

## Fine Structure and Systematic Position of *Enchelyomorpha vermicularis* (Smith, 1899) Kahl, 1930, an Anaerobic Ciliate (Protozoa, Ciliophora) from Domestic Sewage

Wilhelm FOISSNER and Ilse FOISSNER

Universität Salzburg, Institute für Zoologie und Pflanzenphysiologie, Salzburg, Austria

**Summary.** *Enchelyomorpha vermicularis* is a pouch-like ciliate having not only a holotrichous ciliature but also many short, tentacle-like processes on the cell surface. Its systematic position thus varied, depending on the characters used, between the suctorians and the haptorid gymnostomes, and a recent investigation using refined light microscopic methods could also not clarify the situation. We thus examined some stages of the life cycle of *E. vermicularis* and studied its fine structure with the transmission electron microscope. The results definitely show that *E. vermicularis* is the swarmer of a small, globular suctorian with tentacles irregularly distributed on the anterior body half. The tentacles, which are not associated with barren basal bodies, contain the barrel-shaped haptocysts and the two concentric microtubule cores typical of "good" suctorians. Furthermore, the cortex possesses small pits and the fine structure of the somatic kinetids is also very similar to that known from other suctorian swarmers, although the kinetodesmal fibre is anchored to the epiplasm and subkinetal microtubules are probably absent. The kinetids are rotated 90° counter-clockwise with respect to the longitudinal body axis causing the ciliary rows to extend transversely. Usually, two swarmers are produced simultaneously by endogenous budding. *Enchelyomorpha vermicularis* lacks a scopuloid and mitochondria but possesses cytoplasmic inclusions resembling hydrogenosomes, indicating that it is a true anaerobic ciliate, which is in accordance with most faunistic data. The mode of swarmer production, the organization of the swarmer, and the fine structure of the tentacles show that *Enchelyomorpha* is an entotrochid suctorian, belonging to the suborders Acinetina or Discophryina. Acinetid affinities are indicated by the fine structure of the tentacles and the general organization of the swarmer, whereas swarmer production resembles *Cyathodinium* in the suborder Discophryina. Obviously, *Enchelyomorpha* has a highly distinct combination of characters which suggests at least separation at family level, i.e. maintenance of the family Enchelyomorphae Augustin and Foissner, 1992.

**Key words.** Anaerobic protists, Ciliophora, *Enchelyomorpha vermicularis*, sewage, Suctorida, ultrastructure.

### INTRODUCTION

*Enchelyomorpha vermicularis* is a peculiar ciliate which has not only cilia but also many tentacle-like processes described by Smith (1899) as "fairly stout

hispid setae". Smith (1899) assigned the organism to the genus *Enchelys* without, however, discussing his classification. In 1926 Kahl described the same organism as a "swarmer of an unknown suctorian". Later, Kahl (1930, 1931) recognized identity with *Enchelys vermicularis* Smith and put the species in a new genus, *Enchelyomorpha*, which he provisionally assigned to the family Actinobolinidae, together with *Actinobolina* and *Dactylochlams*, which also possess tentacle-like struc-

Address for correspondence: W. Foissner, Universität Salzburg, Institut für Zoologie, Hellbrunnerstrasse 34, A - 5020 Salzburg, Austria; Tel. (0662) 8044-5615; FAX (0662) 8044-5698

tures on the cell surface. A few years later, Liebmann (1936) observed *E. vermicularis* feeding on bacteria by its anterior end, seemingly confirming the "simple apical oral aperture" mentioned by Smith (1899). More recently, Corliss (1979) definitely assigned *Enchelyomorpha* to the Actinobolinidae, a family of tentacle-bearing haptorids originally established by Kent (1881) and later nomenclaturally corrected by Kahl (1930). A light microscopic reinvestigation of *E. vermicularis* by Augustin and Foissner (1992) revealed sufficient details to establish a new family, Enchelyomorphidae, but could also not clarify its systematic position. We thus studied the fine structure of *E. vermicularis* with the transmission electron microscope and re-examined the protargol slides of Augustin and Foissner (1992) for developmental stages. The results definitely show that *E. vermicularis* is the swarmer of a globular suctorian with tentacles irregularly distributed on the anterior body half.

## MATERIALS AND METHODS

The material used is the same as described in Augustin and Foissner (1992), viz. sewage from a trickling filter near Salzburg, where *E. vermicularis* was fairly abundant. Some sewage containing *E. vermicularis* was fixed *in toto* for the light and electron microscopical preparations. Most light microscopical data mentioned in this study are from Augustin and Foissner (1992), who used various light microscopical and silver impregnation techniques to reveal the infraciliature and other details. The protargol slides prepared at that time were re-examined for adult and budding stages. These were indeed found, but were difficult to recognize among the sludge masses which might explain why Augustin did not find them.

Transmission electron microscopy was performed as described in Foissner et al. (1988).

## RESULTS

We shall show in our study that *Enchelyomorpha vermicularis* is the swarmer of a globular suctorian. This causes some problems with the orientation of the cell, which must thus be defined before the results are described and discussed. Generally, the orientation of suctorian swarmers is a complicated and controversial matter (Batisse 1994, Kormos and Kormos 1957), which has been almost completely ignored in the electron microscopic literature. It is, however, essential to understand the orientation of the kinetids and ciliary

rows as well as the ontogeny and phylogeny of ciliates in general (Bardele 1989, Kormos and Kormos 1957).

*Enchelyomorpha vermicularis* has a distinct longitudinal body axis and swims with the narrowed end ahead (Figs. 1, 6). Thus, this end has been considered as anterior and the opposite one, which contains the contractile vacuole, as posterior (Augustin and Foissner 1992). However, the electron microscopic investigations indicate just the opposite, because the postciliary microtubules extend to the narrowed end. Thus, this region is very likely the morphologic posterior, as postciliary microtubules usually extend rearward (Lynn 1981). Furthermore, the kinetid pattern suggests that the ciliary rows extend transversely, i.e. at right angles to the longitudinal body axis.

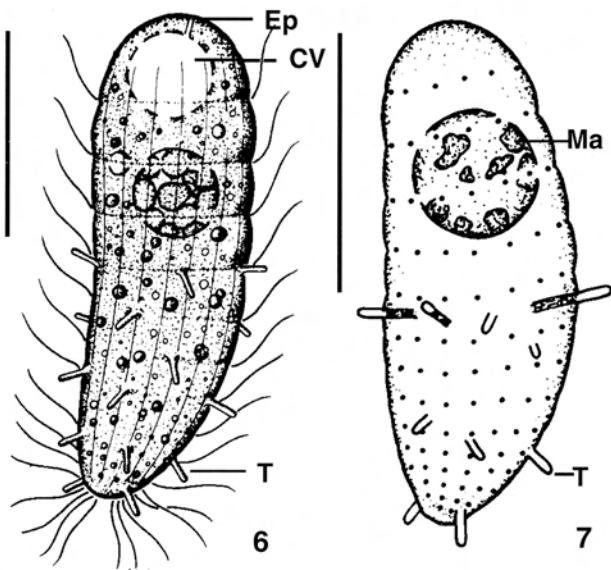
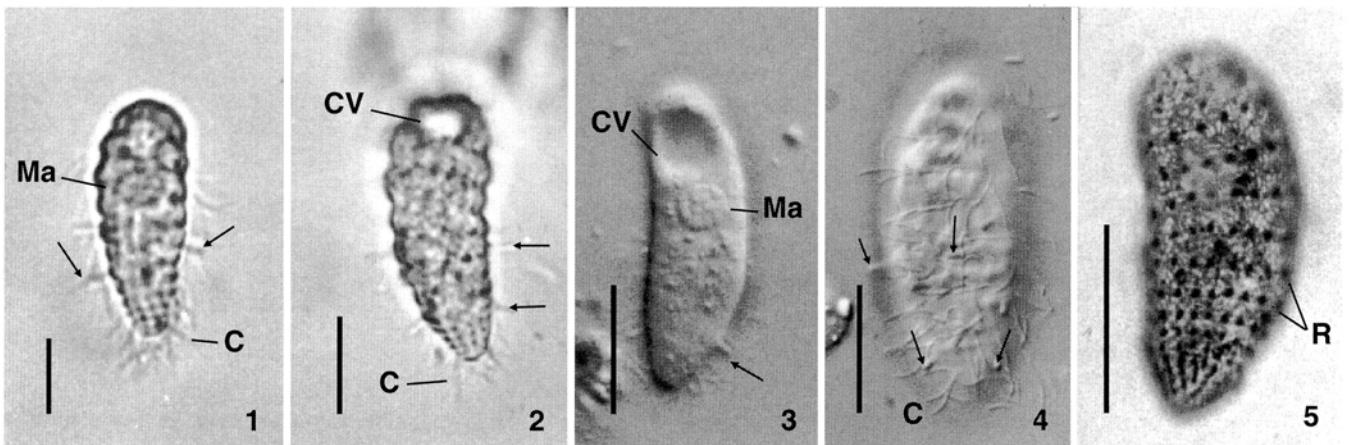
We thus use the following orientation in the description and figures: the broadened end of the cell is anterior, the narrowed is posterior; the right side of the kinetid is defined, as usual, by the kinetodesmal fibre, the posterior by the distal portion of the postciliary microtubules.

### Light microscopic description of *E. vermicularis*

A detailed light microscopic characterization of *E. vermicularis* and a review of the older literature have been published by Augustin and Foissner (1992). Thus, only a brief description and some figures will be given here for orientation purposes.

Size *in vivo* 25-45 x 10-17  $\mu\text{m}$ , pouch-like and slightly asymmetric, gradually narrowed from broadly rounded anterior end to short-truncate posterior end (Figs. 1-3, 6). Macronucleus about 7  $\mu\text{m}$  in diameter, in anterior half of cell (Fig. 3). Contractile vacuole in anterior end, with single excretory pore (Figs. 2, 3). Cortex distinctly furrowed transversely by somatic kineties (Figs. 1, 2). On posterior half about 15-20 acontractile processes (tentacles) 2-3  $\mu\text{m}$  long with distal end inconspicuously knobbed (Figs. 1-4, 6, 7). Moves slowly and clumsily with narrowed posterior end ahead. About 13 transverse ciliary girdles, forming about 17 longitudinal ciliary rows, commence at posterior end of body and extend anteriorly leaving pole region free of cilia (Figs. 5, 7). Silverline system finely and irregularly meshed (Fig. 5).

Adult cells, not described by Augustin and Foissner (1992), were recognized in protargol stains only. They are globular with a diameter of 15-20  $\mu\text{m}$  and have short tentacles in the body half opposed to the contractile vacuole (Fig. 29). No stalk is recognizable in the stained cells and may in fact be absent as no scopuloid



Figs. 1 - 7. *Enchelyomorpha vermicularis* from life (1-4, 6) and after dry silver nitrate (5) and protargol (7) impregnation. Figures 3-7 are from Augustin and Foissner (1992). 1, 2, 3, 4 - bright field (1, 2) and interference contrast (3, 4) light micrographs of typical specimens with many small tentacles (arrows) and distinctly furrowed body; 5, 7 - the ciliary rows form a longitudinal and circular pattern, and a very fine-meshed silverline system extends throughout the cortex; 6 - slightly schematic drawing showing general organization of cell. C - cilia, CV - contractile vacuole, Ep - excretory pore of contractile vacuole, Ma - macronucleus, R - ciliary rows, T - tentacles. Bars 15  $\mu$ m

could be identified in the swarmer, i.e. in *E. vermicularis*.

**Electron microscopic description of *E. vermicularis***

Morphometric data shown in Table 1 are not repeated in this section.

**Cortex:** The cortex of *E. vermicularis* is about 54 nm thick and of usual structure, i.e. has a cell membrane followed by alveoli, which are very flat and small, and a thin layer of finely granular epiplasm (Fig. 23). The

alveolar content is strongly osmiophilic and slightly thickened at the triangular alveolar junctions, which contain finely granular material, possibly epiplasm or a substance to which silver nitrate adheres, producing the silverline system (Fig. 5). As in other suctorians, there are small (about 160 x 130 nm) pits in the cortex covered only by the cell membrane and some epiplasm (Fig. 10). These pores strongly resemble the parasomal sacs around the cilia and are distinguishable from these only in the anterior pole region of the cell where no cilia occur.

**Tentacles:** The short cortical processes in the posterior half of the body each contain a slightly elliptical axoneme composed of two concentric microtubule cores (Figs. 1, 3, 6, 13; Table 1). The axonemes, which are not associated with barren basal bodies but often located near somatic cilia, originate deep in the cytoplasm and extend almost to the distal end of the tentacles where haptocysts are anchored (Figs. 16, 18). The microtubules of the outer core are linked to each other and to those of the inner core by strands of fibrogranular material. The microtubules of the inner core are very close together and carry distinct projections (arms) on the concave side of the axoneme, facing the lumen of the tentacle which is filled with cytoplasm containing dumb-bell shaped membranous structures (Figs. 12-16). The distal end of the tentacles, the knob, is covered by the cell membrane and the cortical alveoli which becomes evident in transverse sections (Fig. 11).

Three regions can be distinguished along the axoneme. Its distal portion is slightly conically dilated and shows five widely separate microtubule lamellae each in the outer and inner core (Fig. 12). In the middle region of the axoneme the number of microtubules increases slightly and those of the outer core become fairly evenly spaced. Those of the inner core remain

grouped to five lamellae, each composed of seven microtubules and parallel to the outer core (Figs. 13, 14; Table 1). However, their lamellar arrangement becomes inconspicuous in the proximal region of the axoneme, where the microtubules are almost as evenly spaced as those of the outer core (Fig. 15). No specializations were found around and at the base of the axoneme.

**Haptocysts:** These organelles are found mainly in the distalmost portion of the tentacles (Figs. 11-13, 18) but also within the axoneme (Fig. 19) and in the cytoplasm (Fig. 17). The cylindroid shaft is rounded anteriorly and composed of a finely granular coat, which is apparently continuous with the granular substance composing the barrel-shaped posterior bulb, and a heavily osmiophilic tube separated from the bulb by a strongly osmiophilic granulum (Figs. 17-19, Table 1). Very likely, radial structures project from the shaft and the bulb into the cytoplasm (arrows in Figs. 11, 17, 19), as described by Mogensen and Butler (1984) in *Trichophrya rotunda*.

**Kinetids:** In contrast to the tentacle axonemes, which were well preserved by the method used, the basal bodies and their fibrillar associates were not. However, the quality of the material was sufficient to analyze the kinetids in some detail. They are rotated 90° counter-clockwise to the kinety axis (see discussion), i.e. the ciliary rows extend transversely to the longitudinal body

axis, which must be taken into account in the following description.

The cilia and basal bodies are of usual structure and size (Figs. 20, 21; Table 1). Each kinetid has associated 2-3 postciliary microtubules at triplet 9, a kinetodesmal fibre at triplets 6 and 5, a transverse fibre at triplet 3, and 2 parasomal sacs (Figs. 20-22, 24).

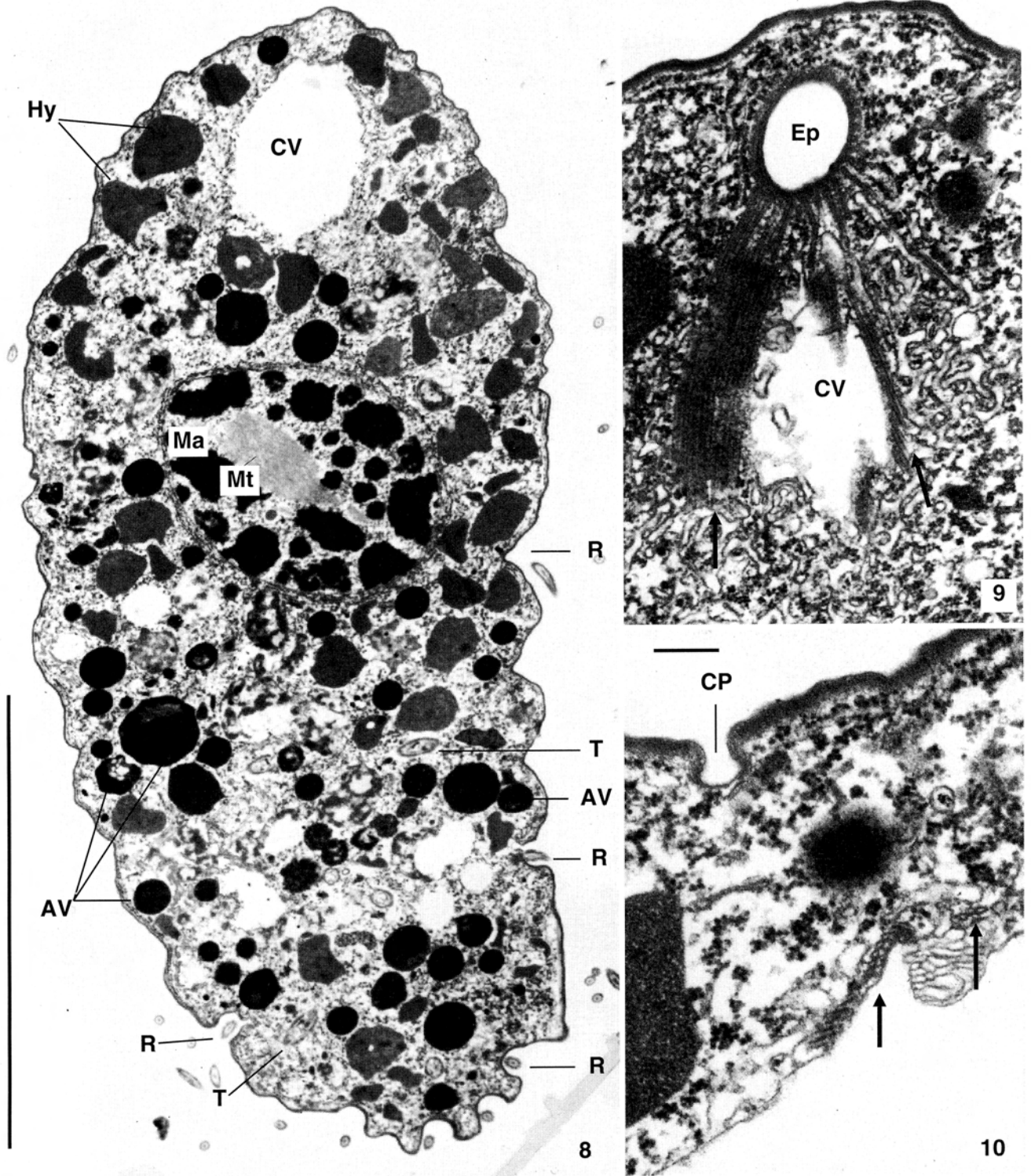
The postciliary microtubules are slightly convergent and extend horizontally into the intrakinetal ridge, where they curve sharply backwards to the narrowed posterior end of the cell. They are rather long and several bundles thus overlap to form a loose array of 8-19 microtubules bridging 3-6 ciliary rows (Figs. 21, 22, 24; Table 1). Those of the last kinetids extend on the posterior pole area, where they form a dense, rather irregular sheet. The flattened kinetodesmal fibre is of usual structure and extends steeply upward to the centre of the interkinetal ridge, where its distal end becomes frayed and anchored to the epiplasm (Fig. 20). The kinetodesmal fibre is about 1300 nm long and extends anteriorly, seemingly connecting the kinetids of neighbouring rows (Fig. 22), as does the inconspicuous transverse fibre, however, in posterior direction. The parasomal sacs are at the posterior end of the kinetid, rather distant from the basal body and slightly oblique to the transverse body axis. The anterior sac is near the kinetodesmal fibre, while the posterior sac is between the postciliary

Table 1

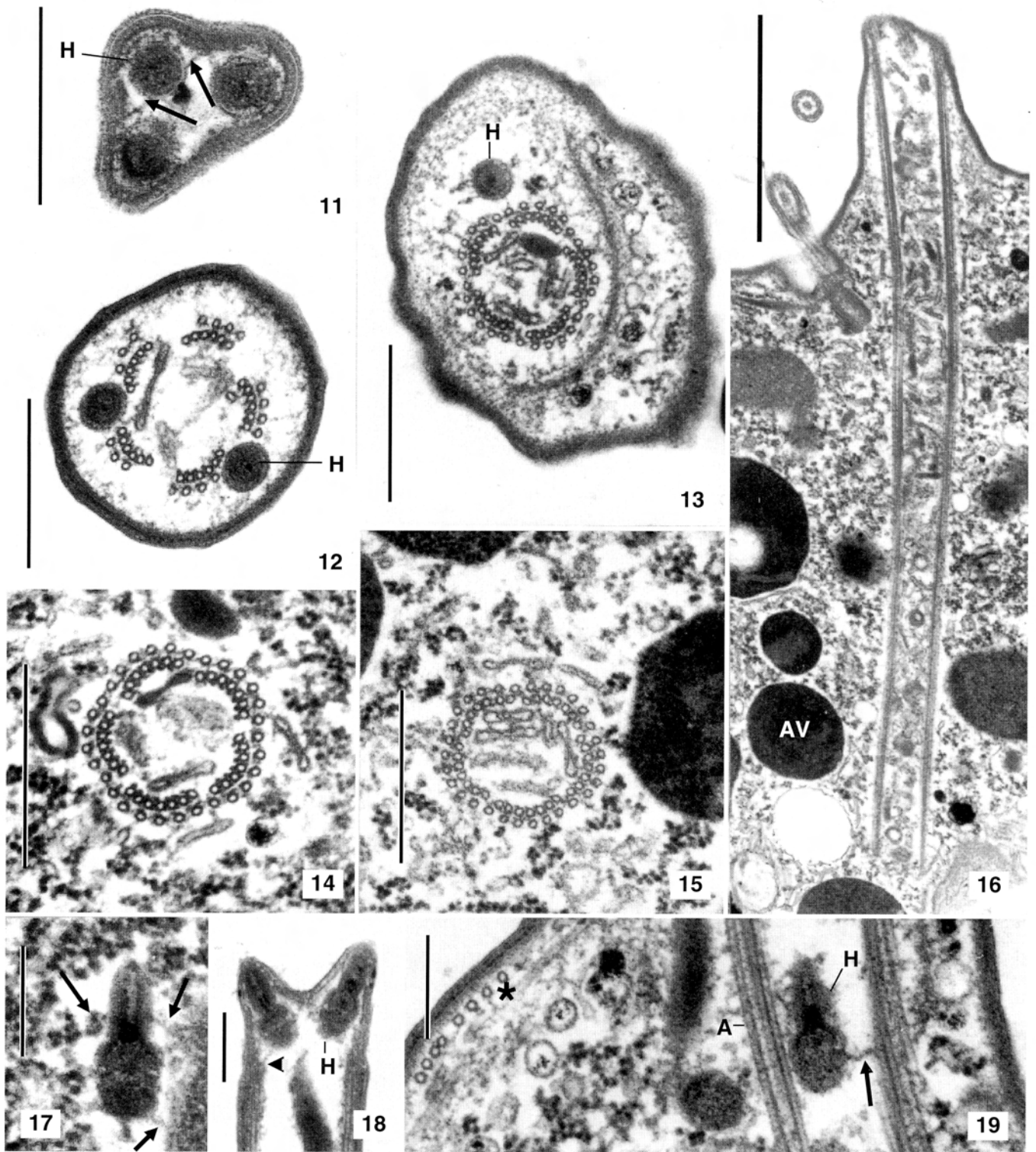
Electron microscopic morphometric characteristics of *Enchelyomorpha vermicularis*\*

Character	$\bar{x}$	M	SD	SD $\bar{x}$	CV	Min	Max	n
Axoneme, diameter long axis	452.6	469.0	40.6	9.8	9.0	375	500	17
Axoneme, diameter short axis	413.7	417.0	40.2	9.8	9.7	344	469	17
Axoneme, number of microtubules in outer core	30.0	30.0	0.0	0.0	0.0	30	30	16
Axoneme, number of microtubules in inner core	35.0	35.0	-	-	-	34	35	18
Axoneme, number of microtubule ribbons in inner core	5.0	5.0	0.0	0.0	0.0	5	5	16
Axoneme, number of microtubules in ribbon of inner core	7.0	7.0	-	-	-	6	7	18
Cortex, thickness (with epiplasm)	52.0	54.0	9.0	2.7	17.4	31	63	11
Epiplasm, thickness	14.5	13.0	2.3	0.7	16.1	12	20	11
Basal body, length	334.8	344.0	17.8	5.4	5.3	313	366	11
Basal body, diameter	198.3	195.0	12.1	3.6	6.1	188	218	11
Parasomal sac, deepness	178.2	187.0	39.6	13.2	22.2	122	250	9
Parasomal sac, diameter	144.2	150.0	20.0	6.7	13.9	122	172	9
Postciliary microtubules, number per bundle in cortical ridges	12.9	12.0	3.7	1.4	28.8	8	19	7
Haptocyst, length	327.4	322.0	13.7	4.8	4.2	312	346	8
Haptocyst, width	135.4	135.5	13.8	4.9	10.2	122	154	8
Hydrogenosome, long axis	1249.0	1109.5	523.9	102.7	41.9	667	2688	26
Hydrogenosome, short axis	937.8	938.0	265.5	52.1	28.3	437	1344	26
Autophagous vacuole, long axis	743.7	703.0	214.1	36.7	28.8	437	1437	34
Autophagous vacuole, short axis	690.6	640.5	203.9	35.0	29.5	406	1437	34

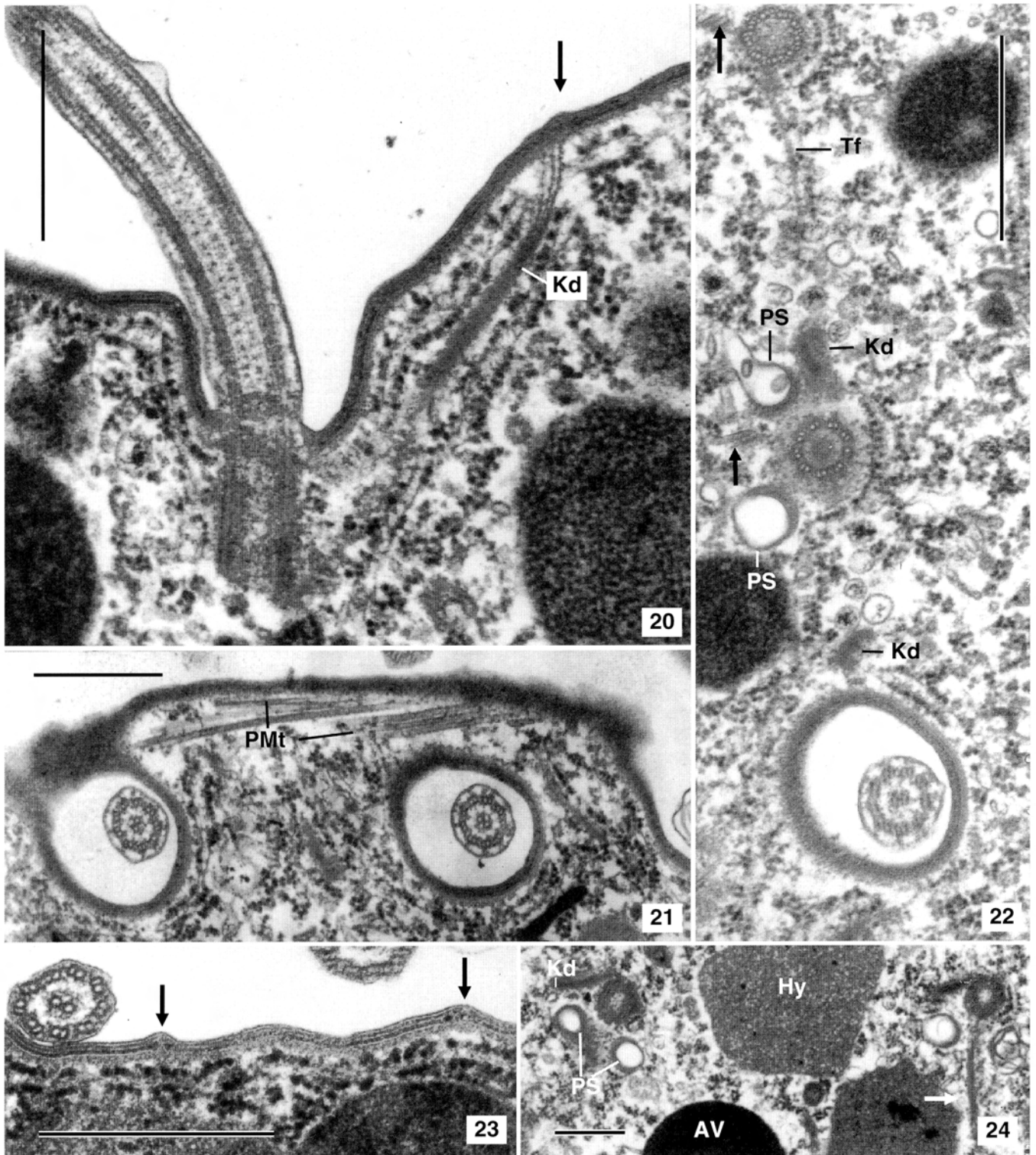
\* All data based on ultrathin sections of 4 cells fixed with glutaraldehyde and osmium tetroxide. Measurements in nm. Abbreviations: CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, SD - standard deviation, SD $\bar{x}$  - standard deviation of the mean,  $\bar{x}$  - arithmetic mean



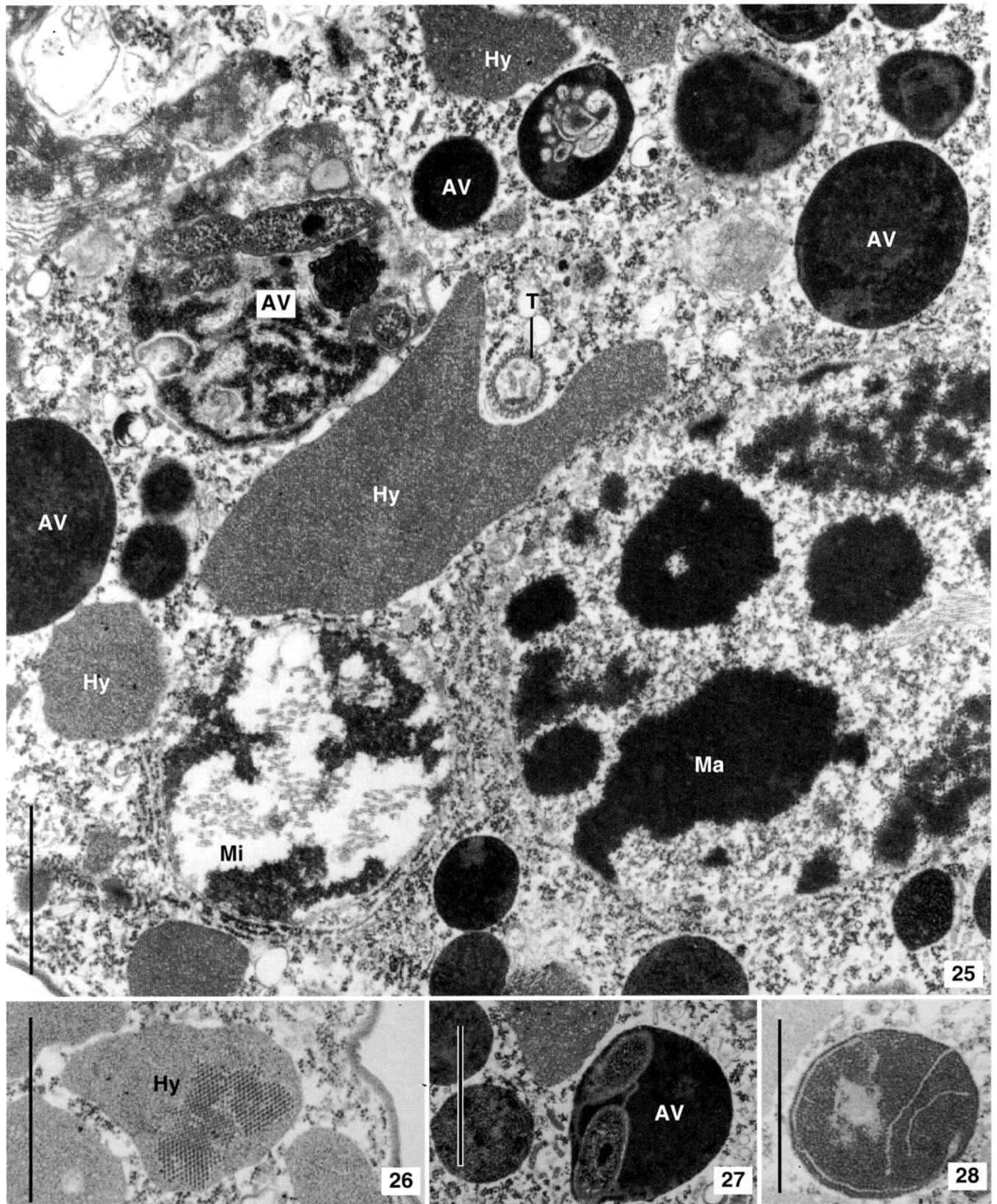
Figs. 8 - 10. Electron micrographs of the general organization and the contractile vacuole of *E. vermicularis*. 8 - longitudinal section showing the macronucleus (Ma) with a distinct microtubule bundle (Mt) inside, the contractile vacuole (CV) in the anterior end, transversely extending ciliary rows (R), tentacle axonemes (T), hydrogenosomes (Hy) and autophagous vacuoles (AV); 9, 10 - longitudinal sections through the anterior end showing a cortical pit (CP) and many microtubule bundles (arrows) originating at the excretory pore (Ep) and surrounding the contractile vacuole (CV) which is embedded in spongy cytoplasm. Bars - 10  $\mu$ m (Fig. 8), 200 nm (Fig. 9), 1  $\mu$ m (Fig. 10)



Figs. 11 - 19. Electron micrographs of the tentacles and haptocysts of *E. vermicularis*. 11, 12, 13, 14, 15 - transverse sections of tentacles arranged from the distal to the proximal end. The tentacles contain haptocysts (H) with radial projections (arrows) and a conspicuous axoneme composed of two concentric microtubule cores (Bars - 500 nm); 16 - longitudinal section of a tentacle filled with granular and membranous material. Some dark-stained autophagous vacuoles (AV) are recognizable in the cytoplasm (Bar - 1500 nm); 17, 18, 19 - haptocysts (H) occur in the cytoplasm and axoneme (A) and have a barrel-shaped posterior bulb. The arrows mark radial projections of the haptocysts, the arrowhead points to the distal end of the axoneme, and the asterisk marks transversely sectioned postciliary microtubules of the somatic kinetids. Bars 250 nm



Figs. 20 - 24. Electron micrographs of the somatic kinetids and the cortex of *E. vermicularis*. 20 - longitudinal section of a kinetid showing the frayed distal end of the kinetodesmal fibre (Kd) anchored to the epiplasm (arrow); 21 - oblique section showing postciliary microtubule ribbons (Pmt) extending into an interkinetal ridge; 22, 24 - transverse sections of somatic kinetids showing kinetodesmal fibers (Kd), a transverse fibre (Tf), postciliary microtubule ribbons (arrows) extending between the parasomal sacs (PS), hydrogenosomes (Hy) and autophagous vacuoles (AV); 23 - transverse section showing alveolar junctions in the cortex (arrows). Bars - 500 nm



Figs. 25 - 28. Electron micrographs of *E. vermicularis* showing cytoplasmic inclusions, viz. the macronucleus (Ma), micronucleus (Mi), a tentacle axoneme (T), autophagous vacuoles (AV) and hydrogenosomes (Hy) which sometimes have crystalline inclusions (Fig. 26). Bars 1000 nm



ribbon and the transverse fibre. Thus, the proximal portion of the postciliary microtubule ribbon extends between the parasomal sacs, causing a characteristic kinetid pattern (Figs. 22, 24).

No subkinetal microtubules or barren basal bodies were found.

**Nuclear apparatus:** *E. vermicularis* has a roundish macronucleus (Figs. 1, 2, 6, 7) and an ellipsoid micronucleus which is difficult to recognize in the light microscope but prominent in the electron microscope due to many loosely arranged microtubules extending in its main axis (Fig. 25). Likewise, the macronucleus contains microtubules which are, however, more densely packed than those in the micronucleus (Figs. 8, 25). Both nuclei are surrounded by about four layers of rough endoplasmic reticulum and their ultrastructure appears similar to that known from other suctorian swarmer (e.g. Bardele 1969, Hauser 1970).

**Contractile vacuole:** *E. vermicularis* has a single contractile vacuole with a prominent excretory pore near the centre of the anterior pole (Figs. 2, 3, 6, 8-10). The vacuole is surrounded by spongy cytoplasm and a basket of microtubules which originate at the excretory pore and form distinct posteriorly extending ribbons (Figs. 9, 10).

**Cytoplasmic inclusions:** Two types of cytoplasmic inclusions were found which clearly differ in size and structure. The first type, probably hydrogenosomes, is rather irregularly shaped and has an average size of 1109 x 938 nm (Table 1). It is surrounded by at least one unit membrane and contains lightly stained,

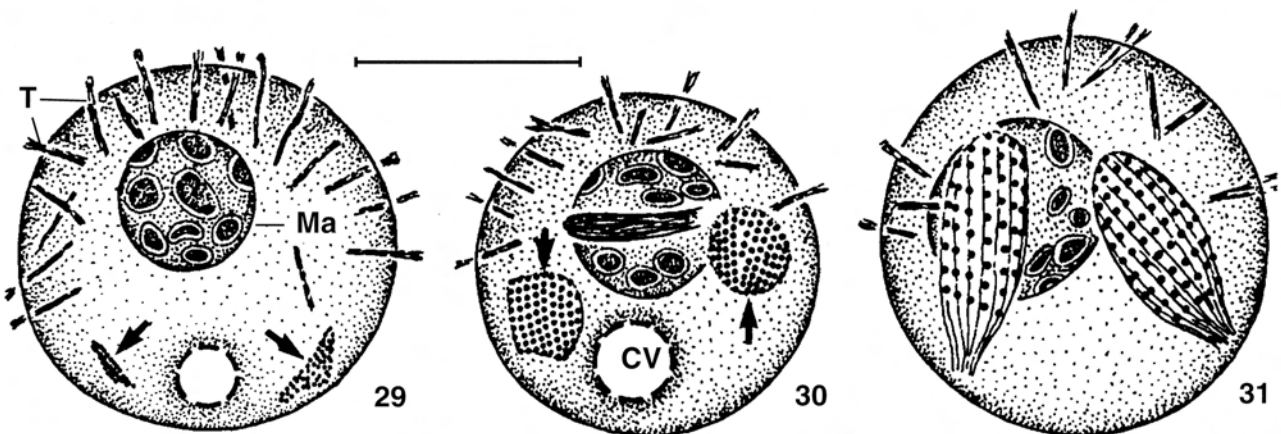
homogenously distributed, finely granular material (Figs. 24, 25). In two cells some of these bodies contained highly ordered inclusions, possibly protein crystals or virus particles (Fig. 26).

The second type of inclusion is more regularly shaped and smaller than type one, viz. has an average size of 703 x 640 nm (Table 1). These bodies are heavily stained and often contain a variety of inclusions, some of which are highly reminiscent of bacteria (Figs. 16, 24, 25, 27). Several stages of condensation can be clearly recognized in Fig. 25, suggesting that these inclusions are degenerating food vacuoles and/or autophagous vacuoles.

### Morphogenesis

Swarmer formation commences by proliferation of basal bodies in small areas, one each to the right and left of the contractile vacuole (Fig. 29). These fields enlarge and become convex, while a distinct bundle of microtubules (?) appears in the centre of the macronucleus (Fig. 30). By continued growth and enfolding the distances between the basal bodies are increased and the margins of each ciliary field become connected, producing two ellipsoid swarmer within an adult (Fig. 31). Only one swarmer was observed in about 10% of the morphogenetic active cells.

Unfortunately, the slides did not contain stages showing either the division of the macronucleus, which is one of the latest events in embryogenesis (Bardele 1970), or the release of the swarmer.



Figs. 29 - 31. Early and middle stages of swarmer formation in *E. vermicularis*. Usually, two swarmer (arrows) are produced, one each to the right and left of the contractile vacuole. CV - contractile vacuole, Ma - macronucleus, T - tentacles. Bar - 10  $\mu$ m

## DISCUSSION

*Enchelyomorpha*, a "good" suctorian

Lynn and Foissner (1994) reviewed the distribution of tentacle and tentacle-like structures in ciliates. This compilation shows that suctorians are unique in having two concentric microtubule cores in their tentacles. The outer core may or may not be complete and the inner core is always composed of ribbons of arm-bearing microtubules. The axonemes of *E. vermicularis* match this pattern perfectly (Figs. 12-16), leaving no doubt as to its suctorian relationship. This is supported by the occurrence of haptozooids, a type of extrusome as yet found only in this group of ciliates (Batisse 1994), and also by its mode of reproduction, viz. endogenous budding.

The position of *Enchelyomorpha* within the Suctoria

Classification of taxa within the suctorians is mainly according to the mode of swarmer production, which is polygemmic and internal in *Enchelyomorpha* (Figs. 29-31). Thus, *Enchelyomorpha* belongs to the Entotropida or Endogenina, depending on the classification preferred (Batisse 1994, Corliss 1979). It is just this order which contains two other peculiar suctorians, viz. *Allantosoma*, living in the cecum and colon of horses, and *Cyathodinium*, inhabiting the cecum of domestic pigs (Batisse 1994). Batisse (1994) uses details of the swarmer formation for subordinal classification. Unfortunately, such details are not known for *Enchelyomorpha*. However, data are sufficient to discuss some af-

finities. A relationship of *Enchelyomorpha* with the Acinetina is indicated by the fine structure of the tentacles and the general organization and ciliature of the swarmer, whereas swarmer production resembles *Cyathodinium* in the suborder Discophryina.

As concerns the tentacle axonemes, those of *Acineta* (Bardele and Grell 1967), *Tokophrya* (Rudzinska 1967, 1970), *Cyathodinium* (Paulin and Corliss 1969) and *Enchelyomorpha* (Figs. 12-16) are extremely similar not only in the number of microtubules comprising the axonemes but also in that the lamellae of the inner core are only slightly curved and thus almost parallel to the outer microtubule core. Unfortunately, other members of the Acinetidae and Tokophryidae have tentacle axonemes which look rather dissimilar to those of *Enchelyomorpha*. Their lamellae are distinctly curved and often composed of hundreds of microtubules, e.g. in *Acinetopsis* (Grell and Meister 1982a), *Trematosoma* (Batisse 1972), *Choanophrya* (Hitchen and Butler 1973a), and *Rhyncheta* (Hitchen and Butler 1974). The fine structure of the tentacle axoneme is thus very likely a weak character for establishing phylogenetic relationships.

We thus place more weight on the symmetry and ciliature of the swarmer. Both *Tokophrya* and *Enchelyomorpha* have monaxone, oblong swarmers and possess circular-transverse ciliary rows. It is easy to imagine how an enchelyomorphid swarmer originates from a tokophryid swarmer simply by increasing the number and spreading of its circular-transverse ciliary rows (Fig. 32). Unfortunately, this attractive hypothesis has some shortcomings. In contrast to *Enchelyomorpha*, *Tokophrya* and *Acineta* swarmers typically have a short, oblique ciliary row near the inflated anterior end and lack tentacles in the free-swimming (larval) stage. Furthermore, the typical brood pouch of the Acinetina is apparently lacking in *Enchelyomorpha* (Figs. 29-31).

There is a striking similarity between *Enchelyomorpha* and *Cyathodinium* during the early and middle stages of swarmer production: both develop two swarmer anlagen at opposite sites of the adult (Figs. 29-31; Cunha and Freitas 1940, Lucas 1932, Nie 1950). Unfortunately, we did not find late dividers of *Enchelyomorpha*, but there is a fair probability that they look similar to those of *Cyathodinium*, i.e. that budding is evaginative and *Enchelyomorpha* thus belongs to the Discophryina.

*Enchelyomorpha* obviously has a highly distinct combination of characters which suggests maintenance of the family Enchelyomorphidae Augustin and

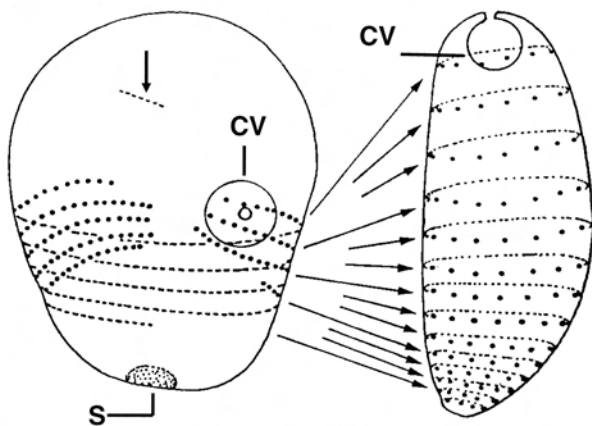
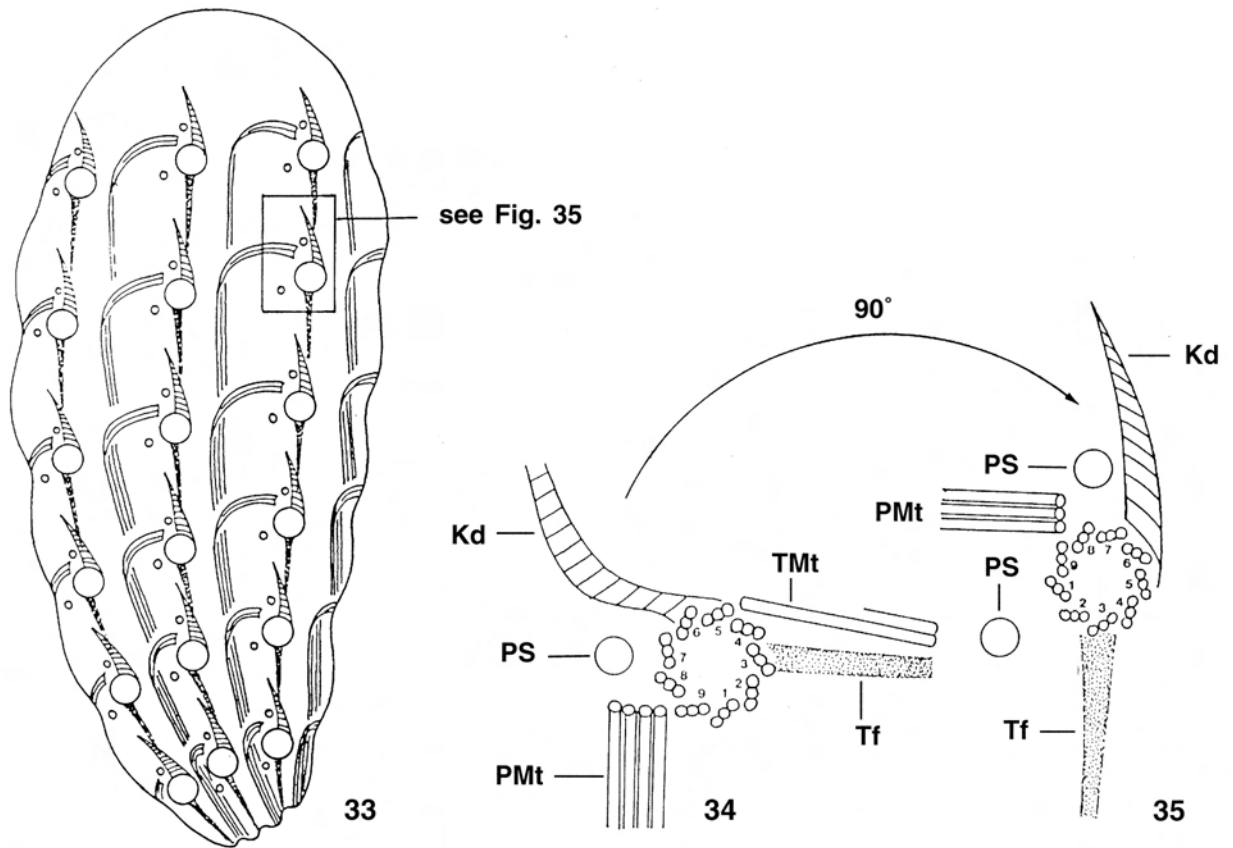


Fig. 32. The enchelyomorphid swarmer can be derived from a tokophryid bud simply by increasing the number and spreading of its circular-transverse ciliary rows. The arrow marks a short, oblique ciliary row lacking in *E. vermicularis*. CV - contractile vacuole, S - scopuloid. Based on figures by Augustin and Foissner (1989, 1992)



Figs. 33 - 35. Schemes of the cortical fine structure of *E. vermicularis* (33, 35) and *Trithigmostoma steini* [34; from Hofmann and Bardele (1987), subkinetal microtubules omitted]. The typical kinetid pattern of *Enchelyomorpha* can be modelled by a 90° rotation of the *Trithigmostoma* kinetid, if it is assumed that the narrowed portion of *Enchelyomorpha* is its posterior end. Kd - kinetodesmal fibre, PMt - postciliary microtubule ribbon, PS - parasomal sac, TMT - transverse microtubule ribbon, Tf - transverse fibre. Numbers designate triplets of basal body

Foissner, 1992. This is strengthened by the lack of mitochondria, i.e. its anaerobic mode of life.

**Fine structural comparison**

Having shown that *E. vermicularis* is a suctorian swarmer, a more detailed comparison of its structure with related taxa seems appropriate. However, most electron microscopic data on suctorians are from adult cells (Batisse 1994), while our investigations on *Enchelyomorpha* concern the swarmer only. This limits a fruitful comparison considerably and it is quite possible that, for instance, the simple tentacle structure of *Enchelyomorpha* is a juvenile character. We thus restrict the comparison to a few main structures, namely the tentacles, the somatic kinetids and the cytoplasmic inclusions.

**Tentacles and haptocysts:** The tentacles of *Enchelyomorpha* appear simple when compared to those

of many other suctorians, e.g. *Trichophrya rotunda* (Mogensen and Butler 1984), *Rhyncheta cyclopum* (Hitchen and Butler 1974), *Discophrya* (Curry and Butler 1976), *Acinetopsis rara* (Grell and Meister 1982a), and *Ephelota gemmipara* (Grell and Benwitz 1984a). The most remarkable feature concerns the distal end, i.e. the tip containing the haptocysts, which is very likely sealed by the full set of cortical membranes, whereas it is covered solely by the cell membrane in other species. However, this might be a juvenile feature, because the tip of the growing tentacles of *Choanophrya infundibulifera* is also covered by the cell membrane and the cortical alveoli (Hitchen and Butler 1973b). Growing suctorian tentacles are usually associated with a non-ciliated basal body which possibly acts as an organizing centre for the microtubules of the axoneme [see Hitchen and Butler (1973b) for short review]. No barren basal bodies have been observed in *E. vermicularis* which

matches results by Grell and Benwitz (1984b) on *Ephelota gemmipara* and *E. plana*.

The haptocysts of *E. vermicularis* look rather simple compared with those of other suctorians (Batisse 1994, Jurand and Bomford 1965, Mogensen and Butler 1984). Specifically, the proximal portion of the shaft is not dilated and the granular material in the bulb appears homogenous. Furthermore, the bulb is not globular, as is usual, but barrel-shaped. Similar haptocysts occur in *Ephelota* spp. according to the micrographs in Grell and Benwitz (1984a).

**Somatic kinetids:** The pioneering study of Batisse (1972) showed a basic similarity between the suctorian, cyrtophorid and rhynchodid kinetids. It provided a strong argument for uniting these groups in a monophyletic taxon, the class Phyllopharyngea (Puytorac et al. 1974), which is still recognized (Lynn 1991, Puytorac et al. 1993). This view has not been contradicted by later studies (Grell and Benwitz 1984a, b, Mogensen and Butler 1984) and is also supported by our investigations. In fact, the enchelyomorphid kinetid pattern can be easily modelled by a simple rotation of the cyrtophorid kinetid (Figs. 33-35). However, detailed studies on the fine structure of the suctorian kinetids are not available, and our own study also has shortcomings. Thus a substantial comparison is difficult and should await more detailed investigations.

*Enchelyomorpha vermicularis* has a holotrichous ciliature with basal bodies so regularly arranged that the ciliary rows can be interpreted as extending longitudinally or transversely with respect to the swarmer axes (Figs. 5, 7). The electron microscopic investigations showed that the proximal portion of the postciliary microtubule ribbon extends in the transverse axis of the swarmer, while the longer distal portion curves backwards to the narrowed end of the cell. Accordingly, the kinetodesmal and transverse fibres extend along the main body axis. If it is accepted that the postciliary microtubules and the kinetodesmal fibres define the orientation of the kinetid, then the ciliary rows extend transversely and the kinetids are rotated 90° counter-clockwise with respect to the longitudinal body axis and the cyrtophorid kinetid pattern. This interpretation appears reasonable because most suctorian swarmers, especially those of the Acinetina to which *Enchelyomorpha* possibly belongs, have circular-transverse ciliary rows with ends almost touching each other (Batisse 1994, Grell and Benwitz 1984b, Guilcher 1951).

In *Trematosoma bocqueti* two parasomal sacs occur in the posterior area of the kinetid (Batisse 1972),

whereas *Acinetopsis rara* has only one sac at the right side of the basal body near the kinetodesmal fibre (Grell and Meister 1982b). In *Trichophrya rotunda* a parasomal sac occurs on either side of the kinetid (Mogensen and Butler 1984). The parasomal sacs of *E. vermicularis* are located as in *T. bocqueti*, if it is taken into account that the ciliary rows extend transversely to the longitudinal body axis (Figs. 22, 24, 33, 35).

A remarkable difference between *E. vermicularis* and other suctorians concerns the kinetodesmal fibre which not only extends close beneath the epiplasm but definitely contacts it *via* the frayed distal end (Fig. 20). This resembles some colpodid ciliates (Lynn 1976).

Whether subkinetal microtubules are really lacking in *E. vermicularis* needs confirmation from better fixed material.

**Cytoplasmic inclusions:** *Enchelyomorpha vermicularis* definitely lacks structures which could be interpreted as mitochondria. This is in accordance with most faunistic records, showing it to be restricted to microaerobic or anaerobic biotopes (Augustin and Foissner 1992). Thus, *E. vermicularis* should possess hydrogenosomes and endosymbiotic (methanogenic) bacteria like other anaerobic ciliates [for review see Fenchel and Finlay (1991)]. We could not identify such organelles unequivocally in the sections. However, the lightly stained, rather irregular bodies distributed throughout the cytoplasm closely resemble hydrogenosomes known from, e.g. *Trimyema* (Finlay et al. 1993), *Plagiopyla* (Berger and Lynn 1992) and an anaerobic suctorian, *Cyathodinium* (Paulin 1973), living in the cecum of pigs, although we could not identify a double membrane surrounding them which might, however, be due to inadequate fixation (Paul et al. 1990).

Structures resembling bacteria (methanogens) were not found in the cytoplasm but often in the dark globules interpreted as autophagosomes (Figs. 25, 27, 28). Likewise, stalk (scopuloid) granules and scopular pores as described in several suctorian swarmers (Bardele 1970, Grell and Benwitz 1984b, Grell and Meister 1982b, Hascall and Rudzinska 1970, Mignot and Puytorac 1968) are apparently lacking in *E. vermicularis*. In fact, we could not identify any structure reminiscent of a scopuloid. The adult of *E. vermicularis* is thus very likely stalkless.

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