The African Heterotrich Ciliate, *Stentor andreseni* sp.nov., and *S.amethystinus* Leidy

A Comparative Ultrastructural Study

By JYTTE R. NILSSON

Biologiske Skrifter **27**

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Abstract

A medium-sized (300 μm), brownish *Stentor* sp. was collected from an artificial pond near Nairobi. The brownish colour was due to the presence of purplish-red pigment granules and green zoochlorellae. The ciliate was a typical *Stentor,* but never extended to the long slender trumpet shape, typical of the genus; it contained two compact macronuclei. In most characteristics, the ciliate resembled *S. amethystinus* as described from Cameroun (DRAGESCO 1970). In the present, mostly fine structural, study, the Kenyan ciliate was compared to *S. amethystinus* Leidy from the Austrian Alps. The latter ciliate was also medium-sized, but more plump in shape, and it contained also zoochlorellae and purplish-red pigment granules. These granules were, however, smaller in size than those of the Kenyan ciliate; moreover, the substructure of the two types of granules differed. Both ciliates showed typical *Stentor* features, such as dikinetids, myonemes and km fibers; the two latter structures are involved in contraction and extension of the ciliates. The km fiber, composed of microtubular bundles arising at the dikinetids, contains bundles from several dikinetids; during changes in body length, these bundles slide within the km fiber and the length of the bundles determines the maximal extension of the ciliates. In the Kenyan ciliate, the microtubular bundles were 8–9 μm long, whereas in *S. amethystinus* they were 5–6 μm long, in good agreement with the greater flexibility of the Kenyan ciliate. The conclusion of the study is that the Kenyan *Stentor* sp. cannot be referred to *S. amethystinus*, or to any other known *Stentor* species, hence it is considered a new species, *Stentor andreseni*.

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Contents

Introduction

Among ciliated protozoa, members of the genus *Stentor* are recognized easily by their wide frontal field, encircled by a prominent adoral zone of membranelles (AZM), and by their typical body shape, being bell-shaped in the swimming state and trumpet-shaped when attached and extended (e.g. TARTAR 1961). Ventrally, the extensive AZM spirals down the narrow funnel-shaped buccal cavity to the cytostome where food vacuoles are formed.

The *Stentor* genus contains several species. In 1961 TARTAR (1961) listed 13 species and since then another 4 species have been added to the list (DRAGESCO 1970, MURTHY and BAI 1974, FOISSNER 1980). These species are characterized by their mean body length, the shape of the macronucleus, and the colour of the ciliates. The size of the ciliates ranges from about 100 μm to more than 1 mm in length with large individual variation, dependent on whether measurements are taken in the swimming, contracted, or extended state, as the capacity of extension and contraction is very great; but also the nutritional state of the ciliate may give rise to individual variations in size. The various species of *Stentor* are generally referred to as being of large, medium, or small size. The shape of the macronucleus may be moniliform or compact (oval or spherical). The colour of the ciliates varies greatly, from blue-green, green, violet-blue, violet-red, pink, to yellow and brown, dependent on the colour of their pigment granules and/or the presence of zoochlorellae, but also colourless species, or strains, are found (KAHL 1932, TARTAR 1961, DRAGESCO 1970). Although the colour of the pigment varies from one *Stentor* species to another, the pigment, named stentorin (LANKESTER 1873) or stentorol (BARBIER *et al.* 1956), belongs to the same group of chemical compounds as the purple pigment, blepharismin (GIESE 1973) or zoopurpurin (ARCICHOVSKIJ 1905), of the related heterotrich ciliate genus, *Blepharisma;* furthermore, both pigments resemble the plant pigment hypericin (BARBIER *et al.* 1956, MØLLER 1962, SEVENANTS 1965).

A medium-sized *Stentor* species of a brownish colour was collected near Nairobi, Kenya, in 1983. The brownish colour is derived from the presence of purplish-red pigment granules and green zoochlorellae. Although the ciliate was a typical *Stentor* in shape, fully extended trumpet-shaped forms were not observed. In size and some general characteristics, but not in shape, the Kenyan *Stentor* resembled *Stentor amethystinus* as described by DRAGESCO (1970) from Cameroun. Since DRAGESCO (1972) found a *Stentor* of the same description in Uganda, there was good reason to assume that the Kenyan *Stentor* belonged to that species.

Stentor amethystinus is well established. It was originally described in 1880, by LEIDY (1880), and the latest description of the ciliate dates from 100 years later when FOISSNER (1980) found it in the Austrian Alps. The Austrian ciliate resembles the classical description of *S. amethystinus* LEIDY also in showing little variation in body length in the different states, a character typically present in the Kenyan *Stentor* sp., but absent in the Camerounian *S. amethystinus* as described by DRAGESCO (1970). These descriptions of *S. amethystinus* are based on light microscopical studies.

In order to characterize the Kenyan *Stentor* species, a comparative, mainly electron microscopical, study was made of this ciliate and the Austrian *S. amethystinus,* kindly collected by DR. W. FOISSNER in 1983 at Hohe Tauern, Grossglockner area. The conclusion of the present study is that, although the Kenyan *Stentor* sp. and *S. amethystinus* LEIDY have several features in common, some major fine structural differences rule out the possibility that they belong to the same species.

Materials and methods

The Kenyan *Stentor* sp. was collected from an artificial fresh-water pond, about 10 miles out of Nairobi, Kenya, in July 1983. The water sample contained numerous specimens of the ciliate and after return to Denmark some of the ciliates were maintained in the original water, whereas an attempt was made to establish some other individuals in laboratory cultures using Pringsheim's salt solution and boiled wheat grains, as described for the African strain of *Blepharisma japonicum* (NILSSON 1967). However, all attempts to cultivate the ciliates failed, although they were feeding in the temporary cultures.

Stentor amethystinus LEIDY was kindly provided by Dr. W. FOISSNER who collected the ciliates in September, 1983, at Hohe Tauern in Austria. Attempts to establish this ciliate in permanent laboratory cultures also failed.

The African strain of *Blepharisma japonicum* from established laboratory cultures was used for comparison in the study for determination of the colour of the pigments of the two *Stentor* species.

Living ciliates were observed light microscopically, using for low power a WILD preparation microscope and for higher magnification a REICHERT anoptral phase contrast microscope, Zetopan.

Within a week after collection from nature, some ciliates were fixed for electron microscopy. The Kenyan *Stentor* sp. was fixed for 10 minutes in 2% glutaraldehyde in O.1 M cacodylate buffer and, after rinsing in the buffer, for 1 hour in 1% osmium tetroxide in the cacodylate buffer. Some of the *S. amethystinus* specimens were fixed in the same manner, but others were fixed only in 1% osmium tetroxide in the cacodylate buffer for 1 hour; of the two fixation procedures, the latter was superior because the ciliates did not contract as seen on fixation in glutaraldehyde. After fixation, the ciliates were washed in distilled water, dehydrated in a graded series of ethanols and finally in propylene oxide before embedding in epon 812. The embedded material was sectioned on a LKB ultratome and the sectioned material was contrasted in zinc-uranyl acetate (VENABLE and COGGESHALL 1965) for 20 minutes and in lead acetate (WEINSTEIN *et al.* 1963) for 3 minutes. The material was examined in a ZEISS EM9 electron microscope at primary magnifications between 1,800 and 20,000 times.

Fig. 1. Outline of the shape of the Kenyan *Stentor* sp. in the swimming (A), contracted (B), and extended (C) states.

Fig. 2. Simplified drawing of the Kenyan *Stentor* sp. indicating the extensive adoral zone of membranelles (AZM), the buccal cavity (BC), the frontal field (FF), the contractile vacuole (CV), and the two spherical macronuclei (MA). The longitudinal lines indicate the position of kineties (rows of cilia). Zoochlorellae (symbiotic algae) and pigment granules are not drawn.

Results

The Kenyan *Stentor* sp. and the Austrian *S. amethystinus* were studied *in vivo* in the light microscope and after fixation in the electron microscope. The observations will be described separately for each species before the main characteristics of both ciliates are summarized in the last Result Section. The terminology used is largely that listed by *Corliss* (1979).

Light Microscopy

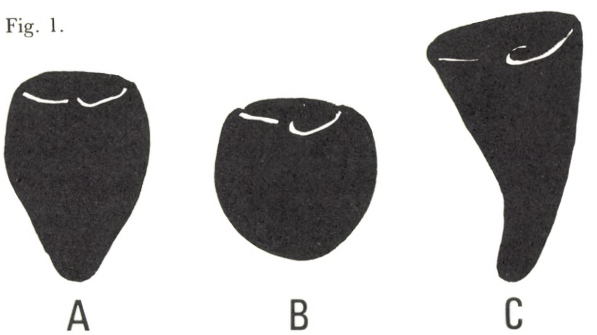
The Kenyan Stentor species

The ciliate was a medium-sized Stentor, measuring about 300 μm in length, and it had a distinct brownish colour when viewed at low magnification. The ciliate appeared plump in shape when swimming, whereas it had a typical trumpet-shape when attached to plant remnants; however, it never displayed the long, slender, extended form typical of most members of the *Stentor* genus. In the contracted form, the ciliate was almost spherical in shape; its outlines in the different states are illustrated in Fig. 1. Numerous specimens were found in the samples collected at the fresh-water pond and because of the size, the ciliates were the most dominant protozoan species; however, the samples were also rich in different small flagellates, the amoeba *Arcella vulgaris,* and ciliates of the genera *Halteria* and *Spirostomum.*

At higher magnifications, the brownish colour of the Kenyan *Stentor* sp. could be resolved into two components (Plate I), consisting of purplish-red pigment granules, resembling in colour that of the pigment granules in *Blepharisma japonicum,* African strain (Nilsson 1967), and numerous green zoochlorellae, as mentioned in the Introduction. The pigment granules were about 1 μm in diameter and they were found in stripes along the kineties (Plate II, 1), but also in large masses at deeper levels within the ciliates. In ciliates kept for some time in dishes on the laboratory bench, the pigment granules of the central region collected as a cap in the anterior end which then appeared brown, whereas the posterior end appeared green, clearly revealing the presence of the symbionts. The number of zoochlorellae was high, in the order of a few hundred per ciliate, and the symbionts were found to be evenly distributed throughout the cytoplasm. The size of the zoochlorellae was about 4 μm in diameter (Plate II, 2) and they resemble the symbionts in, for example, *S. polymorphus* and *Paramecium bursaria.*

The Kenyan *Stentor* sp. had a typical adoral zone of membranelles (AZM). About 150 membranelles encircled the wide frontal field and extended down the narrow, spiralling funnel-shaped buccal cavity (Fig. 2). The ciliates were feeding in the laboratory

Fig. 1.



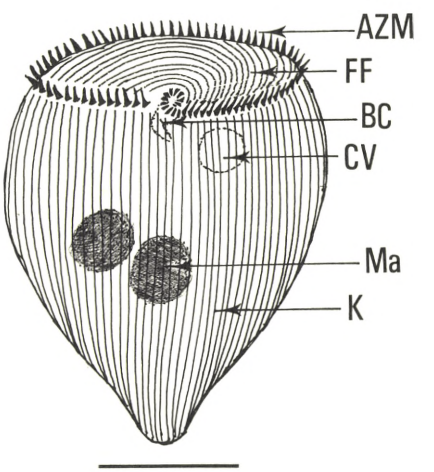


Fig. 2.

cultures; however, they were not multiplying. The number of kineties was about 80 and the diameter of the frontal field was about 150 μm, whereas the width of the AZM was about 10 μm.

In compressed specimens, the cytoplasm was seen to be highly vacuolated. The numerous small vacuoles had a diameter close to that of the zoochlorellae (Plate II, 2), but also larger digestive vacuoles were seen. The macronucleus was of the compact type, being spherical in shape, and at least two, occasionally three, macronuclei were found in all ciliates observed (Plate II, 3). The presence of three macronuclei could indicate division stages; however, as mentioned above, the ciliates did not multiply in culture.

Stentor amethystinus Leidy

The Austrian *S. amethystinus* has recently been described by FOISSNER (1980). It is also a medium-sized *Stentor* which had a length of about 300 μm. It was brownish in colour at low magnification; however, of a lighter shade than that of the Kenyan *Stentor*, thus the presence of the zoochlorellae was more clearly revealed. Generally, *S. amethystinus* is described as being habitually contracted (KAHL 1932, TARTAR 1961) and the ciliate was more plump in appearance than the Kenyan *Stentor*; however, as described by FOISSNER (1980) also sessile forms may be seen. In this attached state, the ciliates appear as very short and wide trumpets (see FOISSNER 1980, Figs. 48, 49) and curiously, and characteristically, the frontal field bulges out, whereas normally stretched trumpet-shaped *Stentor* species have a flat (stretched) frontal field. In the present samples of *S. amethystinus* only a few ciliates were found attached, the most common state was the swimming form, but also contracted, almost spherical forms were observed.

The pigment granules of *S. amethystinus* were of the same purplish-red colour as those of the Kenyan *Stentor*; however, the granules were only about 0.5 μm in diameter. The distribution of the pigment granules was the same as that described for the Kenyan *Stentor*, i.e. in stripes along the kineties (Plate I, 4), as well as in groups within the cytoplasm of the ciliates. Although the overall number of pigment granules of *S. amethystinus* contributed to a lesser degree to the colour of the ciliate, presumably because of the smaller size of the granules (compare Figs. 1 and 4 in Plate II). The zoochlorellae were also abundant in *S. amethystinus* and they resembled the symbionts in the Kenyan *Stentor* with respect to size, about 4 μm in diameter, and general distribution (Plate II, 5).

The AZM in *S. amethystinus* consisted of about 150 membranelles as also stated by FOISSNER (1980). The number of kineties was found to be in the order of about 80, similar to the situation in the Kenyan *Stentor;* FOISSNER (1980) gives a figure of 60 kineties for *S. amethystinus,* but this difference is not considered as a significant feature, since some variation in size, and number of kineties, may occur within single species.

The macronucleus of *S. amethystinus* is also of the compact type (Plate II, 6). However, in this species only a single macronucleus was seen in all individuals examined and the diameter of the nucleus was larger than that of the nuclei in the Kenyan *Stentor*. In compressed specimens of *S. amethystinus,* several small spherical structures were seen around the macronucleus, they had a brim af pigment granules and an overall diameter of about 2–3 μm. The structures resemble those described for this species by KAHL (1932), DRAGESCO (1970), and FOISSNER (1980).

Electron Microscopy

The Kenyan Stentor species

The cortical region of the ciliate revealed the presence of typical *Stentor* features (e.g. HUANG and PITELKA 1973). The kineties, rows of cilia, were composed of dikinetids of which only the posterior kinetosome was ciliated (Plate III, 3). From each dikinetid arose a postciliary fibril, a ribbon of about 20 microtubules, passing posteriorly to the right of

the kinety (viewed from inside the cell) to join with postciliary fibrils from other dikinetids in the formation of the km fiber; thus in cross section the km fiber is composed of overlapping rows of microtubular ribbons (Plate III, 1). The maximum number of microtubular ribbons within the km fiber was 25 in contracted ciliates and in this instance, the distance between the dikinetids was 0.33 μm. From these figures the postciliary fibrils were calculated to be 8–9 μm in length. Several pigment granules were aligned near the surface between the kineties (Plate III, 1, 2); the granules had a diameter of about 1 μm. Most of the pigment granules had a »fluffy« content of finely granular material, and their limiting membrane stands out distinctly. In thickness the membrane of the granules resembles that of the plasma membrane (Plate III, 3); both these membranes are thicker than the membrane of the endoplasmic reticulum (Plate III, 3). The plasma membrane was covered on the outside by a thin "coat" and supported on the inner side by another membrane (Plate III, 3); the somewhat poorly defined cortex may consist of an inner alveolar system of which the outer membrane is fused with the plasma membrane, the double membrane system was not continuous throughout the cortical region. Some distance below the surface, the extensive myonemal system is seen (Plate III, 1, 2); the myonemes are composed of fine filamentous material which showed high electron density in contracted ciliates. The myonemes are not membrane-limited but they are found in close opposition to the membrane of smooth endoplasmic reticulum (Plate III, 1). Moreover, the cortical region contained a high density of mitochondria (Plate III, 1, 2).

The feeding organelle consists, as mentioned earlier, of the extensive adoral zone of membranelles (AZM) which encircles the wide frontal field and spirals down the buccal cavity to the cytostome. The individual membranelles were composed of three rows of kinetosomes in the frontal field, whereas in the buccal cavity the membranelles consisted of two rows only (Plate IV, 3–5). The transition from three to two rows of kinetosomes per membranelle was found to coincide with the site where the AZM enters the buccal cavity (Plate IV, 1, 3); a maximum number of 19 membranelles with two rows of kinetosomes was counted in a single buccal cavity, but the actual number may be higher. Within the membranelles all kinetosomes were ciliated, but from only two of the kinetosomal rows arose nematodesmata composed of about 10 microtubules from each kinetosome. The nematodesmata within a membranelle join to form an extensive nematodesmal fiber in which the microtubules are packed in a hexagonal pattern (Plate IV, 2). Distally, these nematodesmal fibers bend as they meet fibers from adjacent membranelles, forming the so-called "basal fiber" (Randall and Jackson 1958) which passed below the AZM at a distance of about 15 μm. The fan-shaped nematodesmal fibers passed close to the myonemal system, about 1 μm below the interconnected bases of the kinetosomes in the AZM of the frontal field (Plate IV, 2). As the AZM passes into the narrow buccal cavity, the left hand side of the membranelles (viewed from outside) showed a similar relationship to the myonemal system, whereas this system was lacking below the right hand side of the membranelles bordering the buccal cavity wall (Plate IV, 1, 3). Further down the buccal cavity, the myonemes were absent, but pigment granules were still found in rows between the menbranelles (Plate IV, 3, 5). In the cytopharyngeal region, the plasma membrane was subtended by ribbons of microtubules, these cytopharyngeal ribbons (Pitelka 1968) are arranged perpendicularly to the membrane (Plate IV, 3).

The compact macronuclei contained a coarse network of chromatin material, among which numerous nucleoli were interspersed (Plate V, 1). The small micronuclei, about 1.4 μm in diameter, were closely associated with the macronuclei, where they were found in clear macronuclear depressions (Plate V, 1, 4). In one instance, two micronuclei were found associated with a single macronucleus, but the maximum number of micronuclei per ciliate was not determined.

The cytoplasm around the macronuclei contained numerous small granules of about 1 μm in diameter (Plate V, 5). The contents of these granules varied greatly in substructure from distinctly globular to finely granular (Plate V, 2). The globular substructure of the granules appeared either as electron translucent 100 nm globules embedded in finely granular matrix (Plate V, 3) or as images of such globules demarcated by finely granular material (Plate V, 6); the latter configuration appears to represent a transition stage to the finely granular substructure of other granules. The position of the granules corresponds to that of pigment granules, as observed in the light microscope. That the granules are indeed pigment granules was indicated by the finding in the cortical region of pigment granules having images of a globular substructure (Plate V, 7). This observation indicates that the pigment granules undergo maturation after their formation. Since most of the pigment granules in the cortical region had a fluffy content of finely granular material, these granules must represent the mature stage, whereas the granules with the globular substructure may represent the immature stage; the maturation may apply to development of the pigment, or alternatively of the protein/mucus components of the granules.

The zoochlorellae were abundant in the ciliates below the region of the myonemal system (Plate III, 2). The symbionts, mostly as single algae, were enclosed in perialgal vacuoles of a smooth outline; the algae appeared with high electron density due to the highly lamellated chloroplast (see also Section on *S. amethystinus*). A few instances of partially disintegrated zoochlorelae were seen within irregularly shaped perialgal vacuoles (Plate III, 2); these ciliates did not show other signs of autophagy.

Of other inclusion bodies, the Kenyan *Stentor* contained numerous mitochondria distributed throughout the cytoplasm and, as mentioned earlier, concentrated in the cortical region especially near the myonemes (Plate III, 1, 2; Plate IV, 1, 3). In addition, these ciliates contained peroxisomes resembling, for example, those of *Tetrahymena* (Nilsson 1981); the ratio of peroxisomes to mitochondria was about 1 to 20.

Stentor amethystinus Leidy

The cortical structures of this ciliate were also those typical of the *Stentor* genus. As mentioned earlier, two different fixation procedures were used in this instance; in glutaraldehyde fixation, the ciliates were contracted, whereas in osmium fization, they were in the relaxed state. Within the kineties, the dikinetids were close together in contracted ciliates and the km fiber was fairly wide (Plate VI, 1), whereas the dikinetids were wider spaced in relaxed ciliates and the km fiber contained only a few postciliary fibrils (Plate VI, 2). The postciliary fibrils, arising from the posterior, ciliated kinetosome of the dikinetids, were composed of 15 microtubules, which means that each microtubular ribbon of the km fiber is narrower than the ribbons of the km fiber in the Kenyan *Stentor*. The maximum number of microtubular ribbons observed in the km fiber of *S. amethystinus* was 12 in which case the distance between the dikinetids was 0.5 μm, thus the calculated length of the postciliary fibrils was 5–6 μm. Between the kineties, pigment granules were packed below the cortex and as noted in the light microscope, these granules had a diameter of about 0.5 μm. The substructure of the granules varied, in part also according to the fixation procedure; in glutaraldehyde-fixed specimens the granules appeared with little electron density or with finely granular material (Plate VI, 1, 4), whereas in osmium fixation a more gradual transition was seen from quite electron dense, finely granular content to an electron translucent content (Plate VI, 2). Moreover, in osmium-fixed ciliates the membrane of the pigment granules stood out distinctly. The plasma membrane showed an electron dense "coat" in both fixation procedures (Plate VI, 1, 2, 4). The myonemes in this ciliate resemble those seen in the Kenyan *Stentor* sp.; they were also electron dense in contracted ciliates (Plate V, 1, 4), whereas their

filamentous substructure was clearly revealed in the relaxed ciliates (Plate VI, 3; Plate VII).

The adoral zone of membranelles (AZM) was similar to that described for the Kenyan *Stentor* sp.; however, some details of the region were more clearly resolved in osmium-fixed *S. amethystinus*. The membranelles bordering the frontal field were also composed of three rows of kinetosomes (Plate VII, 1) and the kinetosomes within the membranelles were seen interconnected by amorphous material which may be resolved into fine filamentous connections. From the base of the last row of kinetosomes, microtubules, the right, radial ribbon (PITELKA 1963), pass towards the surface of the next membranelles (Plate VII, 1, 2). Moreover, from the base of each kinetosome of two of the rows within the membranelles, the nematodesmal fibers were seen as bundles of 12 microtubules (Plate VI, 1). The nematodesmal fibers within individual membranelles unite with the large fan-shaped fiber described for the Kenyan *Stentor* sp. and the microtubules within the fiber are packed in a hexagonal pattern (Plate VII, 2). This frontal field part of the AZM is underlain by the myonemal system, as also described for the Kenyan *Stentor,* but in the relaxed *S. amethystinus,* the myonemes were found in close opposition to the base of the kinetosomes of the membranelles (Plate VII). At the site where the AZM passes into the buccal cavity, the number of kinetosomal rows per membranelle changes from three to two (Plate VIII, 1), as also shown for the Kenyan *Stentor* (Plate IV, 1); this transition from three to two rows of kinetosomes in *S. amethystinus* is shown in detail in Plate VIII, 2, where also the filamentous connections between the kinetosomes are revealed. The myonemes are also here found only below the left side of the membranelles, towards the buccal cavity wall the myonemal system was absent (Plate VIII, 3, 4). At the cytostomal region, the cytopharyngeal ribbons supported part of the membrane (Plate IX, 1, 2) below which numerous small vesicles were accumulated. The irregular outline of the cytopharyngeal membrane indicates fusion of this membrane with the small vesicles, presumably preparing the formation of the limiting membrane for a future food vacuole. The small vesicles had a heterogenous appearance with respect to outline and content, thus indicating that they are flattened, disc-shaped vesicles occasionally cut tangentially through the irregularly shaped surface. The lumina of the vesicles were electron translucent; furthermore, the vesicles were aligned along fine filamentous extensions perpendicularly to the cross sections of the cytopharyngeal ribbons (Plate IX, 2). The vesicles resemble those found, for example, in *Blepharisma* (JENKINS 1973) and undoubtedly represent membrane-renewal vesicles.

The compact macronucleus resembled in structure that of the macronucleus in the Kenyan *Stentor* sp. (Compare Plate IX, 5 and Plate V, 1). The micronuclei of the two ciliates were of a similar size; however, in *S. amethystinus,* the micronuclei were not found in macronuclear depressions (Plate IX, 5). Moreover, in this ciliate the micronuclei were embedded in closely packed granules of which some had an electron dense core (Plate IX, 6); the size of the granules was about 0.5 μm, corresponding to that of the pigment granules in the cortical region. The configuration of these groups of micronuclei-granules resembled in position that of the small spherical structures observed near the macronucleus in the light microscope. In addition to this special postition of granules around the micronuclei, granules of the same size were abundant in the region near the macronucleus; in osmium-fixed specimens the substructure of the granules was that of rather electron dense, finely granular material, resembling the densest granules shown in Plate VI, 2, and the limiting membrane stood out distinctly. The exact number of micronuclei was not determined; however, reconstruction of the major part of a single macronucleus from serial sections revealed the presence of 6 micronuclei.

The symbiotic algae of *S. amethystinus* resembled those of the Kenyan *Stentor* sp., apart from the appearance of the large inclusion bodies (compare Plate VI, 3 and Plate III, 2). The substructure of the zoochlorellae was most clearly revealed in the

osmium-fixed *S. amethystinus* (Plate IX, 3,4). The symbionts were separated from the perialgal vacuolar membrane by an electron translucent space and usually only a single symbiont was enclosed (Plate IX, 4); however, four division products could be found within a single perialgal vacuole (Plate IX, 3). The zoochlorellae contained a lamellated chloroplast associated with a pyrenoid, a nucleus with a large nucleolus, a mitochondrion, a golgi complex, ribosomes, and large inclusion bodies of varying substructure.

Prominent cytoplasmic inclusions in *S. amethystinus* were the paraglycogen bodies (Plate VI, 3). They resemble the paraglycogen bodies in *Blepharisma* (KENNEDY 1965) and as described for that ciliate, they appeared with high electron density in osmium-fixed specimens. Peroxisomes were observed in *S. amethystinus,* but only occasionally, whereas mitochondria were abundant and their distribution was similar to that described for the Kenyan *Stentor sp.*

Summary of the Observations

The present comparative study of the Kenyan *Stentor* sp. and *S. amethystinus* LEIDY has revealed that the ciliates have several features in common; however, they differ in other features. Some of the characteristics of the two ciliates have been summarized in Table 1; however, only features relevant in taxonomical aspects are included. It is evident that the ciliates differ on major points, such as in general shape, the size and substructure of the pigment granules, the thickness of the km fiber, and the length and number of microtubules in the postciliary fibrils; these differences are sufficient to indicate that the two ciliates belong to different species.

In order to fully characterize the Kenyan *Stentor* sp., the known species of the *Stentor* genus have been listed in Table 2. The information has been compiled from the light microscopical descriptions of the various species presented by TARTAR (1961) DRAGESCO (1966, 1970), MURTHY and BAY (1974), and FOISSNER (1980).

The contents of these two tables form the basis for the following discussion which concludes that the Kenyan *Stentor* must be considered a new species.

Table 1. General Characteristics of the two *Stentor* Species

Feature	The Kenyan *Stentor* Species	*Stentor amethystinus* Leidy
General appearance	typical Stentor in shape, but does not extend fully, about 300 μm long	"habitually conical" (Tartar 1961) with little variation in length, about 300 μm long
Pigment granules	purplish-red, 1 μm in diameter, globular substructure	purplish-red, 0.5 μm in diameter finely granular substructure
Zoochlorellae	numerous, about 4 μm in diameter	numerous, about 4 μm in diameter
Macronucleus	at least 2 macronuclei, spherical in shape	a single macronucleus, spherical in shape
Micronuclei	at least 2 in macronuclear depressions, about 1.5 μm in diameter	at least 6, not in macronuclear depressions, about 1.5 μm in diameter
Km fiber	25 ribbons with dikinetid distance of 0.33 μm	12 ribbons with dikinetid distance of 0.5 μm
Postciliary fibrils	ribbons of 20 microtubules, estimated length of 8-9 μm	ribbons of 12 microtubules, estimated length of 5-6 μm

Table 2. Genus Stentor Oken, 1815

Species	Macronucleus	Pigment	Zoochlorellae	Reference
Large in size:				
S. coeruleus Ehrenberg, 1831	moniliform	blue-green	absent	(5)
S. polymorphus (Müller 1773) Ehrb., 1831	moniliform	absent	present	(5)
S. multimicronucleatus Dragesco, 1970	oval	absent	absent	(2)
S. caudatus Dragesco, 1970	moniliform	pale green	absent	(2)
S. loricata Bary, 1950	veriform	green	absent	(5)
Medium in size:				
S. amethystinus Leidy, 1880	oval	violet-blue	present	(2, 3, 5)
S. niger (Müller 1773) Ehrenberg, 1838	oval	yellow-brown	absent*)	(1, 5)
S. felici Villeneuve-Brachon, 1940	moniliform	yellow	absent	(5)
S. introversus Tartar, 1958	moniliform	blue-green	absent	(5)
S. muelleri Ehrenberg, 1838	moniliform	brown	absent	(5)
S. tartari Murthy and Bai, 1974	spherical	red	present	(4)
S. pygmæus Swarczewsky, 1929	moniliform	?**)	absent	(5)
S. pallidus Foissner, 1980	oval	absent	absent	(3)
S. roeseli Ehrenberg, 1835	moniliform/oval	absent	absent	(5)
Small in size:				
S. multiformis (Müller 1786) Ebrb., 1840	oval	blue-green	absent*)	(5)
S. igneus Ehrenberg, 1838	oval	pink	absent*)	(5)
S. rubra Bary, 1950	oval	pink	absent	(5)

References: 1. Dragesco 1966; 2. Dragesco 1970; 3. Foissner 1980; 4. Murthy and Bai 1974; 5. Tartar 1961.

*) occasionally present **) undetermined: dark in fixed specimens

Discussion

The initial light microscopical observations of the Kenyan *Stentor* sp. revealed features resembling those described by DRAGESCO (1970) for *S. amethystinus* from Cameroun, as mentioned in the Introduction. This conclusion as to the possible identity of the Kenyan *Stentor* sp. was reached on account of its medium size, compact type of macronucleus, and content of pigment granules, as well as, zoochlorellae. Moreover, it appeared likely that a *Stentor* species found in Cameroun and Uganda (DRAGESCO 1970, 1972) would also be present in Kenya, especially since *S. amethystinus,* originally described from New Jersey, U.S.A. (LEIDY 1880), has also been found at various localities in Europe (KAHL 1932, FOISSNER 1980). The *S. amethystinus,* recently described from the Grossglockner area in Austria (FOISSNER 1980), tallies with the classical description of the species, but it differs in some respects from the description of the Camerounian species (DRAGESCO 1970). Thus, as pointed out by FOISSNER (1980), the Camerounian *Stentor* has a higher number of kineties and a more flexible body shape than the Austrian *S. amethystinus* Leidy to which the Kenyan *Stentor* species is compared in the present study.

General Consideration of the Known *Stentor* Species

Before going into a detailed discussion of the present study, the general features of the Kenyan *Stentor* sp. should be considered in relation to the features of the 17 known species of the genus *Stentor,* listed in Table 2. The presence of a compact type of macronucleus in the Kenyan *Stentor* leaves eight species to be considered; however, the medium size of the ciliate excludes consideration of five of these species, leaving *S. amethystinus, S. niger,* and *S. tartari* as possible candidates. The presence of zoochlorellae in the ciliate could, according to most descriptions, exclude *S. niger* form the list; however, this ciliate may be found occasionally with these symbionts (DRAGESCO 1966, 1972; KAWAKAMI 1984), yet *S. niger* is excluded on account of the colour of its pigment, being described as yellow or yellowish brown. The remaining species are *S. amethystinus* and *S. tartari,* the latter ciliate has been described from India only (MURTHY and BAI 1974). The ciliate has red pigment granules, zoochlorellae, and two spherical macronuclei, features which resemble those of the Kenyan *Stentor* sp.; however, *S. tartari* should be a slender ciliate having about half the number of kineties (rows of cilia) found in *S. amethystinus* and the Kenyan *Stentor* species.

This general consideration leaves *S. amethystinus* LEIDY as the most likely species for the Kenyan *Stentor,* a main obstacle, is, however, the interpretation of the colour of the pigment granules. The colour of the pigment in *S. amethystinus* was described originally by LEIDY (1880) as lilac or amethystine and by other authors as violet-blue or blue (TARTAR 1961), violet-red (DRAGESCO 1970), and dark violet (FOISSNER 1980). In the present study, the colour of the pigment in the Austrian strain of *S. amethystinus* was found to be purplish-red, resembling the pigment in the African strain of *Blepharisma japonicum* (NILSSON 1967), and identical to the colour of the pigment granules in the Kenyan *Stentor* species.

The last feature of the living Kenyan *Stentor* sp. to be considered, in this taxonomical aspect, is that the attached ciliate never seemed to stretch into the typical, slender trumpet-shape seen in most members of the *Stentor* genus. This feature also applies to *S. amethystinus,* which is described by TARTAR (1961) not to stretch out but to remain habitually pyriform or conical in shape; however, LEIDY (1880) and FOISSNER (1980) describe some altera-

tion in body length of this ciliate, yet not to the extent described by DRAGESCO (1970) for the Camerounian ciliate, as previously pointed out by FOISSNER (1980).

Consideration of Structural Features of the Kenyan *Stentor* species and *S. amethystinus*

A main difference between the Kenyan *Stentor* species and *S. amethystinus* was found in the size of their pigment granules, a difference which would hardly have been noticed had it not been a comparative study. Pigment granules are small in size and in light microscopical observations, investigators concern themselves more with the colour of the pigment and the general distribution of the granules. The diameter of the pigment granules in the Kenyan *Stentor* sp. is about twice that of the granules in *S. amethystinus,* which means that the volume of the granules differs by a factor of about 8. This fact also explains the observation that, although the distribution and the number of the pigment granules are similar in the two ciliates, the granules contribute less to the general colour of *S. amethystinus* than to the colour of the Kenyan *Stentor* species.

The pigment granules in *S. amethystinus* are of a size corresponding to that of pigment granules in *S. coeruleus* (FAURÉ-FREMIET and ROUILLER 1955; PAULIN and BUSSEY 1971; HUANG and PITELKA 1973; BANNISTER and TATCHELL 1977) and possibly in *S. igneus* (Plate I, a in GRAIN 1968), although the substructure of these granules differs with the different fixation procedures, as also observed in the present study. Moreover, the size of these pigment granules corresponds to that of pigment granules in different species of *Blepharisma* (e.g. JENKINS 1973; LARSEN and NILSSON 1983). The pigment granules of the Kenyan *Stentor* species thus appear to be of an unusually large size; however, KAWAKAMI (1984) describes three types of pigment granules in *S. niger* of which the largest, but most infrequent, ones are also about 1 μm in diameter. These large granules in *S. niger* are described as having a network substructure (Fig. 8 in KAWAKAMI 1984) which might be interpreted as closely packed oblong structures, but not as the globular substructure of the pigment granules in the Kenyan *Stentor* sp.; this difference can hardly be ascribed to different fixation procedures. Although some resemblance is seen between the structure of the pigment granules in *S. niger* and the Kenyan *Stentor* species, the colour of the pigments differs, as mentioned earlier; moreover, the former ciliate is claimed to contain pigment granules of different sizes.

The distribution pattern of the pigment granules is common to the Kenyan *Stentor* species and *S. amethystinus*. In addition to numerous granules in the cortical region, both ciliates have pigment granules throughout the cytoplasm and especially accumulated in the region near the macronucleus where the substructure of the granules may vary, most conspicuously in the Kenyan *Stentor* species where the distinct globular substructure is seen. Transition stages are seen in both ciliates, thus indicating that some maturation of the granular contents occurs, perhaps correlated with development of the pigment. In *S. amethystinus,* KAHL (1932) and DRAGESCO (1970) describe the presence of small spherical structures, or capsules, about 2 μm in diameter near the macronucleus; these structures have a layer of pigment granules around a hyaline centre. Assuming, for example, that the granules with the globular substructure in the Kenyan *Stentor* species are unpigmented, whereas the granules with the "fluffy" content are pigmented, then the hyaline centres could represent sites of synthesis of pigment granules, containing the immature, globular substructured granules, whereas mature granules of altered substructure collect at the periphery. In fact, DRAGESCO (1970) mentions the presence of unpigmented granules between the pigment granules in the Camerounian ciliate. An alternative explanation of the spherical structures will be presented below. Under certain light conditions, some of the pigment granules, but not those present in the cortical region, shift position within the cytoplasm, and accumulate in the anterior end of the ciliate, as also described to be

the case in *S. niger* (KAWAKAMI 1984). This behaviour leads to the question of the biological function of pigment granules, little is known about this role (TARTAR 1961; MØLLER 1962; GIESE 1973). The position of the pigment granules in the cortical region corresponds to that of mucocysts, or homologous extrusive organelles, in unpigmented ciliates, thus indicating that they have some function in common. In reaction to sudden environmental changes, these organelles extrude their contents (HAUSMANN 1978) which in pigmented ciliates is called "capsule shedding" (TARTAR 1961; GIESE 1973) resulting in the formation of a pigmented halo around the ciliate. The light-stimulated migration of the pigment granules in *Stentor* could also have a protective function to shield off the chromatin material from ultraviolet light, as suggested for *Blepharisma* (GIESE 1973); however, the exact biological function of these organelles remains to be clarified.

Both the Kenyan *Stentor* species and *S. amethystinus* had the typical cortical structures of the *Stentor* genus, namely the myonemes and km fibers (RANDALL and JACKSON 1958; GRAIN 1968; BANNISTER and TATCHELL 1968, 1972; HUANG and PITELKA 1973). These structures are responsible for the high flexibility of the ciliates which generally may stretch to reach an extension of several times their contracted length (TARTAR 1961). As elegantly shown by HUANG and PITELKA (1973), the contractile process in *S. coeruleus* involves contraction of the myonemes, associated with structural changes within them (BANNISTER and TATCHELL 1972), concomitantly with a sliding of the microtubular ribbons (postciliary fibrils (PITELKA 1968)) within the km fibers as the dikinetids within the kineties are brought closer together during the contraction, the number of microtubular ribbons increases within the widened km fibers. Stretching, on the other hand, involves a relaxed state of the myonemes and a reversed sliding of the microtubular ribbons within the km fibers as the distance between the dikinetids increases, thus resulting in a decreased number of ribbons with the now narrower km fibers (HUANG and PITELKA 1973). *S. coeruleus* may contract to about ⅔ of its swimming length and extend to about four times its contracted state (Figs. 1–4 in HUANG and PITELKA 1973). In this ciliate, the ribbons within the km fibers are composed of 21 microtubules and the length of these postciliary fibrils was measured to be 15 μm. Moreover, the number of ribbons in the km fibers in contracted and extended ciliates is 44 and 6, respectively (Figs. 14, 15 in HUANG and PITELKA 1973), and with interdikinetid distances of 0.33 and 2.6 μm, respectively. When, as done in the present study, the number of ribbons within the km fiber is multiplied by the distance between the dikinetids, figures of 14.5 and 15.6 μm for the length of the postciliary fibrils are obtained for the respective states of *S. coeruleus,* in good agreement with the above-mentioned measured length.

It is evident that maximal contraction of *Stentor* is reached when the dikinetids are packed closely within the kineties and that maximal extension requires a certain overlap of the microtubular ribbons in the km fibers. In relaxed, stretched specimens of *S. coeruleus,* the km fibers contain 6 microtubular ribbons (HUANG and PITELKA 1973), this figure may provide an estimate for the minimal number of ribbons necessary to maintain a stable structural basis in the extended ciliates. Neither the Kenyan *Stentor* sp. nor *S. amethystinus* extend to the typical, elongated trumpet-shape of *S. coeruleus,* a phenomenon which should be reflected in a lower number, and shorter length, of the microtubular ribbons in their km fibers, as actually found in the present study. The maximum number of microtubular ribbons observed within the km fibers of the Kenyan *Stentor* sp. and *S. amethystinus* was 25 and 12, respectively, and the length of the microtubular ribbons was calculated to be 8–9 μm and 5–6 μm, respectively. According to these figures, and considering the above-mentioned figures for *S. coeruleus,* the Kenyan *Stentor* sp. should be capable of stretching to about twice its contracted length, whereas *S. amethystinus* should be capable of stretching to about 1.5 times its contracted length, which is in good

agreement with observations on the living ciliates, but not with the drawing of the Camerounian *S. amethystinus* (Fig. 57 in DRAGESCO 1970) which indicates that this ciliate may stretch to about 3 times its contracted length. The difference in the structure of the km fibers in the Kenyan *Stentor* species and *S. amethystinus* clearly indicates that the two ciliates cannot belong to the same species.

The extensive adoral zone of membranelles (AZM) in different *Stentor* species is composed of membranelles containing 2 or 3 rows of kinetosomes (RANDALL and JACKSON 1958; GRAIN 1968; PAULIN and BUSSEY 1971; BERNHARD and BOHATIER 1981; KAWAKAMI 1984). From two of these 2 or 3 rows of kinetosomes originate nematodesmal fibers, bundles of about 10 microtubules; these nematodesmata, from the individual membranelles, form a fan-shaped fiber which extends deep into the cytoplasm taking an oblique course as it meets with fibers from other membranelles to unite in the basal fiber which passes below the AZM (RANDALL and JACKSON 1958; GRAIN 1968; PAULIN and BUSSEY 1971; NEWMANN 1974; BERNHARD and BOHATIER 1981). The part of the AZM containing only two rows of kinetosomes per membranelle was found to be that near the cytostome in *S. polymorphus* (RANDALL and JACKSON 1958) and *S. ceoruleus* (BERNHARD and Bohatier 1981), whereas KAWAKAMI (1984) claims that this is the part of the AZM encircling the frontal field in *S. niger*. In the present study, AZM menbranelles composed of two rows of kinetosomes were found near the cytostome in both the Kenyan *Stentor* sp. and *S. amethystinus*. Moreover, the transition between membranelles composed of 3 or 2 rows of kinetosomes was found to coincide with the site where the AZM spirals from the frontal field into the narrow funnel-shaped buccal cavity; this transition was also found to be demarcated by the presence of the myonemal system below membranelles of the frontal field and the absence of this system in the buccal cavity. Should this latter feature be common to all *Stentor* species, then the protion of the AZM in *S. niger*, shown (Fig. 10 in KAWAKAMI 1984) to illustrate the membranelles with two rows of kinetosomes around the frontal field, would actually be from the buccal cavity region because no myonemes are seen below the membranelles.

The nuclear structures were somewhat different in the Kenyan *Stentor* and *S. amethystinus*. Whereas the latter ciliate has one compact macronucleus (DRAGESCO 1970; FOISSNER 1980), the Kenyan *Stentor* species had two compact macronuclei. In this respect, the Kenyan ciliate resembles *S. tartari*, as already mentioned, but this ciliate is more slender (MURTHY and BAI 1974); however, it should be pointed out that DRAGESCO (1970) reports that the Camerounian *S. amethystinus* appeared occasionally with two, or three, macronuclei. Thus, although more than a single macronucleus was a constant finding in the Kenyan ciliate, the finding may not be of taxonomical significance. The micronuclei in the Kenyan ciliate and *S. amethystinus* were similar in size, but their position, and perhaps their number, differed. In the Kenyan *Stentor* species, the micronuclei were found in clear macronuclear depressions, as also seen in *S. coeruleus* (SKARLATO 1982) and *S. niger* (KAWAKAMI 1984), whereas this was not the case in *S. amethystinus* where the micronuclei were found near the macronucleus but not in macronuclear depressions as also indicated in Feulgen preparations of the Camerounian ciliate (Fig. 60, B in DRAGESCO (1970)). Moreover, the micronuclei in the Austrian *S. amethystinus* were enveloped in closely associated pigment granules, a configuration which recalls the previously mentioned spherical structures, or capsules, described by KAHL (1932) and DRAGESCO (1970) in this ciliate; the size of the structures is very similar, but DRAGESCO (1970) says that the presence of a micronucleus could not be revealed within the structures in the Camerounian ciliate. An alternative explanation of the spherical structures has been presented above.

Zoochlorellae were found in both the Kenyan *Stentor* species and *S. amethystinus*. Although these symbionts differed somewhat in substructure, especially with respect to different inclusion bodies,

they resemble in general the zoochlorellae of, for example, *S. niger* (KAWAKAMI 1984) and *Paramecium bursaria* (KARAKASHIAN *et al.* 1968; KARAKASHIAN and KARAKASHIAN 1973; KARAKASHIAN and RUDZINSKA 1981). As shown in these ciliates, only a single symbiont occupies the perialgal vacuole, apart from rare cases where two or four, clearly division products, were found in a common vacuole. Typically, the membrane of the perialgal vacuole was smooth in outline, quite unlike the irregular outline of most stages of digestive vacuoles. In *P. bursaria,* the freeze fractured membranes of the perialgal and digestive vacuoles have been studied by MEIER *et al.* (1984) and the perialgal vacuolar membrane resembles the membrane of old digestive vacuoles with respect to the density of membrane intercalated particles, but is different from the membrane of early digestive vacuoles having lysosomal enzyme activity. The symbiont-containing vacuoles do not fuse with lysosomes (KARAKASHIAN and RUDZINSKA 1981) as do digestive vacuoles, thus indicating that the zoochlorellae cause alteration of the perialgal vacuolar membrane provided by the host ciliate. The apparent control of the symbionts over the perialgal membrane may, however, be lost under unfavourable growth conditions for the host or symbiont, a situation indicated in the present study by the finding of some irregularly shaped perialgal vacuoles containing partially disintegrated, digested, zoochlorellae, although no other sign of autophagy was seen in the ciliates. Usually autophagy involves activity of isolation membranes (e.g. NILSSON 1984), but in the case of digestion of the symbionts, the perialgal vacuole seems to be transformed directly into a digestive vacuole. The population of zoochlorellae is known to be under the influence of the nutritional state of the host ciliate and the symbionts may be digested (MEIER *et al* 1980; REISSER *et al.* 1985); however, little is known about the mechanism by which the host ciliate takes over control in an established symbiotic relationship.

With respect to other cytoplasmic inclusions the Kenyan *Stentor* sp. and *S. amethystinus* showed some difference which are not considered important as taxonomic characteristics. Most prominent was the presence of numerous paraglycogen bodies, a carbohydrate reserve, only in *S. amethystinus*; these bodies resemble those found in *Blepharisma* (KENNEDY 1965; JENKINS 1973). Accumulation of these bodies may be a reflection of the physiological state of the ciliates, for example in *Tetrahymena,* decreased activity in proliferation is correlated with an accumulation of glycogen particles (e.g. NILSSON 1981). Another difference was the finding of more peroxisomes in the Kenyan *Stentor* species than in the Austrian *S. amethystinus,* but again the number of these organelles, in relation to mitochondria, may change with the nutritional state of ciliates (NILSSON 1984). It should be remembered that, although the two ciliates were fixed for electron microscopy about the same time after their collection from nature, their natural habitats, the Kenyan Highlands and the Austrian Alps, differ climatically.

Concluding Remarks

In this attempt to characterize the Kenyan *Stentor* species, the ciliate was compared, mainly electron microscopically, to *S. amethystinus* from the Austrian Alps, because light microscopically the ciliate showed resemblance to the description of this species collected in Cameroun (DRAGESCO 1970). The Austrian *S. amethystinus* (FOISSNER 1980) fits the classical description of this species (LEIDY 1880; KAHL 1932; TARTAR 1961) but differs on some points from that of the Camerounian ciliate (FOISSNER 1980).

Although the Kenyan *Stentor* species and *S. amethystinus* were found to have many fine structurel details in common, some major differences were observed. These differences applied to the size and substructure of the pigment granules, the position of the micronuclei, the capacity for body extension, correlated with the different length of the postciliary fibrils within the km fibers, and a different number of macronuclei. According to these findings, it is concluded that the Kenyan *Stentor* species

cannot be considered identical to *S. amethystinus* LEIDY, whether the ciliate is identical to the Cameroun ian ciliate, described under this name (DRAGESCO 1970), remains to be clarified, especially with respect to the size of the pigment granules, but also with respect to the flexibility of the ciliate.

As far as the evidence goes at present, the Kenyan *Stentor* sp. cannot be referred to any of the known species of the *Stentor* genus. Consequently, the Kenyan ciliate is considered a new species, *Stentor adreseni,* in memory of my deceased teacher, Dr. NILS ANDRESEN.

Diagnosis of the Kenyan *Stentor* (*S.andreseni* sp.nov.)

Stentor andreseni sp. nov.: Medium-sized, brownish *Stentor,* approximately 300 μm long and 200 μm wide, with moderate capacity of extension in the sessile state (length of postciliary fibrils: 8–9 μm). Presence of zoochlorellae and 1 μm large, purplish-red pigment granules causes its brownish colour. About 80 kineties and 150 adoral zone membranelles. Two spherical macronuclei and micronuclei located in macronuclear depressions.

Known habitats: Fresh-water, Kenyan Highlands, altitude: 1.600 metres above sea-level.

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This study of a Kenyan *Stentor* species is dedicated to the memory of my deceased teacher, Dr. NILS ANDRESEN, for his introduction of protozoa to me at the Carlsberg Laboratory in 1950 and later for his enthusiastic encouragement to me as a young student. In 1954, when I went to Uganda, he urged me to look for protozoa, since undescribed species were likely to be found there. However, only during my second visit to East Africa in 1983 did I encounter such a protozoon and it is only appropriate that this ciliate should bear NILS ANDRESEN's name.

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References

Arcichovskij, V. 1905. Über das Zoopurpurin, ein neues Pigment der Protozoa (*Blepharisma lateritium* (Ehrb.)). Arch. Protistenk. *6,* 227–229.

Bannister, L. H. & Tatchell, E. C. 1968. Contractility and the fibre systems of *Stentor coeruleus*. J. Cell Sci. *3,* 295–308.

Bannister, L. H. & Tatchell, E. C. 1972. Fine structure of the M fibres in Stentor before and after shortening. Exp. Cell Res. *73,* 221–226.

Barbier, M., Fauré-Fremiet, E. & Lederer, E. 1956. Sur les pigments du cilié *Stentor niger*. C. R. Séanc. Acad. Sci., Paris *242,* 2182–2184.

Bernard, F. & Bohatier, J. 1981. Ultrasctructure et mise en place des organelles buccaux au cours de la régénération orale chez *Stentor coeruleus* (Cilié Hétérotriche). Can. J. Zool. *59,* 2306–2318.

Corliss, J. O. 1979. The ciliated protozoa. Characterization, classification and guide to the literature. 2nd edition. Oxford, New York, Toronto, Sydney, Paris, Frankfurt: Pergamon Press.

Dragesco, J. 1966. Quelques ciliés libres du Gabon. Biologica Gabonica *2,* 91–117.

Dragesco, J. 1970. Ciliés libres du Cameroun. Ann. Fac. Sci. Yaoundé (hors-série), 1–141.

Dragesco, J. 1972. Ciliés libres de l'Ouganda. Ann. Fac. Sci. Cameroun *9,* 87–126.

Fauré-Fremiet, E. & Rouiller, C. 1955. Microscopie électronique des structures ectoplasmiques chez les ciliés du genre *Stentor*. C. R. Séanc. Acad. Sci., Paris *241,* 678–680.

Fauré-Fremiet, E., Rouiller, C. & Gauchery, M. 1956. Les structures myoïdes chez les ciliés. Étude au microscope électronique. Archs. Anat. microsc. Morph. exp. *45,* 139–161.

Foissner, W. 1980. Taxonomische Studien über die Ciliaten des Grossglocknergebietes (Hohe Tauern, Österreich) IX. Ordnungen Heterotrichida und Hypotrichida. Ber. Nat.-Med. Ver. Salzburg *5,* 71–117.

Giese, A. C. 1973. Blepharisma. The Biology of a Light-Sensitive Protozoan. Stanford University Press, Stanford, California. pp. 157–171 and pp. 266–303.

Grain, J. 1968. Les Systèmes fibrillaires chez *Stentor igneus* Ehrenberg et *Spirostomum ambiguum* Ehrenberg. Protistologica *4,* 27–35.

Hausmann, K. 1978. Extrusive organelles in protists. Intern. Rev. Cytol. *52,* 197–276.

Huang, B. & Pitelka, D. R. 1973. The contractile process in the ciliate, *Stentor coeruleus*. I. The role of microtubules and filaments. J. Cell Biol. *57,* 704–728.

Jenkins, R. A. 1973. The fine structure. In: Blepharisma. The Biology of a Light-Sensitive protozoan (Giese, A. C., ed.), pp. 39–93. Stanford, California: Standford University Press.

Kahl, A. 1932. Urtiere oder Protozoa. I: Wimpertiere oder Ciliata (Infusoria). 3. Spirotricha. In: Die Tierwelt Deutschlands (Dahl, F., ed.), 25. Teil, pp. 457–466. Jena: Verlag von Gustav Fischer.

Karakashian, S. J., Karakashian, M. W. & Rudzinska, M. A. 1968. Electron microscopic abservations on the symbiosis of *Paramecium bursaria* and its intracellular algae. J. Protozool. *15,* 113–128.

Karakashian, M. W. & Karakashian, S. J. 1973. Intracellular digestion and symbiosis in *Paramecium bursaria*. Exp. Cell Res. *81,* 111–119.

Karakashian, S. J. & Rudzinska, M. A. 1981. Inhibition of lysosomal fusion with symbiont-containing vacuoles in *Paramecium bursaria*. Exp. Cell Res. *131,* 387–393.

Kawakami, H. 1984. Ultrasctructural study of an endosymbiotic alga and its host ciliate *Stentor niger*. J. Protozool. *31,* 247–253.

Kennedy, J. R. 1965. The morphology of *Blepharisma undulans* Stein. J. Protozool. *12,* 542–561.

Lankester, E. R. 1873. Blue stentorin. The colouring matter of *Stentor coeruleus*. Quart. J. Microscop. Sci. *13,* 139–142.

Larsen, H. F. & Nilsson, J. R. 1983. Is *Blepharisma hyalinum* truly unpigmented? J. Protozool. *30,* 90–97.

Leidy, J. 1880. Remarks on pond life. Proc. Acad. Nat. Sci. Philadelphia, 156–159.

Meier, R., Reisser, W. & Wiessner, W. 1980. Zytologische Analyse der Endosymbioseeinheit von *Paramecium bursaria* Ehrbg. und Chlorella spec. II. Die Regulation der endosymbiontischen Algenzahl in Abhängigkeit vom Ernährungszustand der Symbiosepartner. Arch. protistenk. *123,* 333–341.

Meier, R., Lefort-Tran, M., Pouphile, M., Reisser, W. & Wiessner, W. 1984. Comparative freeze-fracture study of perialgal and digestive vacuoles in *Paramecium bursaria*. J. Cell Sci. *71,* 121–140.

Murthy, K. V. N. & Bai, A. R. K. 1974. *Stentor tartari* sp. n. from India. J. Protozool. *21,* 505–506.

Møller, K. M. 1962. On the nature of stentorin. C. R. Trav. Lab. Carlsberg *32,* 471–498.

Newman, E. 1974. Scanning electron microscopy of the cortex og the ciliate *Stentor coeruleus*. A view from the inside. J. Protozool. *21,* 729–737.

Nilsson, J. R. 1967. An African strain of *Blepharisma japonicum* (Suzuki). A study of the morphology, giantism and cannibalism, and macronuclear aberration. C. R. Trav. Lab. Carlsberg *36,* 1–24.

Nilsson, J. R. 1981. On cell organelles in *Tetrahymena*. With special reference to mitochondria and peroxisomes. Carlsberg Res. Commun. *46,* 279–304.

Nilsson, J. R. 1984. On starvation-induced autophagy in *Tetrahymena*. Carlsberg Res. Commun. *49,* 323–340.

Paulin, J. J. & Bussey, J. 1971. Oral regeneration in the ciliate *Stentor coeruleus:* A scanning and transmission electron optical study. J. Protozool. *18,* 201–215.

Pitelka, D. R. 1968. Fibrillar systems in protozoa. In: Research in Protozoology, volume 3 (Chen, T.-T., ed.), pp. 280–388. Oxford & New York: Pergamon Press.

Randall, J. T. & Jackson, S. F. 1958. Fine structure and function in *Stentor polymorphus*. Biophys. Biochem. Cytol. *4,* 807–829.

Reisser, W., Meier, R., Görtz, H.-D. & Jeon, K. W. 1985. Establishment, maintenance, and integration mechanisms of endosymbionts in protozoa. J. Protozool. *32,* 383–390.

Sevenants, M. R. 1965. Pigments of *Blepharisma undulans* compared with hypericin. J. Protozool. *12, 240–245.*

Skarlato, S. O. *1982.* Electron microscope study of the micronuclei of the ciliate *Stentor coeruleus* during meiosis. Protistologica *18,* 281–288.

Tartar, V. 1961. The Biology of Stentor. Oxford, London, New York & Paris: Pergamon Press.

Venable, J. H. & Coggeshall, R. 1965. A simplified lead citrate stain for use in elctron microscopy. J. Cell Biol. *25,* 407–408.

Weinstein, R., Abbiss, T. & Bullivant, S. 1963. The use of double and triple uranyl salts as electron stains. J. Cell Biol. *19,* 74 A.

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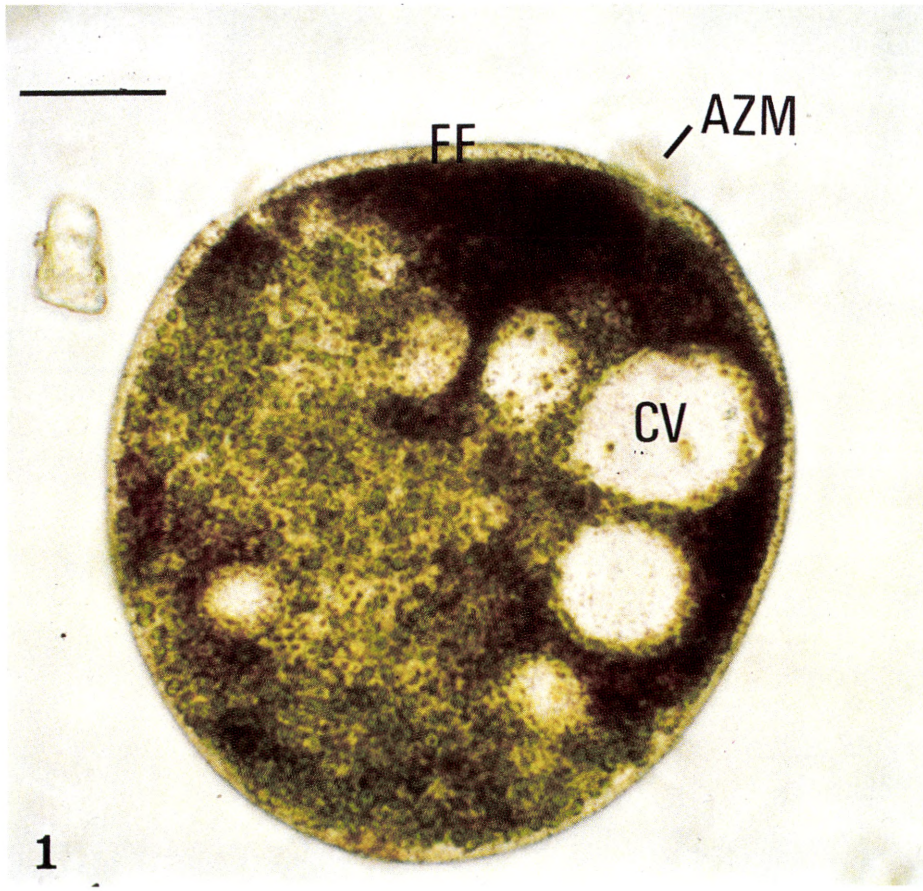
Plates I-IX

Plate I. Colour micrographs of the Kenyan *Stentor* sp.

1. Low magnification of the contracted ciliate illustrating its brownish colour which in part is due to the presence of green zoochlorellae. The adoral zone of membranelles (AZM), the frontal field (FF), and contractile vacuole (CV). Bar indicates 50 μm.
2. Higher magnification of a squashed ciliate showing the green zoochlorellae (symbiotic algae) and the large purplish-red pigment granules (arrows). Bar indicates 5 μm.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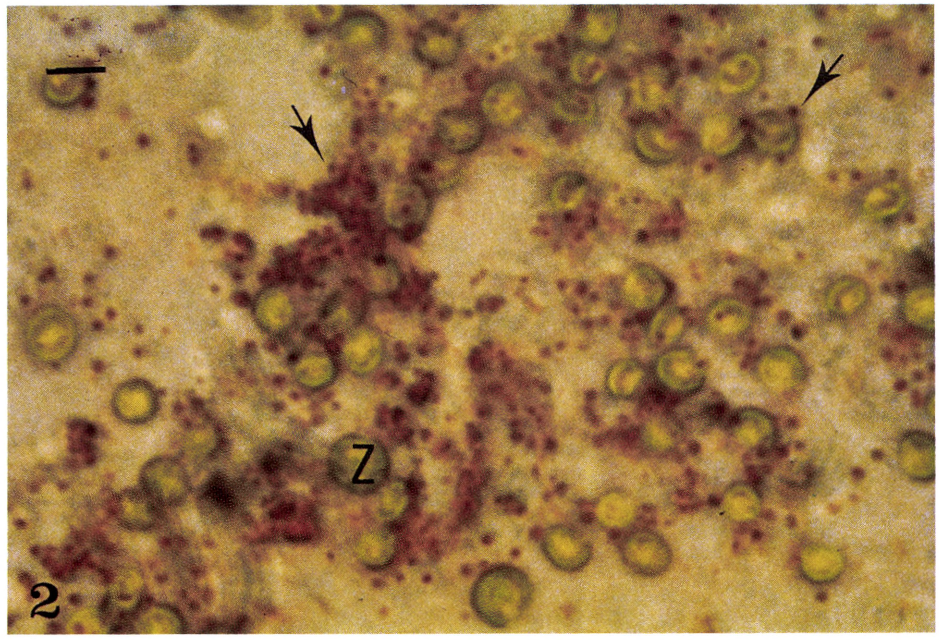
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Plate II. Light micrographs of the Kenyan *Stentor* sp. (*1–3*) and *Stentor amethystinus* Leidy (*4–6*).

1. Phase contrast microscopy of the cortical region (above the contractile vacuole) of the Kenyan *Stentor* revealing pigment granules (arrows) in cortical rows along the kineties. Bar indicates 5 μm.

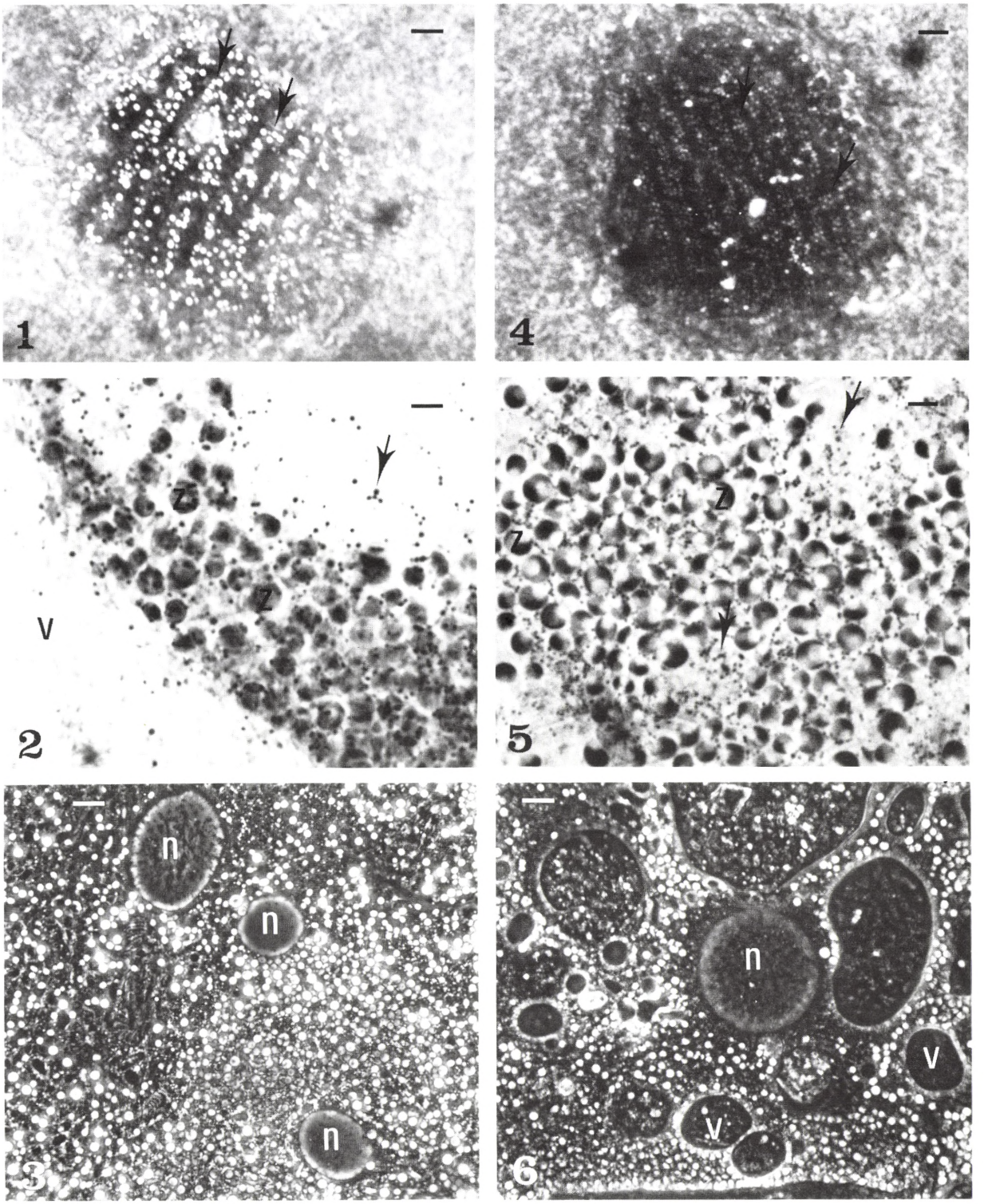
2. Light microscopy of compressed Kenyan *Stentor* showing pigment granules (arrows) and zoochlorellae (z). Note the vacuolated cytoplasm (v) in lower left hand corner of the figure. Bar indicates 5 μm.

3. Phase contrast microscopy of squashed Kenyan *Stentor* revealing 3 macronuclei (n), numerous zoochlorellae (large refractive bodies), and pigment granules (small refractive spots). Bar indicates 15 μm.

4. Phase contrast microscopy of the cortical region of *S. amethystinus*. The small pigment granules are arranged in rows between kineties (arrow). Bar indicates 5 μm.

5. Light microscopy of highly compressed *S. amethystinus* showing pigment granules (arrows) and zoochlorellae (z) with clear indication of the crescent-shaped chloroplast. Bar indicates 5 μm.

6. Phase contrast of a squashed *S. amethystinus* showing the single macronucleus (n), vacuoles (v), zoochlorellae (refractive bodies); the pigment granules are barely visible at this magnification. Bar indicates 15 μm.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6
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Plate III. Electron micrographs of the Kenyan *Stentor* sp.

1. Section through the cortical region revealing the km fibers (km) composed of ribbons of microtubules arising next to double kinetosomes (dikinetids) of the kineties (rows of cilia). Pigment granules (pg) are seen between the kineties. Deeper in the cytoplasm are the myonemes (my) closely associated with the membrane of smooth endoplasmic reticulum (er). Mitochondria (m). X 18,000. Bar indicates 1 μm.
2. Portion of cytoplasm of a contracted specimen showing a kinety of closely packed dikinetids with associated km fiber (arrow); note that only the posterior kinetosome of the dikinetids gives rise to a cilium. Pigment granules (pg) are numerous in the cortical region. The myonemal system (arrowhead) is surrounded by mitochondria (m). Some of the zoochlorellae (z) are in a partly disintegrated state (asterisks). X 5,400. Bar indicates 1 μm.
3. Pigment granules (pg) with fluffy content near the cortex, consisting of the plasma membrane with extraneous coat (arrow) and at least one other membrane. The thickness of the membrane of the pigment granules resembles that of the plasma membrane, whereas that of the endoplasmic reticulum (er) is thinner. X 60,000. Bar indicates 0.5 μm.

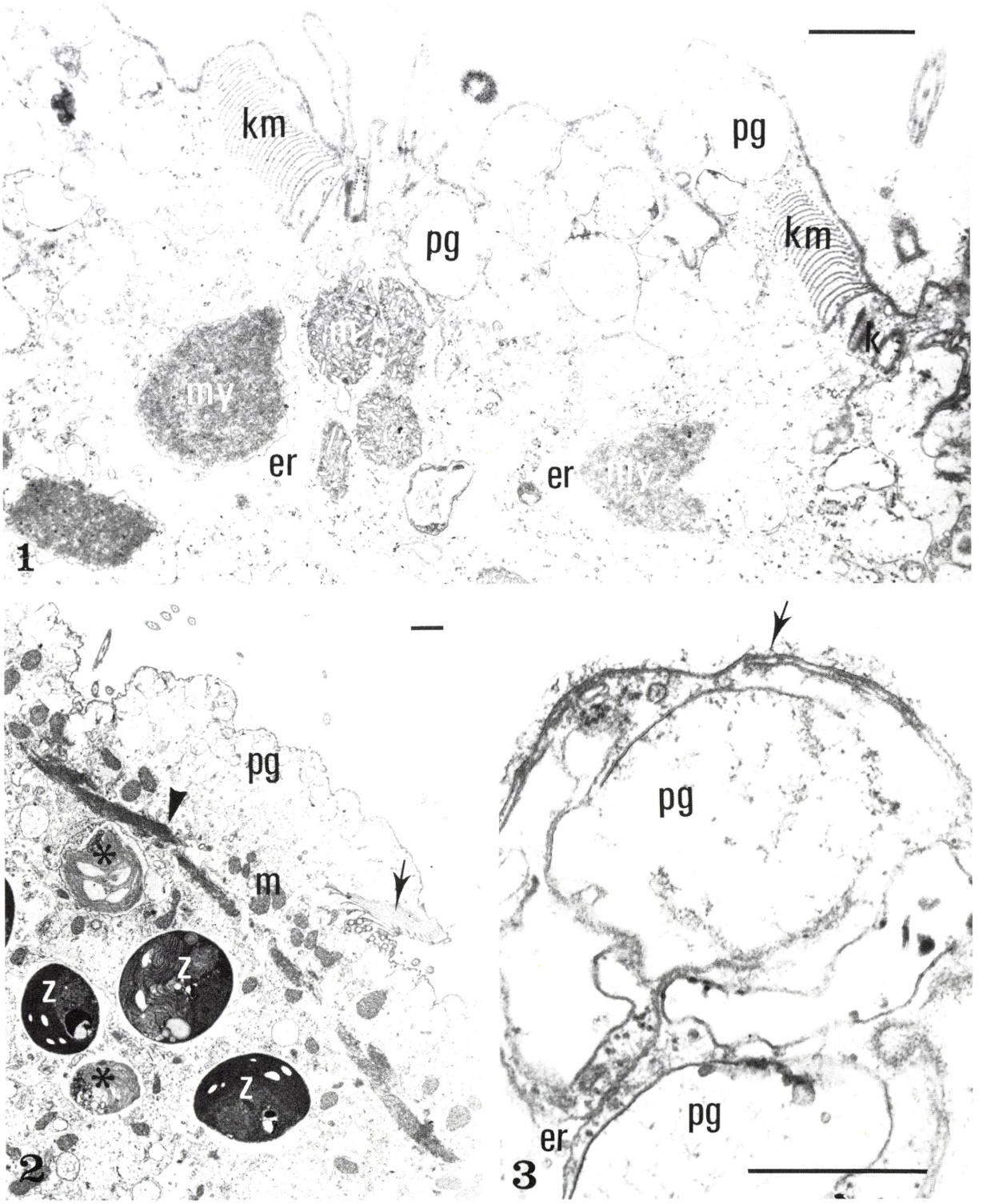
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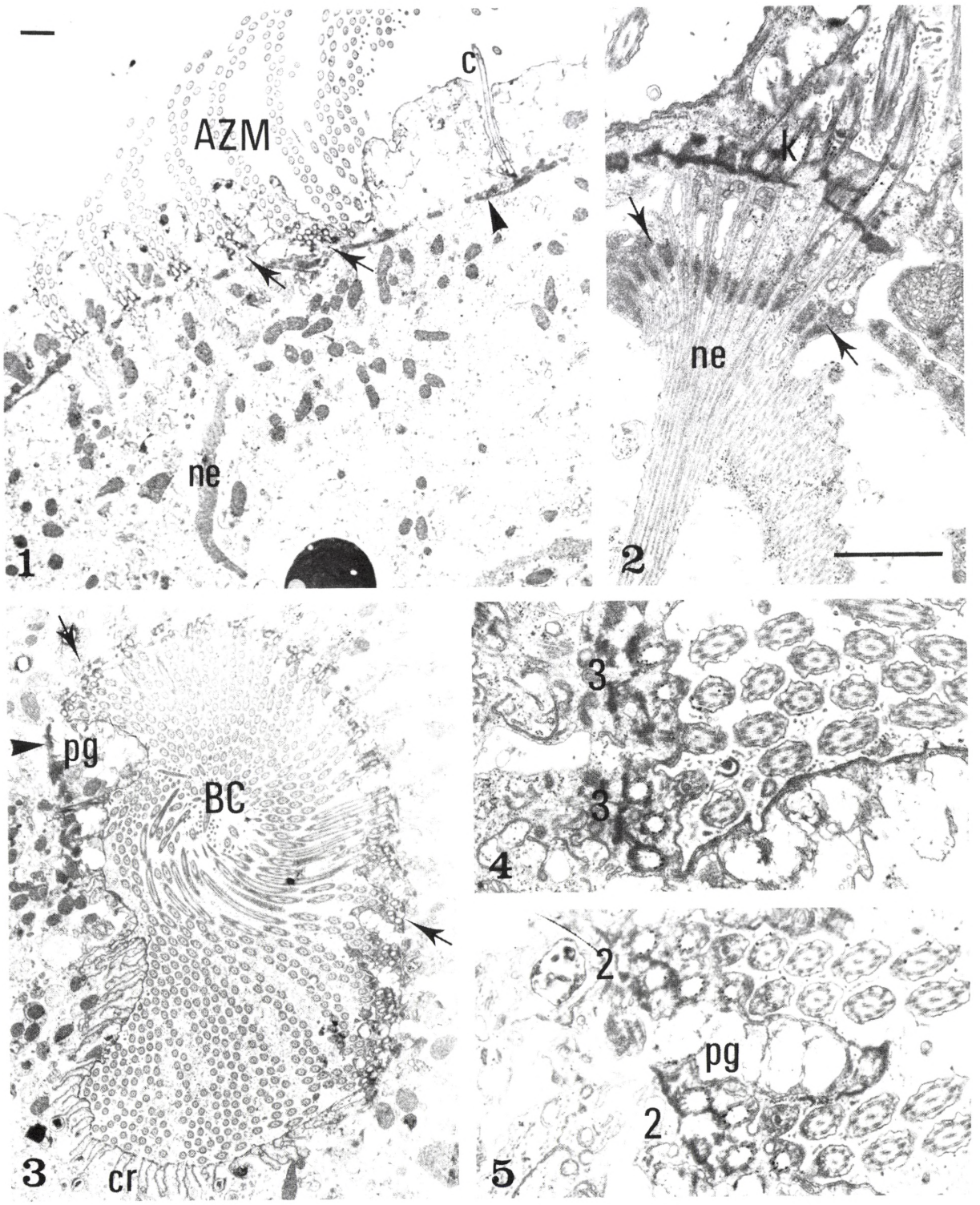
Plate IV. Electron micrographs of the Kenyan *Stentor* sp.

1. Section through the adoral zone of membranelles (AZM) in which the number of rows of kinetosomes changes from 3 to 2 (arrows) at the point where the AZM passes into the buccal cavity. Note the superficial position of the myonemes (arrowhead). The long nematodesmal fibers extend deep into the cytoplasm (ne). A somatic cilium (c). X 5,400. Bar indicates 1 μm.
2. Section through an adoral zone membranelle of which the kinetosomes (k) are basally interconnected by amorphous material. From the kinetosomes extend bundles of microtubules which unite after passing the level of myonemes (arrows), forming a fan-shaped fiber, the nematodesmal fiber (ne). X 18,000. Bar indicates 1 μm.
3. Oblique section through the buccal cavity (BC) showing at superficial level 3 rows of kinetosomes (arrow, upper left) and at deeper level, 2 rows of kinetosomes (second arrow) in the adoral zone of membranelles. Pigment granules (pg) are abundant below upper buccal cavity wall where a trace of myonemes is seen (arrowhead), no myonemes are found at deeper level. Cytopharyngeal ribbons (cr) are found near cytostomal region. X 5,400.
4. Adoral zone membranelles consisting of 3 rows of kinetosomes (enlargement of upper left corner of *3*, turned 45 degrees). X 18,000.
5. Adoral zone membranelles consisting of only 2 rows of kinetosomes (enlargement of *3* at second arrow, turned 180 degrees). Note row of pigment granules (pg) between membranelles. X 18,000.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BC
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3
4
2
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2
5

Plate V. Electron micrographs of the Kenyan *Stentor* sp.

1. Portion of a macronucleus (ma) showing coarse network of chromatin and interspersed nucleoli (n). A micronucleus (arrow) is situated in a macronuclear depression. (See also *4*). X 5,400. Bar indicates 1 μm.

2. Pigment granules (pg) showing varying substructure of content; for overall location in central portion of ciliate, see *5*. X 18,000. Bar indicates 1 μm.

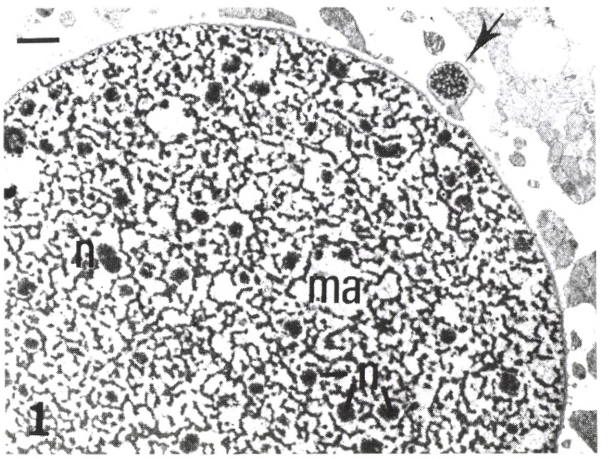
3. Higher magnification of pigment granules, revealing a distinct globular substructure in finely granular material (pg) and a faint image of such a substructure in other, partly shown granules (g). In the cytoplasm, rough endoplasmic reticulum (er) and ribosomes are seen. X 60,000. Bar indicates 0.5 μm.

4. A micronucleus (mi) situated in a macronuclear depression (arrow). Note the electron dense, condensed chromatin in the micronucleus. X 18,000.

5. Cytoplasmic region near a macronucleus (asterisk) containing numerous granules of differing electron density (arrows) believed to be developmental stages of pigment granules. X 5,400.

6. Pigment granules (pg) having a substructure intermediate between the two types shown in *3*. X 60,000.

7. Section through a pigment granule (pg) at the cortex (arrow) with a substructure resembling that of the granules in the central cytoplasm (neighbouring figures), but different from that of the majority of pigment granules in the cortical region (see, Plate III, *3*). Mitochondrion (m). X 60,000.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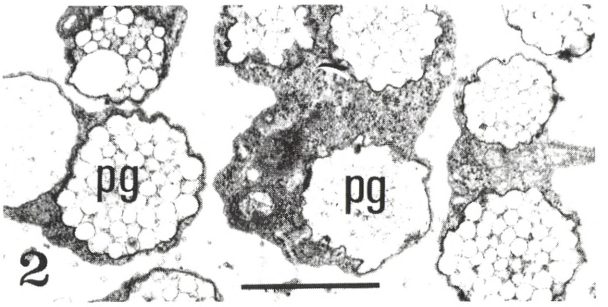
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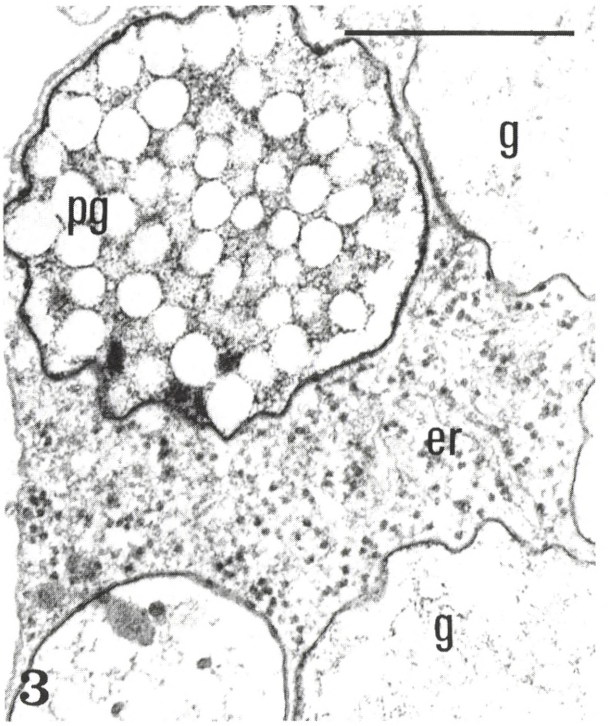
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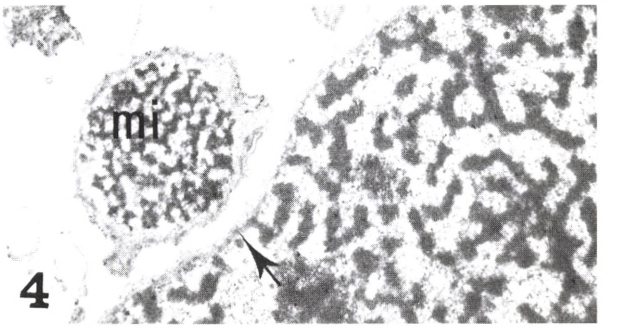
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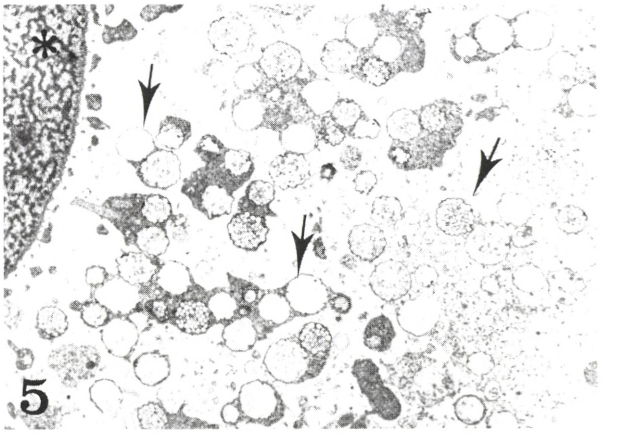
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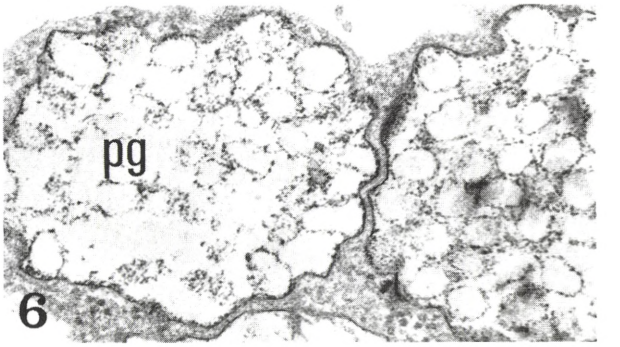
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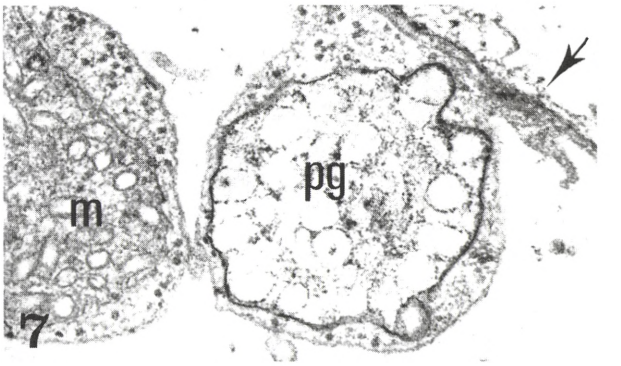
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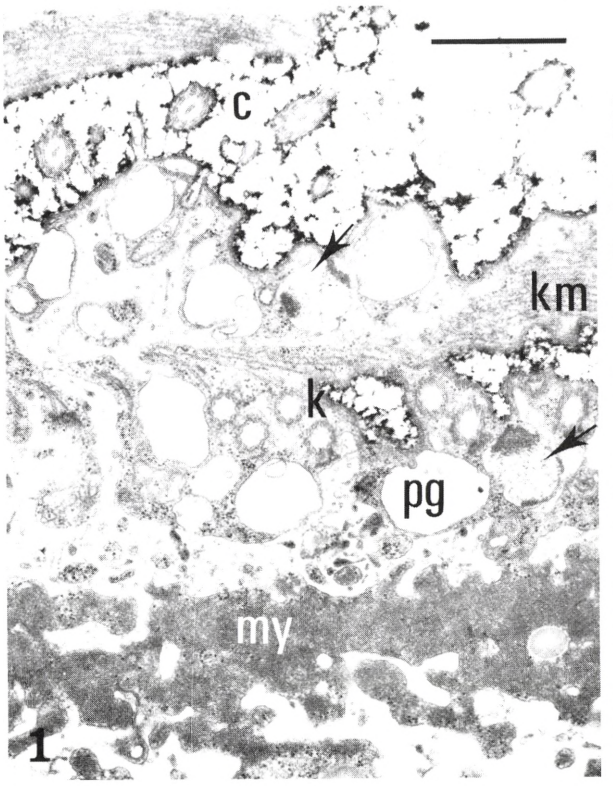
Plate VI: Electron micrographs of *S. amethystinus* Leidy. Osmium fixation: *2, 3.*

1. Section through cortical region illustrating single cilia (c), but double kinetosomes (k), dikinetids, in kineties. Rows of microtubules (postciliary fibrils), arising at posterior kinetosome of dikinetids, form the km fiber (km). Pigment granules (pg) of which some (arrows) have a distinct substructure. Myonemal system (my). X 18,000. Bar indicates 1 μm.

2. The heterogeneity of the substructure of pigment granules (arrows) is clearly revealed in osmium fixation. The large distance between dikinetids (k) indicates the relaxed state of the ciliate; note that the km fiber consists of only a few postciliary fibrils (pcf). Irregularly shaped profiles (asterisks) are cortical depressions and their »fluffy content« is surface coat (co). X 18,000.

3. Portion of a cross section revealing pigment granules in ridges (r) between kineties showing kinetosomes and km fibers. Below the ridges, the myonemal system forms a continuous band (arrow). Zoochlorellae (z) within perialgal vacuoles, numerous paraglycogen bodies (g), and mitochondria (m). X 5,400. Bar indicates 1 μm.

4. Pigment granules (pg) with differing substructure. Their membrane resembles the plasma membrane (arrow) in thickness, whereas the membrane of the endoplasmic reticulum is thinner (er). Cilum (c). Myoneme (my). X 60.000. Bar indicates 0.5 μm.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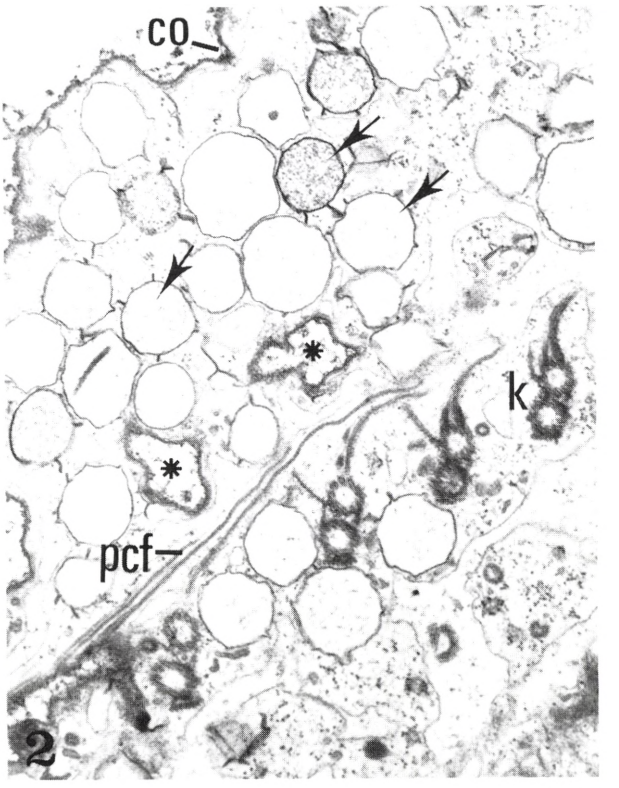
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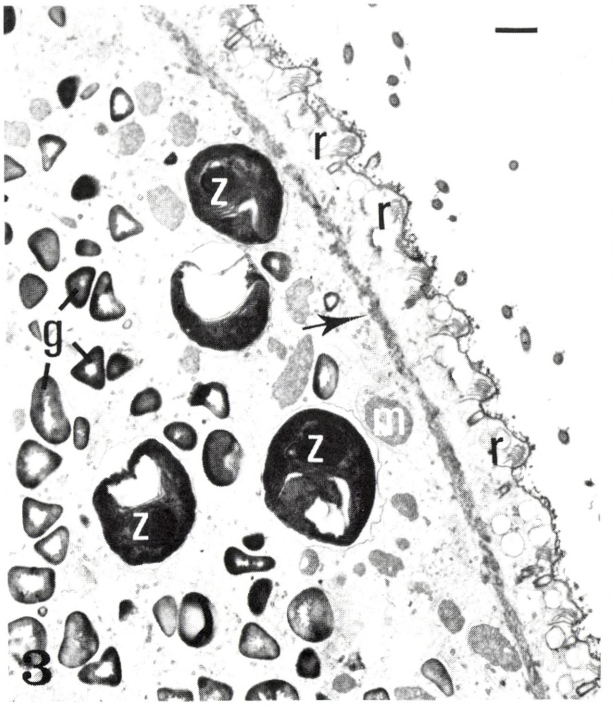
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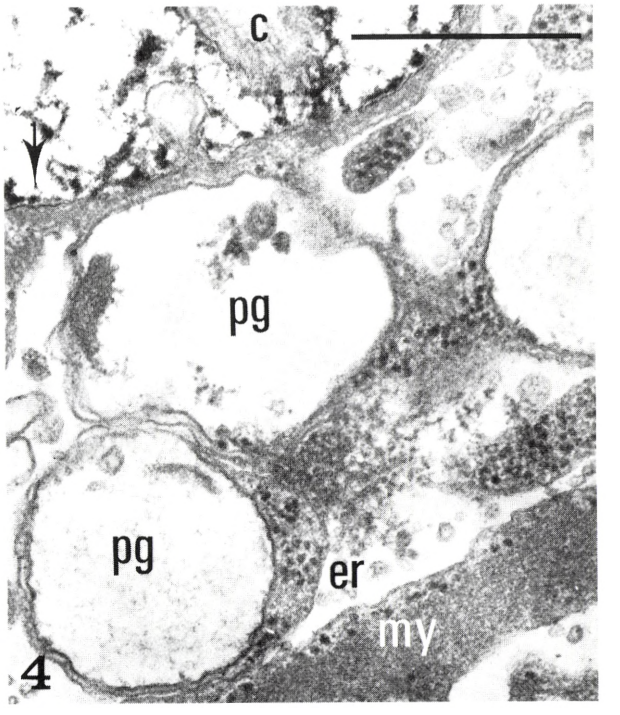
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Plate VII. Electron micropraphs of *S. amethystinus* Leidy.

1. Tangential section through part of adoral zone membranelles (AZM) composed of 3 rows, hexagonally packed kinetosomes which are interconnected by fine filamentous material. Fibrils pass from the base of kinetosomes of the lower row (arrowheads) into the ridges (r) between the membranelles. Nematodesmata, bundles of microtubules, arising at the base of kinetosomes, are shown in cross section (arrows). Presence of myonemal system (my) indicates that the membranelles are from the AZM encircling the frontal field. X 18,000.

2. Cross section through 5 adoral zone membranelles along the frontal field; note the amorphous material at base of kinetosomes (k) and fibrils (arrowheads) passing from the membranelle towards the surface. The extensive nematodesmal fibers are shown in cross, and longitudinal, sections (arrows); the hexagonal packing of microtubules is revealed. Part of a zoochlorella (z) in perialgal vacuole. Mitochondrion (m). X 18,000. Bar indicates 1 μm.

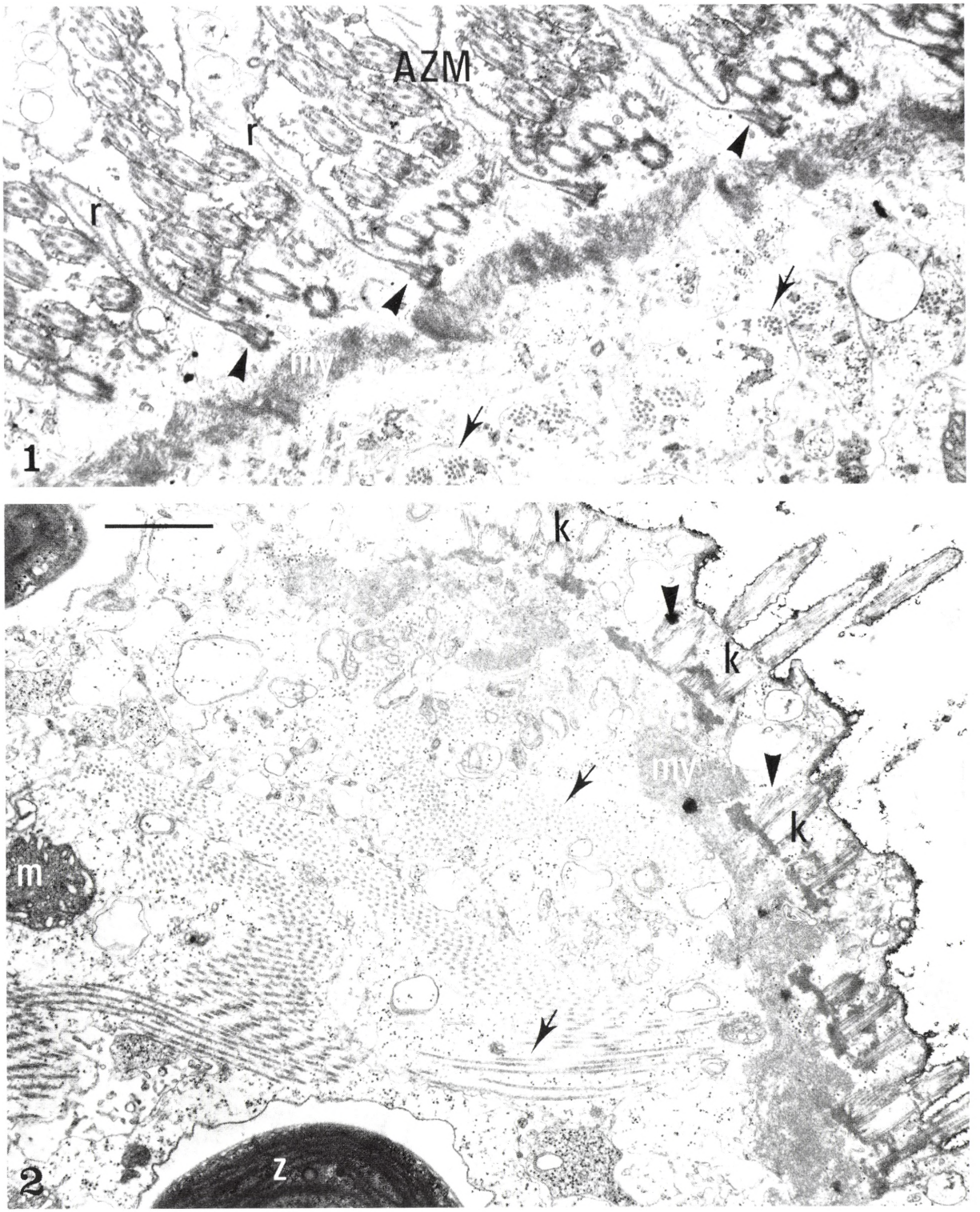
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Plate VIII. Electron micrographs of *S. amethystinus* Leidy. Osmium fixation.

1. Section through the adoral zone of membranelles (AZM) where the number of kintosomal rows changes from 3 to 2 (arrows), believed to represent the site where the AZM leaves the edge of the frontal field to pass into the buccal cavity. The myonemal system (arrowhead) is shown below left hand side of membranelles. Nematodesmal fibers (ne), forming a wavy course towards cytostome where a fraction of a food vacuole (fv) (14 μm in diameter) is shown. Note numerous pigment granules in cortical ridge (r). X 5,400. Bar indicates 1 μm.

2. Enlargement of 2 adoral zone membranelles, labelled by arrows in previous figure, at the transition from 3 to 2 rows of kinetosomes. Note the filamentous connections between the kinetosomes (k) within membranelles. Myonemal system (my). Hexagonal packing of microtubules within nematodesmal fibers (arrows). Mitochondrion (m). X 18,00. Bar indicates 1 μm.

3. Oblique section through upper part of buccal cavity (BC) showing tangential section through part of the frontal field (ff) and adoral zone membranelles (AZM) containing 3 rows of kinetosomes, representing entry of the AZM into the buccal cavity (viewed from inside cell). The basal fiber of the nematodesmata (arrow). X 5,400.

4. Enlargement of the AZM membranelles shown in previous figure; nematodesmal fibers arise from 2 of the 3 rows of kinetosomes. Note the absence of myonemal system. Mitochondrion (m). X 18,000.

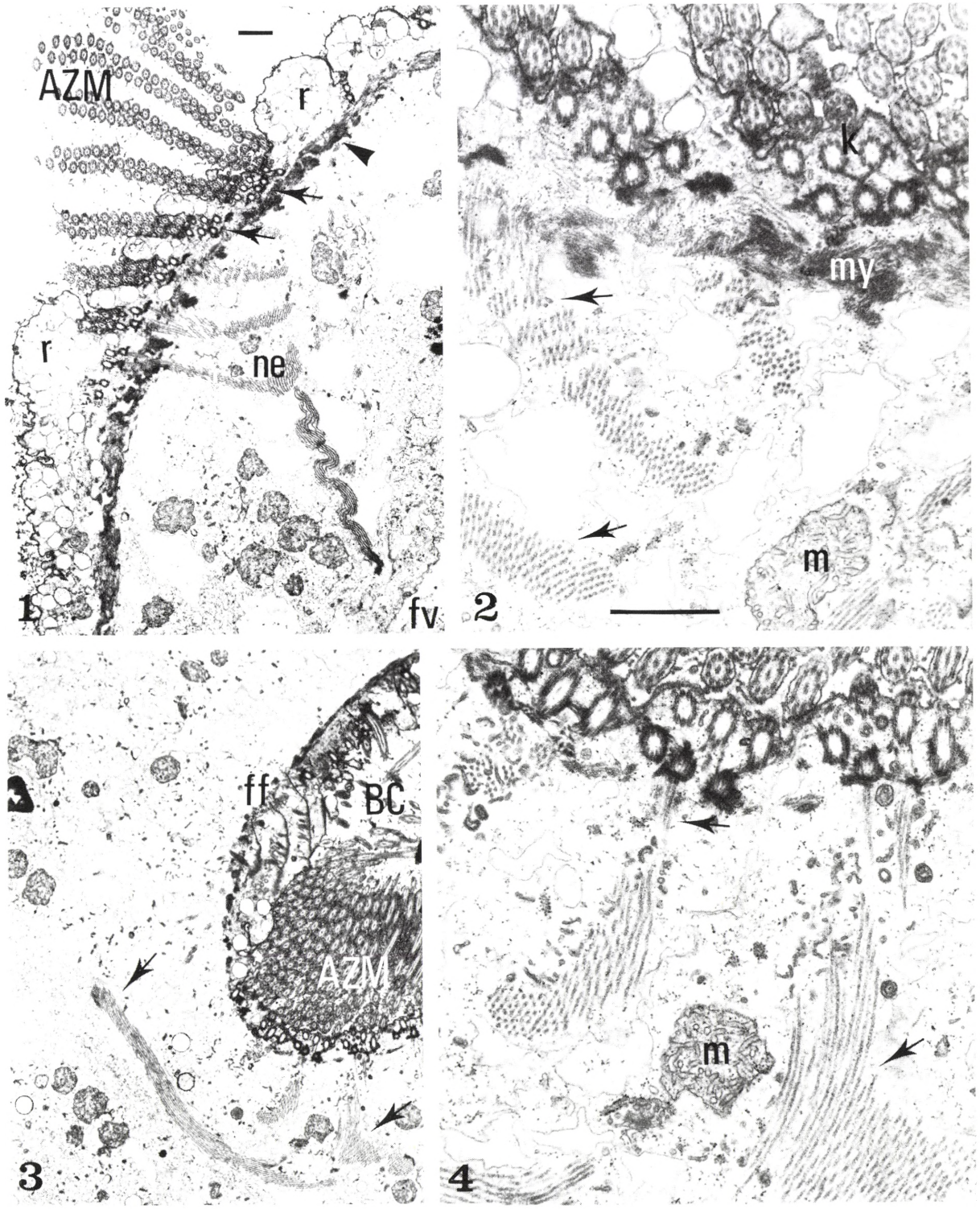
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Plate IX. Electron micrographs of *S. amethystinus* Leidy. Osmium fixation: *1.–4.*

1. Cross section of the cytopharyngeal region (CPh), note tips of cilia from buccal cavity (arrow). Near the irregularly shaped cytopharyngeal membrane are numerous small vesicles (v) believed to provide the limiting membrane of a future food vacuole. Cytopharyngeal ribbons (arrowheads) border most of the cytopharynx. X 5,400. Bar indicates 1 μm.

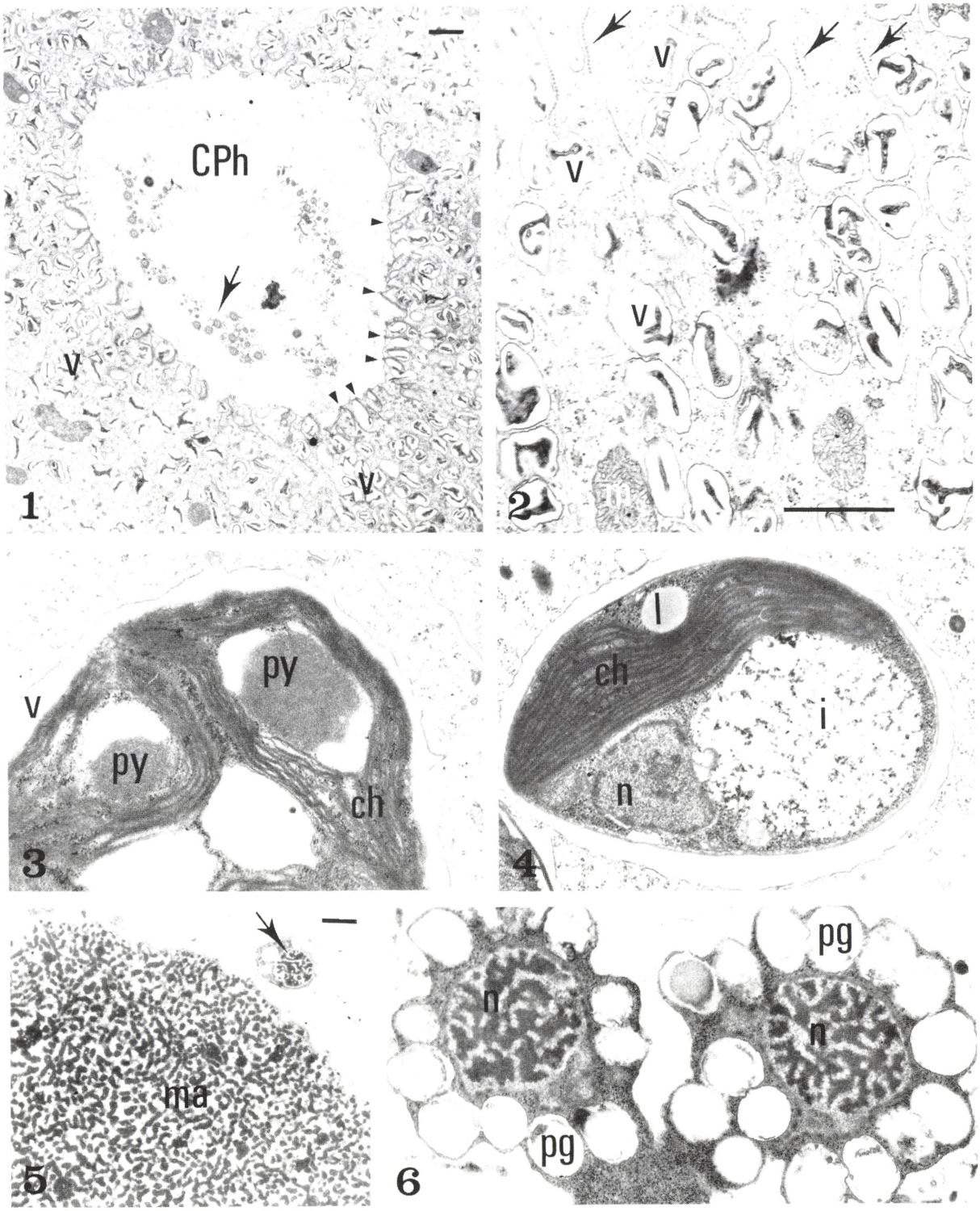
2. Higher magnificatin (neighbouring section to that shown in *1.*) showing the vesicles (v) of the cytopharyngeal region (lumen towards top of figure), they are believed to represent membrane-recycling derived from previous digestive vacuoles. Note cross sections of cytopharyngeal ribbons (arrows). Mitochondrion (m). X 18,000. Bar indicates 1 μm.

3. Part of a dividing zoochlorella in its perialgal vacuole (v) showing 2, of the 4, chloroplasts with pyrenoids (py). This is a rare finding. X 18,000.

4. Typical zoochlorella within perialgal vacuole having a smooth outline. Note the laminated chloroplast (ch), the nucleus (n) with a large central nucleolus, lipid droplet (l), and large inclusion body (i). X 18,000.

5. Part of the macronucleus (ma) with coarse network of chromatin and some distance away a micronucleus (arrow). X 5,400. Bar indicates 1 μm.

6. Two micronuclei (n) with condensed chromatin are surrounded by numerous granules which in size and position correspond to those of pigment granules in the light microscope. Note the differing substructure of the pigment granules. X 18,000.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