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The Unusual, Lepidosome-coated Resting Cyst of *Meseres corlissi* (Ciliophora: Oligotrichea): Light and Scanning Electron Microscopy, Cytochemistry

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Summary. *Meseres corlissi* Petz *et* Foissner, 1992 is a planktonic, spirotrich ciliate closely related to the common *Halteria grandinella*. Pure cultures were established with *Cryptomonas* sp. as a main food source. Thus, resting cyst formation and structure could be studied by light and scanning electron microscopy and cytochemical methods. The resting cyst of *M. corlissi* belongs to the kinetosome-resorbing (KR) type and has a conspicuous coat of extracellular organic scales, here termed lepidosomes, embedded in mucus mainly composed of acid mucopolysaccharides, as shown by the strong reaction with alcian blue. The lepidosomes, which likely consist of glycoproteins, are finely faceted, hollow spheres with a diameter of 2-14 µm. They are formed underneath the cortex and liberated almost concomitantly to the external surface of the cell before the cyst wall is produced. Resting cyst size is dependent on temperature, the average diameter is 46 µm (without lepidosome coat) at 20°C. The cyst wall, which contains considerable amounts of glycoproteins and a layer of chitin, is smooth, 1.5-2 µm thick, and composed, in the light microscope, of two distinct layers highly resistant to various inorganic and organic solvents. The cyst doubles the diameter during excystment and the lepidosome coat is left behind. The occurrence of lepidosomes throughout the ciliate phylum, their genesis and function, and the conspicuous morphological similarity with leafhopper's brochosomes are discussed.

Key words: brochosomes, Cicadellidae, Colpodea, encystment, Haptorida, organic scales, plankton ciliates, protozoa, Trachelophyllida.

INTRODUCTION

Many ciliates form a dormant stage, the resting cyst, when environmental conditions become adverse and/or food is depleting. The cyst is composed of a several µm thick wall protecting the cell from a wide variety of detrimental influences, such as drought, heat, and chemicals (Corliss and Esser 1974, Foissner 1987, Gutiérrez *et al.* 2001). The wall is a complex structure usually composed of three layers, that is, the ectocyst, the mesocyst, and the endocyst. Chemically, these layers consist of varying amounts of carbohydrates (often in form of chitin), proteins, and glycoproteins (Mulisch 1993, Gutiérrez *et al.* 2003, Rosati *et al.* 2004). Usually, ciliate resting cysts are spherical or ellipsoidal and several have a preformed orifice through which the excysting cell leaves the cyst. The external cyst wall morphology is highly different among the various ciliate groups and species, that is, the wall may be smooth,

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tuberculate, faceted, or spiny (for reviews, see Foissner 1993; Berger 1999; Foissner *et al.* 1999, 2002).

Generally, data on ciliate resting cysts are rather incomplete, most coming from only two groups, viz., the spirotrichs (e.g., *Oxytricha*, *Euplotes*, *Blepharisma*; for reviews, see Wirnsberger-Aescht *et al.* 1990, Berger 1999 and Gutiérrez *et al.* 2001) and the Colpodea (for reviews, see Foissner 1993 and Gutiérrez *et al.* 2003). Few data are available from the oligotrichine spirotrichs (for a brief review, see Müller 2000), of which *Meseres corlissi* and the common *Halteria grandinella* are representative examples.

The present study, which consists of two parts, provides the first detailed morphological investigation of the resting cyst of an oligotrichine, halteriid spirotrich, using light and electron microscopy and some cytochemical methods. Such data are urgently needed for comparative purposes and the discussion of the systematic position of halteriid spirotrichs (Foissner *et al.* 2004). In contrast to some other investigators, we perform detailed investigations not only on preserved but also on living cysts because various features, especially morphometrics and colour may greatly change or even remain unrecognized in fixed material, as is the case in various colpodids (Foissner 1993, Díaz *et al.* 2003). Further, such data enhance the interpretation of the electron microscopical findings and the identification of cysts by ecologists. Indeed, the resting cyst of *M. corlissi* is so characteristic that it can be easily identified in field collections of cysts, as performed by ecologists (Müller 2000).

Our investigations show that the resting cyst of *M. corlissi* is unique in having spherical, nicely faceted epicortical scales reminiscent of those found in trophic trachelophyllid ciliates (Nicholls and Lynn 1984, Foissner *et al.* 2002). A detailed literature search showed that epicortical structures occur in trophic and cystic ciliates from various classes. This, however, remained obscure because they have been described under a huge variety of names. Thus, we suggest to collect epicortical structures under a single term: lepidosomes.

MATERIALS, METHODS AND TERMINOLOGY

Origin of cultures and stock culture conditions. Most morphological investigations were performed on a *Meseres corlissi* population collected by Prof. Dr. Walter Till (Vienna University, Austria) in the Dominican Republic, viz., in the tanks of *Guzmania ekmanni*, a bromeliad plant found on a tree of the fog rain forest on the Pico Diego del O'Campo near the town of Santiago. The senior author identified the species according to the original description by Petz and Foissner (1992). The observations on encystment and the cytochemical investigations were performed on cysts from the type population, re-sampled in the city area of Salzburg (Austria) in November 2002.

Raw cultures of *M. corlissi* were established on Eau de Volvic (French table water) enriched with some squashed wheat grains. This raw culture was purified and adapted to an algal diet composed of *Cryptomonas* sp. strain 979-4 (Culture Collection of Algae, SAG, Göttingen, Germany). Both the ciliate and cryptophyte were maintained in modified Woods Hole medium (MWC medium, Guillard and Lorenzen 1972) at 15 ± 1°C and continuous light (100 µmol photons $m^{-2}s^{-1}$).

Induction of encystment and excystment. Cultures in the stationary growth phase were transferred to room temperature (~ 19°C) and starved which caused encystment of most cells within 48 h. Starved cells were pipetted into thin micro-aquaria made of vaseline and a cover glass on a microscope slide and observed for encystment every 30 min. Excystment could be induced by transferring a cyst containing culture from 15 to 24°C.

Temperature response experiments. *Cryptomonas* sp. was offered at saturating food levels to Dominican Republic *M. corlissi* in sterile 200 ml tissue-culture bottles. Samples were taken from experimental containers 48 h after the beginning of the experiment and fixed with Lugol's iodine (final concentration 2% vol/vol). The fraction of cysts was calculated as the number of cysts in a sample, divided by the total number of trophic plus encysted cells. Length and width of cysts were measured by inverted microscopy and image analysis (LUCIA version 4.51, Laboratory Imaging Ltd.). Details of these experiments have been reported elsewhere (Weisse 2004).

Morphological and cytological methods. Cysts were studied *in vivo*, in protargol preparations, and with the scanning electron microscope, using the methods described in Foissner (1991). *In vivo* measurements were conducted at a magnification of ×1000. The nature (organic, inorganic) and chemical composition of the lepidosomes were determined with cytochemical methods described in Adam and Czihak (1964), Tracey (1955) and Romeis (1968), on both, cysts fixed with ethanol (96%) or neutral formalin (5%). Then, the cysts were washed in distilled water, mounted on slides with albumen-glycerol, air-dried, and the albumen hardened in ethanol (100%). For the cytochemical methods, the slides were rehydrated *via* a graded ethanol series. Tests with alcian blue, Lugol's solution, and osmium acid (2%) were made also on unfixed cysts. However, results were similar to those obtained with fixed cysts. This applies also to cysts fixed either with ethanol or formalin. Thus, results are not shown separately in Table 3. Cyst wall solubility was determined with several inorganic and organic solvents mentioned in the results section.

Chitin was detected with the Van Wisselingh colour test, as described in Tracey (1955). This simple, fast method deacetylates chitin with KOH to chitosan which gives a deep, redbrown colour with iodine. Landers (1991) used the method for revealing chitin in the cyst wall of *Hyalophysa chattoni*, a parasitic ciliate on shrimps. Our experiences with cysts of *Meseres* and other ciliates and materials suggest that this test is highly reliable for both, chitin and cellulose. Thus and because it disappeared from the modern literature, we provide the full protocol, as described in Tracey (1955).

"About a milligram of the material to be tested is heated, in a small tube closed with a Bunsen valve, with potassium hydroxide solution saturated at room temperature (dissolve about 120 g KOH in 100 ml water). The contents of the tube are heated by immersing the lower portion in a glycerine bath the temperature of which is raised to 160°C over a period of 15-20 mins. It is held at this temperature for a further 15 mins and allowed to cool to room temperature. If no insoluble material is present then chitin in appreciable quantities is absent. Any insoluble material is transferred manually, or after centrifugation, to 95% alcohol and washed successively with 70%, 50%, and 30% alcohol and finally with water (solutions of glycerol may be used in place of alcohol). Few substances other than cellulose and inorganic material [either adventitious (sand) or intrinsic (barium sulphate in some fungi)] are likely to withstand this treatment. A portion of the material is placed on a slide and covered with a drop of 0.2% iodine in potassium iodide solution. The material will turn brown and this colour will be replaced by a red-violet on the addition of 1% sulphuric acid if chitosan is present. Replacement of the acid iodine solution by 75% sulphuric acid will result in the dissolution of the material. If cellulose is present it will not be coloured by the acid iodine reagent, but will turn blue and swell, instead of dissolving, on the addition of 75% sulphuric acid.

Notes. (1) Washing with progressive dilutions of alcohol has the advantage that less dissolution of the material occurs and that the yield of chitosan appears to be increased.

(2) Many attempts have been made to simplify the alkali treatment by using weaker solutions for longer periods, by evaporating to dryness in alkali, or by boiling in alkali. None gives such satisfactory results as that outlined above.

(3) Some material may have a reddish colour after the alkali treatment. If this is so it is probably as well to relay on the chitosan sulphate test.

(4) Failure to get the colour reaction is not conclusive evidence of the absence of chitin. Delicate structures such as butterfly scales may disperse completely during the normal time of heating, and the iodine colour test may be occasionally negative when the chitosan sulphate test is positive.

(5) It may be desirable to pretreat some materials. Dilute alkali will remove much organic matter thus concentrating chitin in the material to be tested. 0.2 M sodium chlorate at pH 5.0 and 75°C will decolourise the darkest fungal material besides greatly reducing the proportion of non-chitinous organic material."

The sole critical step is the time of KOH treatment. Usually, ciliate cysts dissolve completely if treated for 30 min, as given above. Thus, we used 15-20 min. Further, the complicated heating described above can be simplified by pre-heating 3 ml KOH to 160°C in a glass centrifuge tube and adding the concentrated material directly into the hot KOH solution for 15-20 min. For *Meseres*, the test was performed with the original and the modified protocol with the same result. The modification runs as follows: the cysts were concentrated by centrifugation and the pellet put in 3 ml saturated, hot (160°C), aquaous potassium hydroxide solution (KOH) for 20 min at 160°C. Small, cleaned parts of meal beetle larvae (*Tenebrio molitor*) and some cotton wool served as controls and were added to the preparation. After heating, the probe was diluted with tap water to 10 ml and centrifuged for one min at 3000 x g. The resulting pellet was washed five times in tap water, some drops of 1% sulphuric acid were added to the last wash to dissolve minute (carbonate?) crystals. Finally, a minute drop of the washed pellet was put on a slide, covered with a coverslip, and investigated at a magnification of ×1000 (oil immersion). This revealed many very hyaline, globular cyst "ghosts" which stained reddish to deep redbrown when Lugol´s solution was added from the margin of the coverslip. Controls were treated in the same way and differentiated as described above.

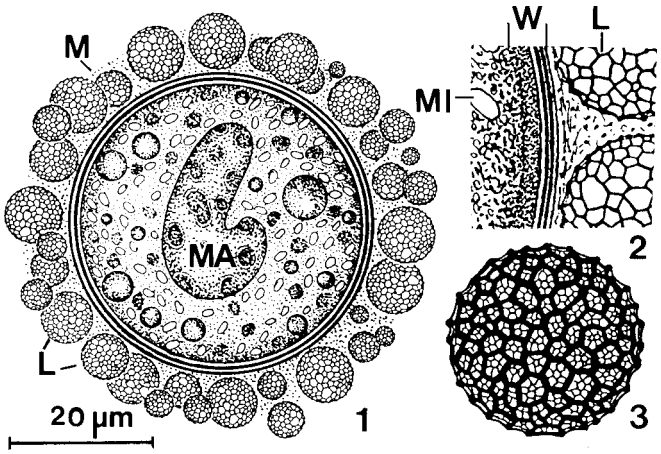
Terminology. Terminology is according to Corliss (1979) and Gutiérrez *et al.* (2003), while the systematic classification follows Lynn (2003) and Agatha (2004). However, we introduce "lepidosomes" (lepidotos Gr. - scaled, soma, Gr. - body) as a new term for all kinds of epicortical (extracellular), organic structures of definite shape produced intracellularly by trophic and/or cystic ciliate species. For details, see first chapter of Discussion.

RESULTS

Trophic cells

The specimens from the Dominican Republic are highly similar to those from the Austrian type population (Fig. 7), but are, both in the field sample and well-growing cultures, frequently distinctly conical and may even have a short tail (Fig. 4). However, conical cells occur also in flourishing pure cultures of the Austrian population. Broadly ellipsoidal cells, as shown in the original description (Petz and Foissner 1992), occur mainly in old and/or oxygen-depleting cultures. The specimens have a size of about 75 × 50 µm *in vivo* and are covered by circa eight equidistant rows of about 16 µm long bristles. The anterior body end is occupied by about 16 collar and 15 ventral adoral membranelles (Figs 4, 7).

Trophic and dividing specimens, including the somatic cilia and adoral membranelles, are coated by a skin-like layer of slime, especially the peristomial bottom, where the layer is thickened. This coat is invisible *in vivo*, but becomes distinct in the scanning electron microscope (Figs 4-6) and when living cells are immersed in alcian blue, especially in the oral area, where flakes of bluish material are released. Depending on the state of preservation, the slime coat appears granular (well preserved) or reticular (poorly preserved), partially exposing the smooth pellicle and the cilia in the scanning electron microscope (Fig. 6). The slime coat is also obvious in the Austrian specimens studied by Petz and Foissner (1992), who did not comment on it. The strong reaction with alcian blue indicates that the coat contains considerable amounts of acid mucopolysaccharides (Adam and Czihak 1964).



Figs 1-3. *Meseres corlissi*, resting cyst of a specimen from the Dominican Republic in the light (1, 2) and scanning electron microscope (3). **1** - Overview, diameter with lepidosome coat about 60 µm; **2** - detail from figure 1 showing, inter alia, the about 1.6 µm thick cyst wall composed of two main layers; **3** - lepidosome at high magnification, diameter 9 µm. L - lepidosomes, M - mucus, MA - macronucleus, MI - mitochondrium (?), W - cyst wall.

Encystment

Of 20 specimens isolated from a non-flooded Petri dish raw culture (Foissner 1987), 17 (85%) produced perfect resting cysts within 12 h. When specimens from the Dominican Republic were cultivated at 19°C and transferred to 4°C, encystment was accomplished within 1 h. In growth experiments, encystment was negligible at temperatures >20°C but increased dramatically if temperature was below 20°C (Table 1). The ciliate did not grow at temperatures <15°C, and food had little effect on cyst production (Weisse 2004). Note that the encystment success may decrease to a few percent in strains cultivated for several months in the laboratory, and many of the cysts may appear defect. Obviously, encystment depends on many factors whose investigation was outside the scope of the present study.

The microaquaria worked quite well, that is, many starved cells produced cysts within 12 h. However, the percentage of non-producers is often near 50%, that is, these specimens do not encyst and most die within 24 h. As the dying specimens become globular, they are frequently not easily distinguished from ordinary, encysting cells. Further, the microscopic observation with oil immersion appears to disturb more or less distinctly the encystment process. Thus, some luck and patience are needed to see and document the following details.

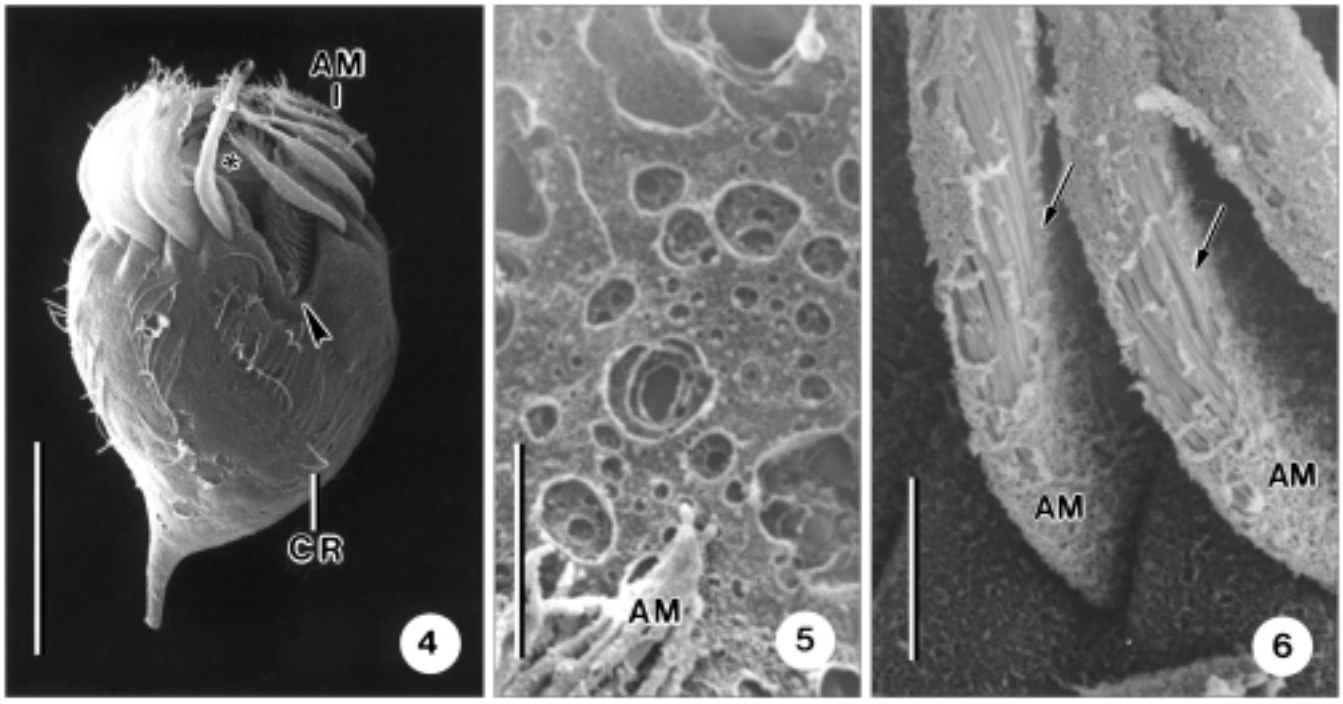
When encystment commences, the cell rounds up and resorbes the somatic and oral cilia and basal bodies (Figs 21, 22). This is not only shown by the transmission electron microscopical investigations (Foissner 2005), but also by observations on protargol-impregnated cysts (no micrographs are provided because they would show only very small areas due to the globular shape of the cyst). 100 well-impregnated cysts each from three different cultures and of three different ages showed the following pattern: 4 days old cysts (99 without, 1 with basal bodies), 9 days old (89/11), 30 days old (97/3). Thus, only 5% of the cysts show basal bodies and their maintenance is not age-related. Only 1 of the 15 cysts with basal body remnants maintained the complete ciliary pattern, though in rather distorted condition, while the others preserved merely small parts of the ventral adoral zone of membranelles. Similar results were obtained with silver carbonate impregnation. Thus, *M. corlissi* belongs to the kinetosome-resorbing (KR) cyst type, according to the classification of Walker and Maugel (1980).

During rounding up, the cell secretes a thin layer of fluffy mucus. Fully developed lepidosomes with faceted wall become visible underneath the cortex when the cell has rounded up (Figs 22–24); frequently, the proximal part of the somatic and oral cilia is still recognizable. Next, the lepidosomes and additional mucus are released almost concomitantly within a few minutes. We could see details of this process only once, and from this it appears that the lepidosomes are released concomitantly by an exocytotic process. We never observed additional lepidosome release in cysts with a lepidosome coat, likely because cyst wall formation commences immediately after release of the lepidosomes (Fig. 25).

Resting cysts

Morphology: Resting cysts are very similar in the Dominican Republic and the Austrian specimens (cp. Figs 15, 16, 27). However, only cysts of the former were studied in detail. The cysts are firmly attached to the bottom of the culture dishes, likely by the mucilage described below. Even strong shaking or water flushes do not detach all from the substrate. Thus, the mucus and the lepidosome coat are usually partially destroyed when cysts are collected for SEM preparations or transferred with a pipette to the microscope slide.

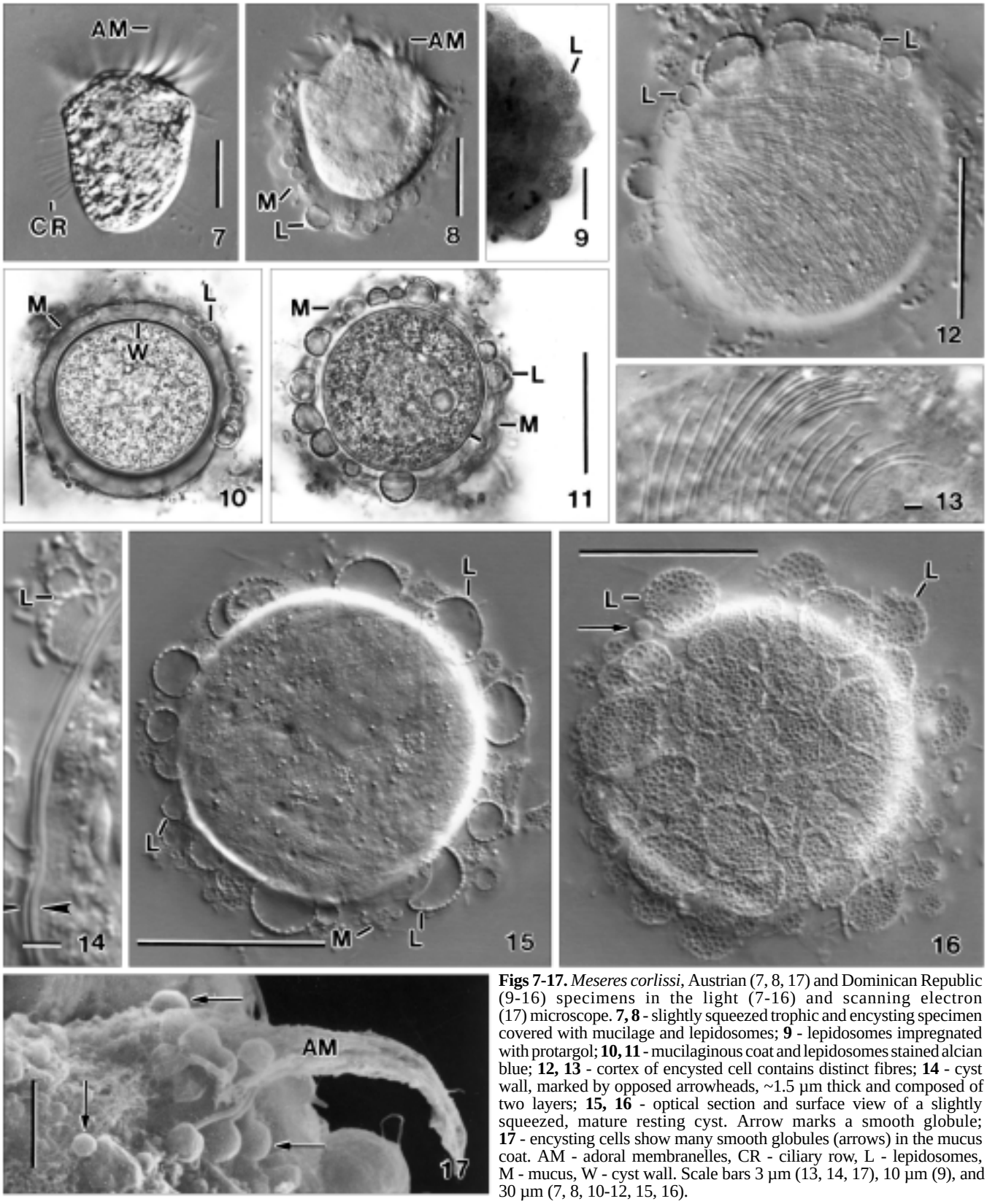
The resting cysts have an average diameter of 58 × 58 µm with and 47 × 45 µm without the lepidosome coat, that is, they are usually spherical, rarely slightly ellipsoi-



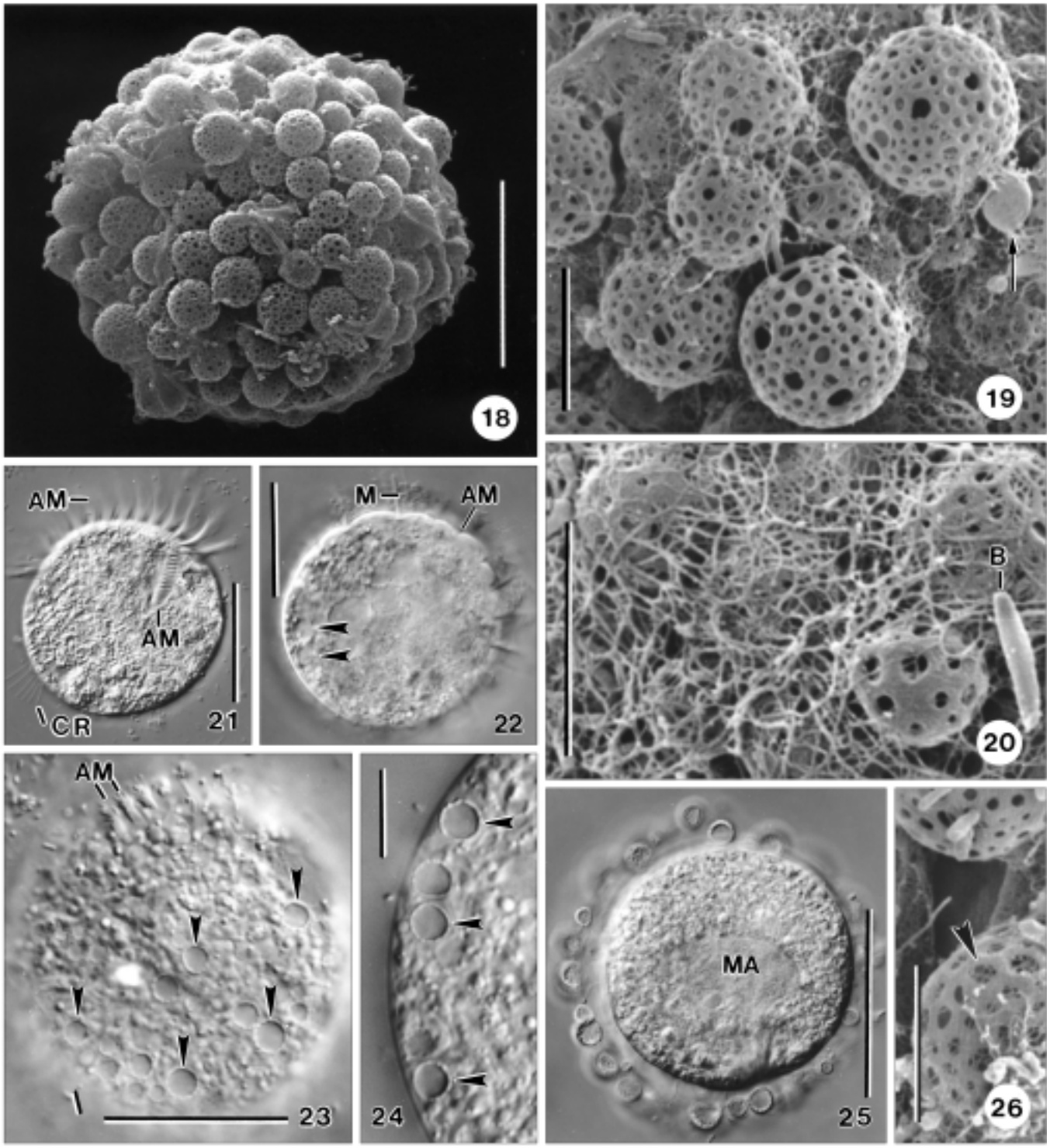
Figs 4-6. *Meseres corlissi*, trophic cells from the Dominican Republic in the scanning electron microscope. These figures show a mucilaginous layer coating the whole cell, including somatic cilia and adoral membranelles. **4** - ventral view of a tailed specimen. The mucilaginous coat is damaged left of the ciliary row (CR). The arrowhead denotes the buccal vertex. The asterisk marks the peristomial bottom, shown at higher magnification in the next figure; **5** - the peristomial bottom is covered by a thicker mucilage, whose circular structures are likely preparation artifacts; **6** - proximal portion of three adoral membranelles which are also covered with mucilage. Arrows mark regions where the coat is damaged exposing the membranellar cilia. AM - adoral membranelles, CR - somatic ciliary row. Scale bars: 30 µm (4) and 5 µm (5,6).

dal with a maximum of 47 × 40 µm (Table 2). Temperature significantly impacted length and width of resting cysts, that is, size increased from 15 to 20°C and declined rapidly at 22.5°C (Table 1). At temperatures >20°C, the few cysts that were formed during the experiments appeared defective. Sizes of live and Lugol-fixed cysts were not different at 19-20°C (cp. Tables 1, 2). The wall is colourless, while the cyst content is more or less distinctly orange due to the cryptomonad food. The cyst wall is smooth, 1.6 µm thick on average, and consists of two flexible layers, viz., an about 0.6 µm thick outer layer and an about 1 µm thick, gelatinous inner layer; the outer zone comprises several sublayers in fortunate preparations and collapses to a single layer under too heavy coverslip pressure (Figs 1, 2, 14). Wall flexibility is high because cysts can be flattened considerably by coverslip pressure without destroying the wall (Figs 14-16). Underneath the cyst's wall, likely in the cortex of the cell, are distinct, radially extending fibres providing the cyst with an axis; they impregnate with protargol and have a granular or smooth appearance *in vivo* (Figs 12, 13). The densely granulated content consists of (i) the centrally located, broadly reniform macronucleus with conspicuously bright nucleoli; (ii) some lipid droplets 1-10 µm across (stain with Sudan red and become blackish in osmic acid); (iii) many pale granules about 3 × 2 µm in size, likely mitochondria; and (iv) many 3-6 µm-sized (autophagous?) vacuoles with granular content (Figs 1, 2, 15, 27).

The cyst is covered by a conspicuous coat of lepidosomes embedded in an about 10 µm thick layer of mucus which is very hyaline and structureless *in vivo*, while composed of many fibres forming a more or less dense reticulum in the scanning electron microscope (Figs 1, 10, 11, 15, 19, 20). The mucus often forms a finely reticular coat from which only the larger lepidosomes stand out as inconspicuous hemispheres. The mucus fibres connect also individual lepidosomes and are 0.06-0.12 µm thick in the scanning electron microscope. We believe that these comparatively thick



Figs 7-17. *Meseres corlissi*, Austrian (7, 8, 17) and Dominican Republic (9-16) specimens in the light (7-16) and scanning electron (17) microscope. **7, 8** - slightly squeezed trophic and encysting specimen covered with mucilage and lepidosomes; **9** - lepidosomes impregnated with protargol; **10, 11** - mucilaginous coat and lepidosomes stained alcian blue; **12, 13** - cortex of encysted cell contains distinct fibres; **14** - cyst wall, marked by opposed arrowheads, ~1.5 µm thick and composed of two layers; **15, 16** - optical section and surface view of a slightly squeezed, mature resting cyst. Arrow marks a smooth globule; **17** - encysting cells show many smooth globules (arrows) in the mucus coat. AM - adoral membranelles, CR - ciliary row, L - lepidosomes, M - mucus, W - cyst wall. Scale bars 3 µm (13, 14, 17), 10 µm (9), and 30 µm (7, 8, 10-12, 15, 16).



Figs 18-26. *Meseres corlissi*, encysting specimens (21-25) and mature resting cysts (18-20, 26) from the Dominican Republic (18-20, 26) and Austria (21-25) in the light (21-25) and scanning electron (18-20, 26) microscope. **18** - overview; **19, 20, 26** - the faceted lepidosomes are embedded in a fibrous mucilage and the facets may have a sieve-like substructure (26, arrowhead). The arrow marks a smooth globule (lepidosome?); **21** - encysting specimens become globular and reduce the ciliary structures; **22-24** - globular, encysting specimen with proximal portion of adoral membranelles still recognizable. The cell is coated with mucilage and contains many lepidosomes (arrowheads) underneath the cortex. The lepidosomes already have a faceted structure (24); **25** - an encysting specimen with lepidosomes just released. The cyst wall has not yet formed. AM - adoral membranelles, B - bacterial rod, CR - somatic ciliary row, MA - macronucleus. Scale bars 5 µm (19, 20, 26), 10 µm (24), 20 µm (18), and 30 µm (21-23, 25).

Table 1. Temperature effects on size (µm) and production of resting cysts of *Meseres corlissi* from the Dominican Republic.

Temperature (°C)	Length[a,b]	SD[c]	Width[a,b]	SD[c]	n[c]	% cysts[d]
15	41.1	2.9	37.7	3.5	66	83.7
17.5	44.6	4.1	41.1	4.5	150	76.9
20	46.3	4.0	43.3	4.2	114	16.0
22.5	36.9	4.8	32.8	4.9	31	0.93

[a]Without lepidosome coat. [b]All value combinations significantly different (one-way ANOVA, Tukey test; P<0.001). [c]SD - standard deviation, n - number of cysts investigated. [d]Denotes the proportion of encysted cells as percentage of all trophic and encysted cells.

Table 2. Morphometric data on resting cysts of *Meseres corlissi* from the Dominican Republic. Data based on living, two-weeks-old cysts from a pure culture. Measurements in µm. CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of cysts investigated, SD - standard deviation, $\bar{x}$ - arithmetic mean.

Characteristics	$\bar{x}$	M	SD	CV	Min	Max	n
Length (with wall)	47.3	47.0	3.3	6.9	42	55	21
Width (with wall)	45.4	45.0	4.0	8.7	40	55	21
Wall, thickness	1.6	1.5	0.4	23.4	1	2	21
Length (with wall and lepidosome coat)	59.5	58.0	4.9	8.3	50	70	21
Width (with wall and lepidosome coat)	58.2	58.0	5.2	8.9	50	68	21
Largest lepidosomes (diameter)	9.1	9.0	1.7	19.1	6	14	21
Smallest lepidosomes (diameter)	3.7	3.5	0.9	25.5	2	5	21

Table 3. Cytochemical analysis of *Meseres corlissi* resting cysts.

Materials to be revealed	Methods	Structures analysed[a]			
		lepido-somes	mucous coat	cyst wall	cyst contents
Carbohydrates (glycogen, polysaccharides)	PAS (A)	–	–	+++	+++
	PAS (B)	–	–	+++	+++
	Bauer-reaction	–	–	±	+ to +++
	Lugol solution	–	±	±	+++
Acid mucopolysaccharids	Alcian blue at several pHs and with/without $MgCl_2$	±	++ to +++	–	–
Cellulose	Chlor-zinc-iodine	–	–	–	+
Chitin	Van Wisselingh test	–	–	++ to +++	–
Proteins	Alloxan Schiff-reaction	+	+	+	+ to +++
Lipids	Oil red	–	–	–	+ to +++
	Sudan red	–	–	–	+ to +++
	Sudan black	–	–	+	+ to +++
	Osmium acid	–	–	–	++
	Acetalphosphatids	–	–	–	+++

[a]Slight (+), moderate (++), strong (+++), uncertain (±) reaction.

fibres are a preparation artifact because they could not be observed *in vivo* and the transmission electron microscope (Foissner 2005).

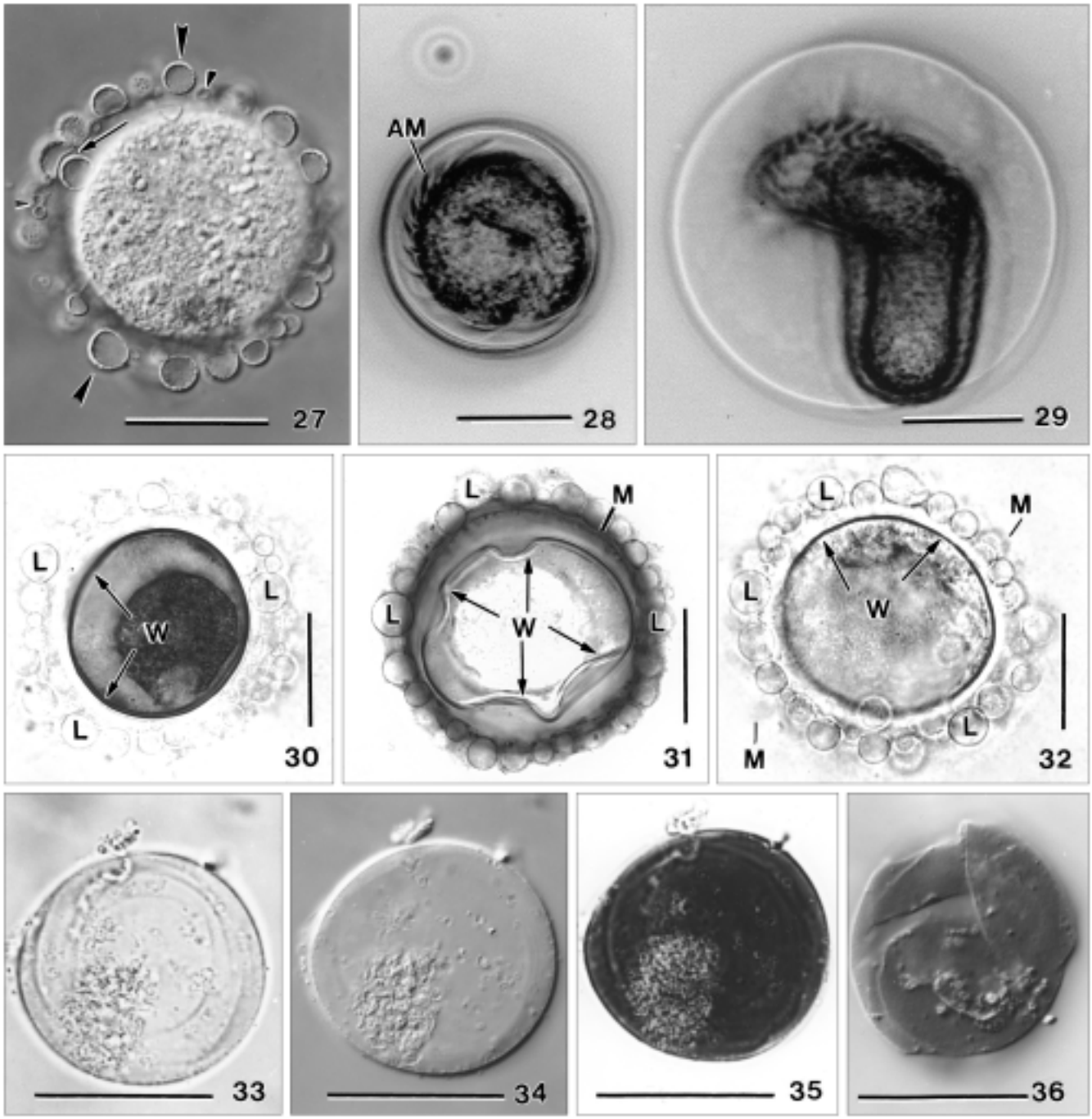
About 200 lepidosomes coat the cyst wall in two more or less distinct layers (Figs 1, 15, 16, 18, 27). When the cyst is squashed, the lepidosomes do not disperse, showing that they are embedded in the mucous coat described above; some can be removed by strong water flushes, for instance, by sucking the cyst through a narrow pipette several times. Most lepidosomes are perfect spheres with an about 0.5 µm thick, polygonally faceted wall; those touching the cyst wall may be hemispherically flattened. The diameter varies highly from about 2-14 µm, with an average of near 6 µm (Table 2). If exposed to strong coverslip pressure, nothing is released from the lepidosomes, perhaps except of water, and they become flattened and deformed, but never break, showing that they are very flexible (Figs 15, 16). The facets, which have a size of about 0.1-1 µm, are recognizable in even the smallest lepidosomes and cause a granular appearance of the wall in optical section; small and large facets occur without any regularity (Figs 12, 15). Frequently, the inner side of the facets is covered by a sieve-like lattice which is possibly very fragile and thus only partially preserved in the scanning electron microscopic preparations (Figs 19, 20, 26). Usually, there are some smooth spheres 0.5-2 µm across between and on the lepidosomes, especially in encysting specimens (Figs 16, 17, 19). We could not clarify, whether these are imperfect lepidosomes, mucus accumulations, or coccal bacteria.

Cytochemical investigations. The data on cyst chemistry are summarized in Table 3. They match the general knowledge that proteins, glycoproteins, and carbohydrates are the main constituents of the ciliate cyst wall (Rosati *et al.* 1984, Gutiérrez *et al.* 2003); rarely, inorganic substances play a role, viz., silicon in *Bursaria* (Bussers 1976) and calcium phosphate or calcium carbonate in a strobilidiid oligotrich (Reid 1987). The cyst content stains distinctly for carbohydrates, proteins, and lipids (Table 3).

Few chemical data are available on the mucous coat of ciliate cysts (Bussers 1976), although it is common throughout the phylum (Foissner 1993, Berger 1999, Foissner *et al.* 1999). This coat, which is usually not identical with that what is called the ectocyst (Gutiérrez *et al.* 2003), adheres the cyst to various substrates, such as plant residues and the walls of the culture dishes. Thus, it is usually partially or completely lost when the cysts are collected for investigations. Additionally, the coat is often very hyaline, as in *M. corlissi*, and thus easily overlooked. In *M. corlissi*, the mucous coat stains deeply with alcian blue under various conditions, showing that acid mucopolysaccharides are a main component (Adam and Czihak 1964, Romeis 1968, Hayat 1989); frequently, acid mucopolysaccharides occur also in the cyst wall (Delgado *et al.* 1987). The mucus is membrane-like and deeply stained around the cyst wall, gradually becoming fluffy and lightly stained distally (Fig. 11); if the stain is applied to living cysts, the coat may collapse to a deep blue, wall-like structure (Fig. 10). The coat shows also a slight stain for proteins and carbohydrates (Table 3), which thus might be minor constituents.

The lepidosomes are of organic nature because they are flexible (Fig. 27) and stain with a variety of histological dyes, such as hematoxylin and silver compounds (protargol, silver carbonate; Fig. 9). Of the cytochemical tests applied, the lepidosomes show a weak reaction for proteins. There is also a slight stain for acid mucopolysaccharids, but this is probably caused by adhering mucus from the mucous coat; however, some stain cannot be excluded because the lepidosome wall is very thin, decreasing staining intensity in general. Thus and because chitin and cellulose can be excluded, glycoproteins are likely a main component of the lepidosomes.

The cyst wall reacts with tests for carbohydrates and proteins, suggesting glycoproteins as a main constituent. This is in accordance with data from other ciliates (Gutiérrez *et al.* 2003). There is also a weak reaction for lipids using sudan black. The significance of this observation is not yet clear, but it matches data from *Didinium nasutum* (Rieder 1973) and other protists (Mulisch and Hausmann 1989). Part of the carbohydrates is chitin, as shown by the strong reaction with the Van Wisselingh colour test (Figs 33-36). The ghosts obtained after hot KOH treatment are very hyaline and structureless globules 25-45 µm across with an about 0.5 µm thick wall. Unfortunately, we could not recognise which part of the wall forms the globule and reacts with the test because all other cyst structures dissolved. The Van Wisselingh reaction for chitin was very convincing, both in the *Meseres* cysts and the control (Figs 33-35). Thus, an artifact can be excluded. Indeed, the presence of chitin is not unexpected because Bussers and Jeuniaux (1974) observed chitin in resting cysts of 14 out of 22 ciliate species investigated. Further, many ciliate loricas contain or consist of chitin (Mulisch 1993).



Figs 27-36. *Meseres corlissi*, Austrian (27, 30-36) and Dominican Republic (28, 29) specimens *in vivo* (27-29, contrast inversed in 28 and 29) and after various cytochemical stains (30-36). **27** - mature resting cyst, diameter 55 µm. Note the very different size of the lepidosomes (arrowheads) and their flexibility (arrow); **28** - when excystment commences, the adoral membranelles become visible and the ciliate begins to rotate; **29** - late excystment stage. The cell increases in size and the cyst swells to double size, likely due to a swelling of the cyst wall and the vigorous movements of the cell; **30** - bright field micrograph of a formalin-fixed, PAS-stained resting cyst. The cyst wall (W) and the cyst content are deeply stained, showing the presence of carbohydrates. The lepidosomes (L) and the mucous coat are unstained and thus very faint; **31** - bright field micrograph of a formalin–fixed, alcian blue-stained resting cyst. The cyst is covered by the deeply stained mucous coat (M), which becomes fluffy and faint distally. The intense staining of the coat causes that the unstained, wrinkled cyst wall (W) and the lepidosomes (L) increase in contrast (cp. Fig. 30); **32** - bright field micrograph of a formalin-fixed, alloxan-stained resting cyst. The mucous coat (M), the lepidosomes (L), the cyst wall (W), and the cyst content are slightly stained (cp. Fig. 30), showing the presence of proteins; **33**-**35** - Van Wisselingh´s chitin test. The same cyst (chitin) wall is shown after KOH treatment in bright field (33), interference contrast (34), and after iodine treatment (35), where the wall becomes deeply redbrown and thus appears black in the micrograph; **36** - same as before, but partially destroyed showing sharp-edged breaks. AM - adoral membranelles, L - lepidosomes, M - mucous coat, W - cyst wall. Scale bars 25 µm.

The cyst wall, the mucous coat, and the lepidosomes are very resistant to a variety of inorganic and organic solvents. They do not change in xylene, pyridine, propylene oxide, acetone, and alcohol. Hydrochloric acid (0.05 N) dissolves the cyst content within 10 min, while the wall, the lepidosomes, and the mucous coat appear unchanged even after 1 h of treatment; the lepidosomes are slightly swollen, but still show the faceted wall. Sodium hydroxide (2 M) dissolves the lepidosomes within 30 min, while the mucilage and the wall, which loses its bipartite structure, are still recognizable. The wall is maintained even after 1 h of treatment, while most of the mucilage disappears or disintegrates to a reticulate, very hyaline material. Concentrated sodium hypochlorite (Eau de Javelle with 12-13% active chlorine) dissolves the lepidosomes and the wall within 5-10 min, while the mucous coat is still recognizable after 1 h of treatment; thus, the cysts are still globular, although they are heavily damaged. Cold, saturated potassium hydroxide does not dissolve the cyst wall and the lepidosomes within 1 h of treatment; however, the lepidosomes often appear wrinkled, and the mucous coat is likely dissolved. Hot (160°C), saturated KOH dissolves the cysts within 20 min, except for the chitinous portion (see above).

These observations match some literature data on the cyst wall of a soil ciliate, *Colpoda cucullus*, which is also very resistant to various solvents (Corliss and Esser 1974, Foissner 1993). Further, the observations agree with data from Gutiérrez *et al.* (1984) on cyst wall lysis of a freshwater ciliate, *Laurentiella acuminata*.

Excystment

To study excystment, a cyst-containing culture from the Dominican Republic was transferred from 15°C to 24°C. On the following day, several cysts in the early stage of excystment were present. With a pasteur pipette, these were transferred to a Sedgewick-Rafter chamber and observed at a magnification of × 500 with phase contrast. Morphologically, three stages can be distinguished. First, the adoral membranelles become visible within the cyst and the ciliate commences to rotate (Fig. 28). Next, the ciliate gradually increases in size, likely through uptake of water, and the cyst roughly doubles its diameter, probably due to partial lysis of the wall, as in *Colpoda steinii* (Foissner 1993), and the vigorous movements of the ciliate (Fig. 29). Finally, the cyst wall ruptures and the ciliate escapes leaving behind the now very thin, wrinkled and shapeless cyst wall. No special emergence pore exists. The mucous coat and the lepidosomes within are lost when the ciliate commences to excyst. Basically, these processes are very similar to those described, for instance, in colpodids (Foissner 1993) and stichotrichine spirotrichs (Gutiérrez *et al.* 1981).

DISCUSSION

The term "lepidosomes"

We define lepidosomes as epicortical, organic structures of definite shape produced intracellularly by trophic and/or cystic ciliate species. Accordingly, the term excludes unstructured, mucous coats and any kind of lorica. In a more general sense, lepidosomes belong to the "scales", as defined by Preisig *et al.* (1994).

We shall show below that lepidosomes likely evolved convergently and possibly have different functions in various ciliate groups. Thus, a common term might be considered as inappropriate. On the other hand, lepidosomes have been described under a bewildering variety of names, making identification in the literature difficult (see next chapter). Thus, the term can be a useful "collector" at the present state of knowledge. Later and if necessary, it can be confined to the scales of trachelophyllid haptorids, the group which sponsored the name (*Lepidotrachelophyllum*; Nicholls and Lynn 1984). For the moment, the term should be understood as purely descriptive, like "coccoliths" for the calcified scales of the coccolithophores.

Distribution and structure of ciliate lepidosomes

Coatings composed of organic scales are widespread among trophic and cystic protists (Margulis *et al.* 1989). In ciliates, they occur rarely and under different names: Schleim, mucilage, gelatinous covering (in some trophic trachelophyllid and encheliid haptorids; Kahl 1930 and earlier authors); foam, organic scales, scale layer, external scale layer, extracellular scales (trophic trachelophyllid haptorids; Nicholls and Lynn 1984); mucilaginous layer, mucilaginous envelope, epicortical scales (trophic trachelophyllid haptorids; Foissner *et al.* 2002); external chalice-like structures (trophic *Peritromus*, Heterotrichea; Rosati *et al.* 2004); curieux éléments, écailles, fines baguettes fourchues (trophic cyrptopharyngid karyorelictids and in *Ciliofaurea*; Dragesco 1960); epipellicular scales (trophic cryptopharyngid karyorelictids; Foissner 1996); network structures (precystic and cystic *Colpoda cucullus*; Kawakami and Yagiu 1963a,b); yellow or brownish globules, brown-

ish discs, highly refractive globules, roundish discs arranged like scales and covered with tri-armed structures (various cystic Colpodea; Foissner 1993 and literature cited therein); papilla-like structures (cystic *Colpoda inflata* and *C. cucullus*; Chessa *et al.* 1994, 2002).

Obviously, lepidosomes have been reported from only few and taxonomically very distant ciliate groups, viz., the Karyorelictea, Haptorida, and Colpodea. Our report of lepidosomes in *Meseres* is the first for oligotrichs. There is evidence that lepidosomes are probably more common in general and particularly in oligotrichs, though they are absent in the common *Halteria grandinella*, a close relative of *Meseres* (Foissner, unpubl.). However, in another, new *Halteria* species from the Dominican Republic, the cyst is covered by conical scales highly resembling lepidosomes (Foissner, unpubl.). Further, cysts of several strombidiids and tintinnids have spine-like processes (for a brief review, see Müller 2000), which might also be lepidosomes. Possibly, they occur, as temporary structures, in *Strombidium oculatum* because Fauré-Fremiet (1948) observed the release of globules, which intensely stained with congo red, in encysting specimens. Likewise, *Didinium*, a relative of the trachelophyllids, secretes so-called clathrocysts, sacculate structures which are involved in mesocyst formation and disappear in the mature cyst (Holt and Chapman 1971). The foamy blisters forming the ectocyst of *Enchelydium*, another relative of the trachelophyllids, also resemble lepidosomes (see chapter below).

Among the trachelophyllid lepidosomes known (Foissner *et al.* 2002), only those of *Spetazoon australiense* Foissner, 1994 resemble the cyst lepidosomes of *M. corlissi*. However, the facets in the lepidosome wall are much coarser in *S. australiense* than in *M. corlissi*.

Few of these epicortical structures have been investigated with transmission electron microscopy. The available data show three organization types. In the first type, represented by trachelophyllids and *Meseres*, the lepidosomes are hollow structures with the wall composed of extremely fine, interwoven fibres (Nicholls and Lynn 1984, Foissner 2005). The second type is found in *Peritromus*, where the lepidosomes are made of organic material forming 60 nm-sided triangular, crystal-like elements (Rosati *et al.* 2004). The third type occurs in many colpodids, where the cysts are covered by small discs or globules composed of a strongly osmiophilic, spongiform material, in which a paracrystalline organization can be recognized (Kawakami and Yagiu 1963a, b; Chessa *et al.* 2002).

Nothing is known about the chemical composition of lepidosomes. Our attempts in *M. corlissi* were only partially successful, that is, show a weak reaction for protein (Table 3).

All these data suggest that lepidosomes evolved convergently in various ciliate groups, which is emphasized by the evidences shown in the last chapter.

Genesis of lepidosomes

Little is known about lepidosome genesis. In *M. corlissi*, the lepidosomes are produced in the cell's periphery within less than one hour. However, the exact sites of genesis are not known and need electronmicroscopical investigations; probably, they are assembled in dictyosomes, as supposed by Lynn and Nicholls (1984) in *Lepidotrachelophyllum fornicis*. In this species, the lepidosomes are generated in large endoplasmic vesicles, and some sections suggest that these vesicles are transported near to the cortex. Here, vesicle extensions may fuse with the plasmalemma and liberate the lepidosomes to the external surface of the cell (Lynn and Nicholls 1984). In *Colpoda cucullus*, the lepidosomes originate from minute, granular, membrane-bound precursors, most located in the cell´s periphery. When completed, they traverse the cell's cortex in the precystic specimens (Kawakami and Yagiu 1963a). These data suggest that lepidosome genesis may be similar in *M. corlissi*, trachelophyllids and colpodids, while *Peritromus kahli* possibly assembles the lepidosomes externally. Rosati *et al.* (2004) did not find lepidosomes or distinct lepidosome precursors in the cytoplasm of *Peritromus kahli* and thus supposed that the "material which composes the lepidosomes may be secreted through cortical invaginations present all over the dorsal surface".

Meseres cysts misidentified as cysts of *Enchelydium*, and lepidosomes of misidentified *Colpoda* species

Foissner *et al.* (2002) described the resting cyst of *Enchelydium blattereri*, a large, rapacious haptorid ciliate discovered in floodplain soil from Australia. The "forming cysts", Foissner *et al.* (2002) described and illustrated (Figs 20h and 306o in their work), are obviously mature cysts of *Meseres corlissi*, which occurred, according to the original notes and protargol slides, together with *E. blattereri* and was present also in the semipure cultures of this species. The resting cyst of *E. blattereri* is, indeed, similar to that of *M. corlissi* (Figs 20i, j in Foissner *et al.* 2002) because the ectocyst is composed of foamy blisters with walls composed of a

fibrous material perforated by minute pores. However, the blisters are not individualized structures, as the lepidosomes of *Meseres* and trachelophyllids, but are an integral component of the ectocyst, that is, cannot be removed without destroying the cyst.

Misidentifications can also explain contradicting results about the occurrence of lepidosomes in *Colpoda inflata* and *C. cucullus*. In a detailed SEM and TEM study on *C. inflata*, Martín-Gonzalez *et al.* (1992a, b, 1994) did not describe lepidosomes, while other investigations showed them by light microscopy (Foissner 1993) and scanning electron microscopy (Chessa *et al.* 1994). Unfortunately, Martín-Gonzalez *et al.* (1992a, b; 1994) described the vegetative specimens very briefly. However, the SEM micrograph in Martín-Gonzalez *et al.* (1992b) indicates that it could have been *C. maupasi* which, indeed, lacks lepidosomes (Foissner 1993). The second case concerns *C. cucullus*, where Kawakami and Yagiu (1963a, b) and Chessa *et al.* (2002) showed lepidosomes by transmission electron microscopy, while Foissner (1993) did not find any in the light microscope. However, *C. cucullus* is a complex of several rather similar species (Foissner 1993), some of which have lepidosomes (*C. lucida*, *C. flavicans*), while others have not (*C. cucullus*, *C. simulans*, *C. henneguyi*). Unfortunately, both studies lack details on the species investigated, and thus *a posteriori* identification is impossible.

Ciliate lepidosomes and leafhopper brochosomes

Leafhoppers (Insecta: Cicadellidae) and occasionally also their egg nests are covered by so-called brochosomes, whose origin and structure is highly reminiscent of the trachelophyllid lepidosomes and the cyst lepidosomes of *M. corlissi*. Brochosomes are synthesized in the Golgi vesicles of cells of the Malpighian tubules, are excreted *via* the anus, and distributed onto the integument by the legs. Size and structure of the brochosomes vary among species, but in the majority of examined leafhoppers they are hollow spheres, 0.2-0.6 µm across, with a honeycomb-like surface; they are insoluble in organic solvents and are rigid and durable in the dry form. Chemically, brochosomes consist of protein and lipid, but details are not known (Rakitov 2002).

Function of brochosomes and lepidosomes

The function of the leafhopper brochosomes is still in discussion. The available evidence suggests that they form a protective coating which (i) repels water and sticky honeydew and/or (ii) prevents fungal infection (Rakitov 2002). However, many other functions have been suggested, such as protection against microbes and desiccation, accommodation of hypothetical pheromones, and even that they are waste products (Humphrey and Dworakowska 2002, Rakitov 2002).

The function of the ciliate lepidosomes is not known. Like in leafhoppers, there is no obvious relation to the organisms' ecology and biology because trachelophyllids and *Meseres* usually occur together with many other ciliates lacking a lepidosome coat. However, the large number and complicated structure of the lepidosomes are energy demanding, suggesting an important function in the organism's life history. Some preliminary experiments with plasticine models, as advised by Padisák *et al.* (2003), showed that the lepidosome coat does not significantly change buoyancy, that is, cyst dispersal by floatation, likely because the gain of surface area is compensated by the increased diameter of the cyst (Padisák *et al.* 2003). Further, any effect of the lepidosomes on cyst buoyancy would be outweighted by the mucous coat in which they are embedded. This very fluffy substance has likely less specific mass than water and thus might indeed increase cyst buoyancy, as in several algae (Ruttner 1940). Fauré-Fremiet (1910) suggests that the lepidosomes of *Mycterothrix tuamotuensis*, a marynid colpodid, are pycnotic food vacuoles because they contain granular material. This is contradicted by data on other colpodids, in which the lepidosomes are clear and homogenous (Foissner 1993), and electron microscopic investigations showing a spongious fine structure (Kawakami and Yagiu 1963a, b, Chessa *et al.* 2002). Kawakami and Yagiu (1963a) suggest that the lepidosomes of *Colpoda cucullus* form a mucous layer attaching the cyst to various substrates. However, lepidosomes in the process of transforming to mucus are not shown. Likely, mucus secretion and lepidosome release are different processes in colpodids (Foissner 1993), as in *Meseres corlissi* (Fig. 22).

Thus, no substantiated hypothesis is available for the function of the ciliate lepidosomes. And the matter becomes even more complex when their distribution in the life cycle is considered: *Meseres* has lepidosomes only in the cystic stage, while they are restricted to the trophic stage in trachelophyllids (Foissner, unpubl.). Further, the occurrence of lepidosomes varies even within a single genus: they are present, for instance, in *Colpoda inflata* and a *C. cucullus* - like species (Foissner 1993; Chessa *et al.* 1994, 2002), while absent in *C. magna* and

C. minima (Foissner 1993, Diaz *et al.* 2003). All these data indicate that lepidosomes may have different functions in various ciliates.

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