THE MORPHOLOGY OF A COMMON SOIL FLAGELLATE, *HETEROMITA GLOBOSA* STEIN (MASTIGOPHOREA: PROTOZOA)

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CONTENTS

SYNOPSIS

Heteromita globosa, a colourless flagellate, was established in clonal culture. The ultrastructure is described for the first time. Two organelles, hitherto undetected in soil flagellates, were observed, but many diagnostic features of Bodonid flagellates were absent. The affinities of *H. globosa* are discussed, and it is suggested that *Heteromita* is a valid genus, clearly distinct from Bodonid flagellates.

INTRODUCTION

Although colourless flagellates of the genus *Heteromita* are common in soils, their ultrastructure is unknown, and their morphology has not been studied with modern microscopes. The last morphological study was by Robertson (1928). Recent systematic accounts do not recognize the genus *Heteromita* Dujardin, 1841 and place these flagellates in the genus *Bodo* Ehrenberg, 1830 (Hollande, 1952; Lepinis *et al.*, 1973). Nevertheless, observations on the external morphology and ultrastructure of a Scottish isolate of *Heteromita globosa* Stein, reported in this paper, suggest that *Heteromita* is a valid genus.

MATERIALS AND METHODS

The flagellate was separated in December 1971 from other protozoa by diluting a soil sample (plough or S layer, Tarves Soil Series, Tarves Soil Association at Tillycorthie Farm, near Udny, Aberdeenshire) to extinction with sterile liquid soil extract and microdiluters (Darbyshire *et al.*, 1974). Five clones of *H. globosa* were isolated by

diluting the original pure culture to extinction with the same method, and by choosing individual cultures in the dilution series with single flagellates. Another clone was isolated by pipetting minute drops from this second dilution series with a fine capillary on to agar squares (about 5 mm^2 of 1% w/v Oxoid agar No. 2) inside sterile Petri dishes (12 agar squares/Petri dish of 10 cm diam.). A small piece of a coverslip was placed on each agar square, and single cell isolates were selected under a light microscope. Subsequently, one agar square with a single cyst was transferred to sterile soil extract and the clonal culture was derived from this cyst. The 6 clonal cultures were subcultured into sterile soil extract at weekly intervals and incubated at either 25 or 10 °C.

Living flagellates were observed in liquid mounts and filmed in agar chambers of Heunert (1962). Eastmann Plus X negative film was used for 16 mm cinephotomicrography. The flagellates were preserved with either the fixative of Nissenbaum (1953) or with a 1 : 6 mixture of saturated $HgCl_2$ and OsO_4 (2% w/v). After the latter fixative, the flagellates were dehydrated in a series of acetone/water mixtures and absolute acetone, before they were mounted in epoxy resin (Spurr, 1969) on microscope slides. Three permanent slides have been deposited in the British Museum (Natural History) and registered with the following numbers: 1976: 1:20:1, 1976:1:20:2, and 1976:1:20:3. After Nissenbaum's fixative, specimens were stained with acridine orange (10^{-4} M in citric acid–Na_2HPO_4 buffer, pH 7), or Giemsa (5% v/v in Sørensen's buffer, pH 7) or toluidine blue (0·5% w/v) in borax (0·5% w/v). Some flagellates were drawn (Fig. 1) with the aid of a Wild M20 microscope and a Treffenberg drawing tube. The dimensions of fixed samples of the clonal cultures are shown in Table 1.

Flagellates, from a 24 hr culture grown at 25 °C, were fixed for scanning electron microscopy in a 1 : 1 mixture of saturated $HgCl_2$ solution and OsO_4 (2% w/v) solution. They were then pipetted on to polycarbonate membrane filters (0.2 μm pore diam. Nuclepore) inside a specimen carrier (Marchant, 1973) and dehydrated in a series of ethanol/water mixtures, absolute ethanol, 'Freon 113'/ethanol mixtures and finally absolute 'Freon 113'. The specimens were dried in a critical point apparatus (Cohen *et al.*, 1968). The polycarbonate membranes were mounted on scanning electron microscope stubs with conductive paint (Silver Dag) and coated evenly with carbon and gold/palladium alloy in an evaporating unit. The stubs were examined in a Cambridge Stereoscan S4 at 30 kV and the results were recorded on Kodak Tri-X Pan film.

Four different fixation methods for transmission electron microscopy were tested involving acrolein, glutaraldehyde and OsO_4; the most satisfactory results were obtained when the flagellates were fixed for 15 min in OsO_4 (2% w/v) in cacodylate buffer (0·1 M, pH 7·1, containing 0·25% w/v $CaCl_2$ and 0·2% w/v NaCl) (Tamm & Tamm, 1973).

For the cytochemical demonstration of catalase, cells were fixed with glutaraldehyde in soil extract (final vol. 2% v/v) for 30 min, washed in half-strength soil extract and incubated in the enzyme medium (10 mg 3, 3'-diaminobenzidine tetrahydrochloride (DAB), 10 ml tris buffer at pH 7·2 and 0·1 ml 0·3% v/v H_2O_2) for 1 hr at a room temperature of 18–20 °C. The control medium lacked DAB but was

otherwise identical to the enzyme medium. The cells were subsequently washed for 1 hr in tris buffer and post-fixed with OsO_4 (1% w/v) in half-strength soil extract for 30 min (Norris & Pearson, 1975).

All the fixed cells were concentrated by centrifugation (150 *g* for 10 min). The pellets were dehydrated in acetone and embedded in a low viscosity epoxy resin (Spurr, 1969). Sections were cut with glass knives on a Reichert OMU 3 ultramicrotome and stained in either a saturated solution of uranyl acetate in ethanol (50% v/v) or uranyl acetate followed by lead citrate (Reynolds, 1963). The sections were examined in an AEI EM6 electron microscope at 75 kV.

For shadow cast preparations, live flagellates were fixed in OsO_4 vapour, on formvar coated grids. The grids were shadowed with nickel/palladium at an angle of 30° in a coating unit.

KEY TO ABBREVIATIONS USED IN THE PLATES AND FIGURES

af	anterior flagellum	lp	lipid droplet
b	bacterium	m	mitochondrion
bb	basal body	mb	microbody-like organelle
cv	contractile vacuole	mt	microtubules
cw	cyst wall	mv	multivesicular area
ed	electron dense deposits	n	nucleus
er	endoplasmic reticulum	nu	nucleolus
f	flagellum	p	pore
fi	striated filament	pf	posterior flagellum
fv	food vacuole	r	semicircular ridge of cytoplasm
G	Golgi apparatus	te	tubular elements
k	kinetocyst-like organelle	vac	vacuole

OBSERVATIONS

LIGHT MICROSCOPY. In the logarithmic phase of growth, the flagellate is either ovoid (5–8 μm long, 3–6 μm wide) or pyroid with the anterior half of the body slightly wider than the posterior region (Fig. 1J; Table 1). Two heterodynamic

TABLE 1

Mean dimensions (μm) of *H. globosa* from 1 mixed and 6 clonal cultures

CULTURE	TROPHOZOITE					THIN-WALLED CYST
	Body length	Body width	Body depth	Flagellum length: Anterior	Flagellum length: Posterior	Diameter
Mixed	6·7	4·0	4·9	8·5	12·4	4·8
Clone 1	6·6	4·0	4·6	8·1	12·8	4·7
Clone 2	7·0	4·0	4·7	9·2	13·9	4·7
Clone 3	6·9	5·3	5·0	7·9	12·6	4·8
Clone 4	6·8	4·3	4·7	8·7	13·3	4·6
Clone 5	6·8	4·5	4·7	8·4	12·8	4·7
Clone 6	7·3	4·6	4·8	8·4	13·4	4·8

Fixed in $HgCl_2/OsO_4$ and mounted in epoxy resin; 50 cells/clonal culture and 100 cells/mixed culture measured.

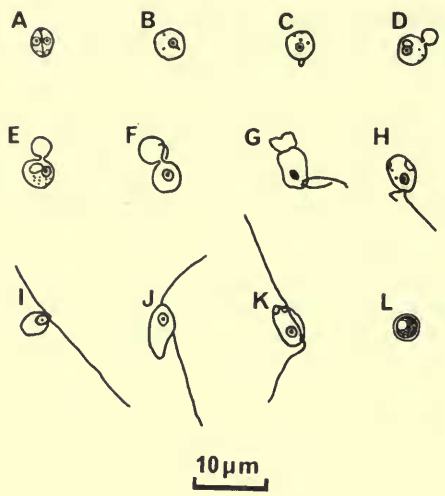

FIG. 1. Camera lucida drawings of *H. globosa*. A & B are thin-walled resting stages, C to G are stages in excystment, H to K are trophozoites (I side view, J dorsal view, K ventral view), L is a mature thick-walled cyst.

acronematic flagella are inserted close together at the anterior extremity of the body (Fig. 1K). The anterior flagellum is slightly longer than the body and beats in an antero-lateral direction in a helical manner from the proximal region to the tip. The posterior flagellum is about twice as long as the other flagellum (Table 1) and often trails passively behind the body. Usually, the flagellate moves forward in a vibratory manner with the anterior extremity of the body and the posterior flagellum in contact with the substratum. The longitudinal axis of the body is maintained at an angle of about 45° to the substratum and at an acute angle to the posterior flagellum (Fig. 1J). As the body moves forward, the longitudinal axis tends to swing from side to side, pivoting on its anterior extremity. When the flagellate is dislodged from the substratum and becomes free-swimming, both flagella move haphazardly. If the body or flagella become attached to organic debris or bacteria, then the movements become more irregular and violent.

The nucleus with its conspicuous central karyosome is anterior and on the longitudinal axis of the body (Fig. 1J; Pl. 1). No kinetoplast was observed near the nucleus, either in living cells or in fixed preparations stained with acridine orange. The single contractile vacuole usually lies slightly posterior to the nucleus near the ventral surface (Pl. 1). Bacteria are rapidly ingested by means of short transient pseudopodia at any point of the body, even during cytokinesis. Sometimes, the bacteria are first entangled with the anterior flagellum and gradually move towards the body, where they are engulfed by pseudopodia (Pl. 1). After ingestion, the bacteria are rapidly transferred to the posterior half of the body.

Reproduction can occur 10–24 hr after the addition of fresh soil extract to an encysted culture at 25 °C and follows the stages described by Robertson (1928). The mother cell becomes quiescent and elongates, as the flagella migrate to opposite ends of the body. A second flagellum develops beside each 'mother' flagellum, the body becomes constricted in the middle, and the two daughter cells pull apart until only a thread of cytoplasm remains between them. This cytoplasmic thread normally

disintegrates ; sometimes the daughter cells coalesce, and a binucleate flagellate is formed. Trinucleate individuals were observed ; these are formed when only one of the daughter cells from the division of a double individual is released. Binucleate cysts were occasionally found in the cultures and were probably derived from binucleate trophozoites. A bicellular (Fig. 1A) cyst was once observed in the cultures.

In addition to a thick-walled cyst (Fig. 1L), *H. globosa* possesses a thin-walled resting stage (Fig. 1B), which was originally described by Sandon (1927). Both resting stages developed in liquid cultures but dried cultures invariably formed thick-walled cysts.

Encystment and excystment of *Heteromita* have been described in detail by Robertson (1928). Encystment in *H. globosa* begins 24–48 hr after inoculation of a batch culture of soil extract at 25 °C. During the initial stages of encystment, the contractile vacuole is very active. Later, the flagellar movements become sluggish, the body becomes spherical and the food vacuoles congregate around the nucleus. The flagella decrease in length and gradually disappear.

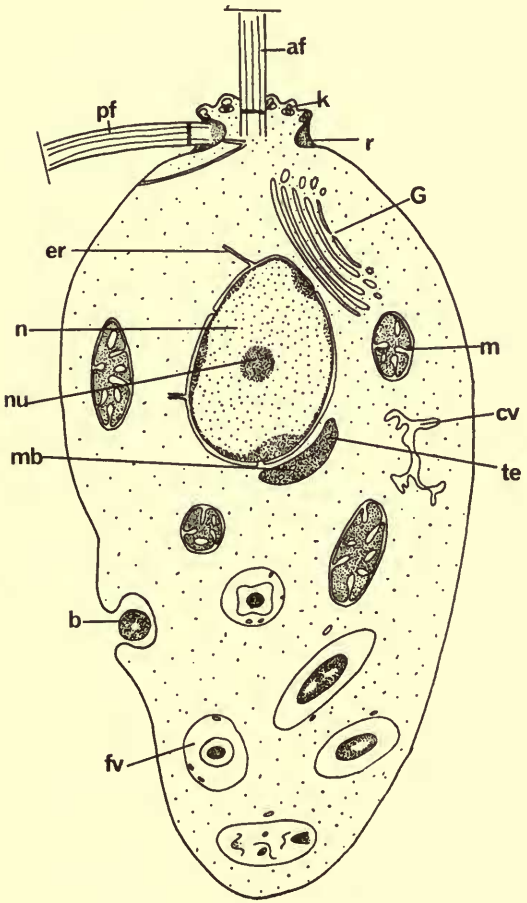

FIG. 2. Diagram of *Heteromita globosa* showing the arrangement of the main organelles.

Excystment of the thin-walled resting stages can be readily induced by the addition of fresh soil extract. Within 30–60 min at 25 °C, the contractile vacuole becomes active and a small translucent pseudopodium, which gradually increases in size, soon emerges from the cyst (Fig. 1C, D, E). The anterior flagellum can be seen outside the cyst before the posterior flagellum (Fig. 1F), although they are both eventually situated close to each other on the pseudopodium. The body is gradually extricated from the cyst by vigorous movement of the anterior flagellum. Sometimes, the trophozoite swims around for several minutes still attached to the empty cyst wall (Fig. 1G). Usually, the recently excysted flagellate is ovoid (Fig. 1H). The whole process of excystment extends over 1–2 hr at 25 °C. Excystment of the thick-walled cysts is a similar process, but 24–48 hr elapses before it is complete at 25 °C.

Electron microscopy. *Trophozoite.* The origin of the anterior flagellum is partly surrounded by a semicircular ridge of cytoplasm at the anterior end of the body. The longer posterior flagellum appears to be continuous with one end of this ridge and is set at right angles to the anterior flagellum (Pl. 2, figs A, B, C). Both flagellar axenomes have the typical 9+2 arrangement of tubules ; the central pair of tubules originates just above the peripherally thickened distal basal plates (Pl. 2, fig. B). No paraxial rods or mastigonemes were observed. Although scanning electron microscope and shadow-cast preparations show that the flagella taper distally, we could not determine if this is due to the absence of tubules. Striated rootlets connect the basal bodies, and several other rootlets run from the basal bodies to the sub-pellicular region of the body (Pl. 2, figs B, D, E). In the extracytoplasmic portion of the basal bodies and proximal to the basal plate, the 9 peripheral doublets are connected to the flagellar walls by strands (Pl. 3, fig. F) ; in the intracytoplasmic portion the peripheral tubules are arranged in triplets.

The semicircular ridge, near the origins of the flagella (Pl. 2, figs A, C), contains a row of small organelles immediately below the plasma membrane (Pl. 2, figs A, D ; Pl. 3, fig. A). Each of these organelles (about 170 nm long and 120 nm wide) has a central conical element with an electron dense core and is surrounded by a unit membrane. These organelles are present beneath the plasma membrane on other parts of the body and the flagella (Pl. 4, figs A, B, C). Similar structures, kinetocysts, have been described in Heliozoa (Bardele, 1972, 1975).

The nucleus is usually ovoid (about 1·7 μm long, 1·3 μm wide) and is enclosed by a double unit membrane (Pl. 3, figs B, E). The nuclear matrix is granular with occasional concentrations of electron-dense chromatin attached to the inner nuclear membrane and a dense central nucleolus (Pl. 2, fig. B ; Pl. 3, figs B, E). Cisternae of the endoplasmic reticulum arise from the outer membrane of the nuclear envelope and ramify in the cytoplasm (Pl. 3, fig. E). Ovoid or spherical mitochondria, with dense matrices and plate-like cristae, are distributed throughout the cytoplasm (Pl. 3, figs B, E, F). A single Golgi apparatus lies directly beneath the flagellar basal bodies and consists of 6–8 saccules, which have both smooth and coated vesicles associated with their margins (Pl. 2, fig. B ; Pl. 3, fig. E). The contractile vacuole is slightly posterior to the nucleus (Pl. 3, fig. C). Numerous food vacuoles are usually present in the posterior half of the cytoplasm. If partly digested food is present, the single unit membranes of these vacuoles are covered with small vesicles. An intermediate

stage in phagocytosis, with a bacterium partly engulfed by pseudopodia (Pl. 3, fig. D), was observed in thin sections of one flagellate.

In the cytoplasm adjacent to the posterior end of the nucleus, there is a microbody-like organelle (160–260 nm wide). This organelle is surrounded by a unit membrane and has a finely granular matrix with tubular elements (Pl. 3, fig. B). Unstained sections of cells incubated in DAB showed no reaction inside this organelle, although there was a strong positive reaction in mitochondria.

In a few sections, a group of 50–60 cross-linked vesicles was observed close to the plasma membrane in a posterior-lateral region of the trophozoite (Pl. 3, fig. E). Each vesicle was about 20 nm in diameter. A similar array of small virus-like particles was reported by Swale & Belcher (1973) in the nucleus of one specimen of the colourless flagellate, *Aulacomonas submarina*.

Cysts. The thick walls of mature cysts appear to be impermeable to acrolein, glutaraldehyde and OsO_4. All the observations on the ultrastructure of the resting stages relate to the thin-walled cysts. During the first stages of encystment, the body assumes a spherical shape and the flagella are resorbed into the cytoplasm (Pl. 6, fig. B). The flagella lie close to the plasma membrane inside the cell but are seldom adjacent to each other (Pl. 5, fig. A ; Pl. 6, fig. B). Food vacuoles are still present at this stage, although they are usually empty or only contain waste material (Pl. 6, fig. B). Vacuoles with single electron-dense spherical particles are present in most cysts (Pl. 5, fig. B). The Golgi saccules are often swollen and appear to be producing smooth and coated vesicles from their margins. Numerous small lacunae are present in the peripheral cytoplasm of the cyst ; these structures are enclosed by unit membranes and are either translucent or contain small central granules (Pl. 5, figs A, B). Bardele (1972) found similar lacunae in Heliozoa and suggested they were repositories for reserve plasma membrane, which can be utilized during the rapid transformation of flagellate to heliozoon. Plasma-membrane material may be stored in *H. globosa* in the same fashion and may become significant during excystment. Some of the lacunae with granular material in *H. globosa* may represent degeneration stages of the kinetocyst-like organelles. Certainly, definite examples of this organelle were not observed in cysts. The mitochondrial cristae in the cysts are similar to those in trophozoites apart from occasional electron-dense particles (Pl. 5, fig. A ; Pl. 6, fig. B). The nucleus in the cyst seems unaltered, but the adjacent microbody-like organelle appears to be reduced. The contractile vacuole collapses in the cyst, although the tubular spongiome usually remains visible. The young cyst wall is a single filamentous layer (Pl. 6, fig. B) in close contact with the cell contents. As the cysts mature, other layers are added to the wall and the points of contact between the wall and cell contents become greatly reduced. Brooker & Ogden (1972) described a similar type of encystment in *Bodo caudatus* with the cyst wall consisting of several filamentous layers. The flagella in the cyst of *B. caudatus*, however, lie in a groove of the cytoplasm close to the cyst wall.

Excystment starts with the emergence of one flagellum from the cytoplasm and with further shrinkage of the cell contents from the cyst wall (Pl. 6, fig. C). The invaginations gradually become more pronounced but fewer in number until there are two deep grooves on opposite sides of the cell. Later, the second flagellum

emerges from the cytoplasm, and both flagella become wrapped around the cell (Pl. 6, fig. A). The flagellate emerges from the cyst by extruding a pseudopodium through a small pore (about 200 nm). Initially, the organelles remain within the cyst (Pl. 6, fig. D) and only later migrate to the pseudopodium (Pl. 6, fig. D). Lipid droplets persist in the cytoplasm for a short period after excystment (Pl. 5, fig. C).

DISCUSSION

The present observations with the light microscope confirm most of Sandon's and Robertson's accounts of the morphology and reproduction of *Heteromita* spp. No perforations in some cyst walls (Sandon, 1927) or rhizoplasts (Robertson, 1928), however, were observed. Although the dimensions of the trophozoites of the Scottish clones in Table 1 are slightly smaller than those reported by Sandon for *H. globosa*, the mean diameters of the resting stages lie within the same range (4.2–5.0 μm). Robertson (1928) identified two isolates of *Heteromita* as *H. globosa* and it is clear from her description that she was studying a flagellate very similar to the Scottish clones. We therefore suggest that the Scottish clones belong to *H. globosa*.

Two organelles, which closely resemble microbodies and kinetocysts, were observed by electron microscopy. These organelles have not previously been reported in soil flagellates. The negative reaction of the microbody-like organelle to the DAB/peroxide test suggests that this organelle is not analogous to the microbodies of *Hartmannella culbertsoni* and *Paramecium aurelia*. A more likely analogy is with those Phytomastigophorean microbodies, which also show a negative reaction for catalase (Müller, 1975). As the trophozoites of *H. globosa* have larger microbody-like organelles than the resting stages, these organelles may be glyoxysomes with the enzymes responsible for the conversion of lipids into carbohydrates. Certainly, large lipid deposits were only observed in the resting forms and recently excysted trophozoites. The positive reaction in the mitochondria with DAB may be caused in *Heteromita* by cytochrome C oxidase. Bardele (1975) suggested that the kinetocysts of centrohelidians may be involved in the selection and transportation of particles in the axopodia. In *H. globosa*, the flagella and pseudopodia are involved in feeding, and the kinetocyst-like organelles just beneath the plasma membrane may play an important role in the capture and ingestion of food. Bardele (1972, 1975) believed that the kinetocysts originate from vesicles close to the dictyosomes and later migrate to the plasma membrane. In *H. globosa* the dictyosome lies close to the base of the flagella, and it may be significant that the majority of kinetocyst-like organelles are found nearby.

The taxonomic position of *Heteromita* within the Zoomastigophorea is uncertain ; the only suborder, which contains zooflagellates with 2 heterodynamic flagella in modern classifications (Hollande, 1952 ; Honigberg *et al.*, 1964), is the Bodonina of the order Kinetoplastida. It is believed that some species in this suborder have lost their kinetoplasts secondarily, and it is possible that such a loss has occurred in *Heteromita* spp. Nuclear division in *Heteromita*, however, is unlike the promitotic division of Bodonids (Robertson, 1927 ; Hollande, 1952). Robertson (1928) showed

that the blepharoplasts in *Heteromita* acted as centrosomes and that the karyosome disintegrated during nuclear division. Recent studies of *Bodo* spp. (Pitelka, 1961 ; Brooker, 1971 ; Burzell, 1975) and *Rhynchomonas* spp. (Burzell, 1973; Swale, 1973) have also shown that the Bodonina have several characteristic ultrastructural features, for example, a flagellar pocket, paraxial flagellar rods, pellicular microtubules, a buccal cavity, a cytopharynx and a single coiled mitochondrion. As many of these features were not observed in *H. globosa*, we consider *Heteromita* is a valid genus and not a synonym of *Bodo* as proposed by Hollande (1952). Similar investigations of other *Bodo*-like flagellates without kinetoplasts, such as *Cyathobodo* (Swale & Belcher, 1975), should clarify the taxonomic affinities of *Heteromita*.

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PLATE 1

Heteromita globosa. Phagocytosis of a bacterium. Nucleus and contractile vacuole are visible. × 1270 phase contrast. Time lapse at 1 sec intervals.

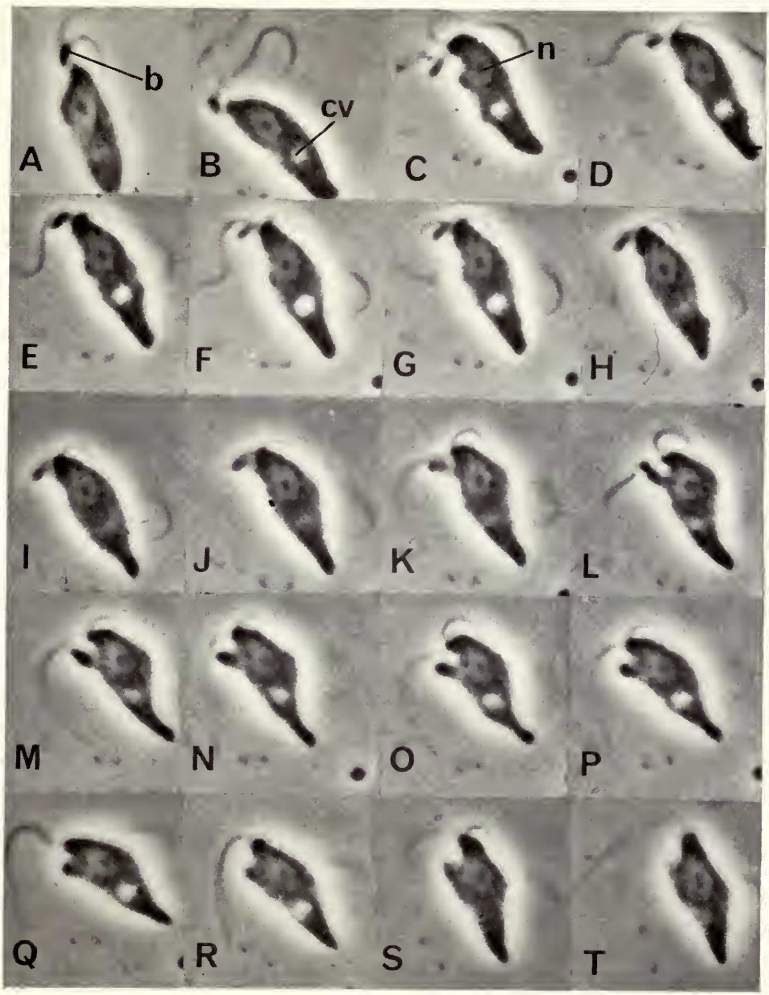
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PLATE 2

H. globosa trophozoite

FIG. A. Section through the anterior end showing the basal body of the anterior flagellum, the semicircular ridge with the kinetocyst-like organelles, the posterior flagellum and microtubules near the kinetocyst-like organelles. × 38 000.

FIG. B. Section showing the position of the Golgi apparatus in relation to the nucleus and flagella. Microtubules run from the base of the posterior flagellum into the cytoplasm. × 24 885.

FIG. C. Scanning electron micrograph showing the posterior flagellum continuous with the semicircular ridge of cytoplasm and the origin of the anterior flagellum inside this ridge. × 3500.

FIG. D. Transverse section through the basal bodies, which are linked by striated filaments. Microtubular rootlets run from the basal bodies to the sub-pellicular region. × 36 000.

FIG. E. Section showing microtubular rootlets near the base of the flagellum. × 39 300.

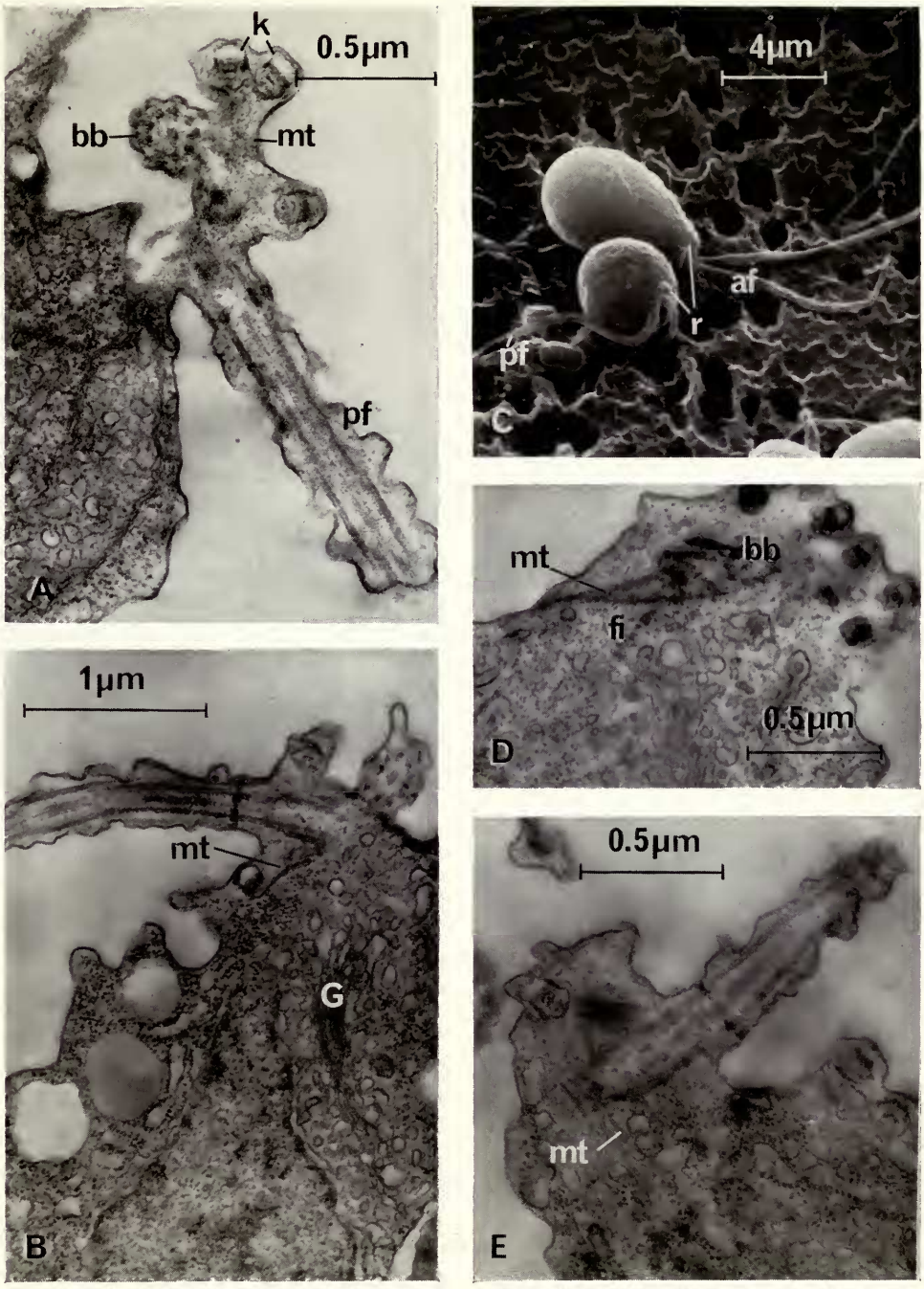
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PLATE 3

H. globosa trophozoite

FIG. A. Longitudinal section through the anterior end showing the kinetocyst-like organelles and the associated microtubules. × 37 100

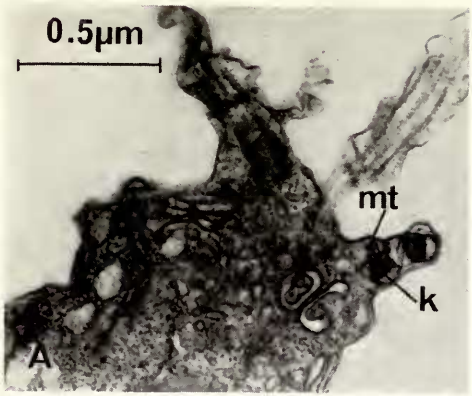
FIG. B. Longitudinal section through the posterior end of the nucleus. An electron-dense septum (arrow) lies across a pore in the nuclear membrane. Tubular elements can be seen in the granular matrix of the microbody-like organelle. × 36 800.

FIG. C. Section showing the contractile vacuole lying on the ventral side of the nucleus. Note food vacuoles, kinetocyst-like organelles, Golgi apparatus and microbody-like organelle. × 11 200.

FIG. D. Oblique section towards the posterior end showing pseudopodia around a bacterium. × 21 000.

FIG. E. Longitudinal section showing the continuity between the outer layer of the nuclear membrane and the endoplasmic reticulum (arrow). The multivesicular area is posterior to the nucleus, the Golgi apparatus is anterior to the nucleus. × 17 625.

FIG. F. Longitudinal section showing the extracytoplasmic portion of the basal body without the two central tubules. Note plate-like cristae in the mitochondrion. × 17 183.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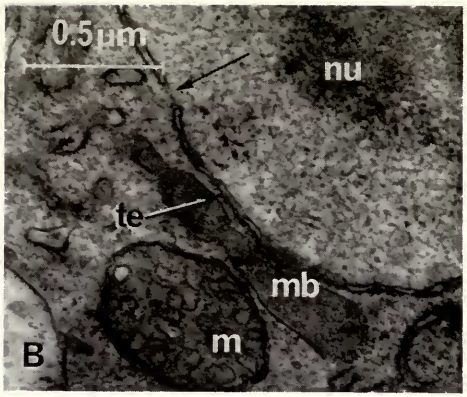
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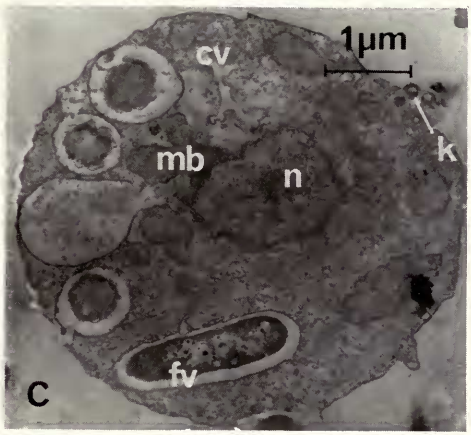
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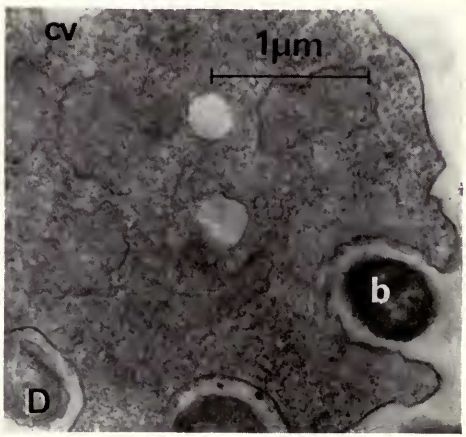
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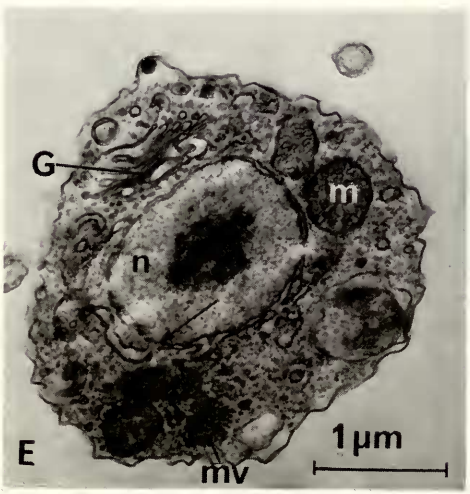
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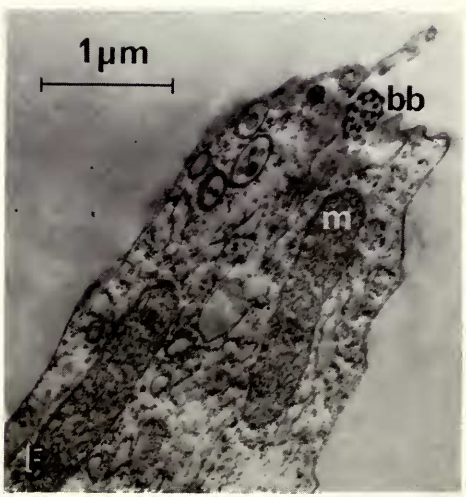
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PLATE 4

H. globosa trophozoite

FIG. A. Kinetocyst-like organelles are present in the semicircular ridge of cytoplasm and elsewhere on the body, below the plasma membrane. × 31 000.

FIGS B & C. Longitudinal sections showing kinetocyst-like organelles below the flagellar membrane. Fig. B × 42 000, Fig. C × 58 000.

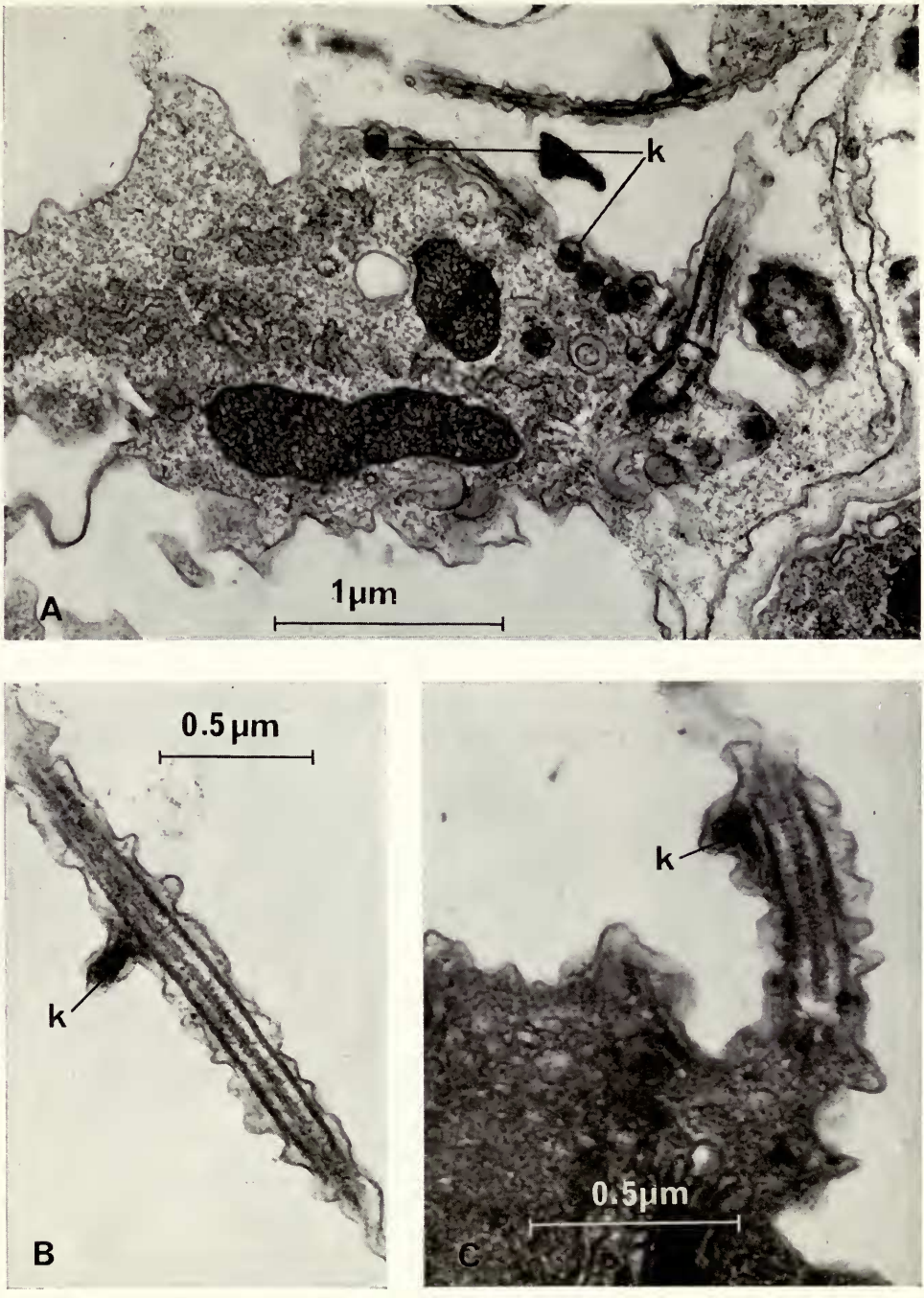
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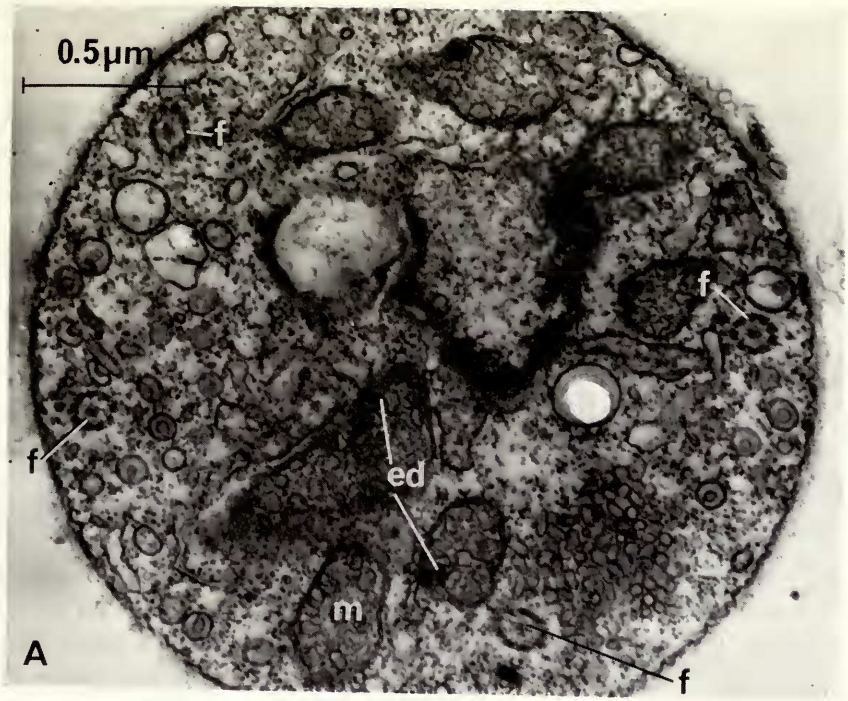
PLATE 5

H. globosa

FIG. A. Transverse section of a resting stage. The mitochondria have electron dense deposits. The flagella are seen in transverse section. × 43 260.

FIG. B. Transverse section of a more mature resting stage with a thicker cyst wall. Small membrane-bound lacunae (arrows) lie around the periphery. Note lipid droplets in cytoplasm, and in a vacuole. × 27 090.

FIG. C. An oblique section through the anterior end of a newly excysted trophozoite with lipid droplets. × 14 100.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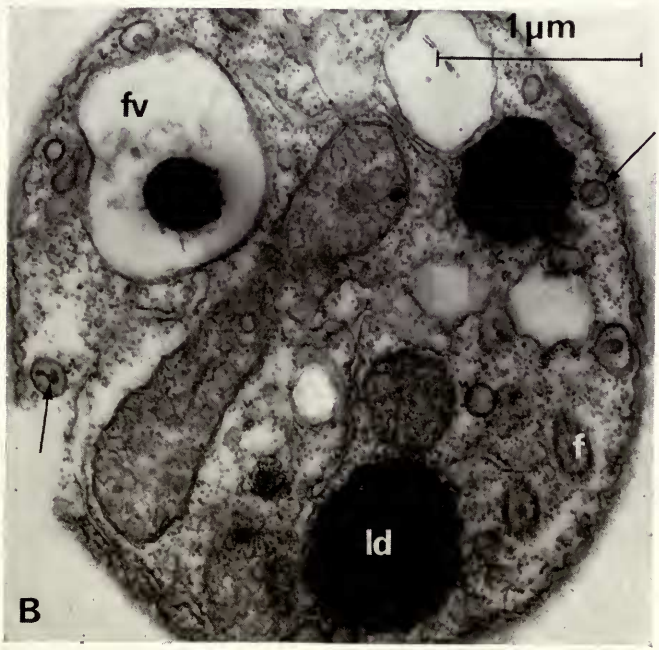
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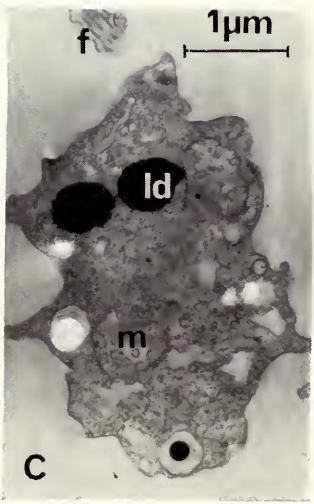
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PLATE 6

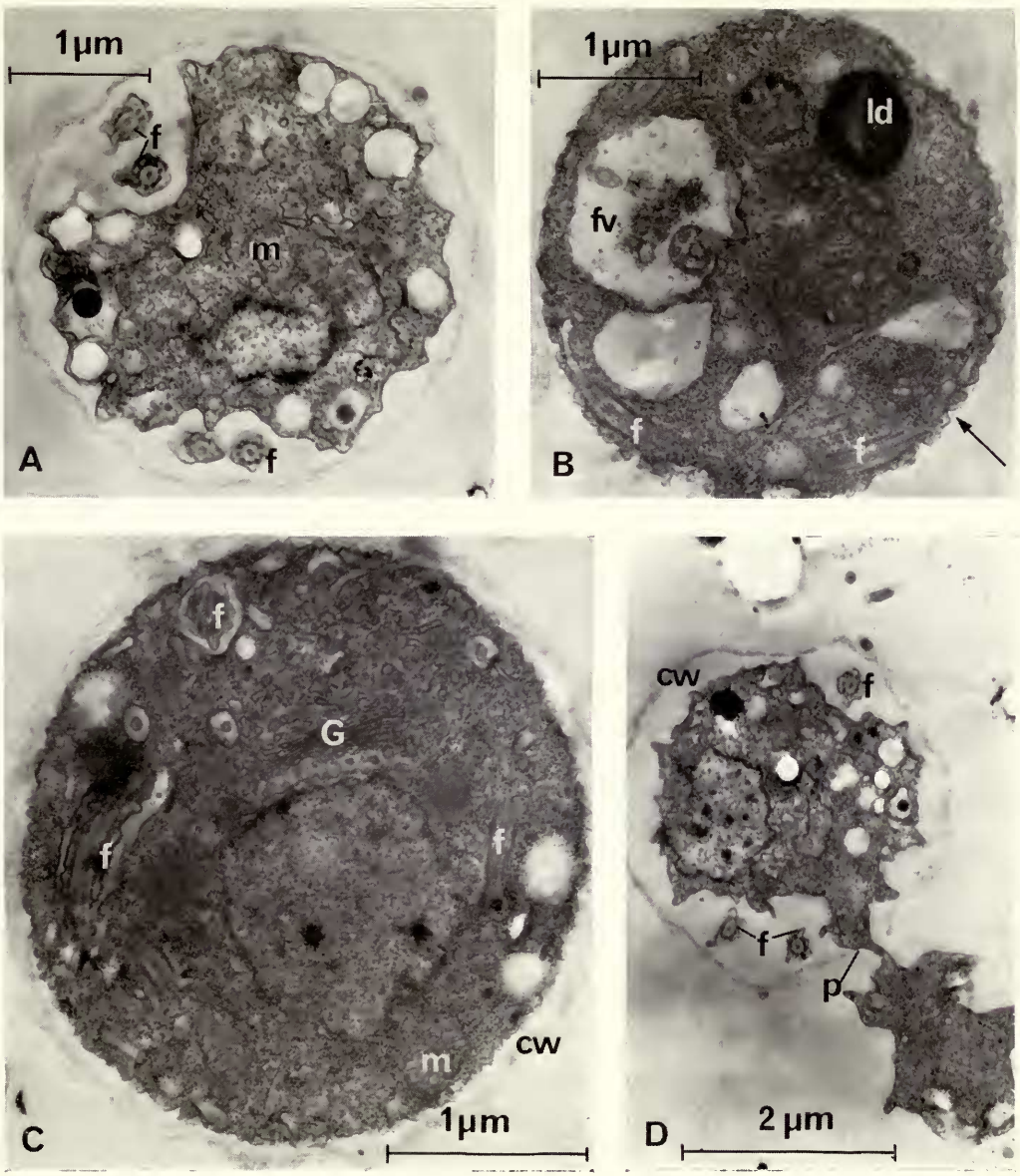
H. globosa resting stages

Fig. A. Transverse section through an excysting cell. The contents have shrunk from the cyst wall and the flagella are lying around the body. × 18 682.

Fig. B. Transverse section of an early resting stage with intracytoplasmic flagella lying around the periphery. Note one vacuole with food remnants and cyst wall with a single filamentous layer (arrow). × 21 000.

Fig. C. Section showing initial stages of excystment. One flagellum has emerged from the cytoplasm. × 26 670.

Fig. D. Section of an excysting cell with the cytoplasm extruding through a pore in the cyst wall. The flagella are inside the cyst. × 14 128.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