

The Hemimastigophora (*Hemimastix amphikineta* nov. gen., nov. spec.), a New Protistan Phylum from Gondwanian Soils

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SUMMARY

The morphology, morphogenesis and ultrastructure of *Hemimastix amphikineta* nov. gen., nov. spec. are described. This species occurred in some Australian and in 1 Chilean soil, but was absent from more than 1000 soil samples from Laurasian localities. Thus, it has probably a restricted Gondwanian distribution. *Hemimastix amphikineta* is a small ($14\text{--}20 \times 7\text{--}10 \mu\text{m}$), colourless organism that looks distinctly Ciliophora-like because of its posteriorly located contractile vacuole and its 2 longitudinal somatic kineties each composed of about 12 cilia-like flagella. These 2 kineties are interposed between 2 large plicated and microtubule-bearing pellicular plates which are arranged inversely mirror-image like ("diagonal symmetry"). *Hemimastix amphikineta* has saccular to tubular mitochondrial cristae and complex extrusomes. It has 2 microtubular systems and a membranous sac associated with each kinetid. The nucleolus persists throughout nuclear division. A permanent cytostome-cytopharyngeal complex, pharyngeal rods, striated fibres, mastigonemes, and a paraflagellar rod are absent. This unique combination of characters dictates a very separate position for *H. amphikineta* within the known protists. Thus, the phylum Hemimastigophora nov. phylum (Hemimastigea nov. cl. and Hemimastigida nov. ord.), is established to include *H. amphikineta* and possibly *Spiromema multici-liatum* Klebs, 1892. The structure of the pellicle and the nuclear apparatus of *H. amphikineta* indicate some relationship with the Euglenophyta. However, clear evidence for a certain affinity is lacking. Thus, the Hemimastigophora are placed in an incertae sedis position within the kingdom Protista Haeckel, 1866.

Introduction

The impact of the electron microscope has greatly influenced our knowledge of single-celled organisms and caused a revival of Haeckel's [33] concept of the "Protista" and the recognition of a structural diversity much greater than thought 30 years before. The new insights dismantled the traditional classifications and stimulated many new suggestions. Cavalier-Smith [11], for instance, recognizes 9 eukaryote kingdoms 7 of which contain unicellular organisms. Corliss [16], on the other hand,

suggests 3 kingdoms and divides the kingdom Protista into 45 phyla.

Most of our knowledge of protists comes from the reinvestigation of species originally described with the light microscope from freshwater habitats. It is still not generally recognized that the soil harbours a large protistan community which is quite different from that of the freshwater [26]. Many strange and phylogenetic important organisms have been found in terrestrial biotopes during the past 10 years. The most remarkable ones are strictly mycophagous ciliates [71], an ectoparasitic flagellated

organism on ciliates which is related to the Sporozoa [27, 43], and aggregative ciliates which develop aerial sorocarps like the Mycetozoa [63]. That these are only the tip of the iceberg, is illustrated by the outstanding flagellated protist from Australian soils described in this paper.

Material and Methods

Small numbers of cells of *Hemimastix amphikineta* were first encountered in a soil sample from a bushland in Brisbane-Waters-National Park (East Australia). Later, we found this organism in some other soils from different regions of Australia and in rather high numbers in a soil sample from Chile (see *Geographic distribution*). The organism appeared in cultures which were made with the non-flooded petri dish method. A petri dish with 15 cm in diameter was filled with air-dried soil and litter which were then saturated but not flooded with distilled water (for further methodological details see ref. [26]). A rich fauna of ciliates, flagellates, and amoebae developed after a few days. *Hemimastix amphikineta* usually appeared 4 days after rewetting the samples and could be found for a period of 1–2 months. Its abundance was, however, highest 4–10 days after rewetting. Obviously, *H. amphikineta* developed from resting cysts. Unfortunately, many attempts to get axenic cultures failed. All isolates died after a few days. Thus, no data about the life cycle can be provided and the raw material has been studied. With exception of the morphogenetic studies, which were mainly done with the Chilean population, material from the type locality has been used for all investigations.

Light microscopy on living cells was carried out with a microscope equipped with phase-contrast and differential-interference-contrast optics. Permanent protargol slides were prepared from both the type population and the Chilean population. The method used was basically the same as that described elsewhere [24]. Fixation, however, was done in hanging drops with osmium tetroxide-vapour for 2 min. Then, cells were directly embedded in albumen-glycerine and processed as usual. Some details were studied on material prepared with the pyridinated silver carbonate method as described by Augustin et al. [2]. Drawings and biometry were performed as described earlier [4].

For electron microscopy cells were fixed in 3.0% (v/v) glutaraldehyde in 0.05 M Na-cacodylate buffer, pH 7.0. After 60 min fixation, they were rinsed briefly in buffer and postfixed in 2% (w/v) osmium tetroxide in 0.025 M Na-cacodylate buffer for 60 min (D. H. Lynn, pers. communication). They were then washed twice in buffer and stored for 8 days in the buffered glutaraldehyde fixative. Then, cells were washed again in buffer,

dehydrated in a graded ethanol series, and transferred through propylene oxide into Epon. Material was embedded in Epon compressed between glass slides that had been coated with Teflon spray. After polymerization, one slide was removed; the thin film of Epon was scanned with the light microscope (oil immersion); individual cells were identified and marked using a steel needle. The cells were cut from the Epon film and remounted on Epon stubs for serial sectioning. Ultrathin sections were cut with a diamond knife mounted on a Reichert Ultracut microtome and stained with aqueous uranyl acetate and lead citrate. Stained sections were viewed and photographed on an AEI Corinth 500 or a Philips EM 400 electron microscope.

Results

Light microscopic description of *Hemimastix amphikineta*

Morphology and feeding behaviour

Hemimastix amphikineta measures in vivo 14–20 × 7–10 µm and is flattened to about half of the width (Tab. 1). It is ametabolic and constantly ovoid with a gently tapered posterior and a truncated anterior end (Figs. 1–5, 13, 14). The anterior region looks mushroom-like, especially in the electron microscope (Fig. 17), and will be referred to as “capitulum”. No left and right side can be distinguished because of the organism’s special symmetry (see *General architecture*). The most prominent features of *H. amphikineta* are 2 rows of flagella which run in lateral furrows. These furrows, each with about 12 flagella, commence at the anterior end and run a slightly twisted course to the posterior end of the cell; thus, they are optically crossed, if viewed from the short axis (Figs. 1, 2). The flagella are about 8 µm long and their distance apart increases from anterior to posterior. They bend stiffly at their base, and not with a sine-wave or helical pattern, causing a characteristic slowly trembling movement. *Hemimastix amphikineta* is, in fact, unable to swim like an ciliate but creeps among detritus. The nucleus is spherical, located equatorially to subequatorially and contains a prominent globular nucleolus. At the posterior end, there is a contractile vacuole which erupts about once a minute; it has no obvious channels draining into it. The cytoplasm is colourless and contains food vacuoles (diameter 1–5 µm) and some fatty shining globules, 0.3 to

Table 1. Morphometric characterization of *Hemimastix amphikineta*^a

Character	\bar{x}	M	SD	SE	CV	Min	Max	n
Cell, length	13.7	13.0	1.5	0.3	6.5	12	17	23
Cell, maximum width	6.6	6.5	0.9	0.2	4.0	5	8	23
Cell, width at anterior end	–	3.0	–	–	–	2.5	3.5	23
Nucleus, length	3.3	3.5	0.4	0.1	1.7	3	4	22
Nucleus, width	2.9	3.0	0.4	0.1	1.6	2	3.5	22
Nucleolus, diameter	–	1.2	–	–	–	1	1.5	23
Number of flagella	24.2	24.0	1.5	0.3	6.6	20	28	23

^a All data are based on the investigation of randomly selected protargol-impregnated specimens from the type population. All measurements in micrometers. Legend: CV = coefficient of variation in %, M = median, Max = maximum, Min = minimum, n = number of investigated individuals, SD = standard deviation, SE = standard error of the mean, \bar{x} = arithmetic mean.

1.5 μm in size, which are often linearly arranged (Figs. 1, 16). Extrusomes and other internal structures are invisible with the light microscope.

Hemimastix amphikineta feeds on small flagellates and probably on bacteria (Fig. 6). The food is ingested at the anterior end, but no special mouth structures are discernible. The food vacuoles appear beneath the anterior end and glide backward, sometimes fusing together. Feeding is frequently incomplete and the prey or a part of it is ejected as a globular mass. Feeding seems to be a difficult process for this organism, probably due to the absence of a permanent cytostome (see *Electron microscopy*). During food uptake, the organism is nearly motionless.

Geographic distribution

Hemimastix amphikineta was discovered in the soil of a bushland in Brisbane-Waters-National Park, near Sydney, East Australia. Later, it was observed in a soil from Moreton Island (near Brisbane, East Australia), in a sandy soil near a salt lake in the centre of Australia (Lake Amadeus, Alice Springs), and in the bark of a tree and in a humic soil from the rain forest near Cairns, East Australia. It was a great surprise to rediscover this species a few weeks later in a soil sample collected near the airport of the Easter Island, Chile.

Hemimastix amphikineta occurred in 5 out of 50 soil samples from Australia and in 1 out of 10 soil samples from Chile. This contrasts sharply with more than 1000 soil samples from the Laurasian region in which this species has never been found [26]. Thus, *H. amphikineta* probably has a restricted Gondwanian distribution like some other protists [26]. The family Spironemidae, to which *H. amphikineta* probably belongs, is however of world-wide distribution because similar genera have been recorded, for instance, from soil of England and from freshwater in Germany ([30, 42]; see Discussion).

Division

Division was studied *in vivo* and in protargol-impregnated slides. It is longitudinal and commences with the duplication of the flagellar rows. This results in 2 parallel rows on each side (Fig. 7). Then, the nucleus and the nucleolus divide. The nucleolus is visible in all stages of the process and is thus not broken down (Figs. 8, 9). Later 2 anterior ends appear and the daughters begin to separate (Figs. 8, 9). During the final stages, the anterior ends lie antipodally apart (Figs. 10, 11).

Electron microscopy

General architecture

Ultrathin transverse sections confirm the light microscopic impression that it is impossible to distinguish between a left and a right or a dorsal and ventral side in *H. amphikineta*. High magnifications show clearly that the cell is composed of 2 large slightly spiraled plates which are arranged in an inverse mirror-image manner. This special "diagonal symmetry" which occurs also in the

Euglenophyta [72] becomes evident from the arrangement of the epiplasm, the subpellicular microtubules, and the fibrillar systems associated with the flagella (Figs. 12, 16, 18, 19, 27, 28, 74). Each plate has a blunt edge which is turned inwards slightly and a thinner edge which is bent inwards strongly (Figs. 12, 18, 27). Between opposed edges of the 2 plates is implanted a flagellar row in a deep channel; each channel is heart-shaped in cross section. On either side of each flagellar row, there is a hemispherical-shaped furrow which is formed by the inwards bent edge of a pellicular plate. The furrow which is associated with the thinner edge of the plate is underlain with highly ordered microtubules (hence, referred to hereafter as the "microtubule furrow"); that which is associated with the blunt edge lacks associated microtubules (hence, referred to hereafter as the "naked furrow") (Figs. 12, 27, 28). The edge of the naked furrow forms a small ridge which surrounds the basal bodies of the flagella (Figs. 27–29). This rather complicated situation is schematically explained in Fig. 12.

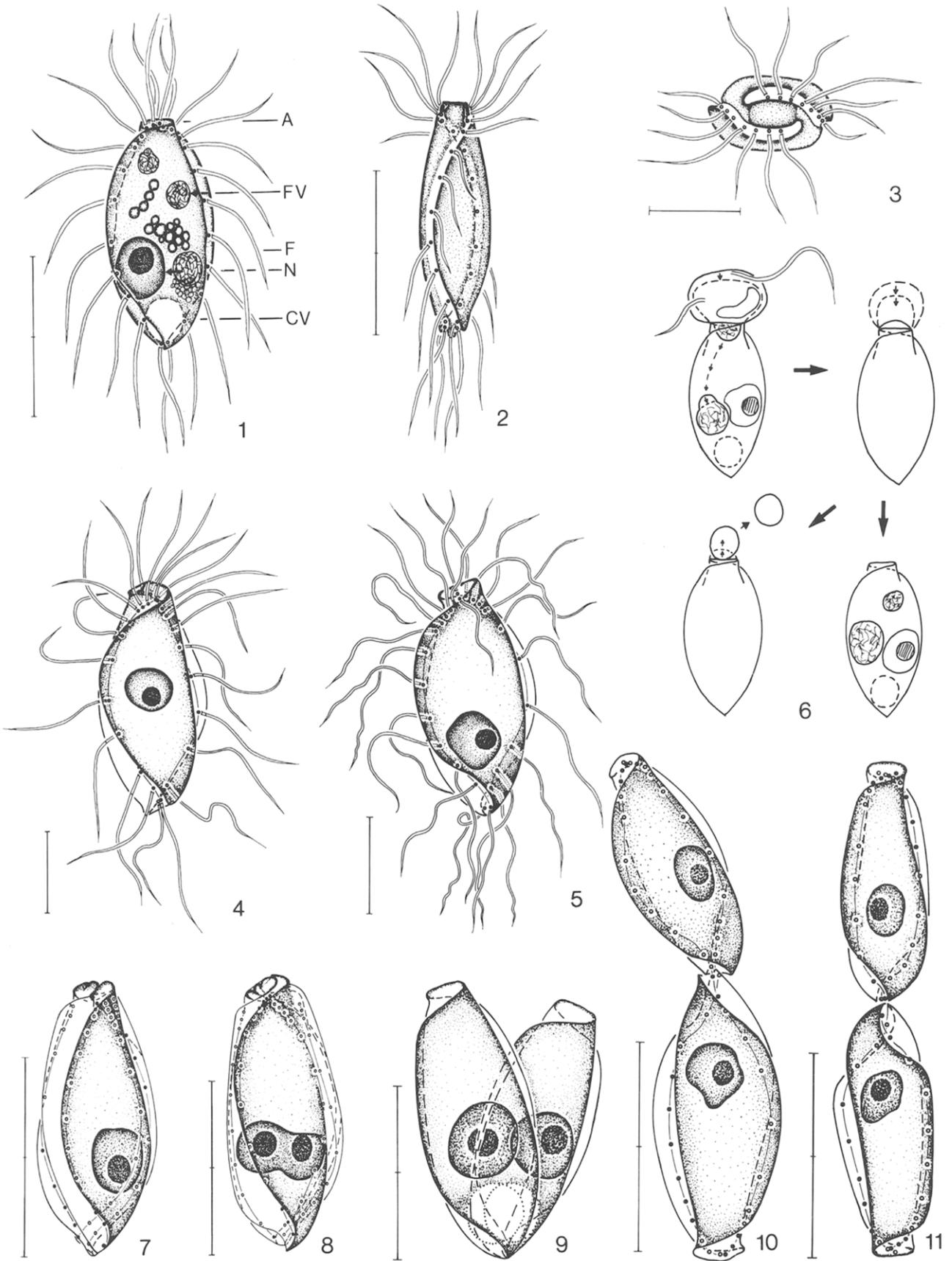
Pellicle and subpellicular microtubules

The pellicle consists of the cell membrane, a thin osmiophilic sheet, and a thick finely granular layer of moderate electron density (Fig. 28). For brevity, the thick layer will be referred to as the "epiplasm" because it looks very similar to the epiplasm of the Euglenophyta and the Ciliophora. These 3 components have a thickness of 40–65 nm ($\bar{x} = 48.3$ nm; $n = 7$). The cell membrane is about 9 nm thick and of typical tripartite structure. The epiplasm and the osmiophilic sheet are absent around the flagella and in the microtubule furrows (Fig. 27). Both are tightly attached to the cell membrane. There are no pellicular alveoli.

The subpellicular microtubules have a diameter of 19–27 nm ($\bar{x} = 22.5$ nm; $n = 6$) and are arranged in 3 distinct groups (Figs. 12, 28, 74). The first group (M 1) underlies the microtubule furrows and contains 21–25 ($\bar{x} = 23.2$; $n = 4$) regularly spaced microtubules which are interconnected by a strand of fibrous material and which are linked to the cell membrane by 2 rows of delicate arms. The second group (M 2) is located at the tip of the thin edge of each pellicular plate and consists of 3–6 ($\bar{x} = 4.2$; $n = 5$) regularly spaced microtubules which are also interconnected by a single strand of fibrous material; they lack, however, arms to either the cell membrane or the epiplasm. The third group (M 3) consists of irregularly spaced armless microtubules along the flat part of the plates.

The number of microtubules in each of these groups decreases toward the ends of the cell where the groups become indistinguishable. Serial sections convincingly show that the microtubules – at least those of the microtubule furrow – extend from one to the other end of the cell. No special structure from which the microtubules originate or any connection with the somatic kinetids were observed.

In the ridge between and around the flagella there is a granular fibre with a diameter of 16–20 nm. Very delicate



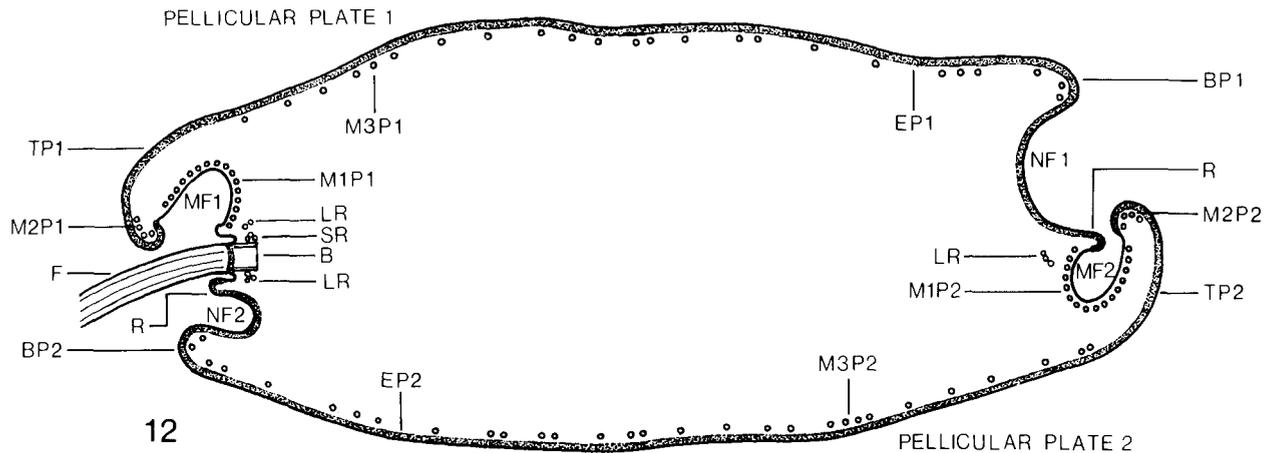


Fig. 12. Schematic transverse section of *Hemimastix amphikineta* (comp. Figs. 18, 19, 27, 28). The cell is composed of 2 large pellicular plates which are underlain with epiplasm with exception of the microtubule furrows. All pellicular components and the structures associated with the flagellar rows (the one on the right is out of the plane of section) are arranged in an inverse mirror-image manner. Thus, no right and left side can be identified. B = basal body, BP1 = blunt end of pellicular plate 1, BP2 = blunt end of pellicular plate 2, EP1 = epiplasm of pellicular plate 1, EP2 = epiplasm of pellicular plate 2, F = flagellum, LR = long microtubular ribbon associated with the basal body, M1P1 = microtubule group 1 of pellicular plate 1, M2P1 = microtubule group 2 of pellicular plate 1, M3P1 = microtubule group 3 of pellicular plate 1, M1P2 = microtubule group 1 of pellicular plate 2, M2P2 = microtubule group 2 of pellicular plate 2, M3P2 = microtubule group 3 of pellicular plate 2, MF1 = pellicular furrow of plate 1 ("microtubule furrow 1"), MF2 = pellicular furrow of plate 2 ("microtubule furrow 2"), NF1 = pellicular furrow of plate 1 ("naked furrow 1"), NF2 = pellicular furrow of plate 2 ("naked furrow 2"), R = ridge between and around the flagella, SR = short microtubular ribbon associated with the basal body, TP1 = thin end of pellicular plate 1, TP2 = thin end of pellicular plate 2.

projections link this structure to the epiplasm (Figs. 27–29, 44). This fibre has no contact with the transition fibres which surround the basal bodies.

The kinetid

The flagellar apparatus of *Hemimastix amphikineta* is abundantly illustrated (Figs. 1, 2, 12–14, 16, 17, 19, 27–31, 33–37, 39–46, 74, 76) because its detailed structure is of paramount importance and shows the exceptional position of this organism among the protists.

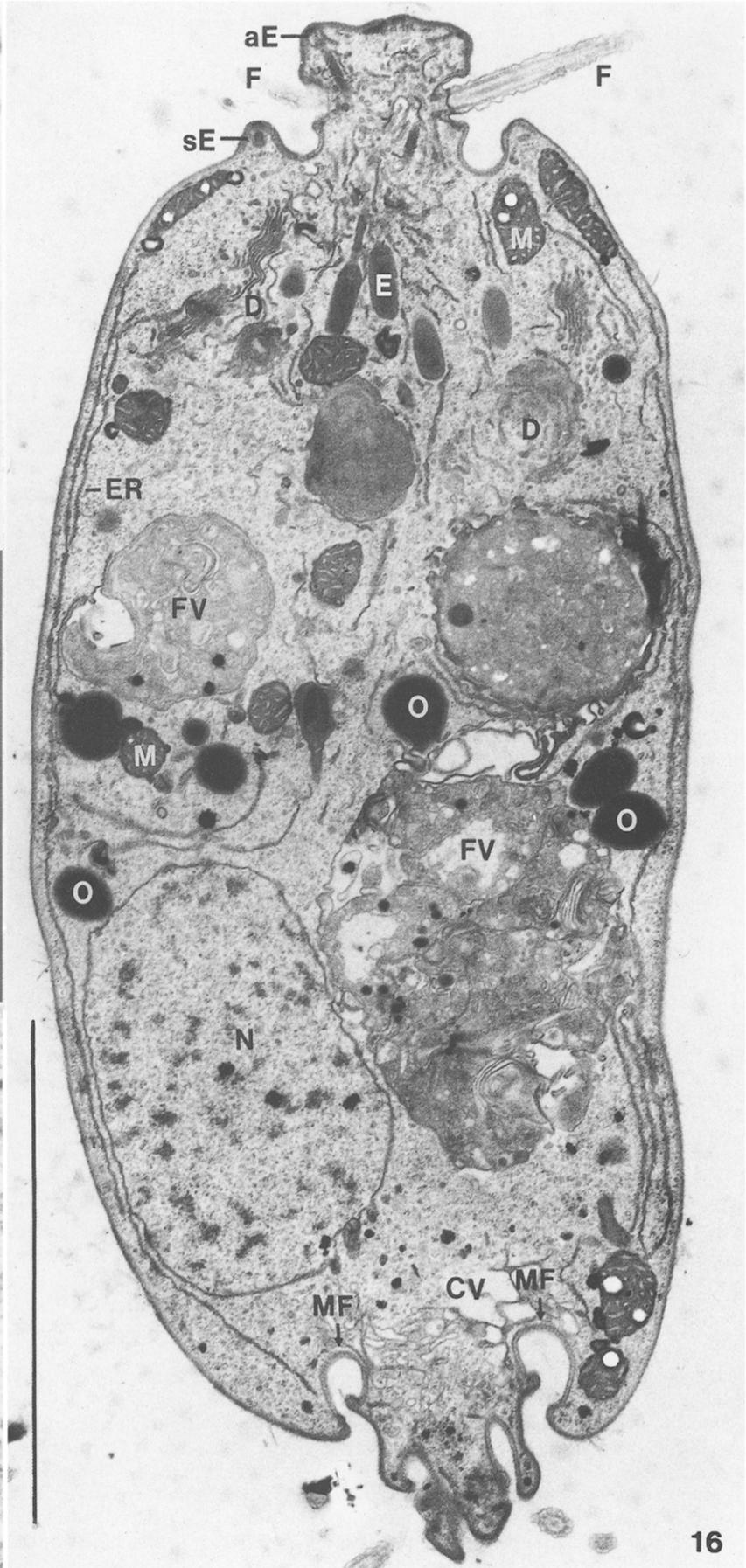
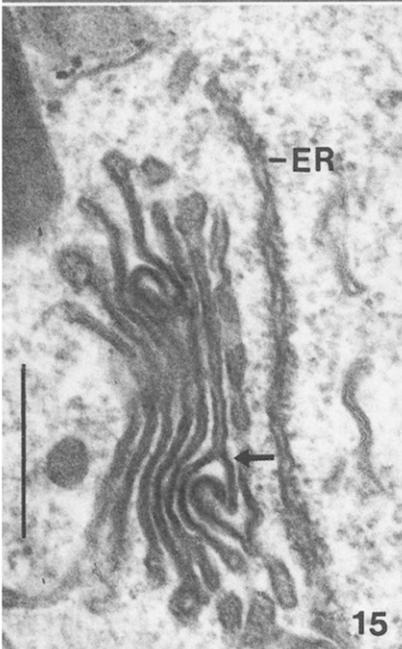
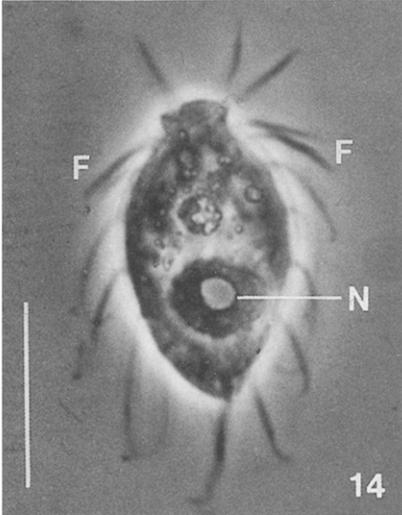
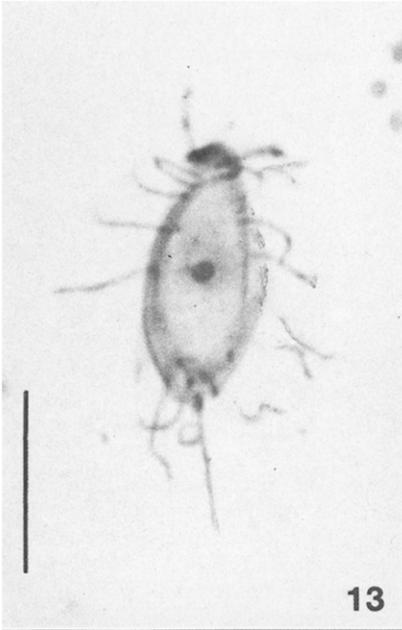
The 2 flagellar rows of *H. amphikineta* will be referred to as "kineties" (Figs. 1, 2, 13, 14, 16, 19). Each kinety is composed of about 12 flagella. A flagellum and its associated structures (basal body, fibrillar systems) form a "kinetid". This general terminology has been adopted from Lynn [52] and Lynn and Small [53]. All kinetids of *H. amphikineta* are monokinetids (= composed of single basal bodies and not of pairs, which are termed dikinetids) and have the same organization. The cell surface is elevated such that there is a pocket around the insertion of each flagellum (Figs. 27, 30, 34, 40). The pocket is

180–310 nm (\bar{x} = 244 nm; n = 8) deep and has a diameter of 400–550 nm (\bar{x} = 486 nm; n = 8).

All flagella of *H. amphikineta* are of the same length (about 8 μ m) and have a diameter of 270–340 nm (\bar{x} = 299 nm; n = 8). They possess a conventional axoneme 180–200 nm (\bar{x} = 191 nm; n = 8) in diameter. Oblique arms link the A-microtubules to the flagellar (cell) membrane along its whole length (Figs. 44–46). The central pair of microtubules terminate at a thick concave transitional plate; one of the two microtubules is slightly shorter than the other (Figs. 27, 30, 31). There is no axosome, no paraxial rod, or mastigonemes (absence of the last confirmed by negative staining). The transitional plate extends between the peripheral microtubules and the flagellar membrane (Fig. 27). A delicate transitional cylinder wraps around the basal region of the central pair of microtubules. This cylinder has a length of about 100 nm and is linked by short projections to the A-microtubules of the axoneme (Figs. 27, 31, 44).

The basal bodies measure 180–250 \times 180–210 nm (\bar{x} = 223 \times 189 nm; n = 9) in length and are thus unusu-

- ◀ Figs. 1–11. Morphology (Figs. 1–6) and division (Figs. 7–11) of *Hemimastix amphikineta* from life (Figs. 1–3, 6) and from protargol-impregnated specimens (Figs. 4, 5, 7–11). Australian type population: Figs. 1–4, 6, 9; Chilean population: Figs. 5, 7, 8, 10, 11. – Figs. 1, 4, 5. View from the broad axis. A = truncated anterior end, CV = contractile vacuole, F = flagellar row, FV = food vacuole, N = nucleus with central nucleolus. – Fig. 2. View from the short axis. – Fig. 3. Frontal view. – Fig. 6. Food uptake. The prey, a small colourless flagellate, is stuck to the anterior end and is propelled backwards (upper left). Feeding frequently ceases and the prey is left (lower right) or a part of it is ejected (lower left). – Fig. 7. Early division stage. The flagellar rows have doubled. – Figs. 8, 9. Middle division stages. The nucleus and its nucleolus divide and the daughters begin to separate. – Figs. 10, 11. Late division stages. The anterior ends of the daughters lie antipodally apart, showing that division is longitudinally. Scale bar divisions = 5 μ m.



ally short (Figs. 27, 31). A third microtubule joins the peripheral doublets close below the transitional plate. The lumen of the basal bodies is filled with a weakly osmiophilic substance. A delicate A-type cartwheel *sensu* Eisler and Bardele [22] may be seen in the most basal region (Figs. 39, 42, 43). A ring of granular material is found slightly above the proximal end of the basal body. Curving arms arise from this ring and extend beneath the membrane of the flagellar pocket. Each arm corresponds to 1 pair of peripheral microtubules. This non-microtubular basket is very conspicuous and does probably not contact the granular fibre in the ridge around and between the flagella (Figs. 27, 31, 41, 42).

A membranous sac with a size of 230–400 × 40–60 nm (\bar{x} = 290 × 49 nm; n = 6) is located posterior to each flagellum (Figs. 30, 34, 35, 40). It opens at the level of the transitional plate and is oriented slightly obliquely to the long axis of the cell and the kinety. Small vesicles seem to pinch off from these sacs.

The proximal end of a basal body is embedded in a ring of granular material which extends posteriorly as a thin layer between the 2 microtubular ribbons (Figs. 27, 28, 39, 43, 74). A long and a short microtubular ribbon originate from this ring and extend posteriorly, slightly ascending, but not touching the pellicle in their path, to the next kinetid (Figs. 33–37, 40–43, 74, 76). Each ribbon consists of 3 to 4 often rather widely separated microtubules which are linked by fine strands of granular material. The long ribbon originates on the naked furrow-side of the basal body and extends obliquely to the microtubule furrow-side of the next kinetid. In appropriate sections posterior to the kinetids there are often 2 groups of microtubules on the microtubule furrow-side (Figs. 27, 29). These and horizontal sections (Figs. 39–43) show that the long ribbons overlap. The degree of overlap varies (anteriorly more pronounced than in the mid-region), and the ribbons are linked by a fine strand of granular material (Fig. 27). The short microtubular ribbon originates on the microtubule furrow-side of the basal body and extends about 600 nm posteriorly (Figs. 33–37, 41, 43, 74).

Mouth structures

Hemimastix amphikineta feeds on small colourless flagellates and bacteria. Large food vacuoles containing remains of these organisms are found at the light- and electronmicroscopic level (Figs. 1, 6, 16). Despite this phagotrophic way of life, a permanent cytostome is certainly absent. The exact site of food ingestion remains unknown. However, light microscopy (Figs. 1, 3, 6) and several ultrastructural details (Figs. 17, 32) show that

ingestion must take place in the centre of the anterior end where the following specializations can be observed. First, the capitulum is completely underlain with epiplasm, that is, the microtubule furrows are not longer recognizable (Figs. 17, 60). It is, however, very likely that the microtubules of these furrows extend to the apical rows of extrusomes (see *Extrusomes*). Second, the microtubular ribbons which accompany the apical rows of extrusomes are underlain with fibrous material. These layers have long projections which extend to the centre of the pole (Fig. 60). Third, the anterior region is filled with phagoplasm and disorganized microtubules about 3 μ m in length (Figs. 17, 32). The phagoplasm consists of about 1000 nm long tortuous tubules with a diameter of about 50 nm (\bar{x} = 50.4 nm; n = 10) and dumb-bell shaped disks. This combination of organelles is strongly reminiscent of the oral structures of gymnostomatous “lower” Ciliophora (e.g., [28]).

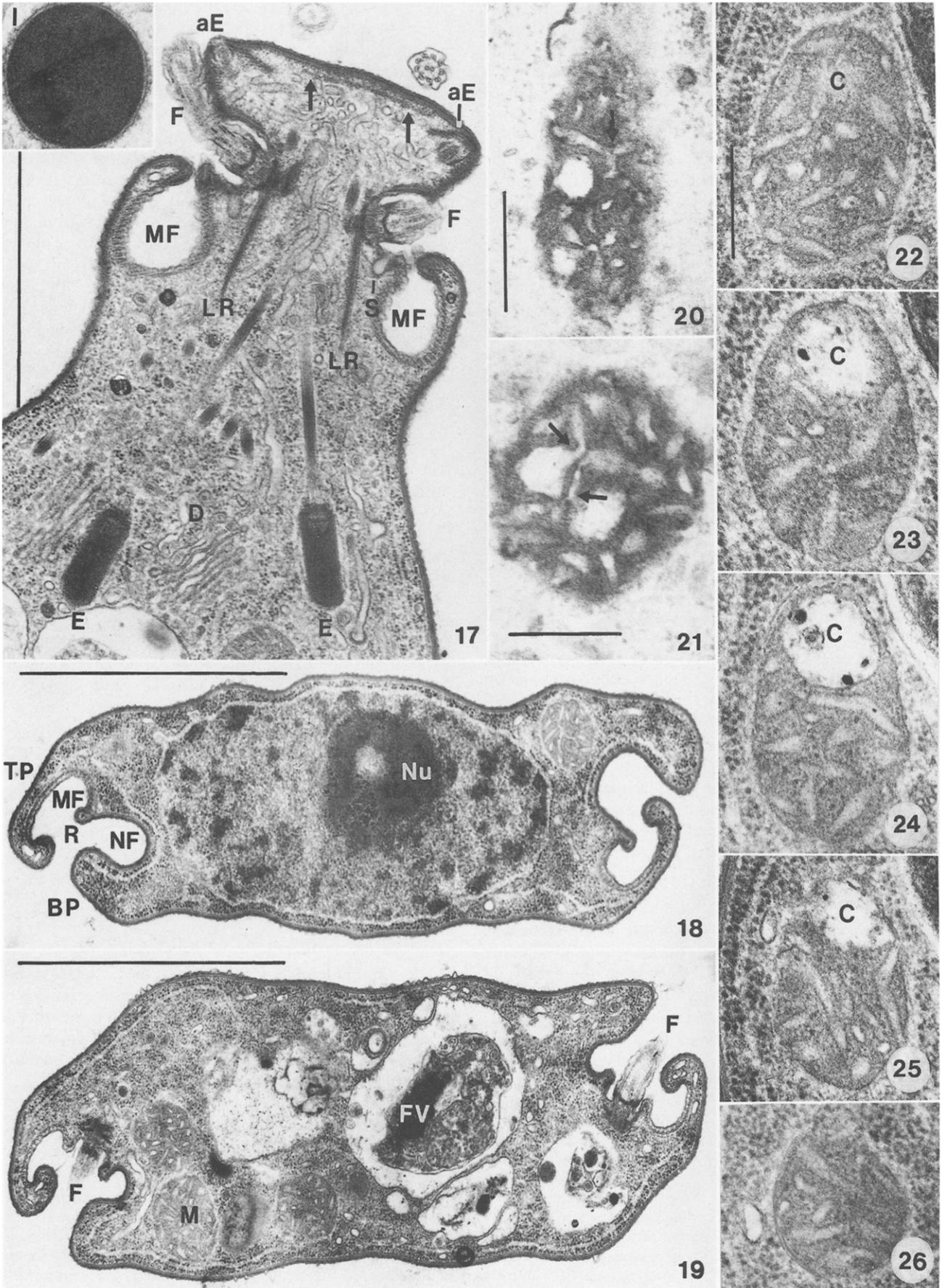
Nucleus

The nucleus of *H. amphikineta* belongs to the “chromosomal” and/or “vesicular” type which are distinguished rather arbitrarily [73]. The nucleus is spherical and contains a conspicuous central nucleolus (Figs. 1, 4, 5, 13, 14). The nuclear envelope is of conventional structure and has conspicuous pore complexes (Fig. 48). The karyolymph is interdispersed with very delicate fibres and contains many compact aggregations which are apparently sections of filamentous chromosomes (Figs. 16, 18, 47, 48). These aggregations which are partly closely attached to the inner side of the nuclear envelope have a size of 100–300 nm (\bar{x} = 168 nm; n = 12) and consist of granules about 20 nm in diameter. The same granules are seen in the nucleolus where they have a clumped distribution, too. Usually, the centre of the nucleolus is nearly free of granules and appears clear (Fig. 18). Similar, but smaller clear zones are found throughout the nucleolus (Fig. 47). Taken all together, the nucleus of *H. amphikineta* has an amazing morphological similarity to that of *Euglena gracilis* (comp. Figs. 18, 47 with Fig. 11 d in [73]), and certain promitotic amoebae, for instance, *Pompholyxophrys punicea* [67].

Contractile vacuole

The contractile vacuole is located at the posterior end of the cell and is surrounded in part by bleb-like vesicles or folds (Figs. 1, 16). Delicate fibres are seen on the outside of the vesicles which suggests a contributory function of the vesicles, although we can not exclude the possibility that they are folds or artifacts.

- ◀ Figs. 13–16. Light (Figs. 13, 14) and electron micrographs (Figs. 15, 16) of *Hemimastix amphikineta*. – Fig. 13. Protargol-impregnated specimen (type individual). Note the heavily impregnated nucleolus in the centre of the cell and the capitulum at its anterior end. Bar = 10 μ m. – Fig. 14. Silver carbonate impregnated (slightly understained) specimen in phase-contrast. Bar = 10 μ m. – Fig. 15. Dictyosome. Note a branched cisterna (arrow). Bar = 300 nm. – Fig. 16. Longitudinal section through the broad axis of the cell. Bar = 5 μ m. aE = apical row of extrusomes, CV = contractile vacuole, D = dictyosome, E = extrusome, ER = endoplasmic reticulum, F = flagellar rows, FV = food vacuole, M = mitochondrion, MF = microtubule furrow, N = nucleus, O = osmiophilic globules, sE = subapical row of extrusomes.



Extrusomes

Hemimastix amphikineta has complex extrusomes which are located in the anterior region of the cell (Figs. 16, 17). They have a length of about 4 μm (\bar{x} = 4.06 μm ; n = 8) and consist of a nail-shaped anterior and a bottle-shaped posterior compartment. The whole organelle, which looks like a pasteur pipette, is surrounded by a unit membrane (Figs. 50, 52, 56). The matrix consists of electron-dense material without any recognizable substructure. The complex structure of these organelles is difficult to describe, so details are documented in a series of micrographs and schematic drawings (Figs. 50–65).

The nail-shaped compartment of the extrusomes measures about 3600 \times 70 nm (\bar{x} = 3574 \times 69 nm; n = 8; without the unit membrane) and is inserted with its head onto the anterior end of the posterior compartment (Figs. 52, 62). The head consists of a smaller anterior and a slightly larger posterior granule which have 2 disk-shaped projections each. These projections link the head with the content of the bottle. In the groove between the granules there is a slightly thicker disk of this material which looks very similar to the content of the bottle. The homogeneous material which fills the shaft of the nail tapers towards the anterior end (Figs. 63, 65).

The bottle-shaped compartment of the extrusomes measures about 700 \times 200 nm (\bar{x} = 750 \times 220 nm; n = 8). About 6 wheel-like structures each composed of some 11 spokes extend between the bulbous dilation at the anterior end of the bottle and the shaft of the nail (Figs. 50, 62). The spokes form a characteristic pattern in transverse section (Fig. 54) and contrast with the disk-shaped projections which link the head of the nail with the bottle (Figs. 52, 62). Similar, but smaller spokes are recognizable along the whole shaft of the nail, indicating that the bottle content continues as a thin layer up to the tip of the extrusomes (Fig. 53).

The extrusomes are anchored in the anterior region of the cell where the kineties approach the anterior end, and around the centre of the capitulum. Thus, there are 2 semi-circular rows of extrusomes in each half of the cell, that is, left and right of the broad axis (Fig. 16). The subapical rows are inserted between the pellicular microtubule groups 1 and 2 at the extremity of the thinner end of the pellicular plates (Fig. 64). Very likely, the same is true for the apical rows because the microtubules which pass the extrusomes show the same characteristic pattern (comp.

Figs. 60, 63, 64). This is, however, uncertain because the capitulum is specialized and is difficult to analyze (for further details see *Mouth structures*). The tip of the extrusome is surrounded by a complex cylindroid structure, termed the “anchorage structure”, which measures about 200 \times 130 nm and consists of fibrous material. Four arm-like projections extend from the central cylinder to the neighbouring extrusomes (Figs. 57, 58, 65). Thus, the anchorage structure looks distinctly like an ice-axe the arms of which are bent downwards and have a triangular cross section (Figs. 57–59, 65). Many fine strands of fibrous material extend from the cylinder to the unit membrane which surrounds the extrusome. The anchorage structure is probably a specialized part of the pellicular epiplasm because there is no distinct border between these 2 components.

Some partly discharged extrusomes have been found (Figs. 51, 61). They look very similar to the resting ones. At the point of protrusion there are 4 wing-like structures probably corresponding to the 4 arms of the anchoring structure.

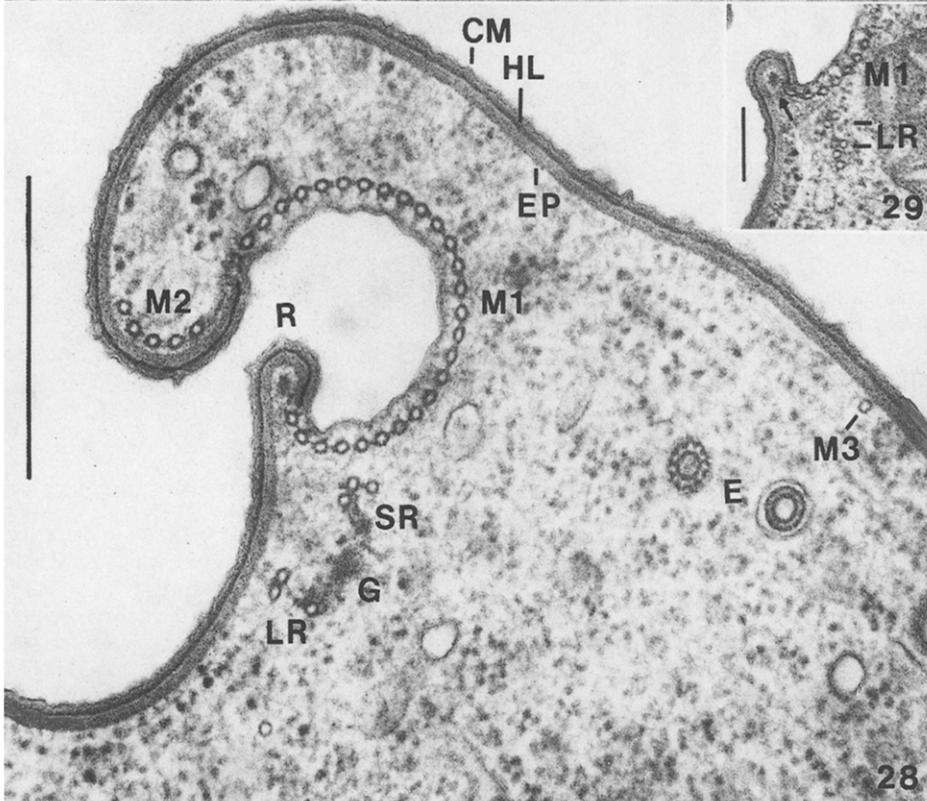
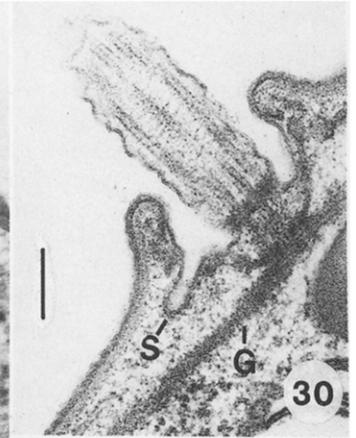
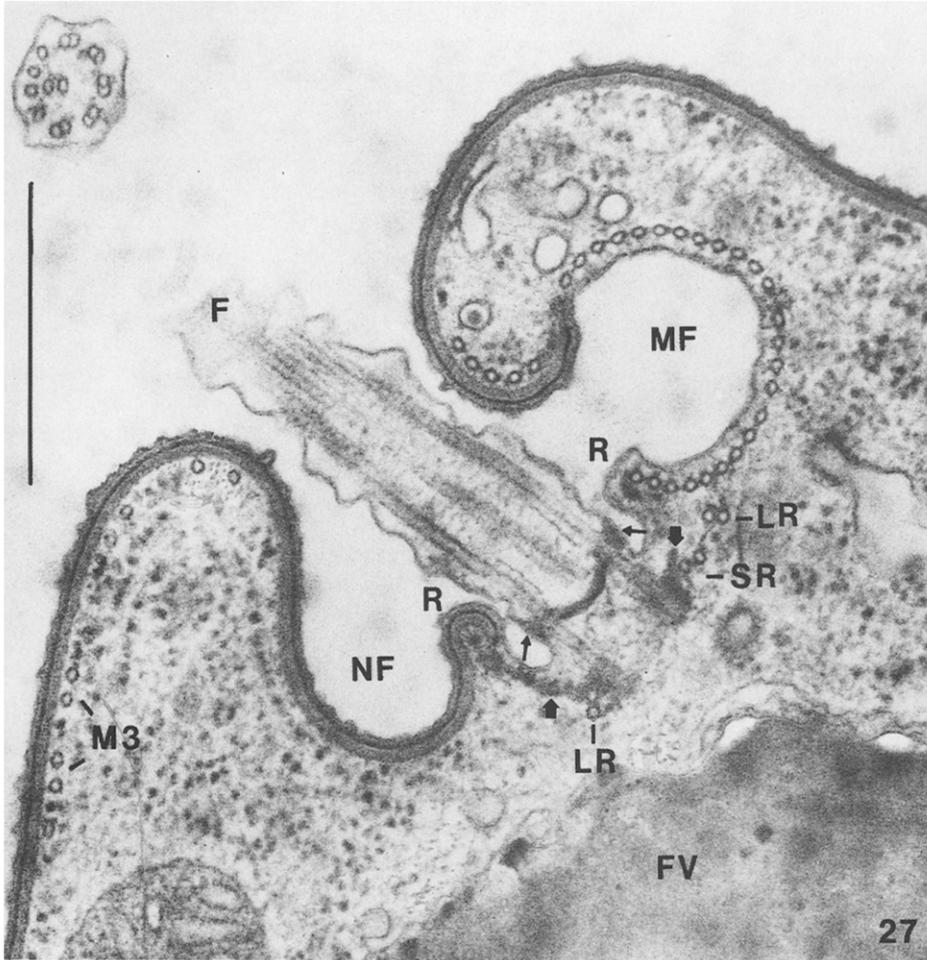
Very rarely, extrusomes or their developmental stages have been found in other regions of the cell; in a single case, the nail-like compartment was enclosed in a food vacuole (Fig. 28).

Other cytoplasmic organelles

The mitochondria, about 1–2 \times 0.4–0.6 μm in size, are mainly located in and near the ends of the pellicular plates (Figs. 16, 18, 19). The exact shape of the mitochondrial cristae is difficult to discern. The most plausible interpretation of serial sections (Figs. 22–26) through 9 mitochondria is that the cristae are strongly tortuous and somewhat flattened saccular tubules with a diameter of 17–60 nm (\bar{x} = 36 nm; n = 15). Certainly, the cristae are not disk-shaped or constricted at the base. Rarely, branched cristae have been seen (Fig. 20). Nearly all mitochondria of *H. amphikineta* contain at least 1 large spherical to egg-shaped caverna (Figs. 22–26). These membrane-bound cavernae are about 85–220 nm (\bar{x} = 146 nm; n = 20) in size and are extensions of long cristae (Fig. 21). They contain often some slightly to heavily osmiophilic granules. A fixation artifact can not be excluded.

In the anterior half of the cell, in front of the nucleus, there are about 10 conspicuous dictyosomes (Fig. 16). They consist of 5–9 cisternae (\bar{x} = 6.4; n = 12) which

- ◀ Figs. 17–26. Ultrastructure of *Hemimastix amphikineta*. – Fig. 17. Longitudinal section through the anterior part along the broad axis of the cell. Note the mushroom-like appearance of the capitulum. The arrows indicate a microtubule ribbon which passes the apical row of extrusomes (aE). Bar = 2 μm . The inset at the upper left shows an osmiophilic globule (comp. Fig. 16) which is surrounded by a distinct unit membrane. Bar = 60 nm. – Fig. 18. Transverse section at the level of the nucleus. This section passes between the flagella. Thus, the ridge (R) which separates the 2 pellicular plates becomes visible (comp. Fig. 12). Bar = 2 μm . – Fig. 19. Transverse section (same specimen as in Fig. 18) slightly posterior the nucleus at the level of 2 flagella (F). Bar = 2 μm . – Fig. 20. Mitochondrion with a branched crista (arrow). Bar = 100 nm. – Fig. 21. Mitochondrion with tubular cristae which open into spherical cavernae (arrows). Bar = 100 nm. – Figs. 22–26. Serial section of a mitochondrion with a large membrane-bound spherical caverna (C). Bar = 100 nm. BP = blunt end of the pellicular plate, C = caverna, D = dictyosome, E = extrusome, F = flagellar rows, FV = food vacuole, LR = long microtubular ribbon of the kinetid, M = mitochondrion, MF = microtubule furrow, NF = naked furrow, Nu = nucleolus of the nucleus, R = ridge between and around the flagella, S = membranous sac, TP = thin end of the pellicular plate.



measure up to 900–1500 nm (\bar{x} = 1045 nm; n = 10) in diameter. An unusual feature is the presence of cisternae which are branched in the longitudinal direction of the organelle (Fig. 15). The branches are distinctly wrinkled and even involuted.

The granular endoplasmic reticulum is of usual structure. One to two remarkable regular cisternae lie closely underneath the pellicle of each plate (Figs. 16, 37).

The cytoplasm contains small and large membrane-bound food vacuoles up to 5 μ m in diameter and differently stained globules, 100–300 nm in diameter, which are also surrounded by a unit membrane (Figs. 16, 17; inset). Rather frequently, small (about 800 nm) vacuoles with 1–2 undamaged appearing bacteria are observed (Fig. 38). A symbiotic function of these often dumb-bell shaped bacteria is, however, rather unlikely because they are also observed in typical food vacuoles and outside the cell in the pellicular furrows.

Discussion

Identification

No organism with the characteristics of *Hemimastix amphikineta* could be found in the literature. Thus, we believe that it is a new species. Some minute free-living flagellates with many cilia-like flagella were described at the turn of the century. Most of these organisms have their flagella quite evenly distributed over the body and thus look rather dissimilar to our species.

Multicilia marina Cienkowsky, 1881 [13] and *M. lacustris* Lauterborn, 1895 [45] are spherical cells with about 40 slowly moving flagella (Figs. 69, 73). It is interesting that Gruber [31] created a genus *Polymastix* in 1884 for a free-living flagellate. In the same year Bütschli [9] used the name for a genus of parasitic flagellates. Use of Bütschli's genus survives to this day, probably because Gruber's *Polymastix sol* (Fig. 70) is synonym with the species described by Cienkowsky [32]. *Multicilia marina* and *M. lacustris* are markedly amoeboid (M. Zölffel, Univ. Berlin, pers. communication), and *M. lacustris* feeds with small pseudopodia [45]. This indicates that they are related to the *Mastigamoeba* – *Mastigella* – *Cercomonas* group, the taxonomic affinities of which are still rather

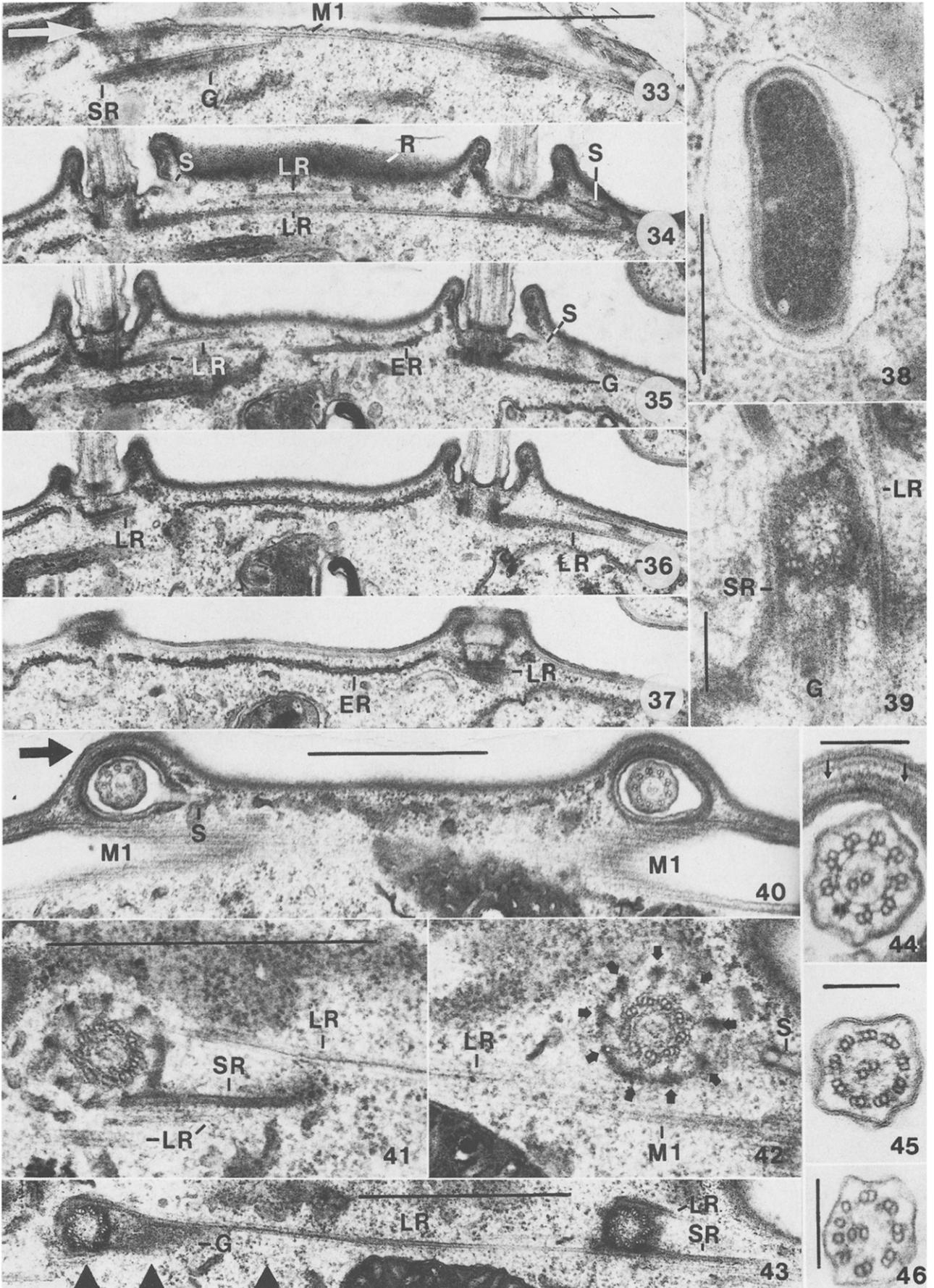
uncertain [46]. *Multicilia* and the other genera in question (see below) are of uncertain affinities and have not been subject to contemporary reinvestigation. Lauterborn [45] erected the suborder “Holomastigina” to include *M. marina* and *M. lacustris*. It might be that the enigmatic genus *Artodiscus* Penard, 1890 [70] belongs to *Multicilia* or is even identical with *M. lacustris* because its pseudopods are flagella (D. J. Patterson, Univ. Bristol, pers. communication).

Trichonema gracile Möbius, 1888 [59] has been found in huge number in a sea-water aquarium (Fig. 72). Möbius [59] included this organism in an unranked taxon “Cilio-Flagellata”, but gave a rather incomplete description. It is impossible to decide whether his organism belongs to the Ciliophora or to the flagellates. Small species of the ciliophoran genus *Cohnilembus* look similar, especially in having a caudal cilium. The same is true for *Schewiakoffia* (formerly *Maupasia*; see [14]) *paradoxa*, a freshwater organism which is considered by several recent authors [15, 19, 40] to belong to the kinetofragminophoran ciliates (Fig. 71). The ventrally located mouth opening of this creature is indeed ciliophoran-like; however, it might well be that *S. paradoxa* belongs to the flagellates because it exhibits active metaboly like *Euglena*, and this is atypical for ciliophorans. Schewiakoff [74] erected the “Mastigotricha” for this unusual organism.

In 1892 Klebs [42] described the freshwater flagellate *Spiroonema multiciliatum*. This peculiar organism is somewhat inadequately figured (redrawn as Fig. 66) but well diagnosed: “Body lanceolate, slightly flattened, with distinct caudal elongation, metabolic. On both sides a shallow, slightly spiraling furrow. Many small flagella arranged in 2 rows at the rim of the furrows. Contractile vacuole at posterior end. Nucleus?” (translated by the authors). It is obvious that this organism is very similar to *Hemimastix* and we believe that it belongs to the same family. The description [42] of the metaboly of *Spiroonema* reminds one of euglenoid movement: “The posterior part of the body and the caudal elongation are rather stiff and hardly change. The anterior part of the body, on the other hand, is strongly metabolic, contracts and extends, bends and curves to all sides” (translated by the authors).

Goodey [30] and M. Zölffel (Univ. Berlin, pers. communication) observed in soil infusions organisms (Figs. 67,

◀ Figs. 27–32. Ultrastructure of the pellicle, the kinetid, and the phagoplasm of *Hemimastix amphikineta*. – Fig. 27. Transverse section of the cell showing a longitudinally sectioned kinetid. The small arrows point to the transitional plate which extends to the ciliary membrane. The thick arrows mark the transitional fibres which are anchored slightly above the proximal end of the basal body. Note the absence of the epiplasm in the microtubule furrow (MF). Bar = 500 nm. – Fig. 28. Transverse section slightly posterior to that shown in Fig. 27 (comp. Fig. 43). The 2 microtubular ribbons are connected by granular material (G). The right extrusome (E) projects from a food vacuole and is apparently lysing. Bar = 500 nm. – Fig. 29. Transverse section of a cell between 2 flagella (comp. Fig. 43). Note the overlapping long microtubular ribbons (LR) of 2 kinetids. The arrow marks the granular fibre in the ridge between the flagella. Bar = 200 nm. – Fig. 30. Section along the longitudinal kinety axis showing the conspicuous membranous sac (S) posterior to the basal body. Bar = 200 nm. – Fig. 31. Longitudinal section of a flagellum and its associated structures. The small arrows mark the delicate transitional cylinder, the thick arrows point to the transitional fibres. Bar = 200 nm. – Fig. 32. The anterior region of the cell is filled with extrusomes (E), microtubules (arrows), tortuous tubules, and dumb-bell shaped disks (comp. Fig. 17). Bar = 200 nm. CM = cell membrane, E = extrusome, EP = epiplasm, F = flagellum, G = granular substance, HL = heavily osmiophilic layer, LR = long microtubular ribbon, M1, M2, M3 = subpellicular microtubule groups 1, 2, 3, MF = microtubule furrow, NF = naked furrow, R = ridge between and around the flagella, S = membranous sac, SR = short microtubular ribbon.



68) which they believed to be *S. multiciliatum* Klebs [42]. These specimens share with *Hemimastix amphikineta* not only the biotope but also the 2 rows of cilia-like flagella though here the rows do not reach the posterior end of the body. Thus, they are certainly not identical with our species but might belong to the same genus. They are probably not identical with *S. multiciliatum* Klebs because their body is completely ametabolic. We believe that they represent a different (new) species or even a new genus. Goodey [30] noted that the nuclear apparatus of his species was most similar to that of *Euglena viridis*.

Doflein [21] erected the family Spironemidae for *S. multiciliatum*. No member of this family has been studied electron-microscopically, with exception of *H. amphikineta*.

Ultrastructural comparison

The free-living *H. amphikineta* (and probably *S. multiciliatum*) has 5 outstanding characteristics the combination of which is unique among known protists: (1) the flagella are cilia-like and arranged in 2 distinct ciliophoran-like kineties; (2) the basal bodies of these kineties are connected by 2 systems of microtubules; (3) a conspicuous membranous sac is present posterior to each kinetid; (4) the mitochondrial cristae are saccular; and (5) the pellicle resembles the structure found in euglenoids (see below).

This unique constellation of characters, shown as a three-dimensional reconstruction in Fig. 74, would make a detailed comparison with a lot of other protists rather meaningless. Some relationship might exist with the Euglenozoa (*sensu* [16]) which include the phyla Pseudociliata (with the unique and sole genus *Stephanopogon* placed in an incertae sedis position [16]; but see [69]), Euglenophyta, and Kinetoplastidea. The comparison of *Hemimastix* with the Euglenophyta (see below) is mainly based on the similarities in the ultrastructure and symmetry of the pellicular plates. These similarities are too conspicuous to be despised, especially the extraordinary inverse mirror-image symmetry which occurs, to our best knowledge, only in the Euglenophyta and in *Hemimastix*.

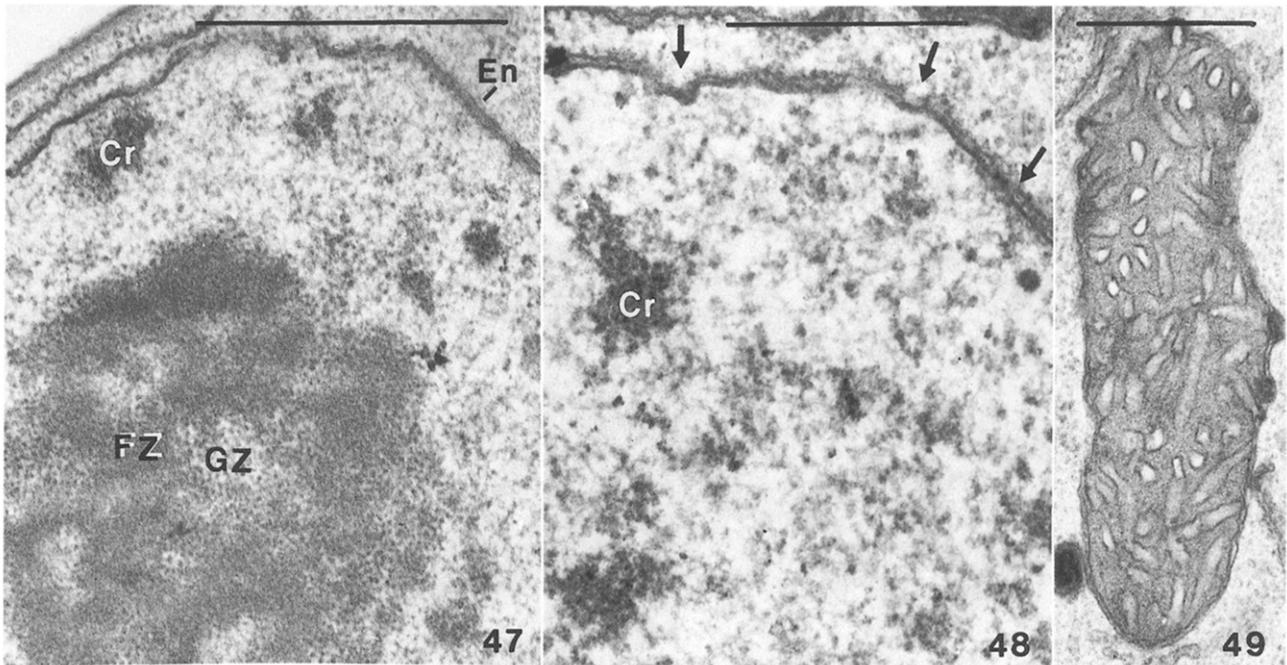
Pochmann [72; p. 515] who studied this “diagonal symmetry” of the Euglenophyta very carefully thinks that it is a special case of bilateral symmetry.

The longitudinal channel at each side of *H. amphikineta* reminds somewhat on the cingulum and sulcus of the Dinoflagellates [83]. We believe, however, that this is an obvious analogy because the cingulum and the sulcus are oriented rectangularly to each other. Likewise, the single flagellar row of filamentous and coenocytic Dinoflagellates like *Protogonyaulax* and *Polykrikos* can be hardly compared with the two flagellar rows of *Hemimastix*. *Hemimastix* is clearly a single cell, whereas the above mentioned Dinoflagellates are chains of more or less fused cells [83]. *Polykrikos* has, however, been used to discuss the origin of the ciliophoran kinetid [64].

A detailed comparison of *Hemimastix* with *Stephanopogon* and the Euglenophyta is given in Table 2. This compilation convincingly shows the distant position of *H. amphikineta*. The discussion of some main characters follows, with exception of the kinetid system, which is treated in the next section.

An important difference between *Hemimastix* and the Euglenophyta concerns the mitochondrial cristae. The discoid cristae of the Euglenophyta have a pedicel which is certainly absent in the tubuli-like cristae of *H. amphikineta*. This distinct difference forbids an inclusion of *H. amphikineta* in the Euglenophyta because the shape of the cristae is considered as an important phylogenetic marker by many students of protistology (see [69] for literature). Page and Blanton [65], for instance, base the class “Heterolobosea” in part on the flattened mitochondrial cristae (see also [23]). Seravin [77] divides the Mastigophora in 2 large assemblages, the Lamellacristata and the Tubulacristata. This distinction has been recognized a few years before by Taylor [82], who first clearly emphasized the fact that the “lower eukaryotes” fall into 2 great camps or lines with regard to possession of either flattened or tubular mitochondrial cristae. The mitochondria of *Hemimastix* show an unexpected similarity to those of cellular slime molds like *Dictyostelium discoideum* and *Perichaena vermicularis* [12, 54] and to lo-

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- ◀ Figs. 33–46. Ultrastructure of the kinetid of *Hemimastix amphikineta*. – Figs. 33–37. Longitudinal serial section of 2 kinetids. The sections proceed from the microtubule furrow to the naked furrow as indicated by the absence and presence of the epiplasm in Figs. 33 and 37, respectively. The arrow in the upper left is directed to the posterior end of the cell. Note the overlapping long microtubular ribbons (LR) of 2 kinetids in Fig. 34 and compare with the corresponding transverse section shown in Fig. 29. Bar = 1000 nm. – Fig. 38. An apparently undamaged bacterium in a small cytoplasmic vacuole. Bar = 400 nm. – Fig. 39. Transverse section through the proximal end of a basal body showing the cartwheel, the origin of the short microtubular ribbon (SR), and an overlapping long microtubular ribbon (LR). Note that Figs. 39, 41, 44, 45 and 46 comprise a complete series (but not from a serial section of a single specimen) from the proximal end of the basal body to the distal end of the flagellum. Bar = 200 nm. – Figs. 40–43. Longitudinal serial section through the short axis of the cell showing 2 transversely sectioned kinetids. The sections proceed from the transitional cylinder through the transitional fibres to the proximal end of the basal body. The arrow in the upper left is directed to the posterior end of the cell. The arrow heads in Fig. 43 indicate the level of the transverse sections shown in Figs. 27–29. Figs. 40 and 43 are of similar magnification and show a membranous sac and a long microtubular ribbon (LR). Figs. 41 and 42 are of higher magnification and show the conspicuous transition fibres (arrows) and the short microtubular ribbon (SR). Bars = 1000 nm. – Fig. 44. Transverse section through a flagellum at the level of the transitional cylinder. The arrows indicate the granular fibre in the ridge of the flagellar pocket. Bar = 200 nm. – Figs. 45, 46. Transverse sections through the middle region and the distal end of a flagellum, respectively. Bars = 200 nm. ER = endoplasmic reticulum, G = granular substance, LR = long microtubular ribbon, M1 = subpellicular microtubule group 1, S = membranous sac, R = ridge between and around the flagella, SR = short microtubular ribbon.



Figs. 47–49. Nucleus and mitochondrion of *Hemimastix amphikineta*. – Figs. 47, 48. Nucleus at low and high magnification. The arrows mark nuclear pores. Bars = 1000 and 500 nm respectively. – Fig. 49. Mitochondrion with tubular cristae. Bar = 500 nm. Cr = chromosomes, En = nuclear envelope, FZ = fibrillar zone in the nucleolus, GZ = granular zone in the nucleolus.

bose and heteroloboseid amoebae like *Acanthamoeba palestinensis* and *Naegleria gruberi* [44, 65]. All have tortuous, slightly branched tubules and large spherical cavities often containing some osmiophilic granules.

The pellicle of *H. amphikineta* consists of 2 large plates which are arranged in an inverse mirror-image manner. Subpellicular microtubules occur mainly where the 2 plates adjoin. In this part of the cell, the conspicuous epiplasm underlying the cell membrane is absent. This situation fits exactly that described for the pellicle of the euglenoids, except that plates do not interlock (comp. [47, 56, 61, 80, 81]). It is amazing that a *Hemimastix*-like organism can be easily modeled by putting together 2 opposite located pellicular stripes of an euglenoid flagellate (Fig. 75). The rather specific arrangement of the subpellicular microtubules and the specific shape of the pellicular plates of *H. amphikineta* do not detract from this model because the euglenoids show a marked diversity in this respect (e.g., [5, 56, 57, 84]). The granular fibre in the ridge between and around the flagella (Figs. 27–29) is similar to the bridges between interlocking pellicular stripes of the Euglenophyta (comp. [5, 80, 81]).

Hemimastix amphikineta has conspicuous extrusomes. As far as we know, such extrusomes have not been reported on other protists, especially not in *Stephanopogon* and the Euglenophyta [35, 69]. The nail-like compartment reminds in transverse section on the microtoxycysts of *Cercomonas*, a colourless amoeboid flagellate with affinities to the chrysophyte algae and myxomycetes [62]. Some similarity exists also with the toxicysts of *Colponema* the nail-like compartment of which is, however, nearly completely submerged in the bottle-shaped part

[58]. These similarities are very likely only superficial because the extrusomes of *Hemimastix* lack the lamellated structure which is typical for those of the above mentioned genera and for toxicysts in general [35]. Some attempts have been made to use extrusomes as phylogenetic markers (e.g., [6]). This is, however, rather uncertain because of the erratic appearance of similar or even identical extrusomes in obviously widely separated taxa. Anchor-like extrusomes occur, for instance, in the ciliophoran subclasses Gymnostomata, Hypostomata, and Hymenostomata [25, 29, 35].

The flagella and the basal bodies of *H. amphikineta* have some attributes which are found in the Pseudociliata and the Euglenophyta. The phylogenetic value of these characters is, however, ambiguous because they occur sporadically also in other protists. Attributes which are shared with the Pseudociliata include the short length of the basal bodies and the absence of mastigonemes and paraflagellar structures. It is remarkable that the basal body is also rather short (about 350 nm) in *Phalansterium digitatum*, a peculiar unflagellated organism of uncertain taxonomic affinities [37]. In contrast, the basal bodies of the Euglenophyta are of usual or greater than usual length (about 1000 nm [47, 57]). A conspicuous feature of the basal bodies of *H. amphikineta* is their basket of curved struts (Figs. 27, 42). Similar transitional fibres occur, however, in a large number of probably widely separated groups, for instance, in *Opalina* [66], *Phalansterium* [37], *Karotomorpha* [7], in trichomonads [39], and in most or all of the Euglenophyta [20, 49, 76]. A microtubular basket around the basal bodies is probably restricted to *Stephanopogon* [69]. The flagellar transition region of

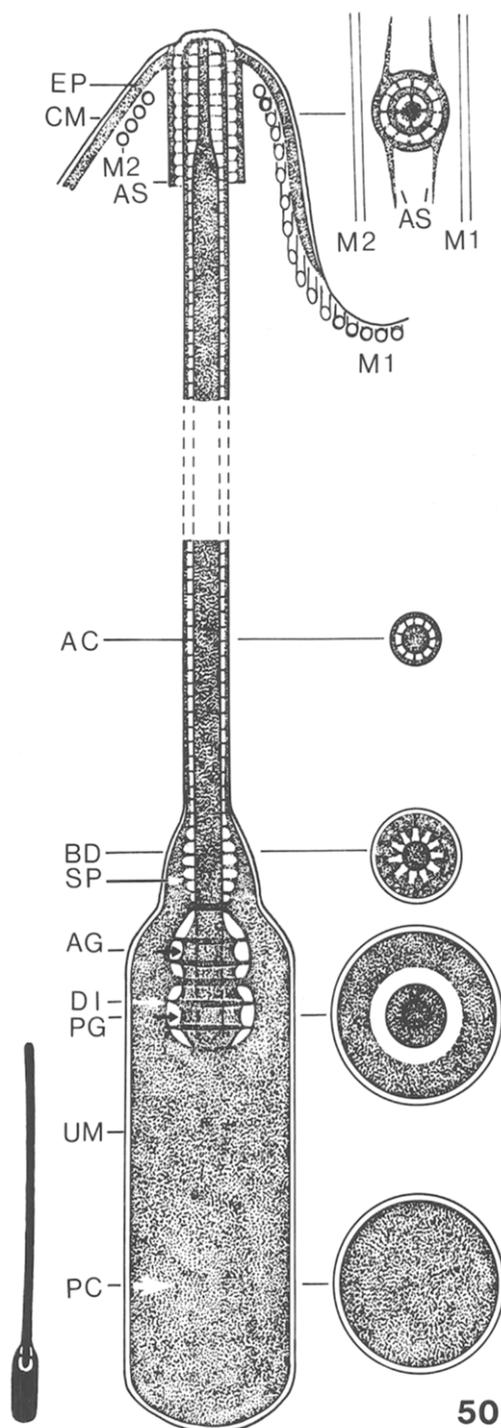


Fig. 50. Schematic drawing of an extrusome of *Hemimastix amphikineta* in longitudinal and transverse sections. In the lower left, a complete organelle is shown at low magnification. For explanation see text. AC = anterior (nail-like) compartment, AG = anterior granule, AS = anchoring structure, BD = bulbous dilation, CM = cell membrane, DI = disk-like projections of the granules, EP = epiplasm, M1, M2 = subpellicular microtubule groups 1 and 2 respectively, PC = posterior (bottle-shaped) compartment, PG = posterior granule of the nail-like compartment, SP = spoke-like structures, UM = unit membrane which encloses the whole extrusome.

H. amphikineta contains a delicate transitional cylinder which is rather similar to that described in *P. digitatum* [37] and in the euglenoid *Entosiphon* though here it probably has a helical structure [57]. The delicate cylinder of *H. amphikineta* is distinctly different from the compact cylinder of *Karotomorpha* [7] and the transitional helix of the Chrysophyceae [36]. The central pair of microtubules in the flagella of *H. amphikineta* have no axosome. This contrasts with *Stephanopogon* which has a distinct axosome [69] but one should note that the euglenoids may or may not have some sort of this structure [41].

The kinetid of Hemimastix and its implication for the origin of the Ciliophora

Hemimastix amphikineta looks distinctly Ciliophora-like because of its 2 longitudinal rows of cilia-like flagella and the posteriorly located contractile vacuole (Figs. 1, 14). This superficial similarity is sustained at the ultrastructural level (Figs. 27–30, 33–43, 74). The internal structures of the flagellar rows of *H. amphikineta* strongly resemble the so-called infraciliature of the Ciliophora, suggesting that the phrase of Corliss [15] "Once a ciliate, always an infraciliature" needs some reconsideration.

The basic component of the ciliophoran cortex is the kinetid or the infraciliary unit which typically consists of a basal body (kinetosome), 1–3 parasomal sacs, a postciliary microtubule ribbon extending posteriorly, a transverse microtubule ribbon, and a striated kinetodesmal fibre [15, 52, 78]. Most of these components of the ciliophoran kinetid can be identified in *H. amphikineta* (Fig. 76). The membranous invagination posterior to each basal body is similar to the parasomal sacs of the Ciliophora, which are, however, located laterally or anteriorly to the kinetosomes. The long and the short microtubule bundle of the kinetid of *Hemimastix* resemble the often long postciliary ribbon and the frequently short transverse ribbon of the ciliophoran kinetid, respectively. The transverse microtubular ribbons of the Ciliophora typically extend perpendicular to the longitudinal body axis, that is, transversely, towards the kinetosomes of the left-most kinety. There is, however, a pronounced variability in the direction of the transverse ribbons [52]. A simple rotation by 90° of the short microtubular ribbon of *H. amphikineta* produces a classical ciliophoran kinetid, with the exception of the missing kinetodesmal fibre (Fig. 76). The absence of any striated fibre in *H. amphikineta* is another unusual feature of this organism because such filamentous roots are widespread among protists, but they are absent also from *Stephanopogon* and the Euglenophyta.

At present it is certainly impossible to decide whether these conspicuous similarities in the kinetid structure of *Hemimastix* and the ciliophorans are analogous or homologous. It is, however, difficult to argue that the microtubular infraciliature of *Hemimastix* is simply a functional requisite, because other organisms with flagella arranged in rows, for instance, *Stephanopogon*, the opalinids and hypermastigids, lack an infraciliature [38, 66, 69]. Their kinetosomes are connected by more or less distinct filamentous and/or striated fibres which occur also in many other flagellates [53]. Bi- and quadriflagellated

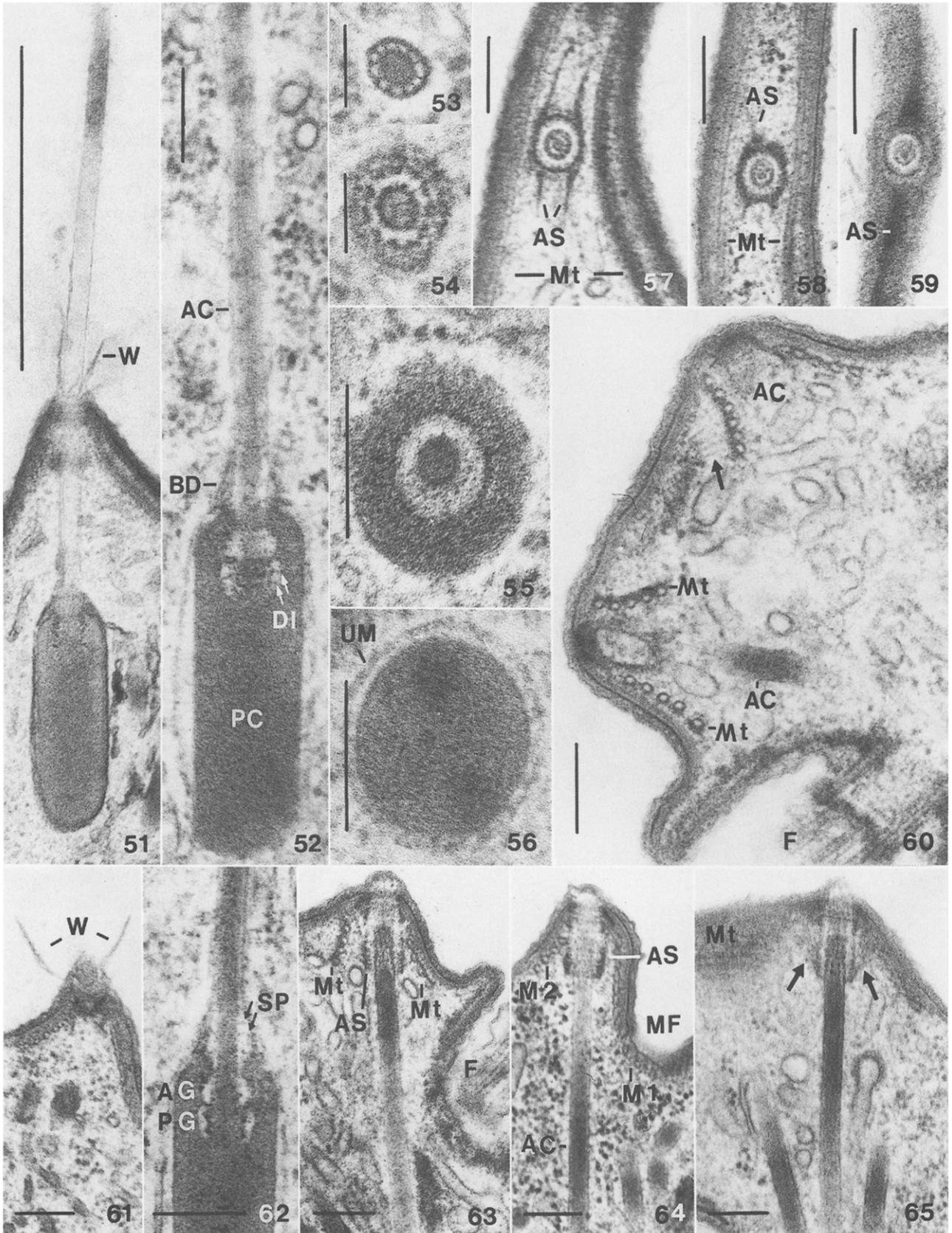


Table 2. Comparison of *Hemimastix* with *Stephanopogon* (Pseudociliata) and the Euglenophyta^a

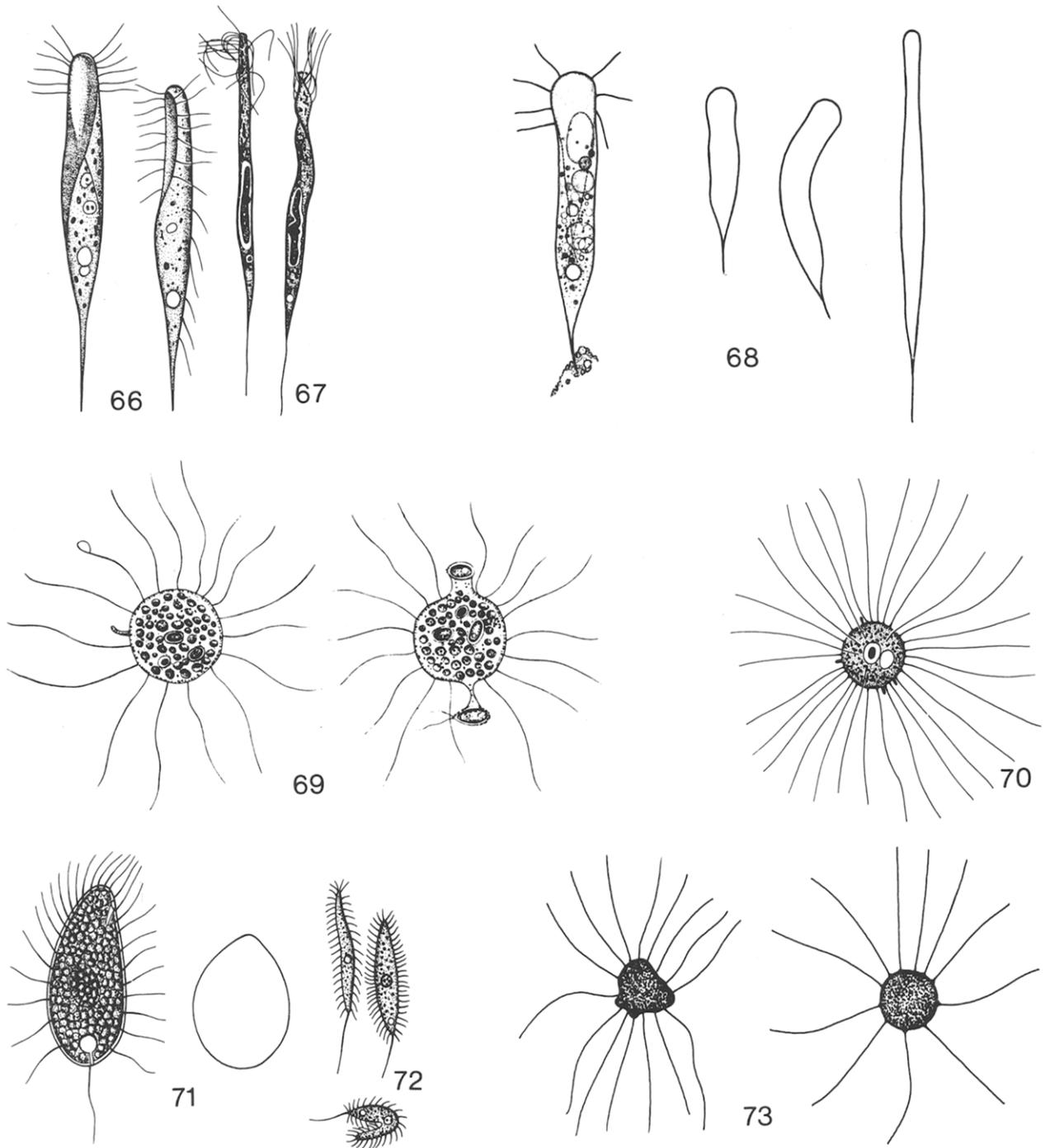
Character	<i>Hemimastix</i>	<i>Stephanopogon</i>	Euglenophyta
Number of flagella	many	many	usually 2, rarely 1 or 7
Arrangement of flagella	rows	rows	localized at anterior end
Length of flagella	all of same length	all of same length	usually 1 long and 1 very short
Mastigonemes	absent	absent	usually present
Paraflagellar rod	absent	absent	usually present
Axosome	absent	present	absent or inconspicuous
Transitional plate	present	present	absent or inconspicuous
Transitional cylinder	cylindroid	absent	helical, single stranded
Length of basal bodies	about 220 nm	about 250 nm	about 1000 nm
Supporting basket of basal bodies	consists of granular material	consists of microtubules and granular material	consists of microtubules and granular material
Membranous sac near basal bodies	present	absent	absent
Parental basal bodies	retained during division	resorbed during division	retained during division
Flagellar connective	microtubules and granular material	granular material	granular material
Striated fibre	absent	absent	absent
Cortex	plicate	smooth	plicate
Cortical alveoli	absent	absent	absent
Cortical microtubules	highly localized	evenly distributed	highly localized
Epiplasm	present	absent	present
Habitat	soil	marine	freshwater, marine, soil
Nutrition	phagotrophic	phagotrophic	phagotrophic and/or autotrophic
Cytostome-cytopharyngeal apparatus	nearly absent; some disordered microtubules	highly organized; many rods composed of microtubules	phagotrophic forms with rods composed of microtubules
Nucleolus	persisting throughout division	persisting throughout division	persisting throughout division
Contractile vacuole	at posterior end	if present, at posterior end	if present, near anterior end
Number of mitochondria	many	many	single to many
Mitochondrial cristae	saccular to tubular	disk-shaped	disk-shaped
Dictyosomes (Golgi bodies)	prominent, ca. 6 cisternae	absent or inconspicuous	prominent, more than 20 cisternae
Extrusomes	complicated; toxicyst-like	muciferous?	muciferous and trichocyst-like

^a Data for *Stephanopogon* from [51, 69]; those for the Euglenophyta mainly from [8, 47, 57].

organisms regularly have microtubular ribbons associated with their basal bodies (for reviews see [41, 53, 55]). In most cases, the location and orientation of these microtubular systems are so different from those of *H. amphikineta* that any homologization would be purely speculative. In addition, the basal bodies of such organ-

isms are rectangularly arranged, which seems to be a very conservative feature in the evolution of the protists and contrasts sharply with the monokinetids of *Hemimastix*. This holds true for the proteromonadid flagellates, too, whose posteriorly directed microtubular ribbons bear some resemblance to those of *H. amphikineta* [7].

◀ Figs. 51–65. Ultrastructure of the extrusomes of *Hemimastix amphikineta* (comp. with schematic Fig. 50). – Figs. 51, 61. Partly extruded extrusomes with wing-like structures (W) at the point of release. Bars = 1000 nm and 200 nm, respectively. – Figs. 52, 62. Fine structure of the anterior and the posterior compartment in longitudinal sections. Bars = 200 nm. – Figs. 53–56. Transverse sections in the middle of the anterior compartment, at the level of the bulbous dilation, the anterior granule, and in the middle of the posterior compartment. Bars 100 nm and 200 nm in Figs. 53, 54 and 55, 56, respectively. – Figs. 57–59. Transverse sections from the subapical row of extrusomes at the level of the anchoring structure (AS). The series proceeds from proximal to distal. The extrusomes lie between microtubular ribbons (Mt; comp. Figs. 63, 64). Note the ridge of the anchoring structure in Fig. 59. Bars = 200 nm. – Fig. 60. Longitudinal section of the capitulum. The arrow points to delicate granular fibres which project from the inner microtubular ribbons to the centre of the anterior pole. Bar = 200 nm. – Figs. 63, 64. Longitudinal sections of the apical and subapical row of extrusomes transverse to the microtubule furrow. The conspicuous anchoring structure is surrounded by 2 microtubular ribbons (comp. Figs. 57, 58). Bars = 200 nm. – Fig. 65. Vertical section of the apical row of extrusomes parallel to the microtubular ribbons (comp. Figs. 58, 63). The anterior compartment of the extrusome and the anchoring structure (arrows) form an ice-axe like structure. Bar = 200 nm. AC = anterior compartment, AG = anterior granule, AS = anchoring structure, BD = bulbous dilation, DI = disk-like projections of the granules, F = flagellum, M1, M2 = subpellicular microtubule ribbons 1 and 2, MF = microtubule furrow, Mt = microtubular ribbons, PC = posterior compartment, PG = posterior granule, SP = spoke-like structures, UM = unit membrane which encloses the whole organelle, W = wing-like structure.



Figs. 66–73. Multiflagellated free-living protists. – Fig. 66. *Spironema multiciliatum*, 14–18 × 2–3 μm (redrawn from [42]). – Fig. 67. *Spironema multiciliatum*, 20–50 × 4 μm (redrawn from [30]). – Fig. 68. *Spironema multiciliatum*, 22–40 × 3–4 μm (Zöllffel, unpubl.). – Fig. 69. *Multicilia lacustris*, 30–40 μm (after [45] from [21]). – Fig. 70. *Polymastix sol*, 20–30 μm (redrawn from [32]). – Fig. 71. *Schewiakoffia paradoxa*, relaxed and contracted, 24 × 10 μm (redrawn from [74]). – Fig. 72. *Trichonema gracile*, 55 × 6 μm (redrawn from [59]). – Fig. 73. *Multicilia marina*, 20–30 μm (redrawn from [13]).

The most distinct characteristics of the Ciliophora are nuclear dualism, existence of an infraciliature, the transverse mode of binary fission, and tubular mitochondrial cristae. The ancestors of the Ciliophora are largely unknown, but flagellated, especially dinoflagellate-like, progenitors are frequently supposed [11, 17, 34, 50, 60,

64, 78]. Organisms like *Colponema loxodes* [58] and many dinoflagellates, which have such ciliophoran characters as cortical alveoli and complex extrusomes, give this idea some support (see detailed discussion in [18]). *Hemimastix amphikineta* possesses only 2 (infraciliature, saccular to tubular mitochondrial cristae) of the 4 main

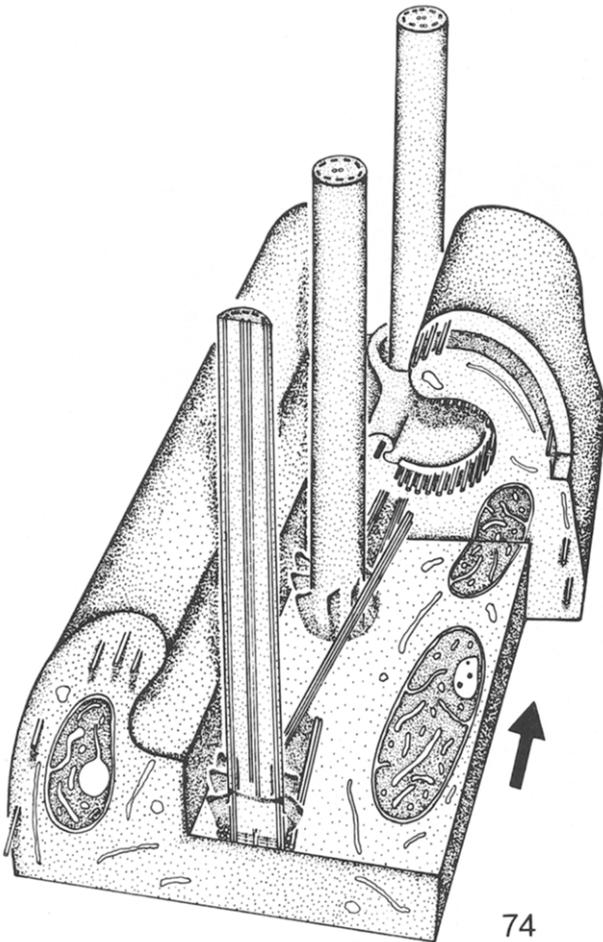


Fig. 74. "Cut-away" ultrastructural view of pellicular and subpellicular organelles in the middle region of *Hemimastix amphikineta*. The arrow points to the posterior end of the organism.

characters of the Ciliophora. After the downfall of *Stephanopogon* as the "eociliate" (it is now considered by some as a separate phylum within the Euglenozoa [16, 17, 51], it would be unwise to raise *Hemimastix* to this throne. It is, however, a strong contender with the dinoflagellates and other candidates discussed by Orias [64] and Corliss [18]. Minor characteristics which support an "eociliate" position of *H. amphikineta* are the presence of complicated extrusomes with a distinct anchoring structure (comp. the similar anchoring apparatus in *Paramoecium* [1, 3]) and the absence of mastigonemes and paraflagellar structures which are common in a lot of flagellates.

Taxonomic position and taxonomic summary

As shown in the preceding sections, *Hemimastix* and very likely *Spiroonema* occupy a unique position within the protists. Certainly, they can not be related confidently to any of the 45 protist phyla proposed by Corliss [16]. Like *Stephanopogon*, *Hemimastix* is best considered as a separate phylum. Such a high ranking might look unjustified,

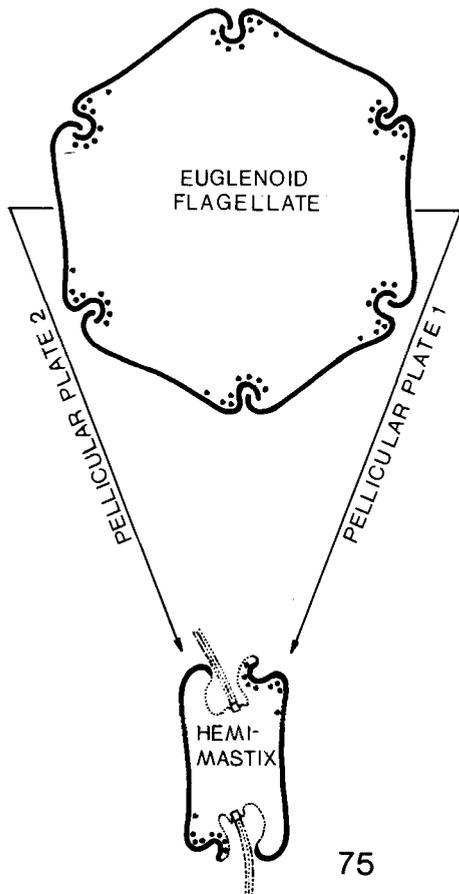
but related forms may exist (see *Identification*). We are just beginning to recognize the diversity of the protists. From our taxonomic research on freshwater and soil ciliates we are convinced that only a small proportion, probably less than 10%, of the existing ciliophoran species are known [26]. The high ranking of *Hemimastix* and *Spiroonema* is not unusual. Twelve out of the 45 phyla listed by Corliss [16] include fewer than 40 species, three are monotypic (single species). Others, however, prefer to leave taxa with uncertain affinities minimally ranked (e.g., genus, family) in an incertae sedis position until their relationships with other taxa are revealed [68, 85]. Most of the colleagues (Bardele, Brugerolle, Casper, Corliss, Kristiansen, Raikov, Vickerman, Zölffel; see Acknowledgements) who reviewed or discussed the paper with us supported the establishment of a new phylum for *Hemimastix*. The others (Mignot, Patterson, Schönborn) would have preferred a lower ranking in an incertae sedis position.

Fundamental differences between the Hemimastigophora and the Euglenophyta include the shape of the mitochondrial cristae, the presence of true kinetids, the basal bodies of which have membranous sacs and microtubular connections, and the absence of a permanent cytostome-cytopharynx complex (note that even the green euglenoid flagellates have a permanent, though inconspicuous cytostome [79, 86]), of mastigonemes, and of a paraflagellar rod. *Stephanopogon* differs from *Hemimastix* in the shape of the mitochondrial cristae, the structure of the cortex, the presence of a permanent cytostome-cytopharynx complex, and by the absence of membranous sacs posterior to the basal bodies and of microtubular connections between the kinetids. In *Stephanopogon* the basal bodies within a row are interconnected by a belt of dense material [51, 69] which resembles the granular substance between the microtubular ribbons of *H. amphikineta*.

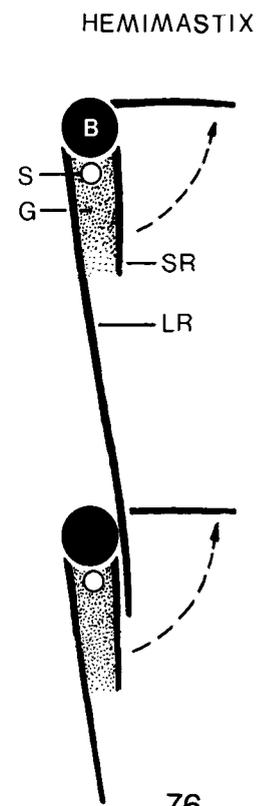
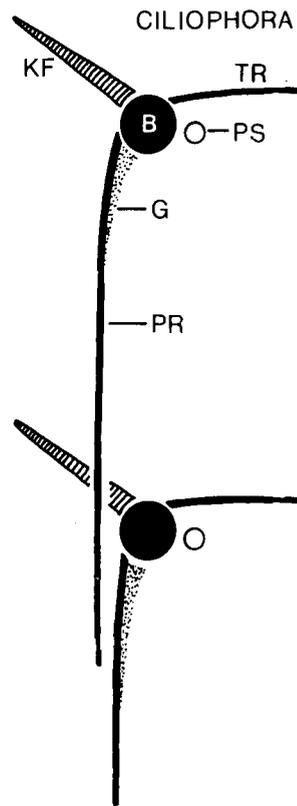
Personally, we think that the Hemimastigophora are a new sister group of the Euglenophyta (cortex!). The evidence for this is, however, too weak (mitochondrial cristae!). We have, indeed, the Hemimastigophora included into the Euglenozoa in the first version of the paper. But this view was strongly opposed by D. J. Patterson (Univ. Bristol) and doubted by G. Brugerolle (Univ. Clermont-Ferrand). Under these circumstances it seems wise to place the Hemimastigophora in an incertae sedis position outside the Euglenozoa and within the kingdom Protista Haeckel, 1866 [33].

Phylum Hemimastigophora nov. phyl.

Cilia-like flagella arranged in slightly spiraling rows. Basal bodies connected by a rather complex system of microtubules and with posteriorly located membranous sac; inconspicuous transitional cylinder. Pellicle plicate (euglenoid?), with distinct epiplasm and underlined with highly localized microtubules. Microphagous, cytostome at anterior pole, not permanent, without pharyngeal rods. Mitochondrial cristae saccular. Complex extrusomes (character probably only of value at ordinal level). Fission in free-swimming condition, symmetrogenic; nucleus with



75



76

Fig. 75. A *Hemimastix*-like organism can be modeled by taking 2 opposite pellicular plates and their associated microtubules (black dots) of an euglenoid flagellate and inserting 2 flagellar rows between them. – Fig. 76. Comparison of structures associated with somatic monokinetids in the Ciliophora and in *Hemimastix amphikineta* (Observer being inside the cell!). A simple rotation by 90° of the short microtubular ribbon (dotted arrow) of *H. amphikineta* produces a nearly (note absence of the kinetodesmal fibre!) ciliophoran kinetid. B = basal body, G = granular substance, KF = kinetodesmal fibre, LR = long microtubular ribbon, S = membranous sac, PR = postciliary microtubule ribbon, PS = parasomal sac, SR = short microtubular ribbon, TR = transverse microtubule ribbon.

large nucleolus which persists throughout division (very likely endomitosis). No paraxial or paraflagellar structures, no flagellar hairs (mastigonemes), no plastids. Two genera (*Hemimastix* nov. gen. and *Spironema* Klebs, 1892) with single species each; placed in an incertae sedis position within the kingdom Protista Haeckel, 1866.

Class Hemimastigea nov. cl. and order Hemimastigida nov. ord. have the same characteristics.

Family Spironemidae Doflein, 1916

Improved diagnosis. Hemimastigida with 2 rows of flagella in slightly spiraling furrows. Extrusomes composed of a short bottle-shaped posterior compartment and a long nail-like anterior compartment which is implanted in the posterior one. Contractile vacuole at posterior end. Two genera known (*Spironema*, *Hemimastix*).

Type genus. *Spironema* Klebs, 1892.

Remark. *Hemimastix* is separated from *Spironema* by its stiff, ametabolic body.

Genus *Hemimastix* nov. gen.

Diagnosis. Very small (less than 20 µm) ametabolic Spironemidae with a single nucleus.

Type species. *Hemimastix amphikineta* nov. spec.

Derivatio nominis. *Hemimastix* is feminine and of Greek derivation. It means, in a broad sense, "an incomplete or atypical flagellated organism".

Hemimastix amphikineta nov. spec.

Diagnosis. In vivo 14–20 × 7–10 µm. Body ellipsoid, about 2 : 1 flattened, the gently tapered posterior end containing the contractile vacuole. Flagellar rows unshortened, each row with about 12 flagella 5–9 µm long. Nucleus spherical, located subequatorially, contains a globular nucleolus.

Type location. Upper soil layer (0–3 cm) of a bushland in Brisbane-Waters-National Park, near Sydney, East Australia.

Type specimens. One slide of holotype specimens and 3 slides of paratype specimens (all protargol-impregnated) have been deposited in the collection of microscope slides of the Upper Austrian Museum in Linz.

Derivatio nominis. "*amphikineta*" is of Greek derivation and means "moved on both sides" which refers to the 2 laterally inserted rows of flagella.

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