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Stomatogenesis in the Ditransversal Ciliate *Homalozoon vermiculare* (Ciliophora, Rhabdophora)

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SUMMARY

H. vermiculare possesses about 10 somatic kineties on the right lateral side, 3 somatic kineties on the left lateral side and a single circumoral kinety. The somatic kineties are composed of monokinetids except for the anterior ends of the brush kineties which are composed of dikinetids. The circumoral kinety consists of paired kinetosomes one of which is nonciliated and associated with a microtubular lamella and a nematodesma.

Stomatogenesis commences when the anteriormost somatic kinetosomes in the opisthe are transformed into the nonciliated kinetosomes of the future oral dikinetid. They lose the somatic infraciliary fibers and the ciliary shaft and each gives rise to a nematodesma and a microtubular ribbon. Adjacent to each of the transformed somatic kinetosomes, a new kinetosome is assembled, thus producing an oral dikinetid anlage. The new anterior kinetosome bears a cilium and becomes the ciliferous kinetosome of the oral wreath of cilia. In protargol stained specimens, proliferation of kinetosomes can first be observed in the left lateral kineties but, eventually, each of the somatic kineties produces one kinetofragment. Thus, *H. vermiculare* has a holotelokinetal type of stomatogenesis. The cirumoral kinety arises from a counter-clockwise rotation (as viewed from outside the cell) of all 15 dikinetid kinetofragments and subsequent "head to tail" fusion of the fragments after cell division has been completed. The oral apparatus of the proter seems to be largely conserved during division.

Some aspects of the evolution of the oral apparatus and the origin of the oral microtubular ribbons are discussed.

Introduction

The fine structure of the oral region in interphasic and feeding cells and the ultrastructure of the extrusomes of *H. vermiculare* have been the subject of several investigations [18, 19, 20]. Despite some light microscopical observations on the formation of the oral region [11, 12, 28], a detailed study on the stomatogenesis is still lacking. To the best of our knowledge, so far no detailed fine structural analysis of stomatogenesis in any ditransversal ciliate[1] has been published. On the other hand, a few detailed light microscopical studies on the morphogenesis of litostomate ciliates are available: *Spathidium muscorum*, *Fuscheria terricola* [2], *Protospathidium serpens* [3], *Bryophyllum tegularum* and *Amphileptus pleurosigma* [11]. Thus, the present study makes a first step towards a fine structural understanding of haptorid stomatogenesis and to compare light- and electron microscopical data. The nuclear changes during division have been the subject of another study [22].

[1] One of the authors (W. F.) would prefer to improve the definition of the subclass Haptoria Corliss and to include *Homalozoon* in that taxon because the Ditransversalia Leipe & Hausmann contain almost only classical haptorids.

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Material and Methods

Preparation Procedures

The protargol method ([8]; Wilbert protocol) was used to reveal the infraciliature. For SEM, cells were fixed in a mixture of OsO_4 and chromic acid, washed in tap water and dehydrated in a critical point dryer. The culture method for *H. vermiculare* and the TEM procedure have been described previously [21, 26]. All stages depicted in the drawings have been seen in at least two individuals.

Morphological Terms

1. *H. vermiculare* is a flattened ciliate which usually creeps along the substrate. The surface turned towards the substrate is densely ciliated (Figs. 1, 4) whereas on the opposite side only three sparsely ciliated kineties are present (Figs. 3, 5). As the asymmetrical position of the cytostome suggests that the narrow left edge corresponds to the "true" ventral side, we refer to the densely ciliated side as the right lateral side. Consequently, the (sparsely ciliated) side bearing the brush kineties is the left lateral side and the kineties on that side are left lateral kineties.

2. The nonciliated kinetosomes of the circumoral kinety are associated with a prominent microtubular ribbon, which lines the cytopharynx. That oral ribbon has been termed microtubular lamella "y" [19]. Unfortunately, the term "y" lamella has been used also for the subcytostomal lamella in nassulid ciliates [5, 27], which is a derivative of postciliary microtubules of the dyads of the paroral membrane [6]. As the oral microtubular ribbon in *H. vermiculare* is thought to be homologous to the somatic transverse ribbon, we will refer to it as the transversal lamella (see also Discussion).

3. Bulge microtubules have been described under various terms (bulge mt, cone mt, accessory mt, widely spaced mt) and are possibly an apomorphous character for the Ditransversalia. The definition given here is consistent with the usage in Foissner & Foissner [9]: Bulge microtubules are single or small groups of non-kinetosome based microtubules, which occur in a number of ditransversal/haptorian ciliates like, e.g., *H. vermiculare*, *Fuscheria terricola*, *Enchelydium polynucleatum* and *Chaenea teres*. They commence in the cortex of the oral bulge and run along the long axis of the cell. They are regularly spaced in the oral area and closely adjacent to the microtubules of the transversal lamellae.

4. The endoplasm of *H. vermiculare* is completely separated from the ectoplasm by a filamentous sheath. That sheath has been termed "tela corticalis" in the somatic area and "fibrous filamentous annulus" in the oral area (see glossary in Corliss [4]) As the tela corticalis and the fibrous annulus cannot be distinguished morphologically and as the filamentous annulus is in no way annular in *H. vermiculare*, we will use the terms "oral filamentous sheath" and "somatic filamentous sheath".

Morphogenetic Terms

In rhabdophoran and cyrtophoran ciliates, the anterior ends of the somatic kineties of the opisthe can participate in the formation of the new oral apparatus. This mode of stomatogenesis is termed telokinetal [4]. However, various subtypes are recognizable within different groups. The definitions given here follow the propositions of Bardele [1] for the holotelokinetal and the merotelokinetal mode and they are largely congruent with the usage in Hiller [13]. In addition, we have expanded the definition on the behaviour of the brush which is considered to be homologous to a part of the oral kineties (adoral organelles) in prorodontid ciliates [13].

Holotelokinetal. The oral kinety arises from the anterior ends of all somatic kineties of the opisthe. The oral kinety can be either a circumoral kinety s.str. (i.e., it is clearly separated from the somatic kineties in an interphase cell) or it may be simply composed of the anteriormost oralized kinetosomes of the somatic kineties. The dikinetids of the brush arise from the anteriormost monokinetids of the opisthe of the same brush kinety (most Ditransversalia).

Monotelokinetal. Each of the two or three (peri-) oral kineties is formed from the anteriormost kinetosomes of a single somatic kinety of the proter. The dikinetids of the brush arise from the anteriormost monokinetids of the opisthe of the same brush kinety (some Ditransversalia, i.e., Pleurostomatida: *Amphileptus*, *Litonotus*, *Loxophyllum*).

Remark: The kinetosomes in the anterior part of the perioral kineties are associated with nematodesmata and transversal lamellae. During stomatogenesis, the posterior (somatic) part of the perioral kineties becomes also associated with nematodesmata and transversal lamellae. As only the posterior somatic part of the perioral kineties is involved in the formation of the perioral kineties of the opisthe, it represents telokinetal rather than buccokinetal stomatogenesis as proposed by several French authors [11].

Merotelokinetal. Only a limited number of somatic kineties take part in the formation of one or several circumoral kineties and the brush kineties. If brush kineties (also termed adoral organelles) are present, they arise from the leftmost stomatogenic somatic kineties of the opisthe whereas the circumoral kinety arises from the rightmost stomatogenic kineties (Cyrtophorida, Prorodontida).

Results

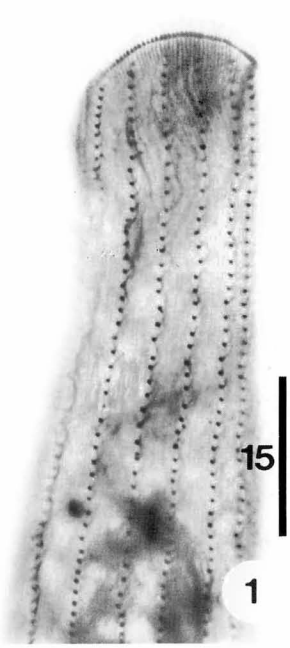
Infraciliature During Interphase

The somatic infraciliature of *Homalozoon vermiculare* comprises 8–11 right lateral kineties and 3 left lateral kineties (Figs. 1–7). The kinetosomes of the right lateral kineties are always ciliated. The kinetosomes on the left side have a short cilium at each 2nd-5th kinetosome, but are always ciliated at the anteriormost part of the cell (Fig. 5; for detailed description see [7]). Adjacent to the anterior portions of the left lateral kineties (1K2 and 1K3), frequently short kinetofragments composed of dikinetids can be found. The circumoral kinety consists of about 130 kinetosome pairs. One kinetosome of a pair is ciliated, the other is barren and gives rise to a long, wavy nematodesma (Fig. 2).

Morphogenesis

Stage 1. Morphogenesis commences with the proliferation of kinetosomes slightly posterior to the middle of the cell within the three left lateral kineties (Fig. 9). The kinetosomes seem to be already paired in that stage. No proliferation can be observed in the right lateral kineties. However, intrakinetal proliferation may be going on as the kinetosomes appear to be more closely spaced than in interphasic cells (Fig. 8 and comp. Fig. 6).

Stage 2. Compared to stage 1, the infraciliature of the left side and the general morphology of the cell are not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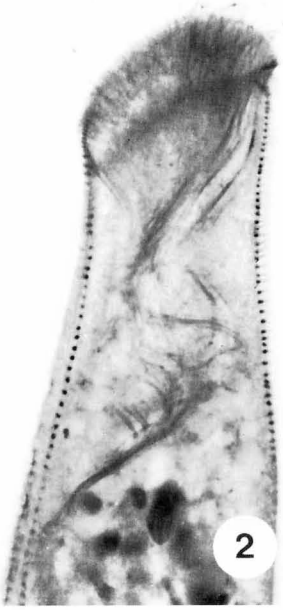
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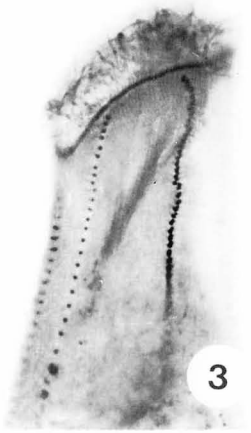
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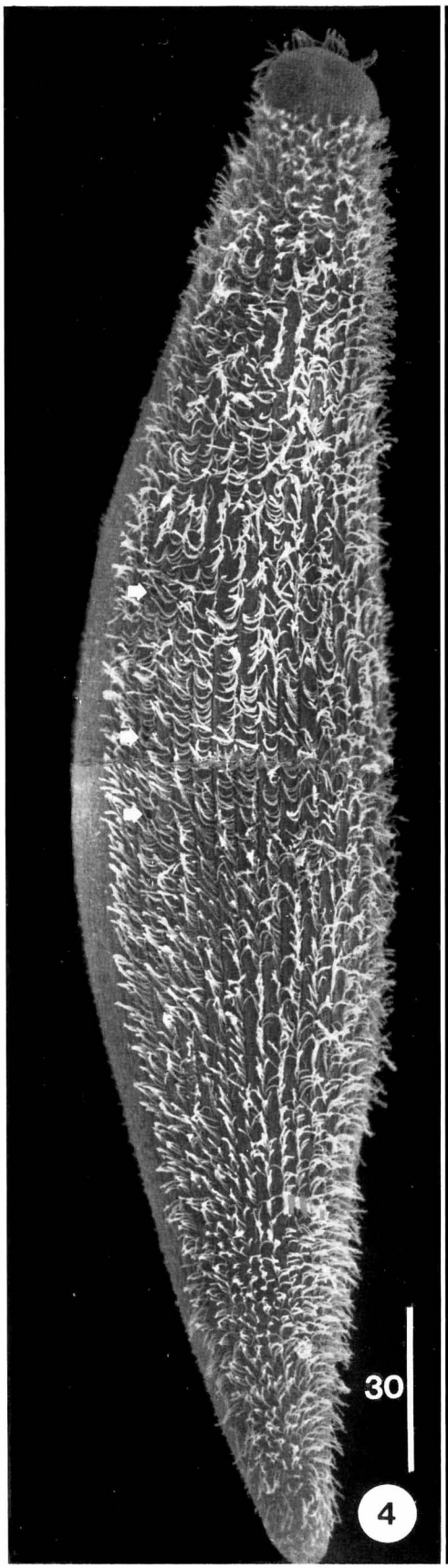
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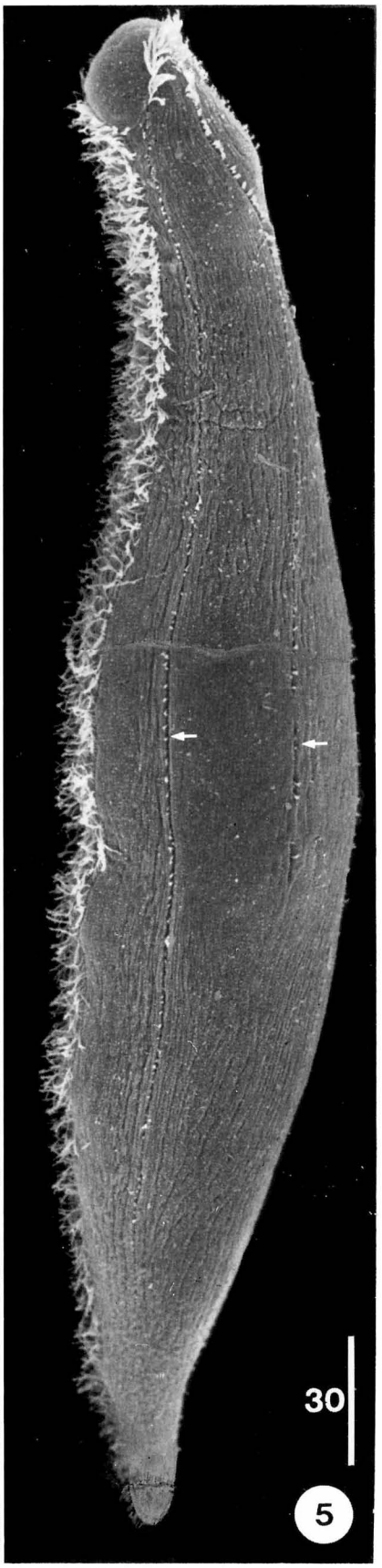
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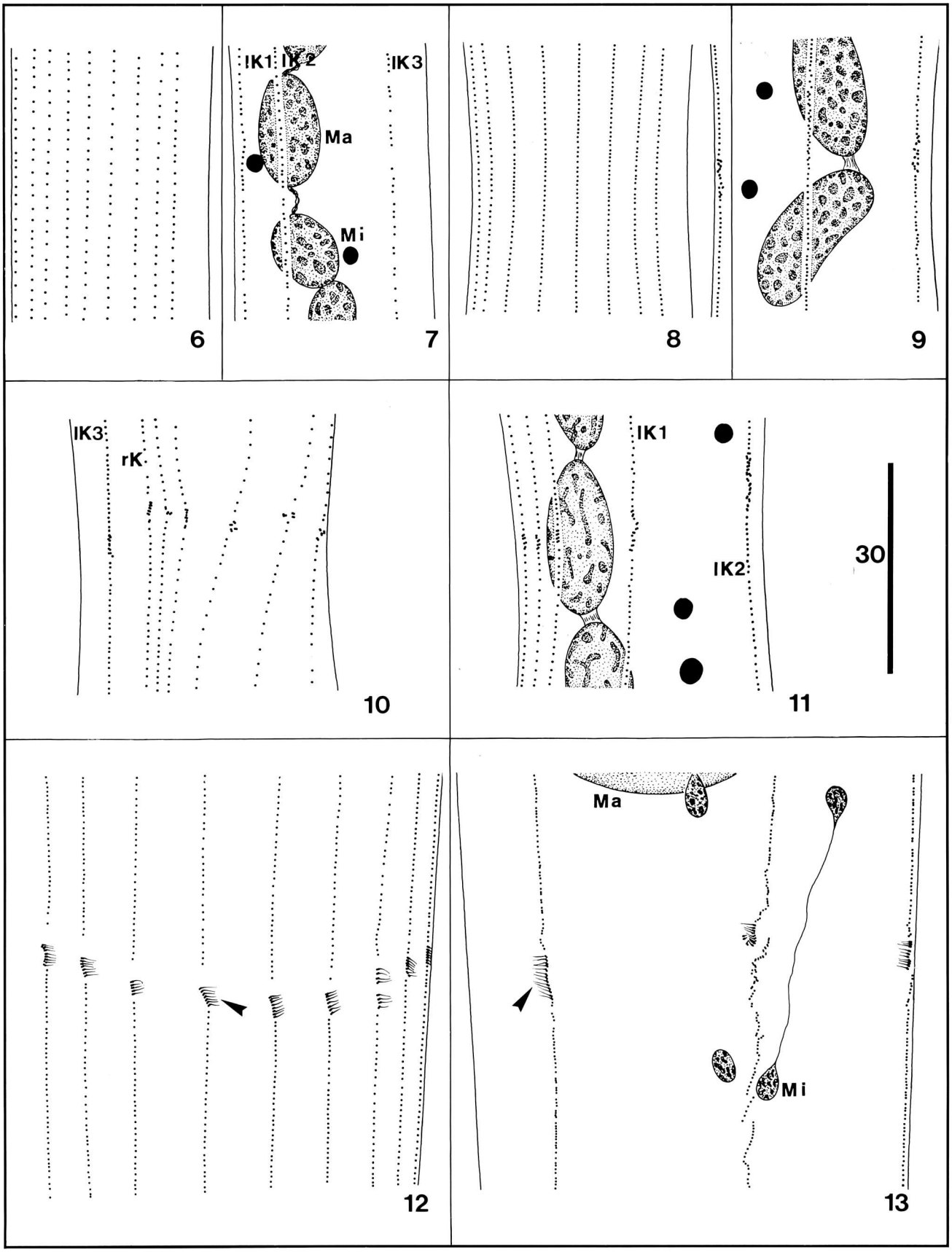
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changed. The right lateral somatic kineties close to the future division furrow begin to proliferate. These kinetosomes can be paired and frequently they show a rather irregular pattern (Figs. 10, 11).

Stage 3. The ongoing proliferation of kinetosomes has produced kinetofragments. The kinetofragments are more or less distinctly separated from the somatic kineties and bear cilia and short nematodesmata (Fig. 12). In the left lateral kinety 2, additional pairs are formed, which can form an additional kinetofragment (Fig. 13, see also stage 6). The macronucleus is condensed and the micronuclei have started to divide (Fig. 13).

Stage 4. The kinetofragments curve to the right and the nematodesmata are always clearly recognizable (Fig. 14). At the anterior end of the brush kineties (1k2, 1k3) of the opisthe, the typical interphasic paired kinetosomes arise. The plane of division becomes oblique to the long axis of the cell (Fig. 15), which corresponds to the course of the oral bulge of the interphase cell, declining ventrally. The macronucleus is condensed and most of the micronuclei have accomplished division. The division furrow can be seen by way of intimation.

Stage 5. The kinetofragments are distinctly semicircularly curved (Fig. 16). They are orientated obliquely to the plane of the division furrow and the axis of the somatic kineties, respectively. The left lateral kinetofragments are about twice or thrice as long as the right lateral kinetofragments (Figs. 15, 16).

Stage 6. The infraciliature can be compared to that in stage 5. The dikinetids are still rather irregularly spaced. Sometimes, in addition to the kinetofragment, a number of dikinetids can proliferate in front of the left lateral kinety 2 (comp. stage 3). The division furrow has become prominent (Figs. 17, 18).

Stage 7. Proter and opisthe have separated. The infraciliature of the proter does not undergo any changes. Thus, it is as that found in the parental cells. In the opisthe, the kinetofragments rotate counter-clockwise thereby closing the circumoral kinety (Fig. 19). That process is finished about 1 hour after the separation of the daughter cells. The number of kinetosome pairs in the newly formed circumoral kinety is about 120 corresponding to the number of kinetosome pairs found in interphase cells. Thus, new kinetosomes were not added after cytokinesis was completed, but kinetosomes proliferate during interphase in the somatic kineties [21].

Ultrastructure

The fine structure of the oral apparatus has been described in detail previously [18, 19]. To facilitate orientation, the most important characters are repeated in the following paragraph.

The circumoral kinety is composed of paired kinetosomes one of which gives rise to the circumoral wreath of cilia (Figs. 5, 20, 21). The pharyngeal basket is made up by many nematodesmata that originate from the nonciliated kinetosomes of the circumoral kinety. Apart from the

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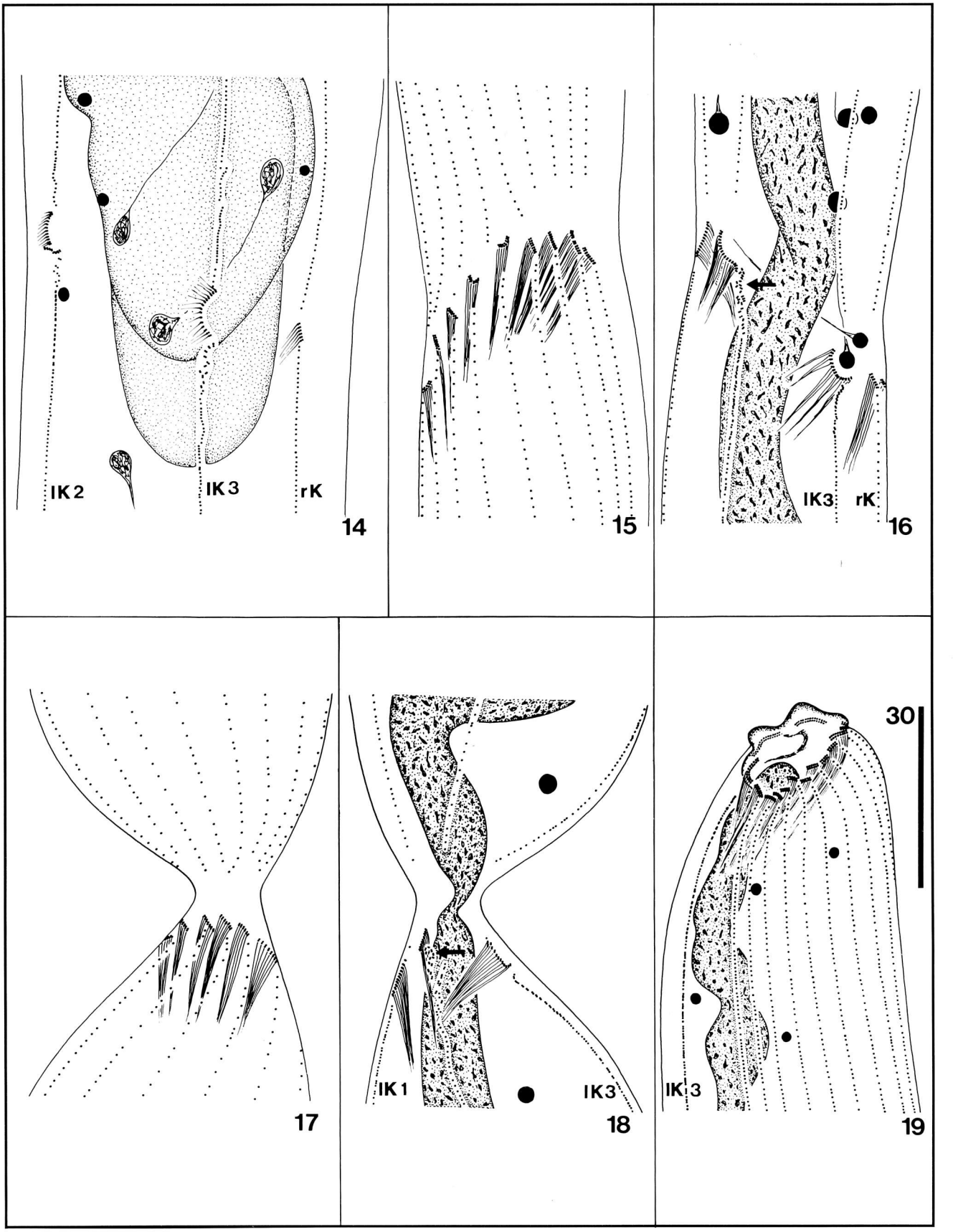
The scale bar represents 1 μm. Bars numbered 5, 10, 15 or 30 represent 5 μm, 10 μm, 15 μm or 30 μm, respectively. An arrowhead pointing to A or P indicates the anterior or posterior side of the cell.

◀ Figs. 1–5. Interphase cells of *Homalozoon vermiculare* after protargol staining and in the scanning electron microscope (SEM). – Fig. 1. Anterior part of the cell showing right lateral kineties. Protargol staining, × 1,300. – Fig. 2. Anterior part of the cell showing the wavy nematodesmata. Protargol staining, × 1,300. – Fig. 3. Anterior part of the cell showing left lateral kineties, × 1,300. – Fig. 4. Right side of the cell. Arrows point to contractile vacuole pores. SEM, × 660. – Fig. 5. Left side of the cell. Arrows point to left lateral kineties. SEM, × 600.

◀ Figs. 6–13. Protargol staining. Early stages of morphogenesis. All figures alternately show the left or right lateral side of the cell in the area of the future division plane. – Fig. 6. Right lateral view of the interphase cell of *H. vermiculare*. – Fig. 7. Left side of interphase cell showing the left lateral kineties (1K1, 1K3), the segments of the macronucleus (Ma) and the micronuclei (Mi). – Fig. 8. Stage 1. Right lateral side at the onset of division. The kinetosome pattern is unchanged. – Fig. 9. Stage 2. The proliferation of kinetosomes in the left lateral kineties has started. The intersegmental parts of the macronucleus are slightly broadened. – Fig. 10. Stage 2. Proliferation of kinetosomes has started in the right lateral kineties (rK). – Fig. 11. Stage 2. The proliferation of new kinetosomes in the left lateral kineties (1K1, 1K2) proceeds. The macronuclear segments start to fuse. – Fig. 12. Stage 3. In the right lateral kineties, kinetofragments with short nematodesmata (arrowhead) have become visible. – Fig. 13. Stage 3. In the left lateral kineties, kinetofragments with short nematodesmata (arrowhead) have become visible. The macronucleus (Ma) is condensed, and the micronuclei (Mi) are mostly in telophase.

Figs. 14–19. Protargol staining. Late stages of morphogenesis. – Fig. 14. Stage 4. The left lateral kineties (1K2, 1K3) start to curve. The ▶ macronucleus is completely condensed, and the micronuclei are in telophase or have completed mitosis. – Fig. 15. Stage 5. A slight constriction in the middle of the cell has appeared. The right lateral kineties start to rotate to the left. The nematodesmata have increased in length. The plane of division forms an obtuse angle with the long axis of the cell and corresponds to the ventrally declining position of the oral rim in an interphase cell. – Fig. 16. Stage 5. The left lateral kinetofragments, which are distinctly longer than the right lateral kinetofragments, have started to rotate. The macronucleus has elongated and the micronuclei have completed fission. – Fig. 17. Stage 6. The division furrow has become more prominent. The kinetofragments have continued their rotation movement. – Fig. 18. Stage 6. The macronucleus becomes constricted in the area of the division furrow. In addition to the kinetofragment, a number of dikinetids has proliferated in front of the left lateral kinety 2 (arrow). – Fig. 19. Opisthe shortly after cytokinesis has taken place. The circumoral kinety is still not completed. The macronucleus is about to renodulate.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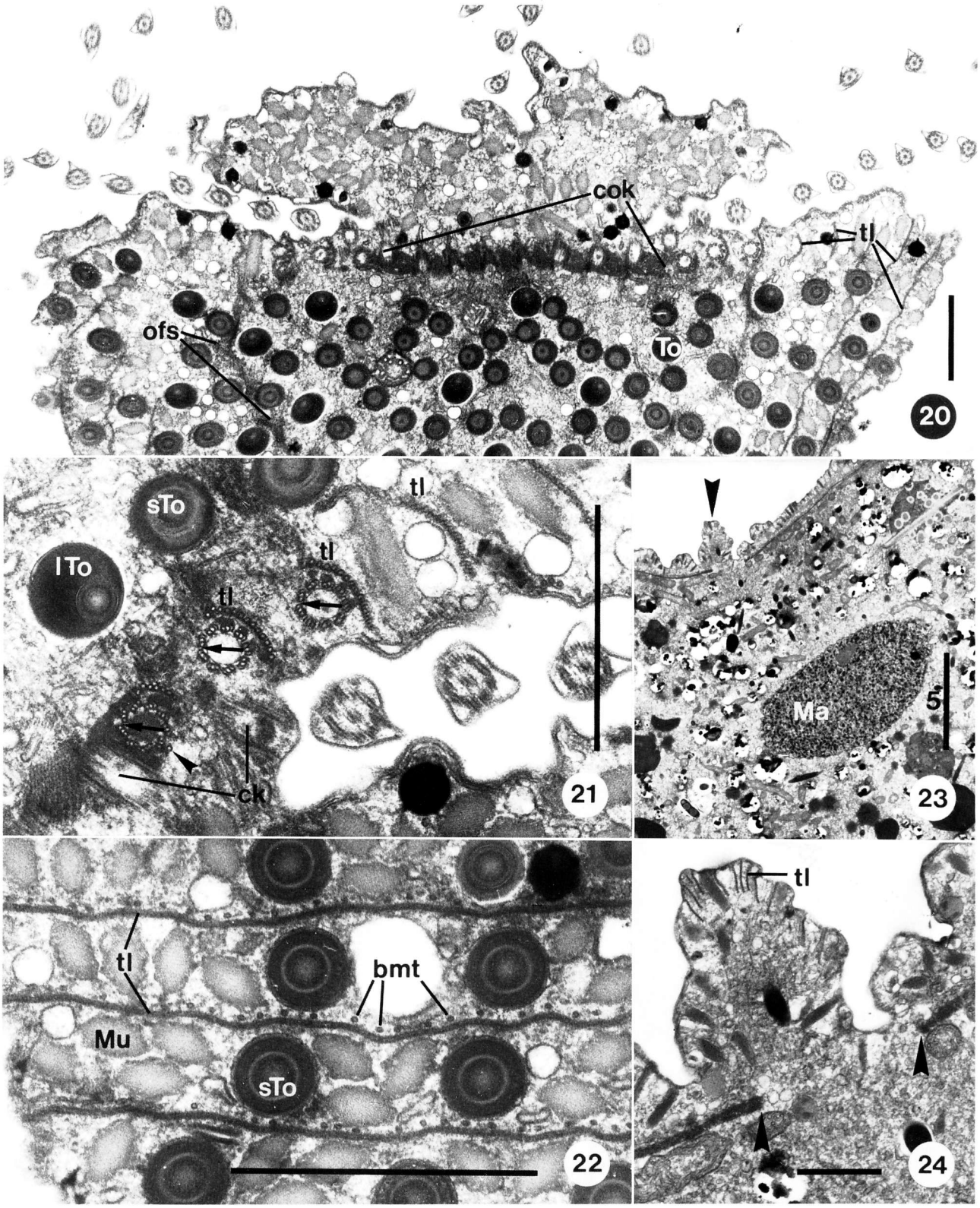
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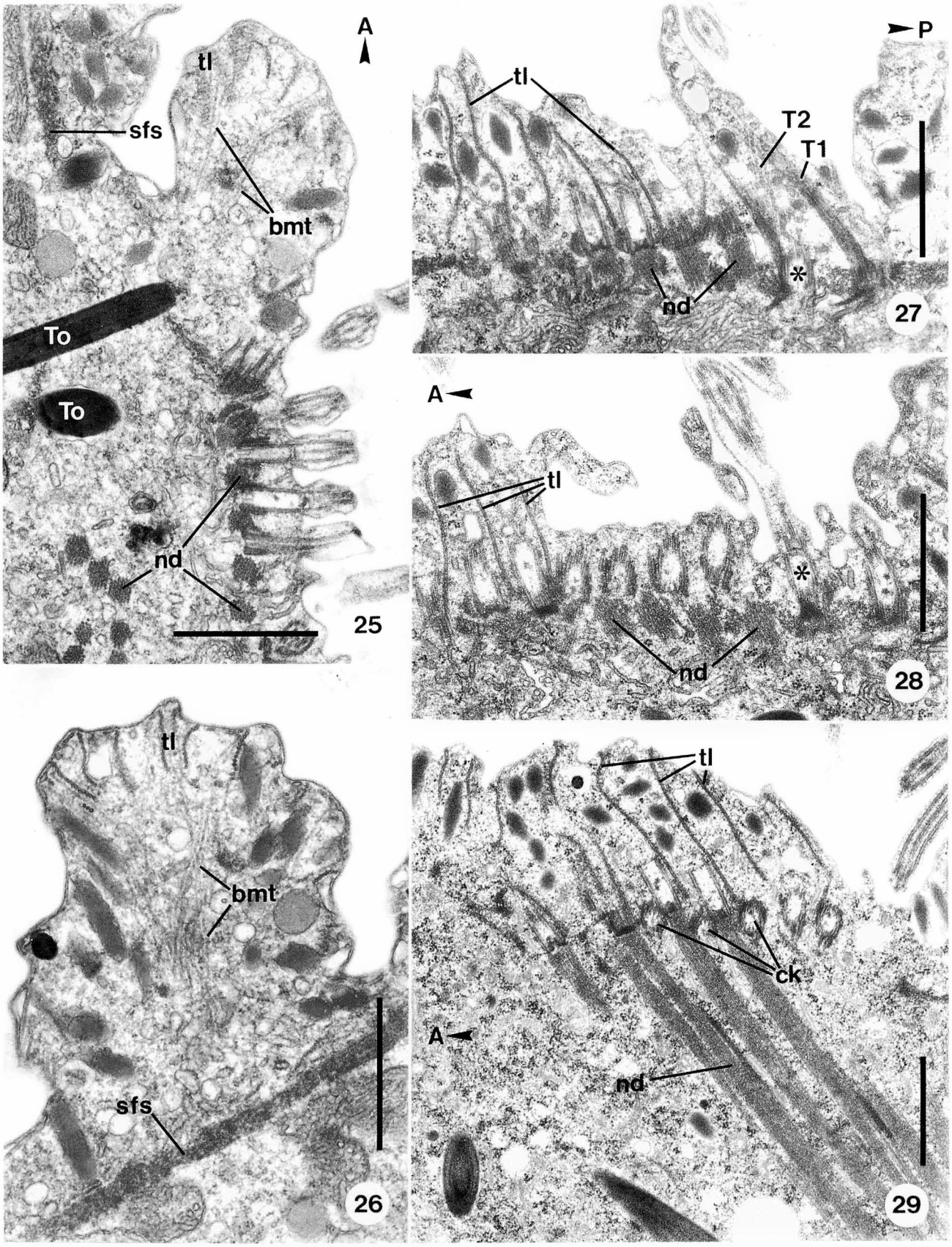
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nematodesmata, each nonciliated kinetosome is associated each with (i) a bundle of filaments that separates the nematodesmata near the origin (not shown here); (ii) the oral filamentous sheath, lying in the periphery of the oral region; (iii) the somatic filamentous sheath which extends along the cell. In addition, a single short microtubule can be observed at the cortical side of the kinetosome, which has not been described before (Fig. 21). Below the plasma membrane, the pharyngeal basket is filled with numerous mucocysts and several hundred toxicysts of two different types (Figs. 20, 21, 22). The apical parts of the toxicysts are surrounded by a lattice made up of transversal lamellae and bulge microtubules (Fig. 22).

At the onset of division, the somatic filamentous sheath disassembles in the middle of the cell and small protuberances appear in the opisthe just posterior to the presumptive fission furrow (Figs. 23, 24, 25, 26). The disintegration of the somatic filamentous sheath seems to start in front of the somatic kineties (Fig. 25), as the sheath is still intact in an early phase of division in those parts of the cell which are not adjacent to a kinety, even though the protuberance can be recognized (Fig. 26). From the very start, microtubules can be found within the protuberances (Figs. 24, 25, 26). These belong either to the bulge microtubules (Figs. 25, 26) or to the transversal lamellae, which surround the apical parts of the extrusomes in an interphase cell. The bulge microtubules are not kinetosome-based in an interphase cell and likewise, no association with kinetosomes could be detected during morphogenesis.

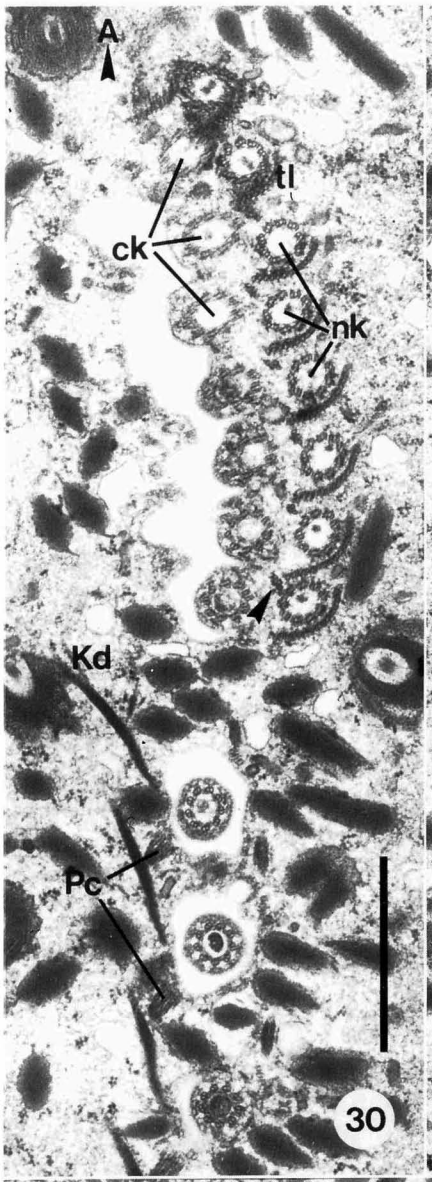
In an early phase of division, when the anlage of the oral kinety is not yet fragmented from the parental somatic kinety, the monokinetids can be seen to be already associated with transversal lamellae and very short nematodesmata (Figs. 27, 28). The kinetosomes of the anlage are very narrowly spaced in that phase and the kinetosomes of the somatic kinety can be recognized by the first and the second transverse ribbon (Figs. 28, 29). The anlage is growing in length when kinetosomes of the somatic kineties situated behind it are separated from their kineties to join the anlage. While morphogenesis proceeds, the nematodesmata associated with the nonciliated kinetosomes elongate (Fig. 29). At the same time, the nonciliated kinetosomes become more widely spaced and new kinetosomes appear in a right angle to the preexisting ones (Fig. 29). These new kinetosomes and their cilia will form the circumoral wreath of cilia in the interphase cell of *H. vermiculare*.

In a tangential section through a kinetofragment, which is still situated in front of the parental somatic kinety, it can be seen to be composed of dikinetids (Figs. 30, 31), corroborating the observations reported in the light microscopical section of the paper (Figs. 12, 13). The left kinetosome of a pair is associated with the semicircular transversal lamella at the posterior left side and a short microtubular ribbon of three microtubules at the anterior right side (Figs. 30, 31). Neither the short microtubular ribbon nor the transversal lamella match with the position and the structure of the postciliary and transversal ribbons

◀ Figs. 20–24. Thin sections. Interphase and early morphogenesis. – Fig. 20. Transversal section through the oral apparatus showing a part of the circumoral kinety (cok) with interoral and cortical cytoplasm. Toxicysts (To), × 15,000. – Fig. 21. Part of circumoral kinety with nonciliated and ciliated kinetosomes (ck). The nonciliated kinetosome is associated with a transverse lamella (tl) and a single (postciliary?) microtubule (arrowhead). Short (sTo) and long (lTo) Toxicysts can be seen, × 43,000. – Fig. 22. Cortical region of oral bulge. Transverse lamellae (tl), bulge microtubules (bmt), mucocysts (Mu) and toxicysts (To) can be seen, × 53,000. – Fig. 23. Section of the middle region of an early divider with a small protuberance (arrowhead), × 3,400. – Fig. 24. Higher magnification of the protuberance area. The filamentous sheath is disassembled (arrowheads) and transversal lamellae (tl) can be seen, × 13,000.

◀ Figs. 25–29. Thin sections. Early stages in morphogenesis. – Fig. 25. Kinetofragment posterior to a protuberance in the opisthe. The somatic filamentous sheath (sfs) is disassembled and very few transversal lamellae (tl) and bulge microtubules (bmt) can be seen in the protuberance. Short nematodesmata (nd) can be seen in a right angle to ciliated kinetosomes, × 26,000. – Fig. 26. Protuberance lateral to that shown in Fig. 25. The somatic filamentous sheath (sfs) is continuous, but bulge microtubules (bmt) and transversal lamellae (tl) are present, × 28,000. – Fig. 27. Section through kinetofragment. The foremost kinetosomes are nonciliated and have oral transversal lamellae (tl) and short nematodesmata (nd). The posteriad kinetosomes still have both somatic transverse ribbons (T1, T2), × 25,000. – Fig. 28. Consecutive section to that shown in Fig. 27. The posteriad kinetosomes are still ciliated, × 25,000. – Fig. 29. Section through kinetofragment. The nematodesmata that originate from the nonciliated kinetosomes have grown out. The ciliated kinetosomes (ck) are arranged in a right angle to the nonciliated kinetosomes, which give rise to the transversal lamellae (tl), × 20,000.

Figs. 30–36. Thin sections. Kinetofragments and autophagous vacuoles. – Fig. 30. Ventral kinetofragment anteriad to the parental somatic kinety. The kinetofragment is composed of dikinetids. The kinetodesmal fibril has been lost. The kinetosomes in the right row of the kinetofragment are interspaced between the nonciliated kinetosomes at the anteriad end of the kinetofragment, × 26,000. – Fig. 31. Consecutive section to that shown in Fig. 30. The kinetosomes in the right row of the kinetofragment are ciliated but show no microtubular associates. The nonciliated kinetosomes have a transversal lamella on the left side and a ribbon of three microtubules at the right anterior side (arrowheads), × 26,000. – Fig. 32. Part of an autophagous vacuole of an early divider situated in the anteriormost third of the cell showing toxicysts (To) and kinetosomes, × 7,400. – Fig. 33. Part of autophagous vacuole with tangential section of nonciliated oral kinetosomes with transversal lamella (tl), × 20,000. – Fig. 34. Part of an autophagous vacuole. Two kinetosomes can be seen one of which is associated with a transversal lamella (tl), × 34,000. – Fig. 35. Oral region of the proter in an early divider. One somatic kinety and part of the circumoral kinety can be seen. The oral kinetosomes are nonciliated and associated with a transversal lamella. The inner rim of the circumoral kinety is indicated by a broken line, × 20,000. – Fig. 36. Oral region of the proter of an early divider. Parts of the circumoral ciliature are lacking (between arrowheads), × 1,900. ▶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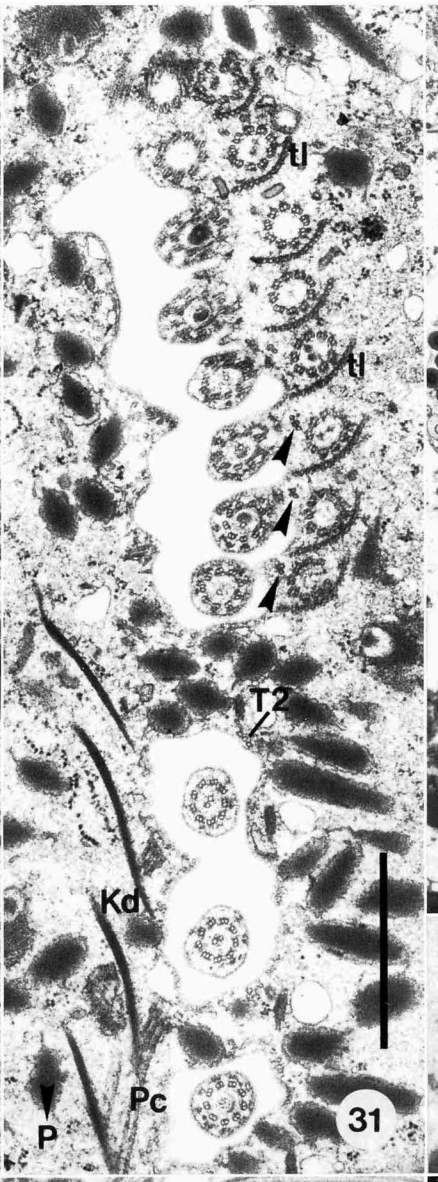
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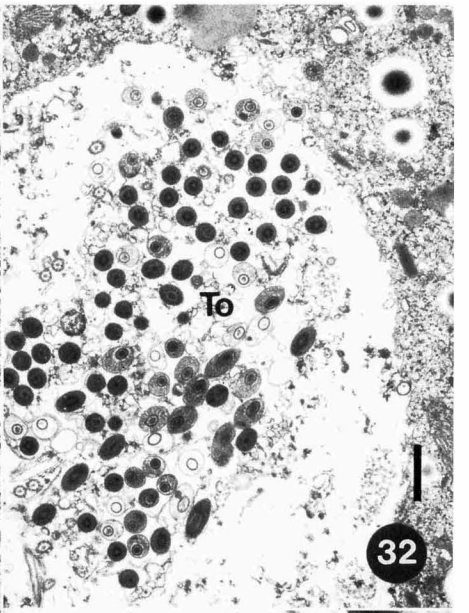
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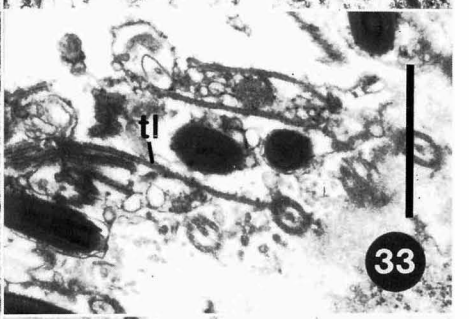
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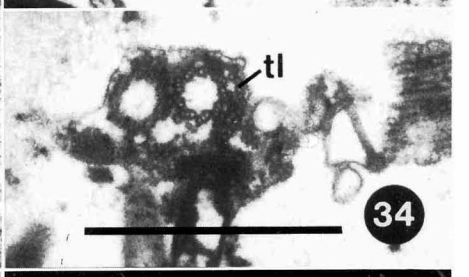
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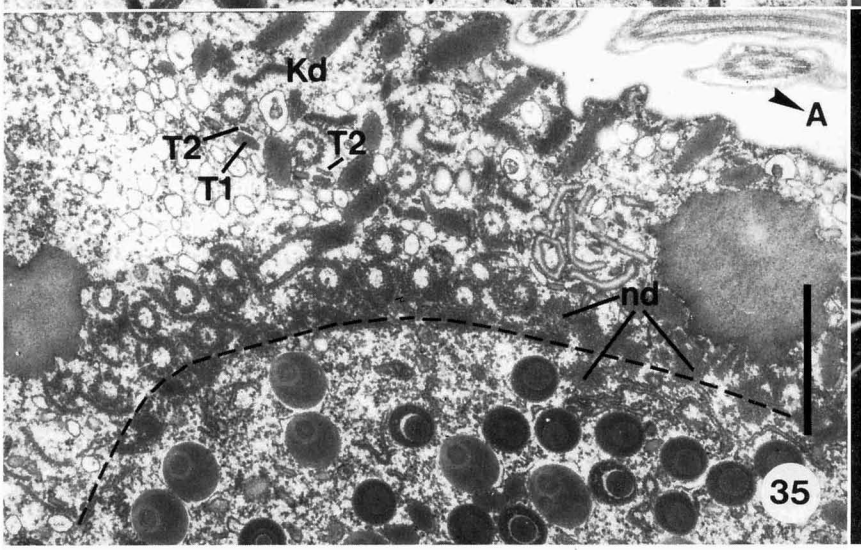
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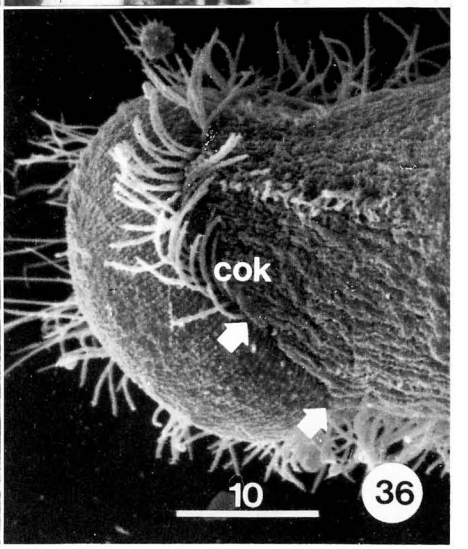
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of the parental somatic kinety (Figs. 30, 31). The right kinetosome of a pair is ciliated and seems to become interspaced between the nonciliated kinetosomes at the anterior end of the kinetofragment (Figs. 30, 31). The main events during the development of the circumoral kinety are summarized in Figs. 41–45.

While a slight division furrow has appeared in the middle of the cell, frequently, autophagous vacuoles which contain numerous toxicysts and kinetosomes can be found in the proter (Fig. 32). Some of these kinetosomes can be identified as oral by the transversal lamella (Figs. 33, 34). In a single case, we have observed paired nonciliated kinetosomes which both give rise to transversal lamellae and nematodesmata in the proter of a divider (Fig. 35). At the same time, no ciliated kinetosomes could be detected in the circumoral kinety. The same phenomenon has been reported for the interphase cell of *H. vermiculare* during regeneration after the oral area has been cut off with a glass needle [17]. However, although the circumoral ciliature does not completely surround the cytostome during morphogenesis (Fig. 36), "bald spots" can usually be found in a non-divider as well (Fig. 5). Therefore, we cannot exclude that some reorganization takes place in the proter, but most probably, these observations correspond to non-division reorganization due to an injury inflicted on the ciliate during prefixation handling. That is, even in the case of non-regular stomatogenesis, the circumoral kinety is reorganized starting with the nonciliated (formerly somatic) nematodesma-bearing kinetosomes. That is corroborated by the observation that a kinetodesmal fibril and nematodesma can be associated with the nonciliated kinetosome at the same time in an early phase of reorganization [17].

When the division furrow has become more prominent, the oral cortex of the opisthe is not yet completely formed. Toxicysts are not very numerous and the oral filamentous sheath is rather incomplete (Fig. 37). Although the oral filamentous sheath is mostly lacking, the pattern of the overlaying bulge microtubules and transversal lamellae can be compared to that found in an interphase cell (Fig. 38). Nevertheless, bulge microtubules still seem to enter the oral region from the endoplasm (Fig. 39). Just before division, the cytoplasmic connection between proter and opisthe is situated within the circular oral field. The oral filamentous sheath is largely closed at that time and the overall organization of the oral apparatus is comparable to that of a differentiated cell (Fig. 40). The most striking character is the large number of highly ordered bulge microtubules in the oral endoplasm (Fig. 40), which are far less prominent in the interphase cell. In all likelihood, the endoplasmatic bulge microtubules act as guideline for the toxicysts, which move towards their launching sites within the oral cortex.

Discussion

Comparison with Related Species

No thorough ultrastructural analysis of morphogenesis in any ditransversal ciliate is yet available. On the light microscopical level, the results on morphogenesis in *H. vermiculare* are similar to those reported by Fryd-Versavel et al. [11], although only three stages have been shown in that study. In addition, morphogenesis in *H. vermiculare* is comparable to that found in other ditransversal ciliates as *Bryophyllum tegularum*, *Spathidium* sp. [11], *Spathidium muscorum* [2], *Protospathidium serpens* [3], and *Fuscheria terricola* [2].

Although hypothetical in some respects, the sequence of morphogenetic events in *H. vermiculare* (and possibly most other Spathidiida) can be summarized as follows:

Phase 1. The anteriormost somatic kinetosomes of the future opisthe are transformed into oral kinetosomes. They lose the kinetodesmal fibril, the postciliary ribbon, the somatic transverse ribbon and the ciliary shaft. Instead they become associated with the newly formed transversal lamella and nematodesmata. Thus, the oral kinety anlage is composed of monokinetids.

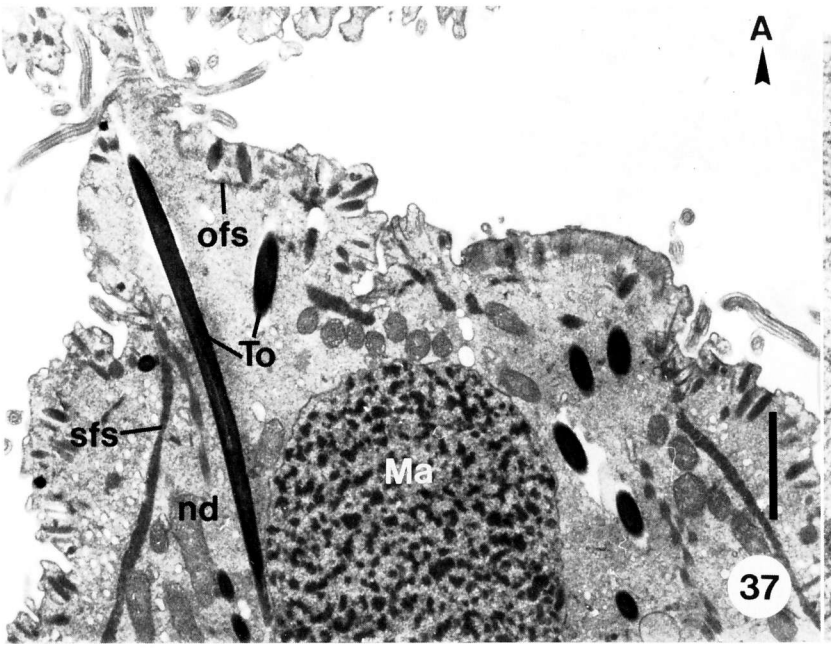
Phase 2. Newly synthesized kinetosomes are interspaced between the transformed somatic kinetosomes. Cilia arise from the newly formed kinetosomes. The nematodesmata associated with the transformed somatic kinetosomes grow out.

Phase 3. The fibrillar associates of the oral anlage are completed and it separates from the parental kinety: the anlage becomes a kinetofragment.

Phase 4. The dikinetid kinetofragments become curved, rotate to the right and eventually, after cell division is completed, join end to end to form a closed circumoral kinety. The events described in phase 4 apply also to *S. muscorum* [2], *B. tegularum* [2, 11], and *P. serpens* [3] except that in the latter no closed circumoral kinety is formed.

Although stomatogenesis starts with proliferation of kinetosomes in the brush kineties in all species of the

Figs. 37–40. Thin sections. Late stages of morphogenesis. – Fig. 37. Tangential section of a cell with a prominent division furrow. ▶ Numerous toxicysts (To) and nematodesmata (nd) can be found in the future oral region of the proter. The filamentous sheath of the oral region (ofs) is still only occasionally detectable, × 13,000. – Fig. 38. Higher magnification of the oral rim. The transversal lamellae (tl), the bulge microtubules (bmt) and part of the oral filamentous sheath (ofs) are present, × 43,000. – Fig. 39. Part of the circumoral kinety. Oral kinetosomes, nematodesmata (nd), transversal lamellae (tl), and bulge microtubules (bmt) can be seen, × 33,000. – Fig. 40. Part of the oral rim of the proter. The oral filamentous sheath (ofs) is almost completed and numerous mucocysts and toxicysts (To) are located beneath the plasma membrane as they are in an interphase cell, × 12,000.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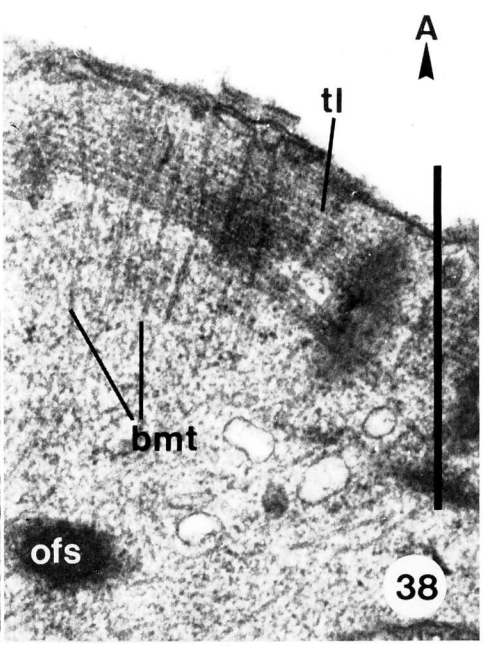
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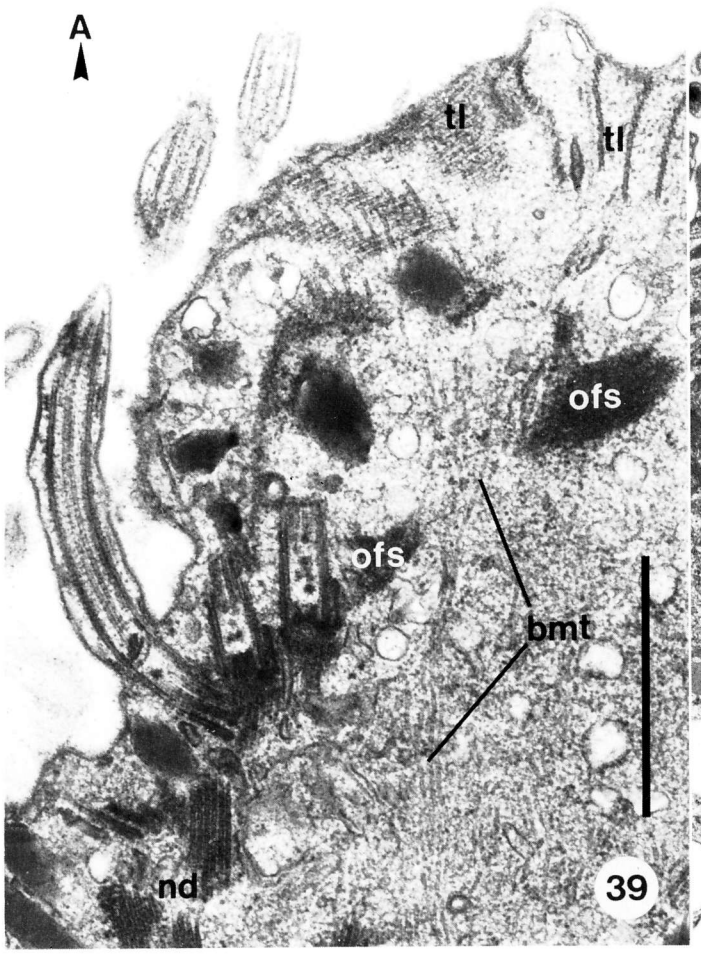
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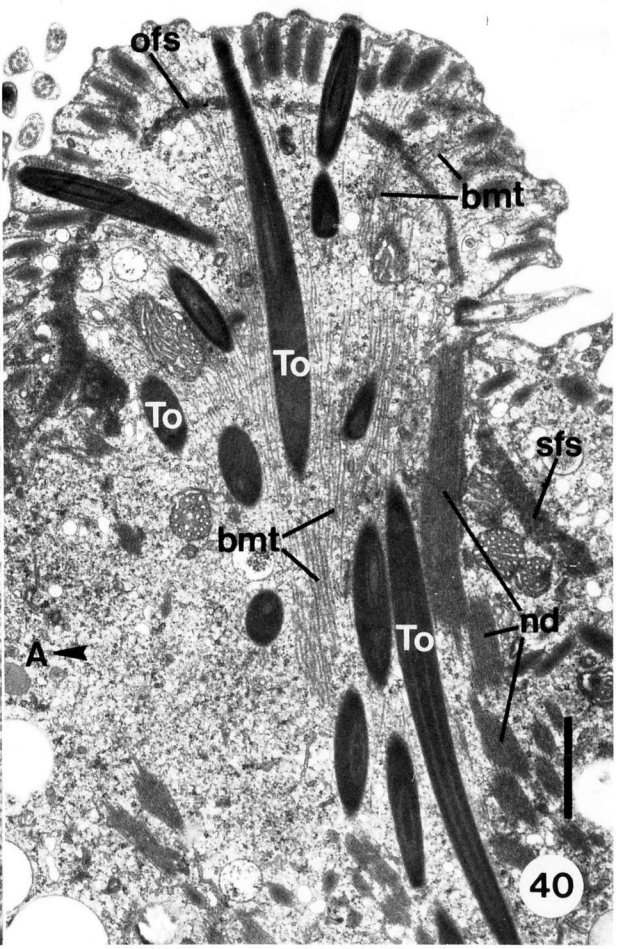
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suborder Spathidiina, later on, virtually all somatic kineties form kinetofragments in the plane of the future division furrow. Thus, stomatogenesis is of the holotelokinetal type. In *H. vermiculare*, the kinetofragments of the brush kineties are about two or three times longer than those on the right side. That is, they make a larger contribution to the circumoral kinety. However, that is no indication of a dominating role of the brush in stomatogenic events. Instead, it is probably due to the disproportionate distribution of the somatic kineties in *H. vermiculare* as there are only 3 kineties on the dorsal surface but about 10 kineties on the ventral surface. In contrast, in *S. muscorum*, where the transverse distance between the somatic kineties is constant, all kinetofragments have about the same size [2].

Comparison with Nonrelated Species

Beside the litostomate ciliates, similar conditions during stomatogenesis and interphase can be found in cyrtophorid ciliates, e.g., *Trithigmostoma steini* and *Chilodonella cyprini*. These ciliates have three circumoral dikinetid kineties, one of which arises by "head to tail" fusion of kinetofragments during morphogenesis [14, 15]. As in the Spathidiida, one kinetosome of each pair is ciliated and the other is nonciliated and gives rise to a nematodesma and a microtubular lamella. In addition, in both groups under question, the dikinetid kinetofragments arise from the anterior end of somatic monokinetid kineties. Likewise, in both groups the ciliated kinetosome becomes newly synthesized whereas the nonciliated nematodesma-bearing kinetosome arises from the ciliated kinetosome of the parental somatic kinety. In addition, a circumoral kinety that arises by head to tail fusion of dikinetid kinetofragments can be found among the prorodontid ciliates [13]. Despite the overall similarity, the circumoral kineties in the Rhabdophora and the Cyrtophora are probably not homologous as there are some important differences:

– Stomatogenesis is merotelokinetal in prorodontid and cyrtophorid ciliates but holotelokinetal or monotelokinetal in ditransversal ciliates.

– The transformed somatic kinetosomes in the oral kineties are associated with a postciliary ribbon in cyrtophorid and prorodontid ciliates but not in ditransversal ciliates.

– The position of the forming cytostome is ventral in cyrtophorid and prorodontid ciliates but apical in ditransversal ciliates (the apicalization of the ventral anlage in prostome ciliates has been subject to two thorough studies [13, 16]).

– The ditransversal ciliates are thought to have derived from a common ancestor that was not equipped with a circumoral dikinetid kinety (see below). That is, the circumoral dikinetids of the ditransversal ciliates are not homologous to the paroral dikinetids of either prorodontid or cyrtophorid ciliates.

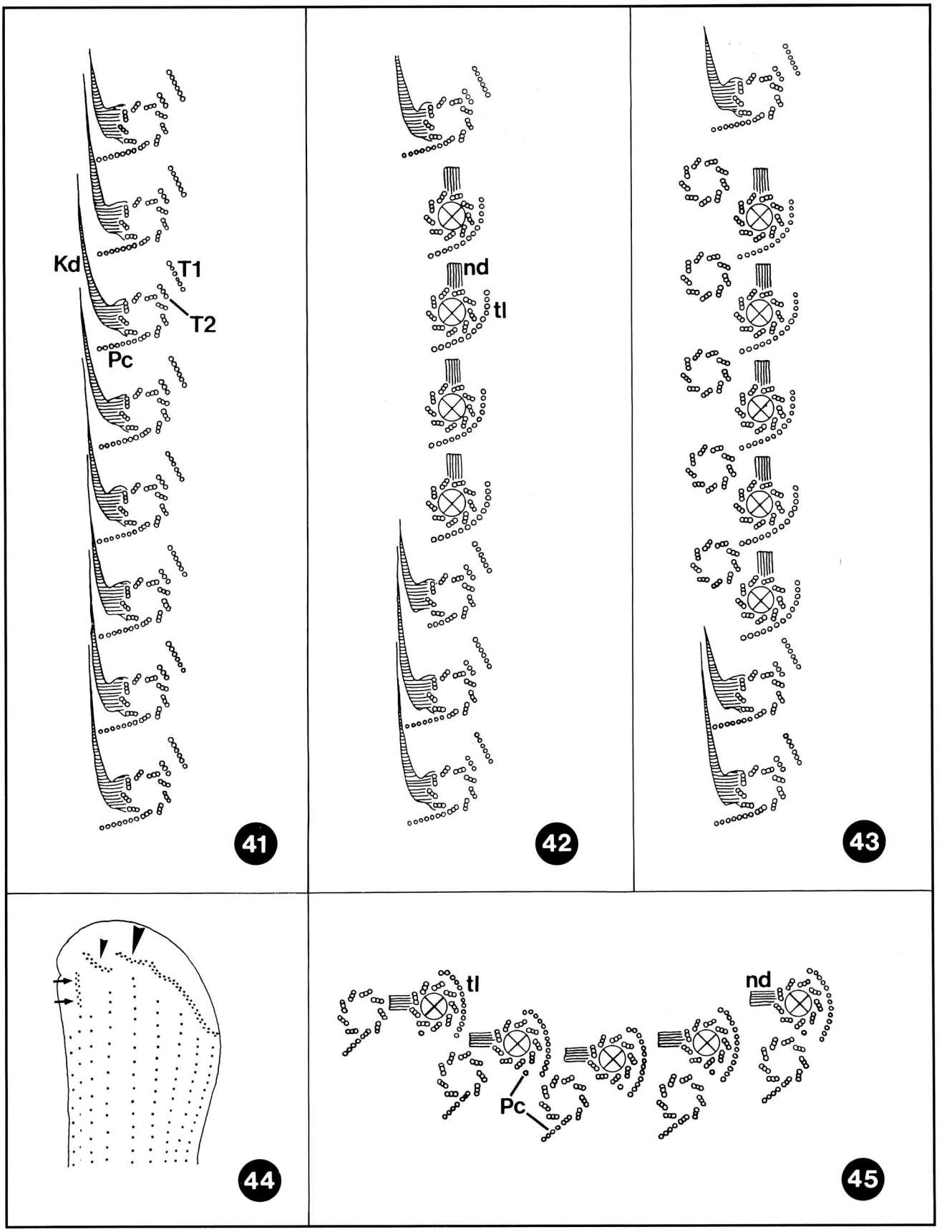
Phylogenetic Considerations

As we have shown, the dikinetids of the kinetofragments and the circumoral kinety, respectively, arise from the anterior ends of all somatic kineties of the opisthe. Except for the brush kineties, all somatic kineties are composed of monokinetids in *H. vermiculare*. As has been shown in an earlier paper [21], the infraciliary pattern of the brush dikinetids is derived from the somatic monokinetid pattern. That is, all oral dikinetids are directly or indirectly derived from somatic monokinetids. Meanwhile, it has been shown that a number of ditransversal ciliates do have oral monokinetids [9, 10 and references therein]. As pointed out above, in *H. vermiculare*, the anteriormost kinetosomes of the somatic kineties are transformed into the nonciliated kinetosomes of the circumoral kinety whereas the ciliated kinetosome of the circumoral kinety is newly formed. Therefore, we conclude that the oral kinety in ancestral ditransversal ciliates originally has been made out of monokinetids associated with nematodesmata and transversal lamellae. The division in labour between nonciliated kinetosomes and their associated fibrils (which strengthen the cytopharynx) and ciliated kinetosomes (which may have sensorial functions) is thought to be a derived character trait, which may have evolved several times independently. Thus, four stages can be hypothesized in the evolution of ditransversal ciliates (see [10] for a detailed listing of characters).

Stage 1. Oralized somatic kinetid stage. No specialized oral kinetids are present. Nematodesmata together with all or most of the somatic components (kinetodesmal fibril, postciliary and transverse ribbons, ciliary shaft) are associated with several of the anteriormost kinetosomes of all somatic kineties (e.g., Archistomatida).

Stage 2. Monokinetid stage. The first somatic kinetosome(s) in each kinety are transformed into oral kineto-

Figs. 41–45. Interpretive and schematic drawings of the development of the circumoral kinety. – Fig. 41. Anterior end of a single right lateral somatic kinety. Kd = kinetodesmal fibril, Pc = postciliary ribbon, T1, T2 = first and second transverse ribbon. – Fig. 42. Somatic kinetosomes become transformed into oral kinetosomes. A cross within a circle indicates a nonciliated kinetosome, the first kinetosome in the row represents the posteriormost kinetosome of the proter. nd = nematodesma, tl = transversal lamella. – Fig. 43. New kinetosomes are replicated adjacent to the transformed (formerly somatic) kinetosomes. Postciliary microtubules are not depicted here because they have not been detected in the anlage but they should hold the same position as in the kinetofragment in Fig. 45. – Fig. 44. Oral kinety anlage (arrows, corresponds to Fig. 43), migrating kinetofragment (small arrowhead) and integration into circumoral kinety (large arrowhead, corresponds to Figs. 19, 45) artificially depicted in a single cell at the same time. – Fig. 45. Kinetofragment as orientated in the interphasic circumoral kinety. Both kinetosomes of a pair are drawn parallel here although they usually are at angles. ▶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somes. They successively lose kinetodesmal fibrils, ciliary shafts and postciliary ribbons (except for a single microtubule?). Thus, a circumoral monokinetid kinety surrounds the cytostome. Nematodesmata still originate from the oral kinetosomes and from oralized somatic kinetosomes (e.g., *Enchelydium, Enchelys*).

Stage 3. Transition stage. Pairs consisting of transformed somatic kinetosomes and newly formed (usually ciliated) kinetosomes surround the cytostome. Nematodesmata originate from the transformed (usually nonciliated) kinetosome of the oral dikinetids and from oralized somatic kinetosomes (e.g., *Acropisthium, Actinorhabdos, Chaenea, Fuscheria*).

Stage 4. Dikinetid stage. Pairs consisting of transformed somatic kinetosomes and newly formed ciliated kinetosomes surround the cytostome. Nematodesmata originate exclusively from the transformed nonciliated kinetosomes of the oral dikinetids (e.g., *Bryophyllum, Homalozoon, Monodinium, Protospathidium, Spathidium*).

Remarks. I. The transition from one stage to the next may have happened several times independently. II. The hypothesis assumes that no distinction between oral and somatic ciliature and infraciliature (i.e., no oral ciliature s. str. at all) has been present in the ancestor of the ditransversal ciliates. Although that doesn't seem to be reasonable at first sight, a similar conclusion has been drawn by Hiller [13] based on an investigation of stomatogenesis in prorodontid ciliates. III. The hypothesis implies that the early circumoral kinety was composed of monokinetids, which were transformed somatic kinetosomes (stage 2, above). However, that does not necessarily mean that this applies to the oral monokinetids of any living ciliate. Hypotheses on the possible homology of oral kinetids in haptorian ciliates have been discussed at length by Lipscomb & Riordan [23]. They have concluded that the oral monokinetids are not homologous to each other and that the ciliated kinetosome of the oral dikinetid has been lost in *E. polynucleatum*. Unfortunately, the authors have failed to discuss their basic assumption but the frequent use of the term "normal haptorid oral dikinetid" indicates that they were assuming that the ancestor of all haptorid ciliates already had a circumoral kinety composed of dikinetids. That is in clear contradiction to the conclusion drawn in our paper although we cannot rule out the possibility that oral monokinetids may have evolved from oral dikinetids in individual cases.

Is the Rhabdophora Concept Still Valid?

The Rhabdophora are defined by the possession of transversal microtubules supporting the cytopharynx. However, the assumed homology between the transversal lamella and the somatic transverse ribbon has been questioned [14, 23]. The relevant findings from our study may be interpreted as follows:

I. In the oral apparatus of an non-divider of *H. vermiculare*, the nonciliated kinetosome bearing the nematodesma and the transversal lamella is associated with a single microtubule. Similarly, a single microtubule, which is thought to be of postciliary origin, can be found associated with the ciliated kinetosome of the oral dikinetids in other ditransversal ciliates like, e.g., *Chaenea teres* [23], *Helicoprorodon multinucleatum* [24], and *L. fornicis* [25]. If that holds true the transversal lamella cannot be homologous to the postciliary ribbon. Instead it is either homologous to a somatic transverse ribbon or it is uniquely derived.

II. If one assumes that the transversal lamella is homologous to the somatic postciliary ribbon in *H. vermiculare*, the somatic postciliary ribbon should detach from triplet 9, move in a tangential position and acquire the slightly bow-shaped form of the transversal lamella. In addition, the nonciliated kinetosome should rotate around its long axis so that the microtubular ribbon is facing the cytostome. However, nothing of the kind has been observed in our study.

III. As described above, the circumoral kinety arises from a rightward rotation of about 15 dikinetid kinetofragments and subsequent "head to tail" fusion of the fragments. If one compares the kinetofragment (Figs. 30, 31) with the circumoral kinety in an interphase cell (Fig. 21), it becomes evident that the orientation, shape and number of microtubules of the transversal lamella remains unchanged during rightward rotation and "head to tail" fusion. Considering the slight rotation of the nonciliated kinetosome, the anterior portion of the transversal lamella is situated at the same place where both somatic transversal ribbons have been before. In addition, there can be little doubt from the published micrographs that the transversal lamella is situated in the same position to the kinetosomal diameter as the somatic transverse ribbons in the oralized and nonoralized somatic kinetosomes of the somatic kineties in *E. polynucleatum* [9].

For the time being, it seems likely that the transversal lamella is either homologous to the somatic transverse ribbon or newly acquired. Thus, in either event, the Rhabdophora concept is still valid. Nevertheless, the transformation into the transversal lamella has not directly been observed and the bow-shaped form of the transversal lamella cannot be compared either to the shape of the first or the second somatic transverse ribbon. In addition, the position of the transversal lamella in the oral kineties anlage may have been misinterpreted due to a possible early rotation of the kinetosome. Thus, further studies on morphogenesis in ditransversal ciliates are highly desirable. It would be a good idea to use a ciliate like *E. polynucleatum*, which has oralized somatic kinetids, as in that case, the position of the fibrillar associates and the axis of the kinetosome can be more easily inferred than in *H. vermiculare*.

Acknowledgements

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Key words: Ciliates – *Homalozoon vermiculare* – Morphogenesis – Phylogeny – Rhabdophora – Ultrastructure

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