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Oral Monokinetids in the Free-Living Haptorid Ciliate *Enchelydium polynucleatum* (Ciliophora, Enchelyidae): Ultrastructural Evidence and Phylogenetic Implications[1]

WILHELM FOISSNER and ILSE FOISSNER[2]

Institut für Zoologie der Universität Salzburg, Akademiestrasse 26 and Institut für Botanik der Universität Salzburg, Lasserstrasse 39, A-5020 Salzburg, Austria

ABSTRACT. Special ultrastructural characteristics of the haptorid soil ciliate *Enchelydium polynucleatum* Foissner, 1984 are the restriction of the parasomal sacs to the area of the "brush" and finger-like projections of the food vacuole membrane into the lumen of the vacuole. The general organization of the infraciliature is similar to that of *Spathidium* and some buetschliids because the anterior ends of the somatic kineties are condensed and obliquely bent. *Enchelydium* is similar to haptorids and buetschliids in possessing monokinetid somatic fibrillar structures with the classical fibrillar associates: 1) a short kinetodesmal fiber; 2) two transverse microtubular ribbons; 3) a long postciliary microtubular ribbon; and 4) a system of overlapping subkinetal microtubules, which seems to be absent in the buetschliids. Unlike *Spathidium* and all other haptorids so far investigated ultrastructurally, serial sections show that there are no oral dikinetids, as in the endocommensal buetschliids and balantidiids. Instead, three to six anterior kinetids in each ciliary row have nematodesmal bundles extending into the cytoplasm and surrounding the cytopharynx. These kinetids lack cilia and all fibrillar associates except enlarged transverse ribbons, which extend anteriorly and inwards to support the cytopharynx. Other similarities between the buetschliids and *Enchelydium* are the conspicuous rough endoplasmic reticulum and abundant sausage-like vesicles in the oral region. As in other haptorids, *Enchelydium* has two types of toxicysts and one type of mucocyst. These observations strongly suggest that *Enchelydium* belongs to the ancestral stock of both the Haptorida and the Archistomatida. The similarities in the somatic and oral infraciliature and ultrastructure of the Haptorida and the Archistomatida suggest that they belong to the same subclass, Haptoria Corliss, 1974.

LYNN (29) emphasized that oral monokinetids probably occur only in the rather specialized endosymbiotic buetschliids and balantidiids. He doubted earlier results of some French workers (for review see 32) who claim that monokinetid-type oral structures are also present in hypotrichs, oligotrichs, and in the gymnostome *Lacrymaria*. Puytorac et al. (34) reemphasized the presence of oral monokinetids in their study of *Stylonychia mytilus*. Lynn's assumption, however, seems to be supported by recent studies (12, 24) which present evidence that the paroral membranes of the hypotrich *Paraurostyla weissei* originate from paired basal bodies and that the lacrymariids possess oral dikinetids. Very probably, the monokinetidal organization of the paroral membrane in some hypotrichs is a derived character and is the result of a longitudinal splitting of originally paired basal bodies (24). It is also possible to generate oral monokinetids by a secondary loss of a basal body from each pair, as is seen in the colpodid *Pseudoplatyophrya nana* (35) and the tetrahymenid *Ophryoglena mucifera* (36).

This paper presents for the first time clear light-microscopical and ultrastructural evidence for oral monokinetids in a free-living "lower" gymnostomatous ciliate.

MATERIALS AND METHODS

Enchelydium polynucleatum Foissner, 1984 was collected from the top soil (0–2 cm) of a meadow near Salzburg (Schaming near Eugendorf) on 24 III 1984. The soils of this area are loamy and slightly acid (pH 5–6). The sample was air-dried for about three weeks, remoistened with distilled water, and investigated after six days. Pure cultures could be established in 1% (v/v) lettuce medium and in pure Eau de Volvic containing bacteria and an undetermined species of the *Tetrahymena pyriformis* complex as food supply. Cultures grew slowly and the specimens became very fragile and smaller, indicating suboptimal conditions. Thus, we used only material from the raw cultures described above.

Cells were processed for transmission electron microscopy following the procedure of Lynn (28, pers. commun.) at room temperature. The ciliates were fixed in 3.0% (v/v) glutaraldehyde in 0.05 M Na-cacodylate buffer, pH 7.0. After a 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. They were then washed twice in buffer and stored for ca. 12 h in the buffered glutaraldehyde fixative. On the next day, cells were washed again in buffer, dehydrated in a graded ethanol series, transferred through propylene oxide into Epon, and flat-embedded in aluminum weighing pans, which were then incubated at 60°C for two days. Individual cells were cut from the flat molds with a heated scalpel and cemented at the desired orientation onto the face of epoxy blocks. Ultrathin sections were cut with a diamond knife mounted on a Reichert Ultracut and stained with aqueous uranyl acetate and lead citrate. Stained sections were viewed and photographed on an AEI Corinth 500 electron microscope.

The light microscopical observations were performed with a microscope equipped with conventional and differential-interference-contrast optics. Protargol slides were prepared from both the type-population (12) and from that described here. They differed only in some minor details which will not be mentioned because they are unimportant in the context of the present paper.

RESULTS

General organization. A detailed light-microscopical and biometrical description of *Enchelydium polynucleatum* Foissner, 1984 has been published earlier (12). Thus, we repeat only the most important features and refer to Figs. 1, 2, 4, 9, 25. This large (in vivo ca. 100–200 × 20–50 μm) soil ciliate is characterized by numerous (100–300) macronuclear segments, many micronuclei, and a cartridge-like body form that often becomes pouch-like in prepared specimens. The oral bulge is circular to elliptical, and depressed in the center; its rim is filled with the pointed protrusions of toxicysts. The center of the cytopharynx contains an argentophilic structure resembling an inverted cone (Figs. 2, 4). The single contractile vacuole, which discharges its

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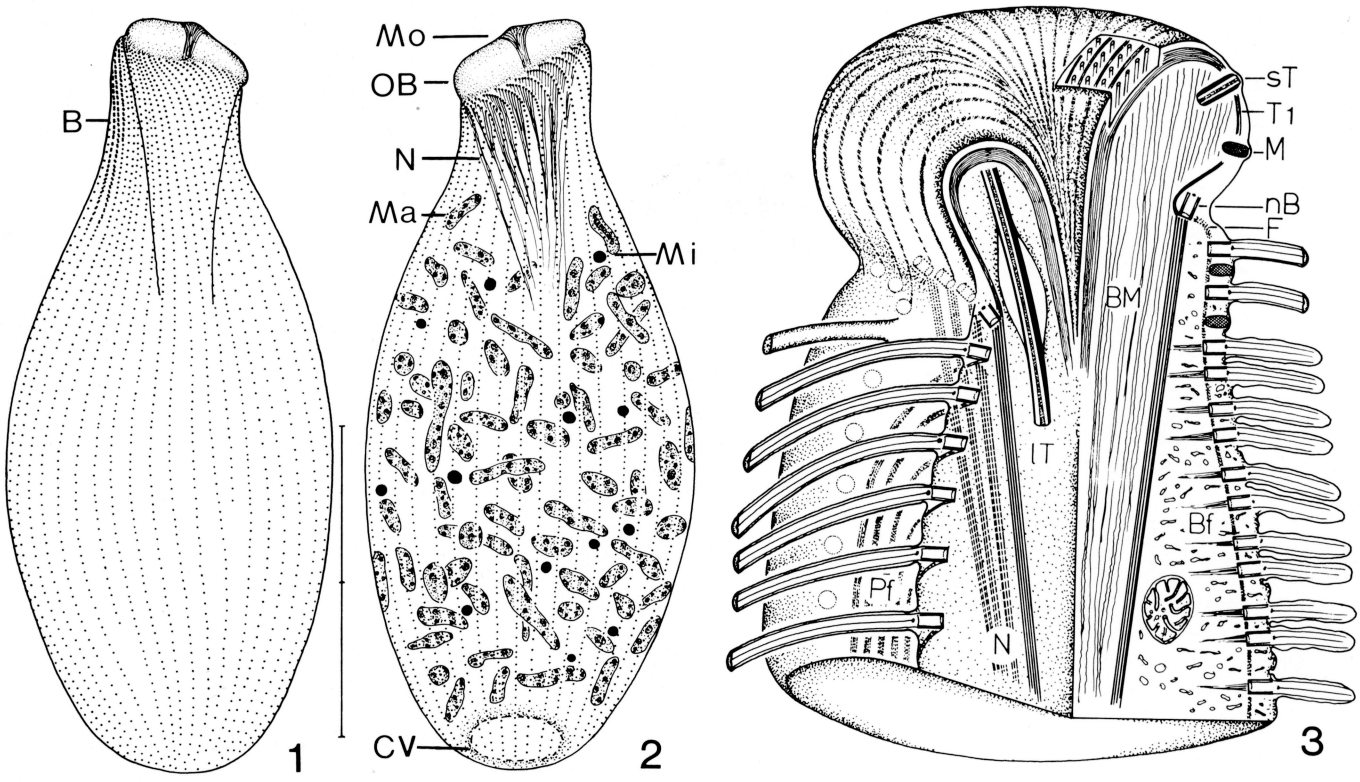

Figs. **1–34**. Infraciliature and ultrastructure of *Enchelydium polynucleatum*. Figs. 1, 2, 4, 9, 25 are drawings and light micrographs from protargol-impregnated specimens; Fig. 3 is a diagrammatic drawing representing a reconstruction of the oral area; Figs. 5–8, 10–24, 26–34 are transmission electron micrographs.

Figs. **1**, **2**. Infraciliature of the right and left side. Nematodesmata (N) are depicted in the left lateral view only. They originate from the anteriormost basal bodies of the somatic kineties, whose anterior ends are condensed and obliquely bent, on the left side more than on the right one. Only a few of the ca. 200 macronuclear segments (Ma) are shown. The brush (B) consists of three kineties with paired basal bodies. The cytostome (Mo) is situated in the center of the oral bulge (OB) and delineated by the first transverse microtubule ribbons. At the posterior end there is the contractile vacuole (CV). Mi, micronucleus. Bar = 50 μm.

Fig. **3**. Reconstruction of the oral area of *Enchelydium polynucleatum*. Cell's right is to observer's left. Two sectors have been removed, exposing pellicular and internal organization. A somatic kinety is sectioned on the left side, a brush kinety on the right side. Only relevant fibrillar components of the somatic kinetids are shown. See legend for structures depicted and text (and following figures) for further explanation of relationships, etc. Bf, brush fibers; BM, bulge microtubules; F, microfibrillar zone (*tela corticalis*); lT, long toxicyst; M, mucocyst; N, nematodesmata; nB, non-ciliated basal body; Pf, postciliary fibers (microtubules); sT, short toxicyst; T1, first transverse microtubule ribbon.

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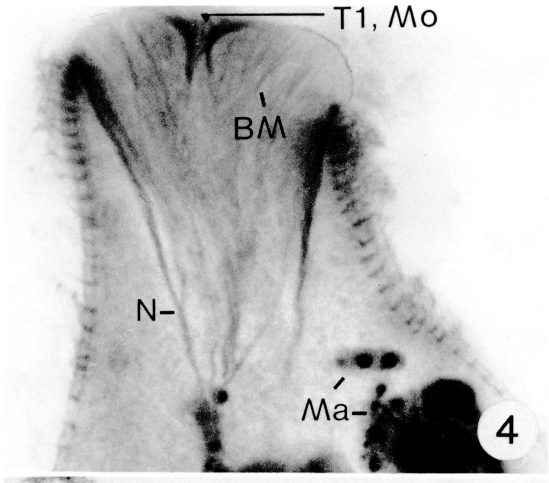
Figs. **4–8**. Structure of the oral area. **4**. An optical section through the anterior region showing the cytostome (Mo), which is delineated by the first transverse microtubule ribbons (T1) and the cytopharynx, which is surrounded by the nematodesmata (N). Note the faintly stained bulge microtubules (BM) and some heavily impregnated macronuclear segments (Ma). ×2000. **5**. Transverse section through the cytopharynx. It is surrounded by densely packed first transverse microtubule ribbons (T1) originating from the non-ciliated basal bodies of the somatic kineties. One bulge microtubule (BM), a mucocyst (M), and some clear vesicles (V) are visible. ×60,000. **6**. Transverse section through the anteriormost part of one half of the oral bulge. Note the highly ordered pattern of first transverse microtubule ribbons (T1), bulge microtubules (BM), and transversely and obliquely sectioned short and long toxicysts (sT, lT). ×22,400. **7**. Longitudinal section through the oral bulge and the cytopharynx. The cytostome is not visible, because the bulge is sectioned at its edge. Note first transverse microtubule ribbons (T1), short and long toxicysts (sT, lT), and many sausage-like vesicles (sV) within the cytopharynx, which is delineated by the nematodesmata (N). ×6300. **8**. Oblique section of the oral bulge. On the left side are the first transverse ribbons (T1) and the cytopharynx (Mo), on the right are nematodesmata bundles (arrows) and somatic kineties. The cytopharynx is filled with sausage-like (sV) and clear vesicles. ×6600.

Figs. **9–15**. Structure of the oral kinetids. **9**. Infraciliature of the left side showing nematodesmata bundles (N) originating from the anteriormost kinetids of the somatic kineties. ×2500. **10**. Transverse section through the cytopharynx. Inside there are nematodesmata (N), sausage-like (sV) and clear (V) vesicles, outside, some mitochondria (Mi). ×54,000. **11**. Longitudinal section of one oral and two somatic kinetids. A compact first transverse microtubule ribbon (T1), which extends anteriorly, and a nematodesmata bundle (N), which projects into the cytoplasm, originate from the non-ciliated basal body (nB). The first transverse ribbon (T1) of the ciliated somatic kinetids is accompanied by a transverse fiber (Tf). ×30,000. Figs. **12–15**. Serial longitudinal sections of some somatic (numbered 1, 2) and oral kinetids (numbered a–h). The oral kinetids are unpaired and lack the kinetodesmal fiber (Kf) and the postciliary ribbons, but have nematodesmata (N) and a transverse fiber (Tf), which is adjacent to the first transverse microtubule ribbon (T1). The ellipsoidal dark bodies are mucocysts (M). ×30,000.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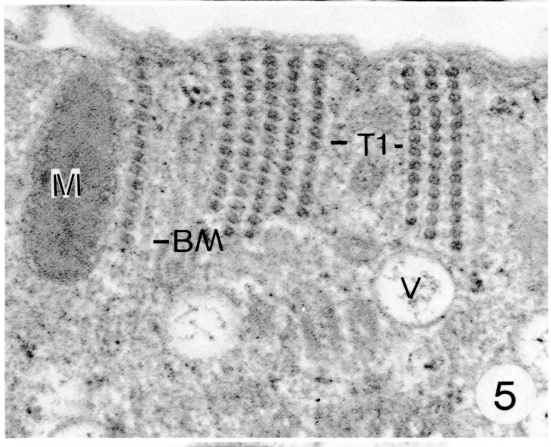
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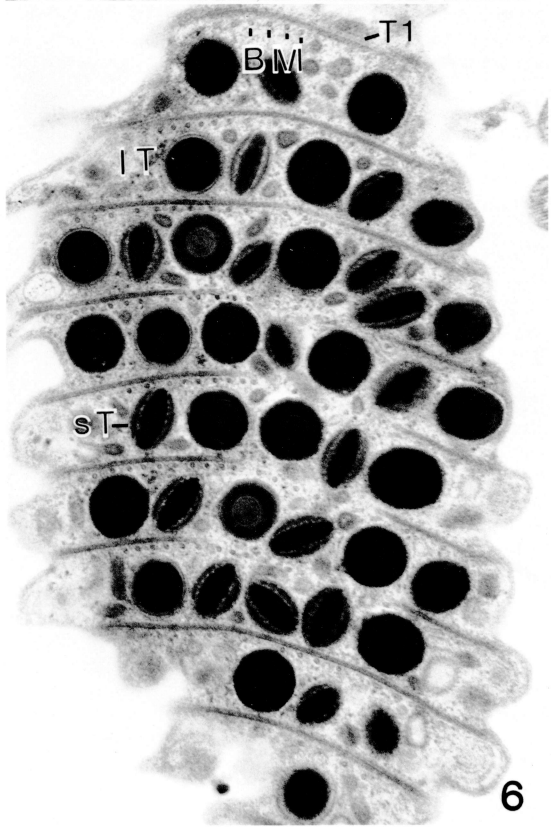
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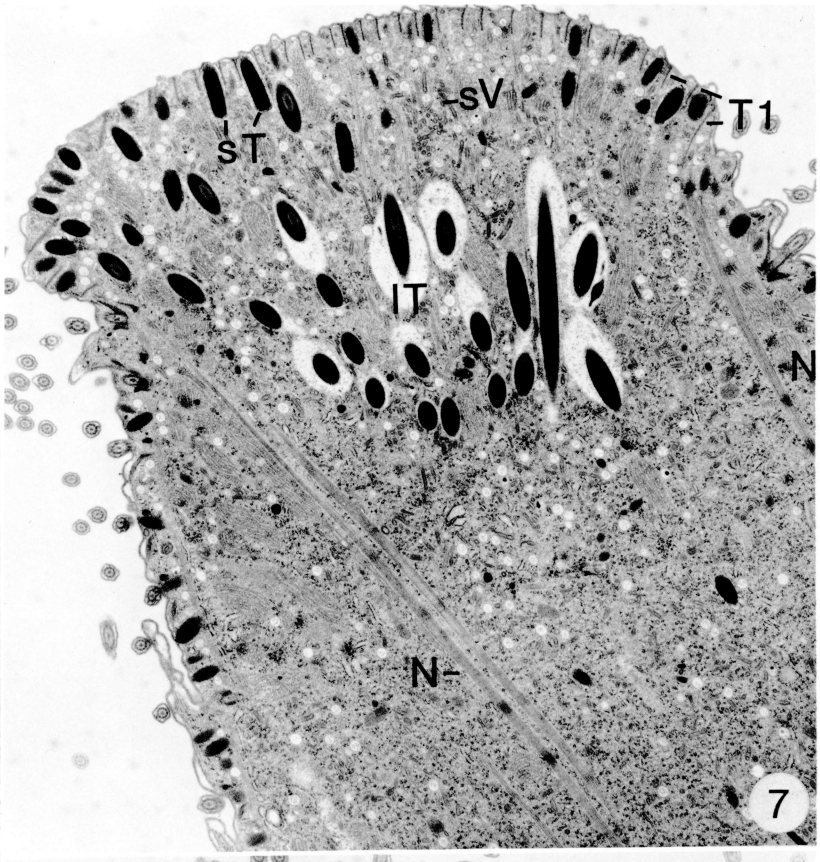
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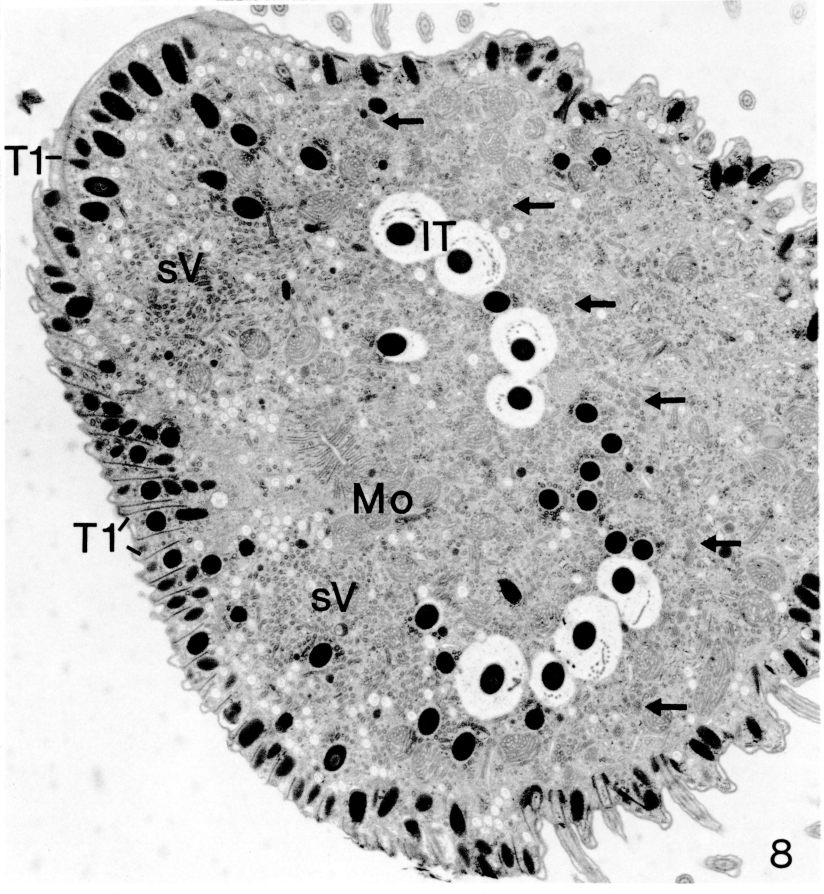
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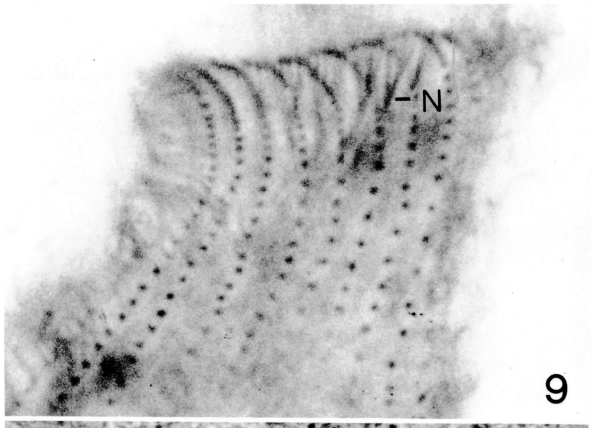
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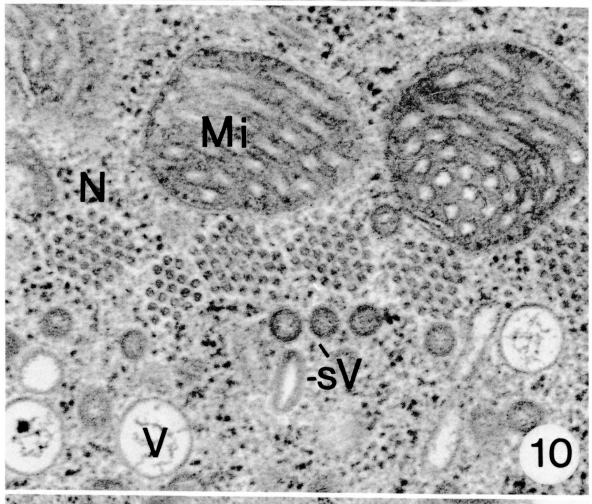
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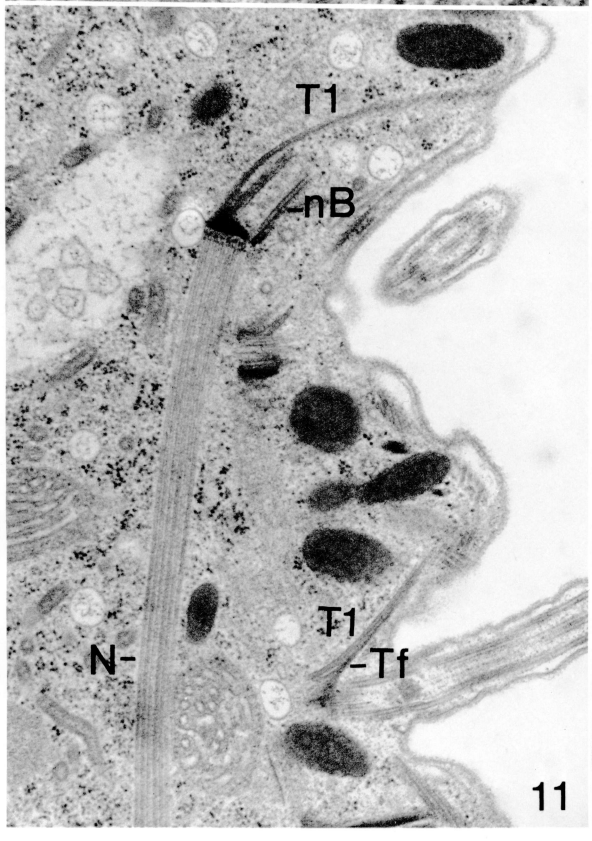
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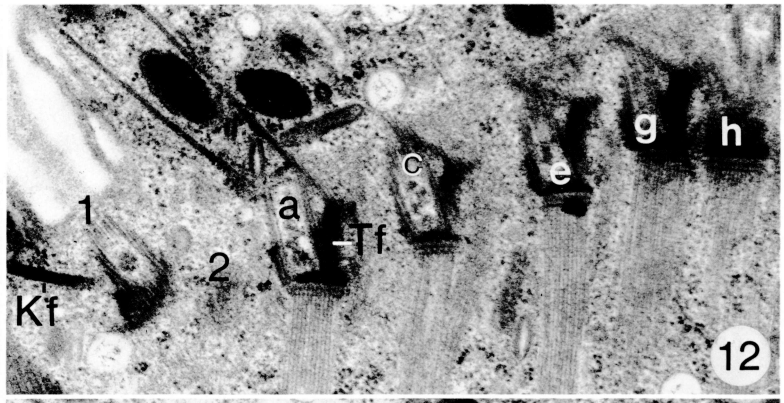
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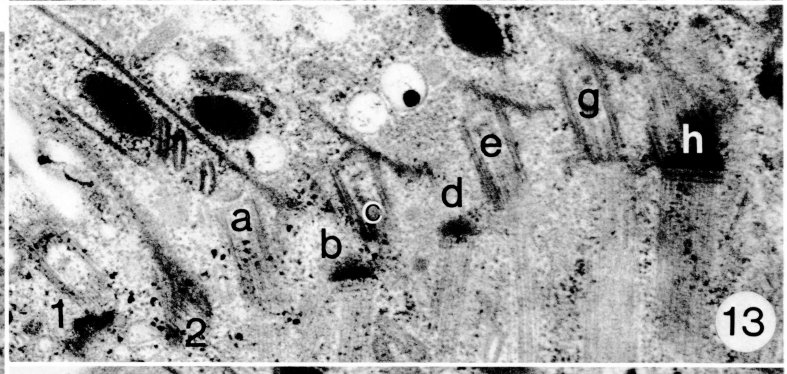
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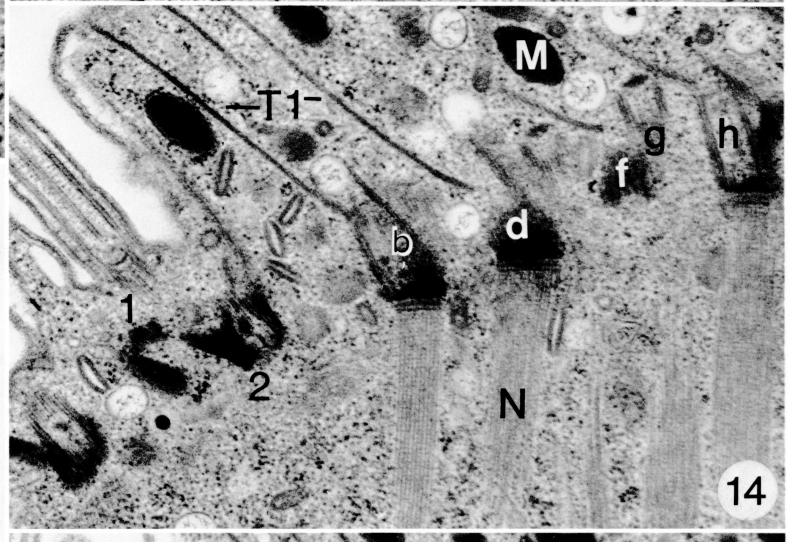
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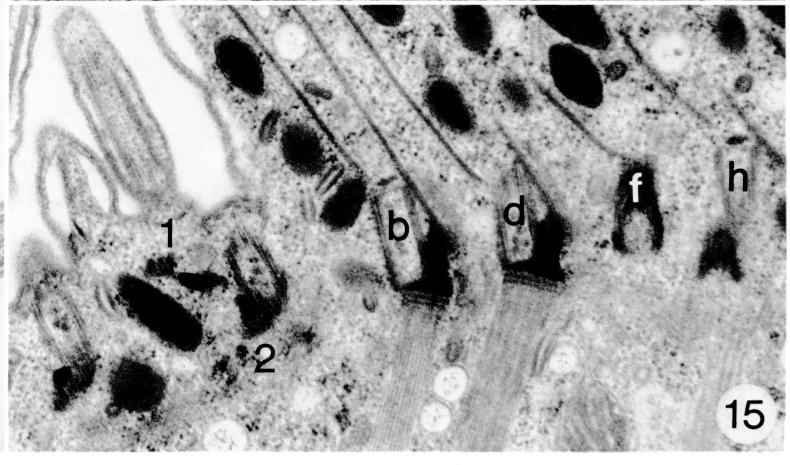
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content through many tubular pores, is located at the posterior end of the body. The infraciliature is very simple: 35–45 ($\bar{x}$ = 39.1; n = 13) longitudinal somatic kineties, which bend counterclockwise (organism viewed from the anterior end) close behind the oral bulge. A special circumoral kinety is absent. The rhabdos is formed by nematodesmata originating from the uppermost three to six basal bodies of each somatic kinety. The "brush" is situated on the dorsal side and consists of three short kineties with paired basal bodies (Figs. 1, 2, 9).

Organization of the oral area. The oral region is dominated by fibrillar derivatives of the somatic kineties. Its bulge-like form and the anterior bending of the kineties (Figs. 1, 3, 4, 9) make it extremely difficult to get appropriate sections. Thus, it is impossible to interpret single pictures and, because the matter is important, we include two sets of serial sections that clearly show all details.

The most prominent structures in the oral area are the large transverse microtubular ribbons that originate from the anterior three to six kinetids of each somatic kinety. These specialized oral kinetids have a terminal plate, an axosome, and nematodesmal bundles, but lack cilia and all of the other fibrillar components characteristic of the somatic kinetids. They are implanted in the ectoplasm and are not connected by desmoses or any other material (Figs. 9, 11–19). The ribbons consist of 8–12 ($\bar{x}$ = 11.1; n = 8) microtubules and are very probably homologous to the first transverse ribbon of the somatic kinetids, which consists of only about six microtubules (Figs. 5, 17). The oral transverse ribbons originate from an electron-dense material (dense fiber of the somatic kinetids) at the inner side of the basal bodies, turn to the rim of the oral bulge, and extend anteriorly to the center of the bulge, where they plunge inwards to support the cone-like cytopharynx. The ribbons surround the cytopharynx in a fan and terminate in the mid-region of the cytopharynx (Figs. 4, 8, 11–15). The nematodesmata originate from the same electron-dense material as the transverse ribbons and are displaced from their basal bodies by the width of one microtubule. They make an obtuse angle to their basal body, are ca. 30 μm long, and consist of 17–35 ($\bar{x}$ = 27.8; n = 12) hexagonally packed microtubules in the upper part of the cytopharynx. Close below the dense material, the nematodesmata show two narrowly spaced dark lines (Figs. 2, 4, 7–15). The third filamentous components of the oral region are vertically oriented long microtubules. They start just beneath the pellicle of the bulge and terminate at the same level as the nematodesmata. In the bulge, they are regularly spaced and are immediately adjacent to the transverse ribbons. Transverse sections of the bulge show a very regularly repeated pattern: longitudinally sectioned transverse ribbons, adjacent transversely sectioned bulge microtubules, and toxicysts (Figs. 3, 6, 24). The regular spacing of the vertically oriented microtubules disappears in the cytopharynx, where they form loosely organized nematodesmata-like bundles.

Figure 3 offers a diagrammatic three-dimensional representation of the oral area of *E. polynucleatum.*

Organization of the somatic cortex. The pellicular and subpellicular organization and the somatic fibrillar structures of *E. polynucleatum* are very similar to those described in *Spathidium spathula* (44), *S. amphoriforme* and *Spathidium* sp. (6), *Lacrymaria* (3, 41), and *Dileptus* (18, 25). Because the above-cited authors, especially Williams et al. (44), have treated the somatic cortex of these species so masterfully, we refer to their descriptions and—for comparison—to our Figs. 3, 11, 12, 14, 19, 23, 26–31. Some details, however, that are different or not mentioned by these authors will be noted.

There are 8–12 ($\bar{x}$ = 9.6; n = 11) flat alveoli and 3–5 ($\bar{x}$ = 3.9; n = 10) mucocysts between two kineties (Fig. 31). We found only one type of mucocyst-like extrusomes and never observed any that appeared discharged. They are ca. 700 nm in length and ca. 250 nm in diameter and have the typical paracrystalline contents (Figs. 23, 27, 31). The microfibrillar *tela corticalis,* which is interrupted at the site of the basal bodies, is ca. 100 nm in thickness and terminates together with the somatic kineties at the oral bulge (Figs. 11, 12, 14, 28, 30, 31). Parasomal sacs occur only in the area of the "brush" (Figs. 28, 30).

The microtubular and fiber systems of the somatic kinetids have the typical haptorid pattern (Figs. 3, 19, 26, 27, 29): a kinetodesmal fiber; about six postciliary microtubules associated with a strut-like structure similar to the condition known for *Spathidium spathula* (44); a long first transverse ribbon that consists of ca. six microtubules arching anteriorly along the electron-dense transverse fiber; a short second transverse ribbon of three (n = 10) microtubules, which projects laterally from the anteriormost part of the first ribbon attachment site; 2–10 ($\bar{x}$ = 4.7; n = 12) subkinetal microtubules running anteriorly, sloping down deeply into the cytoplasm, and usually overlapping the next one or two basal bodies.

The paired basal bodies of the brush are arranged in a zigzag pattern, especially in the anterior region. At the beginning of the brush kineties, which are located between high (ca. 1 μm) interkinetal crests, there are some unpaired normal somatic kinetids. Both basal bodies of a pair have short cilia (ca. 2–3 μm) with a rather irregular ciliary membrane and a disorganized axoneme that lacks one or both central microtubules and sometimes one of the two microtubules of the outer pairs. A nematodesmata-like bundle of microtubules projecting straight into the cytoplasm originates from the proximal center of the basal bodies. The anterior basal body of a pair possesses only these rootlets whereas the posterior one has not only the rootlets but also the normal fibrillar equipment of the somatic kinetids. The second transverse ribbon is perhaps absent (Figs. 28, 30). Further details will be published later.

Internal organization. Enchelydium polynucleatum has long (ca. 14,000 × 370 nm), slightly curved and short (ca. 760 × 280 nm), straight toxicysts, whose structure is similar to those

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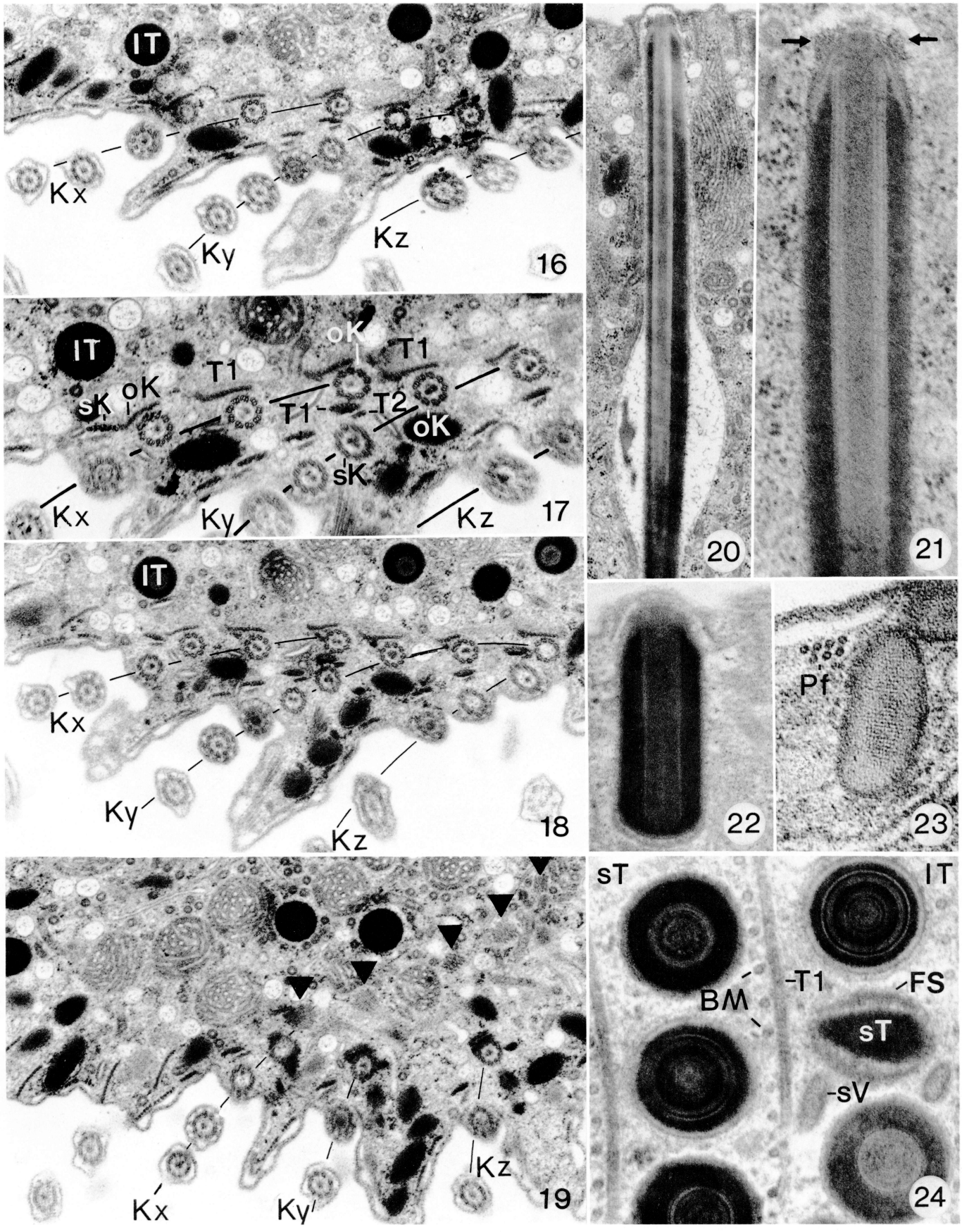
Figs. **16–24.** Fine structure of oral kinetids and extrusomes. **16–19.** Four serial transverse sections of some somatic (sK) and oral (oK) kinetids. Three kineties are shown (Kx, Ky, Kz). To make clear the relationship, their basal bodies are connected by dark lines. Fig. 17 is slightly enlarged to show fine details. The oral kinetids are unpaired and lack the kinetodesmal fiber, the second transverse microtubule ribbon (T2), and the postciliary microtubule ribbon (Pf), but have an enlarged first transverse ribbon (T1), a transverse fiber, and a nematodesmal bundle (triangles in Fig. 19). The dark bodies are mucocysts and long toxicysts (lT). ×25,400; Fig. 17, ×36,000. **20, 21.** Longitudinal sections of long toxicysts. Note the large, clear vesicle at the middle part and the ring of granular material at the tip (arrows). ×24,000, ×66,000. **22.** Longitudinal section of a short toxicyst. It is enclosed by fibro-granular material. ×58,000. **23.** Longitudinal section of a mucocyst. Note its paracrystalline content and the postciliary fibers (Pf). ×56,000. **24.** Enlarged detail from Fig. 6 showing transversely and obliquely sectioned short and long toxicysts (sT, lT) between the first transverse microtubule ribbons (T1) and their adjacent bulge microtubules (BM). The short toxicysts are enveloped by a fibrillar sheet (FS). ×67,000.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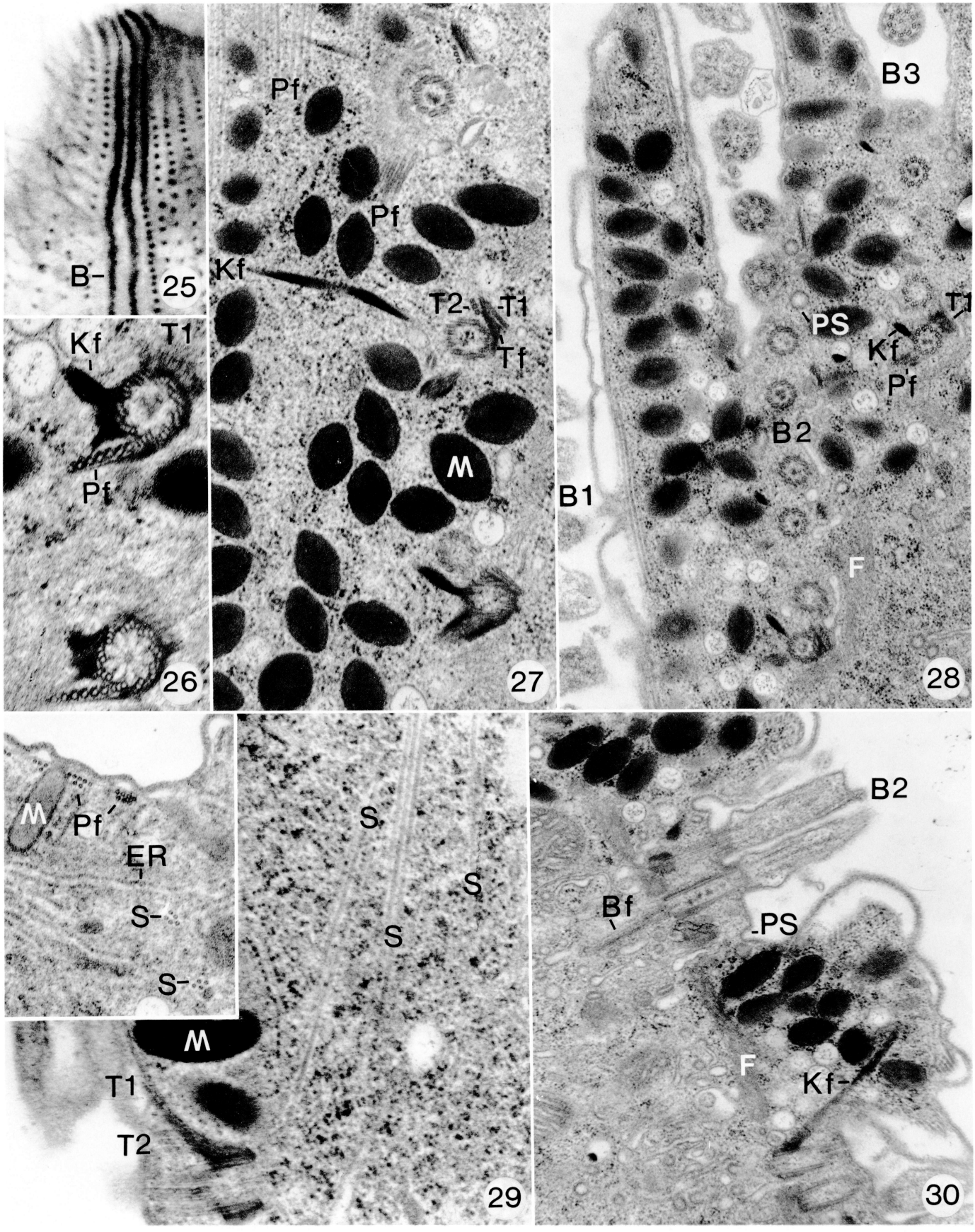
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of other haptorids. Both are membrane-bounded and can be easily distinguished in transverse sections.

The long type shows ca. 10 layers of different electron densities whereas the short type possesses only about four zones. The long toxicysts are surrounded in the middle part by a large vesicle that contains some fuzzy material. They have an arrow-like anterior end, and the tip is encircled by a ring of granular material. The short toxicysts are restricted to the oral region. They are surrounded by a rather prominent (ca. 30 nm) layer of dense material. Their anterior end is arrow-like; posteriorly they are broadly rounded (Figs. 20–22, 24).

The cytoplasm is extremely rich in rough endoplasmic reticulum, but contains relatively few, irregularly distributed mitochondria of typical ciliate morphology (Figs. 10, 31, 32). A remarkable regular cisterna of the endoplasmic reticulum is closely adjacent to the *tela corticalis*. Serial sections indicate that it is interrupted only at the site of the basal bodies. Thus, this plate-like cisterna separates the ectoplasm from the endoplasm (Figs. 29 inset, 31). In the oral region there are many membrane-bounded, sausage-like vesicles whose inner side is covered by fuzzy material (Figs. 6–8, 10, 11). Between these structures are many larger, clear vesicles that contain some granulo-fibrillar material. They have a rather constant diameter of ca. 130–200 nm ($\bar{x} = 171.4$; $n = 16$). The cytoplasm underneath the brush contains many tube- and drop-like vesicles, which seem to pinch off from the parasomal sacs (Figs. 28, 30).

The outer membrane of the macronuclear envelope has its cytoplasmic side densely covered with ribosomes. The macronuclear segments contain many heavily stained masses of chromatin and one or more less heavily stained, granular nucleoli (Figs. 4, 31, 32). The micronuclei are of the "chromosomal" type (38), showing heavily stained sausage-like chromosomes embedded in a less stained granular matrix (Fig. 32).

Enchelydium polynucleatum feeds on ciliates (*Colpoda* spp., *Tetrahymena* sp.). The food vacuoles show many finger-like protuberances especially during the middle stages of the digestion process (Fig. 33). It seems that many or all of the membranous and vesicle-like residues are discharged through the pores of the contractile vacuole (Fig. 34), but it could also be that the membranes in the contractile vacuole derive from another source.

DISCUSSION

Pellicular and cytoplasmic organization. The fine structure of *E. polynucleatum* resembles very much that of haptorids like *Spathidium* (44) and that of buetschliids like *Alloiozona* (15, 16). The somatic fibrillar structures are monokinetids and show a similar arrangement of the classical fibrillar systems. Subkinetal microtubules and a second transverse microtubule ribbon are probably absent in the buetschliids but seem to be widespread among the Haptorida (44). Unlike the trichostomatids and entodiniomorphids (14), the buetschliids and the haptorids do not possess a fibrillar network at the bases of the basal bodies. Similarly, there are no root-like microtubules as in the somatic kinetids of *Balantidium* (31). The organization of the brush is comparable to that of *Spathidium* and *Bryophyllum* (6). Parasomal sacs exist in *Spathidium* (44), but seem to be absent in the buetschliids and in many other haptorids. In *Enchelydium* they are restricted to the area of the brush. The strongly vacuolized cytoplasm below this organelle suggests that the sacs could be sites of pinocytotic activity. A granulo-fibrillar *tela corticalis* is present in all groups and species under discussion. It is more conspicuous in the buetschliids (16) than in *Enchelydium* (Figs. 26, 31) and other haptorids (e.g. 3, 6, 10, 17, 18, 25, 44).

Abundant sausage-like vesicles in the oral area and a very prominent rough endoplasmic reticulum are typical for both the buetschliid *Alloiozona* and the haptorids *Enchelydium* and *Chaenea* (10, 16). In other haptorids they are rather inconspicuous (3, 6, 17, 18, 27). These vesicles may be homologous with the disc-shaped vesicles of *Paramecium* which are involved in the formation of the membrane of the nascent food vacuoles (1, 2). During the recycling process of food vacuoles, the bulge microtubules could act as "guidelines," as is supposed for the microtubular ribbons of *Paramecium* (1, 2). In *Homalozoon,* however, some function during attachment and discharge of the toxicysts has been suggested (27). This is supported by the position of the bulge microtubules between the transverse microtubules and the extrusomes (Fig. 24). The clear vesicles between the sausage-like structures could be phagosomes (cf. 1, 2).

The prominent finger-like folds of the food vacuoles have been found only in *Folliculina* (43), *Phacodinium* (9), *Climacostomum* (11), and *Nassula* (21). Obviously, they occur in widely separated groups and thus are probably more closely correlated with physiological requirements than with phylogenetic trends.

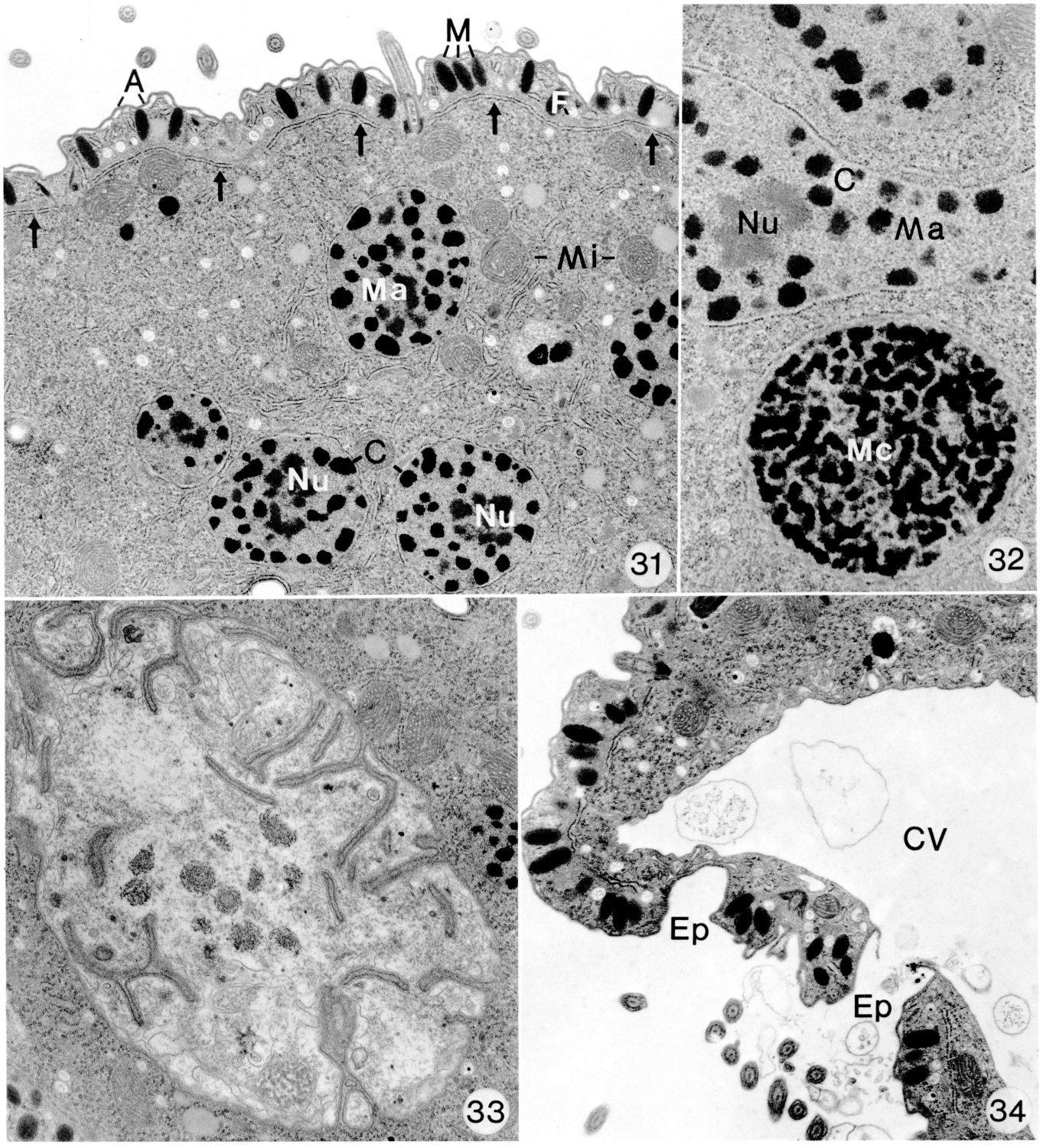
Enchelydium polynucleatum, like other haptorids (19), possesses two types of toxicysts and one type of mucocyst. Electron-opaque mucocyst-like bodies (5, 6, 44) are absent. The structure of the toxicysts is similar to that of other haptorids (3, 5, 6, 17, 18, 20, 26, 33). A more detailed analysis shows some minor differences, especially between the short toxicysts of the two groups. For instance, they are more complicated in *Lagynophrya* (17) and more spongy in *Litonotus* (5) than in *Enchelydium.*

Oral structures and phylogenetic implications. In the following section we refer mainly to the macrosystems of Corliss (8) and Small & Lynn (40). Jankowski (23) proposed a very dissimilar classification, which will not be considered because these and other results (12, 13, 40) strongly oppose some of his ideas.

"There is little doubt from the electron micrographs that monokinetids surround the cytostome of buetschliids and balantidiids. The more anterior kinetids in each ciliary row have nematodesmal bundles extending into the cytoplasm and surrounding the cytopharynx, while the most anterior kinetid in each kinety lacks a kinetodesmal fibril but has the other fibrillar associates; its tangential transverse ribbon, together with the transverse

←

Figs. **25–30**. Structure of the somatic cortex and the brush. **25**. Infraciliature of the dorsal side showing three brush kineties (B) with densely packed paired basal bodies. The somatic kineties consist of widely spaced monokinetids. ×2500. **26**, **27**. Tangential section of some somatic kinetids. They possess a kinetodesmal fiber (Kf), a postciliary microtubule ribbon (Pf), a first transverse microtubule ribbon (T1) with its adjacent transverse fiber (Tf), and a second transverse ribbon (T2). The mucocysts are distributed along and between the kineties. ×64,000, ×37,200. **28**. Tangential section of the brush kineties (B1–B3). Only the posterior basal bodies of the pairs possess kinetodesmal (Kf), postciliary (Pf), and transverse (T1) fibers. Some parasomal sacs (PS), the microfibrillar *tela corticalis* (F), and disorganized brush cilia are recognizable. ×25,000. **29**. Longitudinal and transverse (inset) sections showing a basal body with its first and second transverse (T1, T2) and subkinetal (S) microtubules. Close beneath the pellicle are postciliary microtubules (Pf), mucocysts (M), and the endoplasmic reticulum (ER). ×50,000; inset, ×30,000. **30**. Longitudinal section of brush kinety 2 (B2). Note the brush fibers (Bf), the kinetodesmal fiber (Kf), the *tela corticalis* (F), parasomal sacs (PS), and many cytoplasmic vesicles. ×25,000.



Figs. **31–34**. Fine structure of the cortical region, nuclear apparatus, food vacuoles, and contractile vacuole. **31**, **32**. Cross-sections through the middle region of the cell. The cortex consists of membrane-bounded flat alveoli (A), mucocysts (M), and a microfibrillar *tela corticalis* (F). It is separated from the endoplasm by a large cistern of the endoplasmic reticulum (arrows). The macronuclear segments (Ma) contain dense chromatin bodies (C) and diffusely stained nucleoli (Nu). Note the irregularly formed chromatin of the micronucleus (Mc) and the randomly distributed mitochondria (Mi). ×9300, ×21,600. **33**, **34**. The food vacuoles have many finger-like protuberances. The membranous food residues are discharged through the contractile vacuole pores (CV, Ep) at the posterior end of the cell. ×10,500.

ribbon from the second kinetid in the row apparently extend anteriorly and inwards to support the cytopharynx" (29). This pattern is very similar to that found in *Enchelydium,* with the remarkable deviations that the anteriormost kinetids that bear the nematodesmata lack the cilia and the postciliary ribbons. In all other haptorids whose fine structure has been examined, there are oral dikinetids with one ciliated and one non-ciliated basal body from which the nematodesmata originate (29, 32), with one or perhaps two notable exceptions. The first one is *Acropisthium* (4), where the nematodesmata originate not only from the non-ciliated basal bodies of the oral dikinetids but also from some following somatic kinetids which form a ciliary corona resembling that of *Monodinium* and *Didinium.* But the last-mentioned genera lack ciliated somatic kinetids and their nematodesmata originate only from the dikinetids (39). These dissimilarities justify the recently (12) proposed transfer of *Acropisthium* from the Didiniidae to the Trachelophyllidae. The second case is less convincing. Holt et al. (22) claim that in *Actinobolina* nematodesmata arise from both ciliated and non-ciliated basal bodies and thus sketch a scheme that is similar to *Acropisthium,* but their electron micrographs show no conclusive proof. Very recently, however, Foissner (12) provided light-microscopical observations that *Fuscheria* and *Actinorhabdos* may have a similar pattern. In contrast, the oral structures of *Enchelys* and *Papillorhabdos* are more similar to that of *Enchelydium* (12). Although these observations are not electron-microscopical, the evidence is clear enough to suggest new definitions for some problematic haptorid families (12). Thus, we shall not discuss here the consequences that arise from the present results for the lower taxonomic categories (families and genera).

Can we be certain that *Enchelydium* lacks oral dikinetids? Unfortunately, a negative character is difficult to demonstrate, and the anterior bending of the kineties makes the interpretation of the sections extremely difficult. Lynn (29) and others define the haptorid oral dikinetid as "A 'posterior' ciliated kinetosome with which a convergent postciliary ribbon and a tangential transverse ribbon are associated; an 'anterior' non-ciliated kinetosome with which are associated a tangential transverse ribbon that extends anteriorly to support the cytopharynx." We have never seen such a pattern in *Enchelydium.* The only site where a dikinetid-like pattern occurs is in the zone where the oral kinetids end and the somatic ones begin, that is, about three to six kinetids away from the anterior ends of the kineties. Here the impression of dikinetids is strengthened because all oral kinetids are skewed slightly with respect to the somatic ones (Fig. 17), but this is probably because they are located at the base of the bulge. We cannot exclude the possibility that the last somatic and the first oral kinetid comprise a functional unit. But such a pattern is neither typical haptorid nor known for any other ciliate. Further strong evidence is provided by the protargol-impregnated specimens. They look quite different from ciliates like *Spathidium* and *Enchelyodon,* which possess a heavily impregnated kinety that encircles the oral bulge and is composed of oral dikinetids (12). Such a kinety is obviously absent in *Enchelydium* (Figs. 1, 2, 9). Hence, it is reasonable to conclude that *Enchelydium* possesses oral monokinetids.

Puytorac et al. (37) and Corliss (8) unite in the Prostomatida the suborders Archistomatina, Prostomatina, and Prorodontina. This classification is not convincing, not only because of differences in the fibrillar associates of the somatic kinetids (40) but also in the light of the present results, which show many more similarities between the oral structures of the Haptorida and the Archistomatina than between the Haptorida and the Prostomatina and the Prorodontina. Thus the ideas of Small & Lynn (40) are more plausible. They place the Haptorida and the Archistomatida in the class Litostomea, but unite the Archistomatida, Trichostomatida, and Entodiniomorphida in one subclass, the Vestibulifera, and the Haptorida and the Pleurostomatida in another, the Haptoria. Considering the present results and the fact that the buetschliids lack the typical vestibulum of the trichostomatids and entodiniomorphids, we suggest including the Archistomatina into the Haptoria Corliss, 1974 (7). In fact, some buetschliid genera (e.g. *Curcurbella* [42]) are in many respects so similar to *Enchelydium* and *Enchelys* that they surely would have been classified as Haptorida if they were free-living and did not possess a concrement vacuole. This enigmatic organelle, which occurs also in some trichostomatids (e.g. the Blepharocythidae), but is absent in all haptorids, was probably one reason to unite them in the same subclass (40); however, the concrement vacuole of the Blepharocorythina and the Paraisotrichidae is perhaps not homologous with that of the buetschliids (8). Another interesting point concerns the so-called brush, which occurs in almost all Haptorida (12). Unfortunately, no clear record of its presence is available in the Archistomatina, either in the works of Wolska (e.g. 45, 46) or Grain (15, 16). Some evidence is available that specialized cilia exist in the oral region of *Balantidium* but they seem not to be paired (31).

Ciliates like *Enchelydium,* which lack oral dikinetids, are good candidates for the expected (8) missing links between the present day archistomatids and their free-living ancestors. Perhaps *Enchelydium* is the putative ancestral stock of the endosymbiotic litostomates. The specializations exhibited by the buetschliids can be interpreted as resulting from their adaptation to a symbiotic way of life.

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