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A PARADIGM FOR THE STUDY OF INSECT–CILIATE RELATIONSHIPS: *TETRAHYMENA SIALIDOS* SP.NOV. (HYMENOSTOMATIDA: TETRAHYMENIDAE), PARASITE OF LARVAL *SIALIS LUTARIA* (LINN.) (MEGALOPTERA: SIALIDAE)

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[Plates 1–6]

CONTENTS

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A new species of hymenostome ciliate, *Tetrahymena sialidos* sp.nov., is described and proposed as a model for studies upon insect–ciliate interactions. This ciliate occurs as a parasite in the haemocoel of larval *Sialis lutaria* (Megaloptera) and exhibits field and laboratory characteristics favourable for two particular areas of study, the regulation of invertebrate populations by parasites and insect immunity to parasitic infections. The prevalence of *T. sialidos* infection in *S. lutaria* from a small lake in mid-Wales has been monitored during a four-year period. Each summer, early in the first of their two-year aquatic development, 40–70% of *S. lutaria* larvae become infected. Death of infected larvae due to parasite proliferation is inevitable but does not occur until 11–12 months after infection, when the mean intensity has reached 55000 ciliates per infected host. Ciliates escaping from moribund or dead host larvae undergo a distinct morphological transformation followed by a period of synchronous conjugation. Death of infected host larvae and release of ciliates coincides with the appearance of a new generation of host larvae in which the prevalence of infection rises rapidly. The possible significance of this substantial and persistent regulatory influence upon host population dynamics is discussed in relation to current ideas of parasite regulation of invertebrate populations. *Sialis lutaria* is not able normally to mount a successful host response against *T. sialidos* infections. A laboratory thermal-shock technique is described by means of which parasitic ciliates may be killed *in situ* without incurring physical damage to the host. Infected larvae subjected to thermal-shock at 39 °C for 1 h subsequently encapsulate and melanize the dead ciliates. The use of this technique is discussed in relation to recognition of non-self in insects and survival strategies of parasites infecting insects.

Introduction

The ciliate genus *Tetrahymena* Furgason, 1940 contains a number of species that manifest parasitic tendencies ranging from natural or experimental facultative parasitism to obligate parasitism (Corliss 1973). With the exception of a single species, *T. corlissi*, the parasitic habit always involves invertebrate hosts, most commonly gastropod molluscs or aquatic dipteran larvae (Corliss 1973). These associations represent relatively unexploited models for the study of ciliate biology in general and host–parasite relationships in particular. In a previous paper (Batson 1983) attention has been drawn to a new parasitic ciliate with valuable potential in the context of ciliate morphogenesis. The aim of this paper is to draw attention to another novel ciliate which provides additional exciting opportunities. The selected organism is a new species of hymenostome ciliate which is parasitic in larval alder-flies (Megaloptera) and for which the name *Tetrahymena sialidos* sp.nov. is proposed. Field and laboratory observations upon this association clearly indicate its considerable value in providing insight into two specific areas of host–parasite interaction, namely the role of parasitism in the regulation of insect populations and parasite strategies in the evasion of insect immunity.

Materials and methods

Collection and maintenance of hosts

During the four-year period from early 1980 to early 1984 samples of benthic fauna were collected by hand net from the muddy bottom of Llyn Frongoch, a small oligotrophic lake in mid-Wales. Samples were taken more frequently during the summer than during the winter and the interval between samples varied from one week to several months. Larval *Sialis lutaria* were removed from the benthic sediments and organic debris immediately upon return to the laboratory. Cannibalism was avoided by isolating larvae individually in small containers of lake

water. Larvae were maintained in the dark at 5, 10 or 15 °C in accordance with the prevailing lake temperature, which varied from *ca.* 5 °C in winter to *ca.* 15 °C in summer (Hanlon 1985), and they were fed regularly with chironomid larvae. Specific identity of alder-fly larvae was confirmed by using the key of Elliott (1977).

Determination of the presence of parasitic ciliates required the use of transmitted light microscopy at magnifications of × 25 or more. For each collection the mean number of alder-fly larvae examined in this way was 97 ± 29 (53–136, $n = 29$). To determine the host specificity of *Tetrahymena sialidos* other invertebrates collected in the same samples as *S. lutaria* were routinely examined for the presence of parasitic ciliates.

Estimation of ciliate numbers in parasitized hosts

A modified Fuchs–Rosenthal haemocytometer was used to estimate the mean intensity of ciliate infections in *S. lutaria* larvae at selected times during the year. Freshly collected larvae were washed in distilled water, dried on filter paper and then individually decapitated in *ca.* 0.5 ml Da Fano's fixative (Mackinnon & Hawes 1961). Each body was then finely teased apart to release all ciliates. Fragments of tissues were drained of fluid and removed, and the remaining volume of ciliate suspension was determined. Standard haemocytometric methods were used for estimation of the total number of parasitic ciliates per infected host. At least 250 ciliates were counted from each larva. Ten replicate larvae were used from each age-group. Estimation of ciliate numbers in early infections was made by total parasite counts.

Light microscopy

Observations and photographs of living and preserved ciliates were made with a Leitz Dialux 20 photomicroscope. Living specimens were photographed with the aid of an automatic microflash (Batson & Lloyd 1981).

Successful silver impregnation of parasitic ciliates required slight modification of the Chatton–Lwoff technique (Corliss 1953). The numerous inclusions that pack the cytoplasm of *T. sialidos* rapidly absorbed osmium during primary fixation in Champy's fixative, resulting in a degree of internal staining that obscured the fine cortical detail normally revealed by subsequent silver impregnation. This problem was alleviated by reduction of primary fixation time in Champy's fixative to 10–25 s, rather than the usual 2–5 min.

Nuclear characteristics of the ciliates were studied using the Feulgen nucleal reaction (Pearse 1968). Ciliates were examined for the presence of lipids by staining air-dried smears with oil-red-O or Sudan-black-B (Pearse 1968). Control smears were first extracted with a 2:1 mixture of chloroform and methanol.

Scanning electron microscopy

Ciliates from infected *S. lutaria* were layered upon 0.45 μm Millipore filters, washed with Clark's insect saline (Hale 1976) and then fixed with 0.27 M glutaraldehyde in 0.1 M Dulbecco's phosphate buffer at pH 7.1 for 1 h at room temperature. Following several washes in buffer, secondary fixation was in 10 g l^{-1} osmium tetroxide in the same buffer for a further 1 h at room temperature. After dehydration in a graded ethanol series, specimens were transferred from absolute ethanol through an ethanol–fluorisol series to absolute fluorisol, then dried in a Polaron E3000 critical point drying apparatus. The Millipore filters were attached to metal stubs, coated with gold in a Polaron E5000 coating unit and examined in a Cambridge 150 scanning electron microscope.

Establishment and maintenance of axenic and non-axenic ciliate cultures

Infusions

Infected *S. lutaria* larvae were briefly surface-sterilized by immersion in 70% (by volume) ethanol, washed twice in distilled water, and then teased apart in a small Petri dish containing 3–5 ml of filtered lake water or Prescott's and James's solution (Prescott & James 1955). Large fragments of the host's body were removed. The released ciliates thrived upon the bacterial bloom that developed in the diluted host body fluids and around remaining small fragments of host tissue.

Axenic culture

Isolation of *T. sialidos* for axenic culture was carried out under a dissecting microscope in a horizontal laminar-flow sterile cabinet, with sterile technique observed throughout the procedure.

Following surface sterilization with 70% (by volume) ethanol, infected *S. lutaria* were rinsed in several changes of sterile distilled water and transferred to sterile culture medium. A small incision was made in the abdominal wall of the host, thus producing a stream of haemolymph containing ciliates. Ciliates were transferred with micropipettes through a series of culture medium washes to tissue culture flasks containing 15–20 ml of the culture medium. Axenic cultivation used proteose–peptone–yeast-extract medium comprising 10 g l^{-1} proteose-peptone (Difco) and 2.5 g l^{-1} yeast extract (Difco). Cultures were routinely maintained at 15 °C with subculture at 5- to 30-day intervals.

Experimental infection of alder-fly larvae

Egg batches of *S. lutaria* were collected from vegetation overhanging the margins of Llyn Frongoch. After hatching, young larvae were maintained at 15 °C in the laboratory in lake sediment that had been previously heat-sterilized and to which filtered lake water was added. Larvae in their early instars were exposed to free-living populations of *T. sialidos* prepared in infusions as described above.

Induction of haemocytic encapsulation of Tetrahymena sialidos by thermal shock treatment

Larvae of *S. lutaria* previously acclimated at 5, 10, 15 or 20 °C for at least five days were dried with filter paper and then transferred to pre-heated containers of distilled water at 30, 36 or 39 °C for periods of 0.5–48 h. After return to maintenance temperatures larvae were periodically examined and the effect of thermal shock upon host and parasites was recorded. Some larvae were dissected in Clark's insect saline to permit detailed photomicroscopy.

Results

Description of Tetrahymena sialidos sp.nov.

(figures 2–23, 27–28†, table 3)

Diagnosis

Possesses parasitic and free-living phases.

Parasitic phase. Occurs in the haemocoel of larval *Sialis lutaria*; broadly oval with dense

† Figures 2–5 appear on plate 1, figures 6–14 on plate 2, figures 15–18 on plate 3, figures 19–26 on plate 4, figures 27–32 on plate 5 and figures 33–38 on plate 6.

granular cytoplasm; unimicronucleate; mean somatic dimensions *ca.* 51 μm × 33 μm; dense somatic ciliature; mean total somatic kineties 24.7 (22–27); one or two post-oral kineties; one to five contractile vacuole pores; infection synchronised with host life cycle; infection lethal to host after 11–12 months.

Free-living phase. Elongate pyriform shape with sparsely granular cytoplasm; unimicronucleate; mean somatic dimensions *ca.* 32 μm × 18 μm; undergoes characteristic synchronous conjugation.

Type host

Larvae of *Sialis lutaria* (Linn.) (Megaloptera: Sialidae).

Type locality

Llyn Frongoch in mid-Wales, U.K.

Type specimens

The holotype slide comprising silver-stained ciliates from the haemocoel of *Sialis lutaria* has been deposited in the British Museum (Natural History), London and has the number 1984:5:4:1. A paratype slide of silver-stained material has been deposited in the International Protozoan Type Slide Collection at the Smithsonian Institution, Washington, D.C., U.S.A. and has the number U.S.N.M. 33143.

Life cycle of Sialis lutaria

The life cycle of *S. lutaria* in Llyn Frongoch conforms in general to the account given by Elliott (1977). A brief outline is appropriate here since much of the data concerning *T. sialidos* acquires significance only when viewed against the perspective of the host's life cycle.

In Llyn Frongoch *S. lutaria* has an aquatic larval state that lasts for almost two years. Mature larvae leave the water in April or May to pupate in chambers constructed in the soil or plant litter adjacent to the lake. Adults are usually on the wing in May and June, during which there is a four- to six-week period of egg laying. Batches of eggs are laid on vegetation or other convenient substrates which overhang the edge of the lake, so that hatching larvae fall into the water. First instar larvae are generally regarded to be planktonic, but the remaining nine instars live as predators within the muddy benthic sediments.

Dynamics of natural infection

The prevalence† of *Tetrahymena sialidos* infections in larvae of *Sialis lutaria* from early 1980 to early 1984 is summarized in figure 1. A clear annual pattern emerges from data covering five consecutive annual cohorts of alder-fly larvae.

Within a few weeks of hatching a substantial proportion of young *S. lutaria* larvae become infected with *T. sialidos*. The earliest host stage recorded to be infected was the second-instar larva. No infections were detected in an examination of 470 field-collected first-instar larvae, which are morphologically distinct from later instars (Elliott 1977). By the end of July the prevalence of infection reached a plateau which persisted through the autumn and winter months. The position of this plateau varied from *ca.* 40% prevalence in the 1980 cohort to *ca.* 70% in the 1983 cohort. The 1979, 1981 and 1982 cohorts all bore a prevalence of infection of *ca.* 60%.

† In this paper the use of ecological terms relating to parasitism conforms to that recommended by Margolis *et al.* (1982).

Figure 1 clearly illustrates the synchrony of parasite and host life cycles. In larvae approaching the end of their first year of development there was a dramatic decline in the prevalence of infection, due to parasite-induced host death (see next section). The consequent release of large populations of free-living ciliates coincided with the appearance of the new generation of *S. lutaria* larvae, in which the prevalence of infection increased rapidly (figure 1). This state of flux was brief and transference of *T. sialidos* infection from one host generation to the next was completed within a five- to eight-week period during June and July.

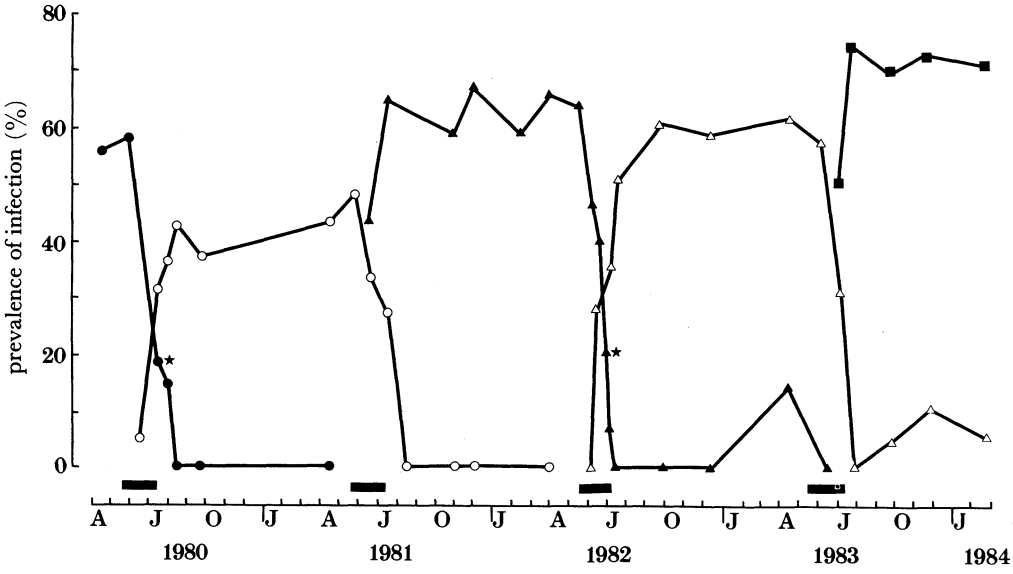

FIGURE 1. Prevalence of *Tetrahymena sialidos* sp.nov. infections in *Sialis lutaria* from Llyn Frongoch between April 1980 and February 1984. Cohorts originating in the following years are represented: 1979 (●), 1980 (○), 1981 (▲), 1982 (△) and 1983 (■). Black bars indicate the period of host egg-laying activity. Asterisks denote collections in which freshly infected second year larvae were recorded (see text for details).

Larvae that escaped infection during the first few weeks after hatching remained uninfected for their first year and could therefore continue into the second year of development. Sometimes, however, second-year larvae were recorded to be infected with *T. sialidos*. Infected second-year larvae never constituted more than 15% of the cohort and the pattern of infection was less clear than in first-year larvae (figure 1). Second-year larvae containing less than 20 ciliates were considered to be recently infected and although very few infections of this kind were encountered (two in July 1980, three in July 1982), it may be significant that they were only recorded during the usual infection period of the new larval generation (figure 1). Infected second-year larvae collected at other times always carried a high ciliate burden (over 2000).

Throughout the study period other invertebrates recovered from the same benthic samples as *S. lutaria* were routinely examined for the presence of parasitic ciliates. More than 800 chironomid larvae and a total of over 550 other larvae from the insect orders Diptera, Ephemeroptera, Odonata and Coleoptera were examined. No ciliate infections were ever recorded.

Pathogenicity

The quantitative development of ciliate populations within larvae of *S. lutaria* is illustrated for the 1982 cohort (table 1). In late July the mean intensity of infection was 147 ciliates per infected host. By December this modest figure had risen to 2180 and continued slow growth

of the ciliate population during the winter months led to a mean intensity of 3200 ciliates per infected host in April 1983. In the brief period from April to June the mean intensity of infection increased dramatically to 55200 ciliates per infected host. The pathogenic effects of such intense parasite proliferation became apparent shortly afterwards, when infected larvae died and the prevalence of infection declined to zero (figure 1).

TABLE 1. INTENSITY OF INFECTION OF THE 1982 COHORT OF *SIALIS LUTARIA* BY *TETRAHYMENA SIALIDOS* SP.NOV.

date	approximate host age/months	mean number of ciliates per infected host	s.d.	range	*n*
July 1982	1	147†	86	25–310	20
December 1982	6	2180	1080	460–3600	10
April 1983	10	3200	1800	1200–5700	10
June 1983	12	55200	15000	29000–76000	10

† Based on total ciliate counts, not haemocytometric estimates. s.d., Standard deviation.

It was necessary to determine whether the declining prevalence of infection in *S. lutaria* was in fact due to the onset of parasite pathogenicity rather than, for instance, the altered behaviour of infected larvae which might preclude their collection, or perhaps due to the host affecting a self-cure. In late June 1982 samples of freshly collected *S. lutaria* were maintained in the laboratory at approximate lake temperature (15 °C) and fed daily with chironomid larvae. Infected and uninfected larvae were isolated in individual containers and arranged in matching pairs of approximately corresponding body size. Survival of these larvae was monitored during a 25-day period corresponding to the recorded decline in the prevalence of infection in Llyn Frongoch. By day 25 all infected larvae had succumbed to the effects of the burgeoning ciliate population and had died, while all uninfected control larvae remained healthy and active (table 2). Infected larvae collected at other times of the year could be maintained in the laboratory at the corresponding lake temperature without mortality (table 2).

Infected second-year larvae appeared to be subject to the same pathogenic consequences of infection as first-year larvae. Five infected second-year larvae collected in May 1983 were maintained in the laboratory at 15 °C and fed daily with chironomid larvae. All three died within 14 days, while five uninfected control larvae remained healthy and active.

TABLE 2. LABORATORY SURVIVAL OF *SIALIS LUTARIA* LARVAE INFECTED WITH *TETRAHYMENA SIALIDOS* SP.NOV. IN COMPARISON WITH UNINFECTED CONTROL LARVAE

	one-year-old larvae collected in June, maintained at 15 °C		six-month-old larvae collected in December, maintained at 5 °C	
time from collection in days	number of surviving infected larvae	number of surviving uninfected larvae	number of surviving infected larvae	number of surviving uninfected larvae
0	50	50	50	50
5	34	50	48†	50
10	22	50	48†	50
18	10	49†	48†	50
25	0	49†	48†	50

† Death of larvae due to accidental desiccation.

The pathogenic events that occurred normally in Llyn Frongoch in June and July could be simulated in the laboratory by raising to 20–30 °C the temperature at which infected larvae were maintained. The consequent acceleration of ciliate population growth caused the premature onset of parasite-induced host mortality within one to four weeks, according to the initial intensity of infection.

The precise cause of death of infected hosts was not determined. Overwhelming numbers of ciliates fill the haemocoel in advanced infections and the parasites may interfere with host gaseous transport, nutrition or other essential processes.

Adult *S. lutaria* collected from vegetation surrounding Llyn Frongoch were never infected with *T. sialidos*. Examination of 55 adults, 23 males and 32 females, collected in June 1980, 1981 and 1982 failed to reveal any ciliate infections.

Ciliates in larval hosts

Living material

Tetrahymena sialidos was confined to the haemocoel of *Sialis lutaria* but was found throughout that ramifying system, from the cavity of the terminal abdominal filament to the small sinuses within the mandibles. The ciliates were observed most easily within the host's legs or tracheal gills (figure 2, 3). Examination of these appendages was particularly useful in older larvae, where increasing pigmentation of the abdominal and thoracic cuticle obscured internal detail.

Microscopic examination at magnifications of × 25 or more was usually necessary to discriminate between infected and uninfected larvae. In advanced infections within a few weeks of the host's death, the large number of ciliates in the haemolymph produced a turbid appearance which was visible with the naked eye. This turbidity was particularly noticeable at the appendage bases and also from the general ventral aspect. Infected larvae remained active and fed voraciously until a few hours before their death and they displayed no overt behavioural abnormalities.

Parasitic *Tetrahymena sialidos* is broadly oval in shape (figures 3, 4) but is capable of considerable deformation when moving between the internal organs of the host. The parasites move freely within the host's haemocoel owing to the combined effects of their own continual ciliary activity and the efficient circulatory currents produced by the host.

The cytoplasm of *T. sialidos* is packed with inclusions which make the ciliates conspicuously opaque in transmitted light (figures 2–4). Many of these inclusions stained intensely with

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Description of plate 1

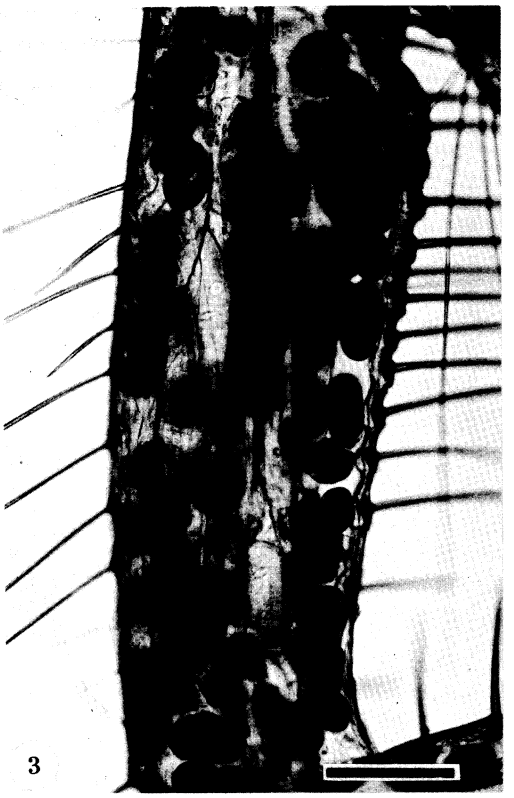
Light micrographs of live *Tetrahymena sialidos* sp.nov. in the haemocoel of *Sialis lutaria* larvae.

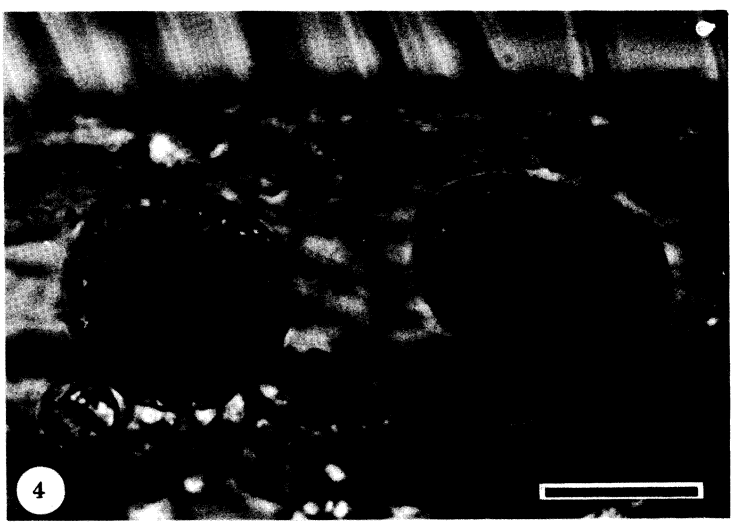
Figure 2. Legs of infected first-year *S. lutaria* larva showing typical intensity of infection with parasitic ciliates in hosts collected during May and June. Microflash. Scale bar, 0.5 mm.

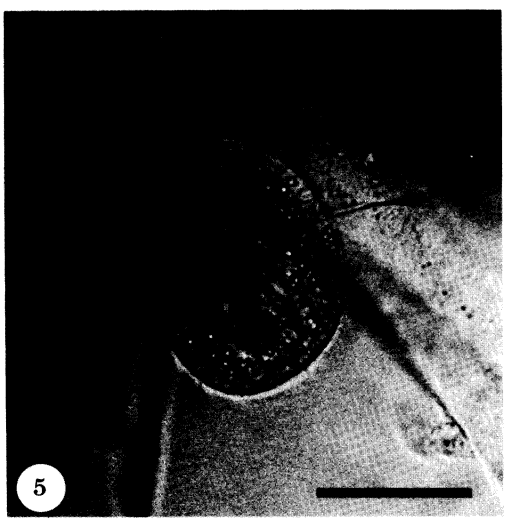
Figure 3. Detail of leg of heavily infected larva showing the high density of ciliates in the haemocoel. Scale bar, 100 μm.

Figure 4. Two ciliates within a tracheal gill showing their broadly oval shape and inclusion-packed cytoplasm. Microflash. Scale bar 30 μm.

Figure 5. One of only two ciliates within a freshly infected young *S. lutaria* larva collected from Llyn Frongoch in July. Notice the translucent cytoplasm. Microflash. Scale bar, 30 μm.







FIGURES 2–5. For description see opposite.

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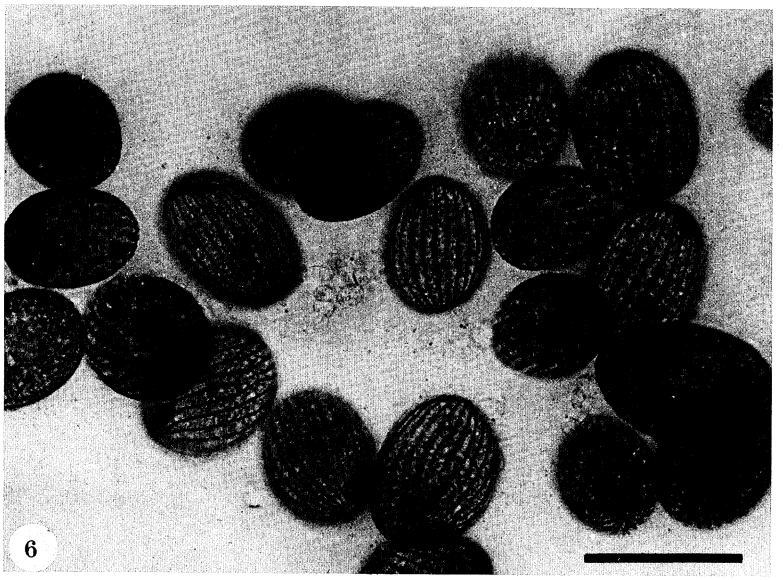
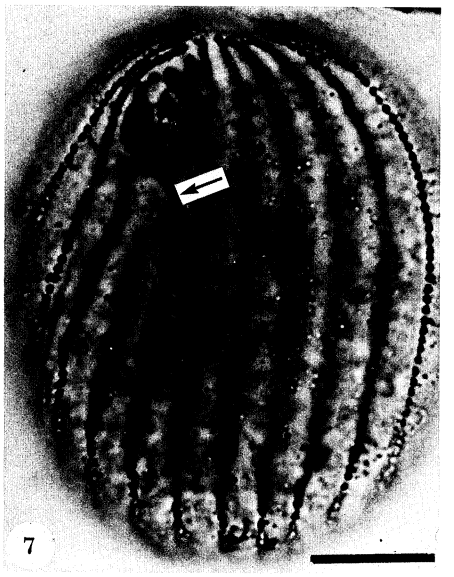
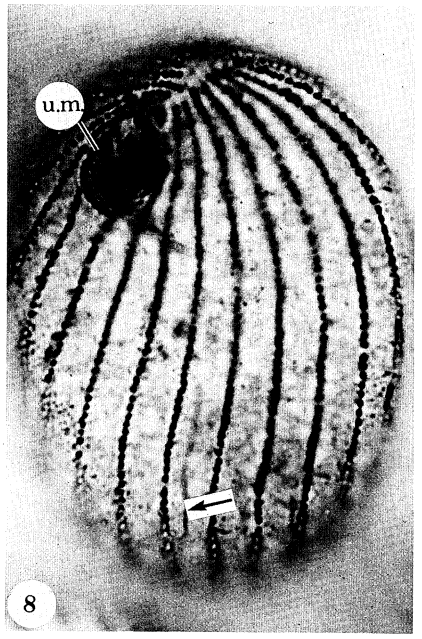
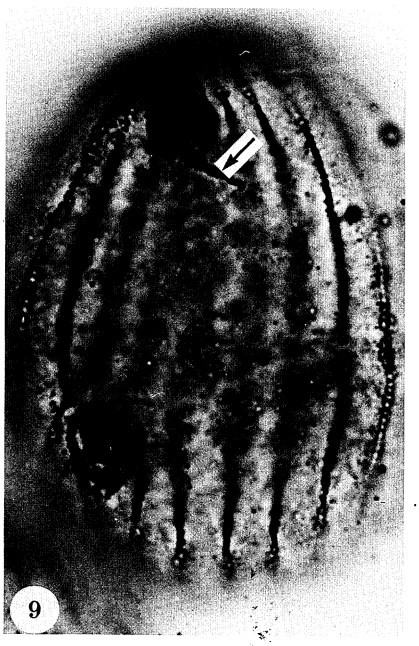
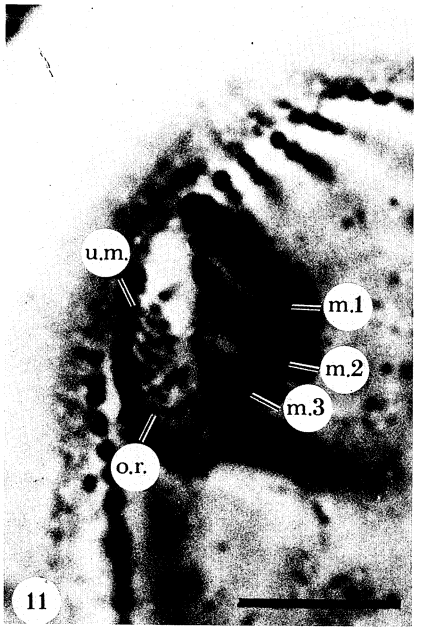
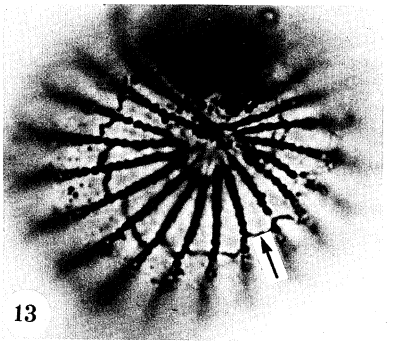


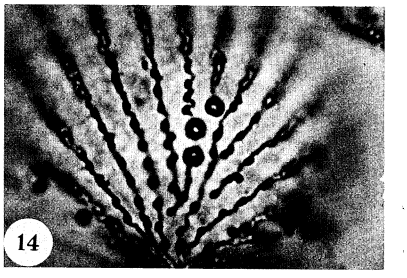












FIGURES 6–14. For description see opposite.

Description of plate 2

Light micrographs of silver-stained *Tetrahymena sialidos* sp.nov. from the haemocoel of larval *Sialis lutaria*.

Figure 6. General view of a typical silver-stained preparation showing ciliates in a variety of orientations. Scale bar, 50 μm.

Figures 7 and 8. Ciliates illustrating the general arrangement of somatic kineties and the position of the oral apparatus. In figure 7 the deep-fibre system (arrow) of the oral apparatus is particularly conspicuous. Two post-oral kineties terminate at the undulating membrane (u.m.) in figure 8. The arrow in figure 8 indicates a fine argentophilic fibre, possibly a secondary meridian. Scale bar, indicating 10 μm, applies also to figures 8–10 and 12–14.

Figure 9. Ventral view of a ciliate approaching cell division. The stomatogenic kinety is number 1. Arrow indicates the deep-fibre system of the cytopharynx.

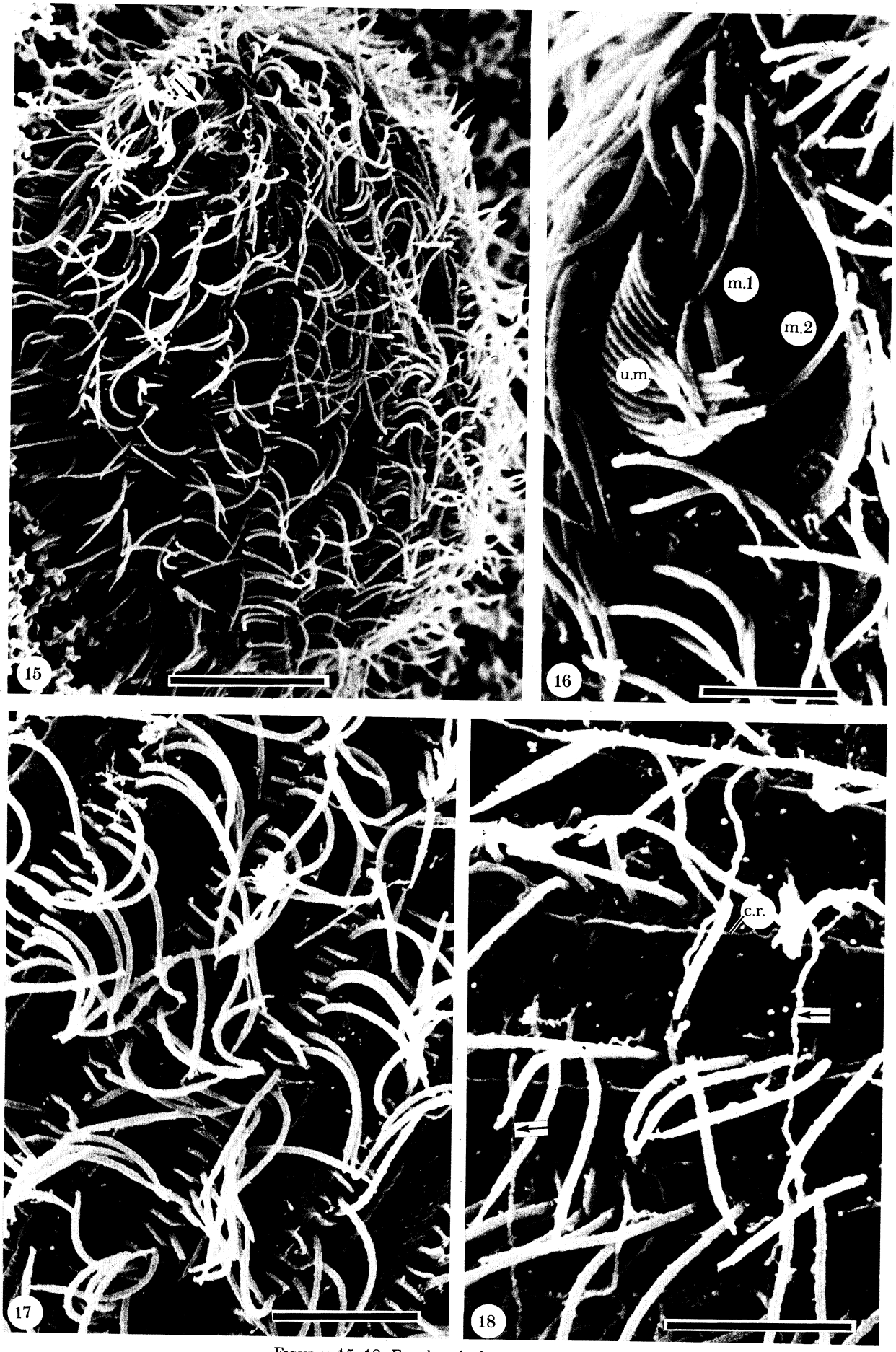
Figure 10. Somewhat flattened specimen demonstrating, in a single focal plane, the densely packed kinetosomes of the somatic kineties.

Figure 11. Detail of oral apparatus showing the 3 membranelles (m. 1, m. 2, m. 3) and the undulating membrane (u.m.). Oral ridges (o.r.) extend from the undulating membrane into the cytopharynx. Scale bar, 5 μm.

Figure 12. Specimen exhibiting an atypical membranelle configuration in which the relative proportions of the membranelles are inverted.

Figure 13. Apical view of cell showing the circumpolar fibrillar ring (arrow).

Figure 14. Ciliate bearing three contractile vacuole pores associated with the posterior ends of kineties 6 and 7.



FIGURES 15–18. For description see opposite.

Description of plate 3

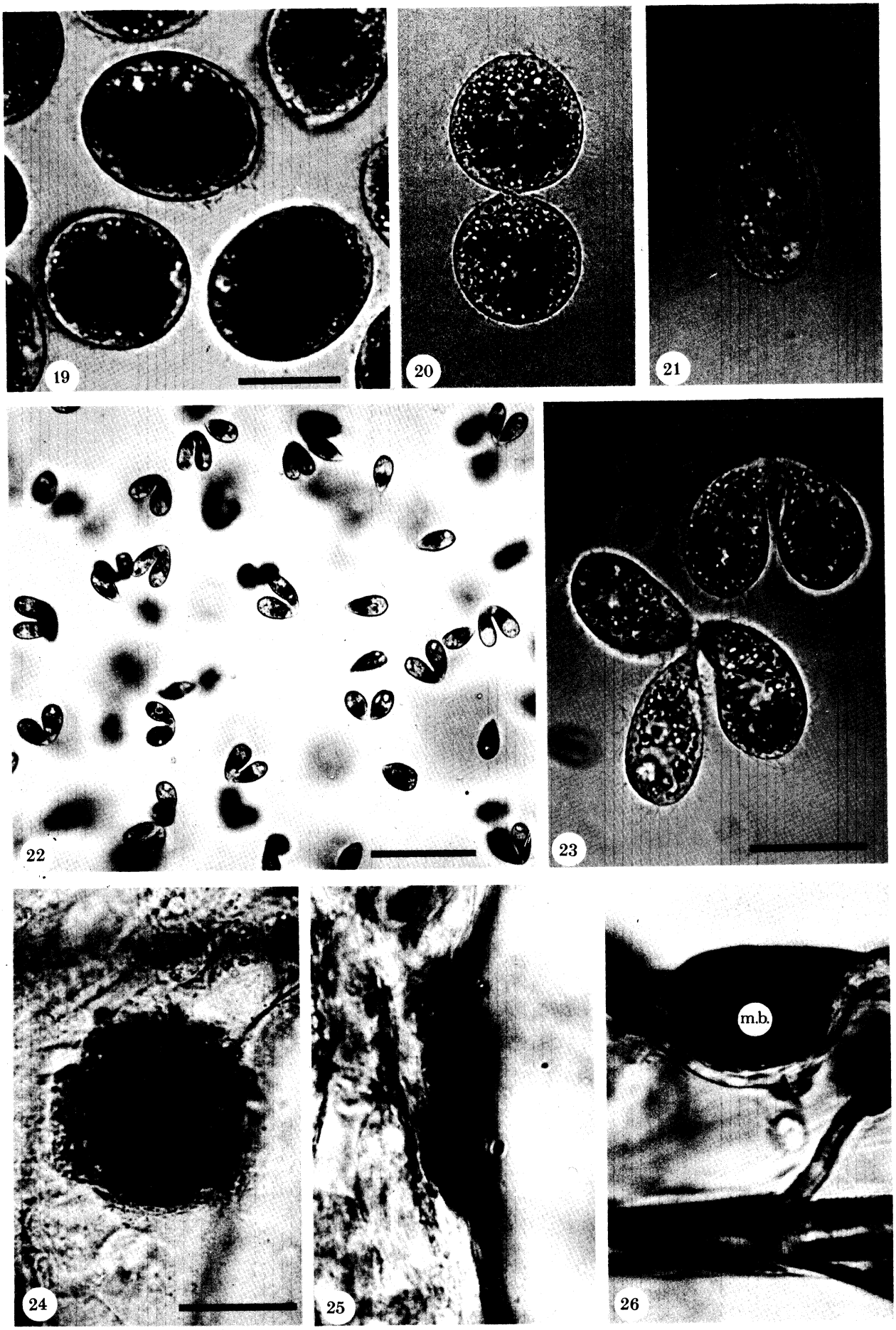
Scanning electron micrographs of *Tetrahymena sialidos* sp.nov. from the haemocole of *Sialis lutaria*.

Figure 15. Entire ciliate showing general body form, arrangement of kineties and position of oral apparatus (arrow). Note the dense somatic ciliature. Scale bar, 10 μm.

Figure 16. Detail of oral apparatus showing undulating membrane (u.m.) and two of the oral membranelles (m. 1, m. 2) within the recess of the buccal cavity. Scale bar, 2 μm.

Figure 17. Detail of densely packed cilia of the somatic kineties. The cilia arise individually from pits separated by narrow cortical ribs. Scale bar, 4 μm.

Figure 18. Beaded extrusomes (arrows), possibly trichocysts, appear to originate from the ciliary pits. A narrow cortical ridge (c.r.) flanks each kinety. Scale bar, 4 μm.



FIGURES 19–26. For description see opposite.

Description of plate 4

Figures 19–23. Light micrographs of live free-living *Tetrahymena sialidos* sp.nov. showing the developmental sequence following release from *Sialis lutaria* larvae.

Figure 19. Ciliates immediately after release from the host. Note the distinct rotund shape and dense cytoplasmic granularity. Microflash. Scale bar, indicating 25 μm, applies also to figures 20 and 21.

Figure 20. First free-living division of *T. sialidos* after about 4 h at 15 °C. Microflash.

Figure 21. Typical form of ciliate after two or three free-living divisions. Notice the reduced cytoplasmic granularity in comparison with figure 19. Microflash.

Figure 22. Synchronous conjugation of ciliates approximately 30 h after release from the host at 15 °C. Microflash. Scale bar, 100 μm.

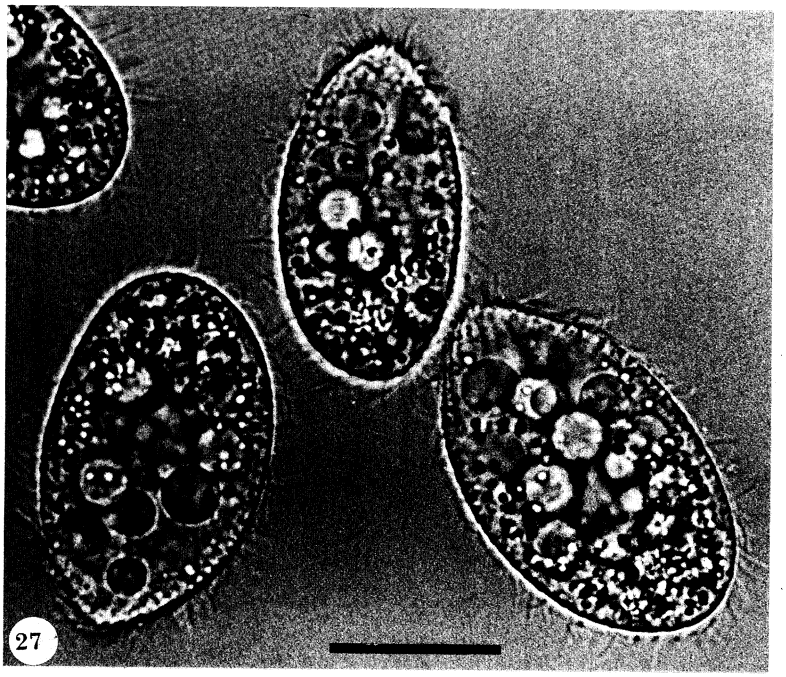
Figure 23. Detail of conjugating ciliates showing normal couplet and unusual triplet configuration. Microflash. Scale bar, 25 μm.

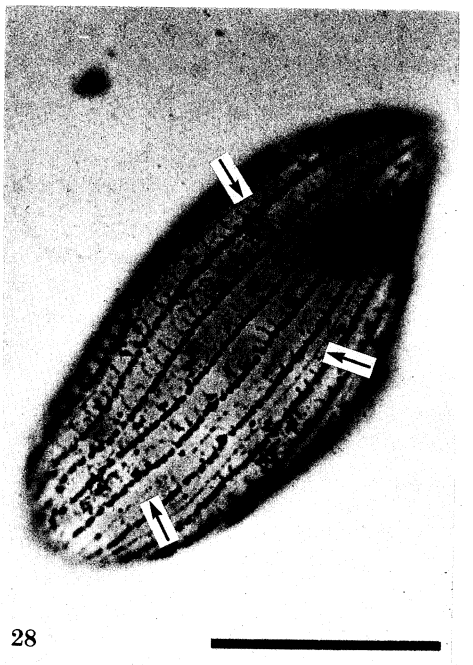
Figures 24–26. Light micrographs of cuticular wounds (ciliate penetration wounds?) in field-collected early-instar larvae of *Sialis lutaria*.

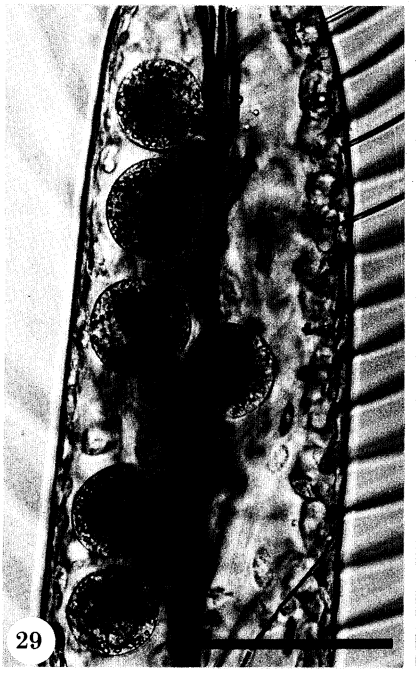
Figure 24. Surface view of abdominal wound. Scale bar, indicating 25 μm, applies also to figures 25 and 26.

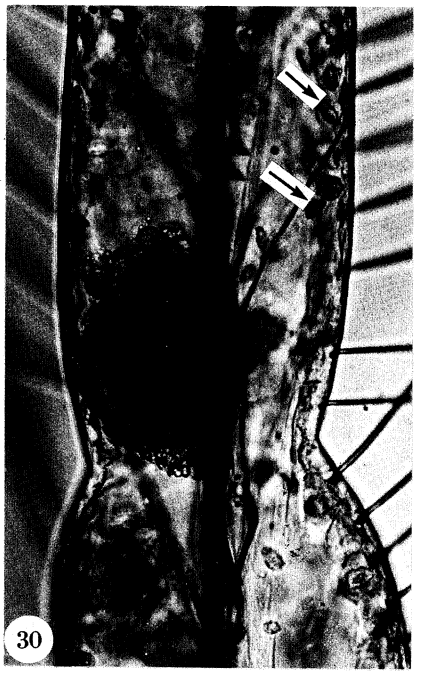
Figure 25. Lateral aspect of wound illustrated in figure 24.

Figure 26. Melanized body (m.b.) (dead ciliate?) beneath a cuticular wound in a tracheal gill of *S. lutaria*.









FIGURES 27–32. For description see opposite

Description of plate 5

Figures 27 and 28. Light micrographs of *Tetrahymena sialidos* sp.nov. cultured in proteose–peptone–yeast-extract medium.

Figure 27. Living ciliates showing elongate body form and sparsely granular cytoplasm. Microflash. Scale bar, 20 μm.

Figure 28. Silver-stained specimen exhibiting some secondary meridians (arrows) between the somatic kineties. Scale bar, 20 μm.

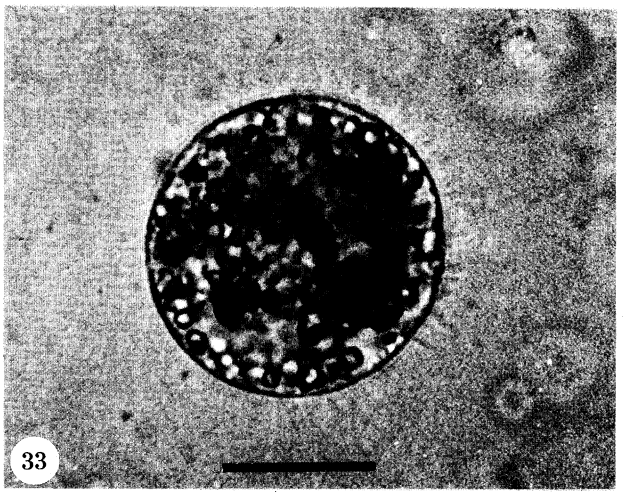
Figures 29–31. Sequence of haemocytic encapsulation of heat-killed *Tetrahymena sialidos* within a single tracheal gill of the host. Scale bar, indicating 100 μm, applies to all three figures.

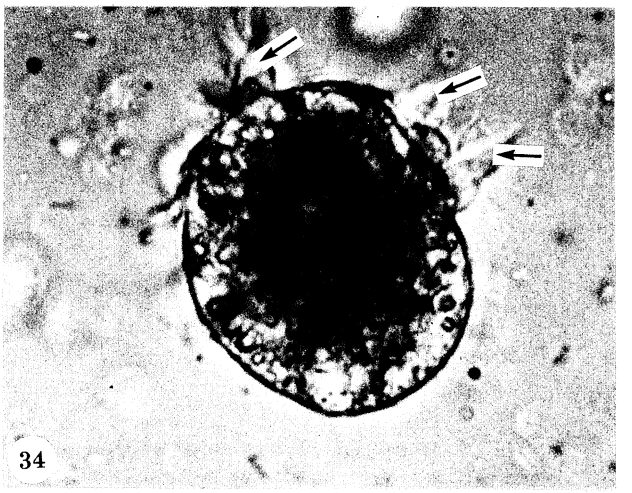
Figure 29. Immediately after thermal shock, the ciliates become rounded but retain their integrity.

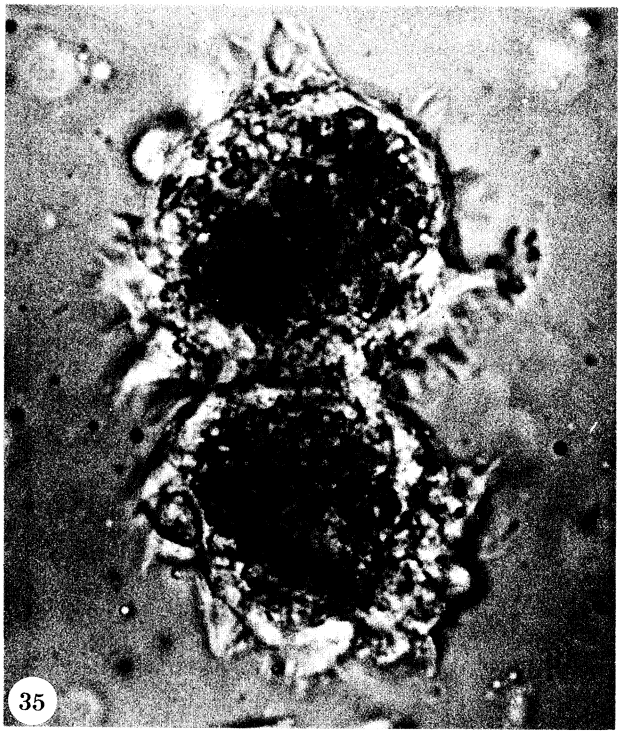
Figure 30. After 24 h several ciliates have been incorporated into a large clump containing many host haemocytes. Free haemocytes are indicated by arrows.

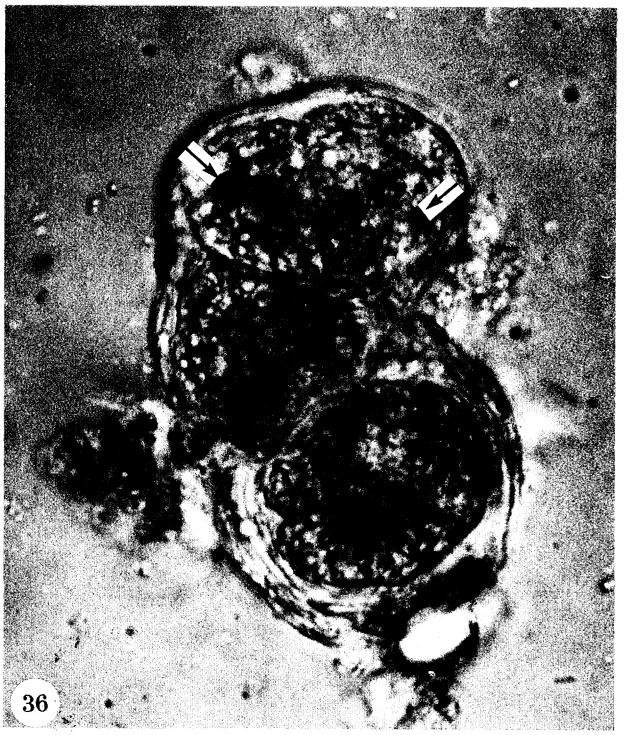
Figure 31. After ten days the aggregate is considerably condensed and has a heavily melanized core surrounded by a flattened layer of haemocytes.

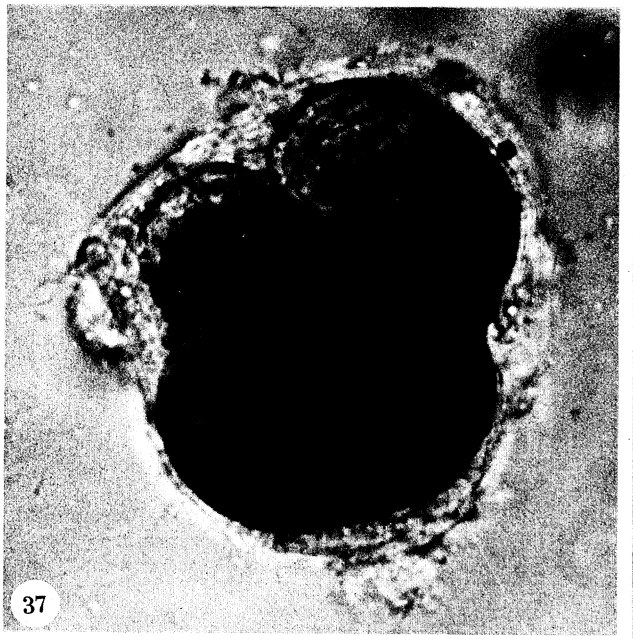
Figure 32. Caudal region of an infected *Sialis lutaria* larva 12 days after thermal shock treatment showing several large melanized aggregates of ciliates within the haemocoel. Scale bar, 200 μm.

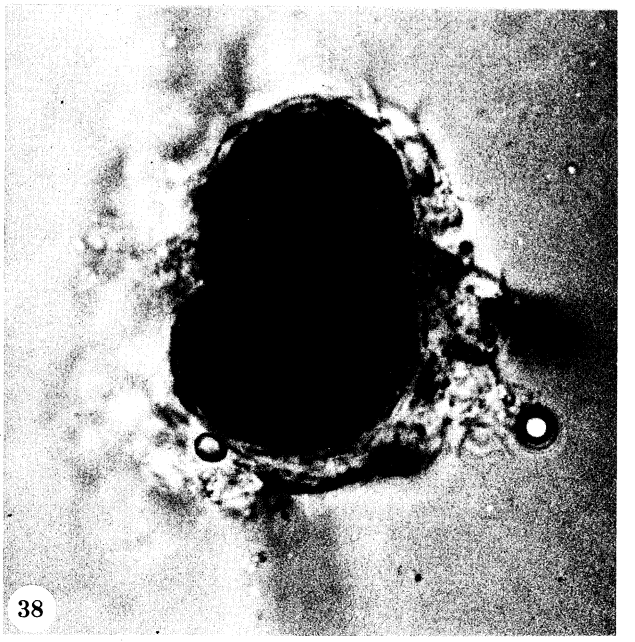












FIGURES 33–38. For description see opposite.

oil-red-O or Sudan-black-B, indicating the presence of significant quantities of lipid. In freshly infected *S. lutaria* the ciliates are relatively translucent (figure 5) but they soon become characteristically opaque as cytoplasmic inclusions accumulate.

Cortical features

Silver-stained specimens of *Tetrahymena sialidos* retain the broadly oval body form of the living ciliates (figures 6–10). Morphometric data from silver-stained preparations are summarized in table 3.

The total number of somatic kineties ranges from 22 to 27. They run from pole to pole of the cell except where truncated by the oral apparatus (figure 8). Secondary meridians are not present, although short lengths of fine argentophilic fibrils are observed occasionally between the somatic kineties (figure 8). An anterior circumpolar ring (figure 13) is the only other interkinetal argentophilic structure.

The somatic kineties consist of closely packed arrays of kinetosomes (figures 7, 8, 10, 12) and high kinetosome density often extends for the entire length of the kinety (figures 8, 10, 13). Scanning electron microscopy demonstrates that many of the kinetosomes do indeed bear cilia, resulting in a cell which is very densely clad (figures 15, 17, 18). Cilia originate singly from cortical pits which often are so closely apposed that only narrow ribs of cortex separate them (figure 17). Each kinety is flanked on its right-hand side by a fine continuous cortical ridge (figures 17, 18). Discrete extrusomes, possibly trichocysts, are sometimes stimulated to extrude during the preparation of *T. sialidos* for scanning electron microscopy. These filamentous bodies exhibit a beaded appearance and originate from sites close to or within the ciliary pits (figure 18).

The oral apparatus of *T. sialidos* is typically tetrahymenine in structure, comprising three ciliary membranelles on the left and a single undulating membrane on the right (figures 7, 8, 11, 16). Oral ridge striations originate at the undulating membrane and descend into the buccal cavity (figure 11). The deep-fibre system of the cytopharynx is conspicuous in many specimens (figures 7, 9). The relative proportions of buccal membranelles are inverted occasionally (figure 12).

Description of plate 6

Detailed sequence of encapsulation of *Tetrahymena sialidos* sp.nov. following thermal shock treatment. To permit detailed photomicroscopy ciliates were dissected out from host larvae into Clark's insect saline, a process that did not cause any noticeable change in the appearance of developing capsules. Scale bar, indicating 25 μm, applies to all figures.

Figure 33. Dead ciliate 15 min after completion of thermal shock.

Figure 34. Dead ciliate 2 h after thermal shock. Several haemocytes (arrows) are securely attached to the surface of the ciliate.

Figure 35. After 24 h large numbers of haemocytes have accumulated around the dead parasites. Here two ciliates are firmly linked by the adhering haemocytes.

Figure 36. By 48 h the haemocytes begin to form flattened concentric layers around the ciliates. Three ciliates are surrounded by such a laminated structure. Initial foci of melanization are visible (arrows).

Figure 37. Almost complete melanization of a small group of encapsulated ciliates nine days after thermal shock treatment.

Figure 38. Heavily melanized capsule after approximately 21 days.

Usually two post-oral kineties are present (82.5%, $n = 40$) (figure 8), sometimes just one (17.7%, $n = 40$). Stomatogenesis in *T. sialidos* is parakinetal and the stomatogenic kinety is number 1 (figure 9).

Tetrahymena sialidos possesses one to five contractile vacuole pores (figure 14), usually two (56%, $n = 70$) or 3 (31%, $n = 70$). These cortical features are associated with the posterior termini of kineties 5–8. A distinct cytoproct has so far not been seen.

Nuclear characteristics and sexuality

In Feulgen-stained preparations of *T. sialidos* the single macronucleus measures 14.0 μm ± 2.5 μm (10.1–19.0 μm, $n = 20$) by 12.2 μm ± 2.0 μm (10.1–16.8 μm, $n = 20$). Macronuclear chromatin extrusion bodies, measuring 3–7 μm in diameter are present in *ca.* 25% of the ciliates. The single, almost spherical, micronucleus has a diameter of 2.6 μm ± 0.2 μm (2.5–3.0 μm, $n = 20$).

Conjugation was never observed to take place within the haemocoel of the host.

Free-living ciliates

Tetrahymena sialidos continued to proliferate after death of the host for up to three days at 15 °C. All ciliates died by the end of that period if the host remained intact. Usually, however, natural breaches in the cuticle and gut permitted ciliates to escape from the decomposing body. It is unlikely that moribund or dead *S. lutaria* larvae remain undisturbed in their natural habitat. In the laboratory moribund infected larvae were subject to cannibalism by other *S. lutaria* larvae, a dismembering process which released dense clouds of ciliates into the surrounding water. Simulation of this environmental change was achieved by decapitation of live infected larvae followed by shredding of the body with fine needles. Ciliates released in this way into filtered lake water or Prescott's and James's solution suffered considerable osmotic and ionic shock and some ciliates died as a result, although the numbers dying could be minimized by release at low temperature (5–10 °C).

Recovery of ciliates from osmotic and ionic shock required 3–6 h following release from the host at 15 °C. Thereafter two or three cell fissions took place in rapid succession, during which the characteristic cytoplasmic granularity of parasitic ciliates was reduced (figures 19–21) and cell shape changed from globose to a more elongate form (figure 21). This change in body form is reflected in a greater somatic length:somatic width ratio (table 3).

On completion of the post-release fissions a remarkable period of synchronous conjugation took place. A peak of conjugal activity was recorded between 30 and 36 h after release from the host and during this period over 80% of the ciliates were directly involved in conjugation (figure 22). Examination of Feulgen-stained ciliates confirmed that all the normal nuclear events associated with conjugation in *Tetrahymena* were in fact taking place. Tripartite participation in conjugation was occasionally observed (figure 23) and rarely four ciliates were involved. Residual cytoplasmic granularity was concentrated towards the anterior of conjugating cells (figure 22). The proportion of *T. sialidos* recorded to be conjugating declined to zero during the period from 36–84 h after release from the host. Ex-conjugants continued to divide, although at a slow rate, and such infusions could be maintained for several weeks. A distinctive feature of the ciliates in infusions older than four days was their tendency to swim rapidly in long shallow spirals.

Conjugation in *T. sialidos* could be induced by artificial release of ciliates from host larvae

TABLE 3. MORPHOMETRIC DATA OF SILVER-STAINED *TETRAHYMENA SIALIDOS* SP.NOV. FROM *IN VIVO* AND *IN VITRO*

	total number of somatic kineties				somatic length				somatic width				ratio mean somatic length: mean somatic width
	mean µm	s.d. µm	range µm	*n*	mean µm	s.d. µm	range µm	*n*	mean µm	s.d. µm	range µm	*n*	
ciliates from the haemocoel of *Sialis lutaria*	24.7	1.1	22–27	60	51.0	4.2	41.3–61.5	50	33.1	3.3	30.2–44.7	50	1.54:1
ciliates after four months culture in p.p.y.e. medium	22.6	0.7	21–24	30	42.8	6.5	33.5–52.5	40	24.3	2.0	22.3–27.9	40	1.76:1
ciliates after 13 months culture in p.p.y.e. medium	20.1	1.0	18–23	30	45.2	8.5	27.9–59.2	40	24.7	2.8	19.0–30.2	40	1.83:1
conjugating ciliates† from bacterized infusion					32.4	1.7	28.6–35.7	40	18.0	1.4	15.7–20.0	40	1.80:1

	length of membranelle 1				length of membranelle 2				length of membranelle 3			
	mean µm	s.d. µm	range µm	*n*	mean µm	s.d. µm	range µm	*n*	mean µm	s.d. µm	range µm	*n*
ciliates from the haemocoel of *Sialis lutaria*	6.2	0.4	5.6–6.7	30	5.4	0.4	4.5–6.2	30	2.0	0.3	1.1–2.2	30
ciliates after four months culture in p.p.y.e. medium	6.8	0.4	6.1–7.8	30	5.7	0.3	5.0–6.1	30	2.0	0.3	1.7–2.2	30
ciliates after 13 months culture in p.p.y.e. medium	6.9	0.5	6.1–7.8	30	5.8	0.4	5.6–6.7	30	2.4	0.5	1.7–3.4	30

s.d., Standard deviation.
p.p.y.e., Proteose–peptone–yeast-extract.
† Refractile to silver staining.

at any time of the year. The distinctive conjugal synchrony described earlier was stimulated most effectively, however, when ciliates were derived from well-established infections containing over 2000 parasites.

Preconjugants, conjugants and exconjugants were consistently refractile to silver impregnation and even the best preparations were too faint to permit definitive statements concerning cortical organization.

Experimental infection of Sialis lutaria

Tetrahymena sialidos did not readily infect *S. lutaria* in the laboratory. No infections were recorded in seven replicates of an experiment in which 20 first-instar larvae were exposed to the combined ciliates released from four to eight heavily infected one-year-old larvae. Exposure continued for three to five days, during which the ciliates underwent transition through all the free-living phases described in the previous section.

In eight replicates of an experiment in which ten second-instar larvae were exposed to ciliates in the same way as described above, a single larva became infected in each of three experiments, giving an overall incidence of infection of approximately 2.7 %. Two of the larvae contained two ciliates each, the third larva had four ciliates. Infection was recorded only after the fourth day of exposure.

Although the process of infection was never observed, the presence of small cuticular wounds may be significant. Melanized cuticular wounds measuring 30–50 μm in diameter were noted in two of the three laboratory-infected larvae and in several of the apparently uninfected larvae. Similar wounds were commonly observed in the cuticle of young field-collected larvae during June and July (figures 24, 25). These melanized areas were recorded both from infected and uninfected larvae. A heavily melanized body was sometimes situated beneath a wound (figure 26). External cysts were never observed to be associated with the cuticular wounds.

In vitro cultivation of Tetrahymena sialidos

Axenic cultures of *T. sialidos* were readily established by passage directly from the host's haemocoel to sterile proteose–peptone–yeast-extract medium. In contrast to the synchronous mating that normally ensued upon release from the host, only a low level (under 5 %) of conjugation was recorded during the first five to six weeks following establishment of a cell line. Conjugation did not occur beyond about the sixth week of culture. Axenic cultures initiated from six separate infections have been maintained continuously, with regular subcultivation, for over three years.

In vitro T. sialidos acquires an elongate body form and sparsely granular cytoplasm (figure 27). Cell size is intermediate between parasitic and free-living stages (table 3). Cultivation in proteose–peptone–yeast-extract medium led to a gradual decline in the mean total number of somatic kineties (table 3). The recorded reduction was from 24.7 in parasitic ciliates to 22.6 after four months in culture and to 20.1 after 13 months in culture. Axenically cultured *T. sialidos* exhibited a greater tendency to produce secondary meridians (figure 28) than did parasitic ciliates. Other argentophilic features showed little change in culture (table 3).

The size of the macronucleus was reduced in cultured *T. sialidos*. Feulgen-stained macronuclei measured 8.8 μm ± 1.6 μm (7.3–12.3 μm, $n = 20$) by 7.7 μm ± 0.9 μm (6.7–9.5 μm, $n = 20$). Micronuclei were also slightly smaller, with a diameter of 2.4 μm ± 0.2 μm (2.2–2.7 μm, $n = 20$). Macronuclear chromatin extrusion bodies were present in less than 1 % of specimens.

Encapsulation of Tetrahymena sialidos by host haemocytes

Parasitic *T. sialidos* were killed within the host haemocoel by selective thermal shock. Infected hosts were subjected to a limited period at an elevated temperature determined to be lethal for the ciliates but not for the host.

All ciliates within an infected host were killed by 1 h exposure to 39 °C, irrespective of acclimation temperature (5, 10, 15 or 20 °C). Parasite mortality at 36 °C became apparent only after prolonged exposure for 4 h or more. No parasite mortality was incurred at 30 °C even after 48 h; the ciliates multiplied rapidly at this temperature.

The following account of the host response to heat-killed ciliates is based upon a series of experiments in which a total of 80 infected hosts previously maintained at 10 °C for one week were exposed to 39 °C for 1 h and then transferred to 15 °C.

Ciliates killed by thermal-shock retained their integrity but became almost spherical (figures 29, 33). Host haemocytes were first seen adhering to the dead ciliates after a lag period of 1–3 h (figure 34). Many more haemocytes had accumulated around the parasites by 24 h after thermal-shock (figures 30, 35). Adjacent ciliates were often incorporated into cohesive clumps usually involving two or three parasites (figures 35, 36). Larger aggregates of 20–30 ciliates were sometimes formed, especially in the more spacious body cavities. During these early stages of encapsulation host haemocytes adhered firmly to the surface of the dead ciliates and were not dislodged by the normal circulatory currents or by the strong gushes of haemolymph caused by sudden muscular contraction.

Gradual consolidation of haemocyte capsules took place during the period 24–72 h after thermal shock treatment. Accumulating haemocytes flattened to form a laminated structure inside which the dead ciliates generally remained clearly visible (figure 36).

Melanization of haemocytic capsules usually commenced approximately 48 h after thermal shock treatment. Small foci of dark brown deposits close to the parasite surface were the first indicators of melanization (figure 36). Many encapsulated ciliates were extensively melanized after about ten days (figures 31, 32, 37, 38).

The timing and final degree of melanization was variable and depended, at least in part, upon the size of the initial clump of ciliates and its position within the body of the host. Melanization was more rapid and more extensive in large aggregates of ciliates located within the main body of the host (figure 32), than in smaller groups located peripherally in the appendages. Melanized capsules were usually more compact than the corresponding initial aggregates of dead ciliates (cf. figures 29, 31), suggesting compression or removal of parasite material during the process of encapsulation.

Uninfected control larvae were subjected to the same thermal shock treatment as experimental animals and examined daily for two weeks after return to 15 °C. Haemocyte morphology appeared normal and there were no indications of haemocyte clumping, indiscriminate melanization or any other signs of heat-induced abnormalities.

Discussion

Relating to the taxonomy of Tetrahymena sialidos

The hymenostome genus *Tetrahymena* currently comprises 27 species, of which 17 belong to the '*T. pyriformis* complex' (Nanney & McCoy 1976; Nyberg 1981). Six of the remaining ten

species are parasitic but only three of these have insect hosts, namely *T. chironomi*, *T. rotunda* and *T. dimorpha* (Corliss 1973; Lynn *et al.* 1981; Batson 1983). The morphology of *T. sialidos* most closely resembles that of *T. chironomi*, a parasite of larval chironomids (Diptera). The number of somatic kineties overlaps in these two species, being 22–27 in *T. sialidos* and 23–28 in *T. chironomi* (Corliss 1973). Secondary meridians are absent from the parasitic phase of both species, although *T. sialidos* tends to manifest these structures *in vitro*. The two species do not differ significantly regarding details of oral apparatus, position and number of contractile vacuole pores, or number of post-oral kineties. The parasitic phase of *T. sialidos* is, however, considerably larger than that of *T. chironomi*. Corliss (1960) gives mean cell dimensions of 40 μm × 23 μm for *T. chironomi*, compared with 51 μm × 33 μm for *T. sialidos*. By using the formula of Seaman *et al.* (1972) these data yield a mean cell volume for *T. sialidos* which is 2.5 times that of *T. chironomi*.

In assessing the taxonomic status of ciliates, morphological characteristics considered alone may be misleading. Despite morphological homogeneity many cryptic species have been recognized in *Tetrahymena* (Nanney & McCoy 1976; Nyberg 1981) and other genera (Sonneborn 1975). The '*T. pyriformis* complex' currently contains 13 micronucleate species formerly identified by their mating-type reactivity, but now also recognized by isozymic characteristics (Nanney *et al.* 1980). Some of the species in the complex are separable by multivariate analysis of morphological characteristics (Gates & Berger 1974).

The nature of the host-parasite relationship is of value in determining the taxonomic status of parasites (Taylor & Muller 1979) and it is therefore significant that *T. chironomi* is found exclusively as a parasite of larval chironomids (Barthelmes 1960; Corliss 1960;Golini & Corliss 1981), whereas *T. sialidos* parasitizes only *S. lutaria*. Chironomid larvae are among the most important invertebrates in Llyn Frongoch, both in abundance and in productivity (Hanlon 1985). The mean population density of chironomid larvae varies from 3000 to 7000 m^{-2} and several species of *Chironomus* are present in addition to members of the Tanypodiinae and Orthocladinae (Hanlon 1985). Despite regular monitoring during a four-year period no chironomid larva was ever found to be parasitized by ciliates. Over 800 chironomid larvae were examined microscopically during this period, many of them in June and July when the infective stages of *T. sialidos* were presumed to be abundant in the lake. The consistent absence of ciliate infections from chironomids and from other invertebrate members of the benthic fauna indicates that *T. sialidos* is distinct from *T. chironomi* and probably species-specific.

Tetrahymena sialidos exhibits an intimacy and synchrony of association with its host that is not apparent in *T. chironomi*. All instars of the chironomid host are liable to infection by *T. chiromoni* and once infection is established the ciliates proliferate in such a way that generally the host is killed within a few days (Corliss 1960). Conversely, the life cycle of *T. sialidos* is intimately synchronized with the developmental pattern of the host alder-fly. The infection process is apparently limited to a brief period each summer and is followed by a protracted association which continues for 11–12 months.

The pattern of sexual events differs in *T. chironomi* and *T. sialidos*. In undisturbed infected chironomid larvae, conjugation of *T. chiromoni* takes place within the body cavity of the living host approximately two days before the host's death. Corliss (1960) regarded this event as a suicidal 'selfing' conjugation since all ex-conjugants died and attempts at *in vitro* cultivation of the ciliates were unsuccessful. Conjugation in *T. sialidos* takes place only after natural or artificial release from the haemocoelomic environment. The exconjugants are viable and

continue to divide, and cultures of *T. sialidos* are readily established in bacterized or axenic media.

Attention has recently been drawn to the generally inadequate nature of most ciliate species descriptions (Berger 1978; Gates 1978). In conjunction with the existence of many cryptic ciliate species (Sonneborn 1975; Nanney & McCoy 1976; Corliss & Daggett 1983), this lends support to the suggestion that descriptions of new ciliate taxa should encompass the broadest data base possible (Corliss 1979). The present description has used a combination of ecological, physiological and parasitological attributes in addition to morphological information. These data indicate that *T. sialidos* is distinct from all other members of the genus, including *T. chironomi*, the species that *T. sialidos* morphologically most closely resembles.

Relating to insect ciliatoses

The literature of the past 50 years contains many brief references to the occurrence of parasitic ciliates in the haemocoel of insects. Most of the observations are incidental, revealing little of the biology of the parasites or of the precise nature of the association. Corliss (1960) comprehensively summarized the pre-1960 literature for insects and other invertebrate hosts of *Tetrahymena*. Since then insect–parasitic ciliates have continued to be noticed, especially in aquatic dipteran larvae (Kellen *et al.* 1961; Clark & Brandl 1976; Corliss & Coats 1976; Yu *et al.* 1978; Corliss *et al.* 1979; Golini & Corliss 1981; Davies *et al.* 1981; Lynn *et al.* 1981). This is the first record of a parasitic ciliate from the haemocoel of a megalopteran larva.

Natural or experimentally induced facultative parasitism of insects by tetrahymenine ciliates has been documented by several authors (Thompson 1958; Seaman *et al.* 1972; Corliss 1973; Grassmick & Rowley 1973), and such predisposition to parasitism may be of importance in the evolution of ciliate endoparasitism (Corliss 1972). Nevertheless, it is becoming increasingly clear that some associations between ciliates and their insect hosts are not simply fortuitous opportunistic instances of parasitism by essentially free-living organisms, but intimate and subtle relationships (Batson 1983). This conclusion is further supported by the results of the present study. The fine synchrony and persistent relationship between *T. sialidos* and its megalopteran host indicate an established association rather than casual opportunism.

Insect–parasitic ciliates do not have a uniform strategy for survival. The protracted nature of the association between *T. sialidos* and *S. lutaria* contrasts with many other insect ciliatoses. Parasitic ciliates possess microbial growth dynamics, in the sense that asexual reproduction provides the potential for logarithmic population growth. Realization of this potential accounts for the rapid pathogenicity generally associated with ciliate infections of insects. In larval mosquitoes and larval chironomids, for example, the host is typically killed within a few days or weeks of infection (Keilin 1921; Corliss 1960). Ciliate infections in certain other invertebrates are also characterized by rapid pathogenicity (Stout 1954). The persistence of *T. sialidos* infections for 11–12 months may be explained partly by the annual pattern of water temperature of Llyn Frongoch. The mean monthly temperature ranges between *ca.* 5 °C and *ca.* 15 °C and for seven months of the year it remains below 10 °C (Hanlon 1985). Assuming that initial infection of *S. lutaria* is by a single ciliate, only 16 cell doublings are required to attain the pathogenic burden of 50000–70000. Data of mean intensity of infection at different times of the year indicate that 11 of the 16 fissions have been completed by November. Less than one further cell doubling takes place between December and March and the remaining four to five fissions occur from April to June. Even during the warmer summer months, therefore

the cell generation time of parasitic *T. sialidos* must be measured in weeks rather than in days or hours, yet upon release from the host two to three fissions occur within 36 h at 15 °C. Factors other than temperature and intrinsic growth rate must therefore be important in determining the development of *T. sialidos* in its megalopteran host. In this context it is pertinent that another member of the genus, *T. dimorpha*, is subject to rigid population control within the larval stage of its dipteran host but proliferates rapidly in the pupal and adult states (Batson 1983), although the nature and location of the regulatory mechanism are unknown.

The life-cycle of many parasites is closely tuned to that of the host (Hawking 1975). The synchronizing influences affecting the interaction between *T. sialidos* and *S. lutaria* require further investigation. Field data indicate that the process of infection of *S. lutaria* is limited to a brief four to six week period each year. Whether this reflects temporal restriction of infective ciliates to that period only, or transient host susceptibility, is not yet clear. Such questions are intimately bound up with the precise method of host infection. Unfortunately, apart from the well-documented penetration cysts of *Lambornella* (Clark & Brandl 1976; Corliss & Coats 1976) the mode of host infection by insect–parasitic ciliates remains enigmatic, although several possibilities have been discussed (Corliss 1960, 1973). Circumstantial evidence indicates that infection of *S. lutaria* by *T. sialidos* may be achieved transcuticularly, although evidence of distinct penetration cysts has not been found. Improvement of laboratory infection procedures for the *T. sialidos–S. lutaria* system will permit resolution of this problem.

Environmental cues for specific developmental events are common requirements of many organisms, including parasites (Smyth 1976). Sexual activity in ciliates is often related to environmental change, among other factors, and a degree of starvation is one of the common prerequisites for conjugation to take place. Nanney (1980) has pointed out the severe selective disadvantage of mating in the presence of plentiful food. The synchronous conjugation of *T. sialidos* seems to be related to the dramatic change from the nutrient-rich haemocoel of an insect to the comparatively nutrient-poor free-living environment. Whatever the biological rationale for this particular genetic economy, the ease of such sexual manipulation in *T. sialidos* provides a convenient laboratory system in which high levels of synchronous conjugation can be reliably induced to order.

Relating to insect immunity

Invertebrates do not possess the highly specific antibody-mediated immunological responses typical of vertebrates. In general they also lack the vertebrate capacity for enhanced immunological responsiveness to foreign substances following previous exposure. Notwithstanding these apparent restrictions insects and many other invertebrates are able to respond to foreign materials, including parasites, in a variety of effective ways. In insects the relevant homeostatic responses reside almost exclusively in the haemolymph and associated cell populations, and involve both cellular elements (phagocytosis, encapsulation) and humoral elements (lysis, agglutination, humoral melanization, precipitation) (Cooper 1974; Maramorosch & Shope 1975; Gupta 1979).

The effectiveness of insect immune mechanisms apparently has led some parasites to select sites where their foreign nature will not be evident, analogous to the immunologically privileged sites occupied by certain parasites of vertebrates. This is illustrated by the mermithid nematode *Filipjevimermis leipsandra* which infects the beetle *Diabrotica u. undecimpunctata*. Upon penetration of the host's cuticle infective nematodes seek a neural ganglion within which to continue

development, but nematodes that linger in the haemocoel are killed by an efficient encapsulation process (Poinar 1968).

Despite the efficacy of immune mechanisms in many insects, a great range of successful parasites are nevertheless able to develop unhindered within the potentially hostile environment of the haemocoel, and a variety of explanations have been proposed to account for this ability. In view of the less sensitive nature of the invertebrate immune system in comparison with vertebrates Lackie (1980) has postulated that approximate antigenic similarity is sufficient to avoid recognition by the host. Such histocompatibilities are argued to be in accordance with the established non-recognition of allografts and certain xenografts in insects (Lackie 1980).

Not all parasites of insects rely upon non-recognition to avoid host immune responses. Sometimes parasite recognition takes place but effective host response is prevented, as in the insect parasitoid *Pseudeucoila bochei* which inhibits the process of haemocyte transformation and thus prevents encapsulation by its *Drosophila* host (Nappi 1975). Evidence is accumulating which indicates that a variety of insect parasites, including bacteria and nematodes, are able to produce immune inhibitors (Siden *et al.* 1979; Flyg *et al.* 1980; Götz *et al.* 1981).

Against this background of different parasitic strategies for survival in the insect haemocoel, the association between *Tetrahymena sialidos* and *Sialis lutaria* provides an exciting opportunity to address the related problems of parasite survival and host recognition of non-self. The *T. sialidos–S. lutaria* system offers several attractive features as a model system in this respect.

First, the association occurs naturally and at high prevalence. Although valuable data have been obtained through experiments involving the artificial implantation of biological or non-biological materials (Ratcliffe & Rowley 1979; Ratcliffe 1982), increasing awareness of the need to relate immunological capacities to natural situations is also apparent (Ratcliffe & Walters 1983). Studies on the cellular responses of insects to injected bacterial suspensions, for example, frequently have involved species of bacteria that are not only unlikely to occur in the insect's natural environment but that also are introduced in quantities unlikely to be encountered naturally.

Secondly the alder-fly host has several attributes which are particularly favourable for experimental studies upon insect immunity. Some of the problems that beset workers in this field arise from the fact that many insect species are too small or delicate to manipulate, too short-lived to use experimentally, or else contain very few blood cells (Ratcliffe 1982). In contrast the larva of *Sialis lutaria* is relatively large, robust, long-lived and possesses an abundant population of circulating haemocytes (Selman 1962). The aquatic larval stage lasts for almost two years and *T. sialidos* infections persist for 11–12 months, much longer than many other parasitic associations. This protracted association together with the ease of laboratory maintenance of infected hosts affords opportunities for long-term immunological experiments.

Finally, this study has established a procedure whereby haemocytic encapsulation of *T. sialidos* may be stimulated by controlled thermal shock treatment. The use of elevated temperature has been effective in the elimination of a variety of symbionts from their association with other organisms, especially insects (Koch 1956; Weis 1982). Successful application of this principle to the *T. sialidos–S. lutaria* system yields an experimentally manipulative technique which avoids interference with the host's physical integrity. The significance of this attribute lies in the fact that haemolymph and haemocytes are involved not only in immunological responses but also in the homeostatic processes of haemolymph coagulation and wound healing

(Grégoire & Goffinet 1979). Most experimental systems involve implantation or injection procedures and consequently difficulties arise in the separation of the above components of response. The initial haemocytopenia induced by injection of bacteria into *Galleria mellonella* larvae and many other insects, for example, is probably due in part to wound healing rather than immunological responses (Ratcliffe & Walters 1983).

Sialis lutaria is known to be capable of mounting effective cellular responses against a variety of naturally acquired parasites. A number of publications record the presence of larval Digenea and nematodes in the haemocoel of alder-fly larvae, and these parasites are almost invariably encapsulated (Thompson 1915; Buttner 1951; Salt 1963; Evans & Higgs 1982). The observation that *T. sialidos* is not normally subject to an effective host response raises fundamental questions regarding the nature of the parasite's ability to occupy successfully and proliferate within the potentially hostile haemocoelomic environment. This study has established that thermally induced death of parasitic ciliates is followed by their cellular encapsulation. It has yet to be determined whether this response reflects host recognition of the parasite for the first time, or the delayed fulfilment of an already primed reaction.

The first phase in cellular encapsulation of a foreign body involves the attachment of certain haemocytes to its surface (Ratcliffe & Rowley 1979), although there is some controversy concerning the relative importance of stochastic and chemotactic events in the establishment of initial contact (Nappi 1974). It may be significant that *T. sialidos* is densely clad in cilia, which maintain ceaseless activity throughout the cell cycle. Such a turbulent physical barrier to initial haemocyte adhesion is possibly sufficient to protect the living ciliates from encapsulation.

Relating to host population dynamics

The influence of parasitism upon host population dynamics is often not readily assessed since many parasites either do not cause host mortality or else they do so only after the host is mature and reproducing. Careful measurement of host growth-rate, death-rate and fecundity is therefore necessary to quantify the effects of parasitism upon host population dynamics (Brambilla 1983). Anderson & May (1981) have drawn together a great deal of hitherto unanalysed data in an attempt to understand the persistence of microparasites within invertebrate populations and the role of parasites in the regulation of those populations. Elsewhere (May & Anderson 1983) they have pointed out the need for more quantitative information from suitable host–parasite systems. The distinctive natural association of *T. sialidos* and *S. lutaria* is proposed as a paradigm by means of which various mathematical models of host–parasite dynamics may be tested. Intensive studies upon the interaction of host and parasite population dynamics in this system have recently commenced in this laboratory, and it is appropriate at this point to discuss briefly the merits and exciting possibilities provided by this model.

The results of this study establish several crucial features of the relationship between *T. sialidos* and *S. lutaria* which are indicative of its value in population dynamics studies. First, data over a four-year period, spanning five consecutive host generations, indicate that the relationship is persistent. The intimacy and synchrony of the host–parasite association suggests an established relationship rather than the transient opportunism that may apply to certain other ciliate infections of invertebrates (Corliss 1972). Secondly, the prevalence of infection is consistently high at 40–70%, usually about 60%, and so far there is no indication that this

high prevalence reflects a transient epidemic phenomenon, although caution must be exercised in the extrapolation of data covering only five host generations. Thirdly, the ciliate is highly pathogenic, killing all infected hosts in their larval stages. The problems of evaluating more subtle effects upon host survival and fecundity are thereby eliminated.

An additional practical advantage is that this model system operates within the relatively closed environment provided by a small lake. Aquatic larval stages of the host are easily sampled; annual larval cohorts are readily distinguished; mature larvae may be trapped as they leave the water to pupate; adults are slow-flying and easily captured and estimation of dispersion and recruitment of adults from elsewhere is feasible; eggs are laid in large batches in predictable locations and thus amenable to quantification. In short, the technical problems involved in determination of important life table data are minor. In view of these features it is perhaps surprising that little quantitative ecological information is available concerning *S. lutaria* (Elliott 1977). However, even less is known about the general role of parasites in the regulation of freshwater invertebrate populations, as most data have been obtained from terrestrial forest and pasture systems (Anderson & May 1981).

A priori reasoning leads to the conclusion that since parasites reduce host survival or reproductive capacity, or both, then selection will favour those individuals with reduced susceptibility to the parasite. The intensity of selective pressures would accordingly be proportional to the prevalence of infection within the host population. *Tetrahymena sialidos* manifests consistently high prevalence within its host population and both field and laboratory data indicate that this parasite has the maximum possible regulatory effect, in that all infected individuals die in their immature stages.

In general insects do not exhibit features of acquired resistance in the classical vertebrate sense (Lackie 1980), and consequently innate resistance may be argued, therefore, to be of prime importance. The genetic basis for innate resistance to parasitic infections is receiving increasing attention (Skamene *et al.* 1981; Wakelin 1983). In insects, genetically determined differential susceptibility is well known. The susceptibility of *Aedes aegypti* to filarial infections, for example, is controlled by a single recessive gene and variations in susceptibility shown by different field populations can be correlated with differences in the frequency of this gene (Macdonald 1976; Briese 1981). In the laboratory it is possible to select for increased resistance in *A. aegypti* (Kartman 1953) and differences in susceptibility are correlated inversely with the ability to encapsulate microfilariae. With these comments in mind, it is reasonable to ask whether *S. lutaria* is potentially resistant to *T. sialidos* and if so what factors are responsible for the apparent lack of natural resistance in at least 40–70% of the population.

Anderson & May (1981) have demonstrated by empirical mathematical modelling that in general the prevalence of infection within a host population is inversely related to pathogenicity. The more pathogenic the parasite, the more likely it is to regulate the host population, yet paradoxically the lower will be its equilibrium prevalence. Highly pathogenic diseases of invertebrates are often characterized by patterns of epidemic outbreak. Such dynamic characteristics are exemplified by many viral pathogens of forest and pasture pests and are in accordance with mathematical models derived by Anderson & May (1981). *Tetrahymena sialidos* is, therefore, of particular interest in exhibiting features of high prevalence combined with high pathogenicity, yet apparently expressed consistently and not epidemically. Significantly, Anderson & May (1982) have dispelled the myth that the only well-balanced ('successful') host–parasite relationship is one in which the parasite causes little harm to the host.

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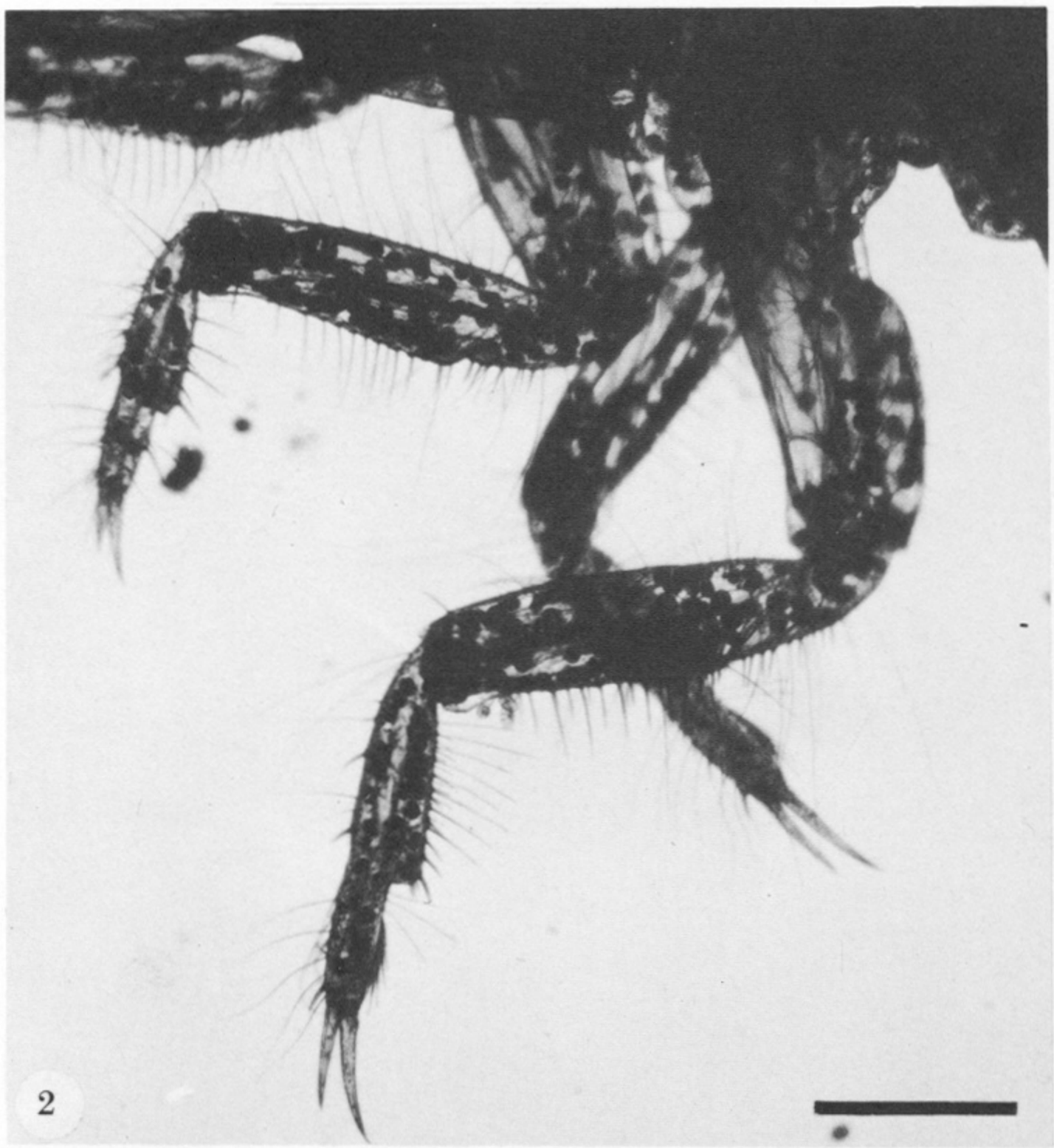
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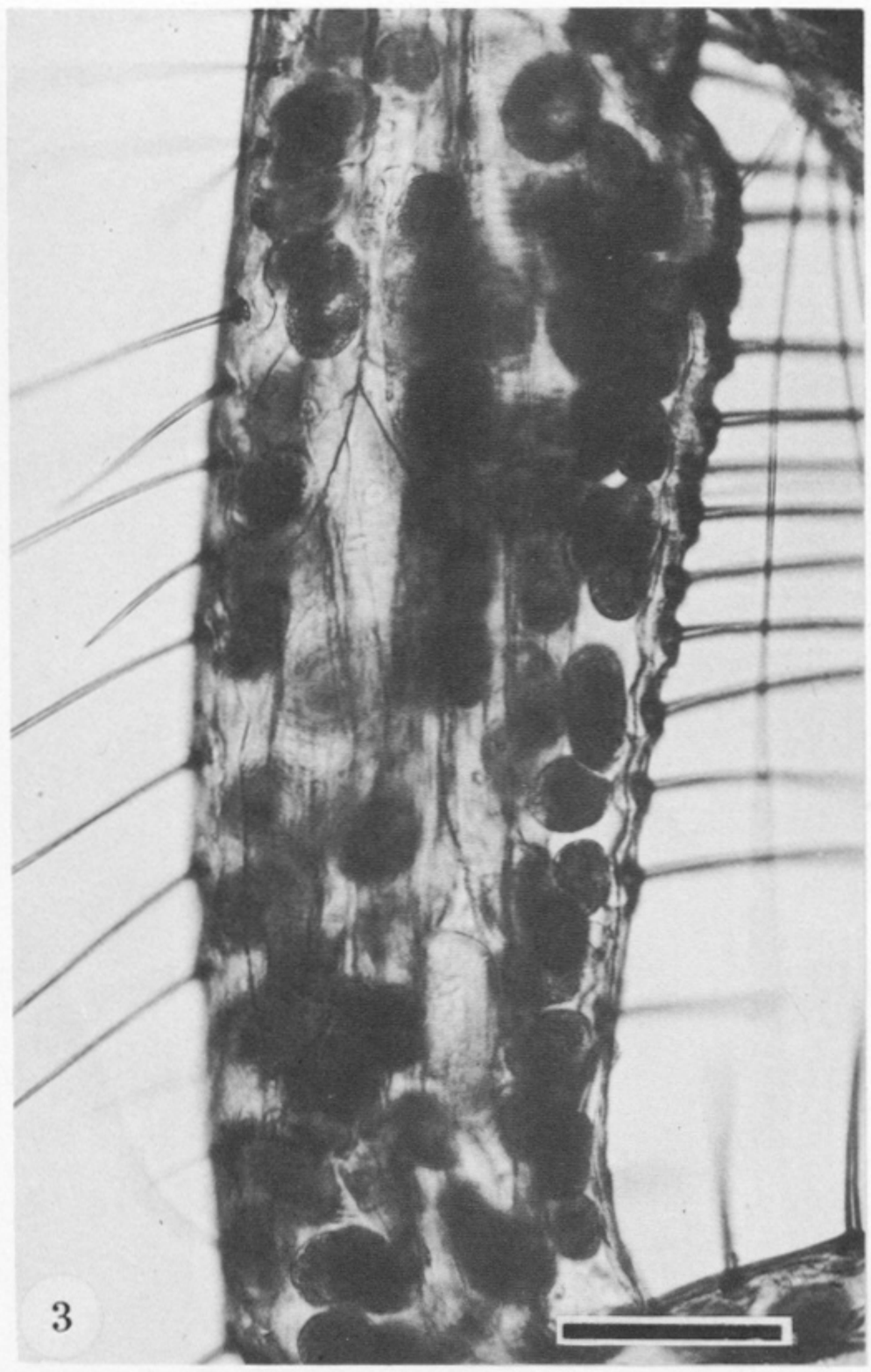
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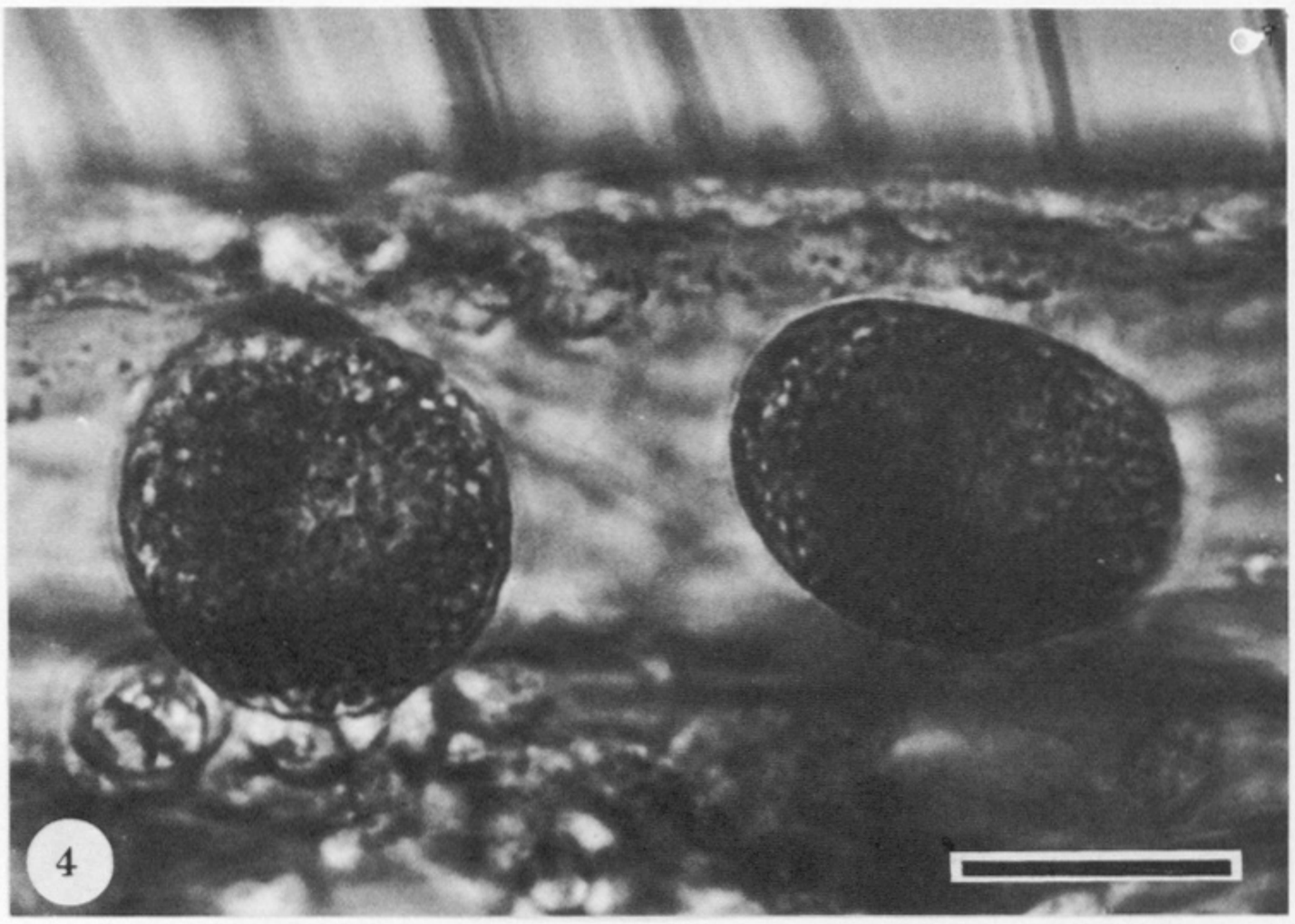
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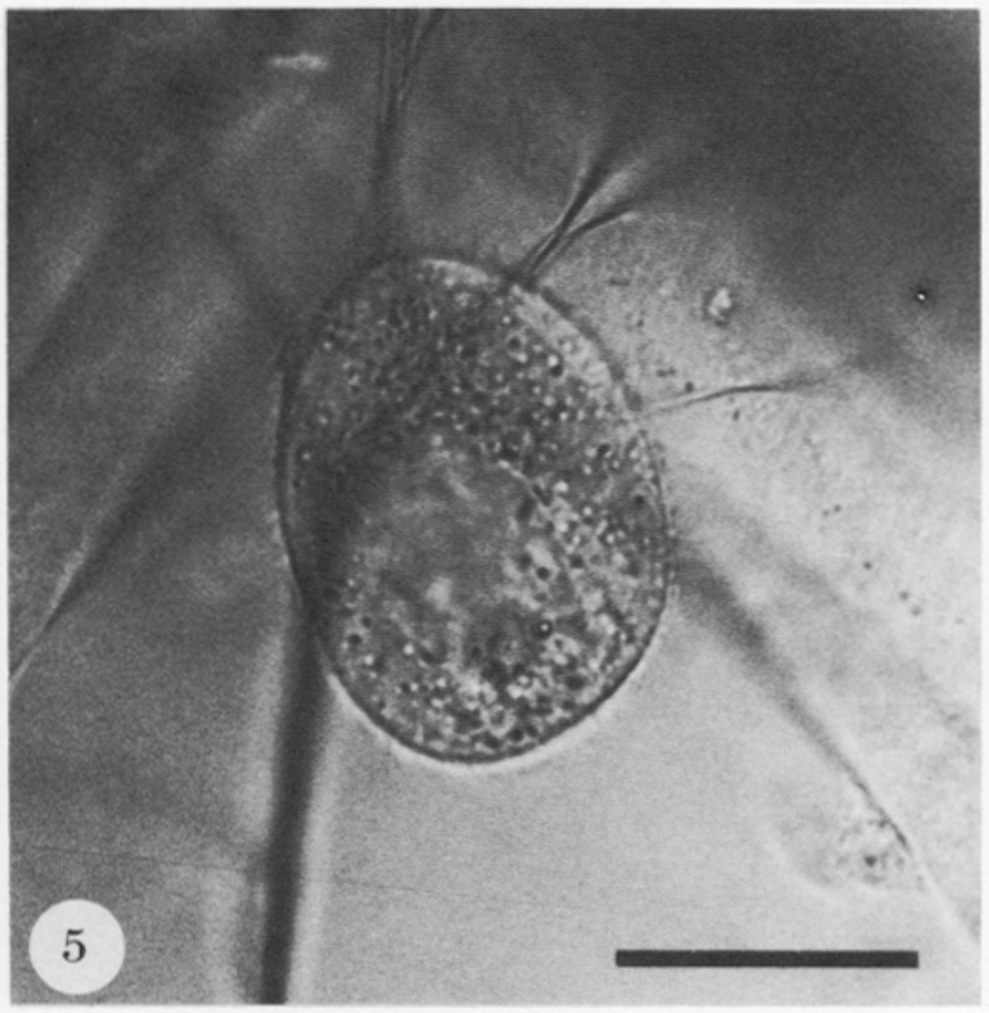
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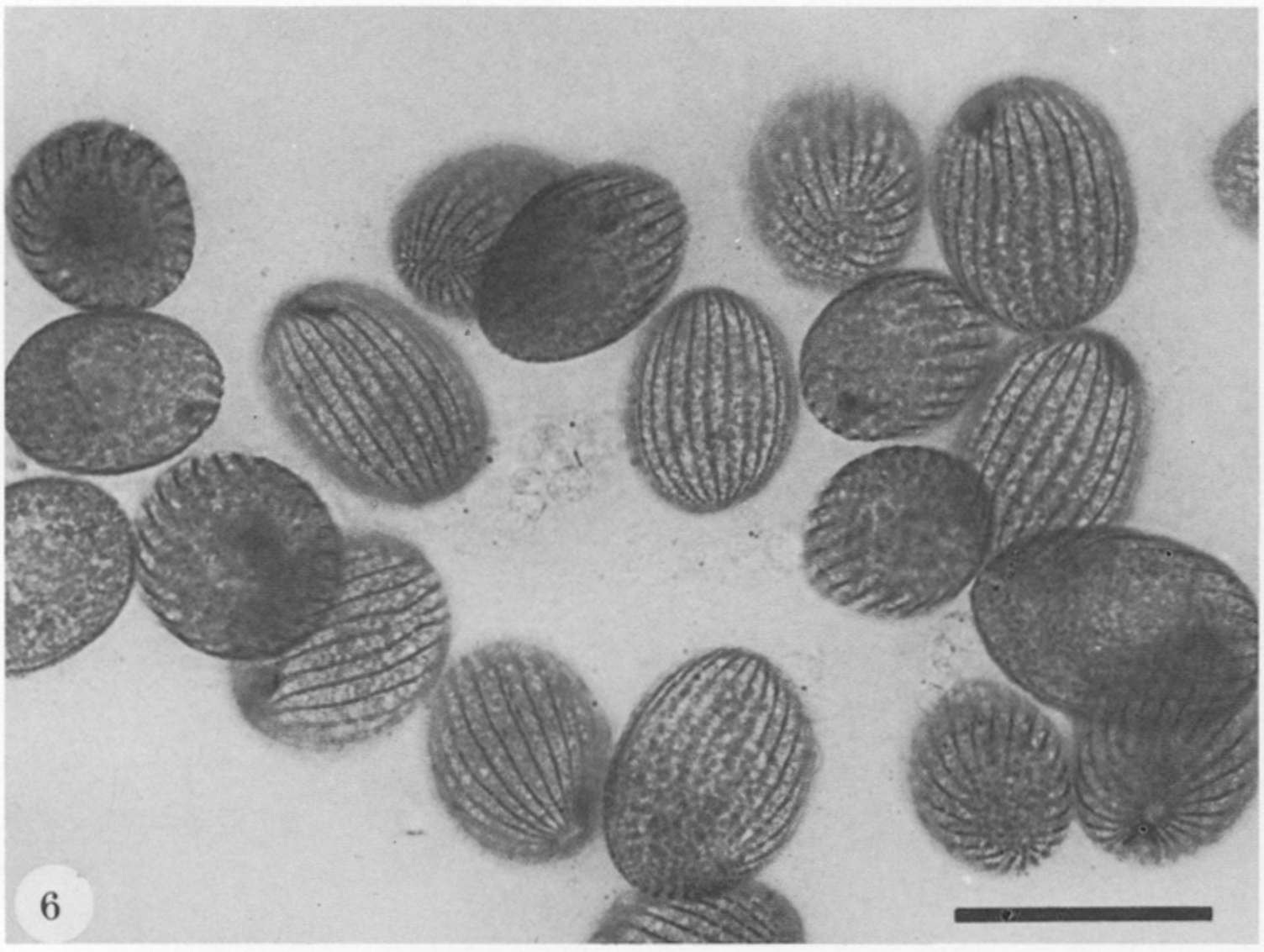
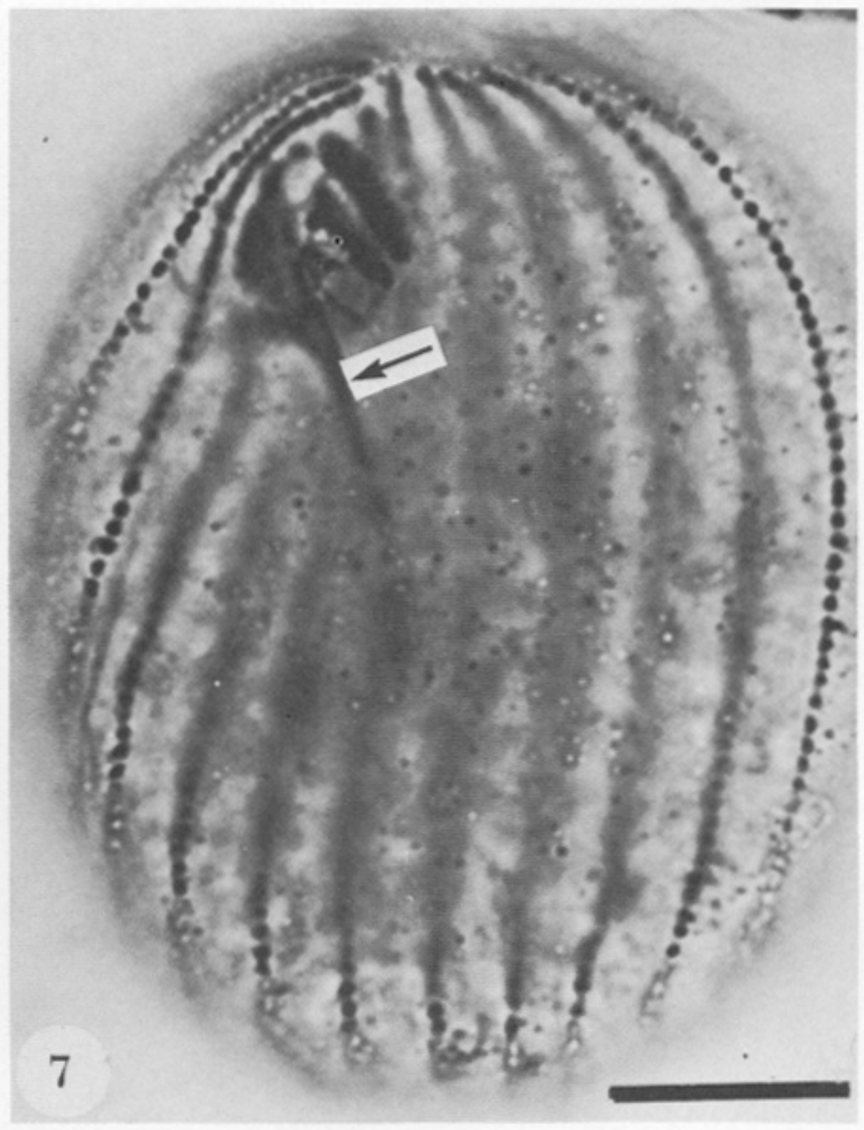
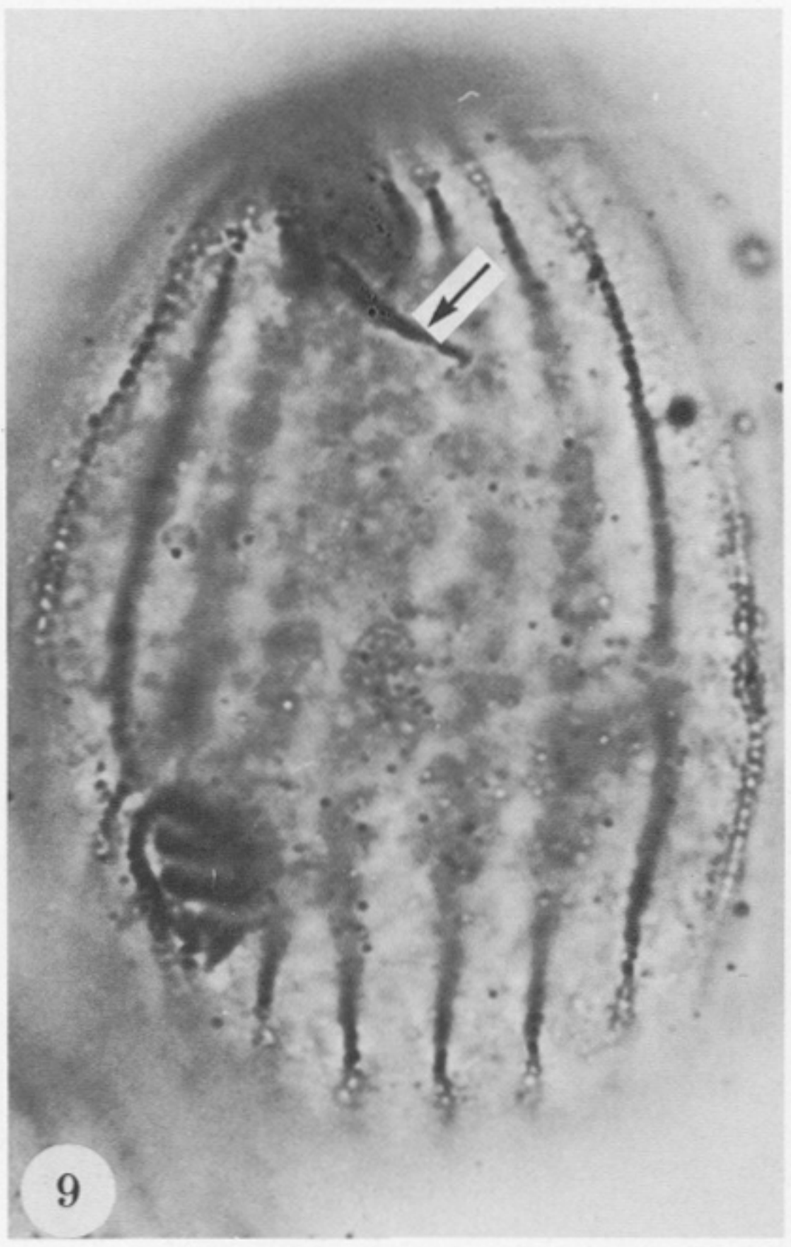
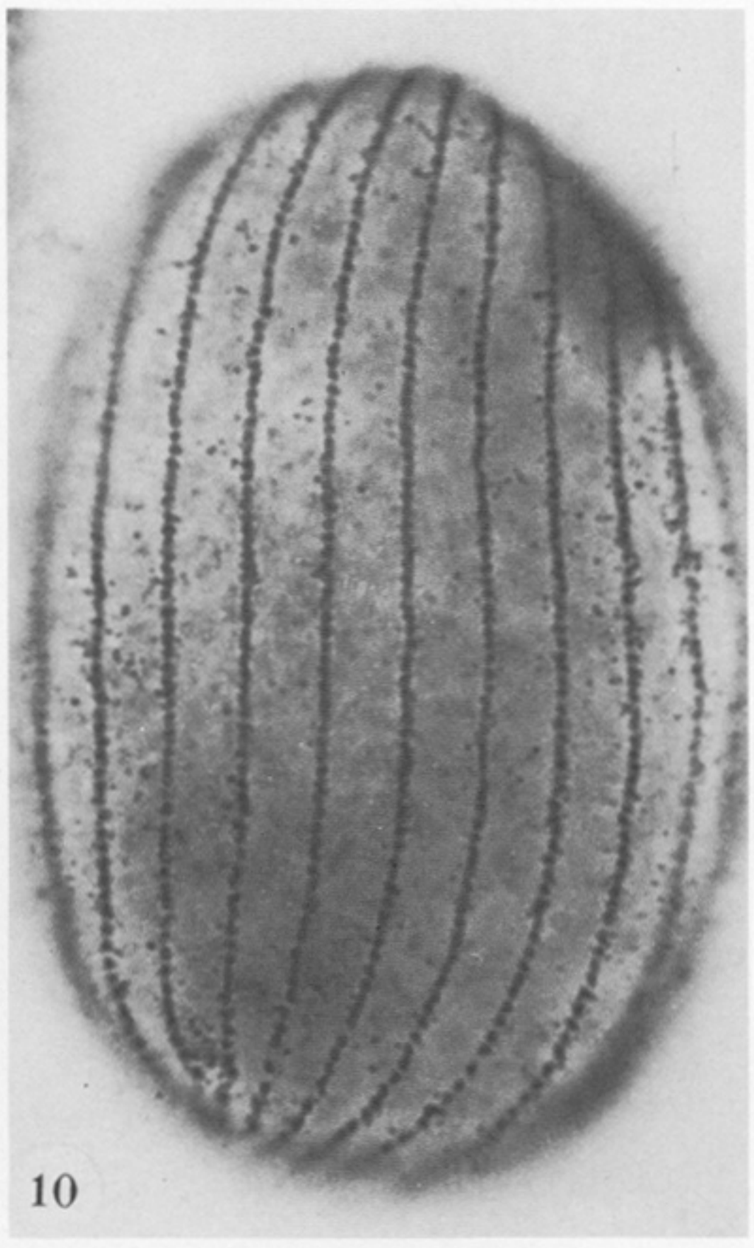
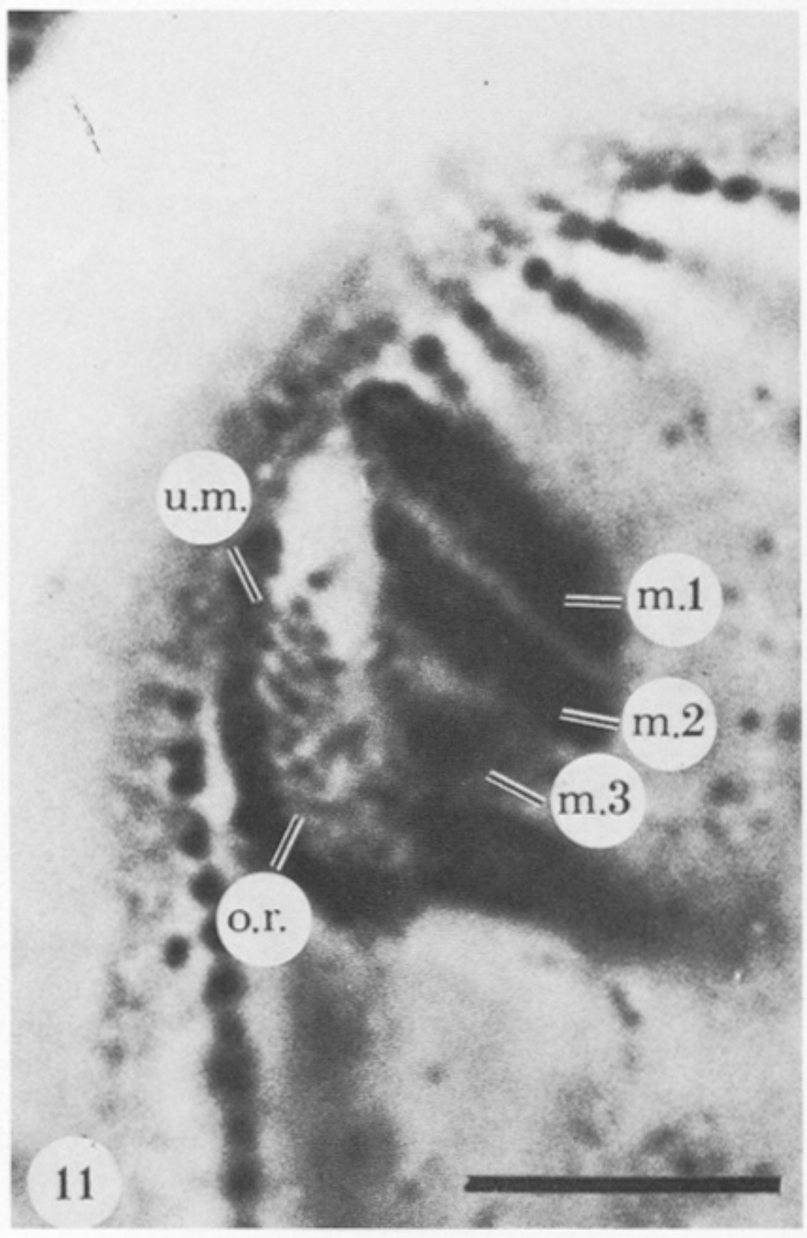
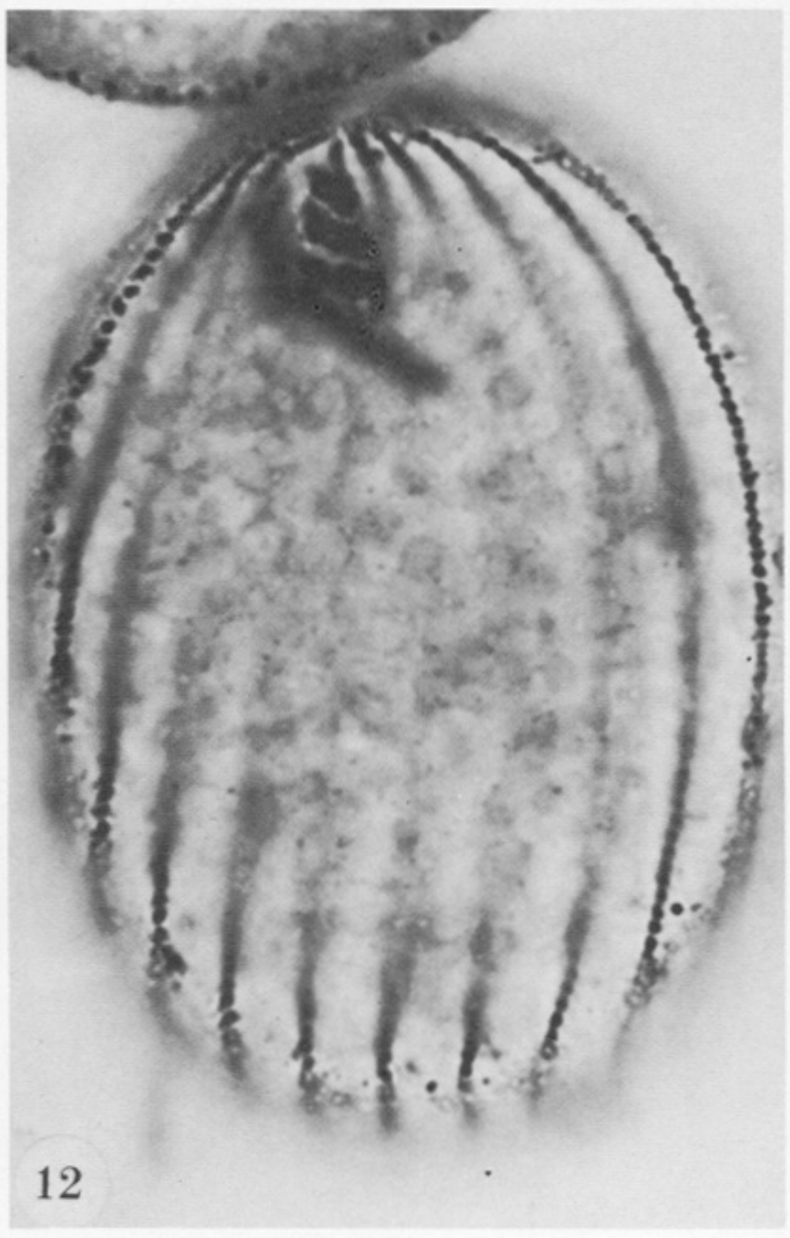


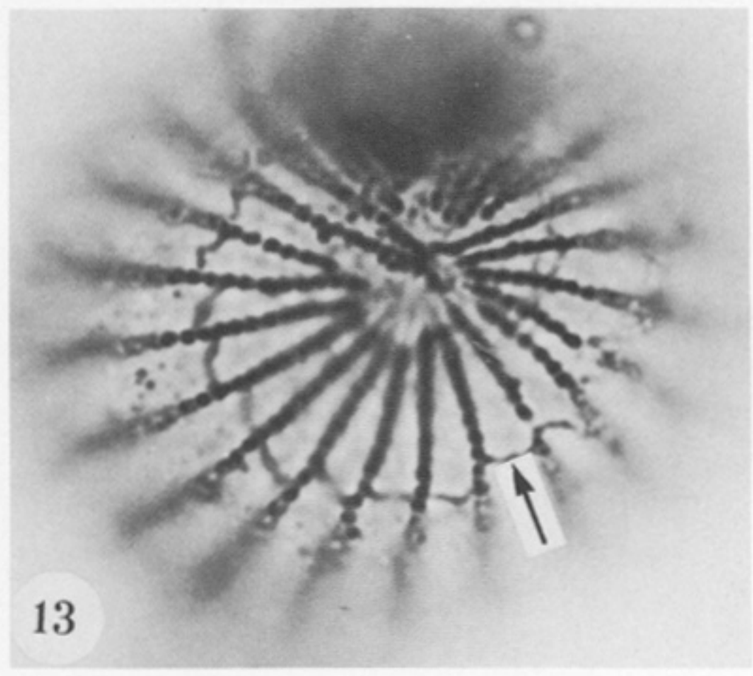


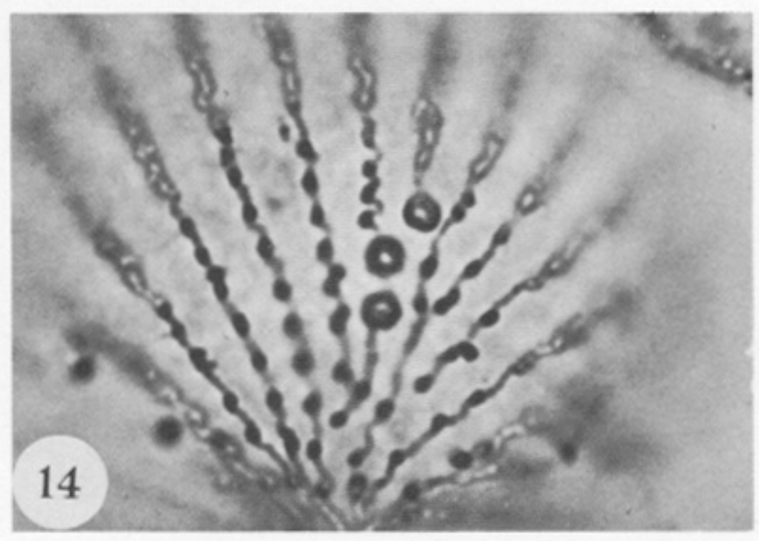




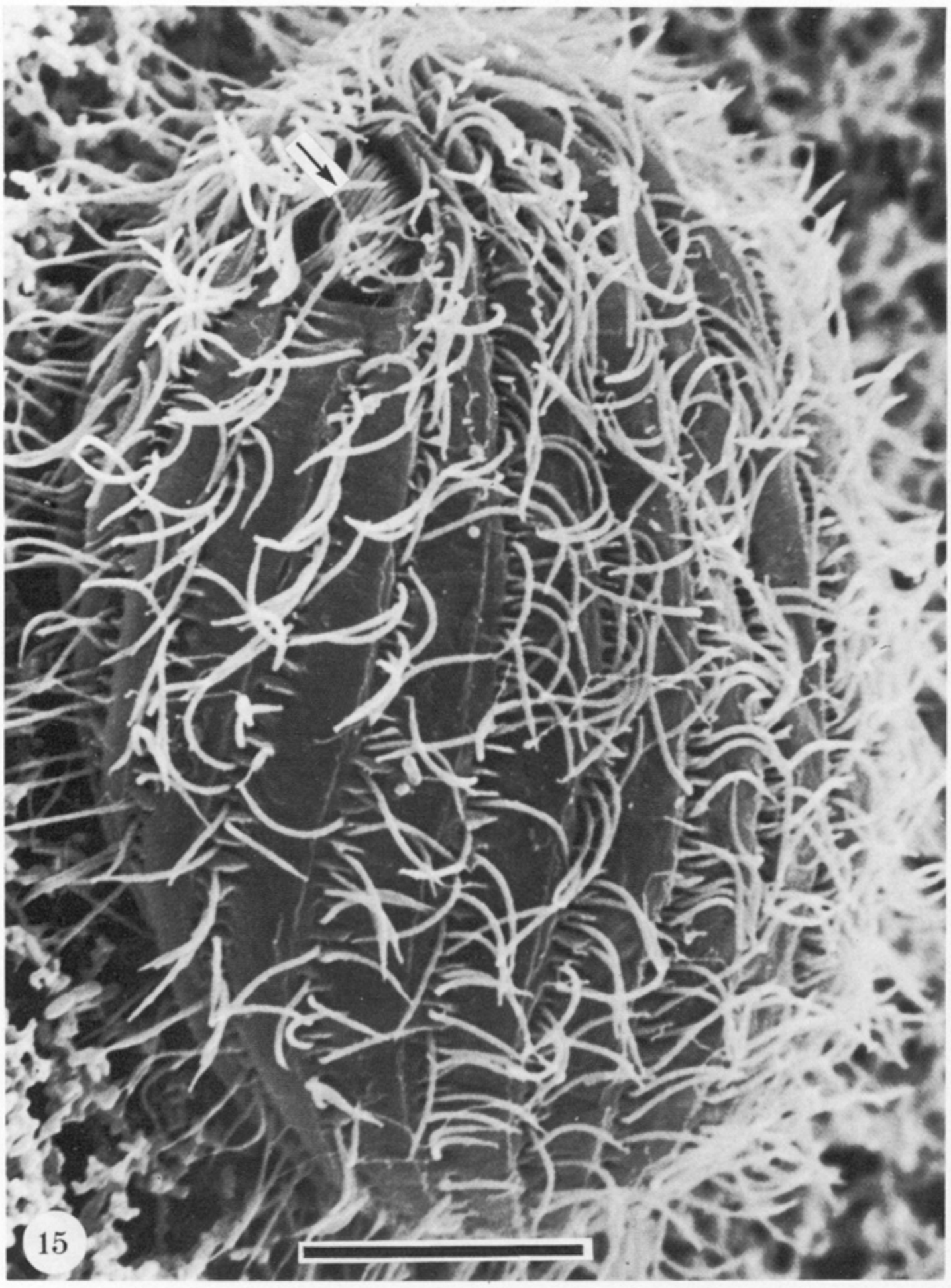




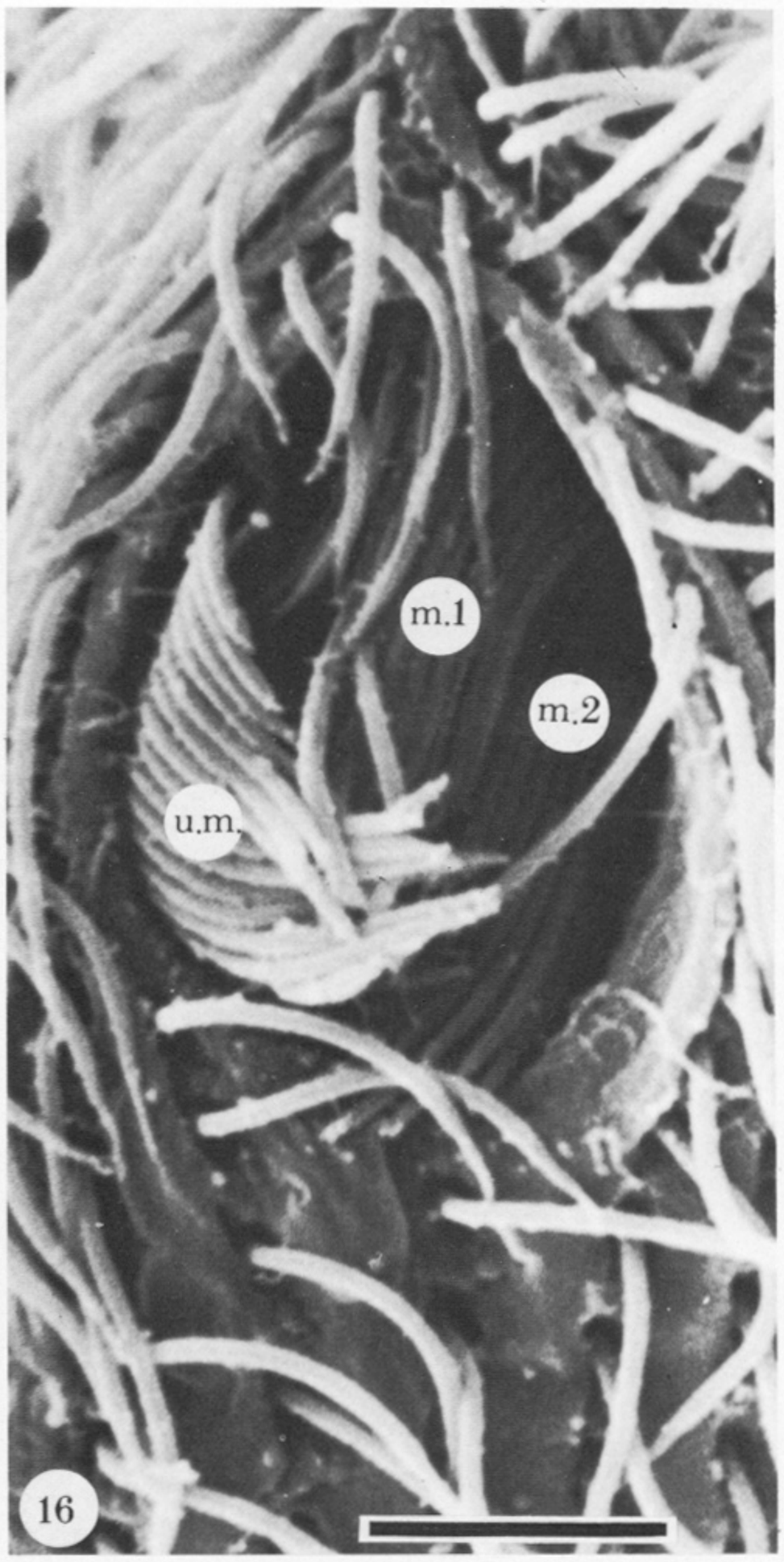




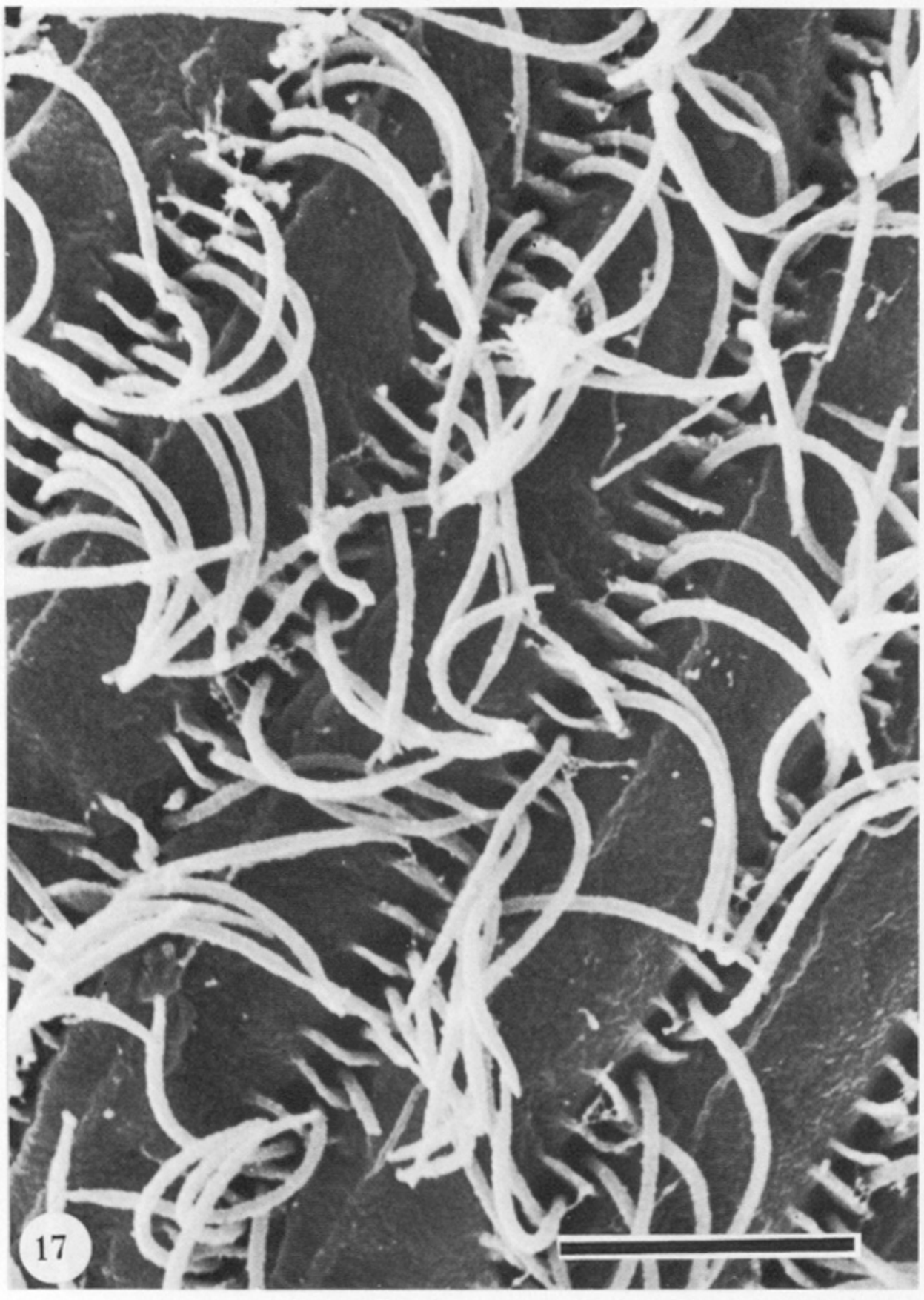
FIGURES 6–14. For description see opposite.



15



16



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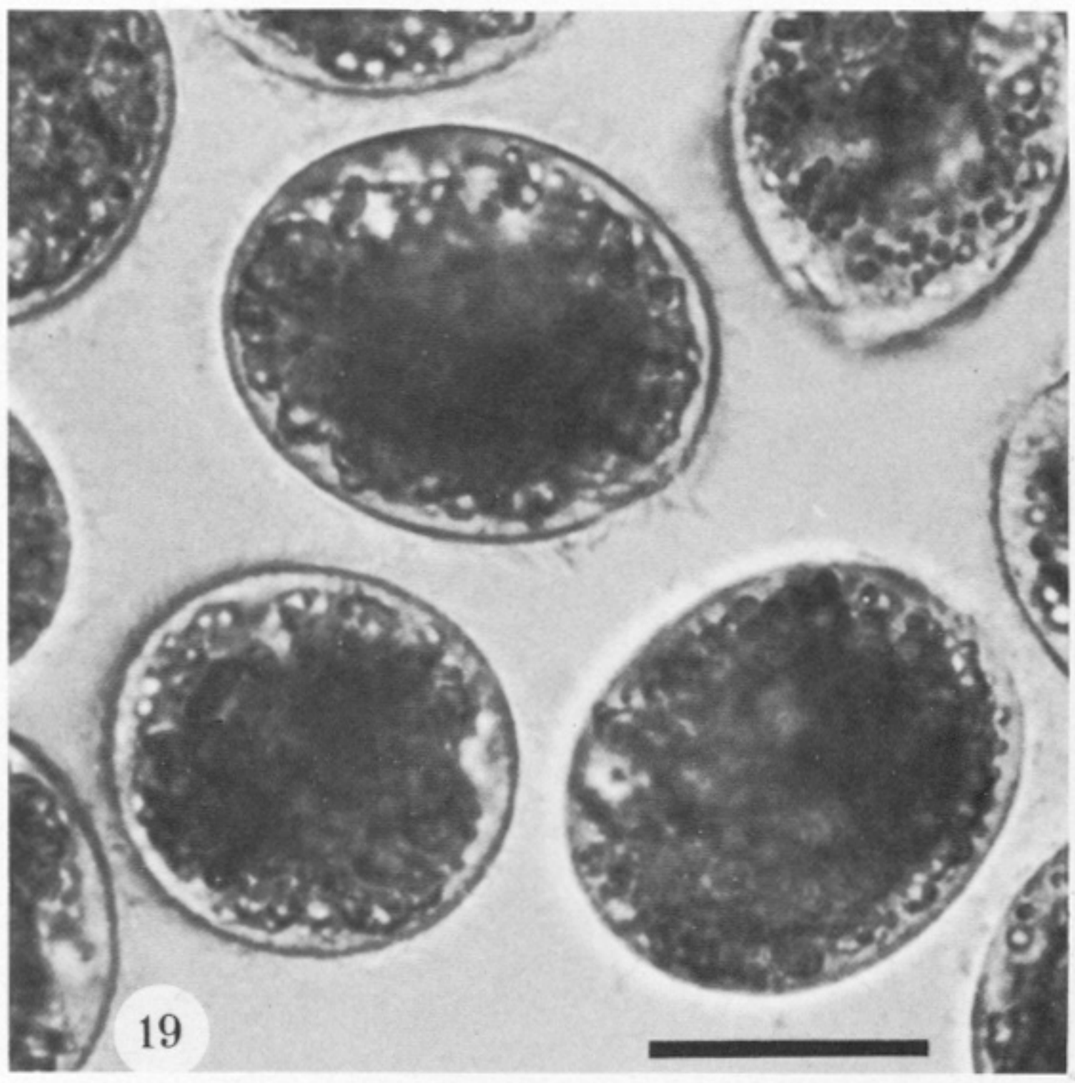
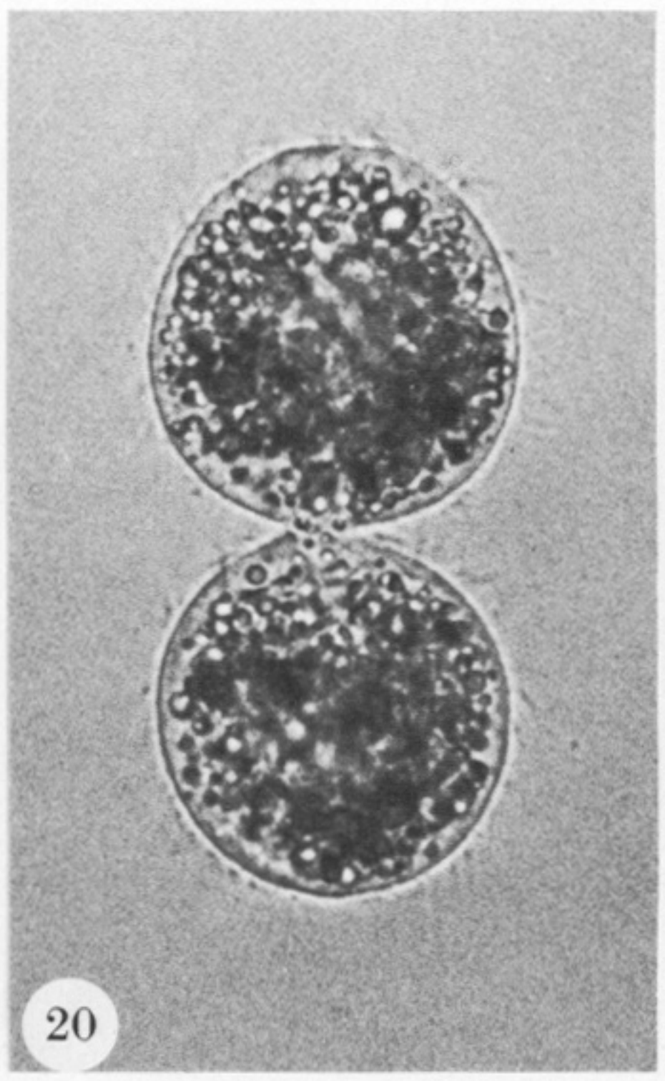
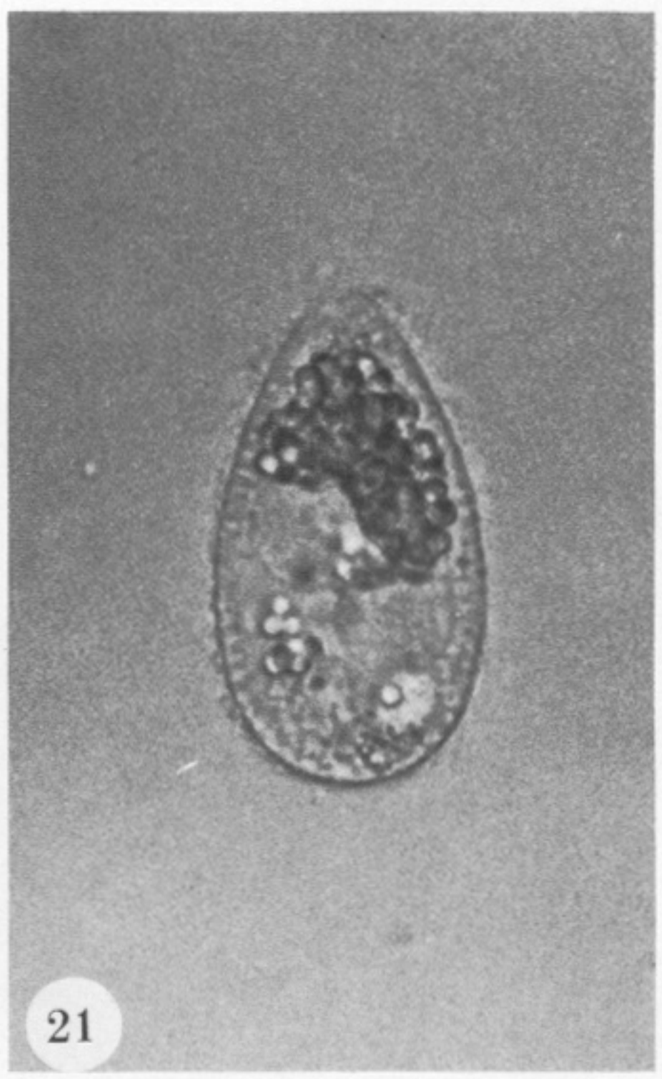
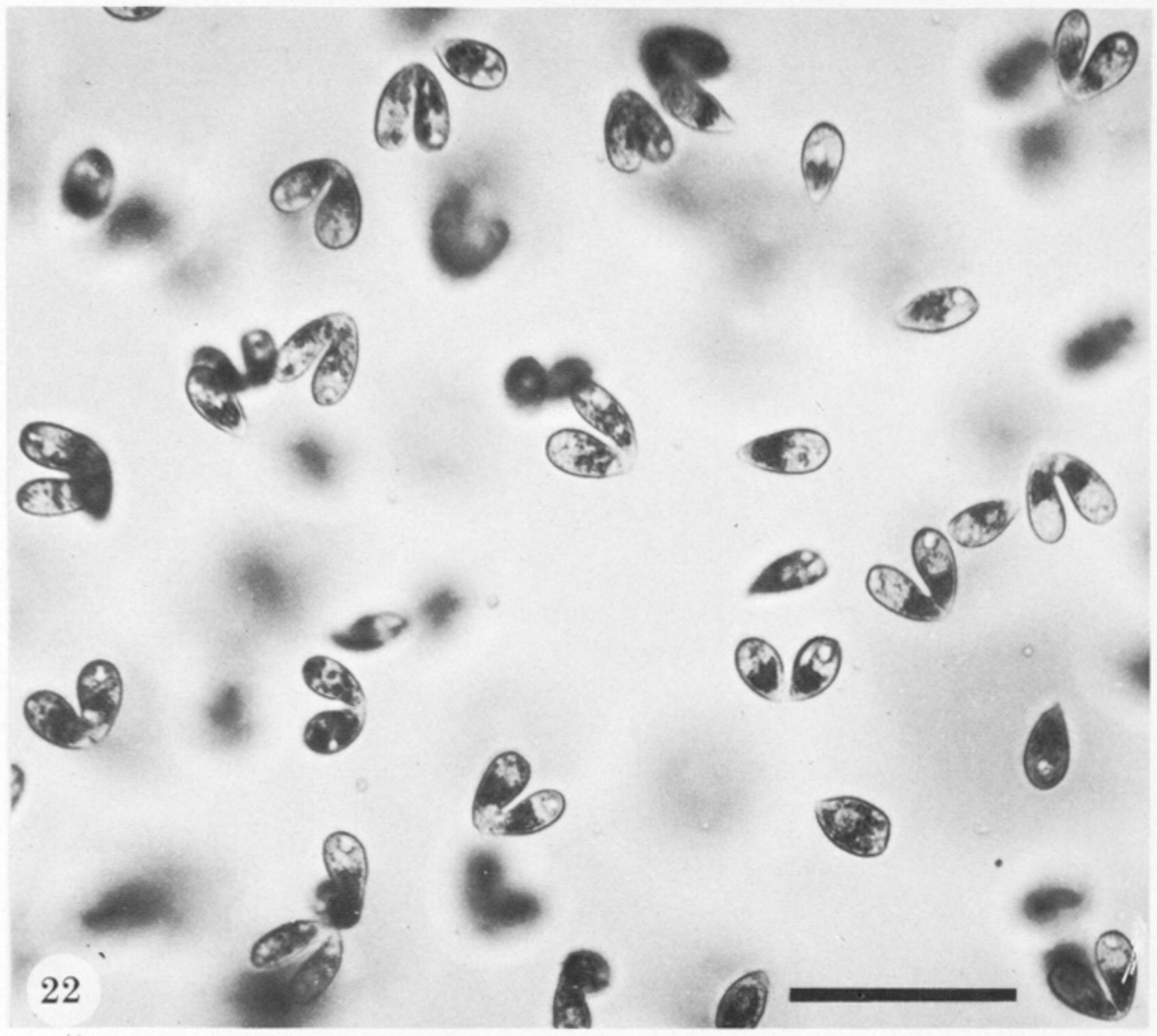
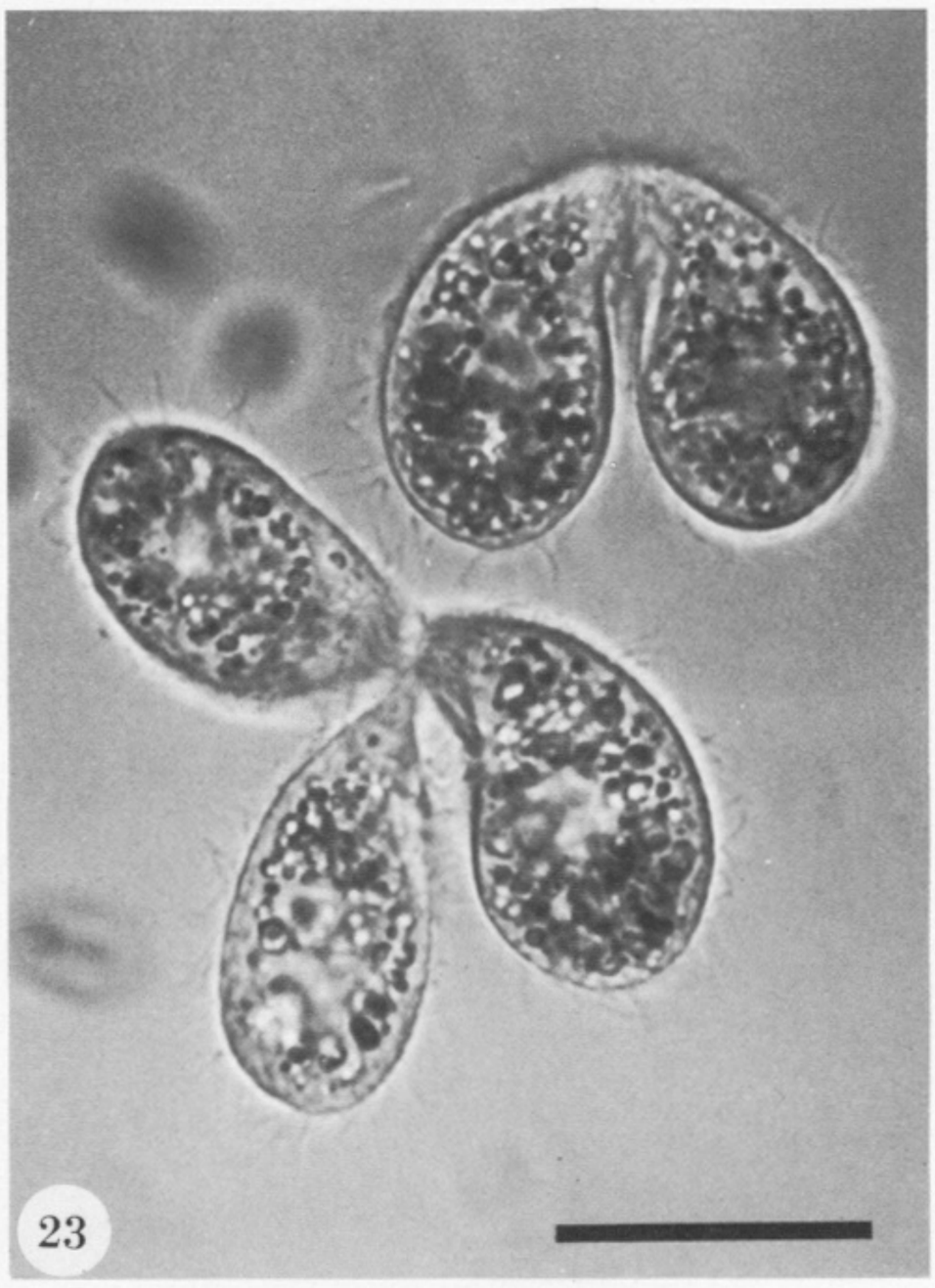
FIGURES 15–18. For description see opposite.

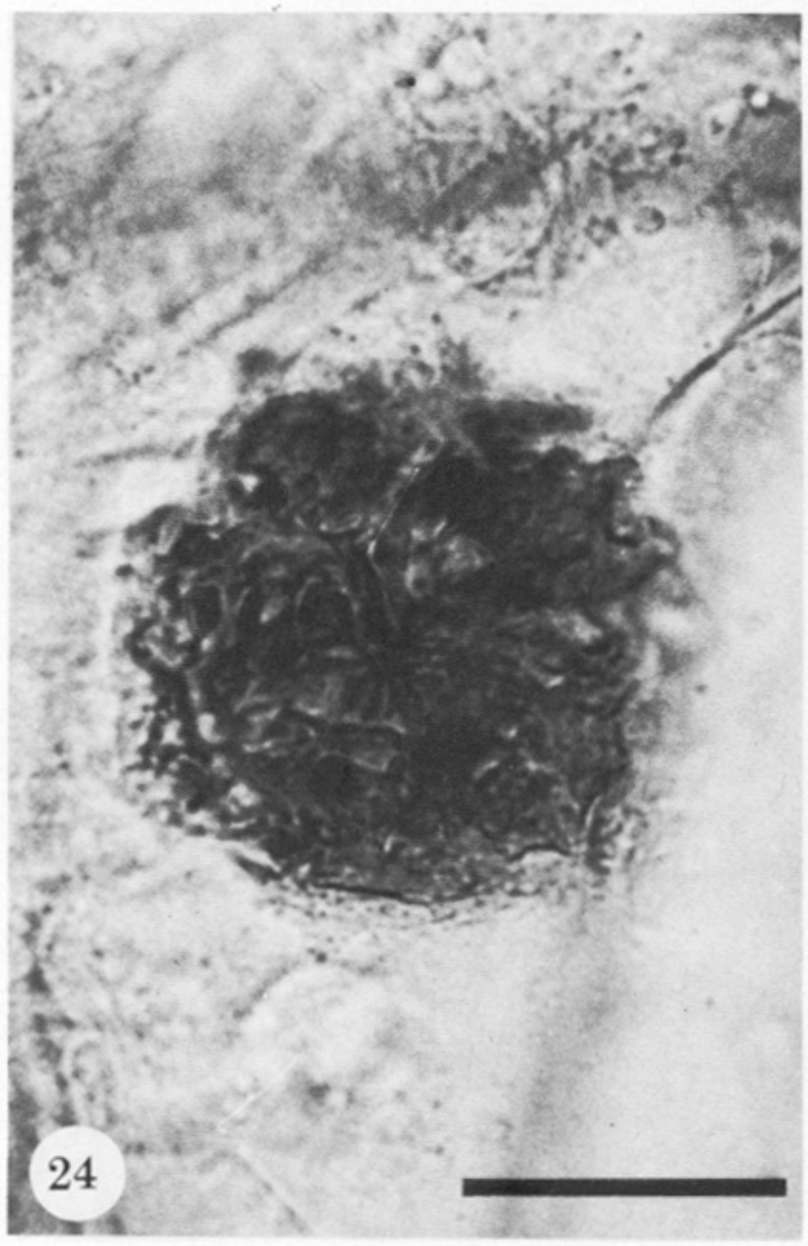


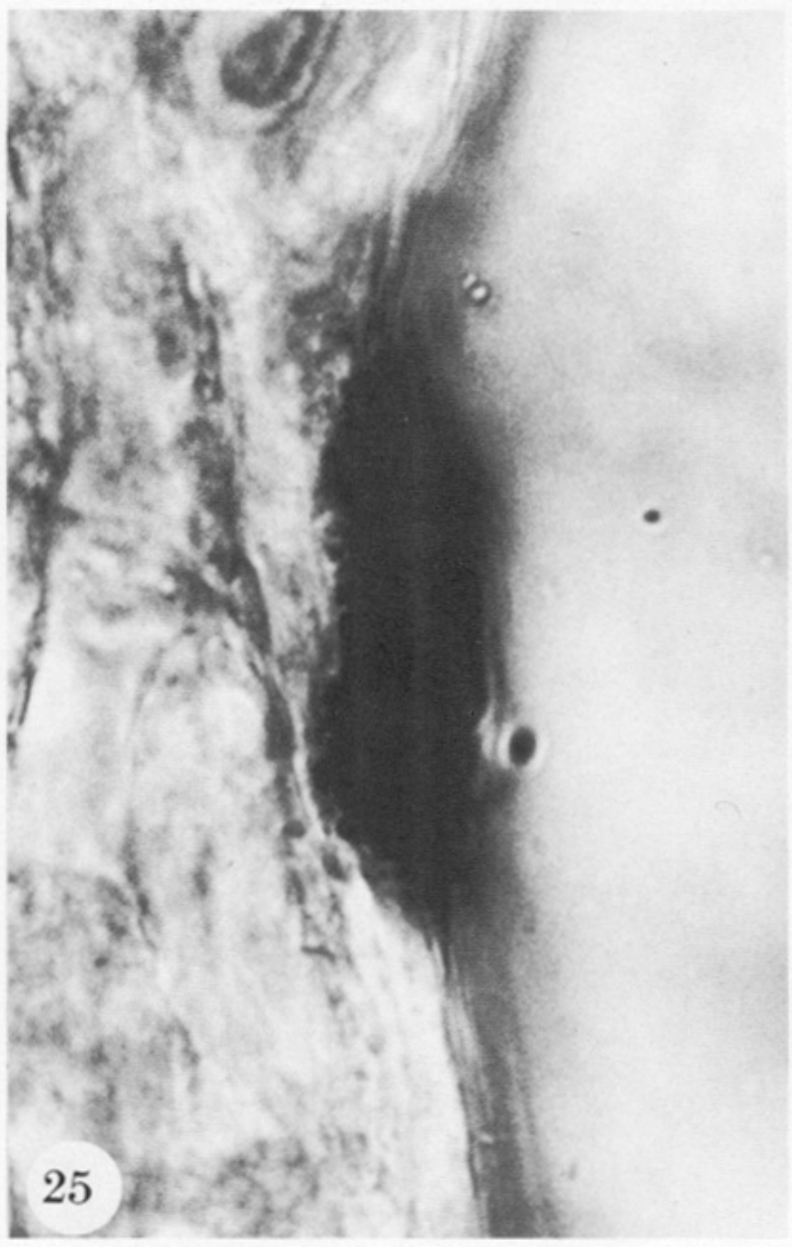


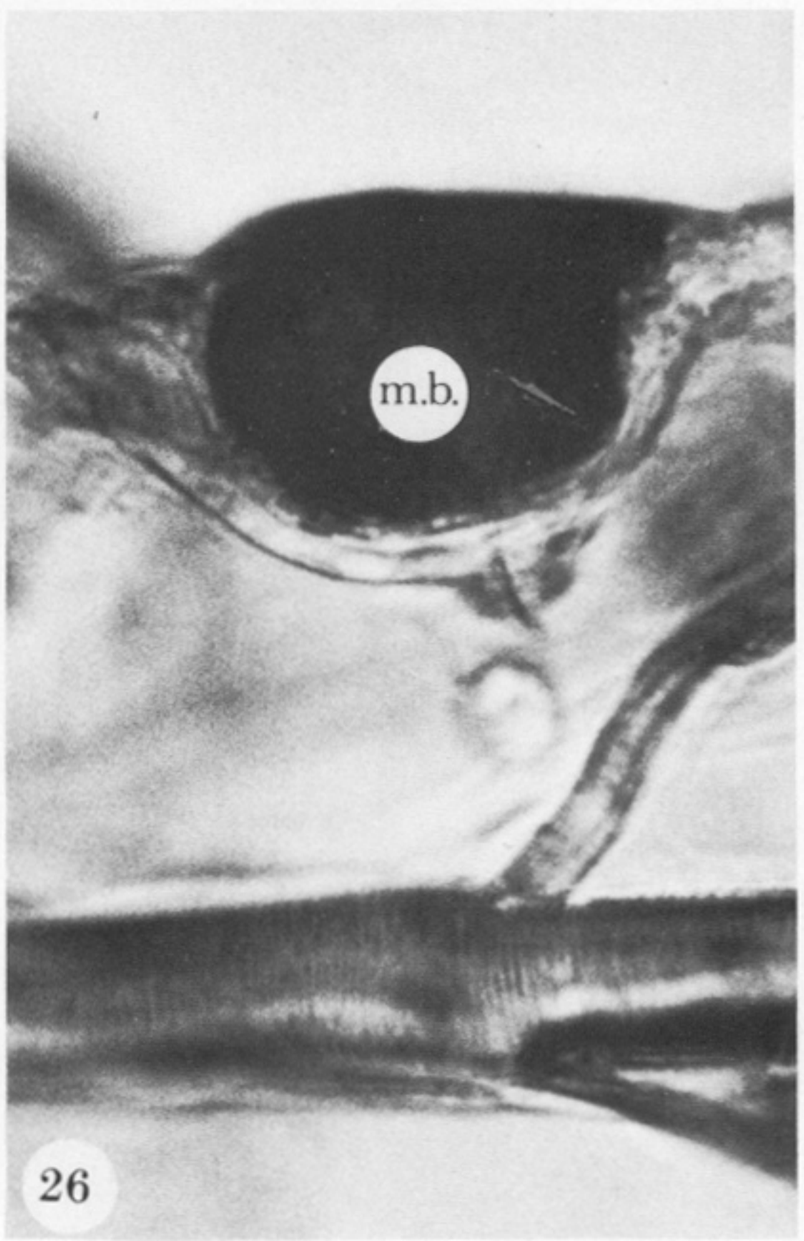




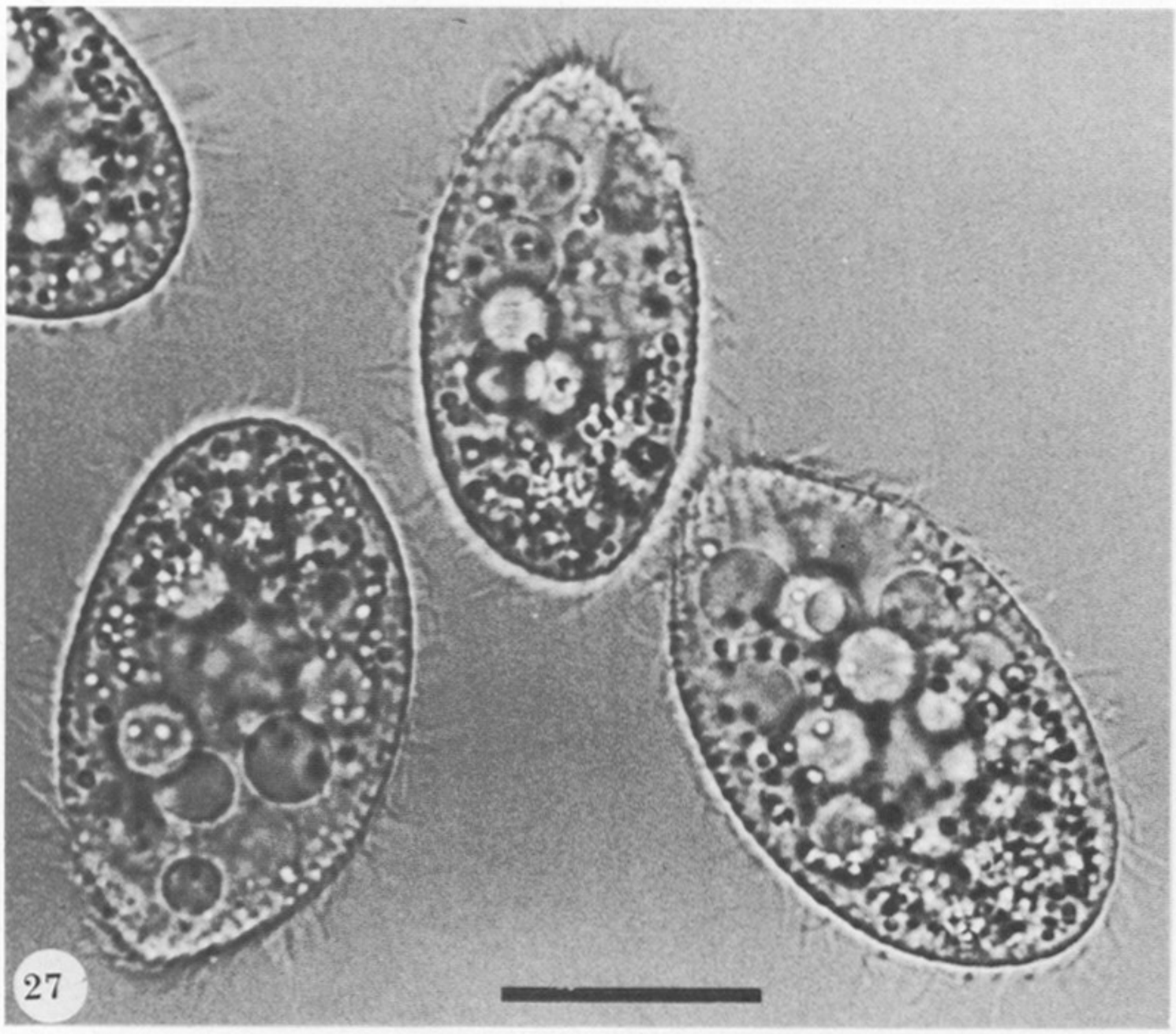








FIGURES 19–26. For description see opposite.



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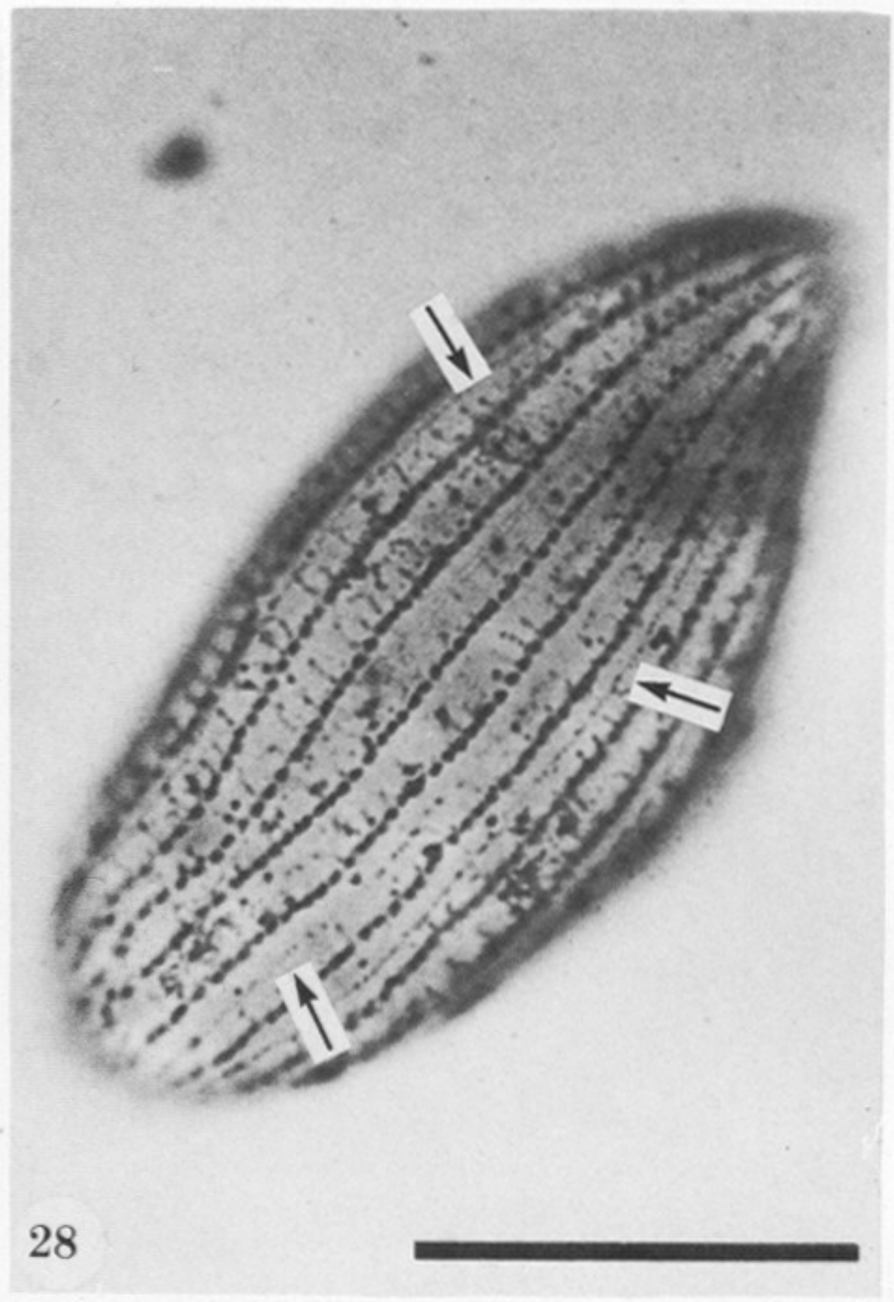
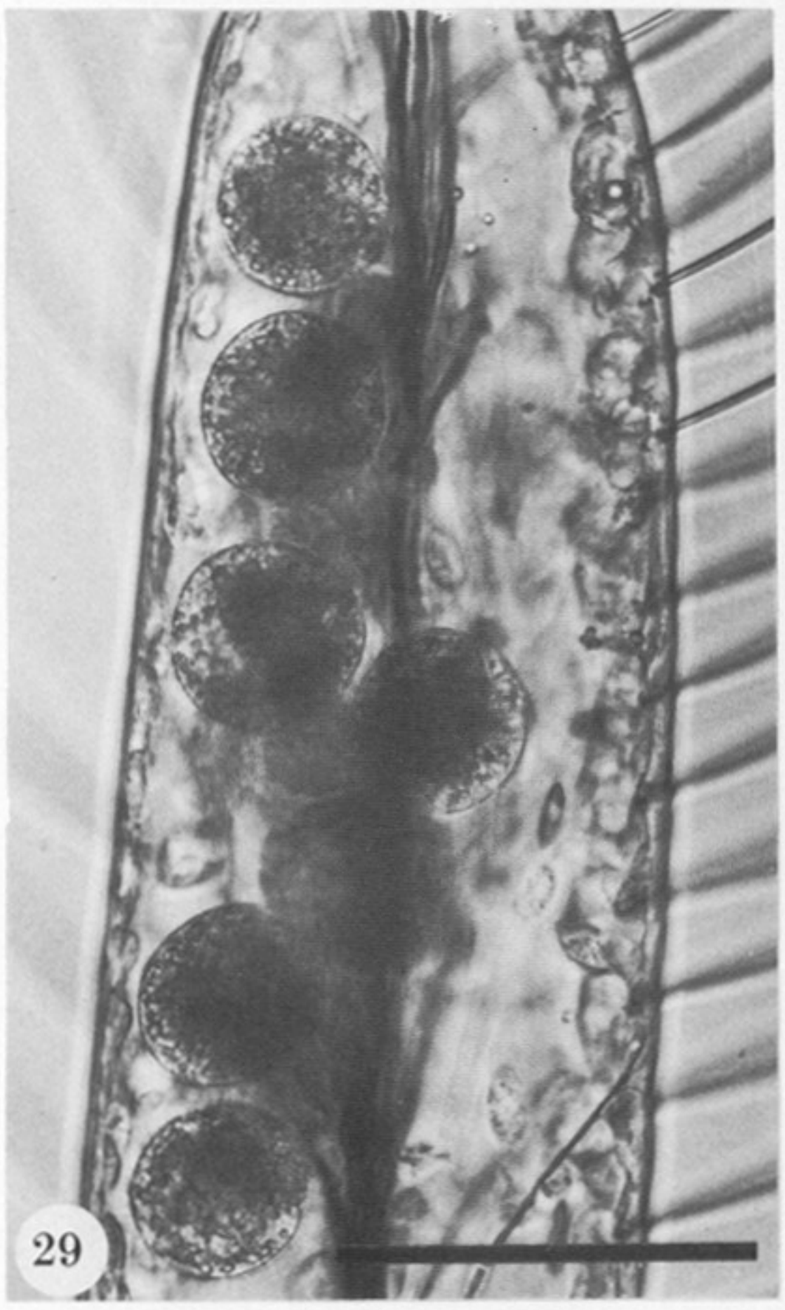
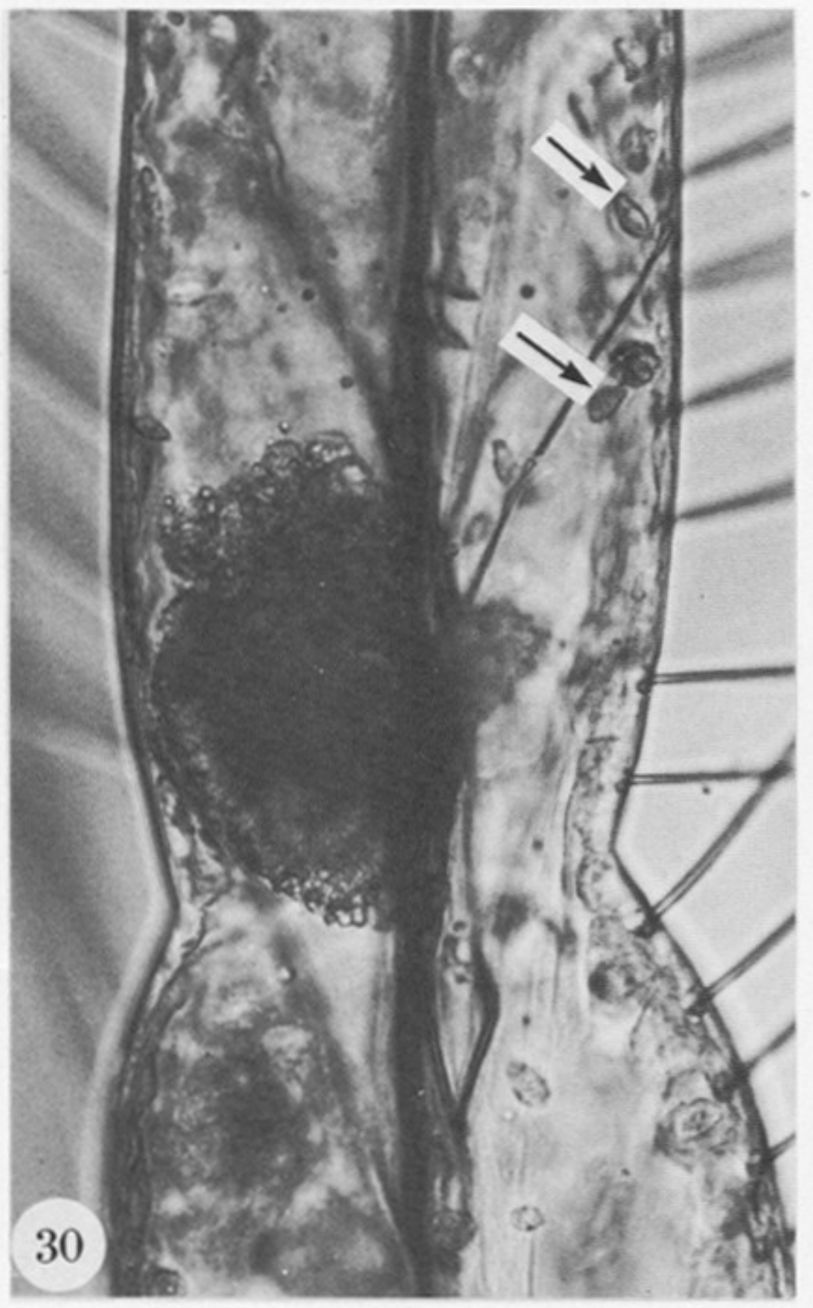


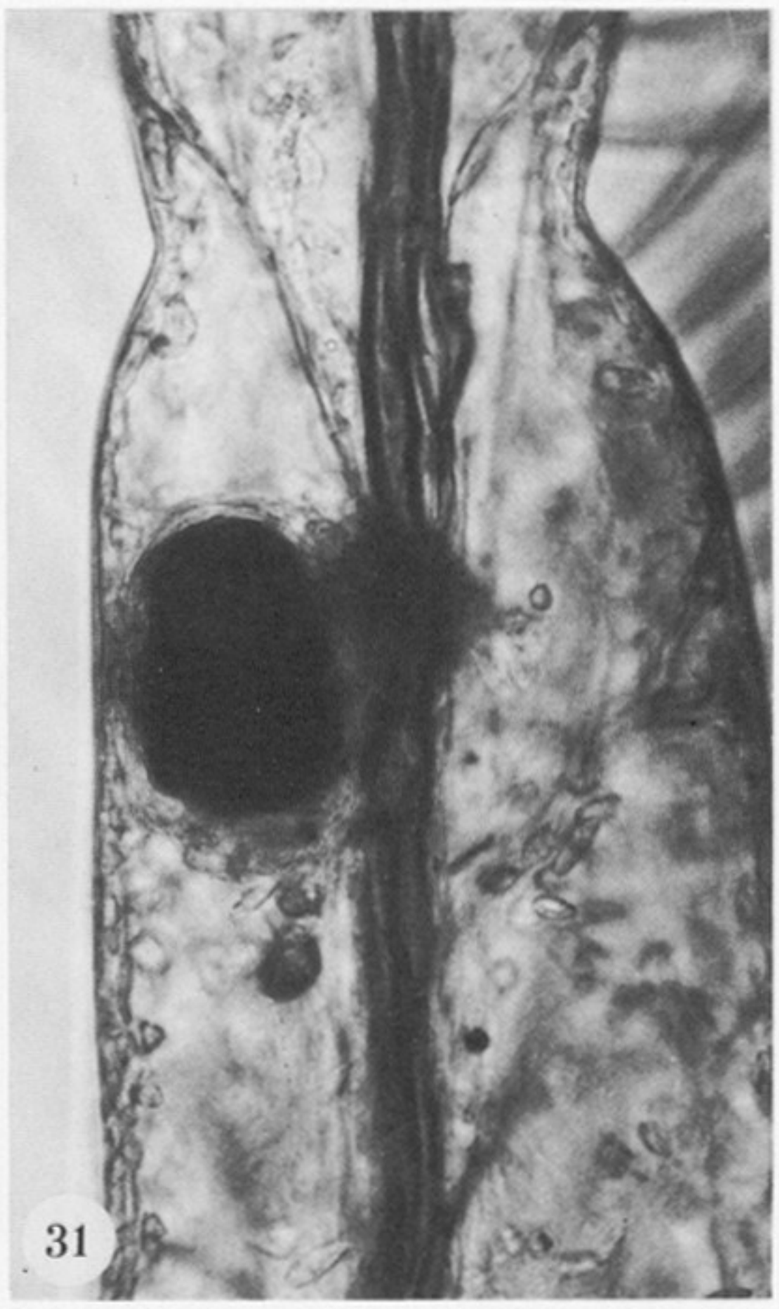
28



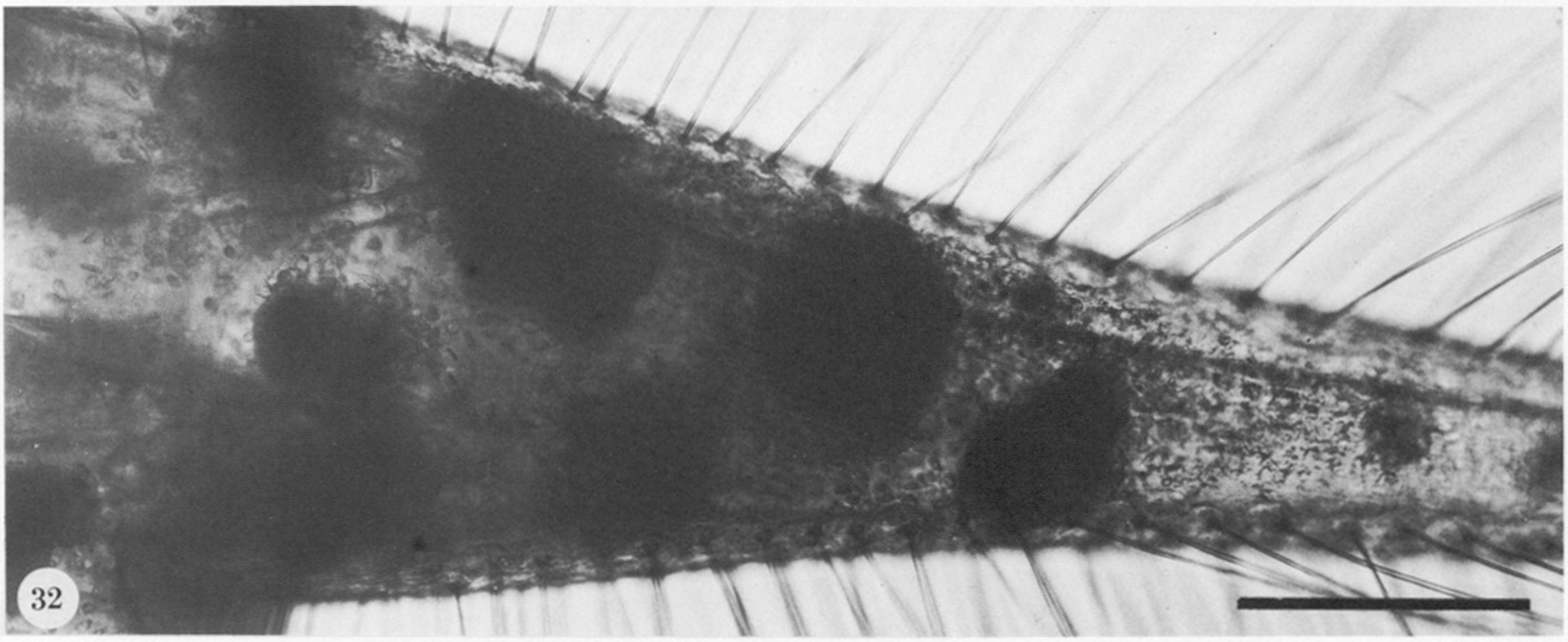
29



30

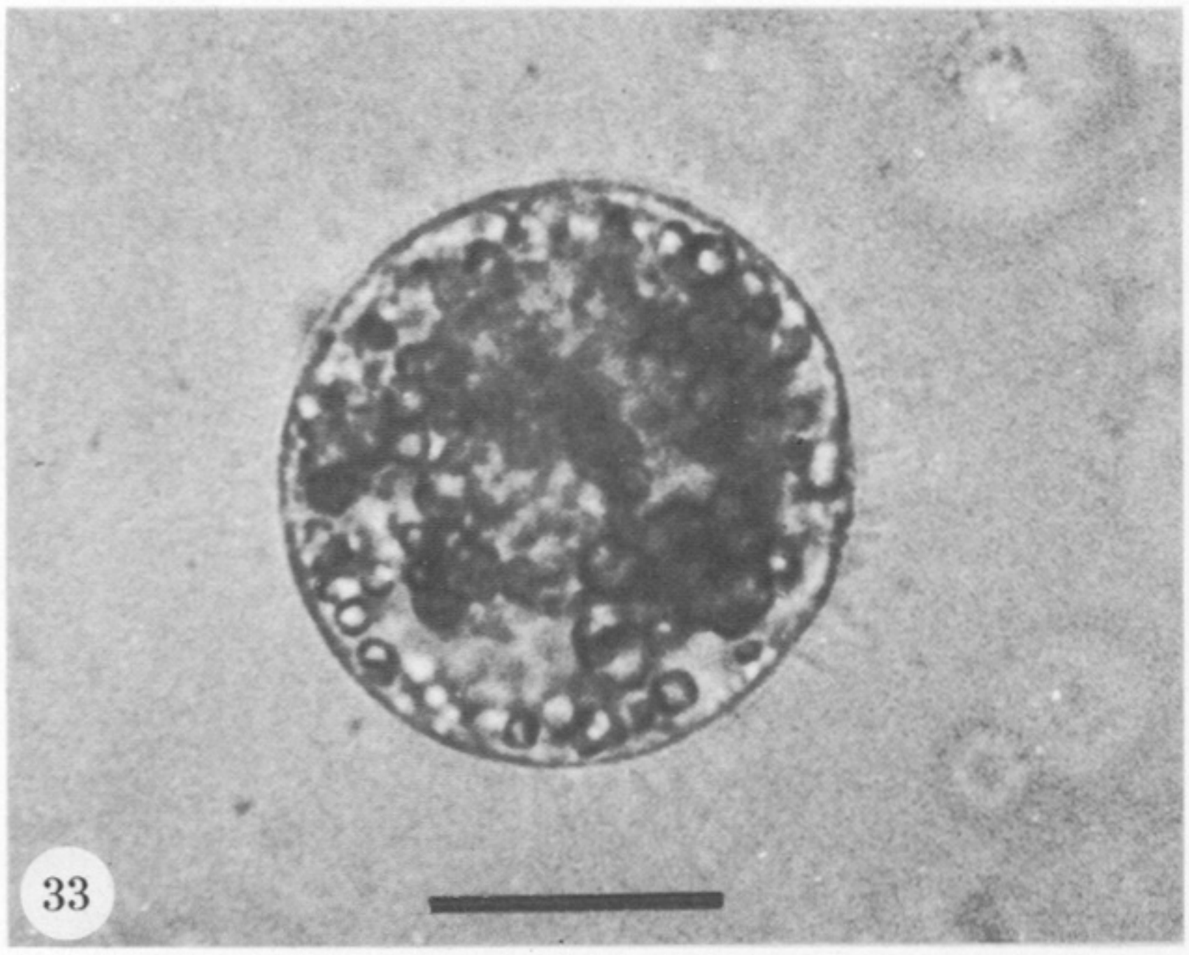


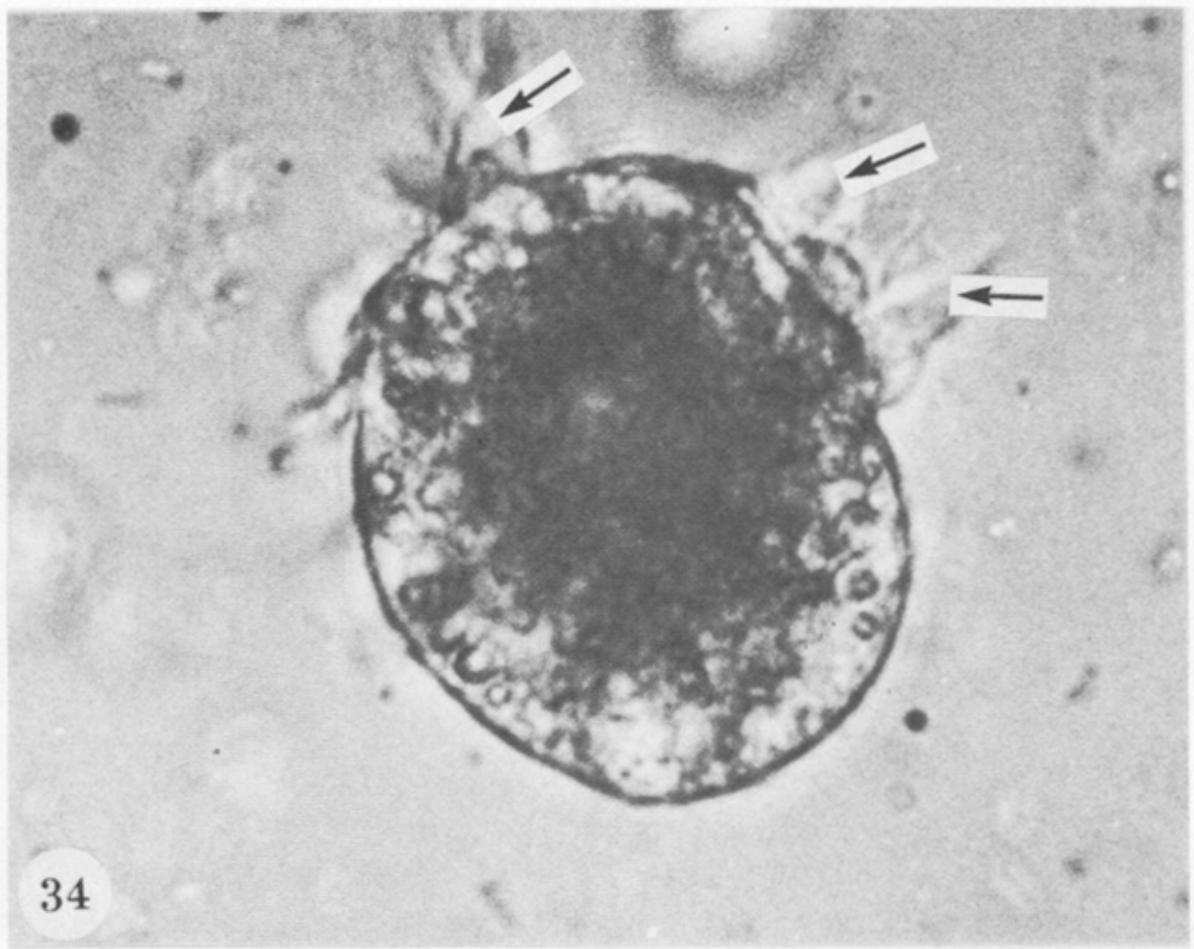
31

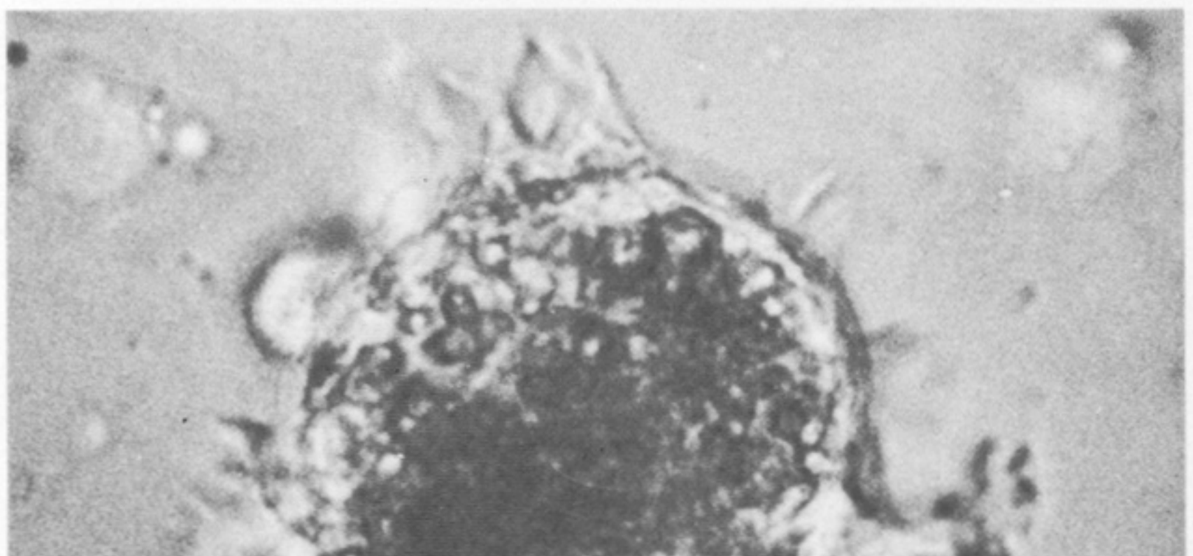


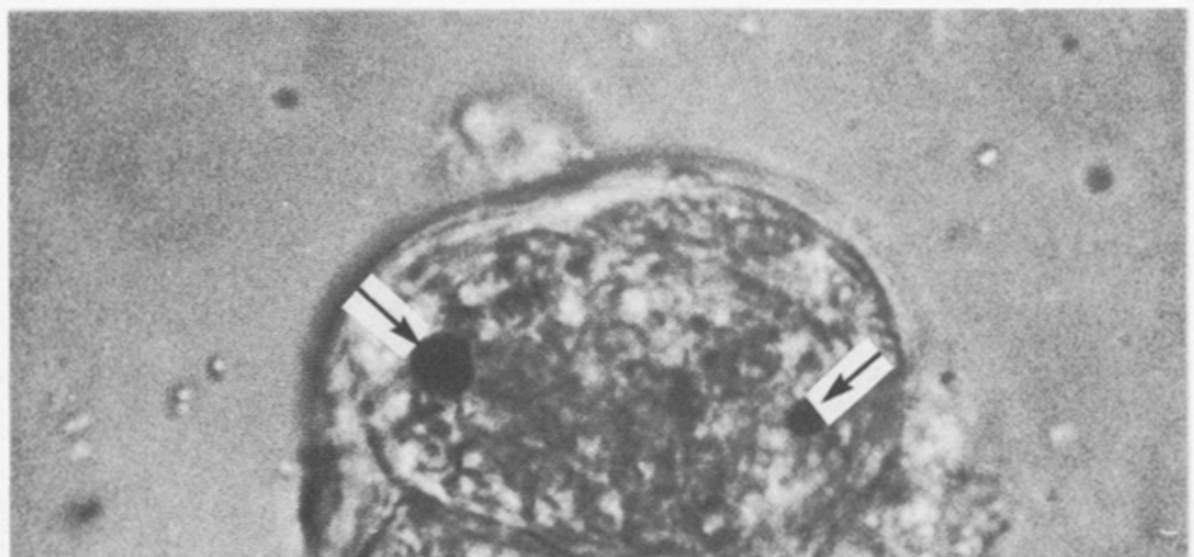
32

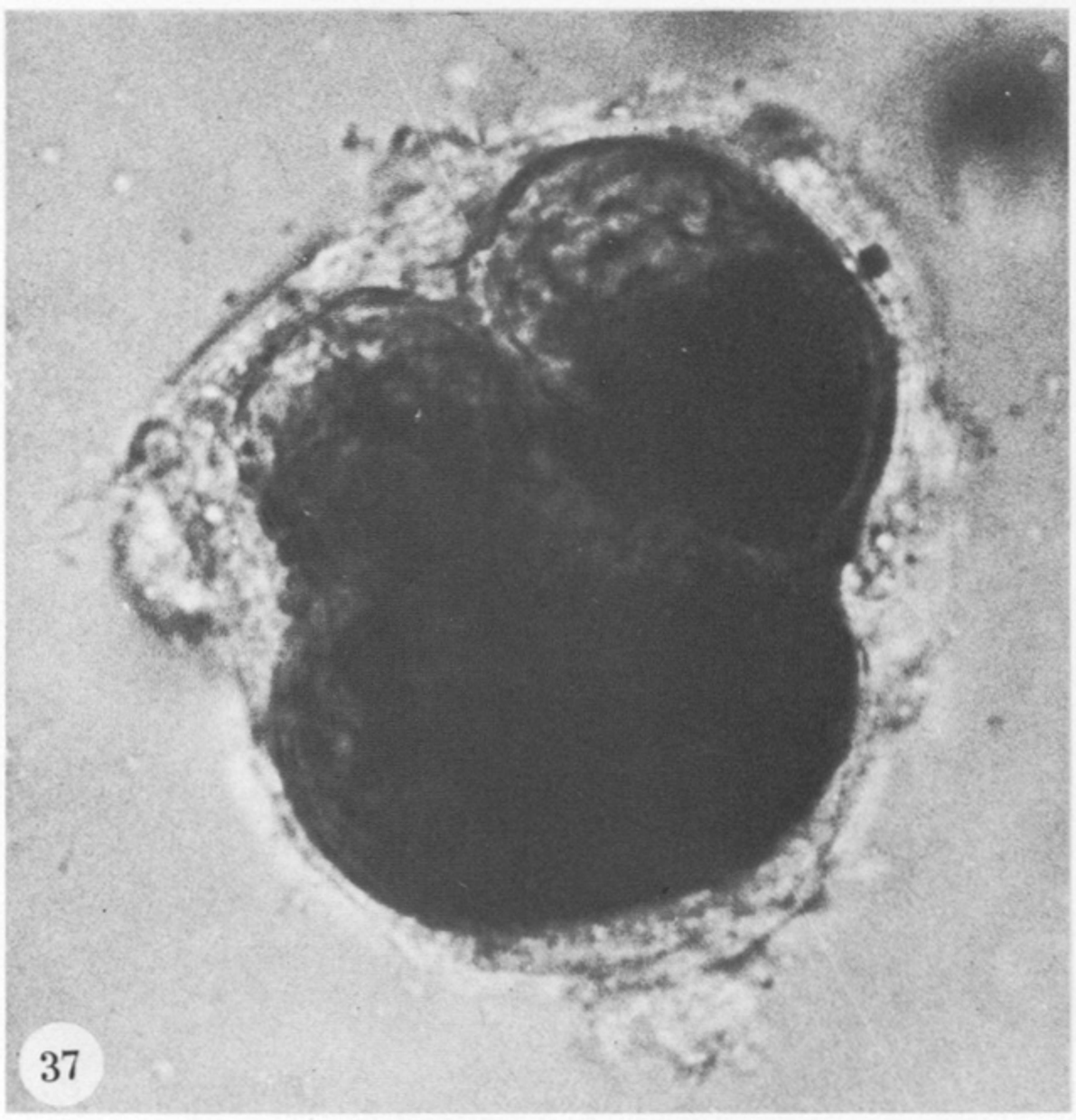
FIGURES 27–32. For description see opposite

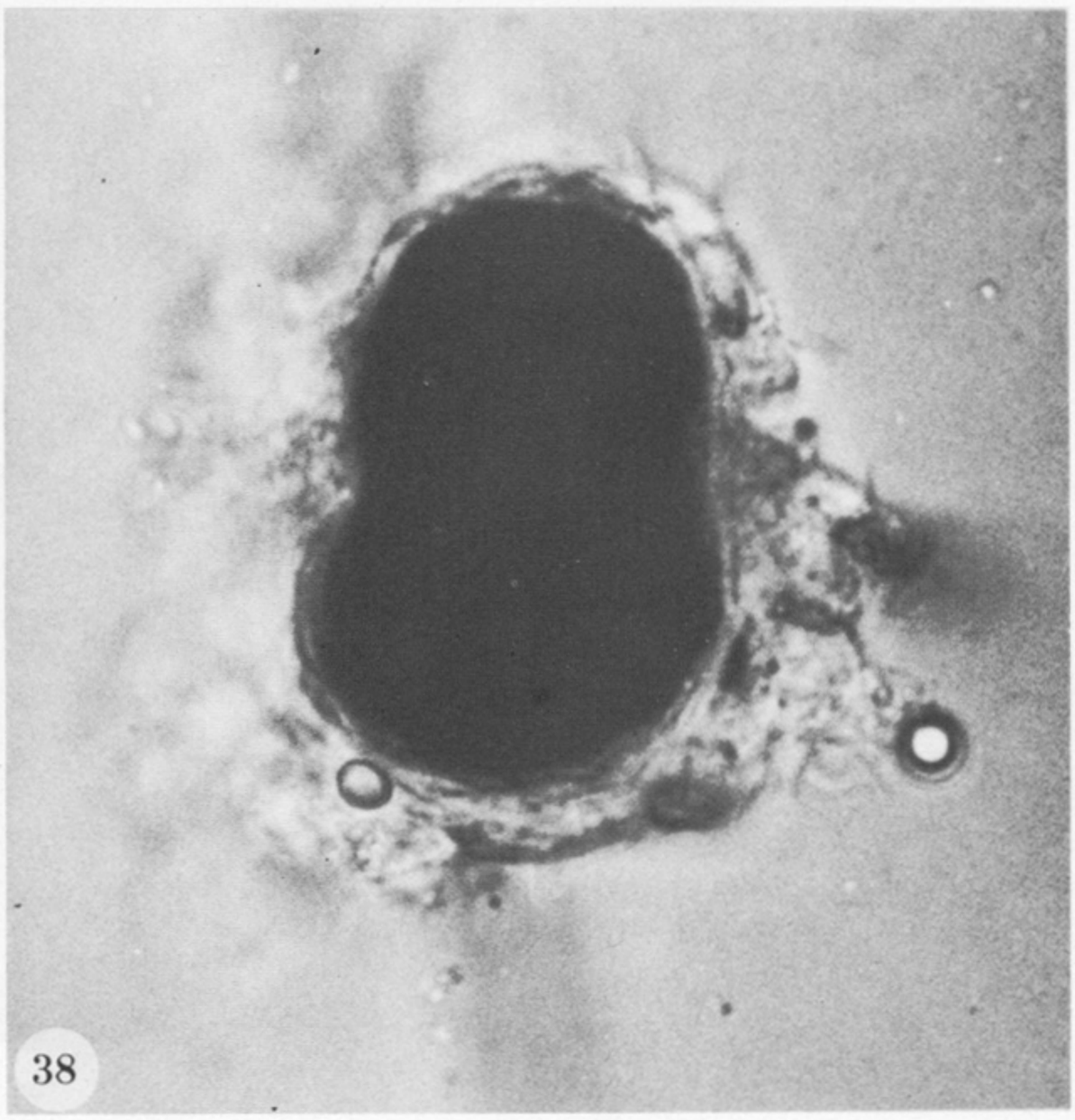












FIGURES 33–38. For description see opposite.