aug.	10 (on bottom)	22	×	×
A6	Fernandina Island	16 Aug.	19	19	—	×
A8	James Island	17 Aug.	15	22	—	×

Further confirmation of the correctness of magnifications cited in the legends is provided by the light microscopy, carried out last by the senior author after completion of the electron microscopy, as on previous occasions. For each organism at least one photograph, reproduced at a low power (× 1000) and taken with a dry lens on the dry preparation, has been illustrated (see figures 1*a*, *b*, 2*a* and 13*a*) but figure 13*b* draws on oil immersion, with Objektol applied to the formerly dry specimen mounted on glass, a procedure impossible with a specimen mounted on a support film. The microscope mainly used has been a Zeiss Photomicroscope 2 set up for phase contrast in the Cytogenetics Unit at the Medical School, University of Liverpool, (courtesy Dr S. Walker) though two photographs (figures 1*a* and 14 *a*) involved a Reichert Zetopan microscope set up for Nomarski interference in the Lancaster Department.

As on previous occasions, the photographic printing was mainly carried out in Leeds on a Leitz Focomat 2 enlarger belonging to the Royal Society but still available personally to the

Downloaded from rstb.royalsocietypublishing.org

senior author. As explained in the previous section the transmission micrographs illustrated have been printed, where necessary, through the back of the negative to combat left–right reversal except where otherwise stated. Contradictions as between light microscopy, scanning microscopy and transmission electron microscopy, have thereby been avoided.

Results

Specimens of *Anoplosolenia* are both larger and more easily damaged than those of *Calciosolenia*. Fragments are therefore more numerous and this in itself is a major advantage. There is the added advantage that Halldal & Markali (1955) constructed a diagram of a single coccolith summarizing their interpretation of transmission electron micrographs. Though we can now see that emendations of several kinds would be needed to bring this diagram fully into line with more recent information, it can nevertheless still provide a useful background to our own observations since some important concepts, introduced then for the first time, have not been outmoded.

1. *Anoplosolenia brasiliensis* (*Lohm.*) *Defl.*

The characteristic general shape of the crescentic cell with its tapered ends is perhaps sufficiently authenticated by figures 1 *a–c*, plate 1. The light microscopy (figures 1 *a*, *b*) had been taken last on a specimen already partly recorded by transmission electron microscopy (see figure 1 *c*, right hand end). The support film in figures 1 *a*, *b* is splitting and rolling back on itself almost, although not quite, obscuring the tip of the cell. Regardless of this degree of damage, comparisons between the various images are rewarding, and indeed necessary, if mistakes of interpretation are to be avoided. Thus the dark patch seen within the cell in figure 1 *b* might have been mistaken for evidence of cell contents had the optical system in use been unremarked or the real position of the shrunken protoplast been unknown. As it is, we can see from figure 1 *c* that the protoplast is much larger than the area occupied by the dark patch in figure 1 *b* which has picked out not the densest area, but the emptiest equivalent. With this information the empty area can be recognized again in figure 1 *a* though this photograph would otherwise have given no information on the cell interior.

The rhombic parallelograms covering the whole cell in a single layer become narrower but not shorter towards the tip (figure 1 *c*). Individual coccoliths can be seen again in figures 2 *a–c* scattered among debris of other cells including diatom fragments, in the field adjacent to an almost intact cell of *Ophiaster reductus* Manton. The disparity in size between single coccoliths in these two taxa is clearly attested by this juxtaposition. In addition it is perhaps noteworthy that almost all the detached coccoliths included in the field of figure 2 *b* can now be diagnosed as lying with their proximal faces uppermost (for the two exceptions see below), although this could not have been deduced from the transmission micrographs alone without knowledge of the other information summarized on plate 2.

The first indication that the rhomboidal coccoliths of *Anoplosolenia* are not flat is obtainable by scanning, provided that the specimen itself is first tilted. As may be seen at a low magnification in figure 2 *c* and in a more spectacular manner on some of the same coccoliths in figure 6, the exposed face of each of these is concave with a somewhat sigmoid central groove. When lying the other way up, as in figure 4, the plate is convex with a central ridge. More highly magnified details of this ridge, as seen by transmission microscopy, are illustrated in figure

Downloaded from rstb.royalsocietypublishing.org

8. The ridge itself consists of the slightly widened but abruptly truncated ends of incoming bar-crystallites which interlock before terminating a little above the distal surface of the plate. This micrograph also shows the compound nature of almost all the bars, as correctly recorded by Halldal & Markali (1955), doubtless on similar evidence.

A fortuitously tilted coccolith seen in side view, as in figure 7 (see also figure 2*b*), gives further valuable information. Thus the main rim is not simply ribbon-shaped but is higher centrally than at the ends. This feature can be recognized again in almost all the less strongly tilted specimens in figure 6 and elsewhere. Another departure from expectation aroused by Halldal & Markali's diagram is the presence of a system of oblique ridges crossing the junction of the plate with the rim when a coccolith is scanned from below. These ridges are numerically equal to the adjacent crystalline bars which, in turn, are attached individually to small crystallites of the lower rim. It is not difficult to show the presence of ridges of this kind on two of the four edges, simply by tilting as in figure 6. A more favourable instance in which tilting can be carried out in opposite directions, as in figures 5*b*, *c*, leaves no doubt that all four edges are similarly constructed. Lastly, figure 3, from a different water sample, more heavily shadowcast and slightly tilted, exposes the posterior ridges in the only way in which they might have been detected in a transmission electron microscope. Though figure 3 itself is a scanning electron micrograph, the shadow would have been at least as distinct in a transmission electron micrograph, the tell-tale feature in both cases being the delicately undulant edge.

Valuable as tilting has proved to be for analysis of these three-dimensional structures, there is one potential drawback which must not be forgotten, namely the unavoidable introduction of foreshortening. Measurements cannot easily be made on tilted specimens and, in extreme cases, mistakes of interpretation could arise if tilt were ignored. Thus a casual inspection of figures 4, or 5*b*, *c*, might cause the objects depicted to be thought of as equilateral parallelograms, which we know they are not. The true shape of the coccolith of figures 5*b*, *c* is shown without tilt in figure 5*a* (see also figure 2*b* bottom right), while that of figure 4 can be seen flat, by transmission microscopy, at top right in figure 2*b*. However, in both these coccoliths the presence of long and short sides can at once be confirmed, even when tilted, by counting the numbers of attached bars on the various edges which are approximately 13:9 in each of these particular specimens.

Such verification of shape is important in more than one context, not least because shape alone, when correctly construed, provides immediate guidance as to which way up a coccolith is lying, no matter what form of microscopy is used. Thus we know from figures 5*a*, 6, etc., that an inverted coccolith will have a long side on the left of the pointed tip if this is considered to be 'forwards' while the long side will be on the right, as in figure 4, if the coccolith is lying the other way up. In the light of these criteria, re-examination of figure 2*b* will at once show that among a majority of inverted specimens, two only are un-inverted, the latter being located respectively at top right and in the short column of coccoliths at top left (see further figures 7 and 9).

The way is now clear for consideration of unmineralized components for which evidence is assembled on plate 3. In this context, scanning electron microscopy is rarely useful although an exception is illustrated in figure 9. This is part of the uppermost coccolith included in figure 7 and in which local surface charging has impeded resolution of the crystallites, almost certainly as a result of chance influences resembling shading conferred by the other coccoliths in the group. There is fortunately no impediment to observations provided by the spaces between the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

bars which, in no less than four instances, show one or more dense dots arranged in a row and necessarily interpretable as small holes through otherwise undetectable material.

Transmission microscopy is more informative regarding this particular feature and a preliminary view of material in the spaces between bars is obtainable from figure 10. In this, a delicate multiperforate membrane occupies all but one of these spaces, the one apparently empty space (lower centre) almost certainly representing a site of local breakage.

Further information about the finely perforated material visible between bars in figure 10 is obtainable from figure 11*b*. This is a more highly magnified tip of a structurally damaged coccolith, shown complete in figure 11*a*, and in which several of the crystalline bars have broken away. As may be seen in figure 11*b* a continuous membrane occupies the area formerly covered by bars although the membrane itself is tending also to split, especially along junctions with extant bar crystallites.

Finally, the most important specimen of all (representing one of many) is illustrated in figure 12*a*, *b*. The first (figure 12*a*) shows a pair of laterally attached coccoliths lying with their lower (proximal) faces exposed, as indicated by scanning after tilting. The more highly magnified transmission micrograph (figure 12*b*) of part of the same pair without tilting has been printed in this case without left–right reversal to safeguard as far as possible the retention of delicate detail. Such details are of three different sorts. The most conspicuous are a system of close-set perforations visible between the crystalline bars. Secondly, the surfaces of the bars themselves can be seen to be almost covered with a finely granular or rugose deposit presumably relating to imperforate parts of a former membrane. Lastly, some delicate sloping lines, present on both sides of the conjoined scale edges (arrows), can just be detected overlying both crystalline and non-crystalline regions.

We conclude from plate 3 (and many other micrographs not reproduced) that the proximal

---

Description of plates 1 and 2

Anoplosolenia brasiliensis (Lohm.) Defl.

Figure 1. Relatively intact specimen from sample 'A1' (Table 1). (*a*) Light microscopy, dry lens, exposure 190.12 (Lancaster), magn. × 1000. (*b*) Light microscopy, phase contrast, dry lens, exposure 185.2 (Liverpool microscope) magn. × 1000. (*c*) Transmission electron micrographs Y_L 7969.10 (Temscan, Lancaster) and Y_N 7965.18 (EM6B, Nottingham), magn. × 3000.

Figure 2. A field bounded on three sides by grid bars and containing an intact cell of *Ophiaster reductus* Manton among periplast fragments of *Anoplosolenia* (A, B, C) and other detritus, including parts of diatoms; from sample 'Darwin 13' (table 1). (*a*) Light microscopy, dry lens (phase contrast), exposure 186.13, magn. × 1000. (*b*) Part of the field containing the *Ophiaster* cell and coccolith groups A and B; transmission electron micrograph Y_L 7971.15 (Temscan, Lancaster), magn. × 3000. (*c*) Coccoliths at C beside a diatom tip; tilted 45°; scanning electron micrograph YB 8226.8 (T20, Portsmouth), courtesy G. Bremer, magn. × 2000.

Figure 3. Isolated coccolith with proximal face exposed (sample 'Darwin 21'), tilted 30° and showing shadow with crenelated edge. Scanning micrograph YO 8312.6 (Lancaster) magn. × 15000.

Figure 4. Coccolith with distal face uppermost, from top right in figure 2*b*, tilted 60°. Scanning micrograph YO 8303.41 (Temscan, Lancaster), magn. × 15000.

Figure 5. Three different views of the detached coccolith at bottom right in figure 2*b*, the proximal face uppermost. (*a*) Untilted, scanning micrograph YO 8303.36 (Temscan, Lancaster), magn. × 15000. (*b*) and (*c*) Tilted 60° in opposite directions; scanning electron micrographs YO 8303.42 and YO 8303.26, magn. × 20000.

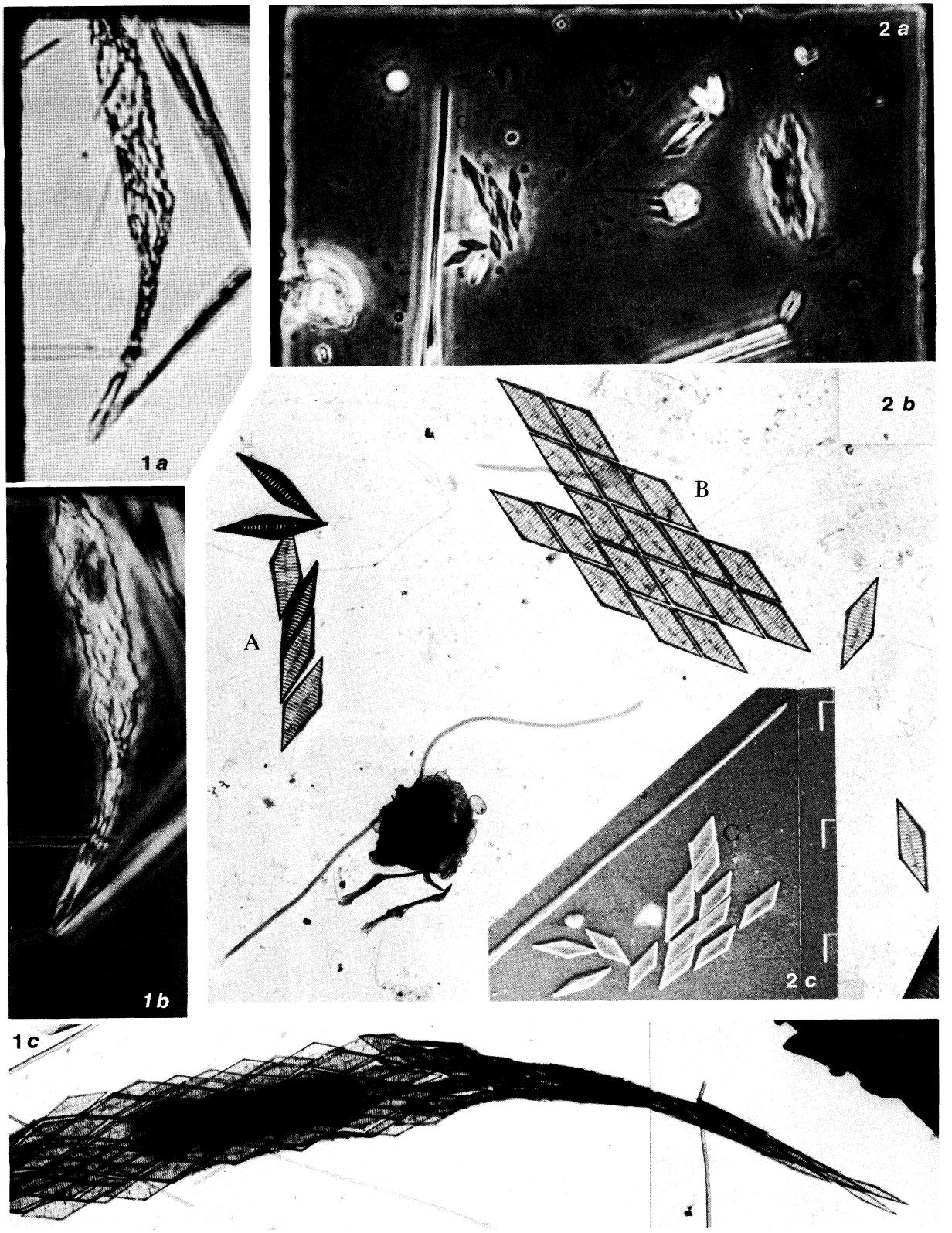
Figure 6. Part of the field of figure 2*c*, tilted 32°, coccoliths with proximal faces uppermost. Scanning electron micrograph YO 8303.6 (Lancaster); magn. × 15000.

Figure 7. Part of the row of detached coccoliths at top left in figure 2*b*, one (uppermost) exposing the distal face and the others inverted or seen edgeways; some further details in figure 9. Micrograph YO 8303.8 (Lancaster) magn. × 10000.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org



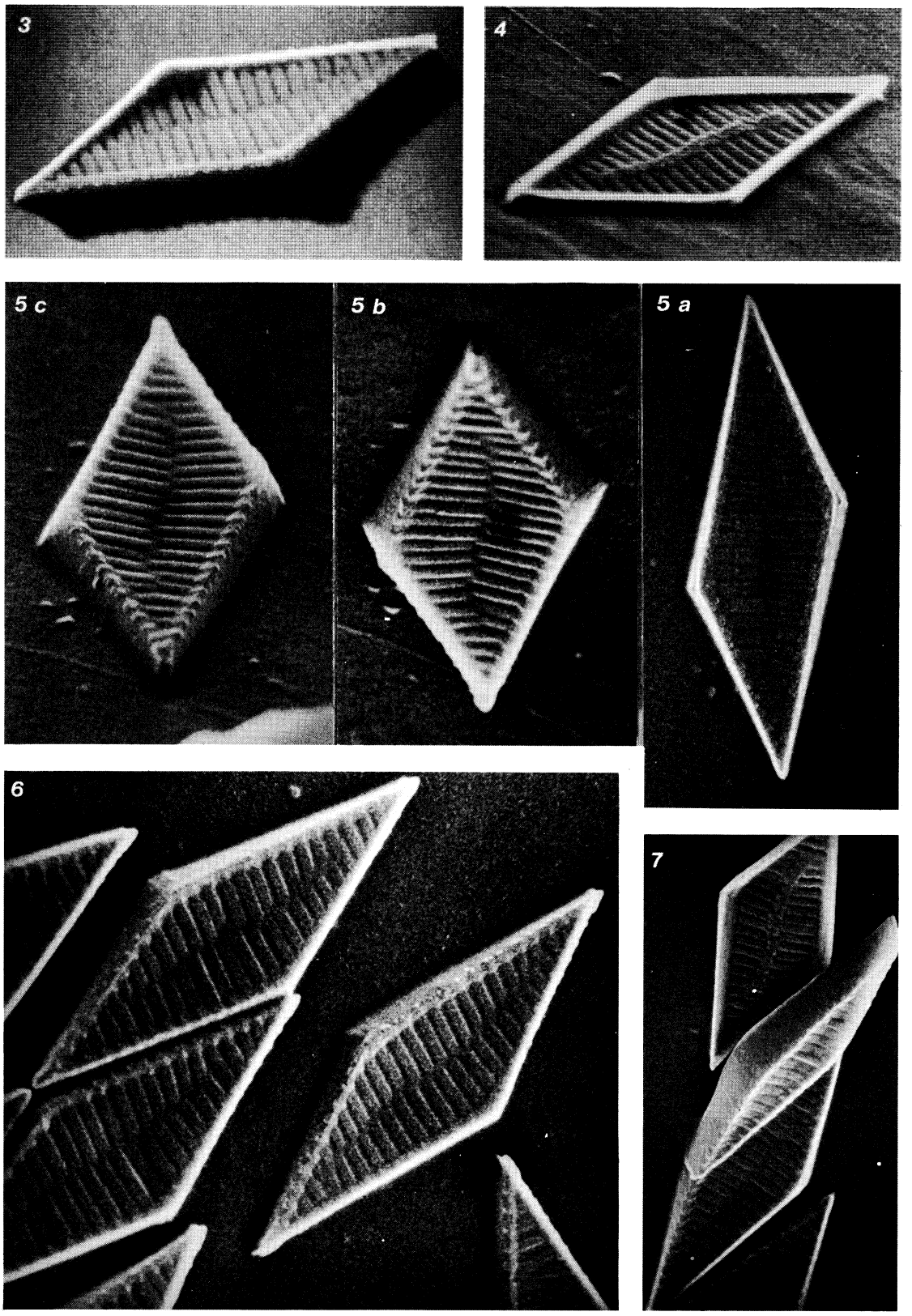
FIGURES 1 AND 2. For description see opposite.

(*Facing p.* 468)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

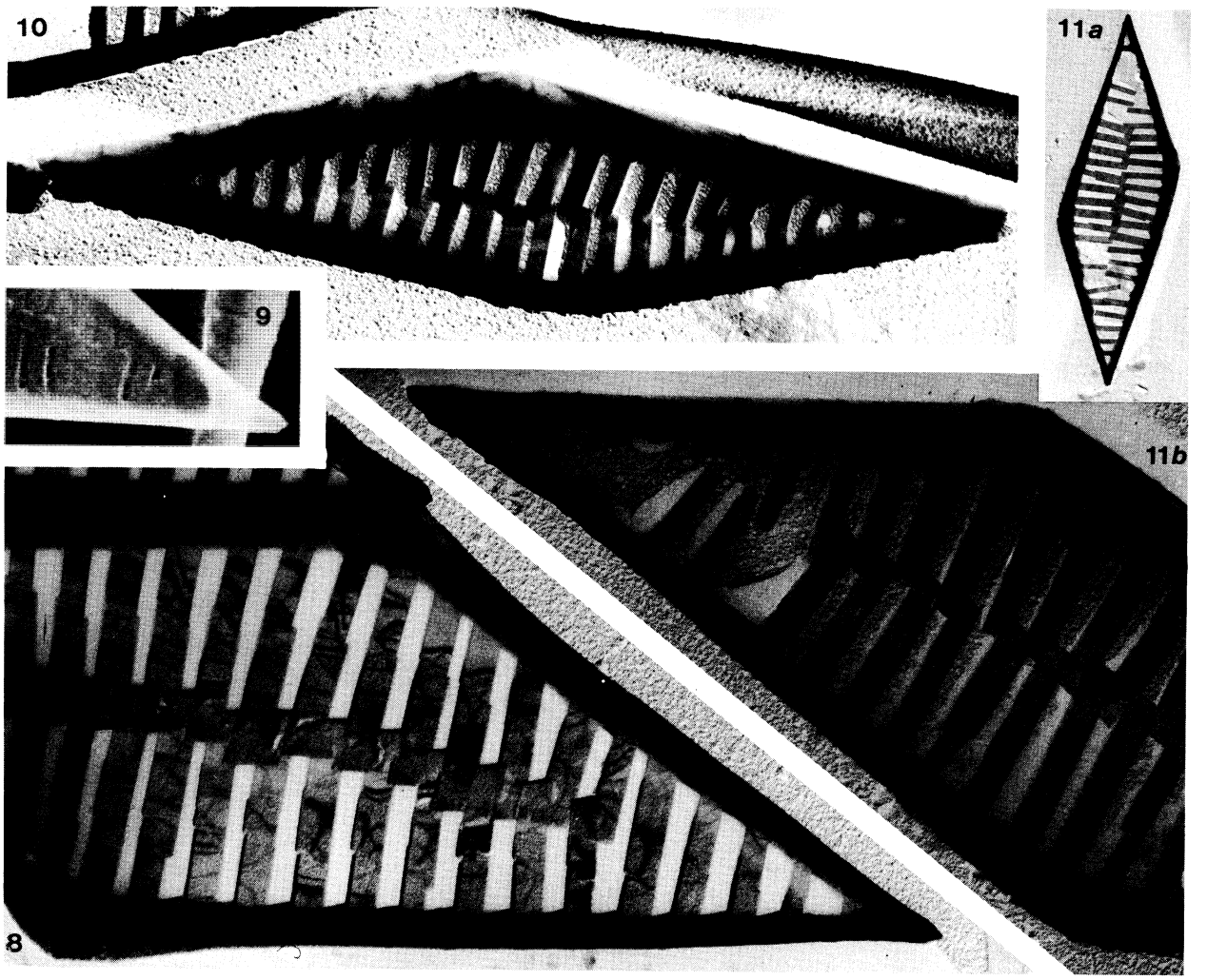


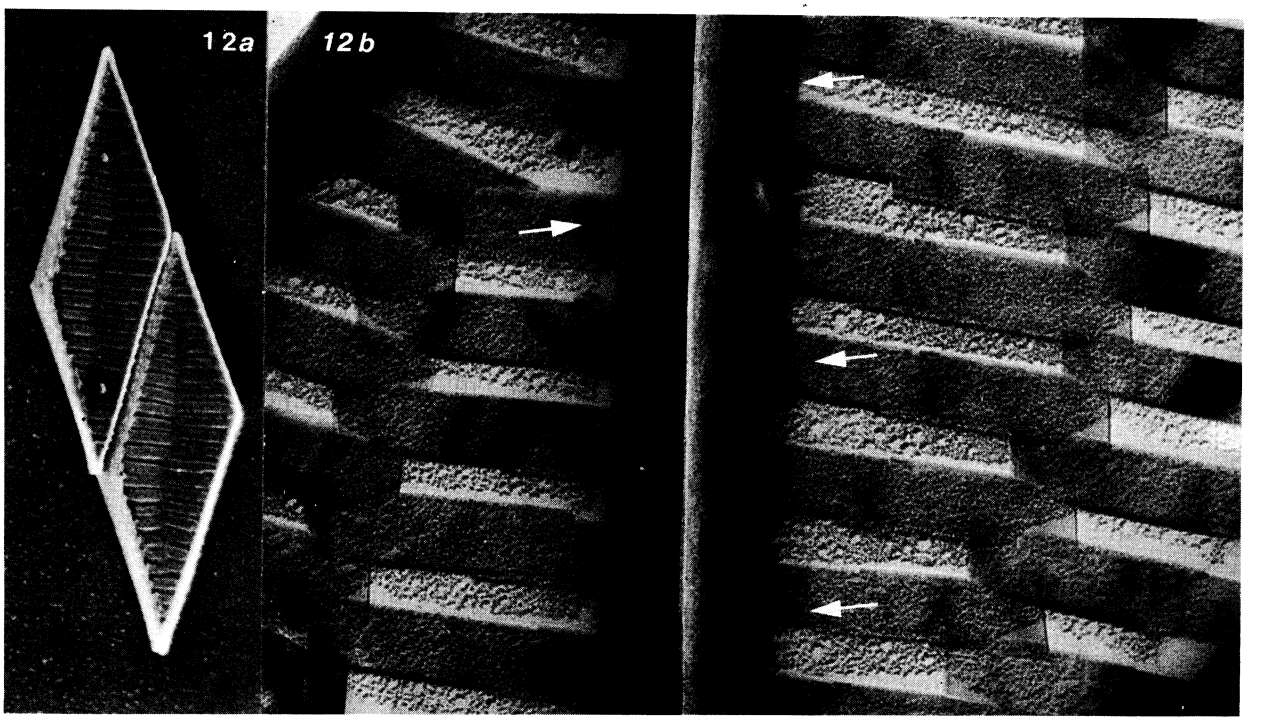
FIGURES 3–7. For description see p. 468.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org



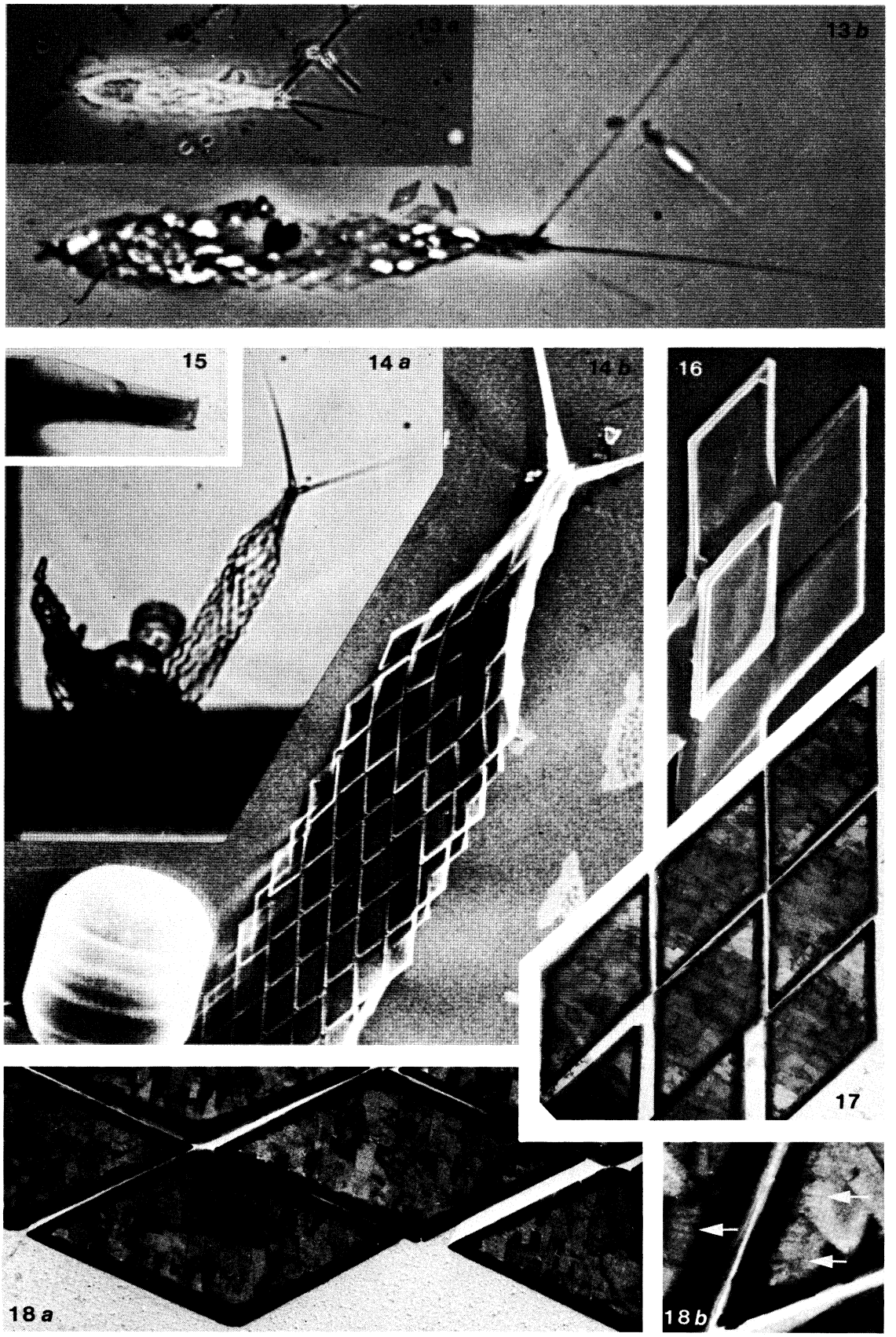


FIGURES 8–12. For description see p. 469.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org



FIGURES 13–18. For description see opposite.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

face of every coccolith in *Anoplosolenia* is initially covered by a continuous but delicate membrane, carrying a highly characteristic pattern of oblique striations peripherally but elsewhere liable to become multi-perforate and eventually to split, when dried. Such breakdown is most easily detectable in relation to the spaces between the bar crystallites of the plate itself.

Finally, the enquiry as a whole, in addition to contributing new facts such as these has drawn attention to something quite different, namely, a mistake in the supposed orientation of these rhomboid coccoliths as reported in the literature. In particular, the well-known diagram compiled by Halldal & Markali (1955), can now be seen to have been left–right reversed: an unavoidable consequence of the use (at that date) of uncorrected transmission electron microscopy (see above). This matter will be further discussed below but it is probably desirable to note this situation at once if only to avoid risk of misunderstanding when the enquiry is extended to the next genus.

2. *Calciosolenia aff. murrayi Gran*

The generic characters of *Calciosolenia* can be seen, at a glance, in plate 4 on which two almost intact cells are illustrated beside fragments of others. The cylindrical shape and terminal spines are easily recognized with the light microscope and, though intrinsically less abundant than *Anoplosolenia* (see table 1), having been found in fewer water samples, the cells of *Calciosolenia*

Description of plate 3

Anoplosolenia: transmission and scanning electron micrographs (Temscan, Lancaster).

Figure 8. Morphological details of central ridge on the distal surface of a coccolith from 'Darwin 21'; transmission micrograph Y 8272.22; magn. × 40000.

Figure 9. Scanned surface of the tip of the uppermost coccolith in figure 7 showing rows of tiny perforations; micrograph Y 8303.20; magn. × 20000.

Figure 10. Narrow coccolith from the tip of a broken specimen in sample 'Darwin 11' with perforated membrane visible between the bars except where broken; transmission micrograph Y 8299.4; magn. × 30000.

Figure 11. Coccolith with several broken bars from sample 'Darwin 21'. (*a*) Transmission micrograph Y 7900.17; magn. × 10000. (*b*) Part of one end showing perforated membrane across areas formerly occupied by bars; transmission micrograph Y 8272.15; magn. × 40000.

Figure 12. Pair of coccoliths in proximal view from sample 'A6', tilted 30°, (*a*) scanning micrograph YO 8315.4; magn. × 10000. (*b*) Detail of the exposed surface with perforated membrane visible between bars and with diagonal striations near conjoined edges (white arrows). Transmission electron micrograph Y 8309.7; magn. × 75000.

Description of plate 4

Calciosolenia aff. murrayi Gran

Figure 13. Phase contrast light microscopy (Liverpool) of a cell from sample 'A1' dried on a glass slide. (*a*) Exposure 195.20, dry lens, magn. × 1000. (*b*) The cell under oil, exposure 196.4; magn. × *ca.* 2000.

Figure 14. Cell from sample 'A1' mounted on an e.m. grid. (*a*) Light microscopy (Lancaster), dry lens, exposure 166.6; magn. × 1000. (*b*) Surface scan (Temscan, Lancaster) showing coccoliths *in situ*; micrograph YO 7984.2; magn. × *ca.* 3000.

Figure 15. Edge view of a coccolith showing rim in face view and in silhouette, from sample 'Darwin 13'; transmission electron micrograph Y 8290.22; magn. × 15000.

Figure 16. Coccoliths from a broken periplast in sample 'Darwin 11' showing distal surfaces (left) and proximal surfaces (right); scanning micrograph YO 8314.1; magn. × 10000.

Figure 17. Proximal view of a detached fragment from 'Darwin 11' showing lighter coccolith tips; transmission micrograph Y 7911.6; magn. × 10000.

Figure 18. Proximal surfaces showing broad bar crystallites but without the localized tip transparency seen in figure 17, from the same water sample 'Darwin 11' but a different set of detached coccoliths. (*a*) Transmission electron micrograph Y 7977.23; magn. × 15000. (*b*) More highly magnified part of the same micrograph showing diagonal striations (arrows) overlying broad bar crystallites peripherally; magn. × 40000.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

are more robust; fragments are in consequence fewer but whole cells more frequent than in the other genus. The numbers of terminal spines range from one to four at each end of the cell or some may fall off. Flagella are sometimes present though they are not always recognizable. Thus figure 13*a*, plate 4; taken with a dry lens from a cell dried on glass might have been interpreted as evidence for three spines, one shorter than the others, but the same cell when immersed in Objektol and re-examined with an oil immersion lens (figure 13*b*) shows that only two spines are in fact present, the third object being much thinner and therefore almost certainly a flagellum. We have no specimens with two recognizable flagella still attached, as illustrated by Gaarder & Hasle (1971), though this difference is unlikely to express more than random operational accidents.

The specific identity of our material with *C. murrayi* Gran is less certain, hence the introduction of *aff.* before the specific name in the title. This dilemma arises from the coccolith substructure (figures 17 and 18*a*) in which the rhombic plates carry calcified bars so broad as to overlap each other laterally leaving no spaces between. The taxonomic value of this character is nevertheless uncertain (see below).

The second intact cell illustrated on plate 4 had not been dried on a glass slide but on the support film of a coated e.m. grid. The full width of a grid bar is included in figure 14*a* and on the surface of this, when scanned, the *Calciosolenia* could be seen to terminate centrally. Here, however, it proved to be too much mixed up with detritus to be usefully illustrated, though at least one attached spine could be seen. Elsewhere, as in figure 14*b*, the beautiful regularity of the rhombic coccoliths is arrestingly clear and there is no difficulty in demonstrating directly that each has a long side on the right of the pointed tip when this is directed forwards, with a short side on the left. When inverted, the converse is of course true, as inferred on other evidence in *Anoplosolenia*.

Individual coccoliths in edge view (figure 15) resemble those of *Anoplosolenia* closely, the dual rim being virtually identical. The posterior rim, in silhouette, resembles a narrow knife-edge, while the anterior rim in face view becomes broader towards the centre of the coccolith, exactly as noted previously in the tilted specimen of figure 7. However, if seen flat, the coccolith outline is marginally different from that of *Anoplosolenia*: the width is about the same but the length some 30% less. Each coccolith of *Calciosolenia* is thus more nearly equilateral compared with those of the other genus, average dimensions being *ca.* 4 μm × 1.6 μm for the one and *ca.* 6 μm × 1.5 μm for the other. The overlapped condition of the crystalline bars makes counting more difficult though a rough estimate of 12:8 can be made on the basis of figure 18*a*. The bar crystallites are not only broader but also thinner than the equivalent in *Anoplosolenia* and it is therefore more difficult to detect absence of flatness in the coccolith as a whole. Faint traces of a ridge on one face and a trough on the other can just be made out in the scanned field of figure 16 though some other details are uninterpretable. In particular we have failed to resolve the expected complications on the posterior angle (compared with figure 5*b*, *c*) but this difference could have some technical explanation since at least one published micrograph (Kling 1975, plate I, figure 11) attributed to *C. murrayi* itself seems to contain some equivalent features to those recorded here in plate 2 for *Anoplosolenia*.

The absence of spaces in the coccolith plates of our material of *Calciosolenia* greatly impedes demonstration of unmineralized components. Fortunately this is not wholly impossible. A membrane is undoubtedly present on the proximal face of each coccolith since peripheral striations, closely resembling those of figure 12*b*, are sometimes detectable (figure 18*b*), visibility being partly dependent on a locally favourable angle of shadowing.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Somewhat unexpectedly, transmission micrographs such as that in figure 17, provide evidence suggesting the presence of a second membrane located on the opposite (distal) face of an inverted coccolith. In this particular field, every coccolith appears to be denser centrally than at the ends. Such an appearance, which is not shared by figure 18*a*, is not uncommon and can be found affecting single coccoliths within a group as well as throughout the group as a whole. The crystallites in such specimens are not disturbed and it is even possible to detect the continued presence of peripheral striations overlying the bars at the coccolith ends thereby indicating the presence of an intact posterior membrane. An interpretation of these appearances in terms of a putative second membrane, spread over the distal surface but liable to tear away preferentially from the coccolith tips, is plausible but of course unproved. Such a membrane, if present, would need to be both fragile and patternless. Further information from sections is thus greatly to be desired and these, if positive with respect to this particular feature, might also explain some, though not necessarily all, of the persistent lack of clarity in the signals recorded by scanning. This is, therefore, potentially one of the more unusual and interesting attributes of this remarkable organism.

3. *Comparison with Navisolenia aprilei Lecal ex Leadbeater and Morton*

Though unknown to Black (1968) (see Introduction) it is now possible to compare the coccolithophorids under discussion with a recently discovered wholly unmineralized organism, able to provide some, if still cryptic, phyletic clues. The organism is *Navisolenia aprilei* Lecal, collected first in surface water off the coast of Israel (Lecal 1965) and found again by B. S. C. Leadbeater in 1971 at various depths from the surface to 10 m in shallow, turbid water in the Adriatic Sea near Split (personal communication, amplifying Leadbeater & Morton (1973)). No other collections are known and nobody as yet has been in a position to study living cells directly. However, field preparations set up by Dr Leadbeater in a manner similar to our own, provided a basis for some excellent transmission electron microscopy, permitting a formal description of the genus and species to be supplied for the first time (Leadbeater & Morton 1973). The diagrams accompanying this description are reproduced again here as figure 20 from which the relevance to *Calciosolenia* will be self evident.

Both the extreme cell shape (figure 20*a*) and its covering with rhombic scales, recall *Calciosolenia* strongly without being identical. Thus spines are absent and the degree of tapering at both ends of the body is slightly different. The rhombic scales also are in some respects simpler than the equivalent coccoliths: they are more nearly equilateral and the rim is consistently more uniform. Indeed it is not difficult to represent the latter by means of the diagram compiled by Halldal & Markali (1955) for *Anoplosolenia* merely by painting out crystallite edges which no longer apply and correcting the left–right reversal that we now know the original diagram to have contained. The rim in such a redesigned diagram (figure 19), shaped like a ribbon placed on edge, accurately represents the equivalent in *Navisolenia* although we now know the former analysis of *Anoplosolenia* in this respect to have been incomplete.

A more surprising resemblance can be found in the under-scale patterning which, as indicated in figure 20*d* is striated peripherally in a manner closely recalling that on the proximal membranes of both *Anoplosolenia* (figure 12*b*) and *Calciosolenia* (figure 18*b*). The tilt in the peripheral striations is described by Leadbeater & Morton (1973) as 'directed towards the short axis of the scale'. This phrase would have been equally appropriate to describe the peripheral striations in both coccolithophorids.

Not unnaturally, there are a few discrepancies, quite apart from the presence or absence of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

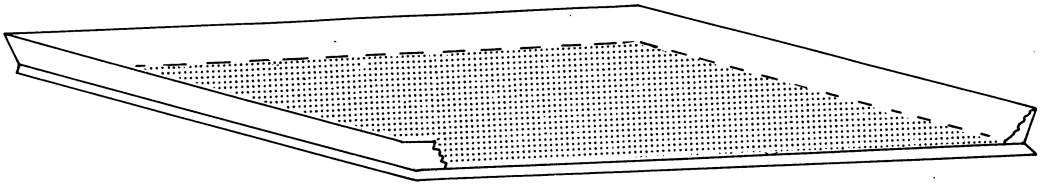


FIGURE 19. Diagram based on Halldal & Markali (1955), originally intended for *Anoplosolenia* but inaccurate for that taxon, now approximately correct for *Navisolenia* after removal of outlines of crystallites and of the left–right reversal unconsciously introduced into the original diagram (loc. cit figure 5, plate XVI).

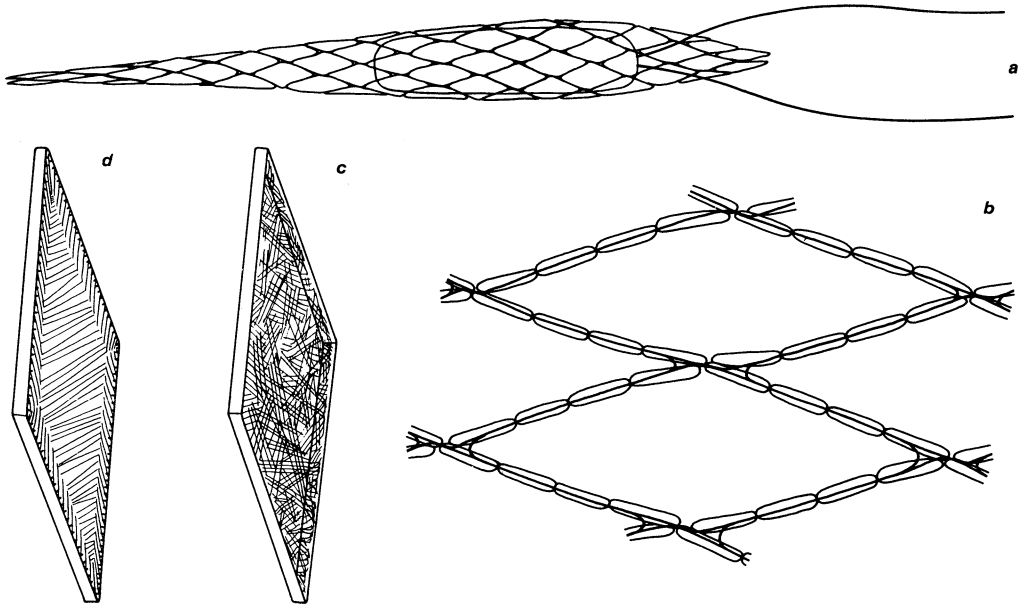


FIGURE 20. *Navisolenia aprilei* Lecal. Drawings reproduced without change from Leadbeater & Morton (1975, figure 1), showing a complete cell at *a*, with more magnified details of underlayer scales in position at *b* and surface marking on the two faces of the ordinary plate-scales at *c* and *d*.

calcite. Thus underlayer scales of a characteristic and unusual kind occur in *Navisolenia*, where they are arranged in a single file beneath the conjoined edges of the rhombic plates (see figure 20*b*). In contrast, no underlayer scales have as yet been detectable in either of the coccolithophorids though had they occurred in a manner similar to that in *Navisolenia*, they could scarcely have been missed.

A second discrepancy is more apparent than real, being photographic in origin. Transmission electron micrographs in Leadbeater & Morton (1973), initially recorded by standard procedures, were subsequently prepared for publication in two different ways. Some, as in their plate 1, are direct prints, exactly comparable in this respect to those of Lecal (1965). In all of these the background is light and the orientation doubtless correct. However, the more highly magnified micrographs assembled in plate 2 of Leadbeater & Morton (1973) are 'reversed prints' (B. S. C. Leadbeater, personal communication) in which the backgrounds have become dark and objects themselves light. This well-known device for increasing the clarity of fine detail involves an intermediate transparency which, if printed in the normal manner, will also introduce left–right reversal. This effect can of course be avoided by printing the intermediate

Downloaded from rstb.royalsocietypublishing.org

transparency through the back though such a procedure is by no means always carried out, unless required by special circumstances, since the risk of a less than perfect final print is a real one. The existence of special circumstances, involving direction, that might become important in the future was of course unknown in 1973. If the necessary corrections are now applied, the suspected anomaly disappears and we are left with clear evidence that scale arrangement in *Navisolenia* is exactly the same directionally as coccolith arrangement in *Calciosolenia* and *Anoplosolenia*. This fact now represents an important addition to the known number of shared characters.

Discussion

Had *Navisolenia aprilei* been a fictitious organism, arbitrarily endowed with supposedly ancestral characters, a description such as that given in the previous section might have been dismissed as perverse exaggeration. As it is, this factual comparison, based on impeccable and independently acquired information has become central to the whole investigation. The morphological characters involved are so peculiar and so extreme that parallel evolution seems highly unlikely or even impossible. A phyletic interpretation linking *Anoplosolenia*, *Calciosolenia* and *Navisolenia* together in some meaningful way would be a highly attractive alternative if only it could be more easily introduced into the taxonomic system.

Further progress in descriptive understanding requires new observations to be based on one or more additional lines of approach. Thus sections, if and when obtained, should clarify the presence or absence of underlayer scales beneath the coccoliths of *Anoplosolenia* and *Calciosolenia* and at the same time should confirm or refute the suggested second membrane postulated on the basis of figure 17 as present on the coccoliths of the latter. In addition, observations on living cells, not as yet carried out on any of these taxa would be even more important on topics such as ecology, motility and life history.

The ecological information we possess at present is virtually limited to the temperatures and depths at which the various samples listed in table 1 were collected. The absence of intact cells of either *Anoplosolenia* or *Calciosolenia* from surface water adds apparent significance to the presence of living specimens of both genera in deeper water, often at or near the bottom of a shallow sea. *Calciosolenia* in the Carribean is said to be similar (Kling 1975). *Navisolenia*, in contrast, was at first reported from 'surface water' off the coast of Israel (Lecal 1965) but when collected personally by B. S. C. Leadbeater in 1971 it was in fact found alive from 0.3 to 10 m under lagoon conditions in shallow turbid water near Split in the Adriatic Sea, and is thus likely to be similar in its ecological requirements to the two coccolithophorids. It may indeed be plausibly suggested that the cigar shape (or equivalent) characteristic of all three taxa could be an ecologically significant adaptation to this kind of habitat by contributing to an optimal orientation of lateral chloroplasts to light. This might be a substantial aid to survival, especially in turbid water.

Locomotion is not a topic that has been studied directly in any of these organisms and we do not as yet know whether swimming in any one of them is habitual or intermittent. The rarity with which flagella have been encountered in the two coccolithophorids may mean no more than fragility during the preparative treatment. Some swimming must of course occur, if only occasionally, to counteract sinking and in this a positive contribution towards ease of swimming is perhaps attributable to the exceptionally smooth outline accompanying the cylindrical cell shape in all three taxa. In many planktonic organisms, a prevailing surface roughness,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

sometimes involving lateral excrescences or even spines, is commonly attributed (see Hardy 1956) to the value of frictional resistance as a means of retarding sinking. The converse must nevertheless obtain in *Calciosolenia* and near relatives in which even roughness caused by overlapping plate edges has been virtually eliminated by the prevalence of straight-sided contacts between adjacent coccoliths (or scales) which are also shaped in such a way as to cover the whole surface of a narrow cylinder without leaving spaces. Moreover their rhombic outlines provide 'streamlining' in a literal sense of the word, since transverse obstructions to surface flow are absent and only obliquely longitudinal ridges remain (figure 14*b*). These may also assist swimming directly if, as is probable, this involves rotation around the longitudinal axis of the cell as a whole. The adaptive significance of these very different facets of external morphology are thus likely to be both more complex and more varied than might at first have been recognizable.

Life histories, including the intricacies of growth and division of cells with periplasts as complex as these, are also as yet unrecorded and they will remain so unless either cultures or unmistakable transitional conditions can be obtained. Enough is nevertheless known about other taxa in culture (including *Emiliania huxleyi*(Lohm.) Hay & Mohler and *Hymenomonas carterae* (Braarud & Fagerland) Braarud) to permit informed comment to be offered against speculations that have already appeared in the literature. Thus a highly improbable speculation, first suggested by Lecal (1965), would treat *Navisolenia* as a juvenile condition of some unknown *Calciosolenia* relative, to which calcite would be added later as the cell matured. We cannot however justifiably presuppose anything so unusual as the gradual calcification, outside the cell, of previously unmineralized scales. There is abundant evidence that so called hetero-coccoliths are formed and fully calcified inside the protoplast within the cisternae of the Golgi system and that no changes in calcite, except perhaps dissolution, take place after liberation, in which case empty organic matrices can sometimes be found. There are no known examples of the converse except with respect to quite different structures (Green & Course (1983) on *Chrysotila*) or under totally different circumstances (B. S. C. Leadbeater and J. Rowson, personal communication). *Navisolenia* cannot therefore be a juvenile condition of any *Calciosolenia* on any evidence at present available.

It is less easy to discount an alternative speculation, namely that *Navisolenia aprilei* might not be a true species but only a self-replicating stage in the life history of a different organism in which other self-replicating stages would resemble *Calciosolenia* sp. The basis of such an idea is of course the morphological resemblance of *Navisolenia* to the latter, as noted in the previous section. This is nevertheless entirely insufficient reason for the inference drawn. It is true that unmineralized self-replicating stages as parts of a complex life history are known in several coccolithophorids (including *Emiliania*, *Hymenomonas* and others established in culture) but in each instance such cells are quite unlike coccolithophorids in shape, motility (or its absence) and periplast characters. Such individuals if encountered in Nature could be (and often have been) assigned to quite different genera, either as species of *Chrysochromulina*, *Apistonema*, or others as the case may be. A morphological resemblance between organic scales and coccoliths cannot therefore be accepted as in any way denoting a shared life history.

Without more positive evidence than anything as yet ascertained, a decision as to whether *Navisolenia aprilei* is or is not an independent species in its own right cannot be reached. It must of course be treated as such, at least for the time being. We can also agree that it is highly specialized in relation to some aspect of the environment shared by *Anoplosolenia* and *Calciosolenia*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

and that, in this, it is slightly less advanced in certain details other than mere absence of calcite. Thus the scales of *Navisolenia*, though rhomboid, are more nearly equilateral and their rims more uniform than in either of the equivalent coccoliths. Moreover, the flat bases, complete with oblique peripheral streaks add further characters which seem unlikely to be either directly adaptive or attainable by a reduction process from coccoliths. The only plausible interpretation is phyletic, in terms of some ancestral condition antedating mineralization but retained in modern times either in a rare relict species or by recapitulation as part of an unusual life history. A choice between these two alternatives, and there seem to be no others, must await further evidence.

Apart from these major problems of taxonomy or ontogeny, or both, a few other matters relating only to coccoliths should perhaps receive brief further attention. Thus the specific identity of *Calciosolenia* as represented in the Galapagos Islands was left uncertain because of one unfamiliar morphological detail, namely the width of the bar crystallites and consequent absence of spaces between them. In the early electron micrographs attributed to *C. sinuosa* Schlauder by Halldal & Markali (1955), lateral spaces between the bar crystallites are as conspicuous as in *Anoplosolenia*. They seem slightly narrower in both these taxa as illustrated by Borsetti & Cati (1972), but Gaarder & Hasle (1971), in reducing *C. sinuosa* to a synonym of *C. murrayi* Gran, do not clearly illustrate this particular feature at all, and it is neither mentioned nor illustrated by Heimdal & Gaarder (1981 p. 44) who merely repeat the citation of synonyms. However some further insight is provided by Nishida (1979), in spite of confused terminology in which *Anoplosolenia* is the only generic name introduced to cover at least two specimens of *Calciosolenia* type, complete with terminal spines (Nishida 1979, plate 14, figures 1*a*, 2*a*). Among the detached coccoliths, illustrated to show the morphological range encountered in the Pacific, *Anoplosolenia brasiliensis* is indeed represented (Nishida 1979, plate 14, figures 1*b*, *c*) but so also are *Calciosolenia*-type coccoliths with exceptionally broad bars. Some of the latter (Nishida 1979, figure 2*c*) are substantially wider and fewer than those shown in our own plate 4 though they overlap laterally and terminally in a similar manner. We can express no opinions on their specific identity (other than dissent from use of the name *Anoplosolenia*) but a necessary conclusion seems to be that more taxa exist in this group, at least in the Pacific Ocean, than have as yet been adequately defined. A decision as to which, if any, of these forms correctly represents the type species of *Calciosolenia* itself is thus less well established than is sometimes supposed. The need for caution in the use of the specific name *C. murrayi* Gran is an obvious consequence of these findings.

Lastly, the special terminology introduced first by Halldal & Markali (1955) on the basis of transmission microscopy only, has perhaps, in this case, outlived its usefulness. After the subsequent introduction of scanning, it had not been foreseen that so much about the basic morphology of these particular coccoliths could have remained unnoticed for so long. In particular no attempts seem to have been made to ascertain the facts for the top and bottom surfaces for their own sake and though the proximal (under) face has in fact been illustrated three times (unconsciously by Halldal & Markali (1955) in *Anoplosolenia*, and incidentally by scanning in Black (1968) and Kling (1975)), no use seems to have been made of these views for morphological interpretation. On the other hand, everything that we have now been able to add about gross morphology (plates 2 and 3 above) has greatly reduced the supposed morphological resemblance of these coccoliths to boats. In consequence the term scapholith as originally defined is scarcely applicable to the taxa under discussion and the fact that it has

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

already been discarded by several authors as already listed (Black 1968; Leadbeater & Morton 1973) is shown to have been a wise precaution. The words 'rhomboid', 'rhombic' or 'parallelogram' carry no unintended overtones liable to mislead and had this practice been more general, one incipient mistake would already have been avoided. Thus an imperfectly known new taxon introduced by Okada & McIntyre (1977), p. 18 as '*Calciosolenia ? bimurata* sp.nov.' seems unlikely to have been attributed even tentatively to *Calciosolenia* had its coccoliths not been described as of 'scapholith type'. The coccoliths in this new form may indeed be boat-shaped but they are not parallelograms. Conversely, rhombic coccoliths exist elsewhere, notably in the circumflagellar region in *Michaelsarsia/Halopappus* (see Manton *et al.* 1984) though in this position they are not normally referred to as scapholiths. For all these reasons it seems to us highly desirable that this word should be either used consistently after re-defining to exclude, or to be limited to, coccoliths of rhombic outline or else, and preferably, be abandoned.

Finally, attention should perhaps be re-directed to the many insidious ways in which left–right reversal can falsify results, even in the hands of skilled and careful workers, unless informed precautions are taken and fuller photographic information provided, as explained above. Greater awareness of this particular hazard is perhaps one of the more important results of the present enquiry.

Conclusions

Removal of errors, especially the unsuspected introduction of left–right reversal in the literature on several different taxa, together with amplified descriptions of the essential coccolith morphology in *Anoplosolenia* and *Calciosolenia* have greatly increased the degree of resemblance between both of these genera and the wholly uncalcified flagellate *Navisolenia*. A phyletic interpretation seems unavoidable and this, as foreseen, provides a potential breakthrough in relation to geological and taxonomic problems summarized by Black (1968) as noted in the Introduction. Developmental information is nevertheless still needed before the concept of Calciosoleniaceae can be further clarified and this remarkable family unequivocally defined.

Financial support from grants towards the cost of visiting the Galapagos Islands, made by the Royal Society and Percy Sladen Trust, are gratefully acknowledged as is the permission from the Ecuador authorities for use of the Charles Darwin Research Station on Santa Cruz Island for two weeks in August 1977. Personal collaborators in the field include Miss Joan Sutherland, Dr Margaret McCully, A. D. Greenwood and Mrs Greenwood while help during the long period involved in working out the results must be acknowledged to all those owners and other users of equipment listed in Material and methods, more especially Professor E. C. Cocking of Nottingham and Dr Stanley Walker of Liverpool. Graham Bremer of Portsmouth has kindly given permission for one of the scanning micrographs taken by him to be reproduced here as figure 2*c*. Finally, Dr B. S. C. Leadbeater of Birmingham has been immensely helpful in supplying not only information on *Navisolenia* but also in many other ways during preparation of the manuscript.

References

Bernard, F. 1939 Recherches sur les coccolithophoridés I. Principales espèces du plancton à Monaco. *Bull. Inst. Oceanograph.* **767**, 1–19.

Black, M. 1968 Taxonomic problems in the study of coccoliths. *Palaeontology* **11**, 793–813.

Borsetti, A. M. & Cati, F. 1972 Il nannoplancton calcareo vivente nel Tirreno Centro-meridionale. *Giorn. Geol. Ser. 2a*, **38**, 394–452.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Gaarder, K. R. & Hasle, G. R. 1971 Coccolithophorids of the Gulf of Mexico. *Bull. mar. Sci. Gulf Caribb.* **21**, 519–544.

Grassé, P. 1952 *Traité de Zoologie*, vol. 1. Paris: Masson et Cie.

Green, J. C. & Course, P. A. 1983 Extracellular calcification in *Chrysotila lamellosa* (Prymnesiophyceae). *Br. Phycol. J.* **18**, 367–382.

Halldal, B. R. & Markali, J. 1955 Electron microscope studies on coccolithophorids from the Norwegian Sea, the Gulf Stream and the Mediterranean. *Avh. norske Vidensk. Akad., Oslo* **1**, 1–30.

Heimdal, B. R. & Gaarder, K. R. 1981 Coccoliths from the northern part of the eastern central Atlantic II. Heterococcolithophorids. '*Meteor*' *Forsch. Ergebn.* **33**, 40–44.

Kamptner, E. 1941 Die Coccolithineen der Sudwestküste von Istrien. *Ann. nat. hist. Mus. Vienna.* **51**, plate XII.

Kling, S. A. 1975 A lagoonal coccolithophore flora from Belize (British Honduras) *Micropaleontology* **21**, 1–13.

Leadbeater, B. S. C. & Morton, C. 1973 Ultrastructural Observations on the external Morphology of some Members of the Haptophyceae from the Coast of Jugoslavia. *Nova Hedwigia* **24**, 207–233.

Lecal, J. 1965 *Navisolenia* N.G. *aprili* N.Sp. *Bull. d'Hist. Nat. Toulouse* **100**, 422–426.

Manton, I., Bremer, G. & Oates, K. 1984 Nanoplankton from the Galapagos Islands: *Michaelsarsia elegans* Gran and *Halopappus adriaticus* Schiller (coccolithophorids), with special reference to coccoliths and their unmineralized components. *Phil. Trans. R. Soc. Lond.* B **305**, 183–199.

Manton, I., Sutherland, J. & Oates, K. 1980 A reinvestigation of collared flagellates in the genus *Bicosta* Leadbeater with special reference to correlations with climate. *Phil. Trans R. Soc. Lond.* B **290**, 431–447.

Manton, I. & Oates, K. 1983 Nanoplankton from the Galapagos Islands: two genera of spectacular coccolithophorids (*Ophiaster* and *Calciopappus*), with special emphasis on unmineralized components. *Phil Trans. R. Soc. Lond.* B **300**, 435–462.

Murray, G. & Hjort, J. 1912 The Depths of the Ocean. Macmillan, London.

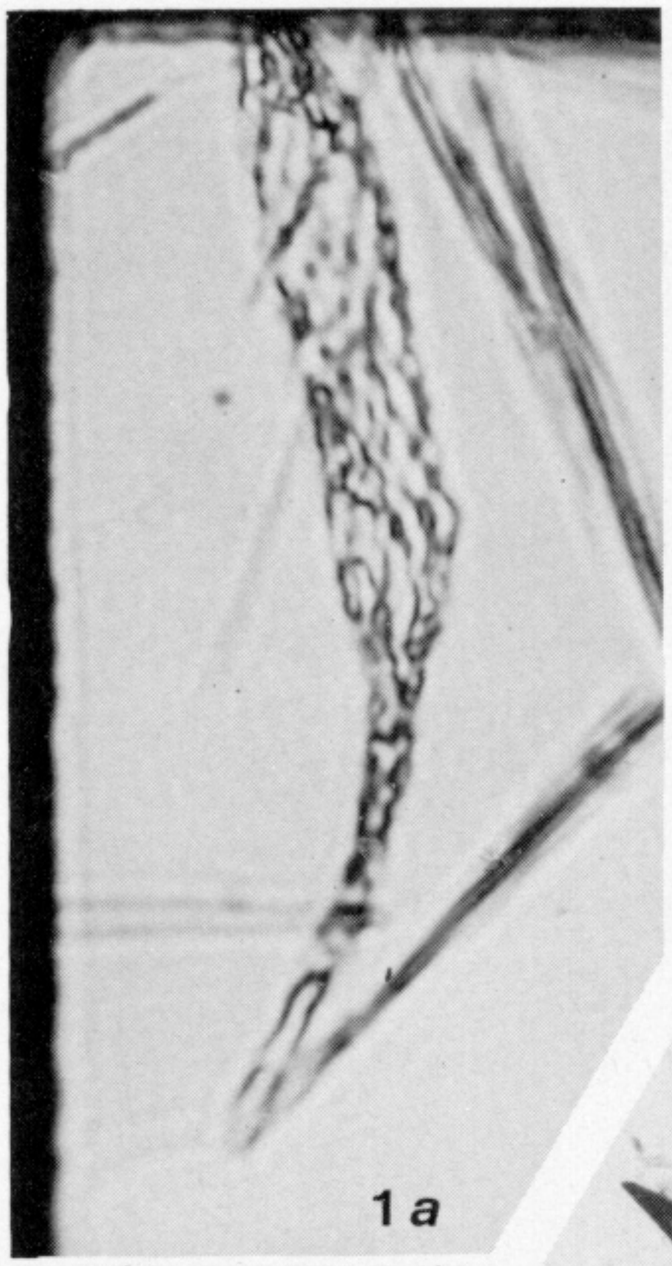
Nishida, S. 1979 Atlas of Pacific nanoplankton. *News Osaka Micropaleont.*, special paper 3, plate 14.

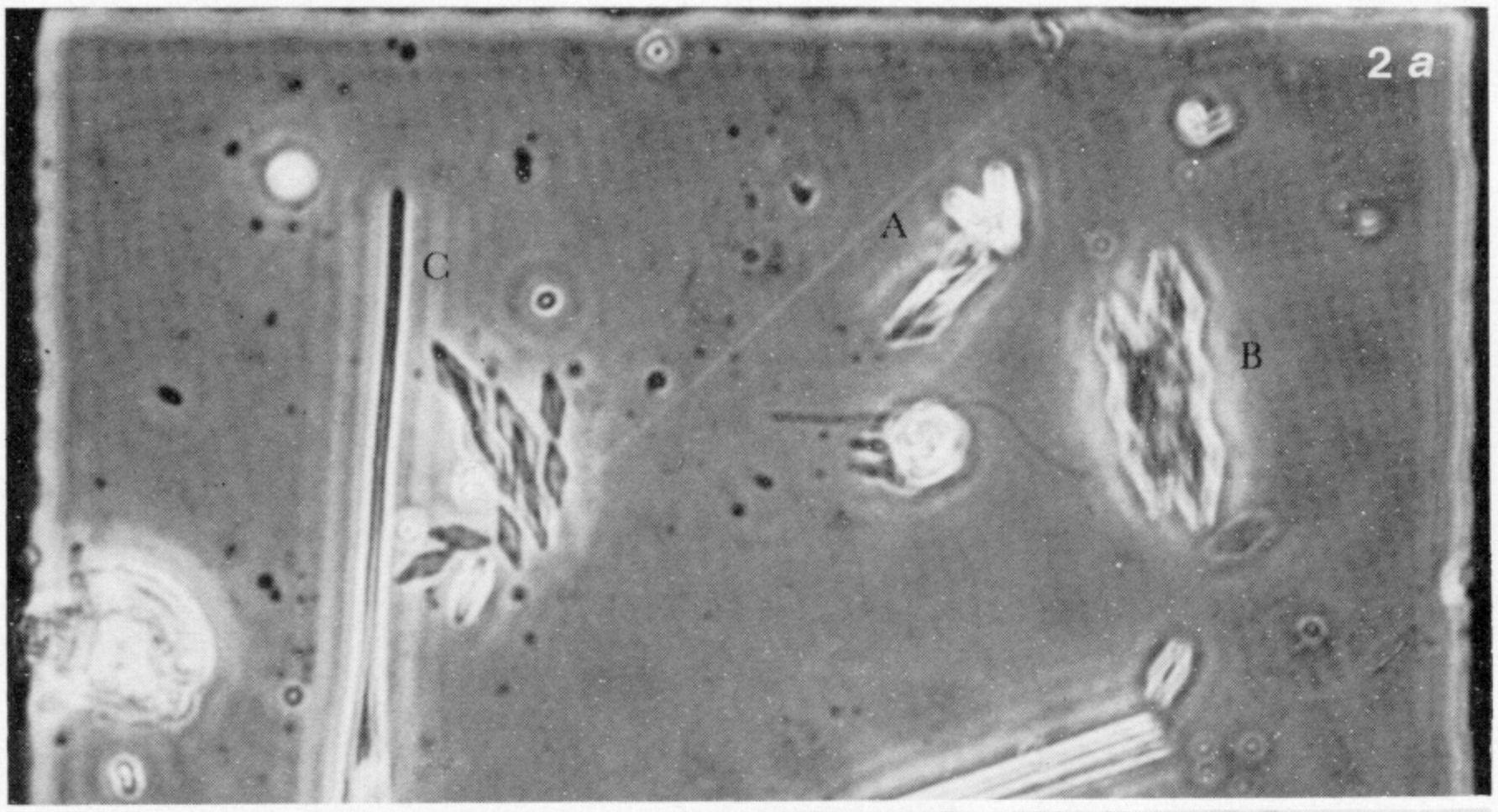
Okada, H. & McIntyre, A. 1977 Modern coccolithophores of the Pacific and North Atlantic Oceans. *Micropaleontology* **23**, 1–55.

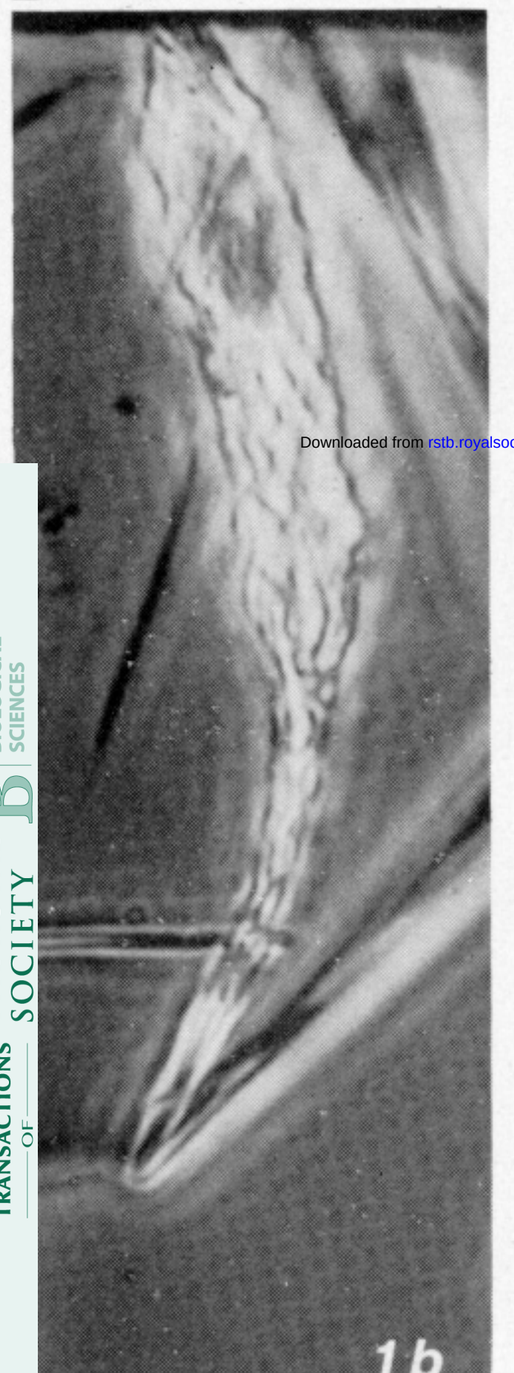
Schlauder, J. 1945 Recherches sur les flagellés calcaires de la Baie d'Alger. Univ. d'Alger Dipl. Fac. Sci.

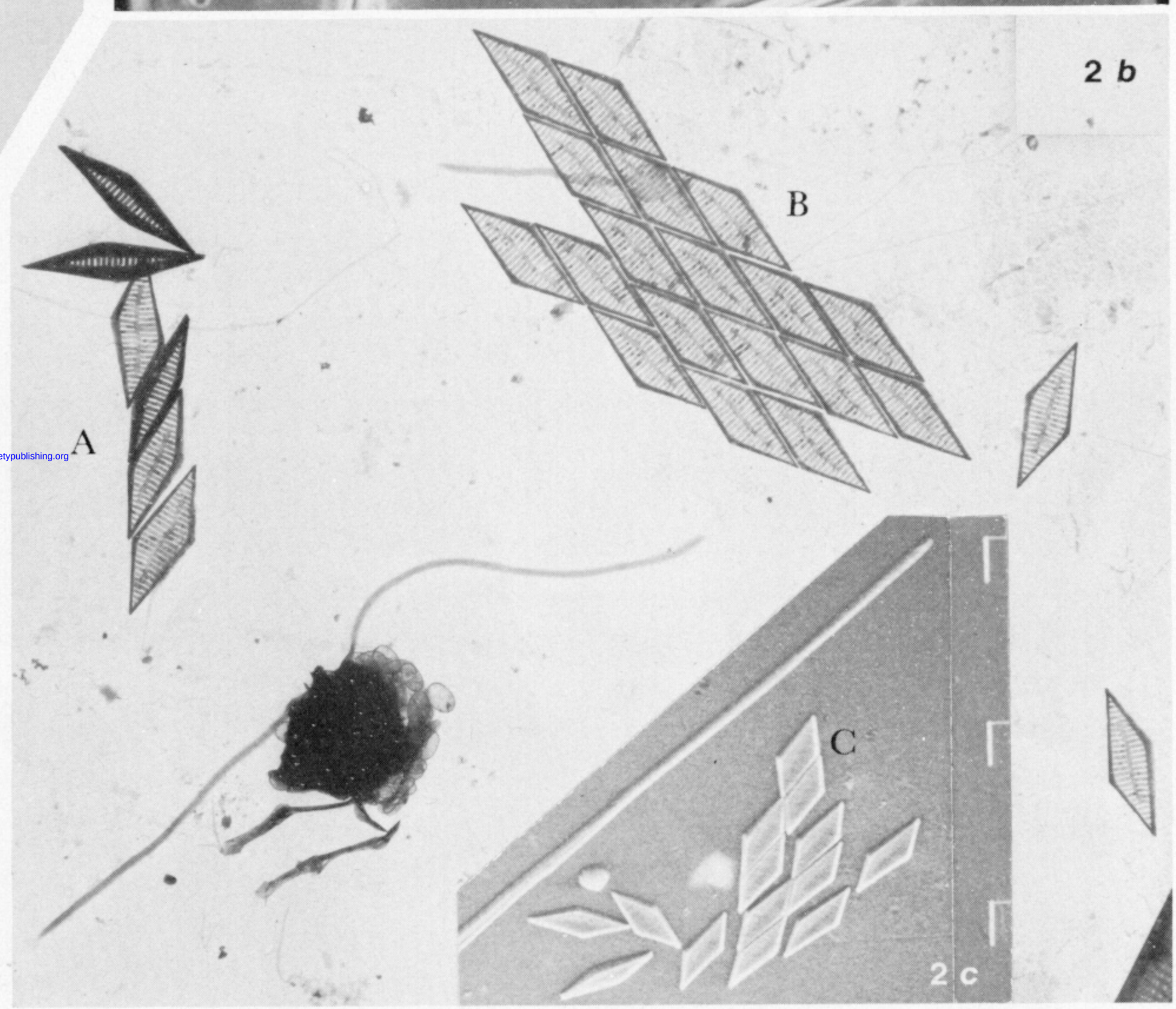
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

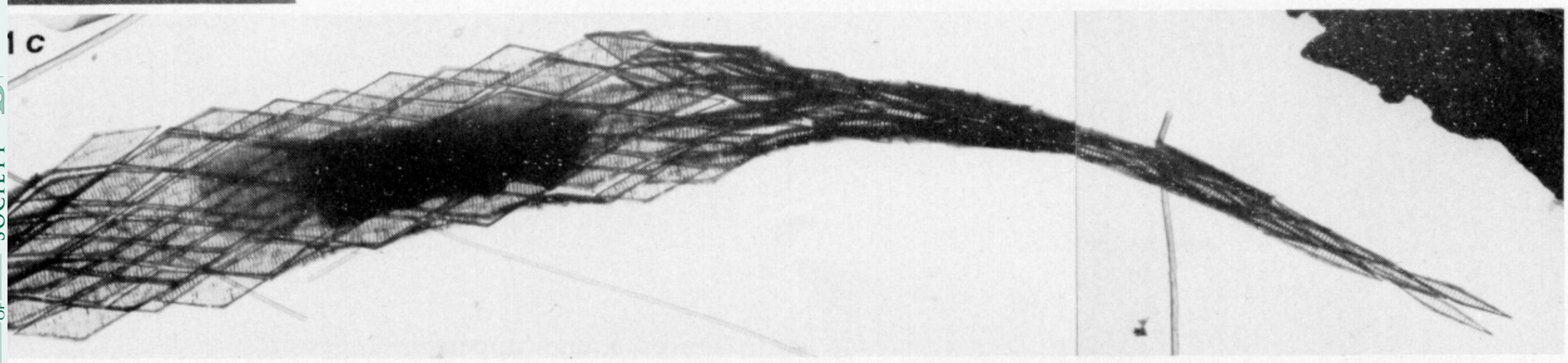
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES







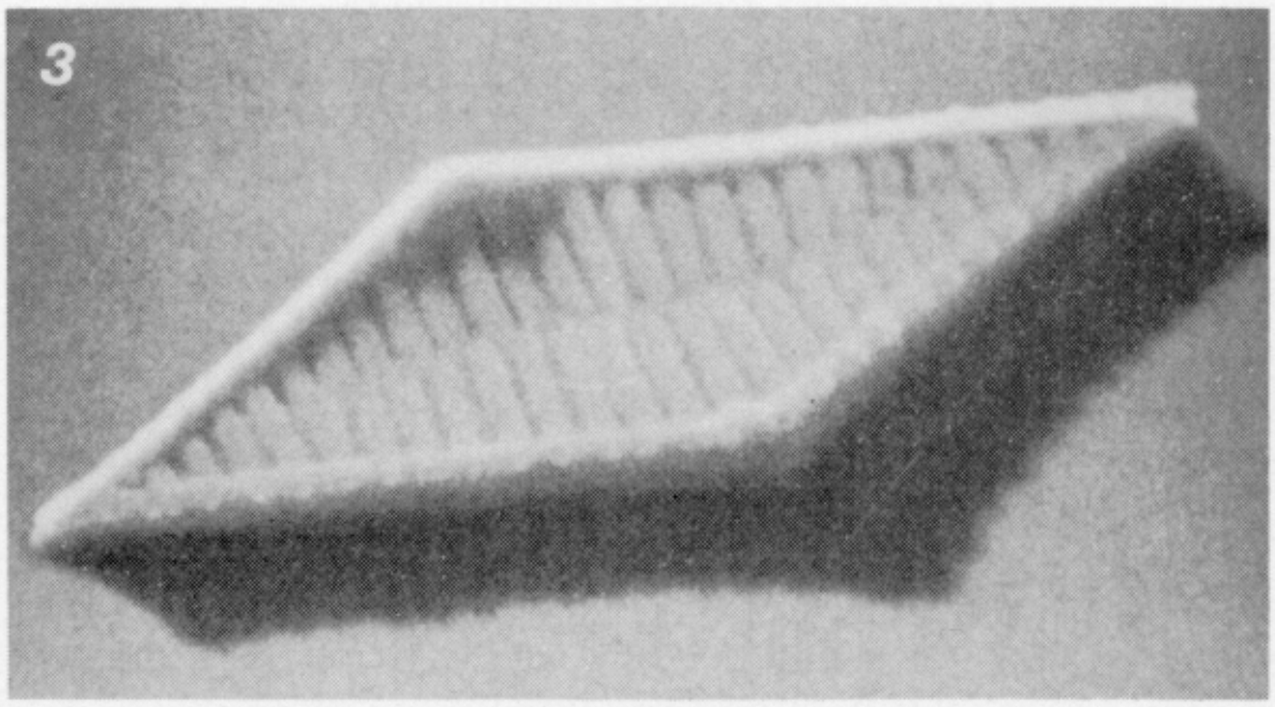


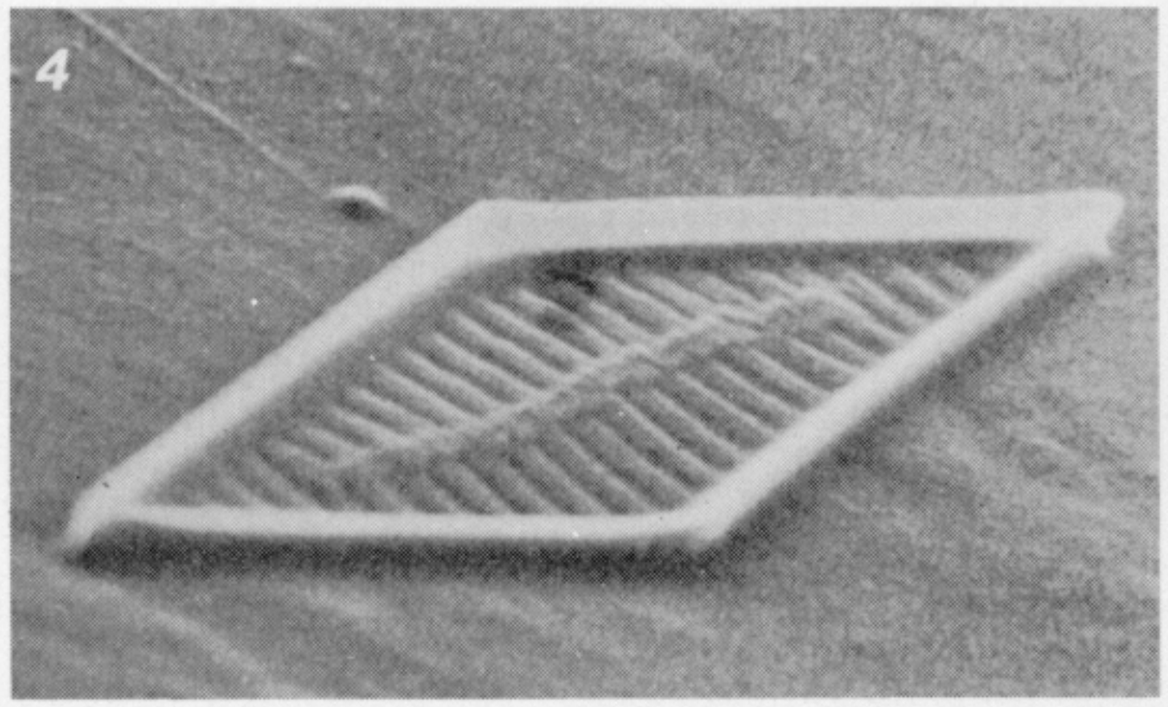


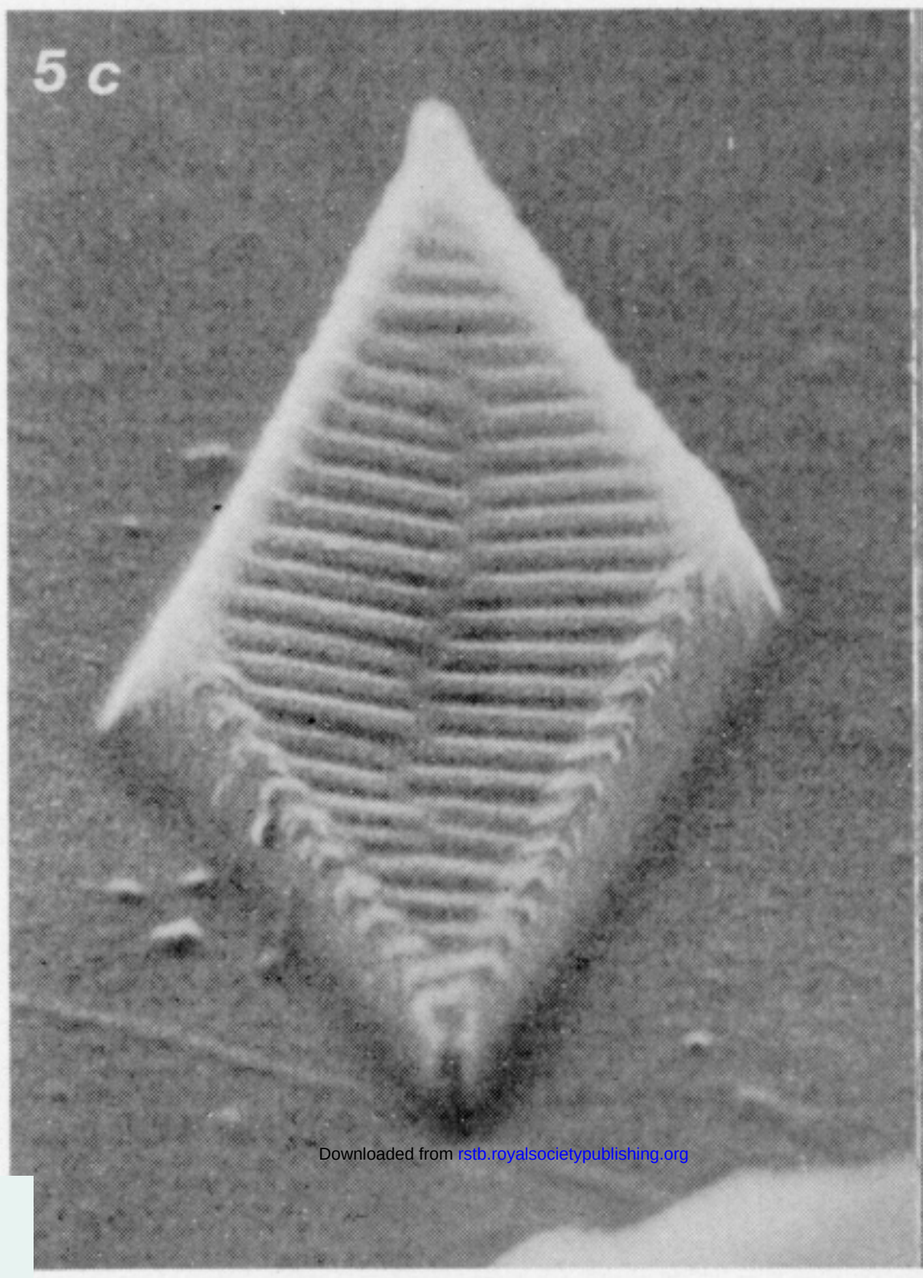
FIGURES 1 AND 2. For description see opposite.

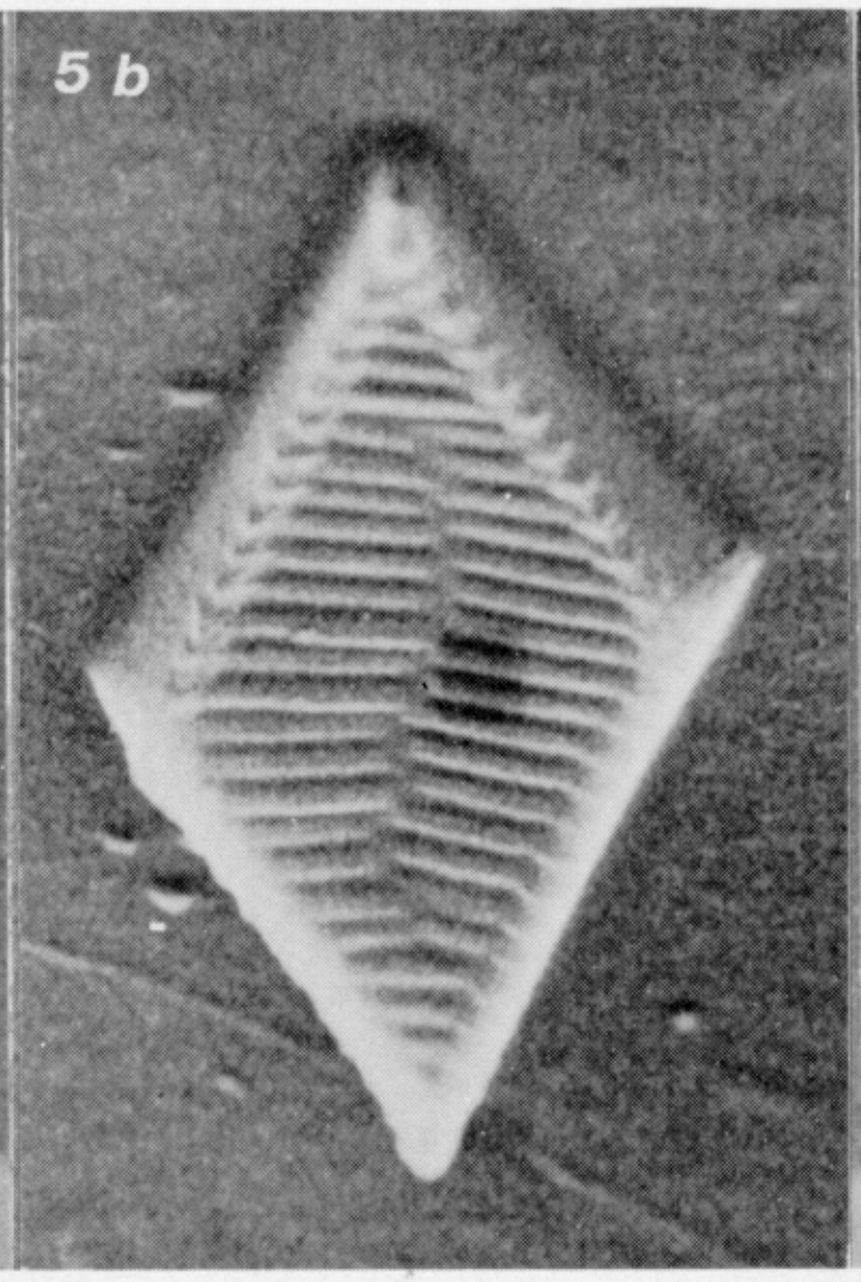
Downloaded from rstb.royalsocietypublishing.org

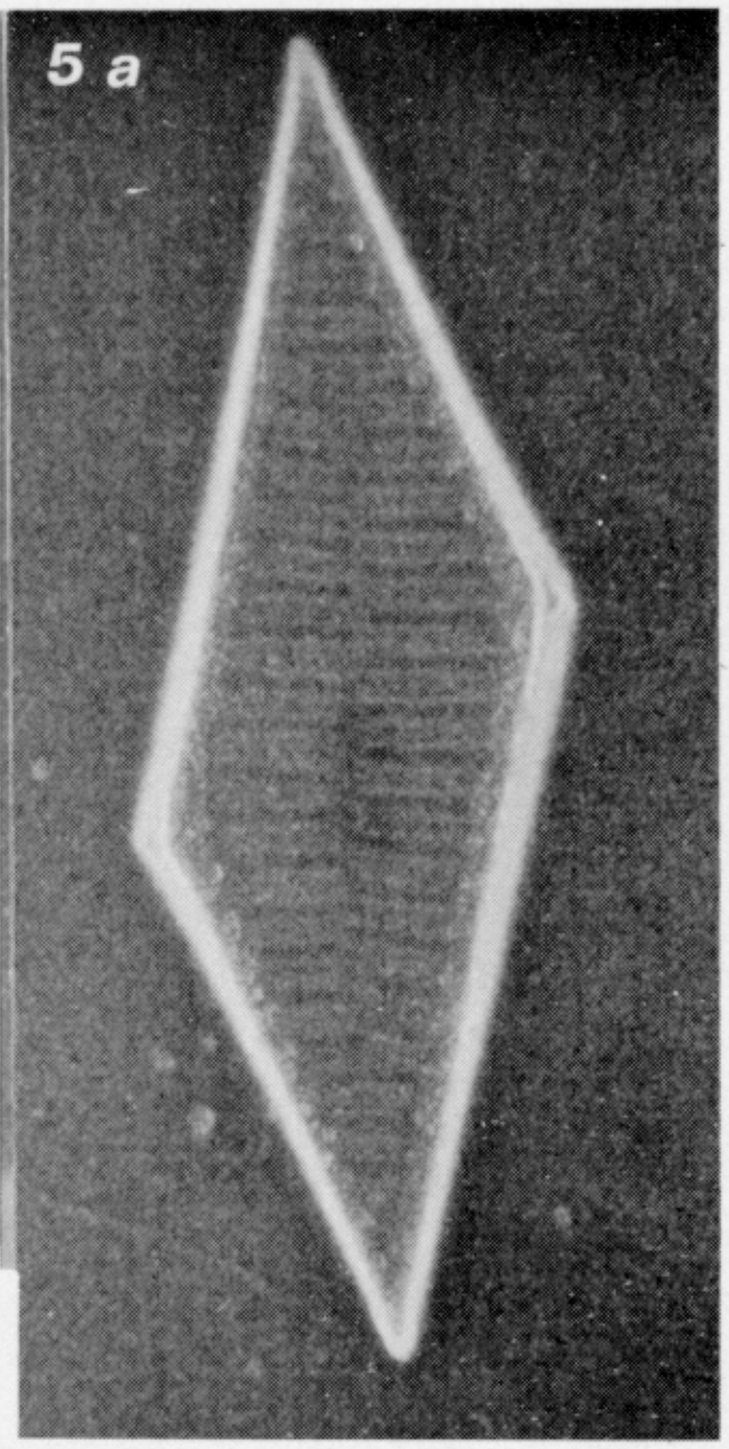
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



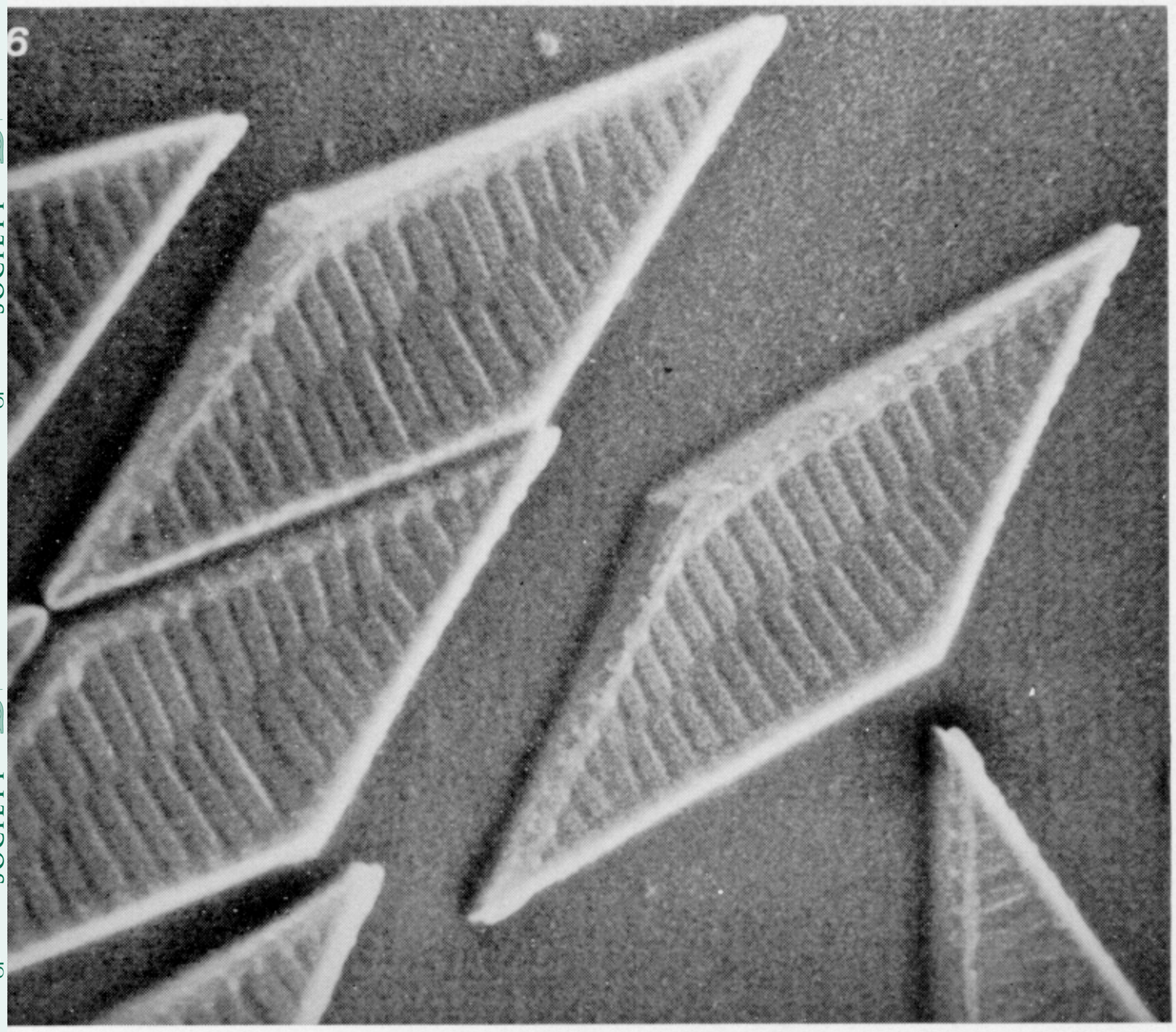


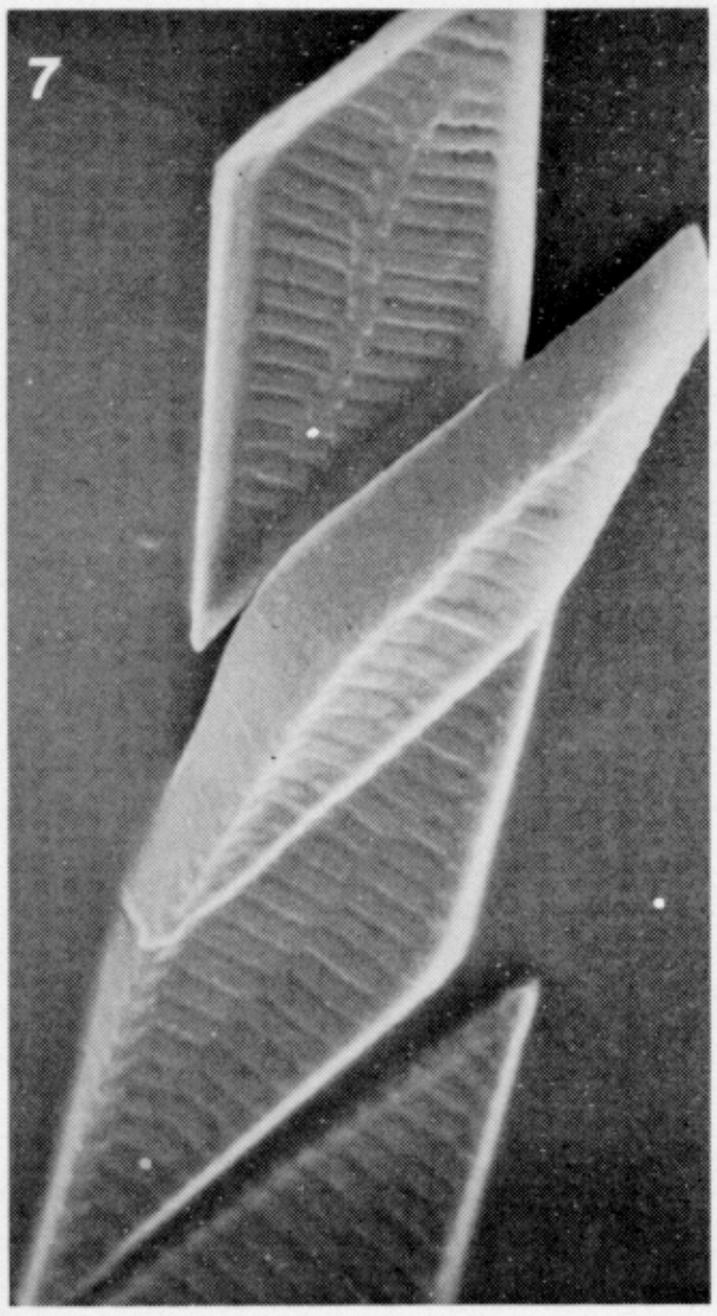






Downloaded from rstb.royalsocietypublishing.org

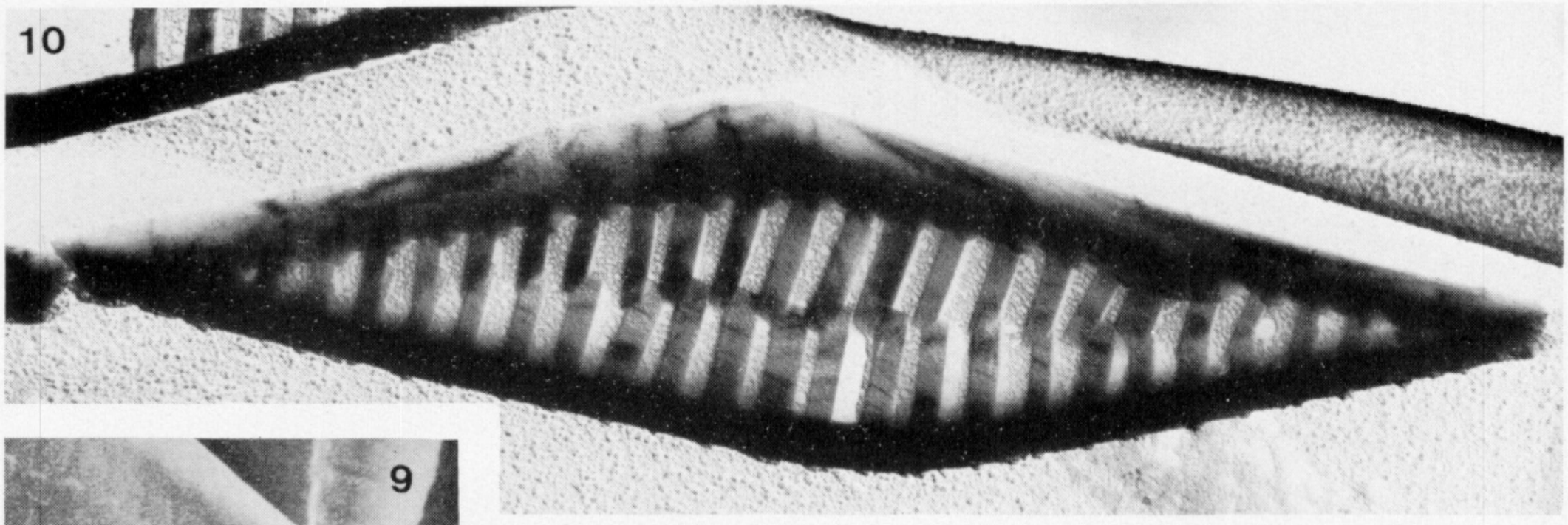


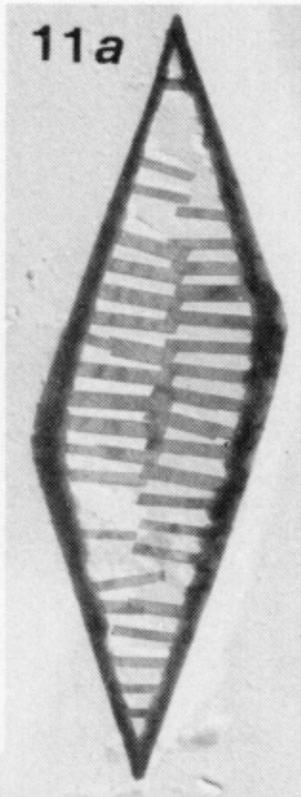


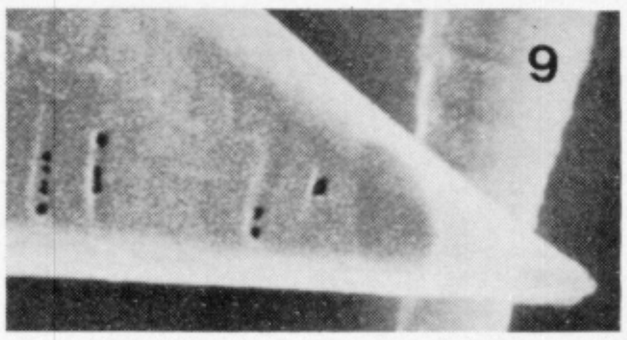
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

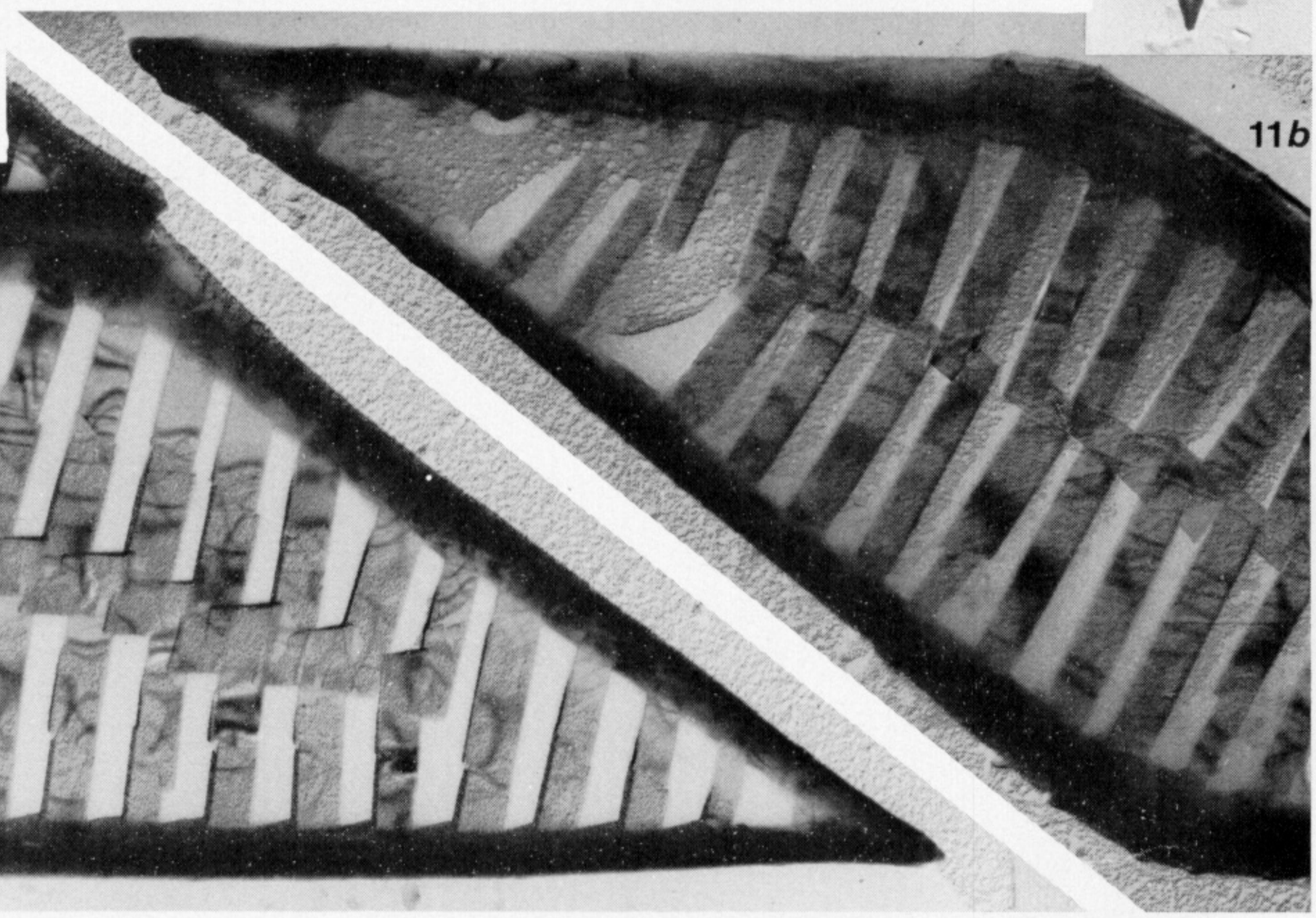
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURES 3–7. For description see p. 468.

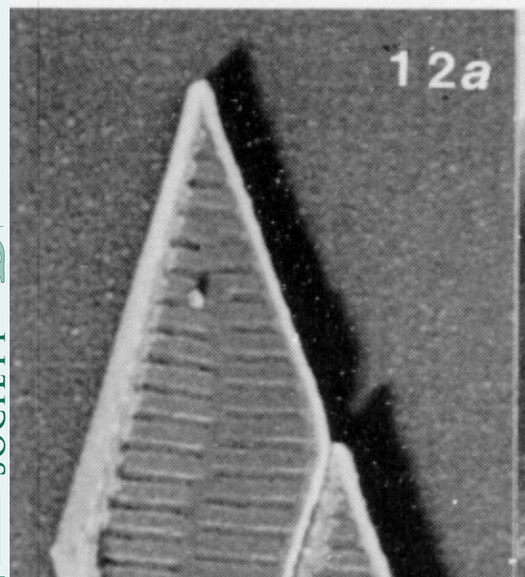


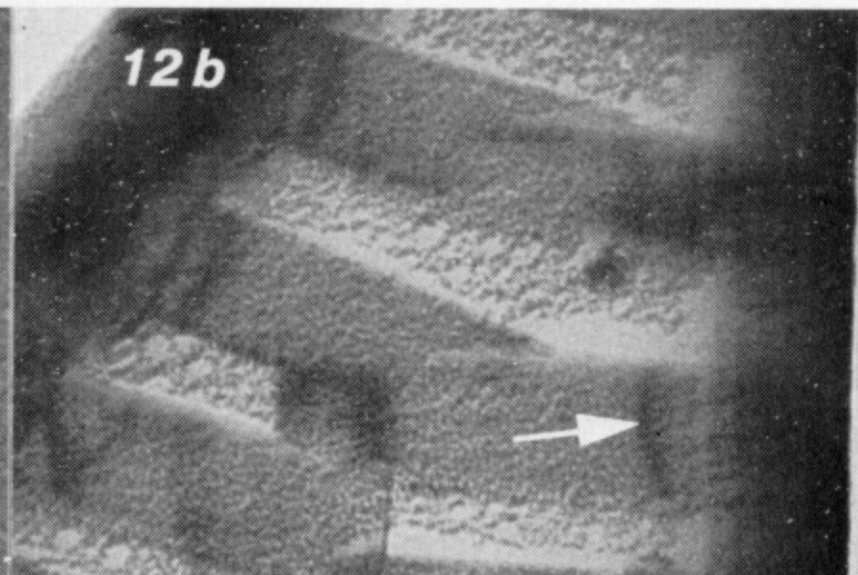






Downloaded from rstb.royalsocietypublishing.org

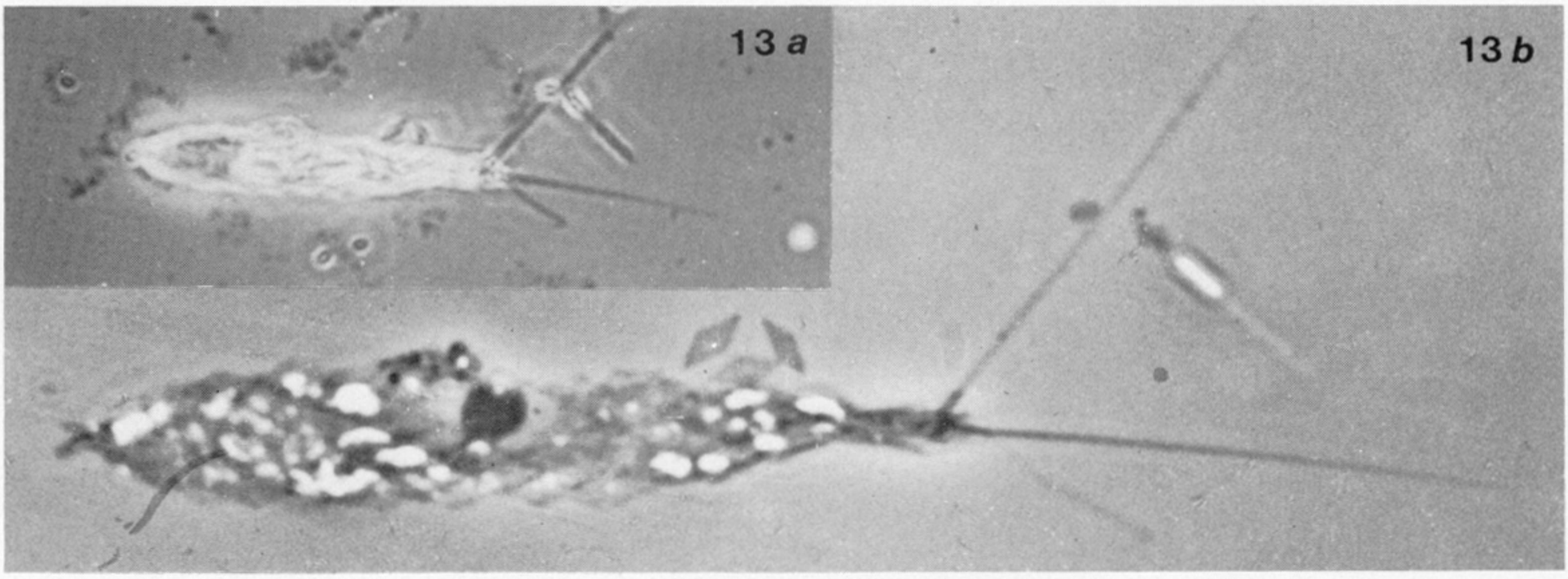


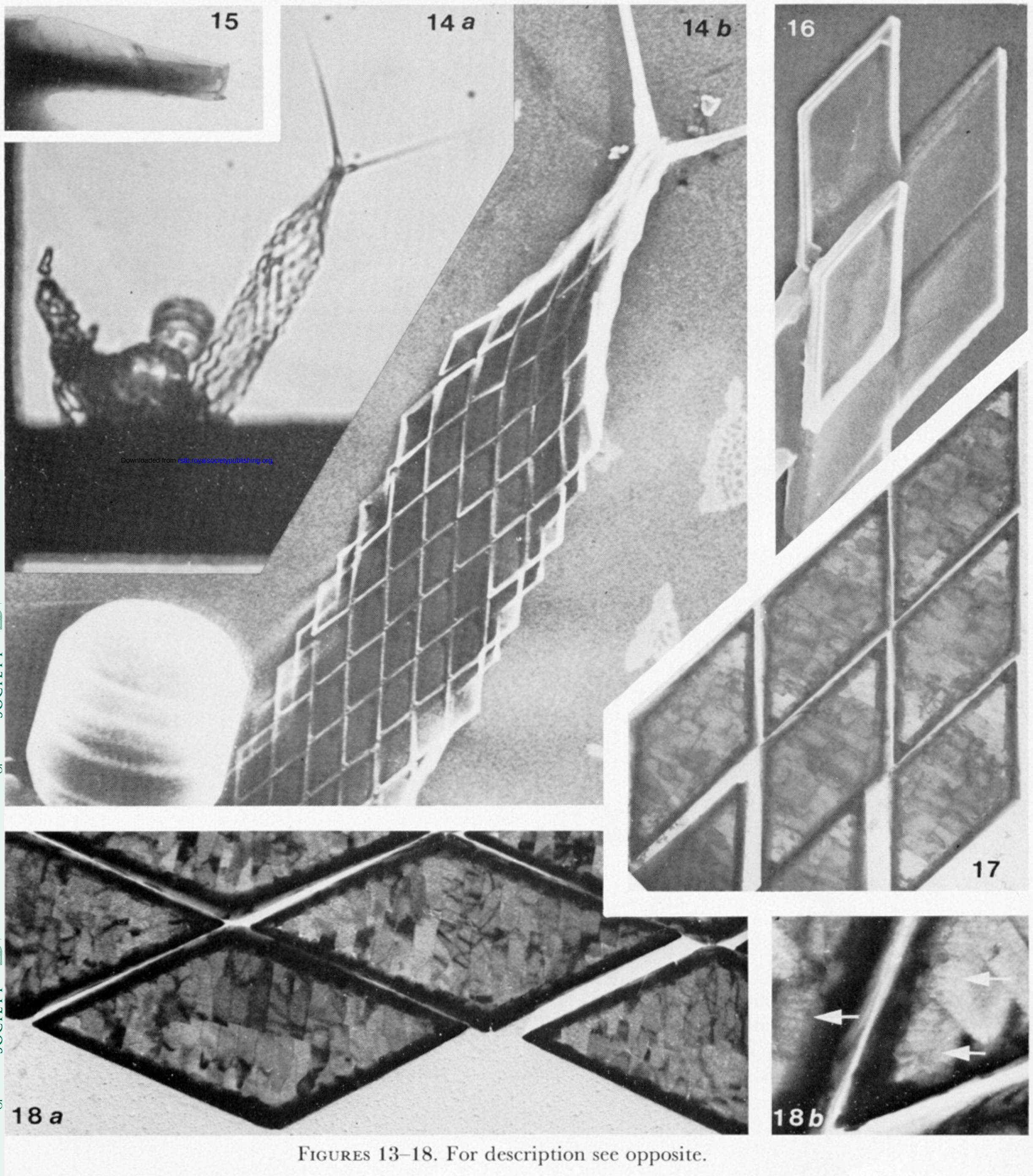


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURES 8–12. For description see p. 469.





Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURES 13–18. For description see opposite.