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Protistology

The fine structure of the tropho-tomont of the parasitic apostome *Chromidina* (Ciliophora, Apostomatida)

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Summary

Scanning and transmission electron microscopy of *Chromidina*'s tropho-tomont stage have revealed new information about this parasitic ciliate of cephalopods. The kineties are uniformly spaced and have a fold of the pellicle (kinetal fold) on the right side of the ciliary row. Kinetodesmal (kd) fibers are well developed along the trunk of the cell and are enlarged at the anterior attachment bulb, extending into the kinetal folds. At the junctions of daughter cells the kd fibers become disorganized and extend randomly into the junctional cytoplasm. The macronuclear (MA) reticulum is connected by microtubular tracts within thin extensions of the MA double membrane. The cytoplasm is filled with various vacuoles and enclosures, some of which are associated with microtubular tracts similar to those associated with the outer pellicle. No mouth or endocytic apparatus was detected except for a pore along the trunk.

Key words: apostome, ciliate, *Illex coindetti*, symbiosis, ultrastructure

Introduction

The ciliate *Chromidina* is an unusual protozoan found within the kidneys of cephalopods. Currently three species have been described: *Chromidina elegans* (Foettinger, 1881), *C. coronata* (Foettinger, 1881) and *C. cortezi* (Hochberg, 1971). Other morphologies have been observed but not formally described (Hochberg, 1971, 1982, 1990). The *Chromidina* tropho-tomont is long (> 1000 μm), uniformly ciliated, and has no mouth (Chatton and Lwoff, 1935). The tropho-tomont attaches to the renal appendages of squid, cuttlefish, and octopus, and hangs its posterior end into the renal sacs where it is bathed in the fluid upon which it feeds. Two division processes occur in the host: 1) monotomy, which produces "apotomites" from the posterior end which form more tropho-tomonts and populate the host kidney, and 2) palintomy, which produces smaller tomites with a unique ciliature and which are reported to leave the kidney to infest an intermediate host (Hochberg, 1971, 1982). To date however, the life cycle is not known and no intermediate hosts have been confirmed.

Chatton and Lwoff (1935) recognized *Chromidina* as an apostome ciliate in their monograph on the Apostomatida, even though this genus is strikingly different from more typical apostome genera

such as *Gymnodinioides, Vampyrophrya*, or *Foettingeria* which feed on exuvial fluid, crustacean tissues, or cnidarian chyme. Their monograph still provides the most complete description of the genus.

Little has been published on the taxonomy or anatomy of *Chromidina* since 1935 (Hochberg, 1971, 1982, 1990), and no reports exist to explain features of the feeding stage using electron microscopy. This first ultrastructural analysis of *Chromidina* provides new information about the mechanism of attachment, the mechanism of feeding, and the structure of the kineties.

Material and methods

The southern short-finned squid *Illex coindetti* was collected from the Gulf of Mexico on the NOAA ship Gordon Gunter (November 2008, 2009) and NOAA ship Pisces (November 2010). The squid renal sacs were punctured and flushed with seawater using a pipette to dislodge the ciliates from the renal and pancreatic appendages. This fluid was withdrawn and the ciliates were pipetted into fixatives for light and electron microscopy. For light microscopy (LM) the cells were fixed in 1-2% unbuffered glutaraldehyde. The cells remained in fixative until processed at Troy University for silver nitrate staining (Chatton and Lwoff, 1935) or for hematoxylin staining using the Heidenhain method (Davenport, 1960). For transmission and scanning electron microscopy (TEM and SEM) in 2008-09 the cells were fixed for 1,5– 24 hr in 2–4% glutaraldehyde buffered with 0.05 M sodium cacodylate, pH 7.5. The ciliates were transferred to buffer for the remainder of the cruise. The ciliates were post-fixed at Troy University in 1–2% OsO_4 for 4 hr and subsequently dehydrated. Specimens for SEM were critical point dried in CO_2 at the University of Georgia's Center for Advanced Ultrastructural Research, and photographed at Auburn University's Research Instrumentation Facility using a Zeiss EVO 50 scanning electron microscope. In 2010 cells for TEM were fixed and post-fixed on board the NOAA ship Pisces using a modified protocol with 2% glutaraldehyde/0.2% tannic acid (2 hr) followed by 0.5% OsO_4/1% potassium ferrocyanide (2 hr) (Landers, 2011). All TEM specimens were dehydrated in isopropanol and embedded in Spurr's epoxy. Thick sections (1–2 μm) were stained with sodium borate-buffered toluidine blue. Thin sections were stained with uranyl acetate and lead citrate, and photographed at Auburn using a Zeiss EM-10CR transmission electron microscope (60 KV). LM photographs of whole stained ciliates and thick sections were taken using a Nikon E600 light microscope and a digital camera. Digital images (LM and SEM) and scanned TEM negatives were adjusted for brightness, contrast, and gamma with Adobe Photoshop® Elements 6.0.

Results

Live observations

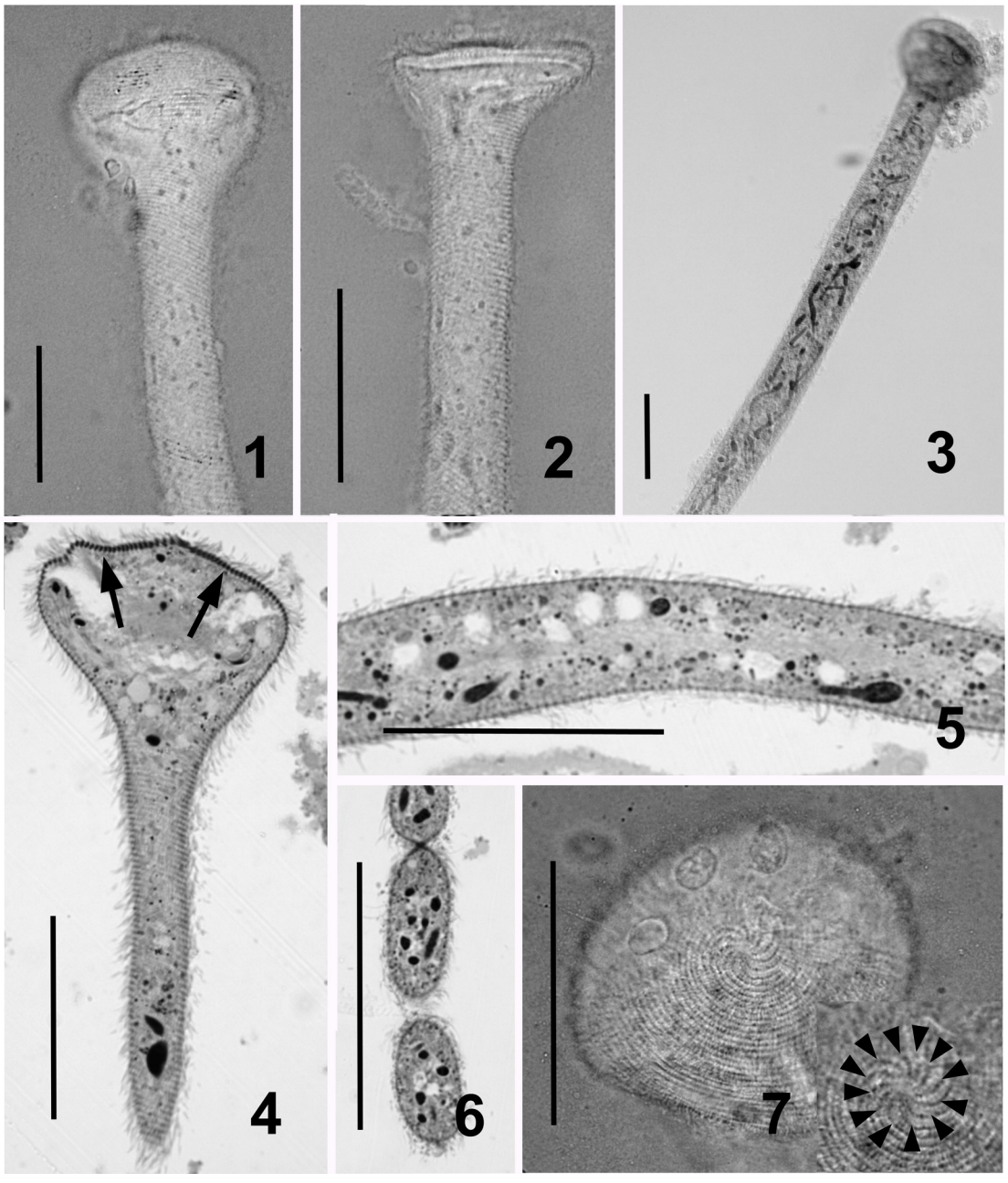
The tropho-tomont stage swam rapidly in seawater after detachment from the host renal appendages. The ciliates often attached to the glass slide or coverslip with their anterior end, as a leech would attach to its host. The anterior bulb flattened completely against the glass or plastic dish to form a disk, making the cell difficult to detach with a jet of seawater from a pipette. In one cell the flattened apex had a diameter of ~108 μm and a trunk width of 25 μm, though the unattached anterior bulbs in other live cells had diameters of ~35–57 μm.

Light microscopy of fixed material

The trunk of the cell averaged 30 μm wide (N= 10) with a length of over 1500 μm. The anterior end averaged 71 μm wide (N= 10). Chatton-Lwoff silver nitrate stains revealed 12 dextrally-spiraled kineties that originated at the apex, and continued uninterrupted down the trunk of the cell (Figs 1, 2, 7). Hematoxylin stains revealed the macronuclear reticulum, which wound throughout the trunk and extended into the anterior end (Fig. 3). Distinct dense fibers associated with the kineties were observed at the anterior attachment bulb in thick (1-2 μm) plastic sections (Fig. 4). These fibers were also associated with the trunk ciliature though they were not as large as they were on the top of the attachment bulb. Internally the cytoplasm was filled with darkly-stained islands of macronuclear chromatin, clear vesicles, darkly-stained vesicles, and osmium-positive oil droplets (Fig. 5). The posterior end of the cell revealed divisional stages that could be either segments arising from monotomy or tomites arising from palintomy (Fig. 6).

Electron microscopy

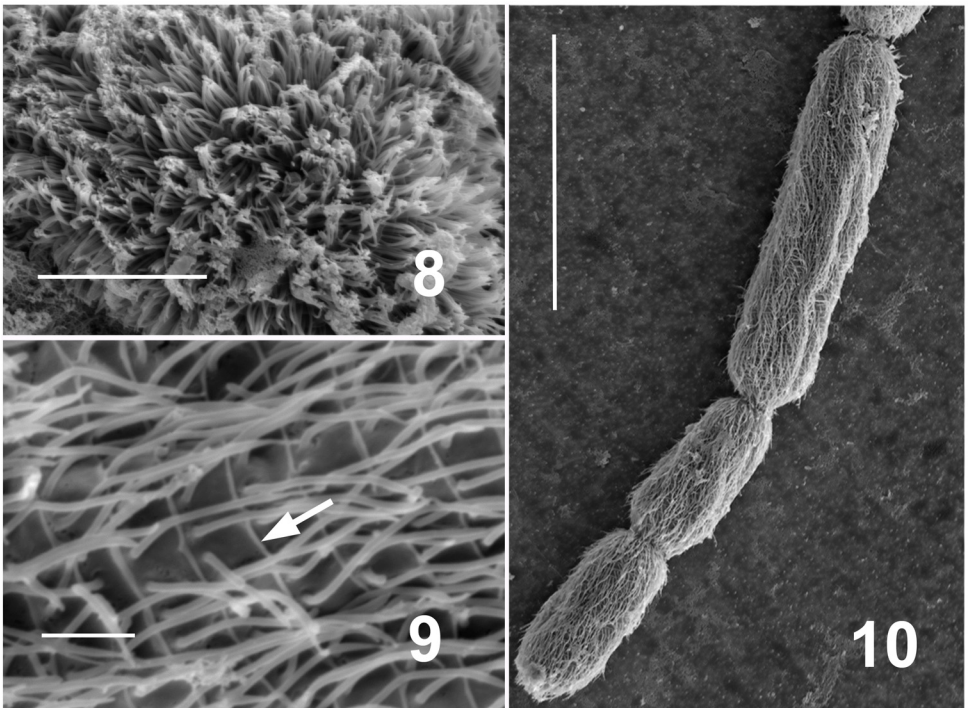
Scanning electron microscopy of tropho-tomonts (Figs 8-10) revealed a uniform ciliation along the trunk of the cell, which extended into divisional junctions between daughter cells. Each kinety was bordered on



Figs 1-7. Light microscopy of Chromidina. 1, 2 - The anterior end of two tropho-tomonts showing the spiraling kineties and the bulbous or cupped morphology of the apex. Silver nitrate stain; 3 - Heidenhain's iron hematoxylin stain of the tropho-tomont. The reticulate macronucleus is stained darkly; 4 - section of a tropho-tomont with large fibers visible at the apex (arrows); 5 - section of a tropho-tomont revealing dark branches of the macronucleus, clear vacuoles, and vesicles of various size; 6 - three daughter cells in section; 7 - the apex of an unsectioned cell showing 12 spiralling kineties and no mouth; inset - 12 kineties are identified. Silver nitrate stain. Scale bars: 50 μm.

the right by a fold of pellicle known as a kinetal fold (Landers, 1990). Ctene folds, or small folds perpendicular to the kinetal fold and located between each cilium, were not observed though they are known from other apostomes. The ciliature at the anterior end was too dense to observe the pellicle and the kinetal folds.

Transmission electron microscopy (Figs 11-26) demonstrated that the kinetal folds had a variable structure based on location. Along the trunk, the folds were located above the kinetodesmal fibers (kd) on the right side of each kinety (Figs 11-13). At the anterior end of the cell, the kd fibers were



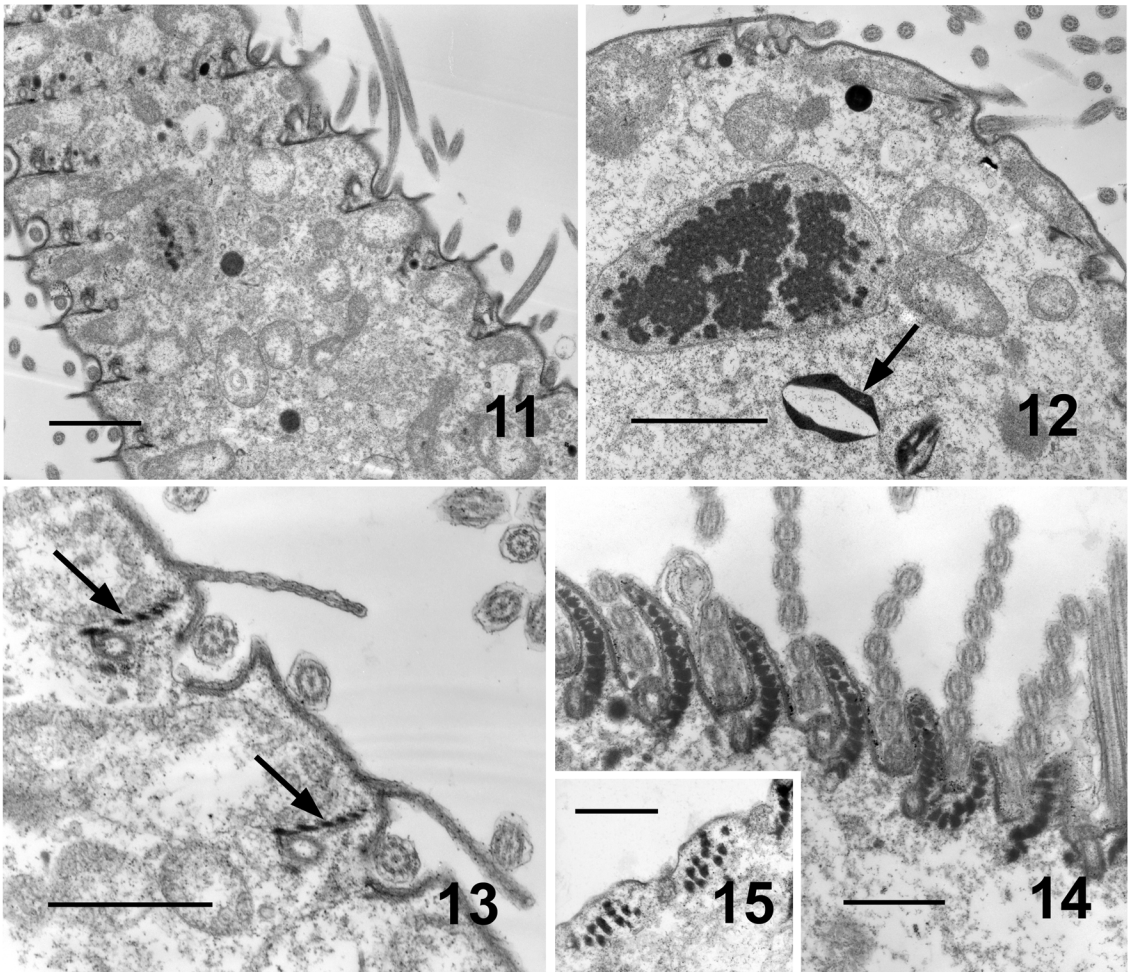
Figs 8-10. SEM of the tropho-tomont. 8 - The anterior end is obscured by the dense ciliation; 9 - high magnification of the tropho-tomont surface, showing the kinetal folds on the right side of each kinety (arrow); 10 - low maginification shows the uniform ciliation of the cell dividing at the posterior end. Scale bars: 8 - 10 μm, 9 - 2 μm, 10 - 50 μm.

enlarged, and formed large stacks that extended into expanded kinetal folds (Fig. 14). Some sections also revealed areas where the kd fibers were disorganized and not stacked on each other, which may indicate areas near division furrows (Fig. 15). Glancing sections of the pellicle confirmed the thin kinetal folds along the trunk, and broad kinetal folds filled with large kd fibers at the anterior end (Figs 16-17). Additionally, glancing sections revealed parasomal pores associated with the cilia and showed that cilia arose from rounded depressions in the pellicle at some locations. Transverse microtubules arose from the left base of each cilium, in groups of 6-8, and extended laterally to the next kinety, projecting into the kinetal fold. Rare sections that either glanced the pellicle or cut through junctional sites revealed numerous subpellicular electron dense fibers, which were apparently disorganized kd fibers extending into junctional regions (Figs 18-19). Pellicular extensions connected the divisional stages at these locations.

The reticulate macronucleus of the tropho-tomont extended throughout the cell. Sections revealed cylindrical extensions of the reticulum as well as islands of chromatin connected by microtubular tracts (Figs 20-23). The microtubules were also present within the masses of chromatin. The cytoplasm of the tropho-tomont stage contained mitochondria, crystalline bodies, and vacuoles (Figs 22-26). The mitochondria were predominantly subpellicular and located between the kineties. Microtubular tracts, similar to those at the kineties, were located around some of the vacuoles. No mouth, nasse, or pharyngeal structures of any kind were observed. In one section a pellicular invagination was observed along the trunk that may have been a site for endocytosis (Fig. 25). Autophagosomes were present, as well as extrusomes which are known from the posterior ends of the tropho-tomont stage (Fig. 26).

Discussion

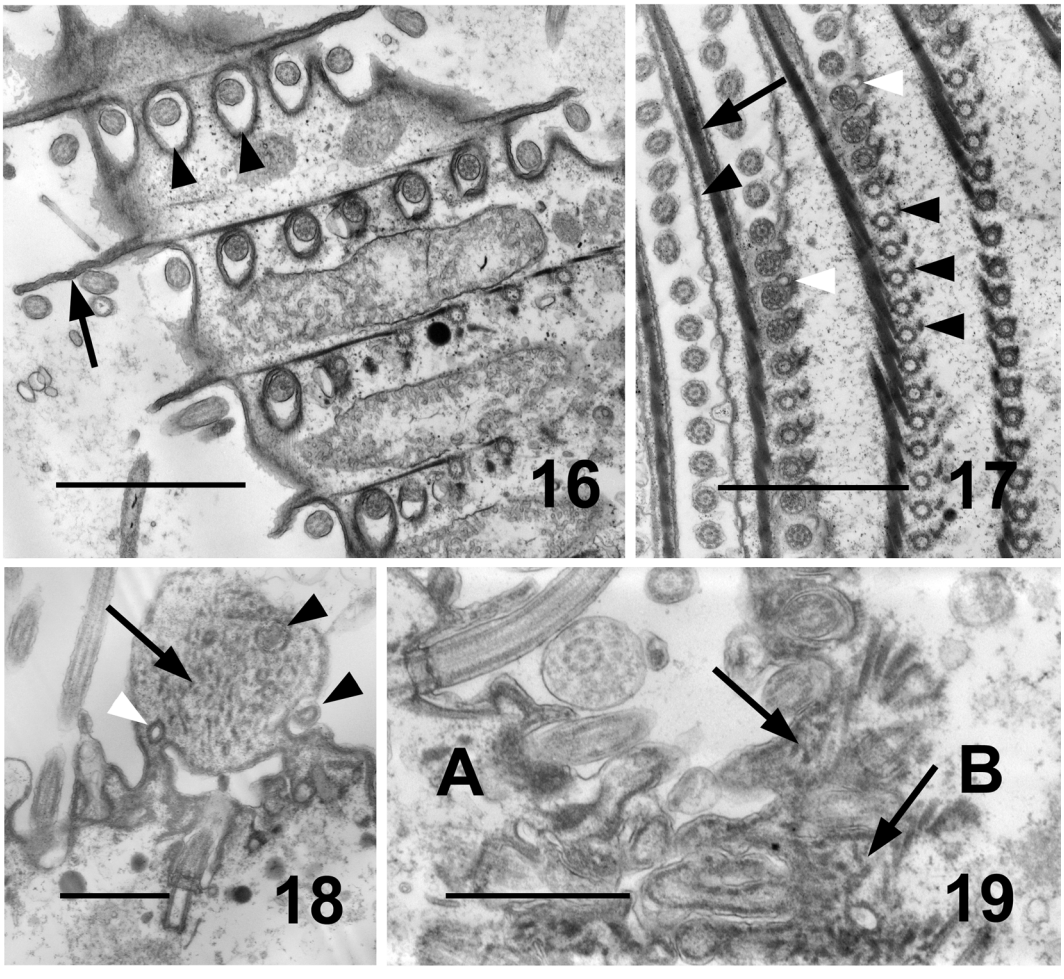
This study has revealed new information about *Chromidina*'s fine structure. The tropho-tomont stage has a distinct infraciliature at the anterior end, with stacks of enlarged kd fibers filling the broad kinetal folds. The folds possibly have a griping function, as the contractile fibers and microtubules inside could provide the mechanism for holding the folds against the host tissue. The kd fibers observed in this study correspond to the contractile fibers illustrated by Chatton and Lwoff (1935) at the apical



Figs 11-15. Kinetal structures and the internal cytoplasm. 11 - Low magnification image of the ciliature; 12 - the macronucleus has rings of dense chromatin (arrow indicates a crystalline body within a vesicle); 13 - the kineties along the trunk have sunken kinetodesmal fibers (arrows) and a thin kinetal fold above the kd; 14 - at the anterior end of the cell the kd fibers are enlarged and stacked within the kinetal folds, which are wide and more well developed than along the trunk of the cell; 15 - the kd fibers forming disorganized bundles. Scale bars: 11, 12 - 2 μm, 13-15 - 1 μm.

papillum of *Chromidina elegans*. Chatton and Lwoff believed that the fibers provided a retractile ability to the papillum, allowing it to function as a sucker. This current study supports their hypothesis and provides an ultrastructural understanding of the apical anatomy. Live tropho-tomonts were able to grip onto glass and plastic substrates strongly, and this same tenacity could hold the parasite against the renal and/or pancreatic appendages of the host's kidney. This would allow the posterior end of the cell to hang in the coelomic renal sac and absorb nutrients from the renal filtrate. Along the trunk of the cell, the kinetal structure was similar to that observed in other apostome genera (Bradbury, 1974a, 1974b; Landers, 1990, 1991a, 1991b; Landers et al., 2007; Rigsby and Bradbury, 1983; Rivaud, 1988) in which kd fibers were stacked under the pellicle and kinetal folds. The folds have been reported under various names such as the bandelette (Chatton and Lwoff 1935), pellicular ribbon, pellicular complex (Rivaud, 1988; Rivaud and Berger, 1988), or lame (de Puytorac and Grain, 1975). The organization of the kd fibers was disorganized near cell junctions and in glancing sections of the pellicle presumed to be near cell junctions. At those sites the kd fibers became unstacked and disorganized, and extended into surface projections from the two separating cells. Their function at those locations could be to facilitate cell division or the division of kineties, or the fibers could be simply caught in that position during division and have no functional ability in that state.

The ability of the cell to take in nutrients along the trunk was demonstrated by Chatton and Lwoff

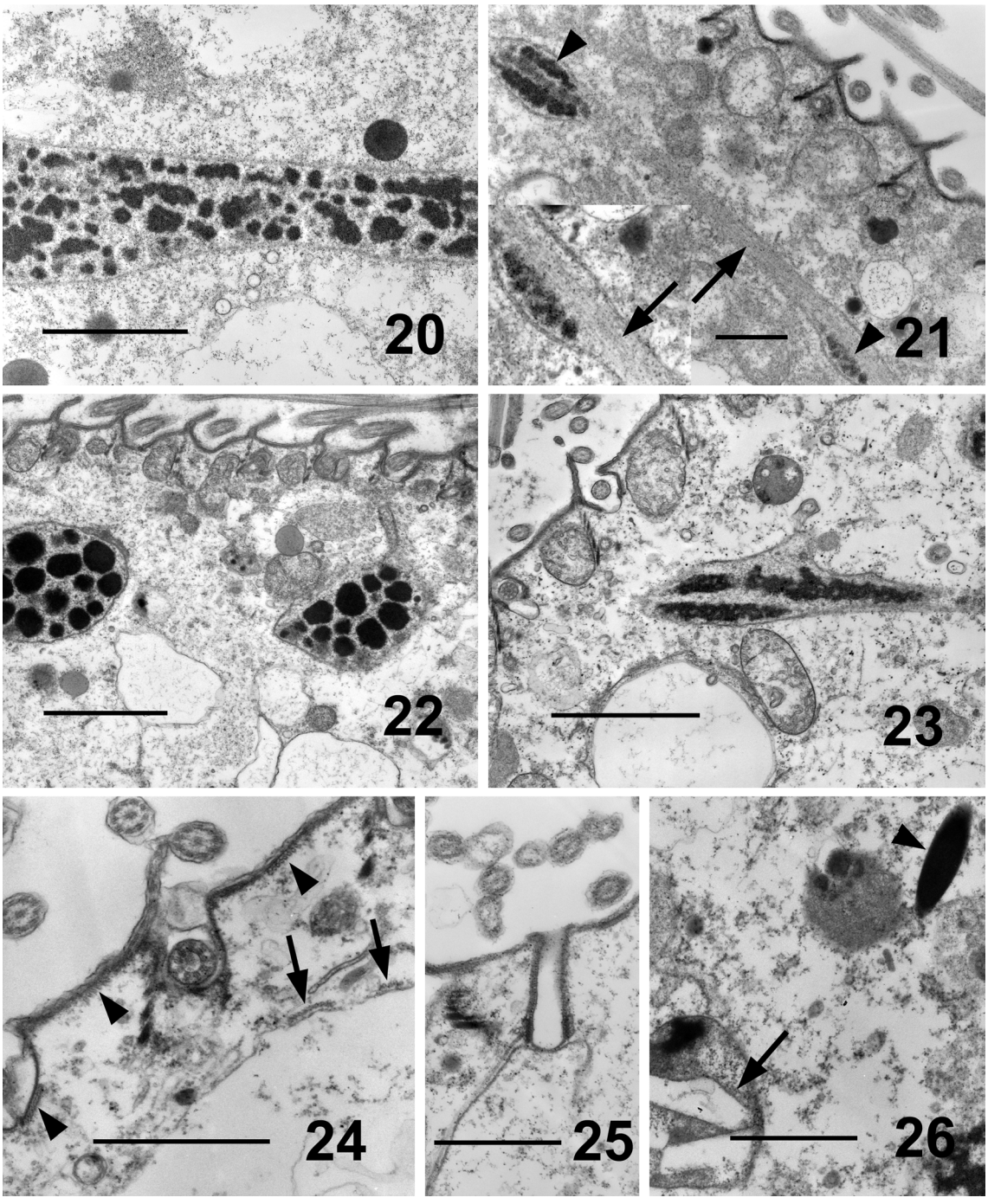


Figs 16-19. Kinetal structures. 16 - Frontal section through kineties along the trunk, showing thin kinetal folds (arrow) and sunken depressions from which the cilia project (arrowheads); 17 - frontal section through kineties at the anterior end (a widened kinetal fold with the kd fiber is indicated at the arrow; parasomal pores - white arrowheads, - and transverse microtubules - black arrowheads - are indicated); 18 - glancing section of the surface showing disorganized fibers (arrow), likely extensions of the kd fibers (sunken depressions of the kinety - black arrowheads - are indicated along with a parasomal pore - white arrowhead); 19 - a connection between two cells (A and B) at a division furrow (pellicular extensions from each cell touch, and are supported internally by disorganized fibers derived from the kd - arrows). Scale bars: 16-17 - 2 μm, 18-19 - 1 μm.

(1935), who put to rest earlier assertions that *Chromidina* possessed a mouth. They reported that black ink was taken into endocytic vesicles along the length of the trunk after long periods of incubation. In this current study no evidence of a mouth or a ciliate pharyngeal apparatus was observed. A rare section revealed a possible endocytic pore and canal along the cell's trunk.

Endocytosis occurring along the cell's trunk explains the absence of a gullet near the anterior end and the presence of food vacuoles throughout the cell. In rare sections, microtubular tracts similar to the pellicular transverse microtubules were observed surrounding internal vacuoles. This may represent a structural connection between the cell surface and the internal vacuoles. Membrane organelles, flattened disks of membrane found in many apostome genera to aid in food vacuole formation (Bradbury, 1973, 1974b; Bradbury and Goyal, 1976; Bradbury et al., 1987; Landers et al., 2007; Ohtsuka et al., 2004), were not observed in *Chromidina*. Similarly, they were not observed in hypertrophonts of *Synophrya*, an apostome ciliate parasitic in crab gills (Landers, 2010).

The reticulate macronucleus of the cell is distinctive, but not the only example of this type of organelle within the Apostomatida. Macronuclear reticula are also found in the endosymbiotic tro-



Figs 20-26. Internal cytoplasmic structures. 20 - The macronucleus cut in a longitudinal section; 21 - microtubular tracts (arrow) connecting two masses of chromatin in the reticulum (arrowheads), the microtubules are indicated in the inset; 22 - large vacuoles and branches of the macronuclear reticulum, mitochondria are located between the kineties; 23 - an internal vacuole and a portion of the macronucleus; 24 - subpellicular microtubules (arrowheads) and microtubular tracts associated with an internal vacuole (arrows); 25 - a surface pore possibly involved in endocytosis; 26 - a crystalline body within a membranous vesicle (arrow), an extrusome (arrowhead). Scale bars: 20, 22-23 - 2 μm, 21, 24-26 - 1 μm.

phont stages of *Foettingeria* (anemones), *Synophrya* (crabs), and *Pericaryon* (ctenophores) (Chatton and Lwoff, 1935). *Chromidina*'s macronucleus contained tracts of microtubules with the chroma-

tin and also in the thin strands of membrane and nucleoplasm that connected the chromatic masses. The microtubular tracts are found in the phoronts of the exuviotrophic apostomes *Hyalophysa* and *Gymnodiniodes* (Bradbury, 1974a; Landers et al., 2007) which do not have reticulate macronuclei. However, the only other apostome macronucleus with a reticulate structure that has been observed by TEM, *Synophrya*, lacks the microtubular tracts (Landers, 2010). This characteristic will be interesting to follow in future apostome studies, to see if a functional significance of the microtubules relates to the general organelle shape.

Many other cytoplasmic components were observed in the tropho-tomont stage, including crystalline bodies, oil droplets, mitochondria, and in some cells, bacteria. The crystalline bodies may be related to their environment in the renal sacs. Chatton and Lwoff (1935) commented that cephalopod urine is a guanine-rich fluid. The crystalline bodies observed within the membrane-bound vesicles of *Chromidina* may be a way for the cell to concentrate some of the compounds taken in by endocytosis.

Species identification

The *Chromidina* species reported herein does not conform to any of the 3 described taxa. The subject of this study has 12 kineties, an enlarged anterior attachment bulb, and lacks a skirt of elongated cilia on the bulb. *Chromidina elegans* has 14 kineties (Chatton and Lwoff, 1935). *Chromidina cortezi* has 12 kineties, but has a pointed apical papillum rather than a bulbous apex (Hochberg, 1971, 1990). *Chromidina coronata* has an unreported number of kineties and a bulbous anterior with a conspicuous crown of elongated cilia (Hochberg, 1971, 1990). Further studies of the current organism may lead to a new species description.

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