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

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Summary. This is the first detailed study on the fine structure of the resting cyst of an oligotrichine ciliate, that is, the halteriid *Meseres corlissi* Petz and Foissner (1992), using a new, "strong" fixative composed of glutaraldehyde, osmium tetroxide, and mercuric chloride. The cyst wall consists of five distinct layers (from inside to outside): metacyst, endocyst, mesocyst, ectocyst, and the pericyst, a new term for all materials produced by the encysting cell and deposited upon the ectocyst. Structurally, the five layers of the *Meseres* cyst are similar to those of the stichotrichine (e.g., *Oxytricha*) and phacodinine (*Phacodinium*) spirotrichs, except of the thin ectocyst which is not lamellar but coarsely granular. The ectomesocyst likely corresponds to the chitin layer demonstrated by the Van Wisselingh colour test. The lepidosomes are within the fluffy, mucous pericyst whose proximal zone forms a reticulate, membrane-like structure on the ectocyst. The mucus consists of very fine filaments with a diameter of about 3 nm, while the lepidosomes are composed of fibres about 20 nm across. The encysted cell is similar to other kinetosome-resorbing ciliate cysts, except of curious inclusions possibly related to the lepidosomes. The data are compared with respect to the classification of the halteriids. While the general wall structure indicates a stichotrichine relationship, the ectocyst, the lepidosomes, and the chitin layer in the cyst wall suggest the halteriids as a distinct group more closely related to the oligotrichine than stichotrichine spirotrichs.

Key words: ciliate classification, metacyst, new fixation method, pericyst, phylogeny, stichotrichs.

INTRODUCTION

In the electron microscope, the wall of ciliate resting cysts conventionally consists of three distinct layers, that is, the endocyst, the mesocyst, and the ectocyst. Depending on the group of ciliates investigated, there might be also a metacyst and/or a pericyst, a new term for all materials produced and deposited upon the ectocyst by the encysting cell, often mucus and/or lepidosomes (Gutiérrez *et al*. 2003, Foissner *et al*. 2005). However, the light microscopical findings show a much greater diversity of cyst wall structures (Reid and John 1983, Reid 1987, Foissner 1993, Berger 1999, Foissner *et al*. 2002), suggesting that refined electron microscopical and cytochemical analyses will show many new structures, such as the lepidosomes in *Meseres* and some other ciliates (Foissner *et al*. 2005).

The unusual, lepidosome-coated resting cyst of *Meseres corlissi* was a good opportunity to contribute to the fine structure of ciliate resting cysts. Transmission electron microscopy of ciliate cysts has been neglected and most descriptions are rather meagre lacking, for instance, detailed morphometrics and a diagrammatic

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presentation. Thus, reliable data are available for less than 30 species (for literature, see Gutiérrez *et al*. 2003 and the present paper). Indeed, the present study is the first which describes in detail the fine structure of the resting cyst of an oligotrichous ciliate, a large group dominating the freshwater and marine ciliate plankton (Lynn *et al*. 1991, Foissner *et al*. 1999). For a general introduction to ciliate resting cysts, see Corliss and Esser (1974), Gutiérrez *et al*. (2003), and part 1 of the study (Foissner *et al*. 2005).

MATERIALS, METHODS AND TERMINOLOGY

Material. Pure cultures of *Meseres corlissi* Petz and Foissner (1992) from the Salzburg type locality were obtained as described in Weisse (2004). Cysts were collected from 3-weeks-old cultures devoid of active cells, that is, were detached from the bottom and wall of the culture dishes by strong shaking.

Methods. Ciliate resting cysts are difficult to fix due to the dense wall structures. Of several attempts made, the following protocol provided reasonable results: 10 ml glutaraldehyde (25%) + 6 ml aquaous osmium tetroxide (2%) + 10 ml saturated aquaous mercuric chloride. Mix just before use, fix for 1h, wash in 0.05 M cacodylate buffer, and transfer to Epon 812 *via* a graded ethanol series and propylene oxide. Flat-embedding in aluminium weighing pans allowed cysts to be investigated light microscopically (× 400) and to select well-preserved specimens for electron microscopy, that is, to cut off from the flat molds and mount onto the face of epoxy blocks. Ultrathin sections were cut with a diamond knife mounted on a Reichert ultracut. Sections were viewed in a Zeiss EM 910, either unstained or after staining with uranyl acetate followed by bismuth (Riva 1974) or, as usual, uranyl acetate followed by lead citrate (U/L staining). The Riva method is referred to "bismuth stain" throughout the paper. The images were digitalized and improved electronically. The results base on 17 sectioned cysts, of which 6 were investigated in detail.

Terminology. General terminology is according to Corliss (1979); systematic categories are based on the new classification by Agatha (2004); and cyst wall terminology is according to Gutiérrez *et al*. (2003). However, I introduce also a new term, viz., pericyst (*peri* Gr. - around/upon ectocyst), which comprises all materials produced and deposited upon the ectocyst by the encysting cell, for instance, mucus and/or lepidosomes in *Meseres* and cortical granules in *Engelmanniella* (Wirnsberger-Aescht *et al*. 1990).

RESULTS

Morphometry and a main artifact

Fixed cysts differ conspicuously from vital ones by an about 5 µm wide, clear zone between ectocyst and pericyst (Figs 4, 22; Table 2). Measurements suggest that this space is caused by about 15% cyst shrinkage, while the pericyst tends to become inflated (Table 1). Epon embedded, unsectioned cysts have a very similar size as those from thin sections, showing the lack of shrinkage in the post-fixational preparation steeps (Tables 1, 2).

Influence of staining and the chitin layer

Unstained sections provide sufficient contrast to be compared with conventionally (U/L) stained specimens. Both look quite similar, except of the endocyst which is more prominent in the unstained sections (cp. Figs 7, 8, 22, 23).

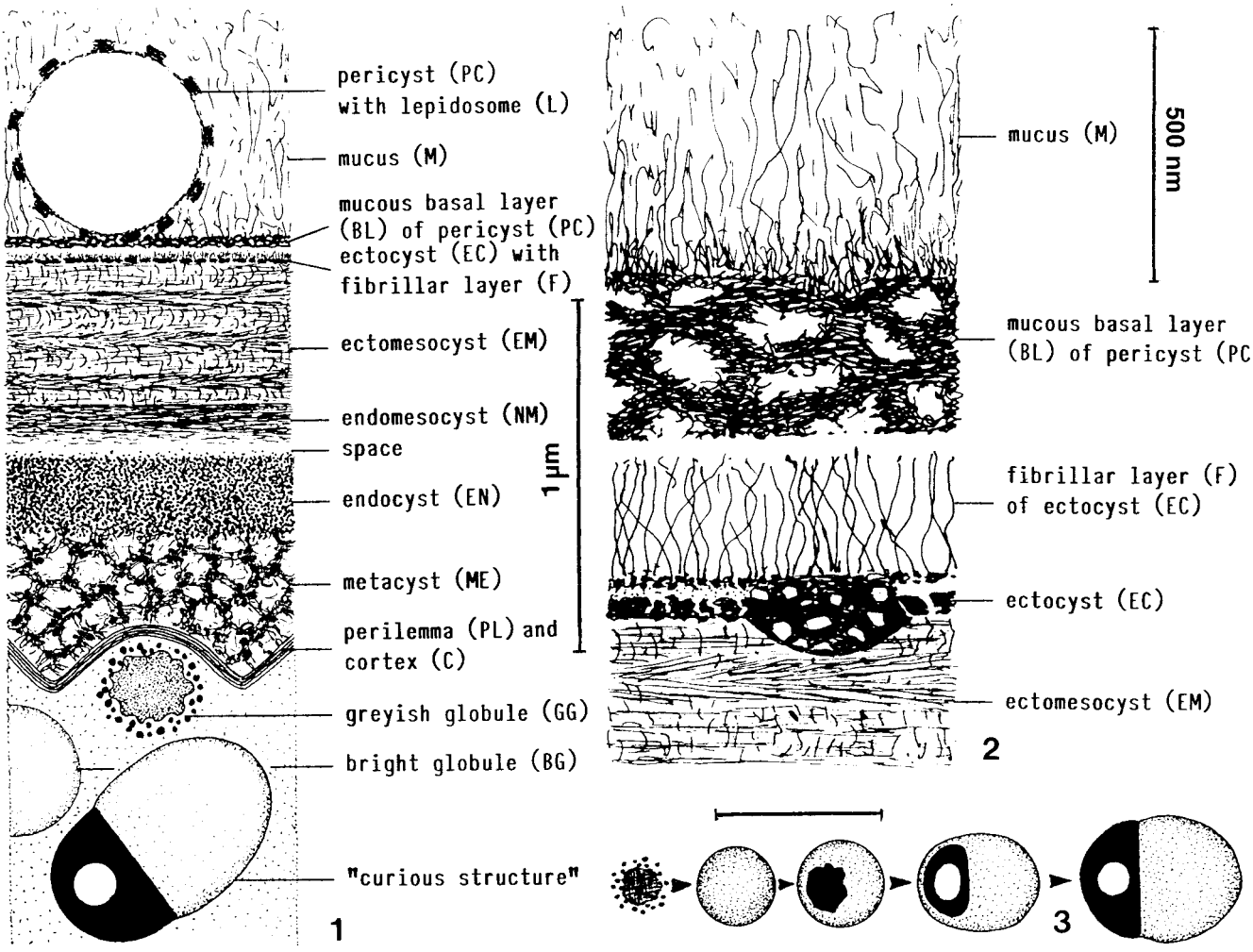
Bismuth staining shows two main differences (Figs 19, 20): the ectomesocyst is stronger stained than the endomesocyst (*vice versa* in U/L stains) and the ectocyst appears not dark and coarsely granular but alveolate with bright, membrane-like boundaries, likely corresponding to the minute spaces between the ectocyst flocs. There are also changes in the cytoplasm: the greyish granules (U/L staining) and the black chromatin bodies (U/L staining) appear bright.

The alkaline bismuth solution applied stains polysaccharides, including glycogen (Hayat 1989). In *Meseres*, the strongest stain is found in the ectomesocyst (Figs 19, 20), suggesting that it comprises the globular chitin layer (ghosts) demonstrated with the Van Wisselingh colour test (Foissner *et al*. 2005). This is supported in that the ectomesocyst is a rather thick layer (~ 350 nm), as is the wall of the KOH ghosts in the Van Wisselingh test. Further, only the ectomesocyst shows a fine structure resembling that of chitin (Westheide and Rieger 1996).

Lepidosomes

The cysts of *M. corlissi* are very sticky and must be detached by strong shaking from the walls of the culture dish (Foissner *et al*. 2005). This causes loss of the lepidosomes and the mucus on the side they are attached (Fig. 4). Most lepidosomes are spherical, except some of those which touch the cyst wall and become hemispherical.

The lepidosomes hardly shrink by the preparation procedures and consist of a thin, about 50 nm thick base from which the polygonal crests (facets) arise, providing the wall with a total thickness of 348 nm on average (Table 2). Both the base and the crests consist of about 20 nm thick fibres likely longer than 1 µm. The individual fibres form minute bundles which unite with other bundles becoming gradually thicker and reticulate. Although the resulting mould appears highly disordered, it produces the conspicuous crests, that is, the polygonally faceted



Figs 1-3. *Meseres corlissi*, semischematic figures of resting cyst structures. All based on transmission electron microscopical investigations and drawn to scale, using average values shown in Table 2, except of the lepidosome in figure 1 which is reduced in size by a factor of 10. **1** - overview with terminology and abbreviations used in this study. Scale bar 1 µm; **2** - details of pericyst and ectocyst. **3** - development of the "curious structures". Scale bars: 1µm (1, 3); 500 nm (2).

lepidosome surface (Figs 1, 4, 5, 9-14, 22; for SEM micrographs, see Foissner *et al*. 2005). Usually, the base is somewhat perforated, and thus bacteria may enter the lepidosomes (Fig. 9). In scanning preparations, the lepidosome base is frequently not preserved (Foissner *et al*. 2005).

Cyst wall

In vivo, the cyst wall is smooth, while minute ridges occur in fixed specimens (Fig. 4). These scattered ridges are shrinking artifacts, which become very conspicuous in poorly fixed (formalin, ethanol) cysts, as used for cytochemistry (Foissner *et al*. 2005). In the electron microscope, the wall consists of five distinct layers and several sublayers, while it is basically bipartite in the light microscope (Foissner *et al*. 2005). The morphometric data show that the inner, slightly thicker layer is composed of the metacyst and the endocyst (together 657 nm average, Table 2), while the slightly thinner, outer layer comprises the mesocyst and the ectocyst (together 566 nm on average and without the delicate microfibrillar layer, Table 2). The semischematic figures 1 and 2 summarize the structures seen.

Metacyst: The metacyst touches the ciliate's cortex and has an average thickness of 450 nm. It consists of fibrous material forming an alveolate mass, the thick-

Table 1. *Meseres corlissi*, morphometric data on resting cysts from life (upper line; from Foissner *et al*. 2005) and after Epon-embedding (lower line) as used for transmission electron microscopy. 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 (without pericyst)	47.3	47	3.3	6.9	42	55	21
	41.1	41	2.0	4.8	38	45	19
Width (without pericyst)	45.4	45	4.0	8.7	40	55	21
	36.8	38	4.5	12.3	28	45	19
Length (with lepidosome coat)	59.5	58	4.9	8.3	50	70	21
	65.6	68	4.3	6.6	52	70	19
Width (with lepidosome coat)	58.2	58	5.2	8.9	50	68	21
	62.0	64	5.4	8.8	48	68	19
Length (distance between basal layer of pericyst)	about as length without pericyst						
	52.5	52	4.2	8.0	43	60	19
Width (distance between basal layer of pericyst)	about as width without pericyst						
	48.8	48	3.8	7.7	40	52	19

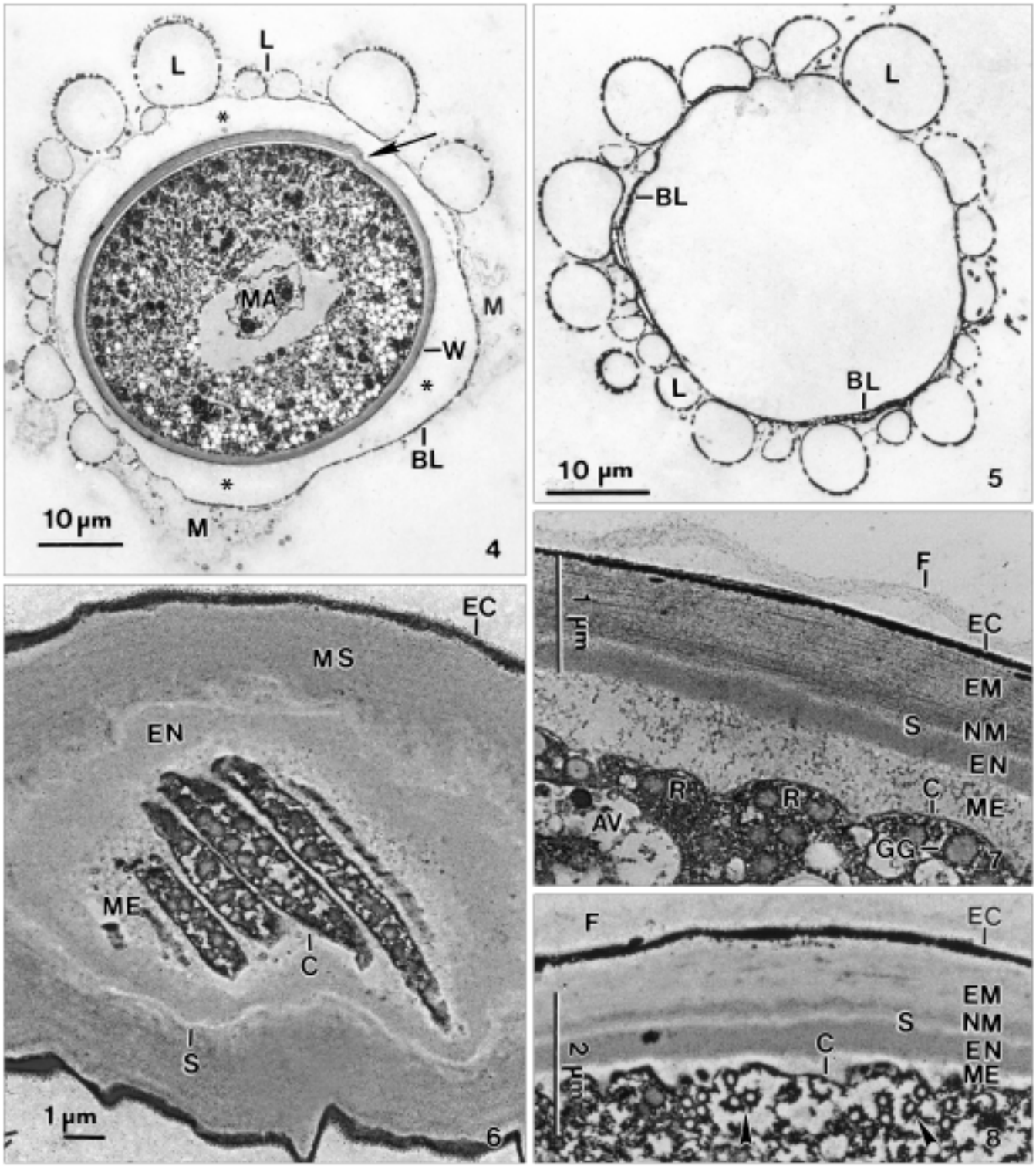
Table 2. *Meseres corlissi*, morphometric data on resting cyst fine structures seen in the transmission electron microscope. CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of cysts (not sections, to avoid pseudoreplication) investigated, SD - standard deviation, $\bar{x}$ - arithmetic mean.

Characteristics	$\bar{x}$	M	SD	CV	Min	Max	n
Largest diameter in middle third and without pericyst (µm)	41.3	42	2.9	7.1	37	45	6
Largest diameter in middle third from pericyst base to pericyst base (µm)	52.0	50	7.0	13.4	47	66	6
Space between ectocyst and pericyst base (µm)	5.3	3	4.3	80.2	2	14	6
Cyst wall, thickness without pericyst (nm)	1240.7	1292	330.0	26.6	750	1792	10
Metacyst, thickness (nm)	455.5	450	184.1	40.4	210	733	11
Endocyst, thickness (nm)	201.5	211	51.8	25.7	120	289	11
Space between endocyst and mesocyst, thickness (nm)	63.1	62	23.5	37.2	27	100	11
Mesocyst, thickness (nm)	478.2	482	143.1	29.9	280	750	10
Ectocyst, thickness (nm)	87.7	80	35.7	40.7	44	158	11
Ectocyst flocs, size (nm)	81.3	100	48.2	59.3	20	180	12
Fibrillar layer on ectocyst, thickness (nm)	306.5	233	254.9	83.2	167	1100	12
Pericyst, thickness of basal layer (nm)	350.7	320	138.5	39.5	185	600	10
Largest lepidosome, diameter (µm)	10.3	10	1.8	17.5	8	13	9
Lepidosome base, thickness (nm)	51.5	50	24.0	46.6	27	100	8
Lepidosome wall, total thickness (nm)	348.0	316	122.2	35.1	200	555	10
Greyish globules in cytoplasm, diameter (nm)	277.6	224	122.9	44.3	200	583	12
Greyish globules in cortex ridges, diameter (nm)	235.8	216	44.2	18.7	187	300	12
Bright globules, diameter (nm)	465.3	500	140.4	30.2	233	667	12
”Curious structures”, length (nm)	979.9	933	51.8	19.1	750	1333	13
”Curious structures”, width (nm)	723.6	700	146.0	20.2	567	1000	13
Mitochondria, largest diameter (µm)	1.3	1	0.3	20.9	1	2	8

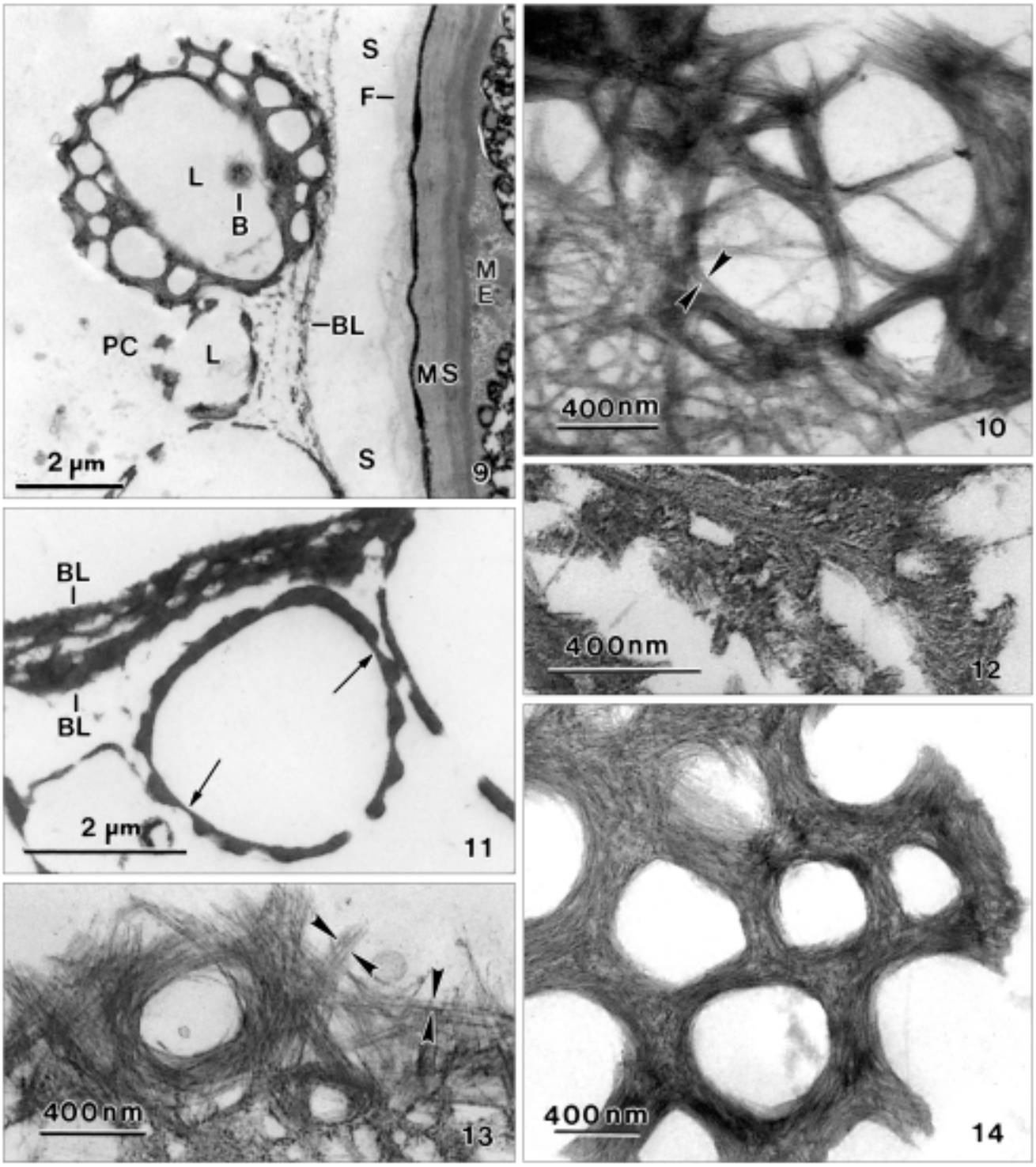
ened edges of which provide the layer with a more or less distinct granular appearance. It is distinctly stained with bismuth (Figs 1, 7, 8, 19, 20; Table 2).

Endocyst: The endocyst has an average thickness of about 200 nm and is usually attached to the metacyst (Table 2); rarely, both are separated by a thin, bright zone. The endocyst appears structureless at even a magnification of ×100 000 (Figs 1, 7, 8) and in bismuth stains (Figs 19, 20). In unstained sections, it has more contrast than the metacyst and the mesocyst, indicating that is more compact (Fig. 23).

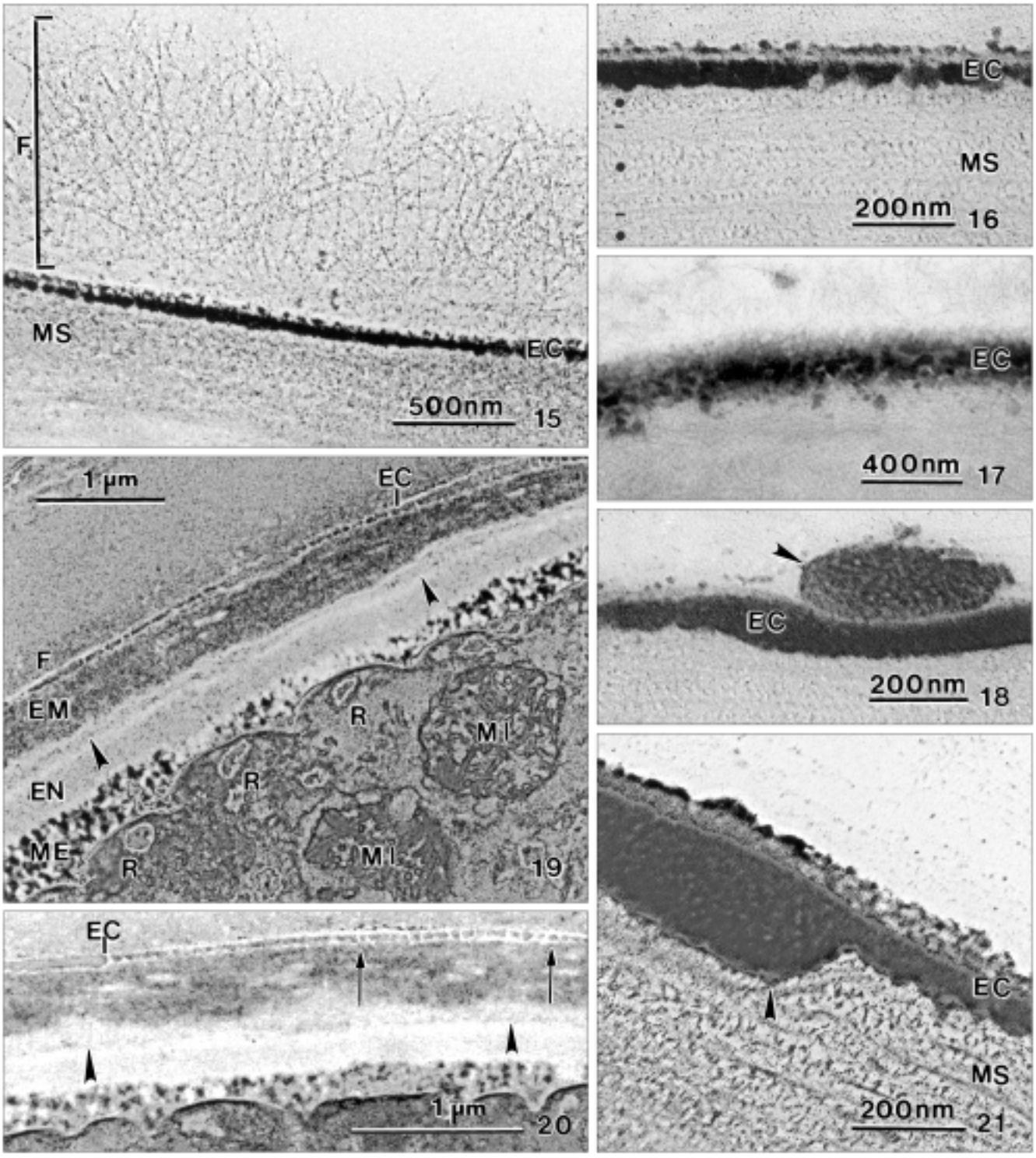
Mesocyst: The mesocyst has an average thickness of 478 nm and is thus the thickest layer of the wall (Table 2). It is separated from the endocyst by a bright, about



Figs 4-8. *Meseres corlissi*, transmission electron micrographs of resting cysts. **4** - overview in middle third. Two main artifacts are recognizable, viz., a shrinkage ridge (arrow) and a wide space (asterisks) between ectocyst and basal layer of pericyst. The lepidosomes were lost during collection on the side the cyst was attached to the culture dish; **5** - grazing section of the pericyst showing the lepidosomes attached to the mucous basal layer; **6** - grazing section showing cortical ridges possibly appearing as fibres in the light microscope (cp. Figure 7); **7, 8** - cyst wall details from two specimens. The cyst shown in figure 8 has maintained some adoral basal bodies (arrowheads). BL - basal layer of pericyst, C - cortex, EC - ectocyst, EM - ectomesocyst, EN - endocyst, F - microfibrillar layer, L - lepidosomes, M - mucus, MA - macronucleus, ME - metacyst, MS - mesocyst, NM - endomesocyst, R - cortical ridges, S - space, W - cyst wall.



Figs 9-14. *Meseres corlissi*, transmission electron micrographs of the lepidosomes coating the resting cyst. **9-12** - overview (9) and details (10-12) of the cyst wall and the lepidosomes. Figure 9 shows the spatial relationship of the various layers of the cyst wall, beginning with the mucous, lepidosome-containing pericyst to the granular metacyst, which is thickened in the area labeled. Gracing sections of the lepidosomes (9, 10) reveal the lepidosome base (Fig. 11, arrows) composed of about 20 nm thick fibres (Fig. 10, opposed arrowheads) forming a finely reticulate sheet (10). In contrast, the basal layer of the pericyst consists of very thin (< 5 nm) fibres forming a coarsely reticulate mould (11, 12); **13, 14** - the facets of the lepidosomes (14) consist of highly interwined, about 20 nm thick fibres some marked by opposed arrowheads in figure 13. B - bacterial rod, BL - basal layer of pericyst, F - microfibrillar layer, L - lepidosomes, ME - metacyst, MS - mesocyst, PC - pericyst, S - artificial space between pericyst and ectocyst.



Figs 15-21. *Meseres corlissi*, transmission electron micrographs of the cyst wall after uranyl acetate/lead citrate (15-18, 21) and bismuth (19, 20) staining. **15 -** the ectocyst consists of a microfibrillar layer (F) attached to an electron-dense, granular sheet; **16, 17 -** the dark (electron-dense) portion of the ectocyst is bilayered and consists of granules and polygonal platelets. The alternating dots and lines in figure 16 mark stacks of helicoidally arranged bundles of microfibrils; **18, 21 -** the ectocyst may have reticulate plaques (arrowheads) on the outer and inner surface. Note the microfibrillar structure of the mesocyst in figure 21; **19, 20 -** bismuth stained sections reveal the ectocyst composed of small and large platelets (arrows). Note the alveolate structure of the metacyst. The endomesocyst (arrowheads) is much lighter stained than the ectomesocyst (EM) and in conventionally contrasted sections (Figs 7, 8). This suggests a different chemical composition. EC - ectocyst, EM - ectomesocyst, EN - endocyst, F - microfibrillar layer, MI - mitochondria, MS - mesocyst, R - cortical ridges.

63 nm thick zone; the separation is not sharp, but both the mesocyst and the endocyst become gradually lighter. In the best preparations, the mesocyst shows two zones, viz., an endomesocyst and an ectomesocyst (Figs 1, 4, 7, 8, 16, 19-21). The endomesocyst, which occupies the proximal fifth, stains slightly darker than the ectomesocyst and consists of very densely arranged, stratified fibres. The ectomesocyst is also composed of very fine fibres connected by countless, minute bridges. The arrangement of the fibres reminds the helicoidal pattern of cellulose and chitin microfibrills in plant and animal tissues (Westheide and Rieger 1996), that is, the layer appears to be composed of stacks of helicoidally arranged bundles of microfibrils. Thus, it is likely this layer which shows the positive reaction for chitin (see above and Foissner *et al*. 2005).

Ectocyst: The ectocyst is complex, that is, composed of an about 90 nm thick, dark (electron-dense) zone from which fine fibres emerge and extend vertically to the pericyst, forming an approximately 300 nm thick, delicate layer (Figs 1, 2, 7, 15; Table 2). Thus, the ectocyst has an average total thickness of about 400 nm. The darker zone, which is electron-dense in both unstained and U/L stained sections, is bipartite consisting of small (mainly in distal half) and up to 180 nm large (mainly in proximal half) granules and polygonal platelets (flocs) with more or less sharp edges; frequently, the zone thickens to or has attached plaque-like accumulations with a coarsely reticulate fine structure (Figs 1, 2, 6-9, 15-23).

The microfibrillar layer on the dark zone likely represents a distinct part of the ectocyst because the fibres are vertically oriented and may form a conspicuous layer, which may detach from the dark zone (Figs 7, 9, 15). Alternatively, it could be part of the pericyst's basal layer which, however, is less likely because the basal layer consists of a dense, reticulate mass of fibres (see next paragraph).

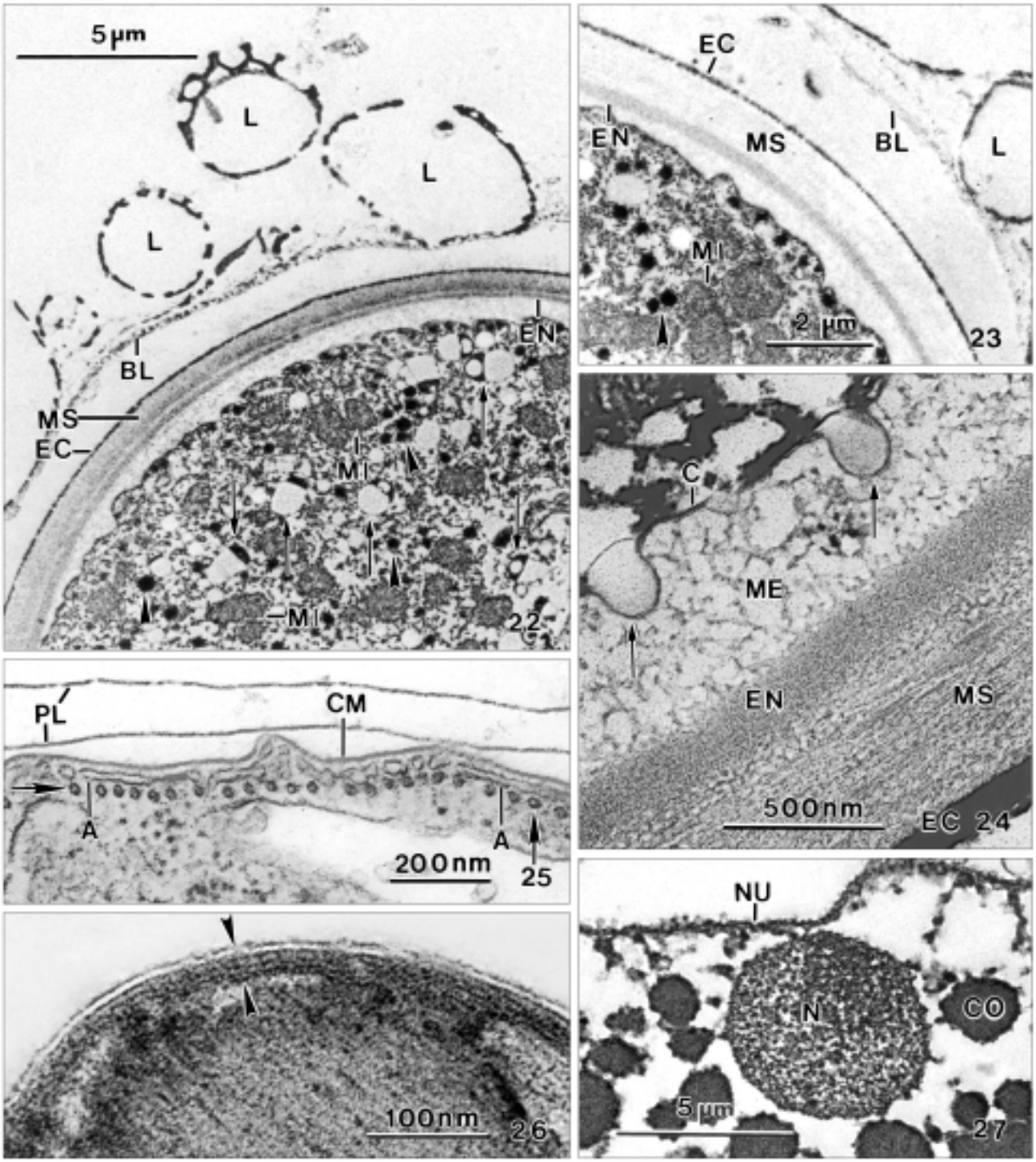
Pericyst: The pericyst consists of the lepidosomes described above and the cyst's mucous envelope, which comprises two more or less distinct zones. The about 350 nm thick basal layer is a dense, reticulate mass of very fine (~ 3 nm), bundled fibres forming a membrane-like cover on the cyst; structurally, it resembles the base of the lepidosomes. However, both are different because the fibres have a different diameter (~ 3 *vs.* 20 nm) and only the mucous layer distinctly stains with alcian blue (Foissner *et al*. 2005). Distally, the basal layer dissolves into a fluffy, microfibrillar (mucous) material extending beyond the lepidosomes and usually colonized by bacteria and small protists (Figs 1, 2, 4, 5, 9, 22, 23; Table 2).

The encysted cell

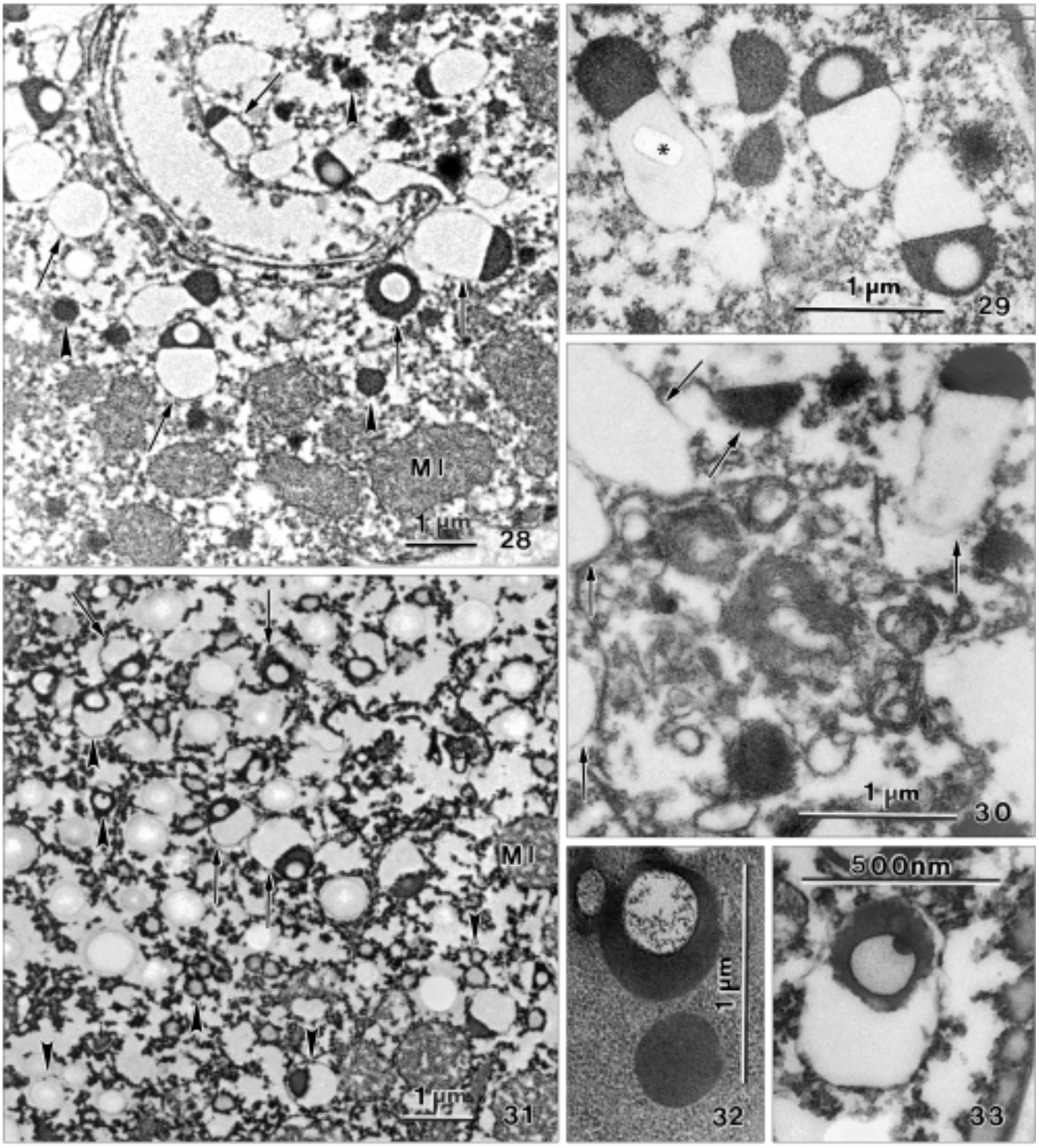
Cortex: In interphase specimens, the cortex is smooth; covered by two to five perilemma layers, each consisting of a unit membrane; and supported by a single sheet of microtubules (Fig. 25). In encysted cells, the cortex has over 200 convex ridges; two perilemma membranes; and all microtubules and ciliary structures disappeared (Figs 4, 7-9, 19, 20, 22, 25, 26). The cortical ridges, which extend between the poles of the cyst, usually contain greyish stained granules arranged one after the other and 236 nm across on average (Fig. 6, Table 2). Likely, the ridges are identical with the "fibres" seen in the light microscope (Foissner *et al*. 2005). The cortical membranes are usually poorly preserved, but some specimens and sections show five of them with a total thickness of about 20 nm (from outside to inside): two perilemma membranes, separated from each other by a 3-5 nm wide, bright zone; the cell membrane; and the outer and inner alveolar membrane (Figs 25, 26).

The entire somatic and oral infraciliature is resorbed. Of six cysts investigated in detail, one showed a small field of scattered basal bodies (Fig. 8). This is in accordance with the light microscopical data showing that 5% of the specimens have remnants of the ventral adoral membranelles (Foissner *et al*. 2005).

Cytoplasm: At low magnification, the cyst's cytoplasm is spotted by bright and dark globules (Fig. 4). The latter are 1-2 µm-sized mitochondria with a tendency to accumulate in the cell's periphery. Most of the bright inclusions, which have an average diameter of 465 nm, are precursors of, or belong to curious structures never seen in ciliate cysts and thus probably related to the lepidosomes. These curious structures, which have an average size of 980 × 724 nm, are composed of a large, lightly stained portion, which rarely contains a crystal-like particle, and a small, electron dense portion with a central, bright globule sometimes containing a filamentous reticulum (Figs 1, 3, 22, 28-33; Table 2). Likely, these strange structures develop from about 278 nm sized, greyish stained, finely granular precursors *via* a globular, bright stage 465 nm across on average; the greyish precursors are frequently wrinkled and usually surrounded by masses of ribosomes (Figs 3, 22, 31; Table 2).



Figs 22-27. *Meseres corlissi*, transmission electron micrographs of resting cysts (22-24, 26, 27) and an active specimen (25); unstained (22, 23) and contrasted with uranyl acetate and lead citrate (24-27). **22, 23** - unstained sections show good contrast, but the microfibrillar layer of the ectocyst and the metacyst are hardly recognizable. The mesocyst and the space between mesocyst and endocyst are recognizable in some sections (22), but not in others (23). The granular layer of the ectocyst and the endocyst are electron-dense and thus well recognizable. Note the furrowed cortex and the curious structures (arrows) and their precursors (arrowheads) shown in detail on the following plate; **24** - the cortex may form spherical protuberances projecting into the alveolate metacyst. Note the finely granular endocyst; **25, 26** - both active (25) and encysted (26, arrowheads) specimens are covered by five unit membranes: two perilemma membranes, the cell membrane, and the outer and inner alveolar membrane. The microtubule sheet (25, arrows) has been resorbed in the cyst (26); **27** - part of macronucleus. A - cortical alveoli, BL - basal layer of pericyst, C - cortex, CM - cell membrane, CO - chromatin bodies, EC - ectocyst, EN - endocyst, L - lepidosomes, ME - metacyst, MI - mitochondria,; MS - mesocyst, N - nucleolus, NU - nuclear membrane, PL - perilemma membranes.



Figs 28-33. *Meseres corlissi*, transmission electron micrographs of unstained sections of the resting cyst cytoplasm. **28, 29, 31, 32** - most resting cysts are packed with curious, ellipsoidal structures (arrows) composed of two bright thirds and a dark (electron-dense) third containing a bright globule sometimes filled with flocculent material (32). There are many precursors (arrowheads) of the curious, about 1 µm long structures, which are likely related to lepidosome production. Rarely, the bright portion contains a crystal-like inclusion (29, asterisk). See figure 3 for an interpretation of the genesis of the curious structures; **30** - an autophagosome surrounded by the curious structures (arrows) shown in figures 28, 29, and 31; **33** - a late developmental stage of the curious structures. MI - mitochondria.

The histochemical data show that there are also lipid droplets and glycogen granules in the cytoplasm (Foissner *et al*. 2005). Both are difficult to identify in the mass of structures described above. There are also some autophagous vacuoles about 2-3 µm across, containing membrane-like structures and other debris (Fig. 30).

DISCUSSION

Terminology: pericyst and metacyst

Light microscopical and electron microscopical resting cyst terminology are neither consistent nor complete, making comparisons difficult and circumstantial. For example, the mesocyst is called endocyst and the endocyst is named intimocyst by Rieder (1973). The unclear terminology also influences the recognition and classification of the individual cyst wall layers, as shown for instance, by the *Colpoda cucullus*-like ciliate (for identification, see Foissner *et al*. 2005) investigated by Chessa *et al*. (2002). In my interpretation, the distal portion of the so-called ectocyst is a pericyst because it is obviously composed of flocculent mucus containing many lepidosomes, as in *Meseres* (Figs 1, 4, 5); further, this interpretation matches the light microscopical findings (Foissner 1993) which were not considered by Chessa *et al*. (2002). The proximal portion of Chessa's ectocyst is likely the ectomesocyst, while the narrow, strongly osmiophilic layer between pericyst and ectomesocyst is the ectocyst, as in stichotrichine spirotrichs and *Meseres* (Figs 1, 7, 8). Thus, this *Colpoda* has a four-layered cyst wall composed of pericyst, ectocyst, mesocyst, and endocyst. A similar re-interpretation needs the resting cyst wall of *Colpoda magna*, where Frenkel (1994) named the pericyst ectocyst and the ectocyst "granular layer". It is beyond the scope of this study to re-interprete the literature which will be often difficult considering the poor fixation and incomplete presentation of the structures.

A first attempt for a more clear cyst wall terminology was undertaken by Gutiérrez and Walker (1983), who introduced the terms "metacyst" (for the "granular layer" between ciliate cortex and endocyst), ectomesocyst, and endomesocyst (for the two layers of the mesocyst). Unfortunately, this attempt largely remained unrecognized likely because it was published as a meeting abstract only.

I not only support the terminology of Gutiérrez and Walker (1983) and Gutiérrez *et al*. (2003), but introduce "pericyst" as a new term for all materials produced and deposited upon the ectocyst by the encysting cell. Usually, this is a more or less voluminous, sticky coat of mucus sometimes containing lepidosomes, as in *Meseres* and various colpodids. The mucous coat is often very hyaline and sticky and thus easily overlooked or lost when cysts are collected; further, it is sometimes partially or completely eroded by bacteria, especially in old specimens.

Martin-Gonzalez *et al*. (1992a) do not consider the mucous coat as a real cyst layer. I disagree, not only because it is produced by the encysting cell and may contain the highly characteristic lepidosomes, but also because it has two important ecological functions: (i) it adheres the cyst to the substrate and (ii) increases buoyancy, that is, cyst dispersal by floatation because the flocculent mucus has a low specific mass very near to that of water (Ruttner 1940, Padisák *et al*. 2003).

Comparative and phylogenetic analyses

Among the halterine spirotrichs, detailed investigations on resting cyst morphology have been performed only in *M. corlissi* (Foissner *et al*. 2005 and the present study). Unfortunately, the same is true for the oligotrichine spirotrichs: only Reid (1987) published three TEM micrographs from the resting cyst of *Strombidium crassulum* (a misidentification; the light microscopic figures of the trophic cells show a strobilidiid oligotrich). Thus, the within-group comparison is very limited. However, if the two examples are representative, then halterine and oligotrichine spirotrichs have very different resting cysts. In the species studied by Reid (1987), the cyst wall is extremely massive and appears to be made of crystalline material (X-ray analysis), similar as in the colpodid *Bursaria* (Bussers 1976), possibly calcium phosphate or calcium carbonate in an organic matrix arranged in 6-11 alternating light and dark laminae. Separated from the main wall by a clear area of variable height is an outer spiny membrane 0.1-0.2 µm in thickness. The spines have solid bases and appear to be made of a similar material to the cyst wall, but details are not recognizable. Reid (1987) emphasized that TEM fixation of the cyst was notoriously difficult, and thus it cannot be excluded that further layers exist but were not preserved.

The halterine spirotrichs, such as *M. corlissi* and *Halteria grandinella*, are a hot spot in ciliate classification (for a review, see Foissner *et al*. 2004): while gene sequences suggest a close relationship with the stichotrichine spirotrichs (e.g. *Oxytricha, Stylonychia*),

morphology and ontogenesis indicate a relationship with the oligotrichine spirotrichs (e.g. *Strombidium, Tintinnidium*). Thus, Foissner *et al*. (2004) called for further morphological and molecular data to solve the puzzle. The *Meseres* study provides four new features, most of which support the classic view.

A five-layered cyst wall, as found in *M. corlissi*, is quite typical for stichotrichine spirotrichs, especially the possession of a metacyst. This seemingly supports the molecular view of a close relationship of halterine and stichotrichine spirotrichs. However, a metacyst occurs also in the euplotine (Walker and Maugel 1980) and phacodinine (Calvo *et al*. 1992) spirotrichs, suggesting that it is a plesiomorphic feature useless for phylogenetic purposes. This is emphasized by *Blepharisma*, which was considered as a typical spirotrich for a long time (Corliss 1979), but now is in a different subphylum (Lynn 2003). *Blepharisma* has a metacyst according to figures 1 and 6 in the paper of Mulisch and Hausmann (1989) and the description of Larsen and Nilsson (1995): "the endocyst of *B. lateritium* is separated from the encysted ciliate by a narrow, amorphous, low density zone". None the less, the *Blepharisma* cyst is rather different from those of stichotrichine and halterine spirotrichs because the ectocyst is composed of fine, weakly staining, fibrous material, while the endocyst is made of electron-dense plates (lamellae) embedded in an amorphous and fine-filamentous matrix (Mulisch and Hausmann 1989, Larson and Nilsson 1995). The opposite pattern occurs in stichotrichine and halterine spirotrichs (Figs 1, 4, 7, 8).

The three other features mentioned above, that is, the structure of the ectocyst, the lepidosomes, and the cyst wall composition relate *Meseres* more distinctly to the oligotrichine than to the stichotrichine spirotrichs. The ectocyst is lamellar in stichotrichine and phacodinine spirotrichs (Calvo *et al*. 1992, Gutiérrez *et al*. 2003), amorphous and weakly stained in euplotine spirotrichs (Walker and Maugel 1980), while coarsely granular and dense in *Meseres* (Figs 1, 7, 15-21). Unfortunately, no detailed data are available, except those of Reid (1987) discussed above, from the oligotrichine spirotrichs, such as *Strombidium, Strobilidium,* and *Tintinnidium.* Thus, the phylogenetic significance remains obscure, but it is obvious that the ectocyst is different in stichotrichine and halterine spirotrichs, giving some support to the hypothesis that the later form a distinct group outside of the stichotrichs (Foissner *et al*. 2004). This is emphasized by the lepidosomes (Figs 1, 4, 5), a special feature not found in cysts and trophic cells of more than 50 stichotrich species (Berger 1999, Gutiérrez *et al*. 2003, Foissner, unpubl. data from more than 30 species), while present in a new species of *Halteria* (Foissner, manuscript in preparation) and likely also in several oligotrichs *s. str*. A SEM-reinvestigation of the cyst of the freshwater *Pelagostrombidium* studied by Müller (2002) showed spines very similar to those of the new *Halteria* species mentioned above, that is, the spines are lepidosomes attached to a filamentous, membrane-like structure distal of the ectocyst (Foissner, unpubl.). The same interpretation applies to the marine species studied by Reid (1987) and discussed above. Thus, the occurrence of lepidosomes is likely a main synapomorphy of halterine and oligotrichine Oligotrichea.

Part of the *Meseres* cyst wall is made of chitin (Foissner *et al*. 2005). Chitin occurs also in the cyst wall of other ciliates, including heterotrichs (e.g. *Stentor, Blepharisma*) and euplotine and phacodinine spirotrichs (e.g. *Euplotes, Phacodinium*), while the cyst of several stichotrichine spirotrichs (*Onychodromus grandis, Oxytricha* sp., and *Urostyla trichogaster*) lacks chitin (Bussers and Jeuniaux 1974). Later, however, chitin was reported in *Oxytricha bifaria*, using a combined protease-chitinase treatment of ultrathin sections (Rosati *et al*. 1984). Thus, some chitin is likely present also in stichotrichine spirotrichs. In general, however, chitin distribution indicates that *Meseres* is more closely related to euplotine and phacodinine than stichotrichine spirotrichs (see also Mulisch 1993). Unfortunately, data are lacking from the oligotrichs *s. str*.

In estimating the phylogenetic significance of resting cyst morphology and composition, it must be taken into account that detailed data are available from less than 30 species! Certainly, this is a very incomplete sample, considering the high light microscopical diversity of ciliate resting cysts (for representative compilations, see Reid and John 1983, Reid 1987, Foissner 1993, Berger 1999, Foissner *et al.* 2002). Thus, any phylogenetic interpretation of cyst morphology and composition is a risky adventure.

None the less, cysts have been suggested to be an important tool in ciliate classification and phylogeny (Walker and Maugel 1980, Reid and John 1983, Rawlinson and Gates 1986, Martin-Gonzalez *et al*. 1992b), referring to the split of the hypotrichs in stichotrichine end euplotine spirotrichs (Lynn 2003). Mulisch (1993) additionally suggested chitin distribution as an indicator of the phylogenetic distance between euplotine (with chitin) and stichotrichine (without chitin; but see above) spirotrichs.

And Rios *et al*. (1988) speculated that the four-layered cyst wall of oxytrichid stichotrichs evolved from the three-layered wall of urostylid stichotrichs by adding the mesocyst. Much phylogenetic significance has been attributed to the infraciliature, that is, whether or not cilia, basal bodies, and cortical microtubules are preserved or resorbed in the resting cyst (Walker and Maugel 1980, Martin-Gonzalez *et al*. 1992b). Three cyst types can be distinguished with this feature: kinetosome-resorbing (KR), partial-kinetosome-resorbing (PKR), and non-kinetosome-resorbing (NKR) cysts. However, phylogenetic relationships are not (yet?) recognizable. For instance, the PKR type occurs in *Colpoda magna* (Colpodea), *Dileptus anser* (Haptoria), and *Kahliella simplex* (stichotrichine spirotrichs).

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