A REINVESTIGATION OF COLLARED FLAGELLATES IN THE GENUS *BICOSTA* LEADBEATER WITH SPECIAL REFERENCE TO CORRELATIONS WITH CLIMATE

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CONTENTS

By combining light microscopy and electron microscopy, the range of geographically linked diversity in lorica size and construction has been recorded for each of the three species of *Bicosta*, on the basis of wild material processed directly from the sea, in many different parts of the world distributed from the high Arctic to the Equator and further

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south. Characteristic differences in responses to climatic pressures occur. The least sensitive species is *B. minor*, present throughout the temperature range (−1 to 22 °C), but with local differences of size depending on environmental factors other than temperature, the smallest cells having been recorded in south (but not north) Alaska and the largest at Portsmouth (England) and in the Galapagos Islands. The other two species are less tolerant of high temperatures and have not been found above 16 °C though they have crossed the Equator. Both are common in the Arctic, where the largest cells characteristically occur. The most elaborate responses were found in *B. spinifera*; these apparently resulted from two different factors, namely environmental selection among genetically predetermined biotypes differing in cell size, and environmentally induced local modifications, probably caused by the slowing down of critical developmental stages under the action of cold. The exaggerated spine length compared with cell length, characteristic of many large arctic specimens, is interpreted in this way, the critical stages involved being late in the replication cycle since both in *B. minor* and *B. spinifera* the costal strips formed first are the short ones. Other biologically significant observations include new information on the structure of the membrane subtending the protoplast and on its mode of attachment to the lorica, which is different in each of the species. Revised taxonomic descriptions summarizing selected parts of the new findings are given at the end of the paper.

Introduction

Collared flagellates are small, unicellular, colourless scavengers living by ingesting bacteria or fragments of organic detritus and collectively forming a major component of marine nanoplankton in coastal waters at all latitudes that have been tested. Because of their small size they have only become prominent in the literature since the advent (during the last ten years) of electron microscopy applied to freshly gathered water bottle samples (as opposed to tow-net samples, in which they are not retained). Once known, they can nevertheless often be effectively recognized and studied, as the present communication will show, by means of the light microscope, since, in marine habitats, though not in freshwater equivalents, the apparent size is greatly increased by the fact that a tiny protoplast is commonly suspended within a much larger basket-like external structure known as a lorica. This is composed of silicified rods known as costal strips, each individually manufactured within the cytoplasm but all collectively assembled outside it, by means that have not yet been fully recorded (see, however, Leadbeater 1979*a*, *b*). The number of component strips varies widely, from less than ten to several hundred, and their arrangement, often in linear sequences known as *costae*, is usually so precise as to constitute the most effective known basis of classification.

The lorica in the genus *Bicosta* is one of the simplest known, being composed of exactly seven costal strips. There are three species so far described (Throndsen 1970; Reynolds 1976; Moestrup 1979), all easily recognized, widespread and often abundant. Nevertheless, in these as in many other organisms of comparable size, basic information has accumulated piecemeal as expertise has grown in the use of modern methods of collecting and observing. The inevitable limitation of many initial descriptions, on which naming depends, to single localities represented by very few individuals, applies to all the known species of *Bicosta*, each of which is at present represented in the literature by little more than four specimens. This limitation precludes understanding of the possible extent, causes and consequences, of intraspecific variability, though the need for such knowledge has recently been expressed several times in the literature in relation to other, more complex, taxa (see, for example, Manton *et al.* 1976; Manton & Leadbeater 1978; Manton & Oates 1979; etc.)

The observations relevant to this topic, summarized below, have accumulated incidentally during the working out of collections of nanoplankton recently obtained during personal visits to a wide range of latitudes extending from the Arctic to the Equator and further south. After appropriate field treatment, even in very remote places, it is now possible to assemble material on a scale not previously available for any organism of this kind, in a form suitable for fine-structural investigation. Evidence on selected problems can then be assembled and collated at will, and the present study arose in this way. The choice of *Bicosta*, mainly because of its simplicity and abundance, was thus not premeditated but followed from the availability of a relatively lavish supply of specimens of varied origin. That these, in turn, have contributed new information potentially affecting the formal taxonomic descriptions, which may have to be amended accordingly, is, in a sense, a disadvantage that must be accepted as incidental to the main enquiry. This is an attempt to assess the part played by climatic influences, as determinants or modifiers of lorica and cell morphology and/or behaviour, in a compact group of three closely related and relatively simple species.

Format of the paper, and nomenclatural history

For reasons that will become obvious, dimensional data in the account that follows will be based on light microscopy, the electron microscope being limited to a few qualitative details that could not have been shown otherwise. This limitation must, nevertheless, not be interpreted as minimizing the initial importance of electron microscopy for establishing basic facts. This is well exemplified by the nomenclatural history of the genus. When observations began on relevant marine taxa by means of the light microscope only (Grøntved 1956; Bursa 1961; Throndsen 1970) there seemed to be no objection to the use of an existing generic name, *Salpingoeca*, introduced by H. James-Clark in 1867 for a freshwater organism. However, reinvestigation of the type species of *Salpingoeca* James-Clark, carried out with the aid of electron microscopy by Thomsen 1977, showed at once that Grøntved's use of this generic name for the marine taxa to which it had been applied (up to and including Reynolds 1976) was mistaken, since James-Clark's type species is wholly without a costate lorica, on which recognition of the marine taxa essentially depends. The consequences of this discovery were set out in detail by Leadbeater (1978), while taking the opportunity of separating the increasing assemblage of marine taxa, formerly subsumed under *Salpingoeca sensu* Grøntved *non* James-Clark, into two new genera, to be called *Calliacantha* and *Bicosta* respectively. Both have subsequently been enlarged until they now each contain three species, all authenticated by at least some electron micrographs.

The essential nomenclatural changes applicable to *Bicosta* have already been carried out by Leadbeater (1978) and need no further attention here, but other emendations, either as additions or corrections to previous descriptions, will be introduced *seriatim* and early for each species in turn. The more important other findings will then be summarized and discussed.

Revised taxonomic diagnoses for all three species, placed at the end of the text for ease of reference by potential users, will be set out in the usual way, starting with the type species, *B. spinifera* (Throndsen) Leadbeater. It will, nevertheless, be convenient in the text itself to begin with the species possessing the smallest cell size, namely, *B. minor* (Reynolds) Leadbeater.

Material and methods

The sources of the material used are listed in table 1 and shown on the map (figure 1). Apart from a single sample from Britain (Portsmouth), processed in 1977 as a means of testing equipment prior to its use in the Galapagos Islands, the material was collected during seven journeys made since 1971.

Table 1. Sources of specimens used

place	latitude	longitude	date	depth	temp./°C
W Greenland (Disko Island)	69° 30′ N	53° 30′ W	June 1972	surface–20 m	4
Hudson Bay (Churchill)	58° 44′ N	94° 00′ W	July 1973	5 m	2
Resolute Bay (Cornwallis Island)	74° 40′ N	95° 00′ W	August 1973	surface–10 m	−1
S Alaska (Homer)	59° 40′ N	151° 35′ W	June 1975	surface–10 m	6–10
N Alaska (Pt Barrow)	71° 23′ N	1° 5′ W	July 1975	4–6 m below ice	0
Portsmouth	50° 48′ N	1° 5′ W	June 1977	surface	16.5
S Africa (Cape Town)	33° 56′ S	18° 29′ E	November 1972	surface–20 m	9
Galapagos Islands	0° 56′ S	91° 0′ W	August 1977	surface–10 m	18–22

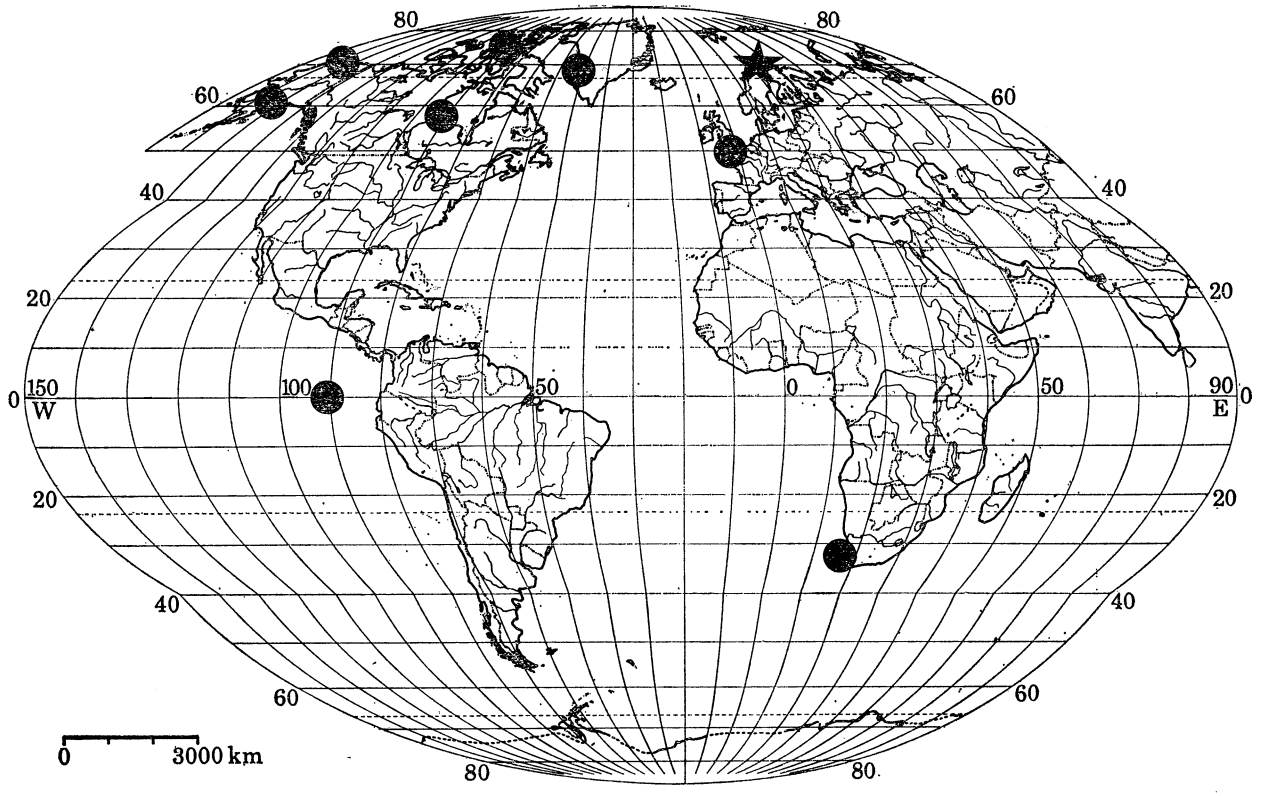


Figure 1. Map showing the main sources of material used in this paper (filled circles), with the type locality for *B. spinifera* (Throndsen) and *B. minor* (Reynolds) indicated by a star.

In each locality, water bottle samples were collected, usually from a small boat and often by means of a van Dorn bottle or home-made equivalent, the depth and temperature being recorded at the time. Each sample, in quantity ranging from 6–10 l, was transferred to a clean polythene container and kept in a cool place for a few hours or overnight. The larger plankton was then removed by pouring the sample through a nylon fabric of approximately 25 μm pore size, after which the nanoplankton was concentrated by means of a Millipore filter and gentle

centrifugation. Drops of the concentrate, positioned either on glass slides or on copper grids carrying carbon-on-formvar support films, were then killed by 30 s exposure to osmic vapour before being dried. Alternatively, bulk fixation of the concentrate by squirting it into 2% osmium tetroxide made up in 0.1 M cacodylate buffer at pH 7, followed by further centrifugation, could be used as a source of drops. After either method, the deposit of salt crystals formed immediately on drying must be removed by gentle washing in distilled water, after which a final drying renders such preparations safe for storage. Shadow casting, as needed for electron microscopy, can be added later, but no further preparative treatment is required.

Routine light microscopy, by means of either phase contrast or Nomarski illumination, has in each case involved use of a $\times 40$ dry lens applied to a dry preparation without a coverslip. Four different microscopes have been involved, namely, a Leitz Universal microscope at Carleton University, Ottawa, two Reichert Zetopan microscopes, respectively at Lancaster University and Imperial College, London, and a Zeiss Photomicroscope II in the Cytogenetics Unit at the Medical School, University of Liverpool. Observations were in each case recorded on 35 mm film (usually Ilford Pan F) and subsequently adjusted to a uniform magnification of $\times 1000$ during printing. This was carried out in Leeds, on a Leitz Focomat 2C enlarger belonging to the Royal Society.

Exceptionally, the use of an oil immersion objective can add significantly to information obtainable from dry preparations. This is exemplified by figure 12*a*, plate 2. The method used here was to put a drop of immersion fluid (Objectol) directly on to a selected cell without adding a coverslip. With the microscope set up for phase contrast the image obtained with a $\times 100$ immersion objective, used directly in this way, is significantly different from that previously obtainable with a dry lens (figure 12*b*). Resolution is slightly improved but, more importantly, a marked difference in the optical properties of silicified costae and protoplasmic parts becomes conspicuous, permitting a clear distinction to be made between a flagellum and a silicified spine of otherwise similar dimensions. This method is difficult to use, demanding accurately parfocal and centred objectives, since otherwise the effect of immersion is to cause objects as inconspicuous as this virtually to disappear, until the Objectol can be removed with amyl acetate.

Electron microscopy has similarly drawn on several different instruments as opportunities have offered. The microscopes responsible for the micrographs reproduced are mentioned in the legends. They include a Siemens Elmskop 1A at Carleton University Ottawa, an A.E.I. 801 microscope formerly in the University of Lancaster, two A.E.I. EM 6B microscopes respectively at Nottingham and Imperial College, London, an A.E.I. 'Corinth' in the University of Nottingham, and, finally, a Jeol Temscan recently installed in the Department of Biological Sciences, University of Lancaster.

Observations

According to the definitions provided by Leadbeater (1978), the lorica in *Bicosta* agrees with the equivalent in *Calliacantha* in being conspicuously spined at each end. The posterior spine in both genera is almost always single, but the anterior spines are two per cell in *Bicosta* and more numerous in *Calliacantha*. The structure of the lorica chamber is also different. Whereas in *Calliacantha* this consists of several (up to six) longitudinal costae converging to a point posteriorly but held together anteriorly by two successive transverse costae, in *Bicosta* there are no transverse costae, and longitudinal costae, each projecting anteriorly as a spine, are limited to two. Since

each longitudinal costa is built up from three successive costal strips, the addition of a single posterior spine brings the total number of costal strips in the lorica as a whole to seven, as already noted.

The protoplast, with its single flagellum surrounded by a ring of tentacles, offers few diagnostic details at the species level, though the facts will be demonstrated with greater precision than hitherto in this genus. A third component, namely the unmineralized membrane within which the protoplast is suspended and attached to the lorica, will be explored for each species to the extent that the material permits, since this component has not previously entered into the descriptions of any of them.

B. minor (Reynolds) Leadbeater (*plates* 1 *and* 2)

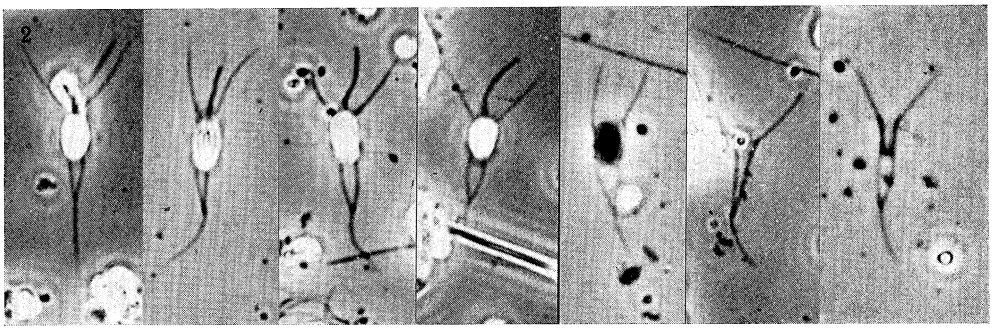
The four specimens illustrated by Reynolds (1976) in describing this taxon were collected in the Barents Sea during a voyage with the British research vessel *Cirolana* in June 1973. Single specimens previously illustrated from Norway (Leadbeater 1972) and Denmark (Thomsen 1973) were accepted as conspecific with these. The new species was shown to differ from '*Salpingoeca spinifera Throndsen sens. strict.*' (now *Bicosta spinifera* (Throndsen) Leadbeater) by a smaller cell size and by the absence of a 'twist' midway along the lorica chamber. Other differences exist but were not noted at the time.

In describing this species, Reynolds 1976 was the first person to notice explicitly the number of costal strips and the positions of the strip junctions in any member of this genus. The facts are perhaps sufficiently authenticated by figure 7*a*, plate 2, with respect to a specimen from Cape Town that can also be used to introduce the protoplast, flagellum, tentacles and part of the subtending membrane, about which further details will be provided below.

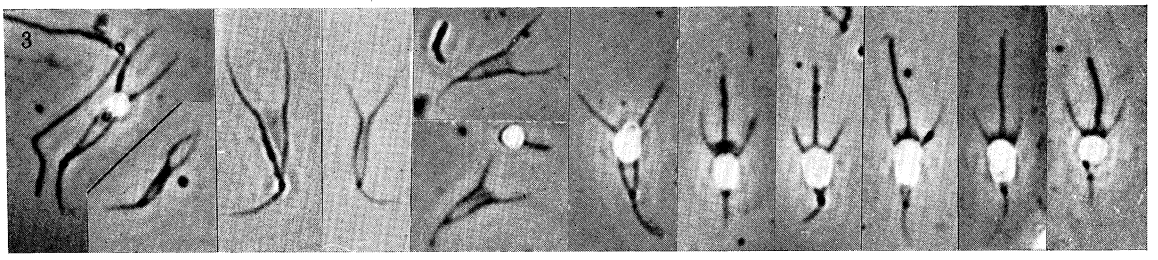
Once these facts are known and in spite of the small size there is no difficulty in exploring the range of sizes and shapes presented by this taxon in different environments, by means of the light microscope alone, as explained on p. 435. When this is done, a surprising discrepancy from the type description at once appears. The overall lorica length given there (Reynolds 1976, p. 13) and presumably based on the four specimens from the Barents Sea, collected at a sea temperature of 7 °C, is 30–45 μm. However, among the 35 specimens illustrated with the light microscope on plate 1, at a uniform magnification of × 1000, none reaches 45 μm and only exceptionally large cells exceed 30 μm. The most probable explanation for this discrepancy is instrumental, involving the calibration of an electron microscope, hence our own preference for light microscopy for this type of measurement.

Size differences of a rather modest kind are nevertheless discernible (plate 1). Thus, Katchemak Bay at Homer in south Alaska (figure 3) stands out as a locality dominated by exceptionally small cells. At Portsmouth (figure 2), on the other hand, the cells are as large as the majority emanating from the three arctic areas (figures 4–6), while in the Southern Hemisphere, the Galapagos Islands, with the highest temperature of all, possess the largest specimens so far identified (figures 12*b*–15, plate 2). Observations such as these rule out any simple interpretation in terms of direct influence by any one climatic factor, such as temperature. A diagrammatic summary of the dimensional details observed will be found on p. 443, at (*a*) in figure 39.

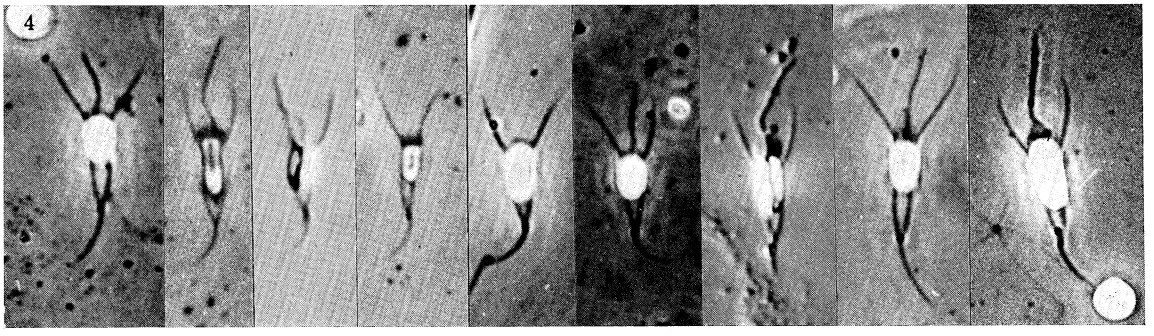
A qualitative detail not explicitly mentioned by Reynolds (1976), though faithfully recorded in his figure 11, is the curvature detectable in almost all the specimens illustrated on plates 1 and 2. This curvature, most conspicuous in the posterior spine, is a simple arc and never the more



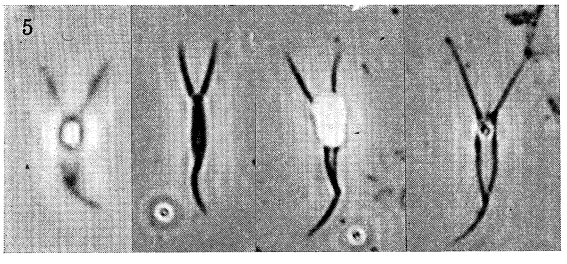
Portsmouth (U.K.), June 1977, sea temperature 16.5 °C



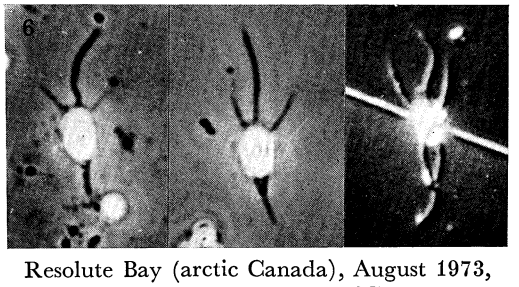
Homer (S Alaska), June 1975, sea temperature *ca.* 9 °C



Godhavn (W Greenland), June 1972, sea temperature 4 °C



Pt Barrow (N Alaska), July 1975,
temperature 0 °C, under ice



Resolute Bay (arctic Canada), August 1973,
sea temperature −1 °C

Description of plate 1

Bicosta minor. Light microscopy (mainly phase contrast) from dry whole mounts of wild material. (Magn. × 1000.)

Figure 2. Seven specimens from a single sample (films 164 and 155).
Figure 3. Three specimens from sample viii (film 163) and nine from sample v (films 161 and 162).
Figure 4. Nine specimens from four samples (exposures on films: 11, 15, 22, 34, 40, 79, 161, 167).
Figure 5. Each specimen from a different sample (films 55, 88, 90, 91).
Figure 6. Each specimen from a different sample (films 36, 94, 112).

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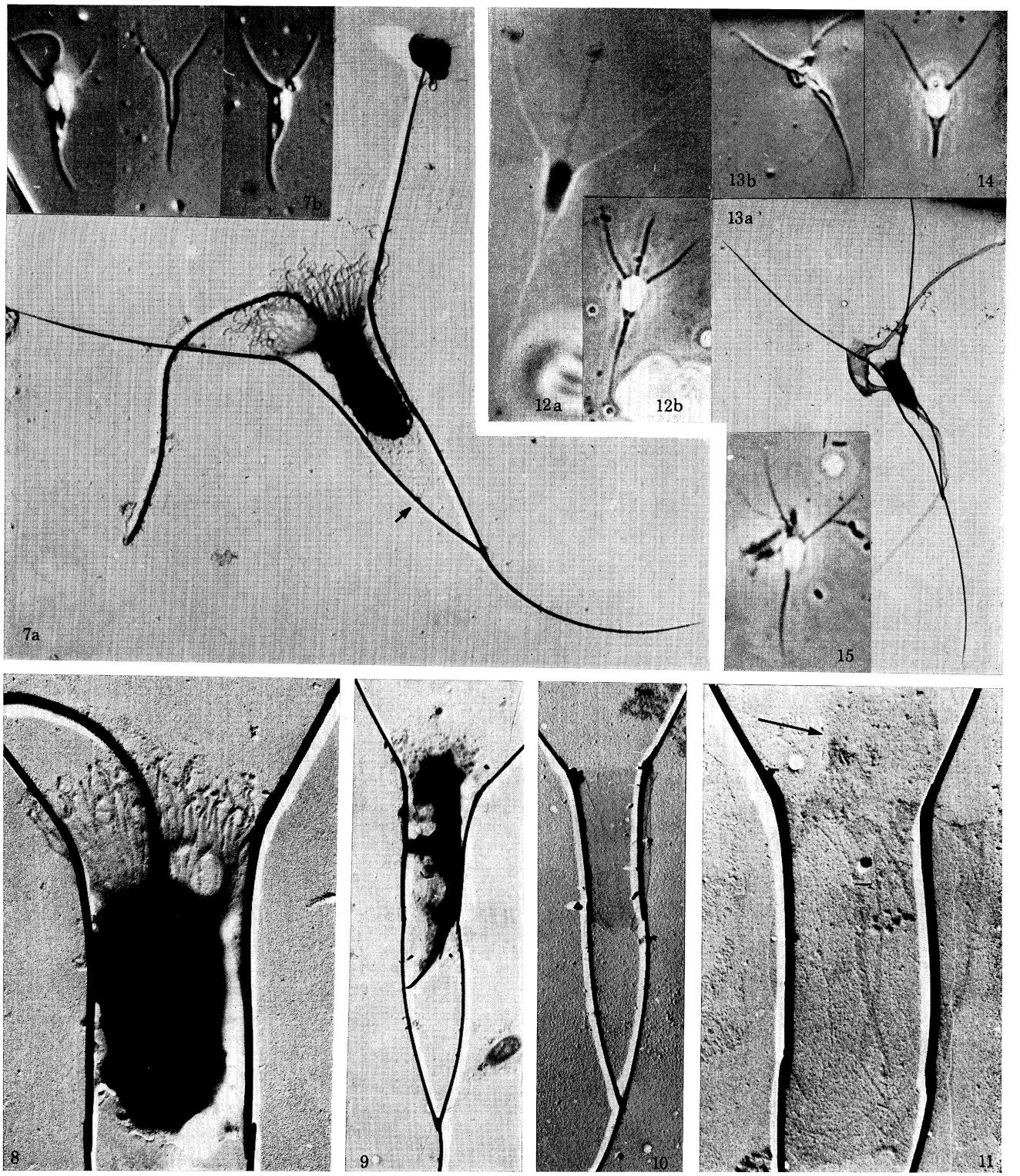

FIGURES 7–15. For description see opposite.

complex configuration characteristic of the next species. It doubtless reflects an aspect of protoplast shape during the formation of new costal strips preceding lorica replication. This relation can be seen in a general way in figure 9, plate 2, in which curved costal strips not yet liberated to the exterior are still present within the cytoplasmic area of a recently dead cell.

When present, the protoplast invariably occupies the anterior end of the lorica chamber. Though rounded posteriorly and perhaps shortened by shrinkage on drying, it never seems to fill the whole chamber as in some other taxa. The flagellum itself is easily shed and as easily regenerated; the length can therefore be less than that of an anterior spine, or much greater. In exceptional cases when the flagellar length is exactly equal to that of an anterior spine, as in figure 12*b*, an oil immersion objective used as explained above may be the only way of clearly distinguishing the costae from the flagellum (figure 12*a*).

Other protoplasmic parts, notably the tentacles, can scarcely be studied at all without electron microscopy. When viewed in this way (figures 7*a*, 8, plate 2) the number of tentacles is of the order of 30, i.e. at the upper limit of the range of numbers listed by Reynolds (1976).

Finally, the unmineralized membrane, surrounding the protoplast in life and responsible for attaching it to its lorica, can be faintly detected with the light microscope on many recently vacated specimens (see plate 1). Usually, however, such membranes are in a reduced and truncated condition, as exemplified in greater detail by figures 10 and 11, plate 2. The membrane may then take the form of a straight-sided tube, exactly equal in length to the middle strips in the longitudinal costae, being apparently attached fairly firmly to the overlapping joins at each end. In life, however, the tube extends much further towards the hind end of the chamber (figure 7*a*, arrow), though even here the truncated bottom edge could indicate tearing. At the anterior end, the bases of the tentacles are covered by a conical extension of membrane so transparent as to be scarcely visible (figure 8). Exceptionally, however, as in figure 11, remnants of

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Description of plate 2

B. minor (cont.). Electron microscopy of shadow-cast whole mounts unless otherwise stated.

Figure 7. (*a*) Cape Town (S Africa), specimen showing lorica and complete protoplast, the bottom end of the subtending membrane indicated by an arrow; electron micrograph Y 4893.1 (801 Lancaster). (Magn. × 5000.) (*b*) Cape Town, three specimens from one sample (Nomarski, light microscopy, exposures 95.28, 84*a*.29, 85.20). (Magn. × 1000.)

Figure 8. Cape Town, another specimen showing tentacles (*ca.* 30); electron micrograph Y 6005 (Siemens, Ottawa). (Magn. × 10 000.)

Figure 9. Homer (S Alaska), lorica chamber with remnants of a protoplast containing nascent costal strips; electron micrograph Y 7835.5 (EM6B, London). (Magn. × 5000.)

Figure 10. Homer, another specimen showing remnants of the membrane formerly subtending a protoplast; electron micrograph Y 7831.4 (EM6B, London). (Magn. × 5000.)

Figure 11. Homer, another specimen, with a more complete membrane, the anterior projection (arrow) formerly covering the bases of the tentacles still present; electron micrograph Y 7828.17 (EM6B, London). (Magn. × 10000.)

Figure 12. (*a*) Galapagos Islands, light microscopy, phase contrast, a specimen immersed as explained on p. 435; exposure 176.27. (Magn. × 2000.) (*b*) The same specimen photographed with a dry lens; exposure 160.11. (Magn. × 1000.)

Figure 13. (*a*) Galapagos Islands, specimen entangled with a diatom fragment; electron micrograph Y 7999A.3 (Temscan, Lancaster). (Magn. × 2000.) (*b*) The same cell taken subsequently with the light microscope (phase); exposure 174.5a. (Magn. × 1000.)

Figures 14 and 15. Galapagos Islands, light microscopy of additional specimens, figure 14 lacking flagellum and posterior spine but figure 15 complete; exposures 159.28 and 147.12. (Magn. × 1000.)

this anterior region are still present (arrow), apparently joined to the rest of the membrane by a straight suture, which, after further decomposition, resembles an edge as in figure 10. Substructural details have not as yet been recognizable in either part of the membrane of this species, though there can be little doubt that they are not uniform, since the anterior region disintegrates much more easily than the rest, after departure of the protoplast.

In geographical distribution, *B. minor* is the most widespread representative of its genus, having been found in all the regions tested (see map p. 434) except Hudson Bay. Even here it is virtually certain that it would have been collected had sampling extended further away from the Churchill River estuary. Elsewhere *B. minor*, though rarely if ever a dominant organism, is at its most abundant in middle latitudes and at moderate temperatures. It can obviously tolerate cold, but in hot climates it can be more tenacious of life than the other two species and can then persist as the sole representative of its genus, though only in very small numbers as in the Galapagos Islands.

B. spinifera (Throndsen) Leadbeater (*plates* 3 *and* 4)

When this species was first described (Throndsen 1970), the main objective was to distinguish it clearly from '*Salpingoeca natans*' Grøntved 1956. Both descriptions relied solely on the light microscope and neither was fully understood in terms of the costate lorica that we now know that both taxa possess. *B. spinifera*, now the type species of the genus *Bicosta* Leadbeater, had been collected in the Barents Sea and studied alive on board ship while the author, J. Throndsen of Oslo, Norway, was accompanying N. Reynolds of Lowestoft, England, on an arctic trip with the British research vessel *Ben Holt* in the summer of 1969. Electron microscopy was added later by Reynolds (1976), following a second journey to the Arctic, as already noted. Micrographs of four somewhat battered specimens of *B. spinifera* sens. strict. were illustrated to contrast with the four specimens used for the description of *B. minor* as a separate taxon. The opportunity was also taken to amplify the description of *B. spinifera* itself by adding details of the positions and numbers of costal strip junctions that had been observed by this author for the first time.

The marked size difference between *B. spinifera* and *B. minor* noted by Reynolds (1976) is at once apparent on comparing plate 3 with plate 1. In plate 3, the larger size of each specimen reduces the total number that can be included on one page, although all the necessary localities

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Description of plate 3

B. spinifera. Light microscopy, mainly phase contrast, but figures 17, 19 to 21*a* Nomarski illumination; all from dry whole mounts of wild material. (Magn. × 1000.)

Figure 16. Homer (S Alaska), sea temperature 9.8 °C, a field with *Calliacantha simplex* (left) and *B. spinifera* (right) among debris; exposure 63.10.

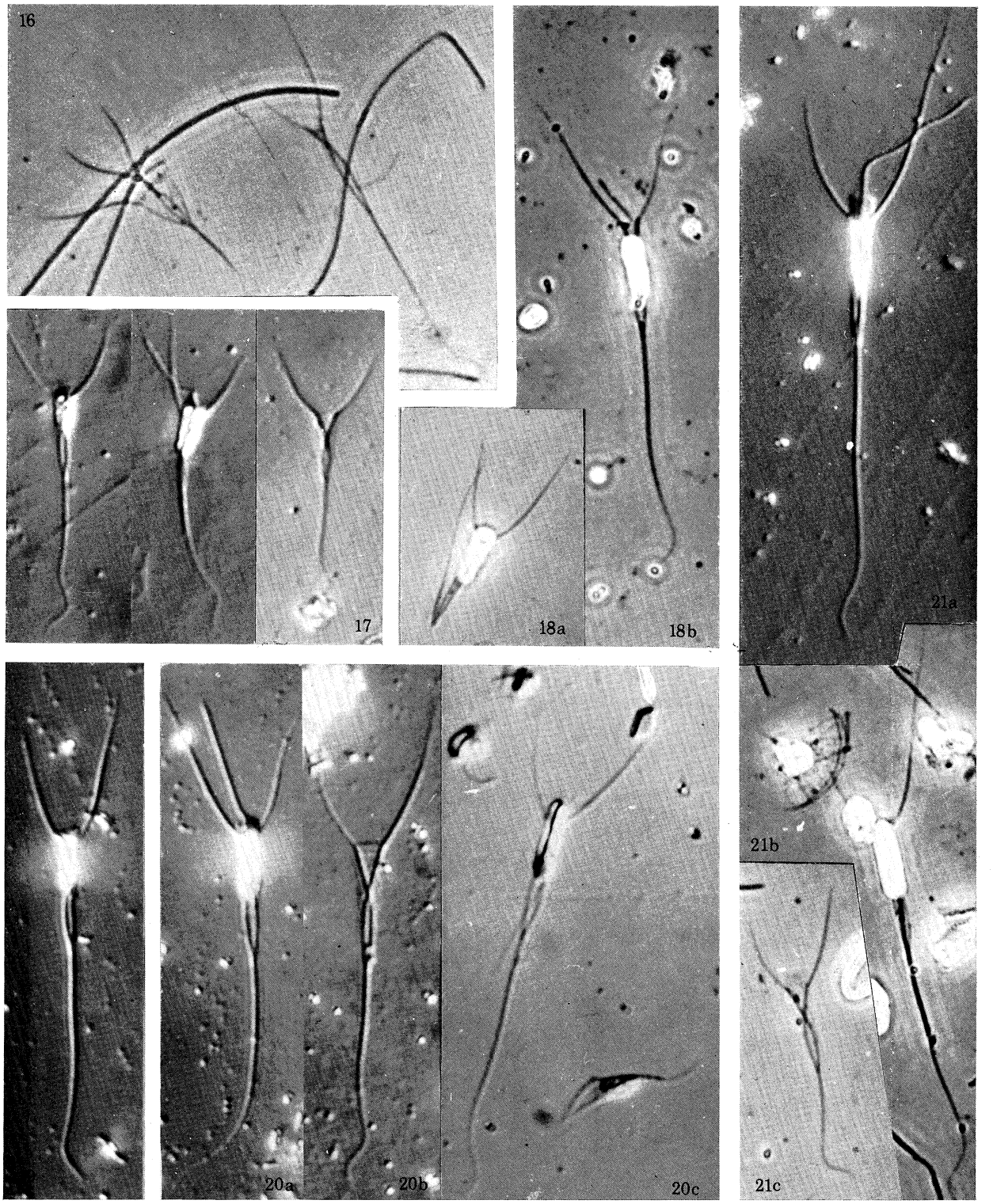
Figure 17. Cape Town (S. Africa), sea temperature 10.25 °C, 3 specimens from one sample (exposures 85.8, 85.6 and 39.21).

Figure 18. Pt. Barrow (N Alaska), beneath sea ice; exposures 51.1 and 53.12.

Figure 19. Churchill (Hudson Bay), sea temperature 2 °C; exposure 26.32.

Figure 20. Godhavn (W Greenland), sea temperature 4 °C, each specimen from a different sample, (*c*) also containing *B. minor*; exposures 29.5, 29.17 and 34.4.

Figure 21. Resolute Bay (arctic Canada), sea temperature − 1°C, each specimen from a different sample, 21*b* also containing *Parvicorbicula socialis* (top left); exposures 36.34, 114.5 and 94.36.



FIGURES 16–21. For description see opposite.

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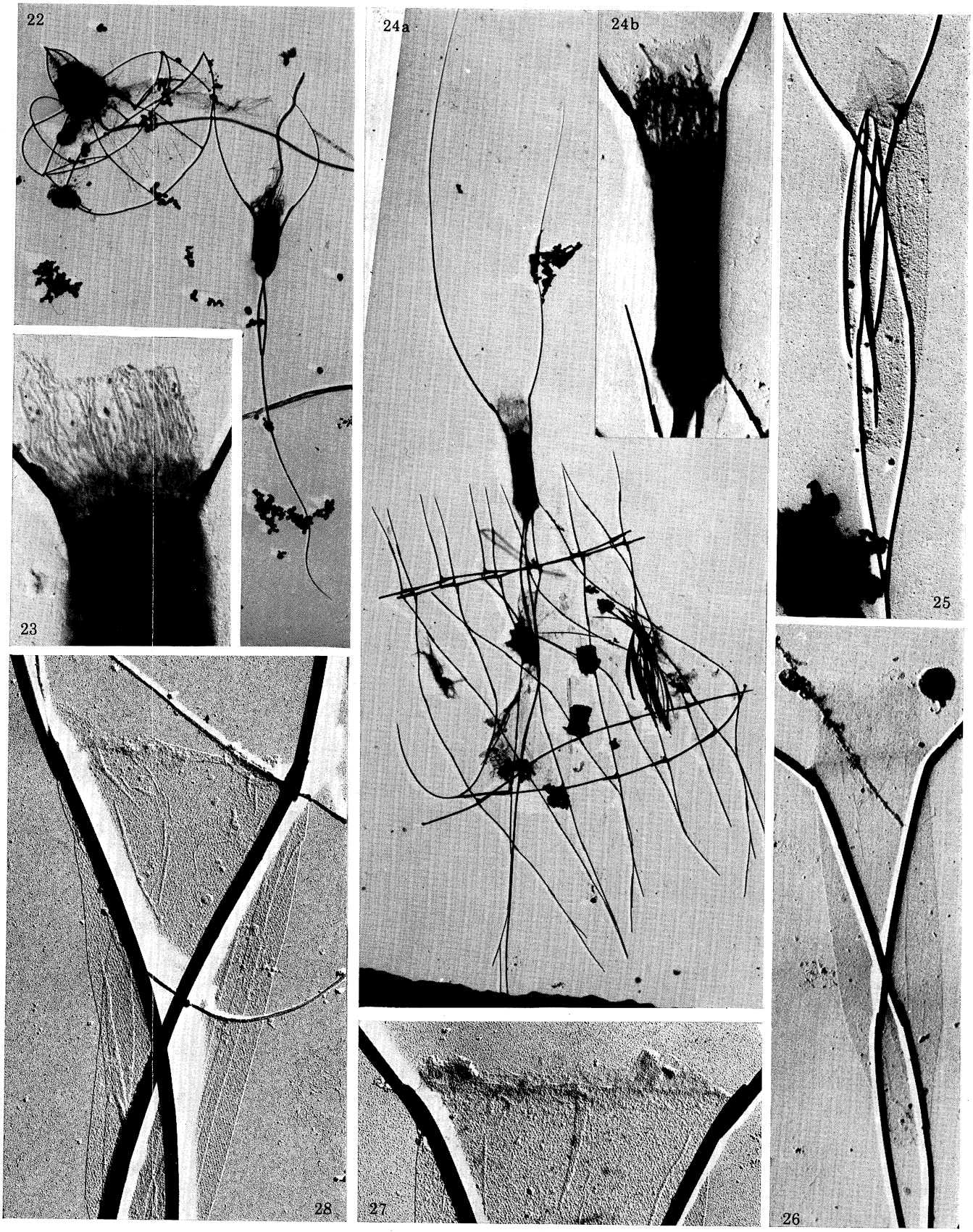

FIGURES 22–28. For description see opposite.

are, in fact, represented. In addition, the larger area occupied by each individual has permitted the occasional inclusion of other taxa within a chosen field, thereby further substantiating the relative sizes. Examples of such additional taxa are: *Calliacantha simplex* (figure 16, plate 3), *Parvicorbicula socialis* (figure 21*b*, plate 3), *Pleurasiga minima* (figure 22, plate 4), *Diaphanoeca aperta* (figure 24*a*, plate 4), and, by a rare chance, *B. minor* itself (figure 20*c*, plate 3), in each case in the same field as a specimen of *B. spinifera*.

Apart from dimensions, about which more is said below, there are several structural features diagnostic of *B. spinifera* sens. strict. The most important of these, as noted by Reynolds, is the crossed relation of the two longitudinal costae midway along the lorica chamber, best seen in specimens from which the protoplast has disappeared, as in those illustrated in figures 16, 17, 20*b*, 21*c*, plate 3; etc. This relation was correctly depicted by Throndsen, though without structural interpretation. It is not, of course, a 'twist', being no more than an expression of obliquity in the longitudinal costae with respect to the long axis of the cell. In other respects, the lorica chamber in *B. spinifera*, though relatively long, is not essentially different from that in *B. minor*.

Additional diagnostic characters, not noticed by either Throndsen or Reynolds, are to be found in the spines. These are more unequal in length than in *B. minor*, the posterior spine being the longest (see plate 3), whereas in *B. minor* (plate 1) the posterior spine tends to be equal to, if not shorter than, the anterior spines. The latter, in *B. spinifera*, are also usually dissimilar within a pair, the longer of the two being frequently demonstrably thicker than its shorter partner (see, for example: figures 16, 18*a*, 18*b*, 21, 22, etc. The degree of difference in length varies considerably, a maximum difference of 20 μm being sometimes recorded in arctic material (see table 2, third column from the right). Exceptionally, in all areas a specimen may be found with apparently equal anterior spines, but such individuals, which can be of any size, occur singly in the populations tested. All spines, of whatever size, taper distally, large specimens sometimes ending in long hair-points so slender as eventually to fall below the limit of visibility with the light microscope (see, for example, figure 24*a*). The posterior spines, in

Description of plate 4

B. spinifera. Electron microscopy of shadow-cast whole mounts of wild material.

Figure 22. Cape Town (S Africa), field containing *Pleurasiga minima* (top left) beside *B. spinifera* complete with flagellum; electron micrograph Y 7766.15 (Lancaster 801). (Magn. × 2000.)

Figure 23. Godhaven (W Greenland), protoplast showing *ca.* 30 tentacles; electron micrograph Y 4002.19 (Nottingham EM6B). (Magn. × 10000.)

Figure 24. (*a*) Resolute Bay (arctic Canada), field with an empty lorica of *Diaphanoeca aperta* transfixed by *B. spinifera*; electron micrograph Y 7736.22 (Nottingham EM6B). (Magn. × 2000.) (*b*) More highly magnified view of the body tentacles and anterior edge of the subtending membrane in the specimen of (*a*); electron micrograph Y 7740.24 (Nottingham 'Corinth'). (Magn. × 10000.)

Figure 25. Homer (S Alaska), lorica chamber of a recently dead cell still retaining remnants of the membrane formerly enveloping the protoplast and still enclosing some nascent costal strips at an early stage of the replication cycle; electron micrograph Y 7816.39 (Lancaster 801). (Magn. × 5000.)

Figure 26. Resolute Bay (Arctic Canada), part of a recently vacated lorica chamber still retaining a virtually complete membrane; electron micrograph Y 7725.17 (Nottingham 'Corinth'). (Magn. × 5000.)

Figure 27. Pt Barrow (N Alaska), beneath sea ice; anterior edge of the membrane with signs of a fibrillar substructure in a recently vacated lorica but with no more than a remnant of the extension previously surrounding the tentacles (see 24*b*); electron micrograph Y 7863.28 (Lancaster 801). (Magn. × 15000.)

Figure 28. As figure 27, another specimen showing spiralized fibrillar substructure, but with the anterior end of the membrane completely removed; exposure Y 7854.8 (Lancaster 801). (Magn. × 15000.)

TABLE 2. *B. SPINIFERA*: SUMMARY OF DIMENSIONAL DATA

		measurements/μm								calculations of proportion of cells with	
		total length		body length		post. spine length		ant. spine		ant. spine at least 25% longer than body (%)	post. spine at least double body length (%)
place and temperature	number of cells	range	mode	range	mode	range	mode	range	difference		
Cape 10 °C	12	39–53	**40**	12–22	**13**	12–26	—	8–17	0–4	0	8
Homer 10 °C	27	45–93	**65**	10–20	**15**	18–45	**30**	7–33	0–13	52	37
Greenland 4 °C	44	65–110	**80**	17–25	**20**	22–53	**45**	11–36	0–20	61	46
Barrow 0 °C	39	50–110	**65**	14–23	**18**	20–56	**35**	10–40	0–15	44	50
Resolute −1 °C	53	50–118	**85**	15–24	**20**	18–50	**40, 45**	12–40	0–20	55	56

contrast, end in a characteristic S-shaped distal extremity that is clearly visible when dry, as in all the specimens illustrated on plate 3, as well as in figure 22, plate 4. In life, on the other hand, such details would certainly be less conspicuous and their absence from Throndsen's camera lucida drawings of living cells, including that designated by Leadbeater (1978) as the holotype (Throndsen 1970, fig. 1*a*), is thus easily explained.

The unmineralized parts of *B. spinifera* compare closely with those of *B. minor*, while showing slight differences. Thus the number of tentacles is similar, namely, approximately 30 (figure 23), and the flagellum, as in *B. minor*, may be missing or short, but when fully developed it can equal, though it rarely exceeds, the length of the longer anterior spine (see, for example, figures 18*b*, 20*a*, 21*a*). The protoplast itself is commonly about three or more times longer than it is wide, whereas in *B. minor* the relative proportions are usually closer to 2:1. The position of the protoplast at the anterior end of the lorica chamber is, nevertheless, similar in both taxa.

The membrane responsible for maintaining contact between protoplast and lorica is so transparent in life as to be difficult to detect except under special circumstances. Exceptionally, however, as in figure 24*b*, a sheath-like extension beyond the tips of the retracted tentacles can be clearly seen. After loss of the protoplast, this, and other parts of the membrane, can be studied more easily. When virtually complete, as in figure 26, the membrane as a whole forms an open-ended tube, which is straight-sided for a distance approximately equal to the length of the middle strips of the longitudinal costae but is slightly tapered posteriorly until abruptly truncated, as if torn, at the bottom end. Anteriorly (figure 26), a straight transverse suture located approximately at the level of the anterior strip junctions marks the change to the extension seen overtopping the tentacles in figure 24*b*. This extension can be seen again in figure 25, but in figures 27 and 28 it has almost or quite disappeared owing to attrition. In these figures, the line of the suture has become almost or quite denuded until it eventually becomes exposed as an anterior edge. Posterior to this edge, in exceptionally favourable specimens, all of which have come from very cold water, diagonal striations suggesting some form of spiralized and fibrillar substructure are faintly discernible (figures 27, 28).

Further insight into the special characteristics of *B. spinifera* can be obtained by more detailed

study of dimensions on the lines summarized in table 2. In spite of the truncated state of the type diagram, Throndsen's numerical estimate (50–80 μm) of the size range in the Barents Sea, at 7 °C, agrees closely with our own figures for south Alaska (45–93 μm), based on a sample that was probably larger but collected at a similar temperature and time of year. In the Southern Hemisphere, at Cape Town, cells are smaller (figure 17), but in colder water the size range increases in the upward direction, although even here a few small cells are also produced (figure 21*c*). These facts can be verified qualitatively by a glance at plate 3 as a whole.

When observations are extended to the relative sizes of individual costal strips, some though not all of which are spines, further points of interest emerge. The necessary comparisons will perhaps be assisted by use of the single figures listed in table 2 in the columns labelled 'mode'. The mode, in each case, is the commonest size ascertainable by counting individuals and not by calculation of arithmetical means. Only once (for the posterior spines in South Africa) was a mode unobtainable since in that small sample no two individuals were exactly alike. Elsewhere, the modes for the main categories vary conformably, all showing a negative correlation with temperature, though with a minor discrepancy in the sample from Pt Barrow (north Alaska). This locality (see table 1) was also exceptional in the long persistence of winter ice, which still dominated the sea at midsummer. Close proximity to the Bering Straits might also have had a significant effect.

When the spines (both anterior and posterior) are compared with the length of the chamber, a new and somewhat unusual situation is exposed. As a glance at figure 7*a* will confirm, all the spines in *B. minor*, as in many other comparable taxa, are approximately equal to the lorica chamber in length. This is because, in each replication cycle, new costal strips of all kinds are formed inside a protoplast that does not normally extend beyond the limits of the chamber. *B. spinifera* is very different, since the spines at both ends can exceed the length of the chamber, often by a considerable amount. The anterior spines are less informative in this respect than the posterior spine since they are also shorter and more confusingly varied (see above). Nevertheless, as indicated arithmetically in the penultimate column of table 2, the longer anterior spine can exceed the chamber length on the same lorica by more than 25% in approximately half the population. The posterior spines are more extreme, being twice the length of the chamber, or more than this, in a very similar proportion of cases. Discrepancies of this order cannot be dismissed as observational errors nor can they easily be explained without postulating some departure from the normal sequence of events during replication.

Direct evidence on replication is, unfortunately, scarce, but figure 25 illustrates the only stage so far encountered in this species that can be recognized with certainty as such. It represents a dead cell still retaining some newly manufactured costal strips within the area formerly occupied by cytoplasm. There are exactly four of these new strips, all short, slightly curved, and therefore obviously intended for use in a new chamber wall. These prove beyond doubt that the shortest costal strips are formed first in the replication cycle, before the parent cytoplasm has changed from its usual size and position. Exactly how the later stages are accomplished remains unknown, though substantial elongation of the protoplast, perhaps for a short time only, is one possibility. Be that as it may, it is perhaps reasonable to infer that the extreme attenuation of the spine ends, including the S-shaped distal tip of the longest (the posterior) spine may be no more than incidental consequences of whatever these changes prove to be.

The only remaining new observations concern geographical distribution. Though among the more abundant and easily recognizable components of the nanoplankton in arctic waters, this

species is apparently unable to tolerate heat. At temperatures above 16 °C it is absent and even at 10 °C it is scarce.

B. antennigera Moestrup (*plates* 5 *and* 6)

This species was first found in west Greenland in 1972, with a frequency roughly equivalent to that of *B. minor* in the same area, though both were less abundant than *B. spinifera*. Thereafter it was collected again in Alaska (both north and south) and arctic Canada, becoming increasingly abundant at temperatures below 4 °C. It was not found by us at Portsmouth, the Galapagos Islands or Cape Town, but was collected in New Zealand by Moestrup (1979), who described and named it.

The characteristic sizes and shapes of members of this species can be ascertained by a glance at plate 5. The overall length, though somewhat variable in each locality, is, nevertheless, roughly intermediate between those of the other two species (see also figure 39), but the essential structural differences depend on a change in the distribution of costal strips in the chamber wall. Whereas in both the other taxa the latter contains two successive short costal strips on each side, the most conspicuous point of attachment for the membrane subtending the protoplast being at the upper overlapping joins, all this in our present species is carried by no more than a single (lowermost) costal strip on each side. The chamber itself thus appears shortly triangular when seen in side view, with the anterior spines disproportionately long since each is composed of two successive costal strips. The presence of an overlapping join along a spine (see, for example, figure 35, plate 6) is thus immediately diagnostic of this species, as in the analogous example of *Callicantha simplex* Manton & Oates 1979. If a difference of costal thickness also occurs on the two sides of such a join, or if it dismembers as in figure 33*b*, plate 5, the two components of a spine can be individually recognized, even with the light microscope, and it is then found that the distal strip terminating the spine is always about twice the length of the proximal strip, as noted by Moestrup. The length of the posterior spine is usually less than that of the anterior costal strips on the same specimen, a condition agreeing essentially with that in *B. minor*. Traces of a crossed condition between the two longitudinal costae are, nevertheless, always present at the posterior tip of the chamber (see, for example, figures 34*a*, 36, 37 inset, plate 6), thereby providing a possibly vestigial character linking the species with *B. spinifera*.

In spite of the short chamber, the protoplast does not seem to have been reduced in size, though it often can be seen to bulge considerably beyond the anterior edge of the chamber (figures 34*b*, 35, plate 6) and to fill it more completely posteriorly. The tentacles can sometimes be counted (figure 35) with exceptional clarity as being approximately 40 in number. The subtending membrane, though different in shape and position (figure 36), is in many other ways similar to those previously described. Traces of a spiralized, fibrillar substructure can sometimes

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DESCRIPTION OF PLATE 5

B. antennigera. Phase contrast light microscopy (except figure 29) from dry whole mounts of wild material of different geographical origin. (All magn. × 1000.)

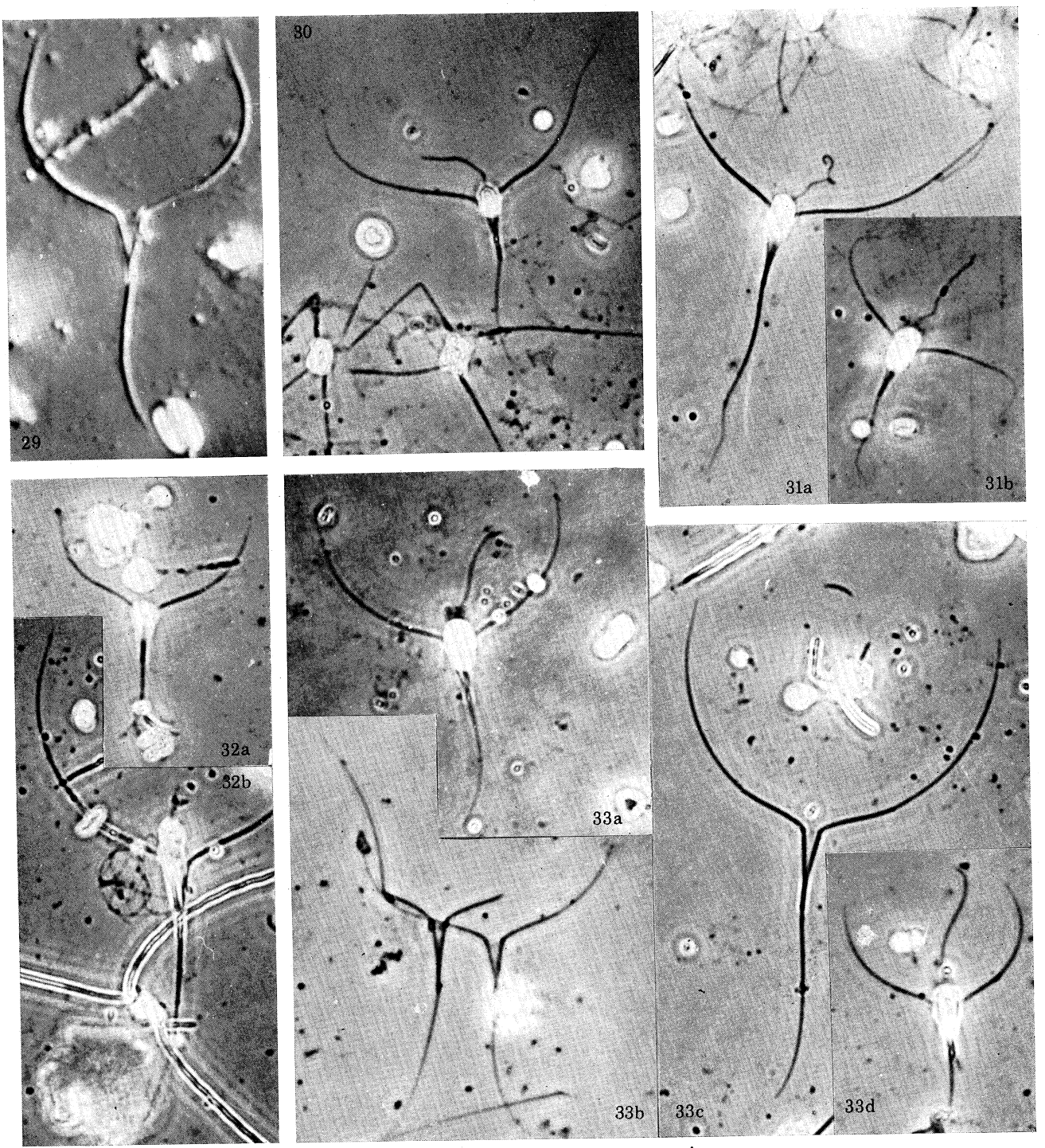
FIGURE 29. Churchill (Hudson Bay), July 1973, sea temperature 2 °C; exposure 26.20 (Nomarski illumination).

FIGURE 30. Homer (S Alaska), July 1975, sea temperature *ca.* 10 °C; exposure 119.33.

FIGURE 31. Godhavn (W Greenland), June 1972, sea temperature 4 °C; exposures 106.6 and 18.9.

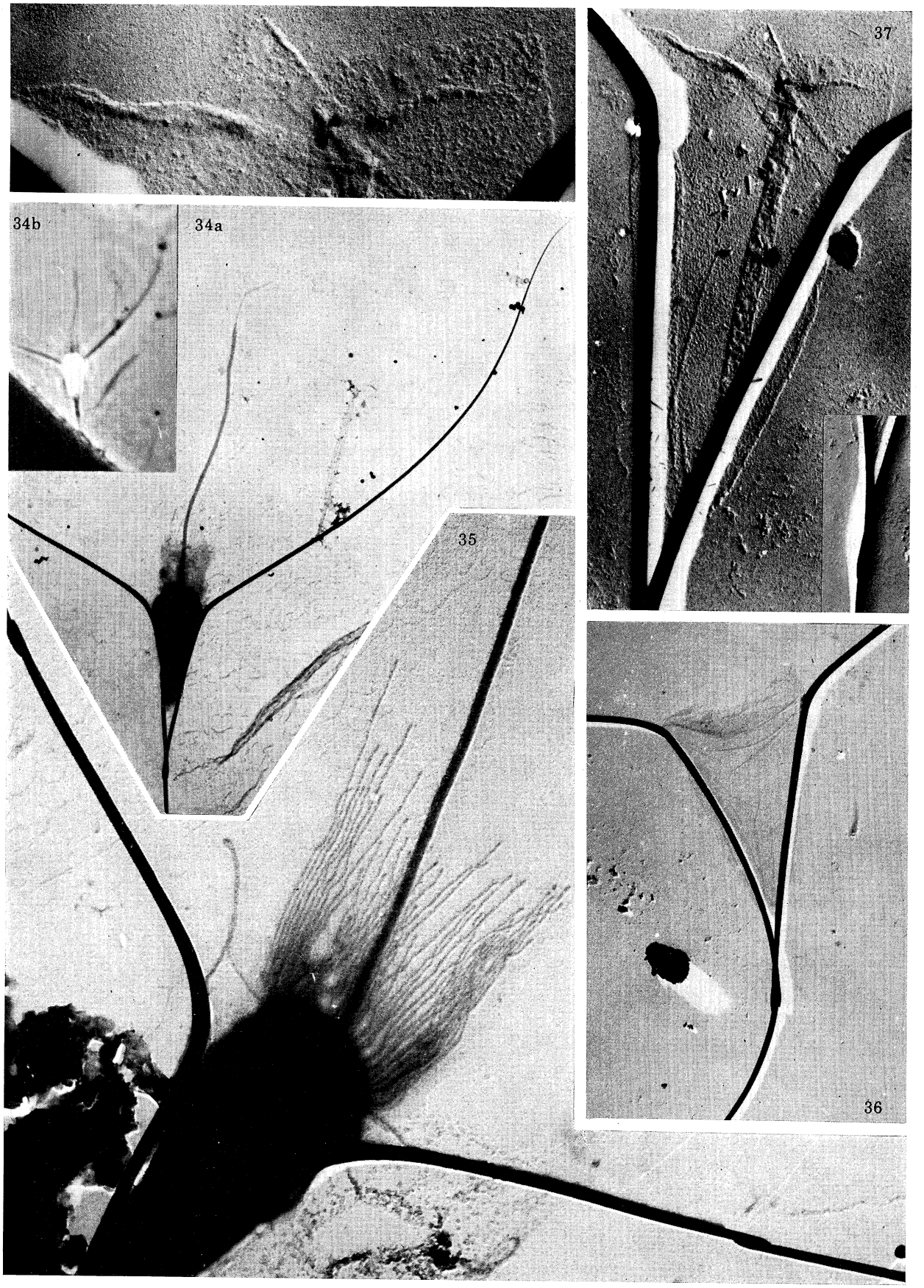
FIGURE 32. Pt Barrow (N Alaska), July 1975, sea temperature 0 °C below ice; exposures 91.38, 90.31.

FIGURE 33. Resolute Bay (arctic Canada), August 1973, sea temperature − 1 °C. Exposures: 115.12A sample II; 8.7 sample III; 108.28A sample I; 100.26 sample VI.



FIGURES 29–33. For description see opposite.

(*Facing p.* 442)



FIGURES 34–38. For description see opposite.

be detected on specimens from very cold water (figure 37, right hand side) and there is a somewhat similar anterior extension (figure 38, plate 6) to that encountered in *B. spinifera*, though, when actually covering the bases of the tentacles, this is commonly too transparent to be easily seen (figure 35).

In the Northern Hemisphere, the geographical range of this species closely follows that of *B. spinifera*, being present at arctic temperatures down to −1 °C but absent at temperatures above 16 °C. In the Southern Hemisphere, it was recorded at 10 °C in the type locality in New Zealand (Moestrup 1979), together with *B. minor*.

Discussion

On the basis of these findings, it is clear that, in spite of the simplicity of the lorica characteristic of this genus, there is considerable diversity between and within species. One of the more obvious variables is size, and a summary of the data for overall lorica length will be found in figure 39.

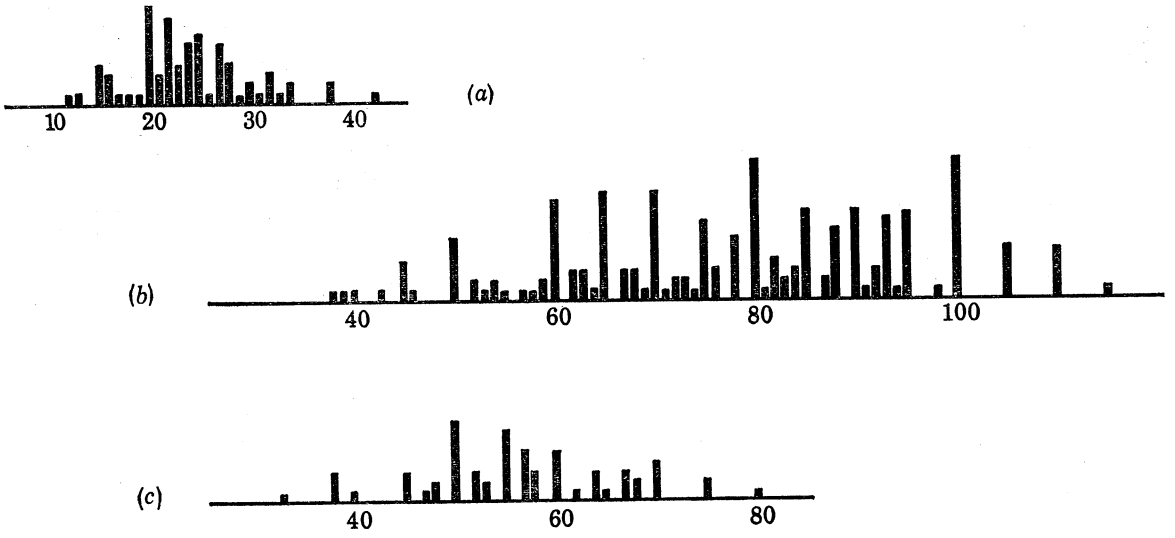


Figure 39. Histograms showing lorica lengths in μm (light microscopy from all localities) in the three species of *Bicosta*. (*a*) *B. minor*, 74 cells. (*b*) *B. spinifera* sens. strict., 182 cells. (*c*) *B. antennigera*, 60 cells.

Description of plate 6

B. antennigera. Electron microscopy from shadow-cast whole mounts, except for 34*a*.

Figure 34. (*a*) Resolute Bay (arctic Canada), part of a specimen with an intact protoplast; micrograph Y 7713.12 (Cambridge 801). (Magn. ×3000.) (*b*) The same as 34(*a*), taken subsequently with the light microscope; exposure 175.17. (Magn. ×1000.)

Figure 35. Resolute Bay (arctic Canada), anterior end of a protoplast, showing expanded tentacles; electron micrograph Y 7600.21 (Lancaster 801). (Magn. ×10000.)

Figure 36. Resolute Bay (arctic Canada), empty lorica showing the characteristic shape of the chamber and membrane; electron micrograph Y 7485 (Ottawa Siemens). (Magn. ×5000.)

Figure 37. Pt Barrow (N. Alaska), anterior end of a chamber from an empty lorica, showing the membrane with folds and faintly fibrillar substructure; electron micrograph Y 7857.15 (Lancaster 801). (Magn. ×10000.)

Figure 38. Anterior edge of the specimen of figure 37, showing fibrillar substructure and the subterminal horizontal suture; electron micrograph Y 7999B.12 (Lancaster Temscan). (Magn. ×20000.)

Before attempting to interpret figure 39 further, it is necessary to remember that size, like any other morphological feature, will be based on determinants of more than one kind, some being genetical and others environmental. These cannot always be separately identified, though inferences are sometimes possible. Thus, the characteristic size-range exhibited by each species must, in the main, be genetically determined, though environmental influences are detectable in various ways, not all of which can be expressed by the histograms illustrated. Thus, extreme environmental pressures leading to reduced numbers regardless of size must be assumed to explain the absence of *B. spinifera* and *B. antennigera* at temperatures above 16 °C, while the equivalent but inverse effect on *B. minor* seems to have led to reduced frequency without change of size at temperatures below 4 °C, as represented in figures 5 and 6. Temperature is, nevertheless, not the only operative environmental factor, since climatic effects unrelated to temperature have to be invoked to account for the concentration of small cells of *B. minor* at 6–10 °C in Katchemak Bay, near Homer (south Alaska), and of unusually large cells of the same species at 22 °C in the Galapagos Islands.

Though the degree of difference in lorica length between the largest and smallest individuals in any one species is of the order of 1:2 or 1:2½ for the majority of cells, the nature of the genetical control of size is not necessarily uniform. Thus, the histograms for *B. minor* and *B. antennigera* (figure 39*a*, *c*) are approximately unimodal, suggesting a relatively simple type of genetical control of size in these. The histogram for *B. spinifera* (figure 39*b*), in contrast, is less obviously unimodal, thereby suggesting that this taxon may, in fact, contain more than one biotype involving size, on which environmental selection might act. If this were true, the presence of a few residual small cells among a majority of large ones in all the arctic regions tested (i.e. west Greenland, Alaska and arctic Canada, as illustrated here by figure 21*c*), or of exclusively small cells in the very different climate of South Africa (figure 17) might denote no more than aspects of environmental selection among pre-existing biotypes.

On the other hand, modifications directly induced by environmental action have also been demonstrated, most clearly so in *B. spinifera*. As summarized in table 2, there is a tendency for maximum size in this taxon to increase inversely with temperature, in a manner not easily explained in terms of mere selection. The same is true of the degree of difference between members of pairs of anterior spines, which vary similarly with temperature, in their relative lengths, thicknesses and degrees of attenuation of the tips. Reduced growth-rate as a direct consequence of cold, leading to lengthening of critical developmental stages, seems likely to be the operative factor here.

Apart from these temperature effects, which have in themselves provided a major objective for the documentation presented, other observations, of general biological interest, include the clear demonstration in *B. spinifera* that costal strips far longer than the lorica chamber, or protoplast in an inactive condition, can sometimes be regularly produced. This was especially conspicuous with respect to the posterior spine in specimens of all sizes, as discussed on p. 441. This capacity is not shared by the other two species and a mechanism for it has not as yet been fully elucidated. A relevant observation that the short costal strips characteristic of the chamber wall are formed first in the replication cycle (see p. 442 and figure 25) nevertheless pin points the later stages as those most in need of further investigation from this point of view.

An unexpected finding was the important part played in speciation by differences in the manner in which the protoplast is held in position. In all three taxa, the membrane responsible is attached to predetermined parts of the lorica, which, in each species, include certain over-

lapping joins on the longitudinal costae. In *B. antennigera* there is only one such join accessible on each side, since the chamber itself, in that case, is delimited by no more than the lowermost longitudinal strips. For the other two species in which major structural significance is vested in a pair of costal strips on each side, the most conspicuous sites for attachment of the membranes are the uppermost joins, although in both of these taxa the lower ones are probably also involved. Such involvement was suggested in *B. minor* by the shape of the truncated tube retained after partial degradation, as in figure 10; but, if it occurred also in *B. spinifera*, a functional explanation for some of the differences between these taxa can perhaps be suggested. In *B. minor*, the two successive joins on each side are superimposed along a straight line (defined by the relevant longitudinal costa). In *B. spinifera*, on the other hand, the crossed relation of the longitudinal costae ensures that opposite ends of their middle strips will alternate with respect to the long axis of the cell. Such an arrangement, if it also affected the major sites of attachment of the membrane carrying the protoplast, might contribute an essential mechanical factor towards achievement of the greater length compared with width of the chamber which is characteristic of this species.

Finally, the membrane itself, not previously introduced into the descriptions of any of these species, has been shown to possess a greater number of functionally significant morphological features, notably its bipartite construction regardless of shape, than could previously have been recognized.

Conclusions

Genetical diversity of many different kinds must underly the numerous structural and distributional differences encountered in and between these three species. Extreme, or even less than extreme, environments are clearly important for exercising selection leading to exclusion, though this occurs to a different degree in different taxa, hence the wider temperature tolerance of *B. minor* compared with the other two species. Other environmental effects, notably those leading to local correlations between lorica and spine length, apparently under the direct influence of low temperature acting on development, are less conspicuous than might have been expected, being clearly demonstrable only in one (*B. spinifera*) among the three species studied. While there are no immediate taxonomic implications of these findings with respect to *Bicosta* itself, they are relevant, as a basis for comparison or contrast, to many superficially similar situations in other genera.

Revised taxonomic diagnoses

Bicosta Leadbeater

Lorica composed of seven costal strips arranged as two longitudinal costae and a posterior spine; no transverse costae. Each longitudinal costa containing three successive costal strips and acting as a spine anteriorly. The protoplast closely invested by a tubular or conical membrane attached anteriorly to the costal strip junctions delimiting the chamber but prolonged beyond this anteriorly into a transparent sheath enveloping the bases of the tentacles.

B. spinifera (Throndsen) Leadbeater, *the type species*

Lorica slender throughout, commonly 45–80 μm long, but can exceed 100 μm under arctic conditions, the anterior spines usually unequal in length and finely pointed, especially in large specimens; the posterior spine also attenuated, generally substantially longer than the chamber

or anterior spines and S-shaped terminally. The chamber wall delimited by the two lower segments in each longitudinal costa, these costae being crossed midway down the chamber. The protoplast membrane, about three times as long as wide, attached to the uppermost strip junctions. Tentacles usually about 30 in number.

Type locality: Barents Sea (Throndsen 1970); also found and often abundant in west Greenland, arctic Canada, north and south Alaska and near Cape Town, but not at temperatures above 16 °C.

B. minor (Reynolds) Leadbeater

Smaller than *B. spinifera* and without the crossed condition of the longitudinal costae. Length commonly 20–30 μm though exceptionally larger or smaller. All spines approximately equal to the chamber in length though the posterior spine slightly shorter and curved but not S-shaped. Protoplast shorter and more rounded than in *B. spinifera* but otherwise similar. Mode of attachment to the lorica also similar though the subtending membrane shorter and more delicate.

Type locality: Barents Sea (Reynolds 1976); also found in Europe, west Greenland, arctic Canada, north and south Alaska, Cape Town, New Zealand (Moestrup 1979) and the Galapagos Islands, the temperatures collectively ranging from −1 to 22 °C.

B. antennigera Moestrup

Lorica length commonly 40–80 μm, the anterior spines strongly curved inwards and resembling pincers, each composed of two successive costal strips, the proximal strip considerably shorter than the distal strip. The posterior spine more or less equalling or slightly shorter than the anterior distal strip, often curved but not S-shaped. The lorica chamber short and conical with only one longitudinal costal strip delimiting each side. The protoplast a little larger than in either of the other species, commonly projecting beyond the anterior edge of the chamber and with up to 40 tentacles. The subtending membrane attached to the posterior strip junction but not at all to the anterior equivalent.

Type locality: New Zealand (Moestrup 1979). Also present in the Northern Hemisphere, from west Greenland to arctic Canada and Alaska (both north and south), at temperatures from −1 °C to 10 °C.

Acknowledgements have already been made several times for practical help in organizing the various journeys. Special thanks must nevertheless be expressed here to Dr Stanley Walker of the Cytogenetic Unit at the University of Liverpool Medical School for use of the light microscope that provided most of the photographs made with phase contrast assembled on plates 1, 3, 5 etc. We are also grateful to Dr Barry Leadbeater of Birmingham University, for helpful discussions in relation to the manuscript.

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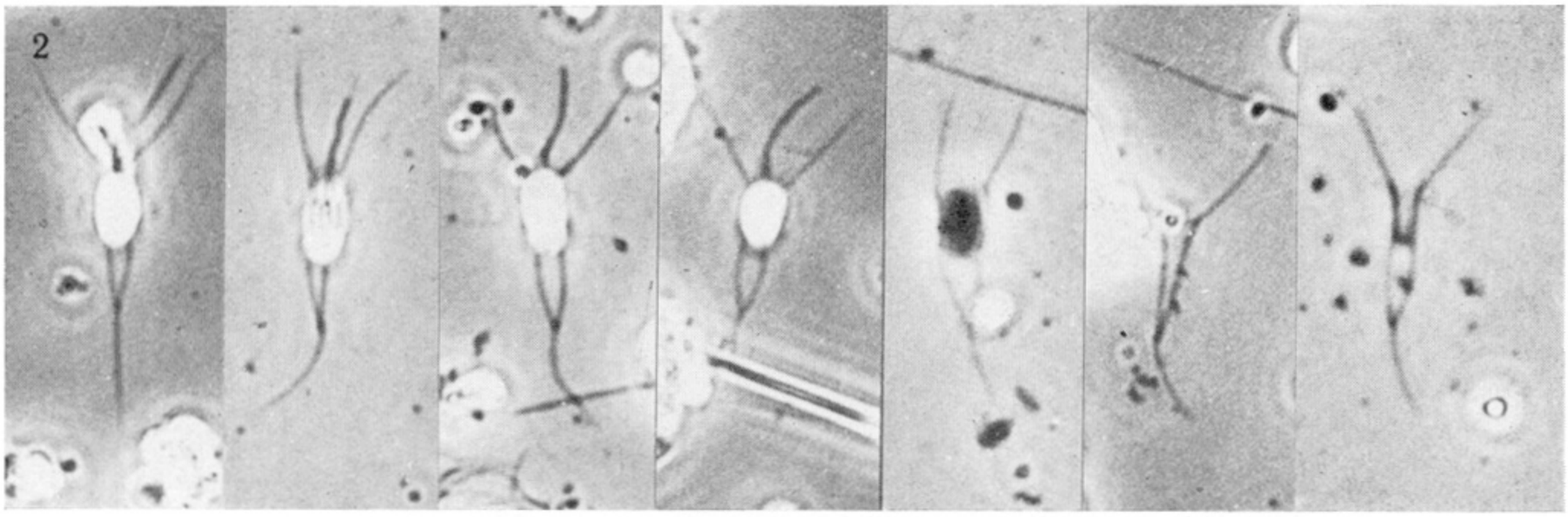
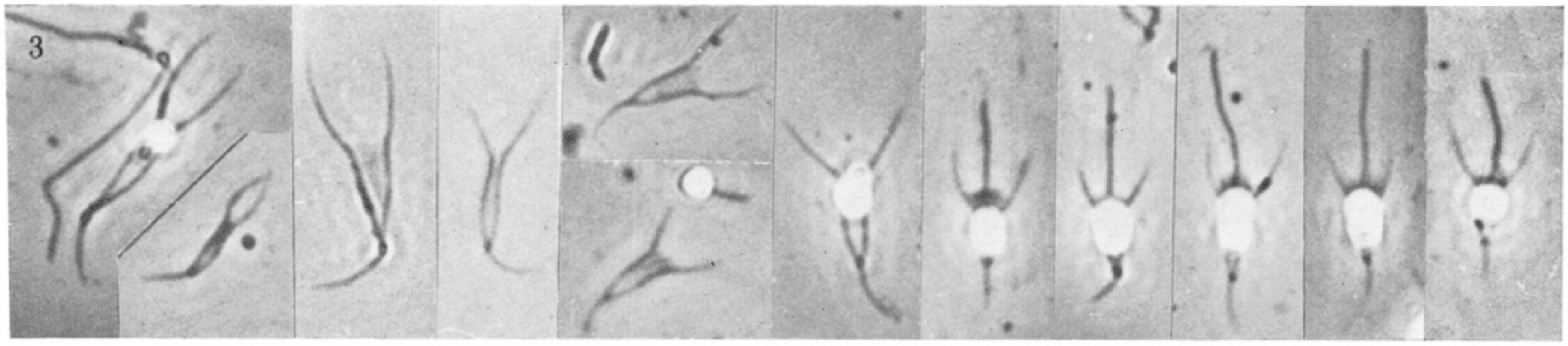
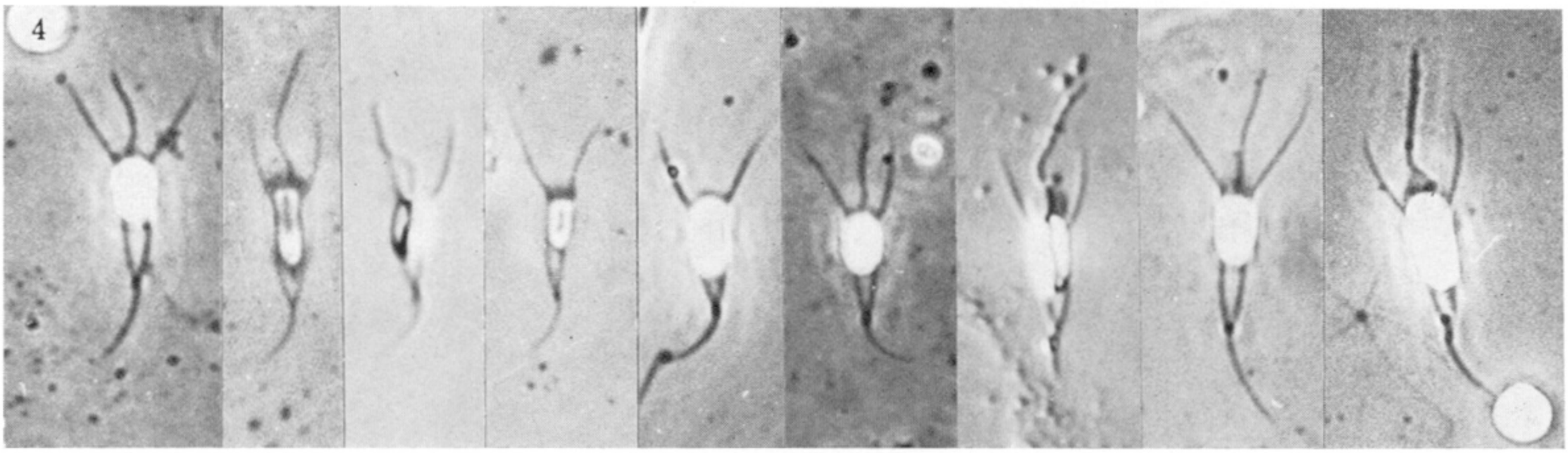


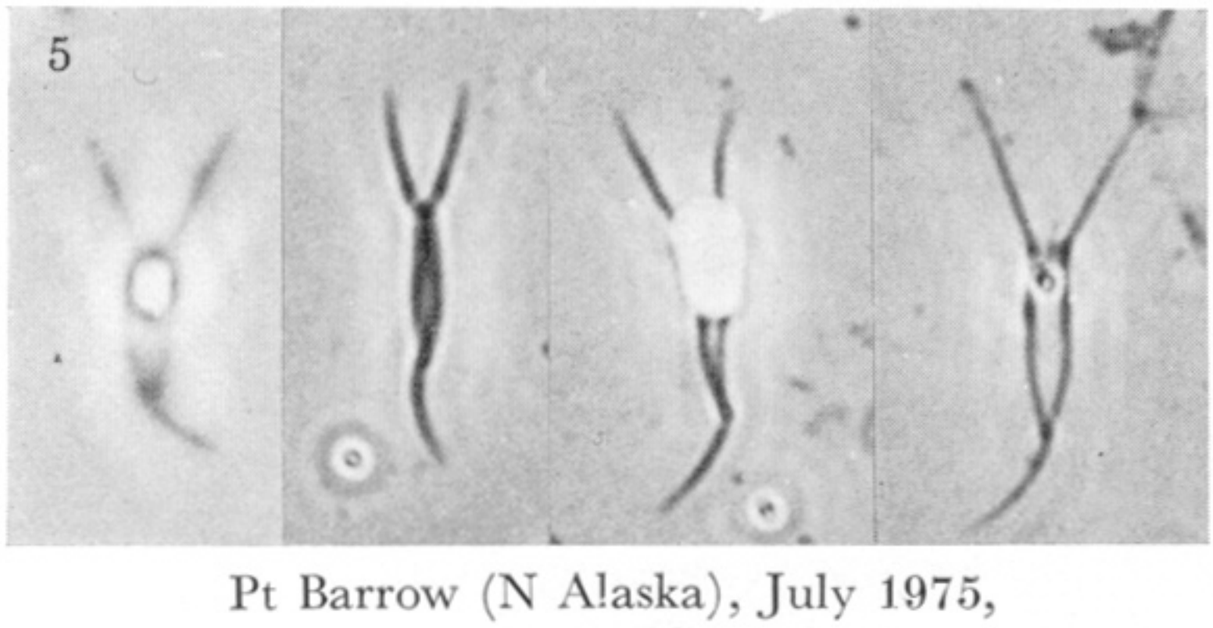
Portsmouth (U.K.), June 1977, sea temperature 16.5 °C



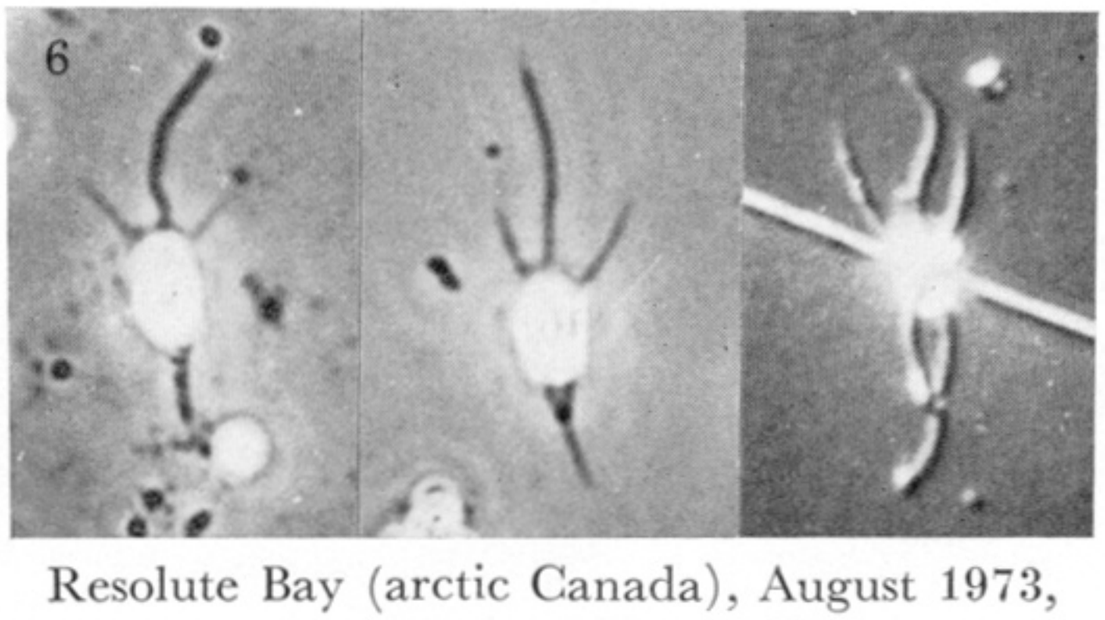
Homer (S Alaska), June 1975, sea temperature *ca.* 9 °C



Godhavn (W Greenland), June 1972, sea temperature 4 °C



Pt Barrow (N Alaska), July 1975,
temperature 0 °C, under ice

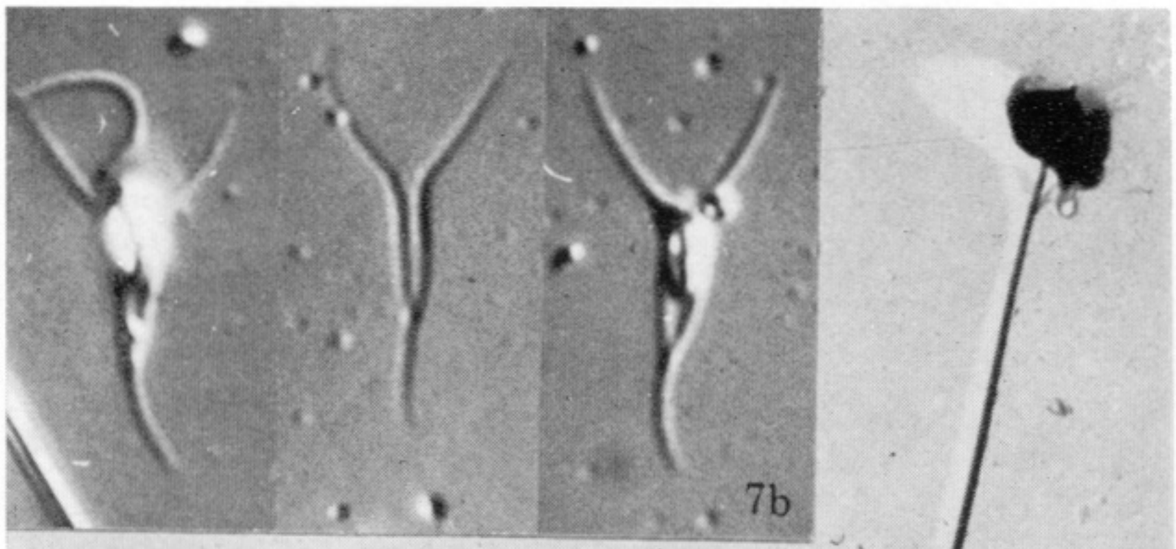


Resolute Bay (arctic Canada), August 1973,
sea temperature −1 °C

Description of plate 1

Bicosta minor. Light microscopy (mainly phase contrast) from dry whole mounts of wild material. (Magn. × 1000.)

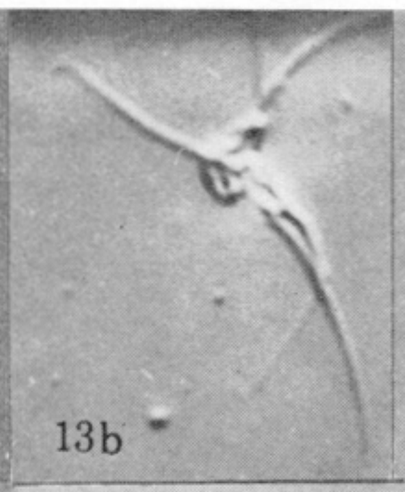
Figure 2. Seven specimens from a single sample (films 164 and 155).
Figure 3. Three specimens from sample viii (film 163) and nine from sample v (films 161 and 162).
Figure 4. Nine specimens from four samples (exposures on films: 11, 15, 22, 34, 40, 79, 161, 167).
Figure 5. Each specimen from a different sample (films 55, 88, 90, 91).
Figure 6. Each specimen from a different sample (films 36, 94, 112).

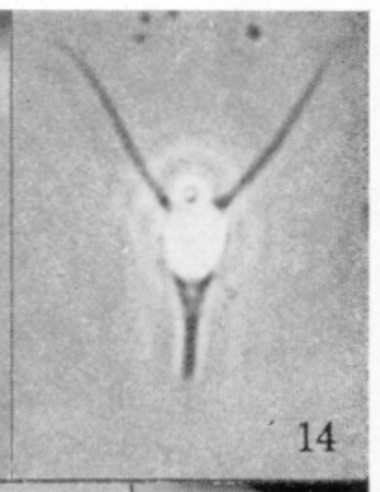

7b

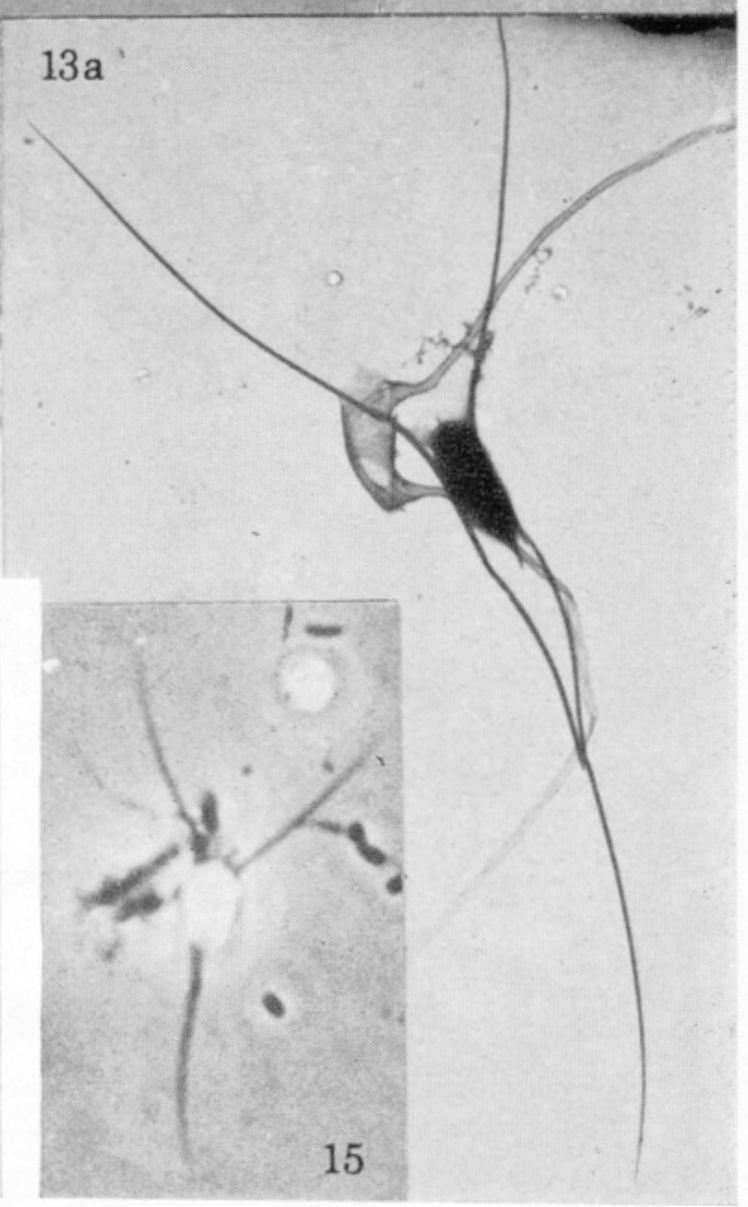
7a

12a

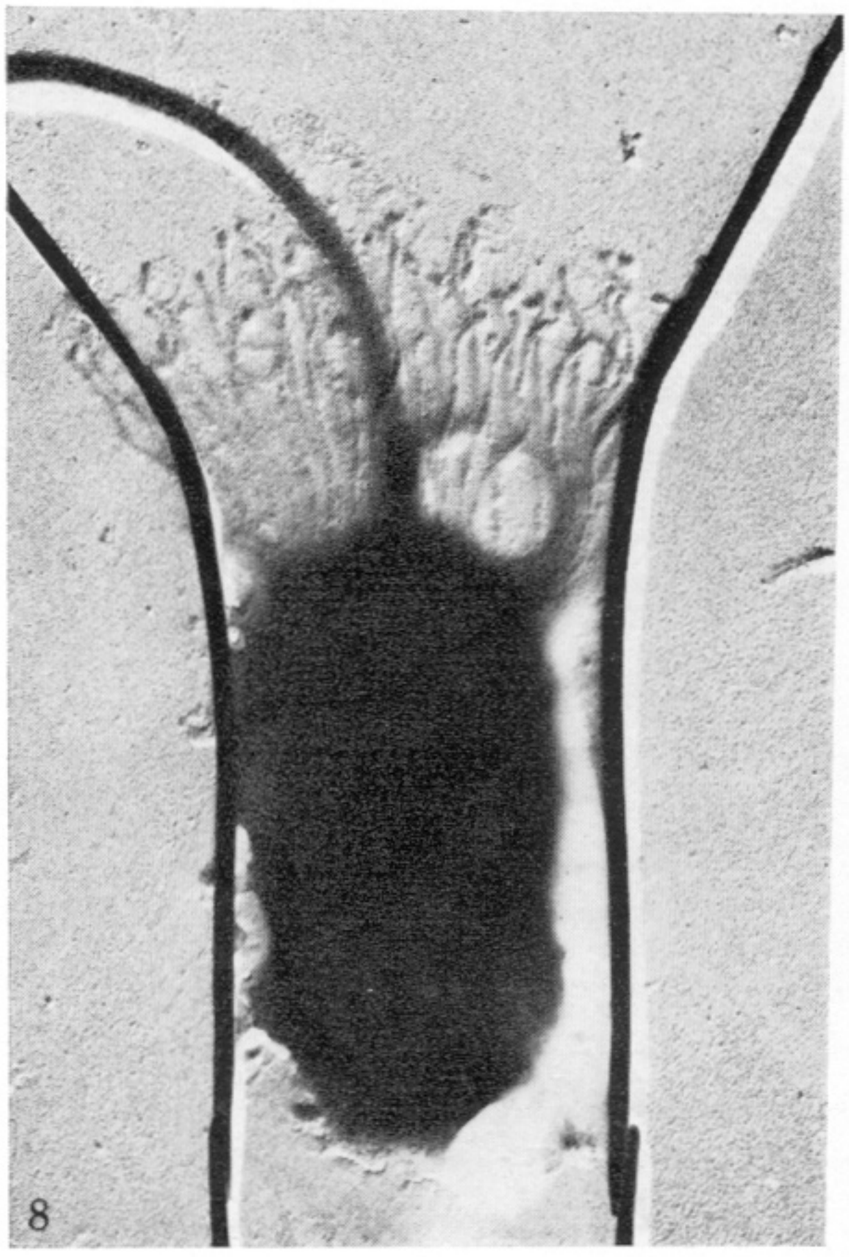
12b

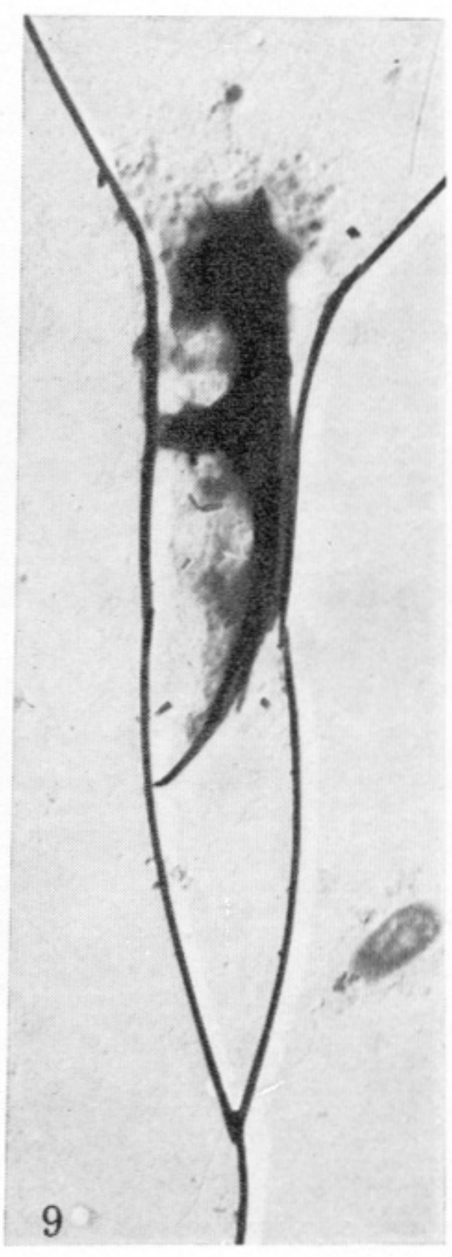

13b

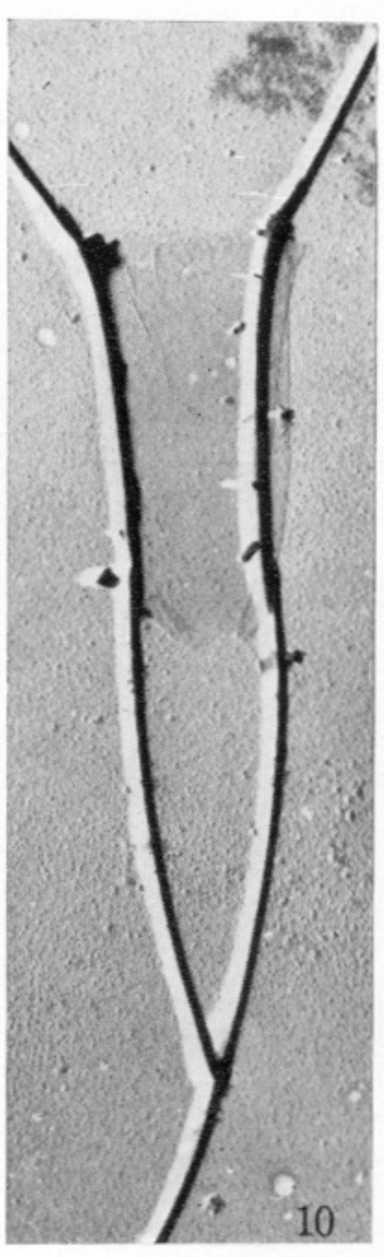

14

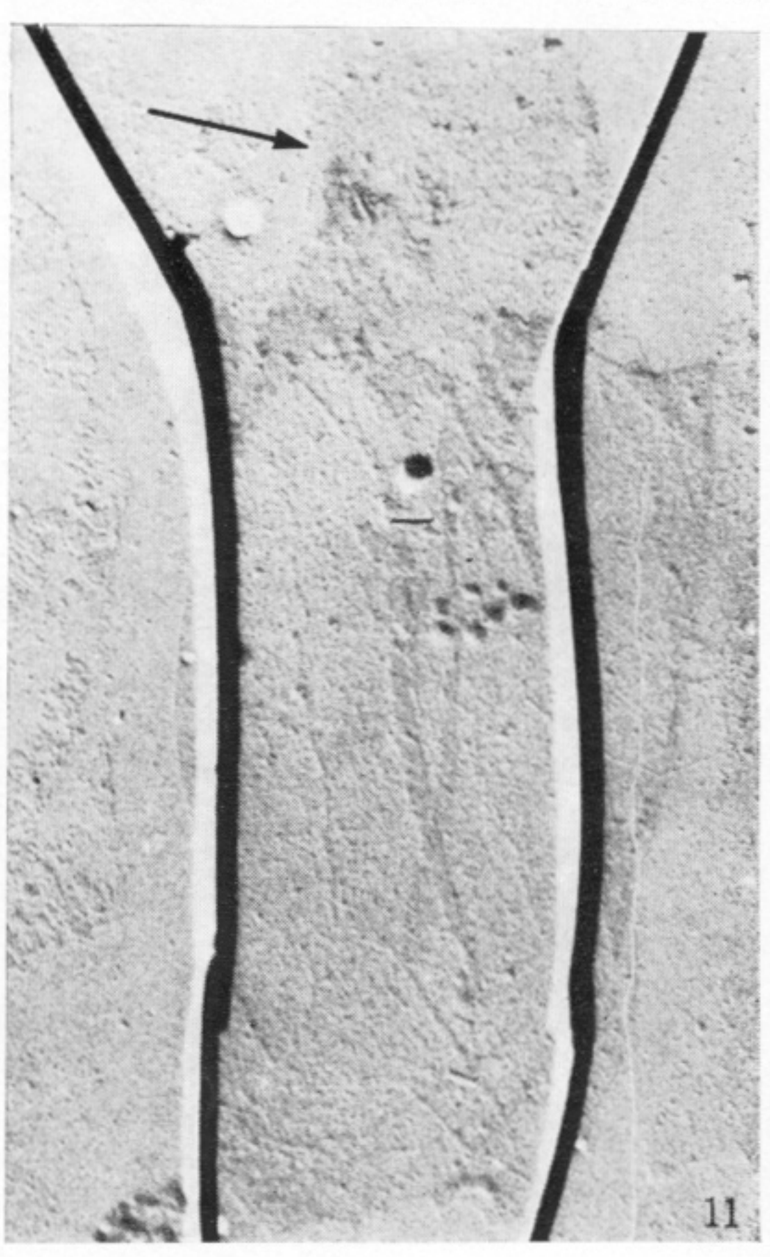

13a

15


8


9


10


11

FIGURES 7–15. For description see opposite.

16

17

18a

18b

19

20a

20b

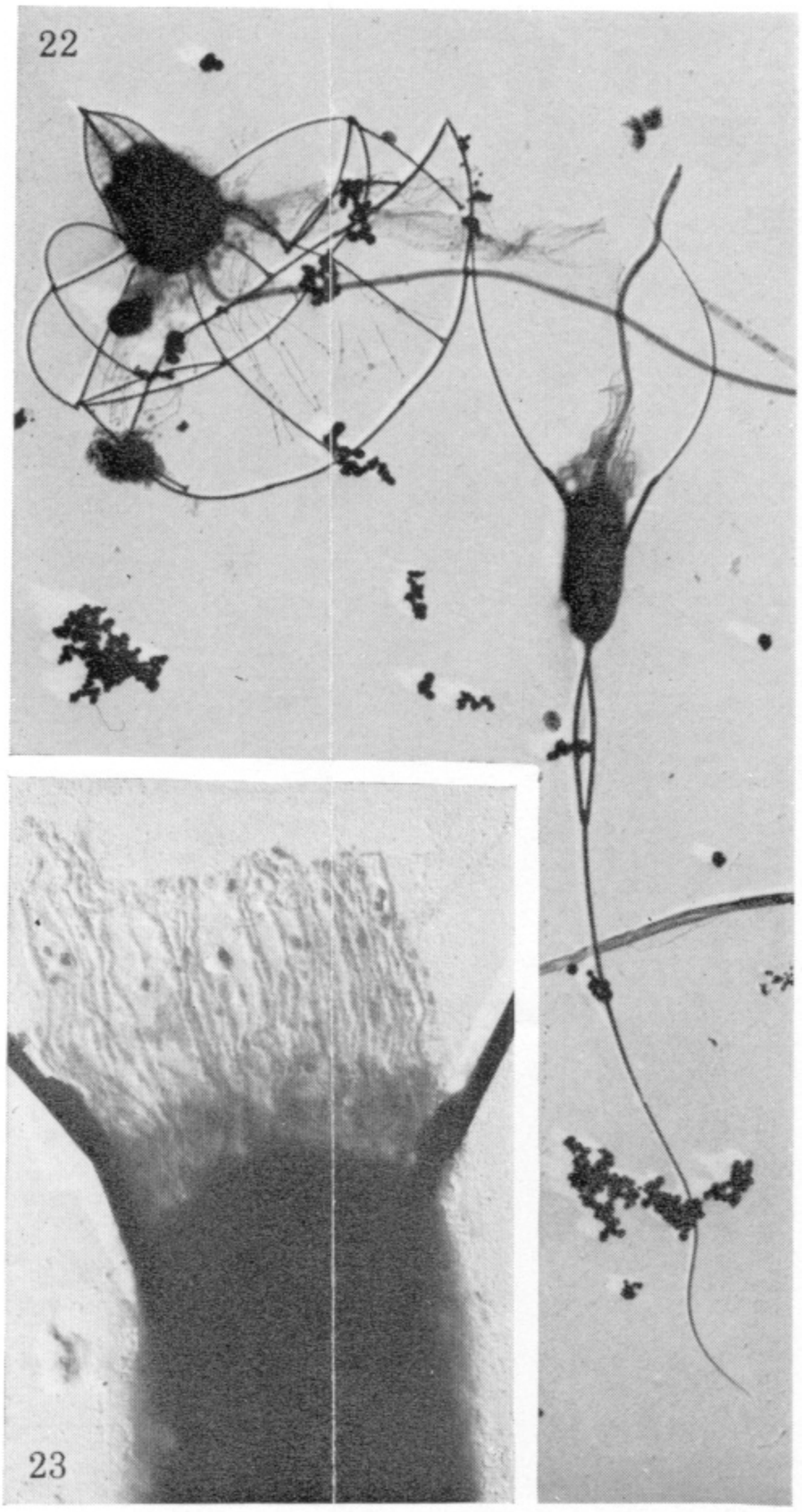
20c

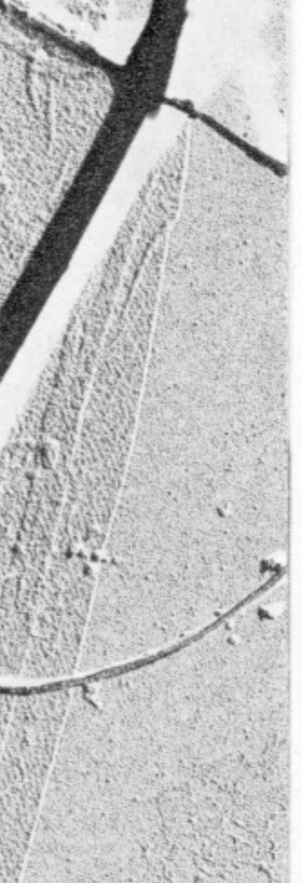
21a

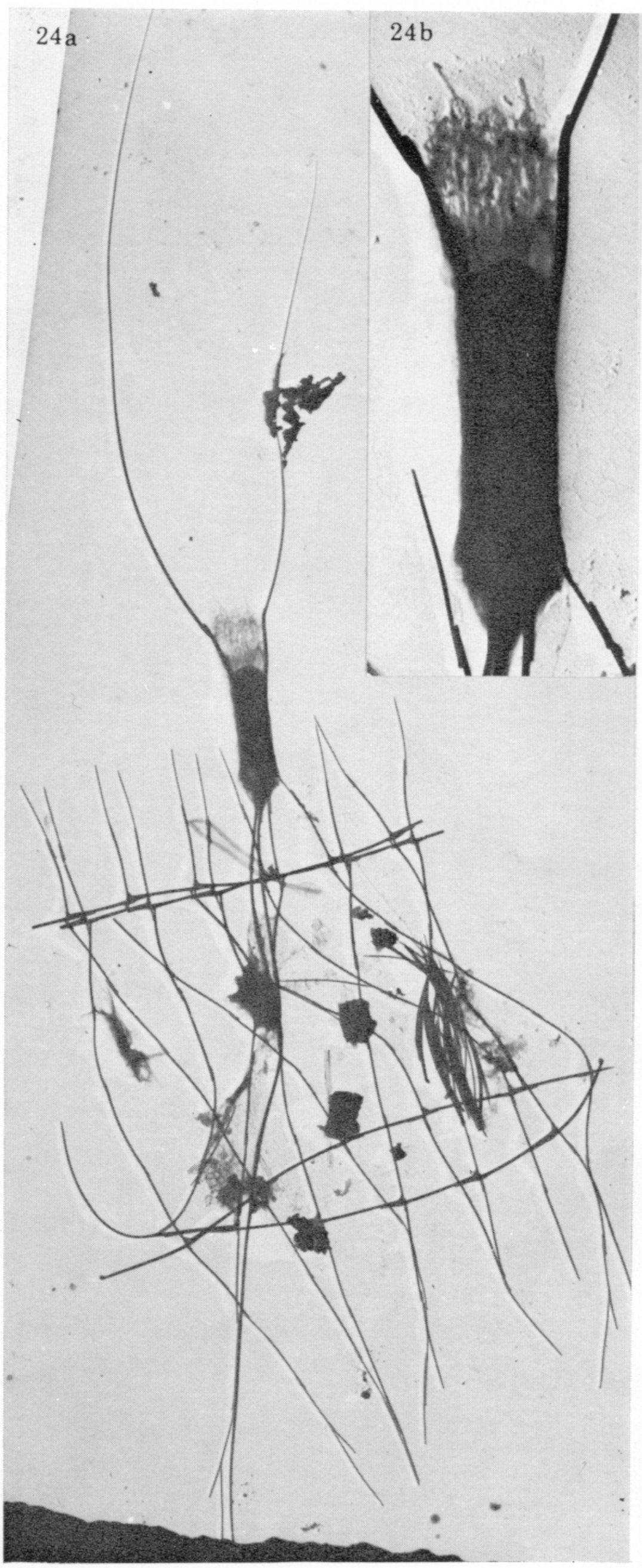
21b

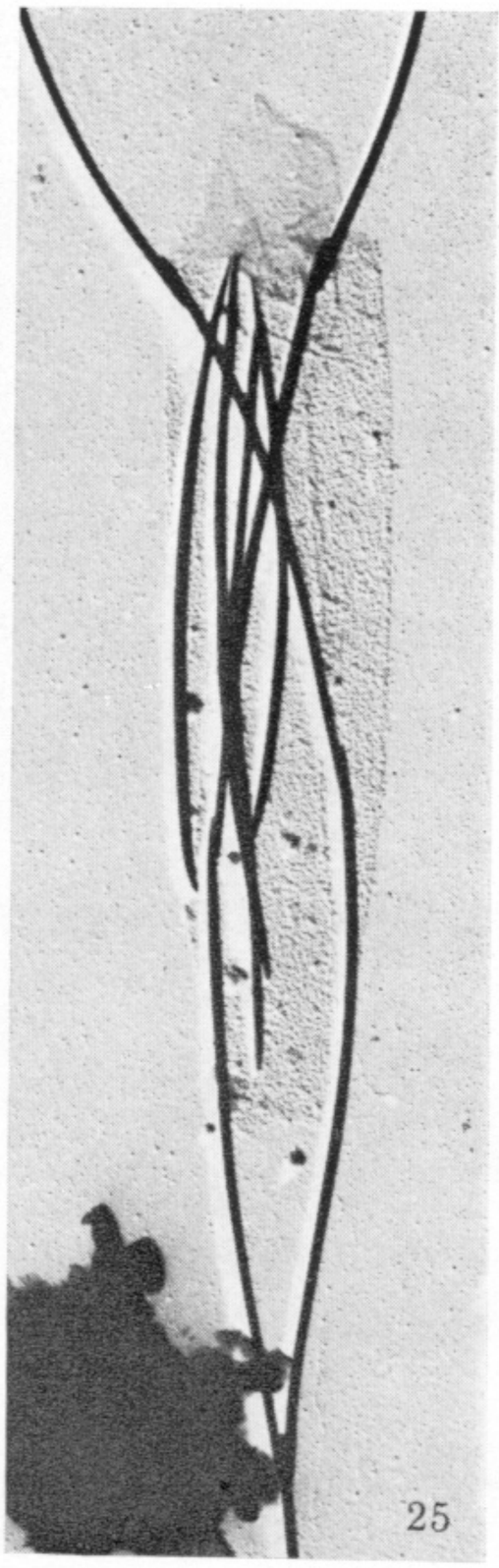
21c

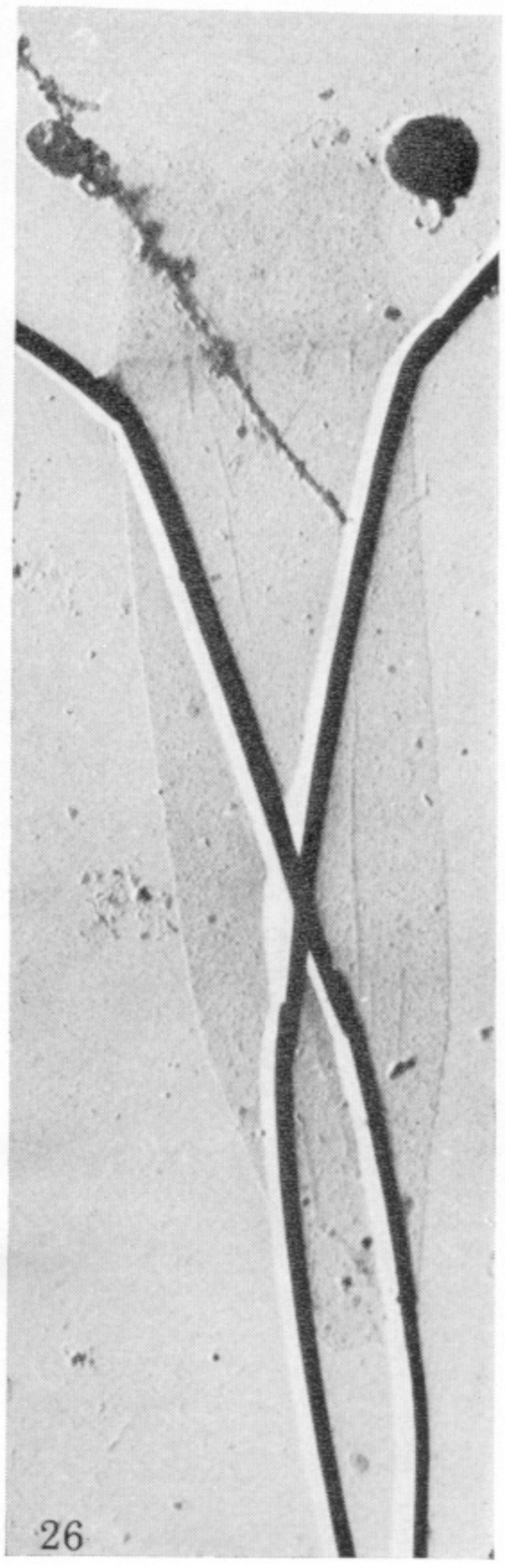
FIGURES 16–21. For description see opposite.



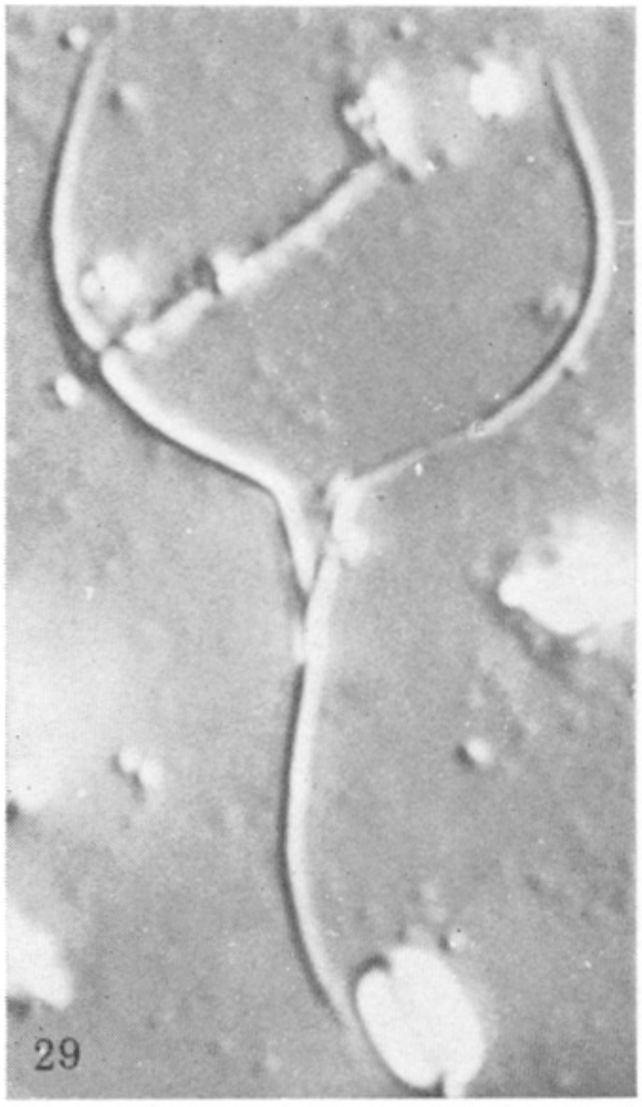


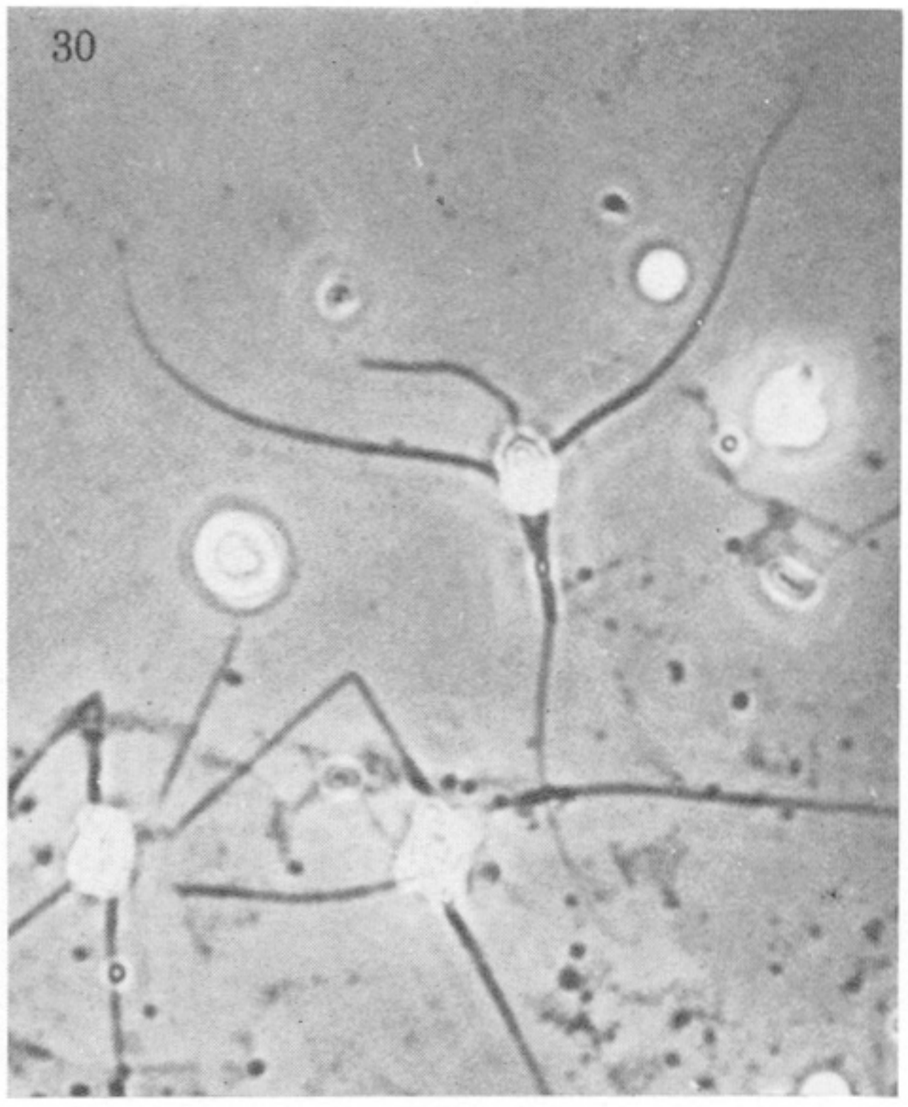


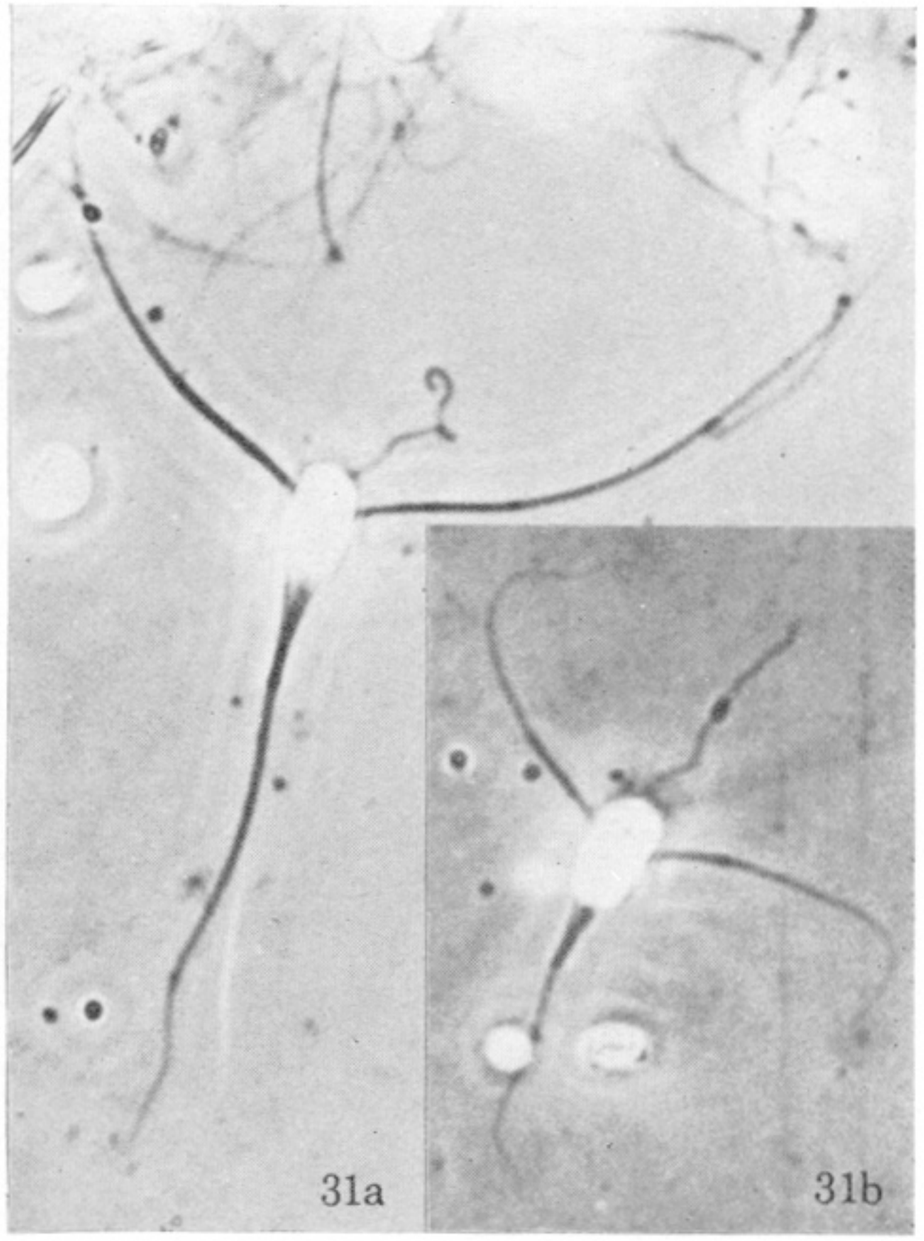




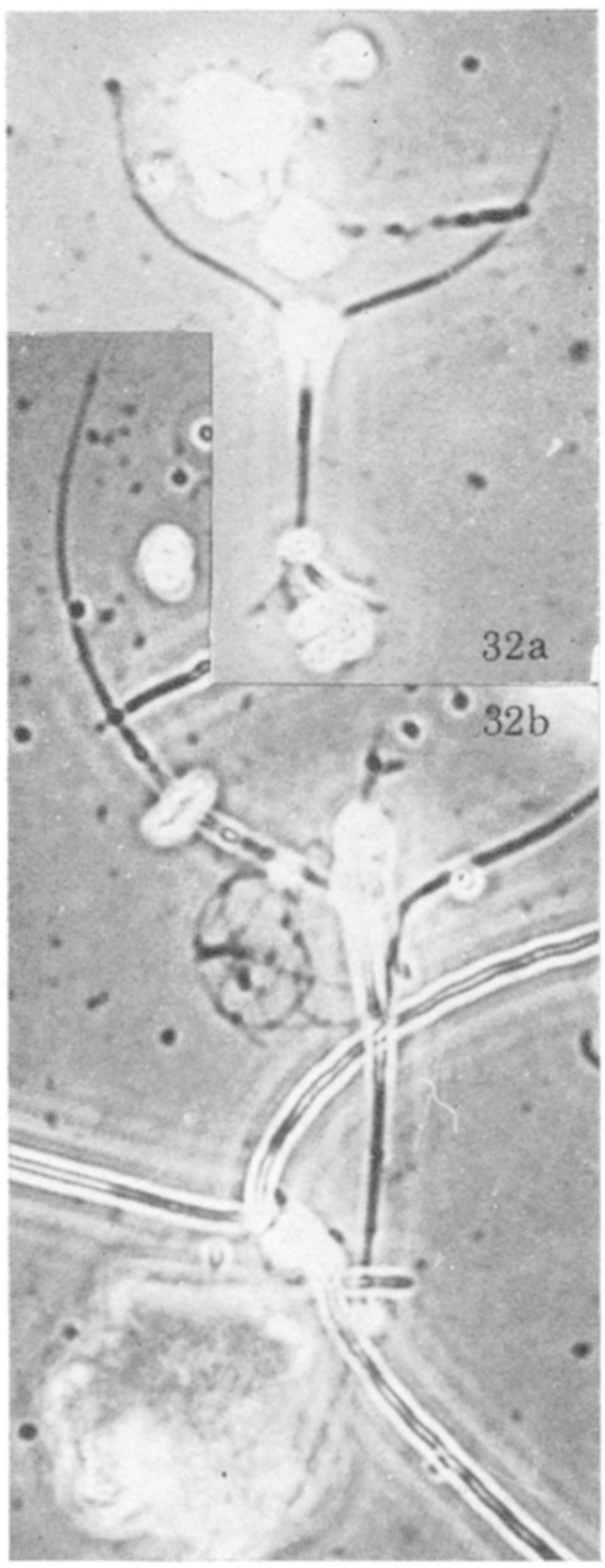
FIGURES 22–28. For description see opposite.


29

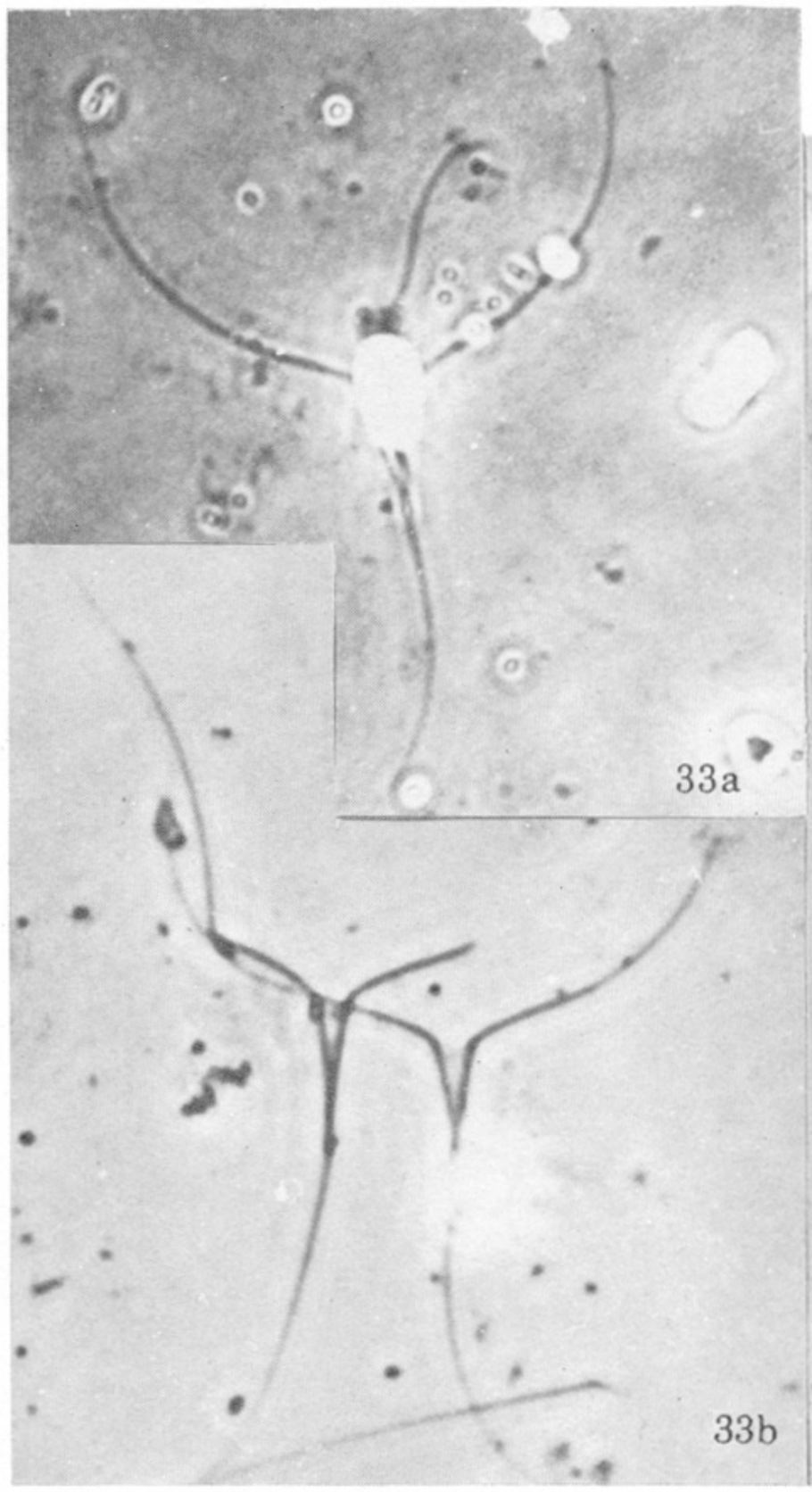

30


31a

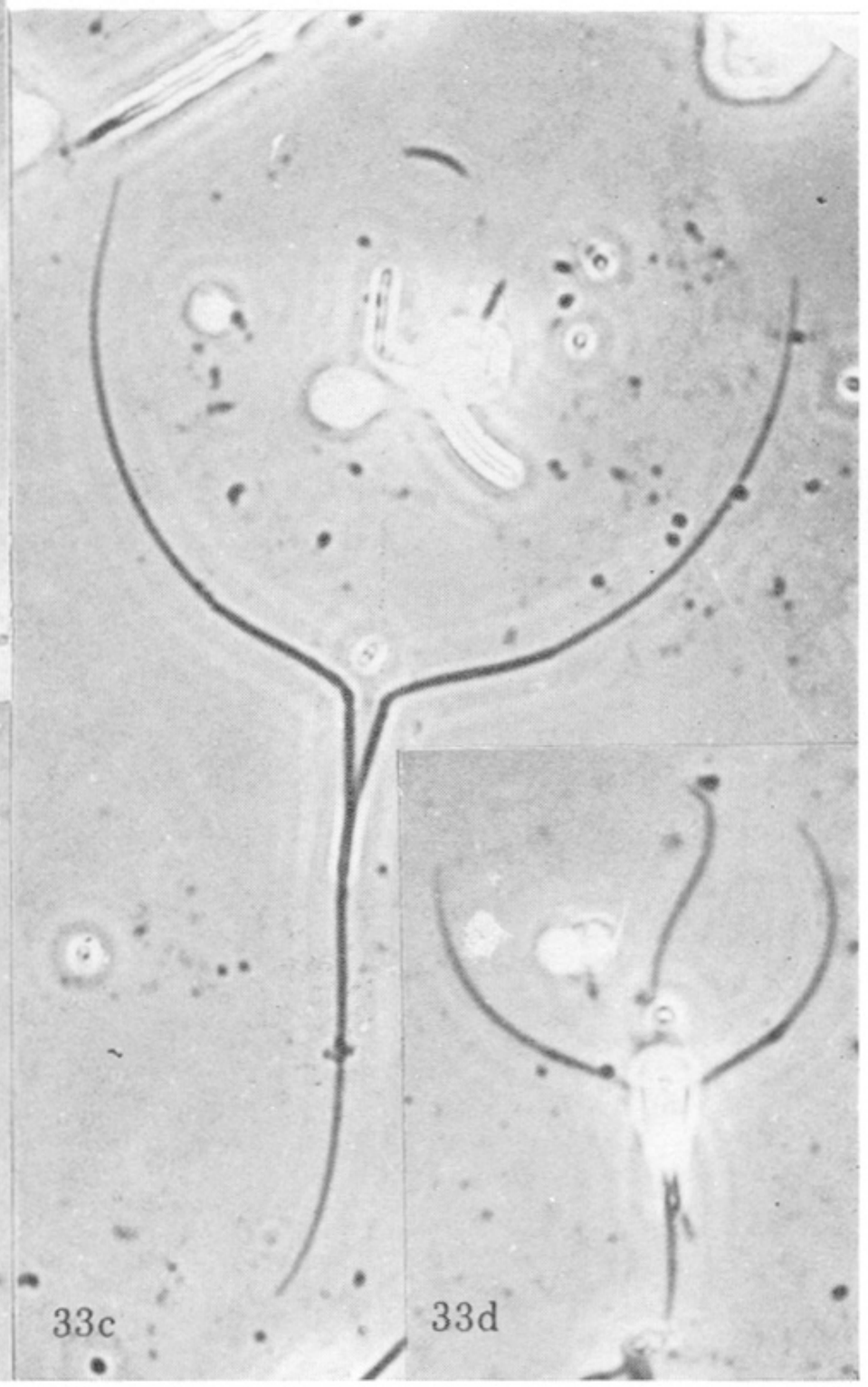
31b


32a

32b

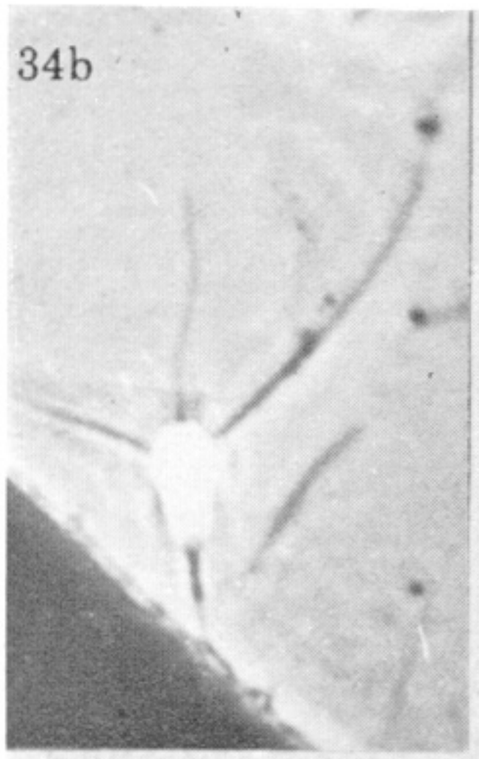

33a

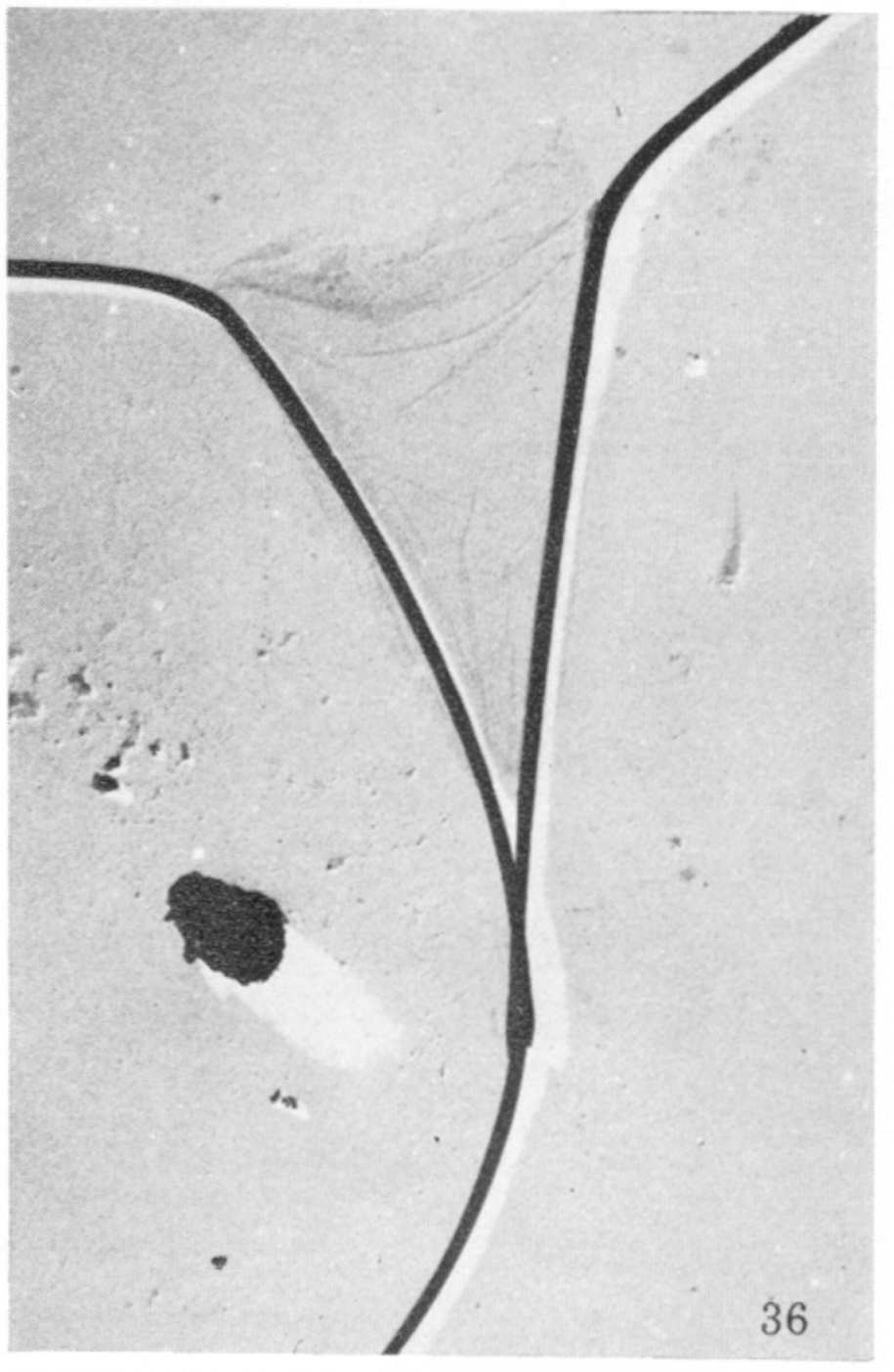
33b


33c

33d

FIGURES 29–33. For description see opposite.





FIGURES 34–38. For description see opposite.