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A comparative fine structural and phylogenetic analysis of resting cysts in oligotrich and hypotrich Spirotrichea (Ciliophora)

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Abstract

So far, neither morphology nor gene sequences have provided a reliable classification of halteriid and hypotrichid spirotrichs. Thus, we performed a comparative study on the fine structure of the resting cysts in some representative species, viz., the oligotrichs *Halteria grandinella* and *Pelagostrombidium fallax* and the oxytrichid hypotrichs *Laurentiella strenua*, *Steinia sphagnicola*, and *Oxytricha granulifera*. Main results include: (i) there are three different, very likely non-homologous cyst surface ornamentations, viz., spines (generated by the ectocyst), thorns (generated by the mesocyst), and lepidosomes (produced in the cytoplasm); (ii) *Halteria* has a perilemma; (iii) *Halteria*, *Meseres* and *Pelagostrombidium* have fibrous lepidosomes, while those of *Oxytricha* are tubular; (iv) the cyst wall structure of *Pelagostrombidium* and *Strombidium* is distinctly different from that of halteriids and oxytrichids, which are rather similar in this respect; (v) cyst ornamentation does not provide a reliable phylogenetic signal in oxytrichid hypotrichs because ectocyst spines occur in both flexible and rigid genera. The new observations and literature data were used to investigate the phylogeny of the core Spirotrichea. The Hennigian argumentation scheme and computer algorithms showed that the spirotrichs are bound together by the macronuclear reorganization band, the subepiplasmic microtubule basket, and the apokinetal stomatogenesis. The Hypotrichida and Oligotrichida are united by a very strong synapomorphy, viz., the perilemma, not found in any other member of the phylum. Halteriid and oligotrichid spirotrichs form a sister group supported by as many as 13 apomorphies. Thus, the molecular data, which classify the halteriids within the core hypotrichs, need to be reconsidered.

Keywords: *Halteria*; Hennigian argumentation scheme; Lepidosomes; *Oxytricha*; *Pelagostrombidium*; Transmission electron microscopy (TEM)

Introduction

Ciliate resting cysts are poorly known; detailed morphological and physiological data are available from less than 40 species (Foissner 2005; Foissner and Pichler 2006; Gutiérrez and Martín-González 2002; Gutiérrez et al. 2003). This ignorance is surprising, not only because the cystic state might be of considerable general interest, but also because resting cysts are the most important dispersal means of protists, a hotly discussed subject in modern ecology (for a review, see Foissner 2006). We suggest that the distribution

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problem (cosmopolitan vs. restricted) is closely related to resting cyst morphology and physiology. For instance, the longevity of resting cysts depends highly on the environment in which they are produced: while resting cysts generated, for instance, in the Namib Desert remain viable in air-dried condition for years (Foissner et al. 2002), those produced in rain forest soil lose viability within a few months (Foissner 1997, 2006).

Resting cysts have a great morphological and ontogenetic diversity. Thus, several studies have suggested that they should contain considerable phylogenetic information (Foissner 2005; Foissner and Pichler 2006; Gutiérrez et al. 2003; Rawlinson and Gates 1986; Reid and John 1983; Walker and Maugel 1980). However, concrete evidence is sparse (Foissner 2005), the best example still being the different resting cysts of euplotid and hypotrichid spirotrichs which, respectively, preserve and resorb the basal bodies (kinetosomes) of the trophic cell (Walker and Maugel 1980). Thus, we selected the resting cysts to investigate another difficult problem, viz., the classification of halteriid and hypotrichid spirotrichs, which has tantalized traditional and molecular taxonomists for a long time (for reviews, see Berger 1999; Foissner et al. 2004). To achieve this goal, several representatives of each group were investigated and the data added to the phylogenetic systems of Agatha (2004) and Agatha and Strüder-Kypke (2007). Surprisingly, this not only confirmed that halteriids are much more closely related to the oligotrichs than to the hypotrichs (Foissner et al. 2004), but also produced overwhelming evidence that Hypotrichida, Halteriina and Oligotrichina form a monophylum.

Materials and methods, terminology

Materials

Several populations of the oligotrich ciliate *Halteria grandinella* have been investigated over the past 15 years. They were cultivated in Eau de Volvic (French mineral water) enriched with some squashed wheat grains to stimulate bacterial growth. Cultures started with about 10 specimens each and were maintained at room temperature and daylight. Population 1 (Figs 1–5, 18–27, 29–36), isolated from meadow soil near to the town of Kefermarkt, Upper Austria, served for live observation, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Population 2 (Fig. 28) was isolated from slightly saline soil from the margin of a mangrove swamp in the Dominican Republic. The ITS sequences of this population differed significantly from those of the North American and European *H. grandinella*, and thus it probably represents a distinct molecular species (Katz et al. 2005). SEM and TEM investigations were performed and served for comparison with population 1. Population 3 (Figs 8–17), isolated from slightly saline soil from the north coast of Venezuela, near to the town of Chiciriviche, served for studying encystment in vivo.

Pelagostrombidium fallax (Figs 41–50) is also an oligotrich ciliate. Lugol-fixed cysts from Lake Mondsee (Salzburg, Austria) were available, as described by Müller et al. (2002). Unfortunately, Lugol's solution is a poor fixative for TEM. However, the cyst wall was preserved fairly well so that important details could be seen. Only Reid (1987) has ever performed a study on the fine structure of strombidiid resting cysts, but obtained only mediocre results, although he used appropriately fixed material, because the inorganic layers of the cyst wall prevented good preservation.

Oxytricha granulifera (Figs 37, 38) is an oxytrichid hypotrich and was isolated from a slightly saline soil from the north coast of Venezuela. It was cultivated as described for *H. grandinella* above. Cirral pattern and resting cyst morphology were as described by Foissner and Adam (1983), while morphometrics were distinctly different, indicating that this population represents a distinct subspecies.

Steinia sphagnicola (Fig. 40) and *Laurentiella strenua* (Fig. 39) are both oxytrichid hypotrichs. The populations studied were isolated from a temporary meadow pond in Salzburg City, i.e., from the type locality of *Meseres corlissi* Petz and Foissner (1992). Raw cultures were established in Eau de Volvic enriched with some ml of the natural sample and a few squashed wheat grains.

Resting cyst sampling

Resting cysts were obtained from old cultures by scraping the mud from the bottom of the culture dishes.

Methods

Morphological analyses: Encysting specimens and cysts were studied in vivo, in the scanning electron microscope (SEM), and in the transmission electron microscope (TEM). For SEM, specimens were fixed and treated as described in Foissner (1991) and Foissner et al. (2002). For TEM, specimens were fixed in a mixture of 10 ml glutaraldehyde (25%) + 6 ml aqueous osmium tetroxide (2%) + 10 ml saturated aqueous mercuric chloride. Cysts of *P. fallax*, originally preserved and stored in Lugol's solution, were washed in tap water and post-fixed in aqueous osmium tetroxide (2%) for 2 h. Specimens were transferred to Epon 812 via a graded ethanol series and propylene oxide. Flat embedding in aluminium weighing pans allowed specimens to be investigated light microscopically (up to × 400) to select well-preserved individuals for electron

microscopy. See Foissner (2005) for further treatment and details.

All cysts were checked for chitin, using the Van Wisselingh colour test, as described in Foissner et al. (2005). Alcian blue was used for revealing acid mucopolysaccharides.

Cladistic analysis: We restrict the phylogenetic analysis to the core Spirotrichea, viz., the Euplotia, Hypotrichida, Halteriina, and Oligotrichina, whose classification is still controversial (for reviews, see Foissner et al. 2004; Lynn 2003). Phylogenetic relationships of the lower oligotrich taxa were exhaustively analysed by Agatha (2004) and Agatha and Strüder-Kypke (2007). No consensus exists on the classification of the lower taxa of the hypotrichs and euplotids (for reviews, see Berger 1999, 2006; Foissner et al. 2004). Lynn (2003) added to the Spirotrichea some special taxa, each containing only a few species, viz., the Protocruziidae, Phacodiniidae and Licnophoridae. Their analysis is outside the goal of the present study.

The phylogenetic relationships were investigated following the method of Hennig (1966; Table 2) and employing the computer program PAUP* ver. 4.0b10 (Swofford 2002; Table 3). The Euplotia were used as outgroup because (i) they are probably the closest relatives of the Hypotrichida and (ii) the nearest relatives of the Spirotrichea are not known. The computed parsimony tree was based on ordered (Wagner/Farris optimization) states in the characters 4, 11, and 14 (Tables 2 and 3). Using the computer program PAUP*, the 50% majority-rule consensus tree was found by the heuristic analyses of equally weighted characters and optimized by the application of accelerated transformation (ACCTRAN). The bootstrap method with heuristic search included 1000 replicates and used the establishment of the starting tree/s by stepwise addition, a random addition of further taxa, and the tree-bisection-reconnection (TBR) branch-swapping algorithm. One tree was held at each step during stepwise addition. The resulting tree was rooted in such a way that the outgroup is a monophyletic relative of the ingroup. Finally, the tree was imported into TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

Terminology

General ciliate terminology follows Corliss (1979); cytological terminology is according to Becker et al. (2006) and Plattner and Hentschel (2002); and cyst terminology follows Foissner (2005), Foissner et al. (2005) and Gutiérrez et al. (2003). The present investigations revealed new substantial differences in various cyst wall structures, requiring further terminological refinements (examples and literature, see Table 4 and Discussion): spines are formed by the ectocyst; thorns are formed by the mesocyst; and lepidosomes are generated in the cytoplasm and transported to the pericyst by exocytosis (Foissner et al. 2006).

Taxonomy and nomenclature of the suborders Halteriina and Oligotrichina follow Agatha (2004) and Agatha and Strüder-Kypke (2007). Nomenclature of the Hypotrichida follows the recent revision of Berger (2006), who maintains the time-honoured naming of Stein and Ehrenberg, and thus abandons the more modern stichotrichs. Accordingly, Berger (2006) distinguishes the Hypotricha Stein, 1859 (e.g., *Oxytricha, Urostyla*; now widely named Stichotrichida) and the Euplota Ehrenberg, 1830 (e.g., *Euplotes, Uronychia*; now widely named Hypotrichida).

In the text, we use, if not ambiguous, the less awkward, rankless vernacular names, e.g., spirotrichs, hypotrichs, euplotids, halteriids, and oligotrichs.

Results

Halteria grandinella (Figs 1–5, 8, 11–36)

Trophic cell and fine structure of cortex (Figs 11, 21, 35, 36)

Halteria grandinella is one of the most common plankton ciliates, occurring in a great variety of habitats globally (for reviews, see Foissner et al. 1991, 1999). However, gene sequence data indicate that it is a complex of morphologically very similar species, possibly occupying different niches and geographic regions (Katz et al. 2005). Briefly, the globular body has a size of about 30–40 μm and bears 7–10 longitudinal, equatorial ciliary rows each comprising four groups of 15–25 μm long cilia (jumping bristles) used to perform conspicuous jumps. The anterior region is occupied by the oral apparatus composed of about 15 collar adoral membranelles encircling the flat peristomial field and about seven buccal membranelles in the small buccal cavity at the right margin of which extends the inconspicuous paroral membrane (Fig. 21). There is an oblong macronucleus, a globular micronucleus, and a contractile vacuole near mid-body (Fig. 11).

The cortex of *H. grandinella* consists of four membranes (from outside to inside): the perilemma and the cell membrane both also covering the cilia; and the outer and inner alveolar membranes, which surround the very flat alveoli (Figs 35, 36; opposed arrowheads). Usually, the perilemma is preserved only in small areas of the cell, probably because it is very fragile and thus difficult to preserve in this species. Underneath the cortical membranes is a single layer of microtubules that form a basket stabilizing body shape, as typical for many spirotrichs (Fig. 35).

Table 1. Morphometric data on *Halteria grandinella* cysts

Characteristics[a]	$\bar{x}$	M	SD	CV	Min	Max	*n*
Length (in vivo), µm[b]	27.9	27.0	2.0	7.0	25.0	31.0	13
Width (in vivo), µm[b]	27.7	27.0	1.8	6.3	25.0	31.0	13
Length (embedded cysts for TEM), µm[b]	24.7	25.0	2.7	10.7	21.0	31.0	21
Width (embedded cysts for TEM), µm[b]	23.3	24.0	2.4	10.2	20.0	28.0	21
Mucous envelope, thickness in vivo, µm[b]	9.1	8.5	2.3	24.9	4.0	12.0	20
Mucous envelope, thickness in TEM sections, µm[c]	6.6	6.5	1.2	17.7	5.0	9.0	8
Cyst wall, thickness without pericyst, nm	659.8	652.0	107.8	16.3	468.0	847.0	8
Metacyst, maximum thickness, nm	187.5	190.0	42.5	22.7	137.0	260.0	8
Endocyst, thickness, nm	92.3	86.0	25.7	27.8	60.0	140.0	8
Mesocyst, thickness, nm	308.8	320.0	65.2	21.1	190.0	380.0	8
Ectocyst, thickness, nm	70.8	70.0	6.7	9.4	60.0	80.0	8
Longest lepidosome, length, µm	3.4	3.5	0.9	26.8	2.1	4.5	8
Longest lepidosome, base width, µm	2.2	2.1	0.6	25.4	1.5	3.2	8
Lepidosomes, length: width ratio	1.6	1.5	0.3	19.3	1.2	2.1	8
Lepidosome strands, width, nm	13.1	13.0	2.8	21.3	9.0	18.0	8
Fibres of mucous envelope, diameter, nm	7.0	7.0	1.2	16.5	5.0	8.0	7
Dark cytoplasmic globules, diameter, nm[d]	216.5	211.0	41.3	19.1	167.0	283.0	8
Curious structures, total diameter, nm	636.4	490.0	240.0	37.4	368.0	1000.0	18
Curious structures, vacuole contained, diameter, nm	182.4	160.0	78.0	42.8	120.0	368.0	9

[a]Data based, if not stated otherwise, on TEM sections of 8 specimens (5 from Kefermarkt, 3 from Dominican Republic). CV – coefficient of variation in %, M – median, Max–maximum, Min–minimum, *n* – number of specimens investigated, SD – standard deviation, $\bar{x}$ – arithmetic mean.
[b]Cysts from Austrian (Kefermarkt) population. Lepidosomes excluded.
[c]Cysts from Dominican Republic population; thickest site measured.
[d]With surrounding membrane.

Encystment (Figs 8–17)

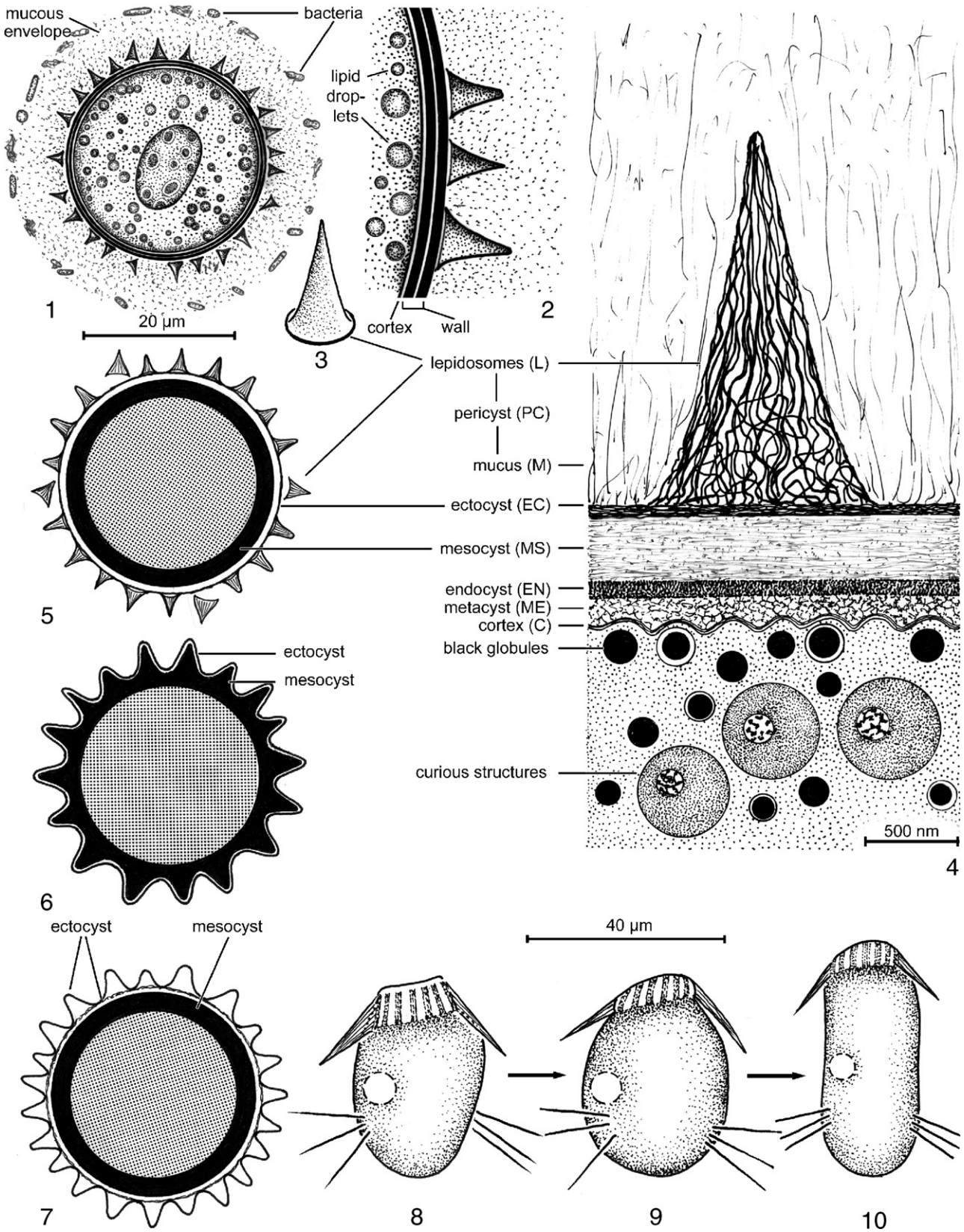
Encysting specimens show conspicuous changes: the globular body becomes cylindrical (Figs 11–14, 21) and conical structures appear in the cytoplasm (Fig. 15). Cell elongation almost doubles body length and occurs mainly in the anterior half of the body, causing the ciliary rows to become displaced nearer to the posterior end of the body. Further, the buccal cavity flattens so that the buccal membranelles become located on the body surface. In this stage, the cells look like another genus or like the swarmer of a peritrich ciliate (Fig. 14). Indeed, *Halteria oviformis* Gelei and Szabados (1950) probably represents an encysting *H. grandinella.* Conspicuous changes occur in the cytoplasm, where many conical structures, up to 5 µm long, appear (Fig. 15).

After the cylindrical stage, the encysting cell rounds up and extrudes a voluminous, mucous envelope, and the conical structures produced in the cytoplasm become attached to the ectocyst (Figs 16, 17). Thus, they are lepidosomes, as in *M. corlissi* (Foissner et al. 2006). Details of these processes and of cyst wall formation were not investigated.

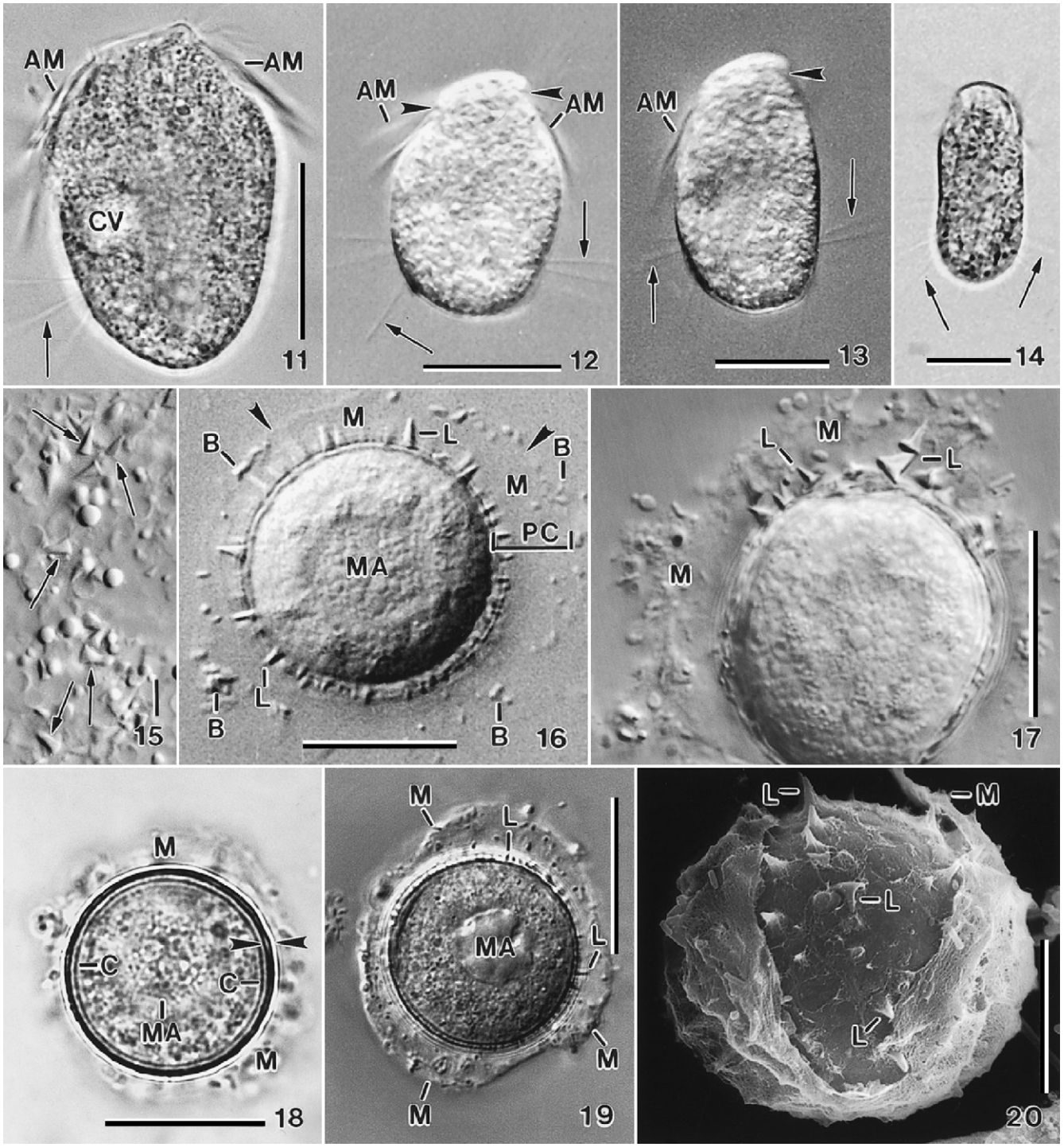
Resting cyst (Figs 1–5, 16–20, 22–34)

General description and phenetic variability (Figs 1–3, 16–20, 25). The cysts of *H. grandinella* are firmly attached to the bottom of the culture dish or, in older cultures, adhere to floating debris accumulating on the culture surface. Attachment occurs by the slimy envelope, up to 12 µm thick, which is very hyaline and often lost when the cysts are scraped from the bottom of the Petri dish. A further conspicuous feature is the conical lepidosomes, up to 5 µm long, which are attached to the ectocyst surface. The cyst wall is 1–2 µm thick and yellowish to brownish in vivo; the macronucleus is ellipsoidal and has coarse nucleoli; and the finely granular cytoplasm contains some lipid droplets up to 3 µm across (Figs 1–3, 16–20, 25–27, 29).

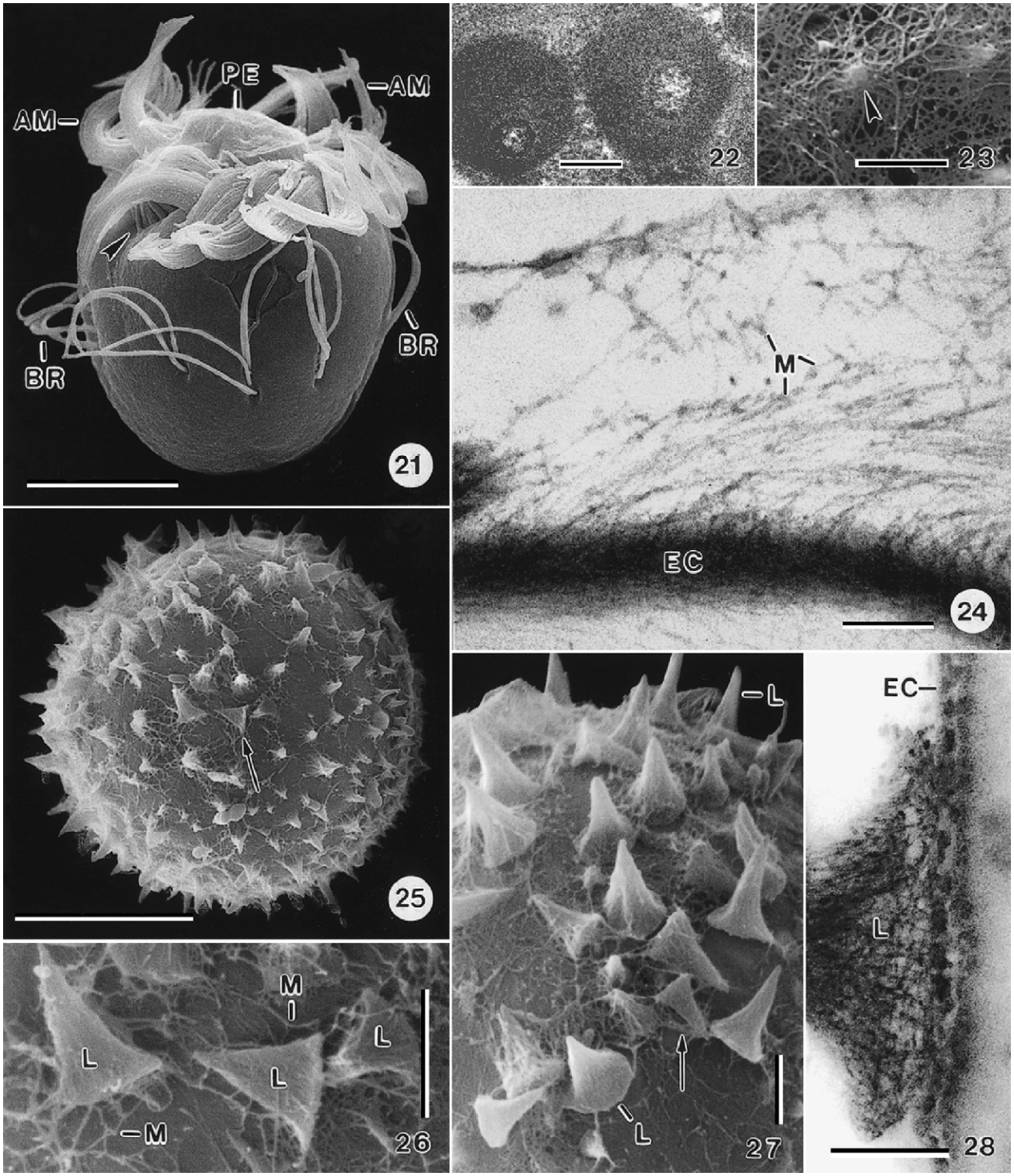
The basic features mentioned above are very constant, that is, are present in over 10 populations from Europe, Asia, Africa, and Australia investigated over the years. Details, in contrast, are highly variable, depending on population and culture age. For instance, the cysts of the Dominican population had an average diameter of 34 µm ($n = 20$) when cultivation started, but decreased to 26 µm after 2 months of cultivation. Likewise, the length of the lepidosomes, the thickness and distinctness of the slimy envelope, and the colour of the cyst wall may change greatly. Indeed, the yellowish to brownish cyst wall turns almost colourless, the lepidosomes become very small (< 1 µm) or even disappear, and the ability to form resting cysts at all usually dramatically decreases after prolonged laboratory cultivation. Thus, the following data refer, if not mentioned otherwise, to cysts from a young culture of the Austrian population from Kefermarkt. Morphometric data are shown in Table 1 and will be repeated only when appropriate.



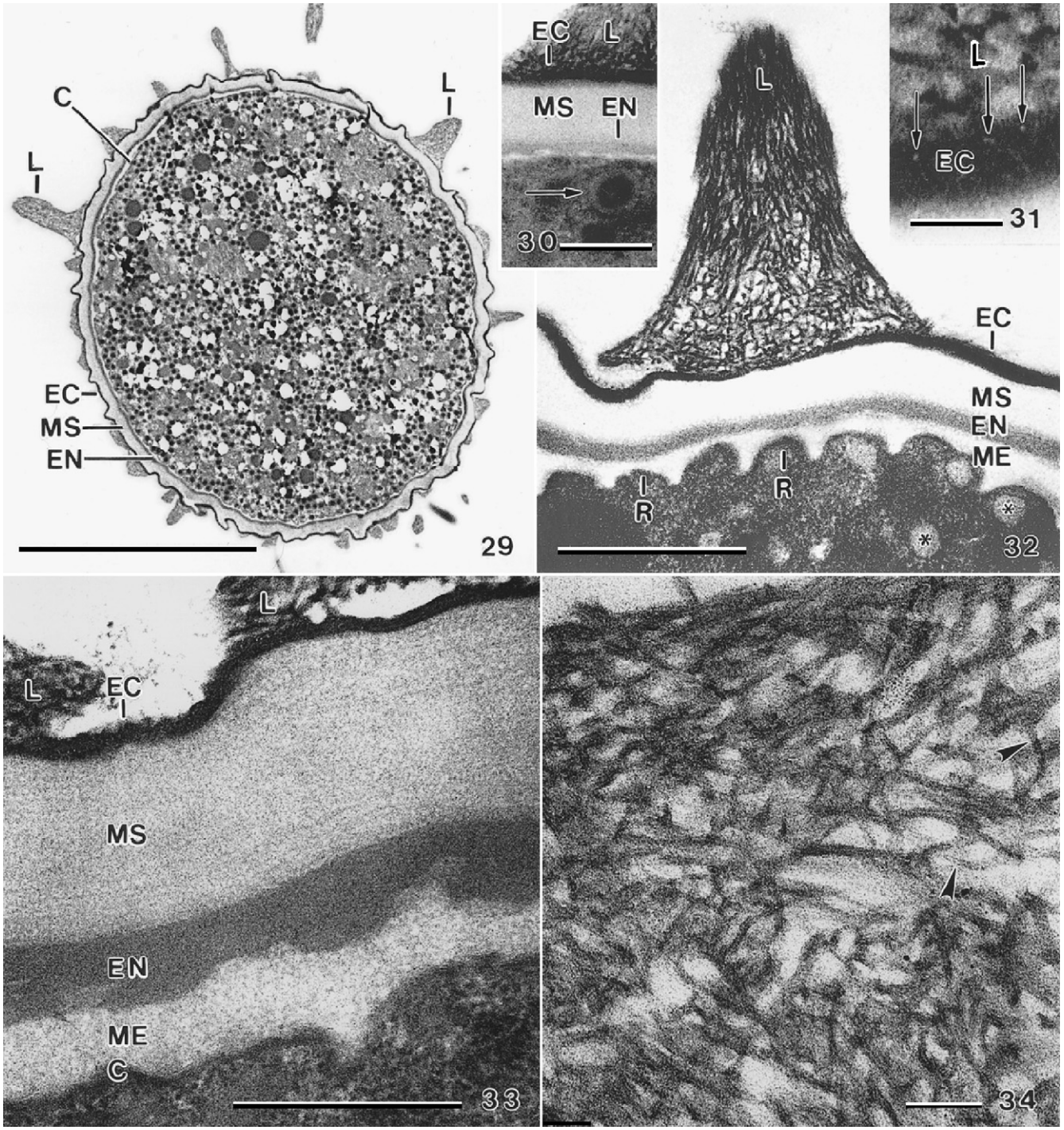
Figs 1–10. Resting cysts (1–7) and encystment (8–10) in some oligotrich (1–5, 8–10) and hypotrich (6, 7) spirotrichs. **1–4**: *Halteria grandinella* in the light (1–3) and electron (4) microscope. **5–7**: Schemes of the origin of the spines in *Halteria*, *Laurentiella* and *Steinia*. **8–10**: *Halteria grandinella*, early (8, 9) and middle (10) phases of encystment.



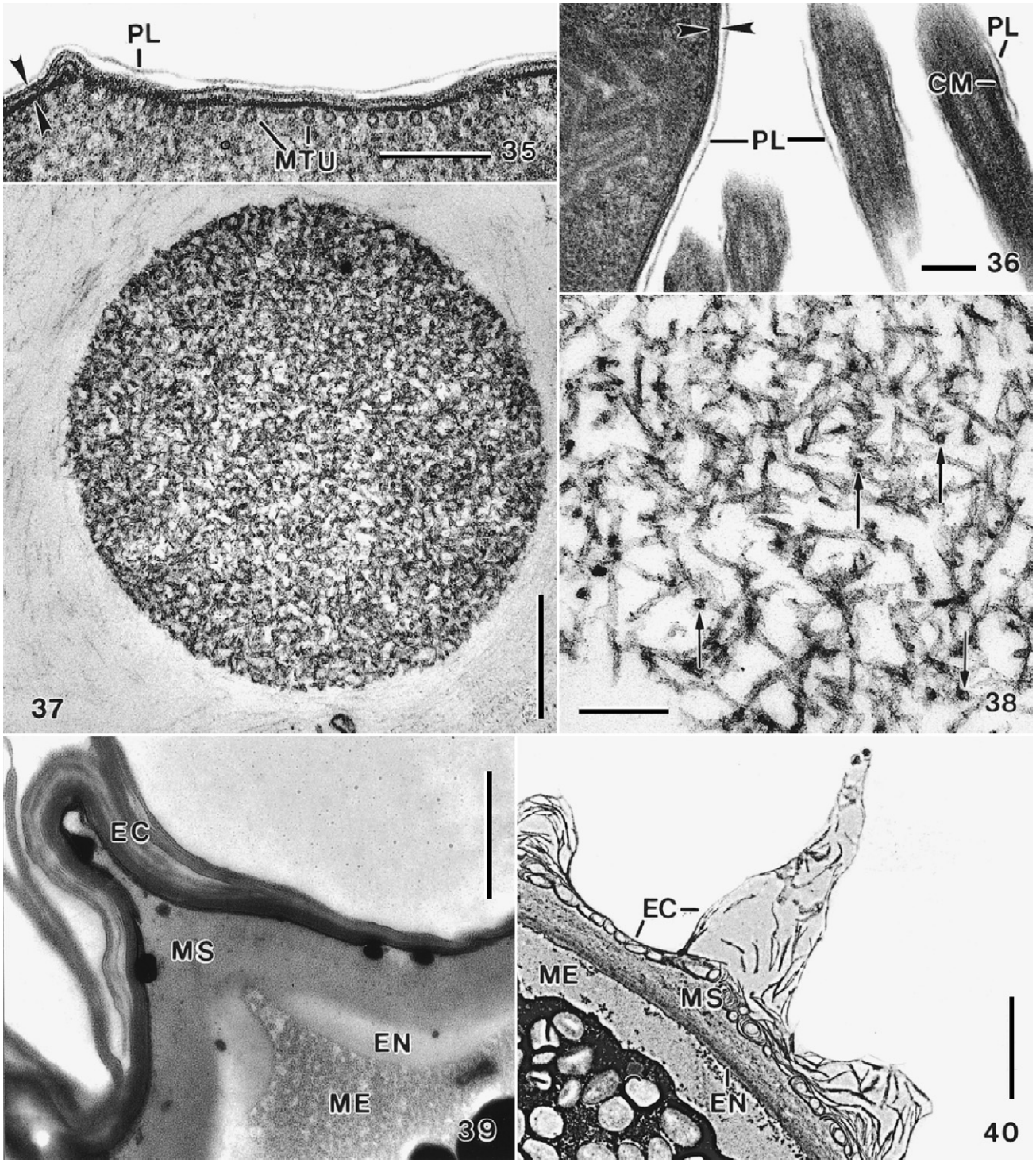
Figs 11–20. *Halteria grandinella*, encystment (11–15) and resting cysts (16–20) in the light (11–19) and scanning (20) microscope (11–17, Venezuelan specimens; 18–20, Austrian specimens). **11–14**: The globular trophic cells (see Fig. 21) become oblong, dislocating the equatorial ciliary rows (Fig. 21) subterminally (arrows). The arrowheads mark the head-like separated peristomial area. **15**: When encysting specimens (12) are squashed, many conical lepidosomes become recognizable in the cytoplasm (arrows). **16**: A young cyst with a very hyaline mucous coat (arrowheads), becoming recognizable due to adhering bacteria (B). **17**: When squashed, the lepidosomes (L) become detached from the ectocyst. **18, 19**: Optical sections. The cyst wall (opposed arrowheads) is distinct under bright field illumination (18), while the mucous coat and the minute lepidosomes are recognizable only with interference contrast (19). **20**: A cyst with mucous coat opened, showing the lepidosomes. AM – adoral membranelles, B – bacteria, C – ciliate cortex, CV – contractile vacuole, L – lepidosomes, M – mucous coat, MA – macronucleus, PC – pericyst. Scale bars 5 μm (15), 10 μm (20), 20 μm (11–14, 16–19).



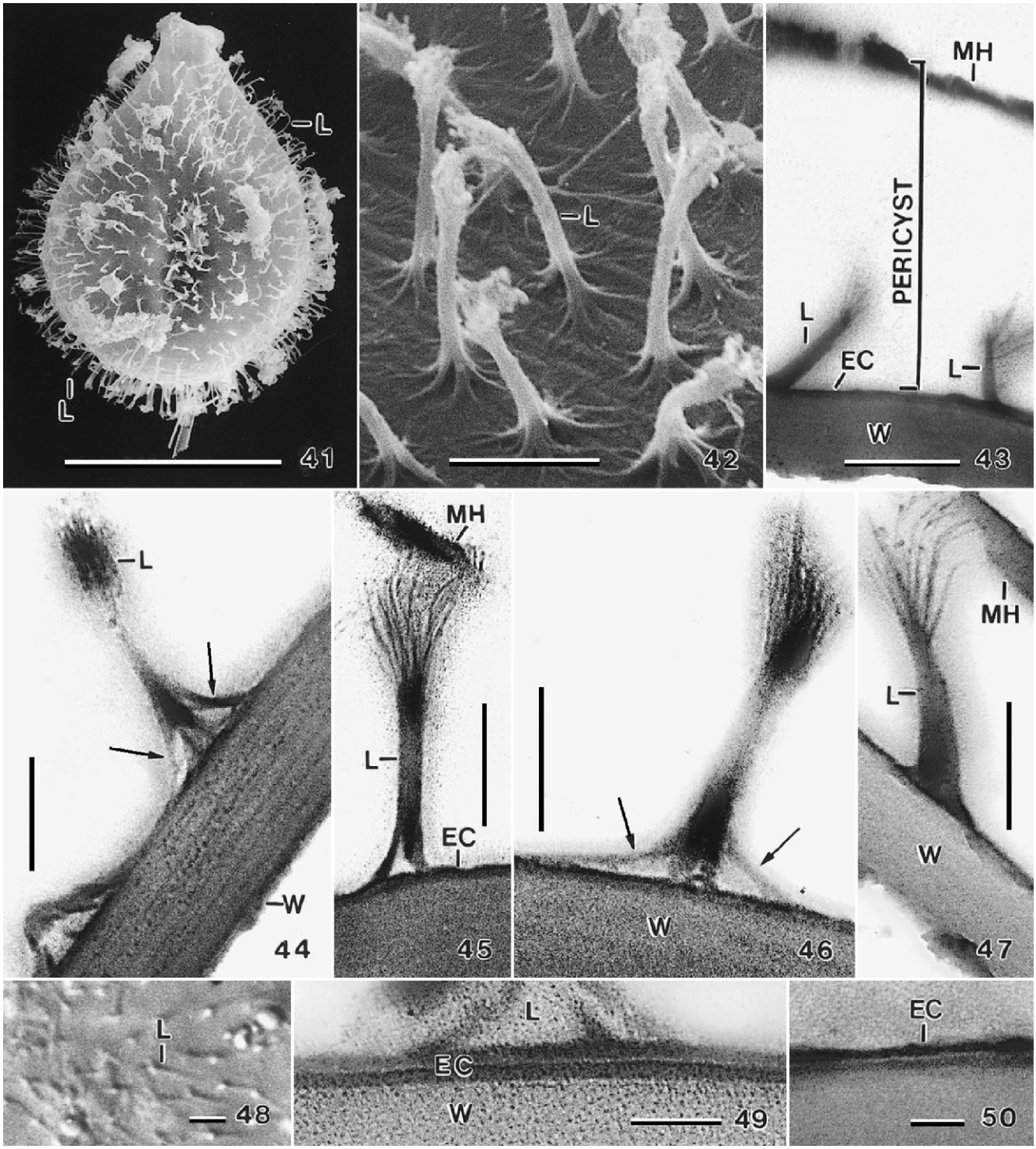
Figs 21–28. *Halteria grandinella*, trophic (21) and cystic (22–28) specimens in the scanning (21, 23, 25–27) and transmission (22, 24, 28) electron microscope (21–27, Austrian specimens; 28, Dominican specimen). **21**: Ventrolateral overview with buccal adoral membranelles marked by arrowhead. **22**: The cytoplasm contains globular ''curious structures''. **23**: The mucous envelope consists of fibres. Arrowhead marks the top of a lepidosome. **24**: A late stage of cyst wall formation, showing that the mucous coat is made of fibres about 13 nm thick that are attached to, or even form, the ectocyst. **25–27**: When the mucous coat is removed, the conical lepidosomes become recognizable. The lepidosomes are attached to the ectocyst by mucous fibres forming an irregular reticulum on the ectocyst; some are fallen down (arrows), showing that the contact is weak. **28**: A decaying cyst, showing that the ectocyst is composed of two sheets of polygonal platelets (flocks). AM – adoral membranelles, BR – bristle (ciliary) rows, EC – ectocyst, L – lepidosomes, M – mucous fibres, PE – peristomial field. Scale bars 10 μm (21, 25), 2 μm (23, 26–28), 200 nm (22, 24).



Figs 29–34. *Halteria grandinella*, transmission electron micrographs of resting cysts. **29**, **30**: Overview and detail showing one of the many dark granules (30, arrow) contained in the cytoplasm. **31**: The darkly stained ectocyst contains many minute, bright areas (arrows). **32–34**: Structure of cyst wall and lepidosomes. The cyst wall consists of five layers (33): the mucous pericyst with lepidosomes, ectocyst, mesocyst, endocyst, and the metacyst. Fig. 32 is from an unstained section, showing the high osmium affinity of the lepidosomes, the ectocyst, and the endocyst. Note also the bright vacuoles (asterisks) which show a dark globule after uranyl acetate and lead citrate staining (Fig. 30). The lepidosomes (34) consist of strands of highly osmiophilic material. The strands, which have a width of about 11 nm (34, arrowheads), are not tubular or fibrous because ring-shaped or circular transverse profiles were very rarely observed. C – ciliate cortex, EC – ectocyst, EN – endocyst, L – lepidosomes, ME – metacyst, MS – mesocyst, R – cortical ridges. Scale bars 10 μm (29), 1 μm (32), 500 nm (30, 33), 100 nm (31, 34).



Figs 35–40. Transmission electron micrographs of trophic *Halteria grandinella* (35, 36) and cystic *Oxytricha granulifera* (37, 38), *L. strenua* (39), and *S. sphagnicola* (40). **35, 36**: Both transverse sections of the body (35) and longitudinal sections of the cilia (36) show that *Halteria* has a perilemma. Opposed arrowheads mark regions where the cell membrane and the two membranes of the very flat alveoli are recognizable. **37, 38**: The lepidosomes of *O. granulifera* are embedded in mucous material and have a diameter of 0.5–3 µm. They lack a surrounding membrane (37) and consist of highly wrinkled tubules with a diameter of about 14 nm (38, arrows). **39, 40**: In hypotrichs, the conical processes of the cysts are formed by the mesocyst (39, *Laurentiella*) or by the ectocyst (40, *Steinia*), while the oligotrichs *Halteria* (Figs 29, 32) and *Pelagostrombidium* (Figs 42–46) produce processes by lepidosomes, that is, structures generated in the cytoplasm and then released by exocytosis. CM – ciliary membrane, EC – ectocyst, EN – endocyst, ME – metacyst, MS – mesocyst, MTU – cortical microtubules, PL – perilemma. Scale bars 4 µm (40), 1 µm (39), 500 nm (37), 200 nm (35, 36, 38).



Figs 41–50. *Pelagostrombidium fallax*, resting cysts in the light microscope (48) and in the scanning (41, 42) and transmission (43–47, 49, 50) electron microscope. All figures from poorly preserved specimens, as described in the material section. Thus, the brush-like distal end of the lepidosomes could be an artifact. **41, 42, 48**: The flask-shaped cyst has 1–5 μm long processes (lepidosomes) and is covered with an only partially preserved, membrane-like material (41). The lepidosomes are attached to the ectocyst by fibrous processes forming a broadly conical base resembling the conditions in *Halteria* (Figs 25–27). **43**: *Pelagostrombidium* has a several μm thick pericyst made of lepidosomes and a membrane-like coat. **44–47**: A thick, compact layer, which usually shows alternating bright and dark zones (44), is underneath the thin ectocyst. Arrows mark the broadened lepidosome base (cp. Fig. 42). **49, 50**: At high magnification, the ectocyst often appears to be composed of two dark layers separated by a narrow, bright zone. EC – ectocyst, L – lepidosomes, MH – membranous sheet(s), W – part of cyst wall (mesocyst ?). Scale bars 30 μm (41), 2 μm (42, 48), 1 μm (43), 500 nm (44–47), 100 nm (49, 50).

Cyst wall. In the light microscope, the cyst wall is composed of two distinct layers: the slimy pericyst with the lepidosomes; and a 1–2 μm thick, dense, bipartite structure (Figs 1, 2, 18, 19) obviously corresponding to the ectocyst, mesocyst, endocyst, and metacyst recognizable in the electron microscope (Figs 4, 32, 33). The light microscopic bipartition of the wall possibly marks the border between mesocyst and endocyst.

Pericyst (Figs 1–4, 16–20, 23–29, 32, Table 1): The pericyst consists of the mucous envelope and the lepidosomes. The mucous envelope is up to 12 μm thick, depending on population and age of the culture, as described above. Usually, it is thicker and more compact in cysts from young cultures. Compactness varies greatly, for instance, the envelope is very dense and cannot be removed from specimens from Botswana. However, this population probably represents a distinct species, according to the molecular data (Katz et al. 2005). Probably, the mucous envelope is secreted in two to three batches and is more compact distally than proximally. It stains with alcian blue, indicating that acid mucopolysaccharides are a main component; it does not stain with methyl green–pyronin, in contrast to the slimy envelope of *M. corlissi* which stains red. This indicates considerable differences between these closely related genera. In the TEM, the envelope is very loose and composed of more or less wrinkled, weakly stained fibres with an average diameter of 7 nm (Fig. 24, Table 1). In the SEM, the fibres have an average diameter of 45 nm and form a dense, irregular, three-dimensional reticulum (Figs 23, 25–27). The reason for the great difference in fibre diameter is not clear; possibly, it is caused by different shrinkage of the fibres during the preparation procedures and by the sputter coating that covers the fibres with gold particles.

The lepidosomes, which are yellowish to colourless in vivo, are rather hyaline cones with a length of up to 5 μm and a base width of up to 4 μm (Table 1); rarely, some are cylindrical, claviform, or indistinctly pyramidal. Lepidosome size greatly varies within and between populations and even in a single cyst (Figs 16, 19, 25, 27). Further, they may disappear after prolonged laboratory cultivation, as described above. When the lepidosomes are small and hyaline, they are easily overlooked at low and medium magnification (100–400 ×). Thus, their presence/absence must be confirmed with an oil immersion objective (1000 ×). The lepidosomes are attached to the ectocyst, probably with the same mucous fibres as those that compose the slimy envelope. Since attachment is weak, they easily detach when the cyst is slightly pressed between slide and coverslip (Fig. 17) or scraped from the Petri dish and observed in the SEM (Figs 25–27). The lepidosomes do not stain with alcian blue but become red when methyl green–pyronin is applied, suggesting that they are composed of proteinaceous material. In the TEM, the lepidosomes lack a surrounding membrane, though a cover is indicated by the smooth appearance in the SEM (Figs 26, 27), and are composed of a highly wrinkled and interwoven mass of "fibres" orientated more or less longitudinally in the distal half. A detailed analysis of many sections showed the "fibres" as long, flat strands with an average width of 13 nm, similar to the strands composing the lepidosomes of *Meseres* (Foissner et al. 2006). Circular and ring-shaped transverse profiles occur, but so rarely that they are probably produced by chance, especially when compared with the lepidosomes of *Oxytricha granulifera* (Fig. 38) and mitochondria, where ring-shaped transverse profiles are frequent.

Ectocyst (Figs 1, 2, 4, 18, 19, 24, 28–33, Table 1): The ectocyst is an electron dense layer about 70 nm thick, often appearing structureless in the TEM. Frequently, it contains some irregularly distributed, minute, bright areas (Fig. 31). The best micrographs and a late developmental stage (Fig. 24) suggest that it is composed of very fine fibres (< 5 nm), possibly "condensed" fibres of the mucous envelope (see also below). However, a different structure is suggested by old, decomposing cysts without contents. In such specimens, the ectocyst is sometimes composed of two dark layers separated by a weakly stained zone about 5 nm wide. The dark layers appear composed of small, irregular platelets and granules (Fig. 28). Decomposing cysts also show that the ectocyst, the lepidosomes, and the mucous envelope are the most stable components of the wall, persisting after the mesocyst, endocyst, and metacyst have disappeared.

Mesocyst (Figs 1, 2, 4, 18, 19, 29, 30, 32, 33, Table 1): The mesocyst is about 300 nm thick and of moderate electron density. Usually, it is very finely granular; rarely some lamination is recognizable, suggesting that it is composed of very fine fibres.

Endocyst (Figs 1, 2, 4, 18, 19, 29, 30, 32, 33, Table 1): The endocyst is about 100 nm thick and more electron dense than the mesocyst and the metacyst, thus forming a conspicuous zone. The distal margin is smooth, while the proximal margin is slightly to distinctly irregular. The endocyst is structureless or very finely granular.

Metacyst (Figs 1, 2, 4, 18, 19, 29, 30, 32, 33, Table 1): The metacyst is about 200 nm thick and of similar electron density to the mesocyst. It is finely to coarsely alveolar and never shows a granulation.

Cytoplasm (Figs 1, 2, 4, 16–19, 22, 29, 30, 32, Table 1). The encysted cell has a distinctly furrowed periphery (Fig. 32) and has resorbed the oral and somatic infraciliature, including the cortical microtubule basket. The cytoplasm has two remarkable inclusions, viz., numerous electron dense granules and some more lightly stained "curious structures". The dense granules, which are usually surrounded by a membrane at a rather wide distance, have an average size of 216 nm and give the cell

a spotted appearance (Fig. 29). In unstained sections, the globules appear as fairly bright vacuoles with finely granular contents (Fig. 32). The curious structures were found in three specimens and probably originate from autophagic vacuoles, as in *M. corlissi* (Foissner and Pichler, manuscript in preparation). They are globular to broadly ellipsoidal and have a rather different size in the three specimens found, ranging from 368–1000 nm (Table 1). These structures consist of a finely granular, moderately electron dense matrix and a rather darkly stained, non-central vacuole with granular contents and an average diameter of 182 nm (Figs 4, 22).

Resting cysts of *Pelagostrombidium fallax* (Figs 41–50)

As mentioned in the material section, only insufficiently preserved material was available. Thus, we cannot provide such detailed data as for *Halteria*. A light microscopical description of the cysts investigated is found in Müller et al. (2002), and a population from Lake Constance in Germany was studied by Müller (1996). Briefly, the flask-shaped cysts are about 55×40 µm in size and have a clear plug at the narrower end. The surface is covered with numerous processes (''spines''), 1–5 µm long, and a membranous sheet (Figs 41, 42, 48).

TEM shows three distinct layers (Fig. 43): a conspicuous pericyst; a thin ectocyst; and a thick, lamellar ''wall'', probably representing the mesocyst. Probably, there is at least one further layer proximally, but it was too poorly preserved to be described.

The pericyst is composed of the lepidosomes and a membranous structure extending on the top of the lepidosomes (Figs 41–48). The lepidosomes consist of fibres with an average diameter of 9.6 nm (8–11 nm, $n = 5$). Proximally, the fibres form several thin, star-like spreading bundles anchoring the lepidosome to the ectocyst; distally, the fibres are spread, brush-like, which, however, might be an artifact caused by the poor fixation and long storage of the cysts. The membranous cover is structureless and probably composed of several very thin sheets (Figs 43, 45–47). The ectocyst is 26 nm thick on average (16–40 nm, $n = 5$) and composed of two homogenous layers separated by a very thin ($\leqslant 5$ nm), bright zone (Figs 45, 49, 50). The outer layer is slightly thicker and more darkly stained than the inner one. The ''wall'' (mesocyst ?) is 457 nm thick on average (307–633 nm, $n = 5$) and composed of an average of 6.6 (5–8, $n = 5$) alternating zones, that is, thin, dark layers and thick, slightly brighter zones (Figs 44, 46).

Resting cysts of *Oxytricha granulifera*, *Laurentiella strenua*, and *Steinia sphagnicola* (Figs 37–40)

The cysts of these species were studied for comparison of main features, that is, they will later be described in detail. Light microscopical data are reviewed in Berger (1999). Fine structural and cytochemical data on the cyst of *L. strenua* are available under the name of its junior synonym (Berger 1999) *L. acuminata* (Gutiérrez et al. 1983a, b; Jareño 1985).

The cyst of the Venezuelan *O. granulifera* has a diameter of about 44 µm and is covered by globular, slightly orange-coloured lepidosomes embedded in a slimy matrix. The lepidosomes are 0.5–3 µm in size and consist of tubes with a diameter of about 13 nm. The tubes are highly wrinkled, forming a dense reticulum. The lepidosomes lack a membrane and are embedded in a slimy matrix consisting of fibres with a diameter of < 5 nm (Figs 37, 38).

The cyst of *L. strenua* has a diameter of about 85 µm and has many rather compact, cone-shaped processes. The processes are formed by the cyst wall, mainly by the thick mesocyst (Fig. 39); thus, they are thorns, according to our terminology.

The cyst of *S. sphagnicola* has a diameter of about 53 µm and has many hyaline, cone-shaped processes. The processes are formed by the ectocyst lamellae (Fig. 40); thus, they are spines, according to our terminology.

Chitin testing

The Van Wisselingh chitin test was negative for the cysts of the oxytrichid hypotrichs and populations 1 and 2 of *H. grandinella*.

Table 2. Character states and coding used for the Hennigian argumentation scheme (Fig. 51)

No.	Apomorphic	Plesiomorphic
1	Cell shape usually globular to obconical (coded 1)	Cell shape usually distinctly flattened dorso-ventrally (coded 0)
2	Usually planktonic (coded 1)	Usually benthic (coded 0)
3	Adoral zone of membranelles mainly apical (coded 1)	Adoral zone of membranelles mainly ventral (coded 0)
4[a]	30–50% (coded 1) or 0% (coded 2) of adoral membranelles four-rowed	>90% of adoral membranelles four-rowed (coded 0)
5	Loss of paroral membrane (coded 1); convergent in several Euplotia	Paroral and endoral membrane present (coded 0)

Table 2. (*continued*)

No.	Apomorphic	Plesiomorphic
6	Undulating membranes usually diplo- or polystichomonad (coded 1)	Undulating membranes invariably monostichomonad (coded 0)
7	Cirri present (coded 1)	Cirri lost (coded 0)
8	⩾40% of somatic kineties shortened (coded 1)	Somatic kineties extend whole body length (coded 0)
9	Macronucleus with reorganization band (coded 1)	Macronucleus without reorganization band (coded 0)
10	With perilemma; now also found in *Halteria*, Figs 35, 36 (coded 1)	Without perilemma (coded 0)
11[a]	With subepiplasmic microtubules in single layer (coded 1) or in doublets or triplets (coded 2)	Without subepiplasmic microtubules (coded 0)
12	Cortex with protein platelets (coded 1)	Cortex without protein platelets (coded 0)
13	Division enantiotropic (coded 1)	Division homeotropic (coded 0)
14[a]	Stomatogenesis epiapokinetal (coded 1) or hypoapokinetal (coded 2)	Stomatogenesis parakinetal or buccokinetal (coded 0)
15	Posterior portion of oral primordium curves rightwards (coded 1)	Anterior portion of oral primordium curves rightwards (coded 0)
16	Undulating membrane(s) originate de novo (coded 1)	Undulating membrane(s) originate from oral primordium or cirral anlagen (coded 0)
17	Entire somatic ciliature originates de novo (coded 1)	Somatic kineties originate at least partially by intrakinetal proliferation of basal bodies (coded 0)
18	Proter and opisthe somatic anlagen (kineties) develop in between the parental kineties (coded 1)	Proter and opisthe somatic anlagen (kineties) develop within the parental kineties (coded 0)
19	Interlocking arrangement of conjugants (coded 1)	Parallel or oblique arrangement of conjugants (coded 0)
20	Parental basal bodies not resorbed, i.e., NKR cyst type (coded 1)	Parental basal bodies partially or completely resorbed, i.e., PKR or KR cyst type (coded 0)
21	Resting cyst with operculum (coded 1)	Resting cyst without operculum (coded 0)
22	Ectocyst bipartite and granular (coded 1)	Ectocyst a single microfibrillar or membranous layer (coded 0)
23	Resting cyst wall with inorganic layers (coded 1)	Resting cyst wall without inorganic layers (coded 0)
24	Lepidosomes with tubular (coded 1) or fibrous (coded 2) fine structure	Without lepidosomes (coded 0)
25	Resting cyst plasm with "curious" structures (coded 1)	Resting cyst plasm without "curious" structures (coded 0)
26	Halteriid cyst wall precursors (coded 1)	Hypotrich cyst wall precursors (coded 0)

[a]Ordered characters (Wagner/Farris optimization).

Phylogenetic analyses

Character states (Table 2): Most characters used in the phylogenetic analyses have been discussed by Agatha (2004), Agatha and Strüder-Kypke (2007), Foissner (1996), and Petz and Foissner (1992). Thus, we only treat the features not contained in these studies.

Character 2 (usually planktonic): Most Oligotrichida are planktonic ciliates (Foissner et al. 1999), while most euplotids and hypotrichs are benthic organisms, typically creeping on the mud surface (Foissner et al. 1991). Even *H. grandinella,* which frequently occurs in benthic samples, is a planktonic organism because, as we observed, it never sits or glides on the bottom of culture dishes. We suggest that the planktonic mode of life caused the globular or obconical body shape and the apicalization of the adoral zone of membranelles so typical of the oligotrichs. Further, we consider these peculiarities apomorphies because most ciliates are sessile or creeping, benthic organisms.

Character 11 (subepiplasmic microtubule basket): The four ciliate groups under consideration have a striking feature in common, viz., subepiplasmic microtubules which form a basket with the shape of the cell (Fleury et al. 1992). These microtubules, which are not associated with any somatic or oral basal bodies, have been found in the euplotids (e.g., Foissner 1978), hypotrichs (e.g., Wirnsberger and Hausmann 1988; Wirnsberger-Aescht et al. 1989), halteriids (e.g., Foissner 2005; present paper, Fig. 35) and various oligotrichs (Grim 1987; Laval 1972). Subepiplasmic microtubules, which probably provide structural support for the cell shape, are absent in most other ciliates, except for the phyllopharyngeans (e.g. *Chilodonella*), *Spirochona*, the entodiniomorphids, and some suctoria (Lynn and Corliss 1991). However, these taxa are far away from the spirotrichs, suggesting convergent evolution of the microtubule basket.

Character 12 (cortex with protein platelets): The cortical alveoli of the euplotids contain a dense,

Table 3. Distribution of character states among the taxa cladistically analysed with the PAUP* computer program (Fig. 52)

Taxa/characters	5							10						15						20						25	
Euplotia	0	0	0	0	0	1	1	0	1	0	2	1	0	2	0	0	0	0	0	1	0	0	0	0	0	0
Hypotrichida	0	0	0	0	0	1	1	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
Halteriina	1	1	1	1	0	0	0	1	1	1	1	0	1	1	0	1	1	1	1	0	0	1	0	2	1	1
Oligotrichina	1	1	1	2	1	0	0	1	1	1	1	0	1	2	1	1	0	0	1	0	1	1	1	2	1	1

amorphous or microfibrillar protein which lend form and rigidity to the cell surface (Kloetzel 1991). Thus, each alveolus consists of a minute, membrane-bound protein platelet. Between adjacent platelets, there is a microfibrillar substance with strong affinity to silver nitrate (Foissner 1978). The euplotid protein platelets must not be confused with the polysaccharide platelets occurring in the oligotrich families Strombidiidae, Tontoniidae, Cyrtostrombidiidae, and Pelagostrombidiidae (Agatha 2004). Both types of platelet are considered apomorphies because of their restricted occurrence.

Character 18 (anlagen in between parental ciliary rows): The halteriids are unique in that the somatic ciliary pattern of the opisthe originates de novo. Further, the somatic anlagen develop in between the parental ciliary rows, and separate anlagen are generated in proter and opisthe (Petz and Foissner 1992; Song 1993); the last feature resembles that in hypotrichs. However, this is very likely to be a superficial similarity because the hypotrichs develop their somatic anlagen within and from parental ciliary rows, i.e., by intrakinetal proliferation of basal bodies (Berger 1999, 2006).

Character 20 (cyst type): With respect to the kinetosomes, there are three types of resting cysts (Gutiérrez et al. 2003; Rawlinson and Gates 1986; Walker and Maugel 1980): kinetosome-resorbing (KR) cysts, partial-kinetosome-resorbing (PKR) cysts, and non-kinetosome-resorbing (NKR) cysts. KR and PKR cysts probably represent the plesiomorphic state because they are most common. They occur in hypotrichs (Gutiérrez et al. 2003), halteriids (Foissner 2005; present paper), and oligotrichs (unpubl. data on *Rimostrombidium*). Thus, the NKR cysts of the euplotids very likely represent the apomorphic state.

Character 21 (cyst operculum): A cyst operculum occurs in many groups of ciliates and probably evolved convergently several times (Foissner 1993; Reid and John 1978, 1983). In the ciliates under investigation, an operculum occurs only in the Oligotrichina. Thus, we consider the operculum an apomorphic feature.

Character 22 (fine structure of ectocyst): In many ciliates, the ectocyst is a single microfibrillar or membranous layer (for a review, see Gutiérrez et al. 2003), for instance, in the hypotrichs (Figs 39, 40). Thus, we consider the bipartite, granular structure found in *Meseres* (Foissner 2005) and *Halteria* (Figs 28, 31) the apomorphic state. Most likely, the bipartite ectocyst of *Pelagostrombidium* is also granular (cp. Figs 28, 49, 50), but detailed investigations on other Oligotrichina are required.

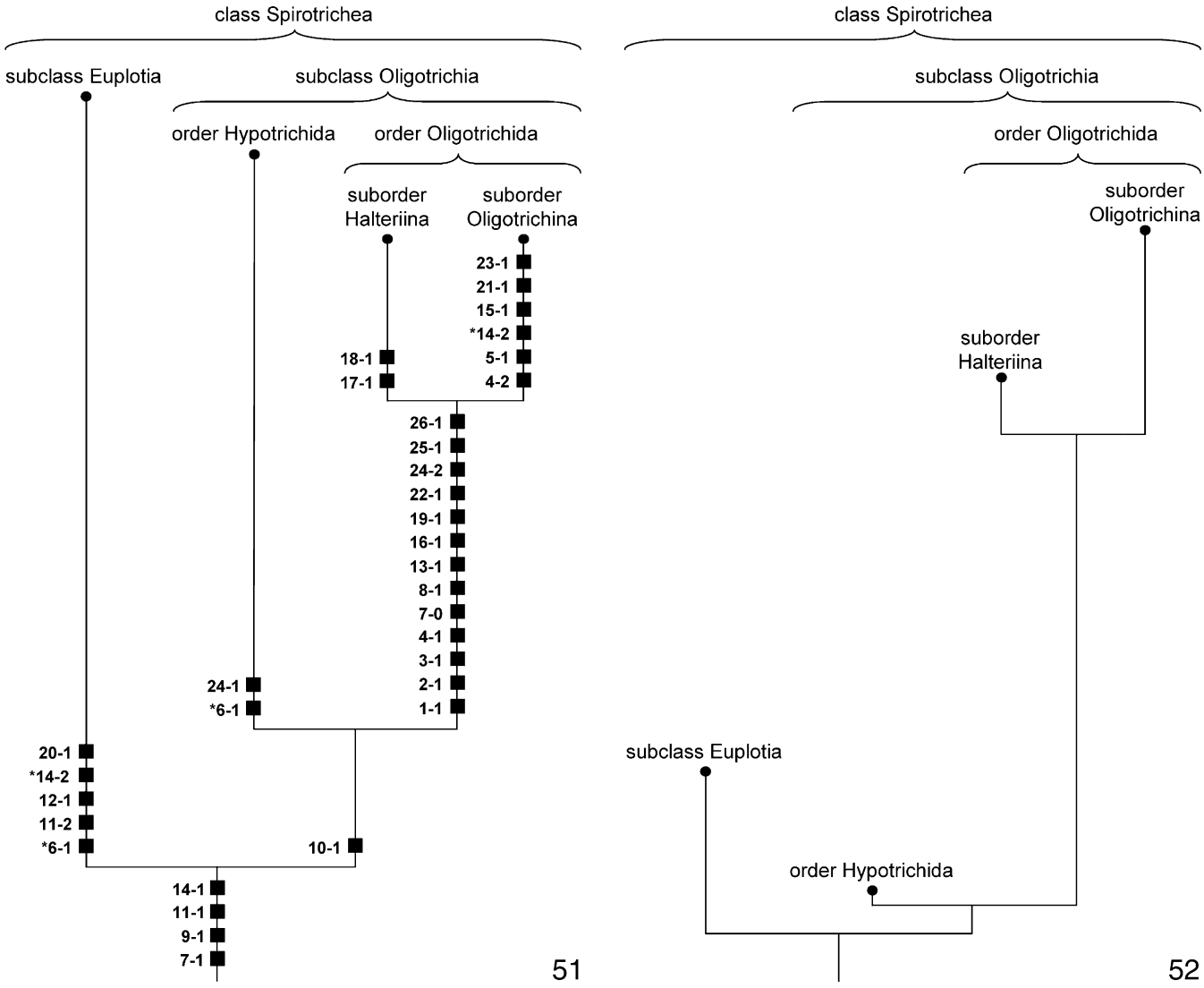
Character 23 (resting cyst wall with inorganic layers): Inorganic layers, probably calcium carbonate, are a unique feature of the cyst wall in ciliates of the suborder Oligotrichina. Such layers were found in a marine *Strombidium* (Reid 1987), a freshwater *Pelagostrombidium* (Figs 44, 45), and in a brackish *Rimostrombidium* (Foissner et al., unpubl.). Thus, we consider the possession of inorganic layers in the cyst wall the apomorphic state.

Character 24 (fine structure of the lepidosomes): Lepidosomes occur in various groups of ciliates and probably evolved convergently several times (Foissner and Pichler 2006; Foissner et al. 2005). In the ciliates investigated, lepidosomes occur in the hypotrichs, halteriids, and oligotrichs. However, their fine structure is completely different. In the hypotrichs, where lepidosomes are very rare, they are composed of minute, interwoven tubules (Fig. 38), while the halteriid and oligotrichid lepidosomes consist of very thin sheets of organic material, providing the lepidosomes with a fibrous appearance (Foissner and Pichler 2006; Figs 34, 45). We assume that the fibrous type is plesiomorphic because it is more common and less organized than the tubular one.

Character 25 (curious structures): Characteristic "curious structures" are prominent in the cyst plasm of *Meseres* (Foissner 2005) and *Halteria* (Fig. 22), while absent in the many hypotrich cysts investigated so far. Thus, these structures, which very likely originate from autophagic vacuoles (Foissner and Pichler, unpubl.), are considered an apomorphy of the halteriids.

Character 26 (cyst wall precursors): The development and structure of the cyst wall precursors is entirely different in hypotrichs and halteriids; indeed, those of *Meseres* are unique throughout the phylum (Foissner and Pichler 2006). Thus, we consider this feature a "strong" apomorphy of the halteriids. Unfortunately, no data are available from the Oligotrichina.

Cladograms (Figs 51, 52): The cladograms are based on the findings of Agatha (2004), Agatha and Strüder-



Figs 51, 52. Phylogenetic relationships of the core Spirotrichea, using a manually constructed Hennigian argumentation scheme (**51**) and a computer-generated PAUP* cladogram (**52**). The Hypotrichida and Oligotrichida form a monophylum, based on a single but very "strong" synapomorphy (10–1), viz., the perilemma. See Tables 2 and 3 for characters and character states. Asterisks indicate "convergences".

Kypke (2007), Foissner (1996), Foissner and Pichler (2006), Foissner et al. (2004), Petz and Foissner (1992), and the present results. Both the classic Hennigian argumentation scheme (Fig. 51) and the computer-produced cladogram (Fig. 52) provide very similar results because we used mainly "strong" apomorphies for the taxa investigated.

The spirotrichs are bound together by the macronucleus reorganization band, the subepiplasmic microtubule basket, and the apokinetal stomatogenesis. These features are present also in the euplotids that gave rise to the Oligotrichia, the latter being bound together by a unique structure with 100% bootstrap support: the perilemma. Although the function of the perilemma is not known (Foissner and Pichler 2006), it seems to be an excellent phylogenetic marker, especially because it has been found now also in the halteriids (Foissner 2005; Figs 35, 36). The suborders Halteriina and Oligotrichina have 13 synapomorphies, showing their close relationship.

The relationships mentioned are evident not only from the manually constructed Hennigian argumentation scheme but also from the computed PAUP* algorithm. Of the 26 characters, 1 character is constant and 11 variable characters are parsimony-uninformative. The tree has a length of 28 steps, a consistency index of 96.4, a homoplasy index of 3.57, a retention index of 92.9, and a rescaled consistency index of 89.5. The sister group relationship of Halteriina and Oligotrichina with 13 informative characters has 100% bootstrap support.

Discussion

Cyst spines, thorns, and lepidosomes

Most ciliates have a smooth or slightly wrinkled resting cyst surface. When surface ornamentations are present, they often occur in the form of spines or spine-like processes looking highly similar in the light microscope (Figs 5–7, 16; for reviews, see Berger 1999, 2006; Foissner 1993; Foissner and Xu 2007; Reid and John 1983).

The electron microscopic investigations show at least three different, most likely analogous cyst surface ornamentations that we differentiate also terminologically (Table 4). Spines are generated by the ectocyst lamellae and are thus usually rather irregular and weak (Figs 7, 40). Thorns are generated by the mesocyst and are thus usually rather regular and rigid (Figs 6, 39). Lepidosomes are generated individually in the cytoplasm and transported to the pericyst via exocytosis and the forming cyst wall (Figs 5, 15–17, 27; Foissner et al. 2006). These ornamentations can be used to investigate some recent phylogenetic hypotheses, viz., the *Halteria* paradox and the split of the hypotrichs into a flexible and a rigid group (see last section).

The term ''lepidosomes'' is synonymous with the very general term ''scales'', and might thus appear superfluous. However, in ciliates scales have been described under a bewildering variety of names, and thus Foissner et al. (2005) suggested ''lepidosomes'' as a valuable ''collector term'' at the present state of knowledge. Scales are common also in autotrophic protists, where the extracellular layer formed is frequently called the ''periplast'' (Manton 1986).

Comparison of resting cyst structure in *Halteria* and *Meseres*

Halteria and *Meseres* are closely related morphologically (Petz and Foissner 1992) and genetically (Katz et al. 2005). This is sustained by their resting cysts, especially the occurrence and fine structure of the lepidosomes: they are made of fibre-like strands of strongly osmiophilic material whose composition, unfortunately, could not be determined by the cytochemical tests applied (Foissner et al. 2005). Further, the cysts share a great overall similarity, that is, the wall is composed of five layers with similar fine structure (cp. Fig. 4 with Fig. 1 in Foissner 2005).

However, there are also conspicuous differences: (i) the lepidosomes are spherical in *Meseres* (Foissner et al. 2005), while conical in *Halteria* (Figs 1, 4, 16, 27); (ii) the lepidosomes of *Meseres* are located in a slimy basal layer (Foissner 2005), while those of *Halteria* are attached to the ectocyst (Figs 4, 28, 29, 32); (iii) *Meseres* has a bright zone between mesocyst and endocyst (Foissner 2005), while both are close together in *Halteria* (Figs 4, 33); and (iv) *Halteria* lacks the chitin present in *Meseres*, a surprising result considering the close morphologic and genetic relationship (Katz et al. 2005; Petz and Foissner 1992). However, the same probably occurs in some oxytrichid hypotrichs (Rosati et al. 1984). Foissner (2005) and Foissner et al. (2005) could not localize the chitin layer in the cyst wall of *Meseres*. Now, it is tempting to speculate that it is contained in the bright zone between mesocyst and endocyst because this zone is lacking in *Halteria*, where chitin is absent.

Table 4. Origin and structure of cyst ornamentation in oligotrichid, hypotrichid and euplotid spirotrichs and some other ciliates. All data based on transmission electron microscopy

Species[a]	Ectocyst spines	Mesocyst thorns	Lepidosomes		Literature
			Shape	Structure	
Oligotrichs					
Halteria grandinella			Conical	Fibrous	This study
Meseres corlissi			Globular	Fibrous	Foissner (2005)
Pelagostrombidium fallax			Conical	Fibrous	This study
Strombidium crassulum[b]			Conical	Fibrous	Reid (1987)
Hypotrichs					
Laurentiella strenua		+	Lacking		This study
Laurentiella acuminata[c]		+	Lacking		Gutiérrez et al. (1983b, 1984)
Coniculostomum monilata		+	Lacking		Kamra & Sapra (1993)
Gastrostyla steinii		+	Lacking		Walker et al. (1980)
Oxytricha bifaria[d]	+		Lacking		Verni et al. (1984)
Pleurotricha sp.	+		Lacking		Matsusaka (1976)
Steinia sphagnicola	+		Lacking		This study
Oxytricha granulifera			Globular	Tubular	This study
Paraurostyla weissei	+		Lacking		Delgado et al. (1987)

Table 4. (*continued*)

Species[a]	Ectocyst spines	Mesocyst thorns	Lepidosomes		Literature
			Shape	Structure	
Euplotids					
Euplotes muscicola	+		Lacking		Bussers et al. (1986)
Other ciliates					
Colpoda inflata			Globular	Tubular	Foissner, unpubl.
Colpoda lucida			Globular	Tubular	Foissner, unpubl.
Protospathidium sp.			Pustulate	Fibrous	Foissner, unpubl.

[a]For authorship and dates of species, see literature cited as well as Berger (1999) and Foissner (1993).
[b]Very likely a *Rimostrombidium* mixed with a *Strombidium*, according to the figures.
[c]A junior synonym of *L. strenua*, according to Berger (1999).
[d]Very likely *Stylonychia pustulata*, according to Berger (1999).

Comparison of cyst structure in halteriid, strombidiid, and hypotrichid spirotrichs

The sparse data available on oligotrichine resting cysts suggest a widespread occurrence of spine-like wall ornamentations (Agatha et al. 2005; Müller 1996, 2002; Müller and Wünsch 1999; Reid 1987; Reid and John 1978). No data are available on the nature of the spines; obviously, these authors considered them as a differentiation of the cyst wall proper. However, our electron microscopic observations show that the spines of *P. fallax* are lepidosomes whose fine structure resembles that of the lepidosomes in *Meseres* and *Halteria* (Figs 25, 32, 42, 46). This is sustained by the location of the spines, viz., attached to the ectocyst by slimy or membranous material (Figs 42, 44), highly resembling the conditions in *Halteria* (Figs 27, 32). Further, our interpretation is sustained by the micrographs of Reid (1987) which show the spines above the ectocyst in a marine *Strombidium*. Thus, we conclude that structurally similar lepidosomes occur in *Halteria*, *Meseres*, *Strombidium*, and *Pelagostrombidium*.

In contrast, the fine structure of the cyst wall is markedly different in halteriid and strombidiid oligotrichs, except for the ectocyst which is a strongly osmiophilic, thin, bipartite layer in *Meseres* (Foissner 2005), *Halteria* (Fig. 28), and *Pelagostrombidium* (Figs 49, 50). Probably, the main part of the strombidiid cyst wall is composed of alternating layers of organic and inorganic materials (Reid 1987; Figs 44, 46), while several distinct organic layers can be distinguished in halteriid and hypotrichid spirotrichs, viz., the mesocyst, endocyst and metacyst (Foissner 2005; Gutiérrez et al. 1983b; Figs 33, 39, 40). A further unique feature of the strombidiid resting cyst is a membrane-like structure covering the lepidosomes (Müller 2002; Reid 1987; Figs 43, 47). All data on strombidiid cysts are incomplete due to fixation problems. Thus, a more detailed comparison is impossible. However, there is no doubt about the occurrence of fibrous lepidosomes and a bipartite ectocyst in both halteriids and strombidiids.

Halteria has a perilemma

The perilemma is a tripartite, membrane-like structure covering body and cilia of the core spirotrichs (Fauré-Fremiet and Ganier 1970; Laval-Peuto 1975; Modeo et al. 2003; Wirnsberger-Aescht et al. 1989), except for its reported lack in *Halteria* (Bardele 1981; Grain 1972), a close relative to *M. corlissi* (Katz et al. 2005), in which the perilemma often forms two or more layers (Foissner 2005; Foissner and Pichler 2006).

Our investigations show that a perilemma is present also in *Halteria* (Figs 35, 36), although it is difficult to preserve, often leaving only small remnants. Very likely, the perilemma escaped former investigators because they used conventional fixatives. Accordingly, the perilemma is indeed the main autapomorphy of oligotrich and hypotrich spirotrichs.

Phylogenetic implications

The monophylum Oligotrichia. The phylogenetic analyses show that the Hypotrichida and Oligotrichida form a monophylum, viz., the subclass Oligotrichia, based on a single but very strong synapomorphy, i.e., the perilemma, a unique structure not found in any other ciliate group (Figs 51, 52). The Oligotrichia are sister to the Euplotia, and these two form the core of the class Spirotrichea. Previously, the Halteriina were the great exception, seemingly lacking a perilemma (Grain 1972; Petz and Foissner 1992). Our investigations show that a perilemma is present in two main species of the halteriids, i.e., in *H. grandinella* (Figs 35, 36) and *M. corlissi* (Foissner 2005). Thus, the perilemma becomes a "very strong" synapomorphy present only in the Hypotrichida and Oligotrichida.

The monophylum Oligotrichia is recognizable also in several recent molecular trees (Agatha and Strüder-Kypke 2007; Foissner et al. 2004; Lynn 2003; Modeo et al. 2003), but for unknown reasons escaped definite recognition, and remained undiscussed.

We name the monophylum "Oligotrichia", although the hypotrichs were created earlier (Berger 2006). However, the hypotrichs are burdened with nomenclatural problems (see Terminology section and Berger 2006). Based on Foissner et al. (2004), Berger (2006) recognized that hypotrichs and oligotrichs form a monophylum and suggested a new higher category. However, he did not name it because "higher taxa do not exist in nature". Actually, a new category is not necessary, but only some change in ranking (Figs 51, 52).

The Halteria paradox. Our phylogenetic analyses (Figs 51, 52) basically match the molecular data, except for the Halteriina which gene trees invariably classify within the core hypotrichs (Agatha and Strüder-Kypke 2007; Foissner et al. 2004; Lynn 2003; Modeo et al. 2003), while the present and former data (Foissner et al. 2004; Petz and Foissner 1992) suggest a classification within the Oligotrichida. This relationship is evident not only from the manual Hennigian argumentation scheme but also from the computed PAUP* algorithm which suggests that the Halteriina and Oligotrichina are sister groups with 100% bootstrap support and sharing 13 informative characters. Thus, the molecular classification should be reconsidered.

Classification of hypotrichid spirotrichs. For a long time, the classification of hypotrichs, such as *Oxytricha*, *Stylonychia* and *Urostyla* have tantalized classical and molecular taxonomists (Berger 1999, 2006; Foissner et al. 2004; Schmidt et al. 2007). Recently, even the best apomorphy, viz., cortex structure (flexible or rigid) has been broken: *Rigidothrix goiseri*, a conspicuous hypotrich from the Niger floodplain, is rigid although it has urostylid midventral rows and an 18SrDNA sequence very similar to that of *O. granulifera* (Foissner and Stoeck 2006). Thus, we hoped that the different ways cyst ornamentations are formed could shed light on, at least, the relationships of genera within the large family Oxytrichidae (for a review, see Berger 1999). Unfortunately, this is hardly the case: ectocyst spines occur in both flexible (*Paraurostyla weissei*) and rigid (e.g. *S. sphagnicola*) oxytrichids (Table 4), and both spines and thorns occur in the rigid oxytrichids *Laurentiella acuminata* (Fig. 39) and *S. sphagnicola* (Fig. 40). However, mesocyst thorns as yet have been described only in rigid oxytrichids, and lepidosomes as yet have been found only in a flexible species, i.e., *O. granulifera* (Fig. 37 and Table 4).

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