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Resting Cysts in the Ciliate Class Polyhymenophorea: Phylogenetic Implications[1]

P. C. REID and A. W. G. JOHN

Natural Environment Research Council, Institute for Marine Environmental Research, Prospect Place, The Hoe, Plymouth PL1 3DH, England

ABSTRACT. Distinctive organic-walled resting cysts of at least three different types with a highly conservative morphology appear to characterize specific orders or groups of genera within the Class Polyhymenophorea (Protozoa, Ciliophora), contrasting markedly with the great diversity of form seen in trophic stages. Polyhymenophorean ciliates have been considered in the past to form a cohesive class within the Phylum Ciliophora and, possibly, to represent the pinnacle of ciliate evolution. Evidence from cysts challenges the cohesive nature of the class, suggesting that the hypotrichs should be subdivided and that they have a different phylogenetic origin from the heterotrichs, tintinnids, and oligotrichs.

A summary of information on the little known cysts of the Polyhymenophorean suborder Tintinnina (26) confirmed that the formation of resting cysts by tintinnids may be more common than previously believed. The distinctive flask-shaped resting cysts of this suborder vary little in morphology between different genera in contrast to the loricae of the trophic stages which have greatly differing shapes. This striking conservativeness in the morphology of cysts from different genera suggests that it may be a primitive evolutionary feature and may have importance in determining phylogenetic relationships between different groups of ciliates. To assess this possibility, a brief survey of cyst forms in the Polyhymenophorea is presented. A need for such comparative studies of cyst formation and its relation to the evolution of the species involved was highlighted by Corliss & Esser (3) in 1974.

MATERIALS AND METHODS

This work is a product of a literature survey of encystment in all free-living Protozoa. Within the Polyhymenophorea, cysts have been described from many geographical locations in habitats ranging from hypersaline lakes, marine plankton, tidal pools, salt marshes, freshwater lakes and ponds, mosses, soils, and as endo-ectocommensals on invertebrate and vertebrate hosts. Only a few of these reports include details of the ultrastructure of cysts (11, 12, 33, 34, 35). To this end, comments in the text must be qualified; apparent homologies based on the light microscope need to be confirmed using the electron microscope. Included in this study is reference to our own observations of cysts in samples taken by the Continuous Plankton Recorder. This machine, which is towed at 10 m depth by merchant ships, has been used in a monthly synoptic survey of the plankton of the northeast Atlantic and the North Sea since 1948 (5).

RESULTS

The Polyhymenophorea (2) has one subclass, the Spirotrichia, which is divided into four orders, the Heterotrichida, Odontostomatida, Oligotrichida, and Hypotrichida in the classification of the Protozoa by Levine et al. (20). The Tintinnina is one of two suborders within the Oligotrichida, the other being the Oligotrichina. Representative cysts are drawn in Fig. 1 for each of the orders except for the odontostomatids, cysts of which are presently unknown.

Immediately apparent is the strong similarity between the cysts of heterotrichs (Figs. 1–4), tintinnids (Figs. 6, 7), an oligotrich (Fig. 8) and the possibly related cysts in the *incertae sedis* group, papulifères (22, 26, 27) (Figs. 9, 10). All, with the exception of the supposed cyst of *Phacodinium* (25) (Fig. 5), are characterized by distinctive oval to spherical resting cysts with a protrusion at one end marking the site of the excystment aperture, which is closed by a hyaline plug (24, 26). In the papulifères (Figs. 9, 10) this opening may at times be covered by a reticulated 'bud-like' extension, which may be equivalent to the 'frothy plug' of *Strombidium* (6) (Fig. 8).

Flask-shaped cysts are now known for at least eight genera of tintinnids: *Acanthostomella* (24), *Eutintinnus* (Heinbokel, pers. comm.), *Favella* (26) (Fig. 6), *Helicostomella* (26) (Fig. 7), *Leprotintinnus* (26), *Metacylis* (Reid & John, unpub.), *Parundella* (26), and *Tintinnopsis* (26); one genus of oligotrich: *Strombidium* (6) (Fig. 8), and five genera of heterotrichs: *Blepharisma* (10, 28) (Fig. 4), *Fabrea* (1, 19) (Fig. 2), *Stentor* (30) (Fig. 1), *Nyctotherus* (21) (Fig. 3), and *Paraclevelandia* (18) but are not known in other classes of the Ciliophora. The cysts range in size from approximately 30 to 140 μm and have smooth walls that appear to be made up of two main layers and a third fragile, at times membranous, outer layer. The majority of forms are resistant to immersion in strong acids and alkalis (1, 24, 26, 27).

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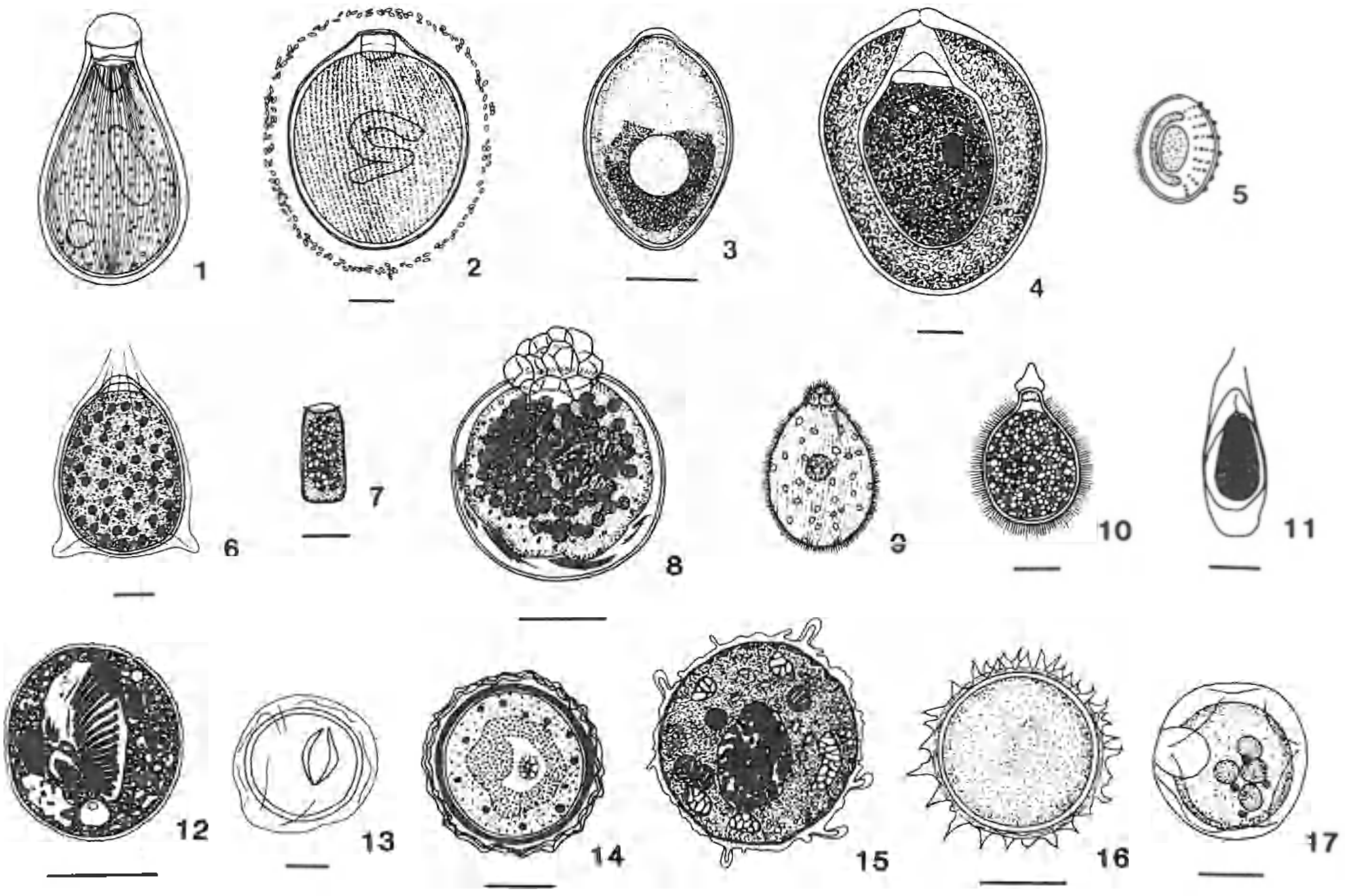

Figs. **1–17**. Resting cysts of the Polyhymenophorea s.l. redrawn from the listed authorities or from original material. Scale bar = 20 μm. **1.** *Stentor polymorphus* (Ehrenberg): elongate macronucleus and spherical contractile vacuole in a granular cytoplasm with alternate green and colorless longitudinal stripes as in the trophic stage (after Stein, 1867). **2.** *Fabrea salina* Henneguy: rod-like bacteria adhering to outer gelatinous layer (absent in older cysts). S-shaped macronucleus. cytoplasm with alternate light and dark bands (after Kirby, 1934). **3.** *Nyctotherus ovalis* Leidy: spherical nucleus in spindle-shaped 'reserve food bodies,' surface striae on cytoplasm (after Lucas, 1928). **4.** *Blepharisma stoltei* Isquith: section of thick-walled cyst, inner wall enclosing protoplasm and plug (after Repak, 1968). **5**. *Phacodinium muscorum* Prowazek: granular surface ornamentation on one pole, crescent-shaped nucleus enclosing a yellow mass of reserve products (after Penard, 1922). **6.** *Favella serrata* (Möbius): unstained specimen, granular cytoplasm, cyst surrounded by fine membrane (as seen within lorica, not shown) (after Reid & John, 1978). **7.** *Helicostomella subulata* (Ehrenberg): cylindrical cyst as seen within lorica (not shown), Continuous Plankton Recorder sample 32RV4. **8.** *Strombidium oculatum* Gruber: 'frothy plug' covering a circular pylome, cilia still visible at margin of cytoplasm (after Fauré-Fremiet, 1948). **9.** Papulifère indéterminé: ornamented by spines, capped by a frothy plug or papula, spherical nucleus (after Meunier, 1910). **10.** Cyst type R: granular cytoplasm, membranous papula covering plug, Continuous Plankton Recorder sample 242G17 (after Reid & John, 1981). **11.** *Chaetospira mulleri* Lachmann: cyst stained in Heidenhain's iron hematoxylin within a lorica (after Froud, 1949). **12.** *Diophrys scutum* Dujardin: TEM section showing intact adoral zone of membranelles (after Walker & Maugel, 1980). **13.** *Euplotes taylori* Garnjobst: empty cyst showing slit through which organism escaped. three wall layers present, outer layer wrinkled (after Garnjobst, 1937). **14.** *Gastrostyla steinii* Engelmann: section, outer wall folded (after Weyer, 1930). **15.** *Oxytricha fallax* Stein: TEM section showing folded outer wall, central nucleus, and outer ring of mitochondrial bands (after Grimes, 1973b). **16.** *Pleurotricha lanceolata* (Ehrenberg): outer cyst wall folded into spine-like projections (after Ilowaisky, 1926). **17.** *Stylonethes sterkii* Garnjobst: wrinkled outer wall, wide space between wall and protoplasm (after Garnjobst, 1937).

Some cysts attributable to the papulifères may be ornamented by solid spines or membranous folds. Cytological changes during encystment have only been studied for a small number of species; dedifferentiation of ciliary and oral structures appears to take place (1, 28). To our knowledge, cysts have not so far been recognized in the heterotrich suborders: Armorphorina, Plagiotomina, and Licnophorina. Possible resting cysts in the suborder Coliphorina (elongate with a membranous outer wall) have only been recorded once (13); the type of aperture and method of excystment is not known.

In marked contrast to the cysts of the previous groups, the Hypotrichida have simple spherical cysts, without any projection or pylome, and dehisce via a split in the cyst wall (Figs. 12–17). An exception is the pear-shaped cyst of the loricate genus *Chaetospira* (8) (Fig. 11), which has an unknown method of excystment. Cyst formation is common in the hypotrichs and is known for at least fifteen genera in eight of the eleven families recognized in the classification of Levine et al. (20): *Aspidisca* (Curds, pers. comm.), *Chaetospira* (8) (Fig. 11), *Diophrys* (33) (Fig. 12), *Euplotes* (7, 9) (Fig. 13), *Gastrostyla* (35, 36) (Fig. 14), *Histriculus* (4), *Keronopsis* (25), *Onychodromus* (16), *Oxytricha* (11, 12) (Fig. 15), *Paraholosticha* (32), *Pleurotricha* (15, 17) (Fig.

16), *Strongylidium* (23), *Stylonychia* (15, 34), *Stylonethes* (9) (Fig. 17), *Urostyla* (31). Cysts of these genera are from 15 to 70 μm in diameter. Ultrastructural studies have shown that the cyst wall may be composed of up to four layers (35). The outer layer is smooth or may be folded to form ridges or 'spine-like' projections which are merely extensions of the outer wall and not rigid structures. Little is known regarding the chemical characteristics of the cyst wall: cysts of *Euplotes* are resistant to acids and alkalis (7).

Hypotrichs have in the past been considered to represent the peak of ciliate development or morphological differentiation and to comprise a closely knit assemblage of species (2). This traditional view has recently been placed in doubt (29), with the suggestion that *Euplotes* and related genera should be distinguished from the hypotrichs *sensu stricto*. Evidence from cytological and fine structural studies of cysts supports this division; striking contrasts are evident between cysts of the euplotid ciliates *Diophrys* and *Euplotes* and other hypotrichs such as *Gastrostyla* and *Stylonethes* (7, 9, 33, 35). The cysts of *Diophrys* and *Gastrostyla* are distinguishable on a number of criteria, the most characteristic of which is whether the kinetosomes are resorbed or maintained within the resting cysts; in hypotrichs *sensu stricto* mature cysts are completely dedifferentiated and contain no kinetosomes or cilia (33).

DISCUSSION

Major differences in shape, excystment aperture, wall structure and cytology are evident between the cysts of both the heterotrichs and oligotrichs compared to the hypotrichs, and in two separate groups within the hypotrichs. These sharp contrasts, in what appear to be highly conservative morphological traits, imply a different phylogenetic origin for each of these cyst groups and suggest that the Polyhymenophorea as construed by Levine et al. (20) is polyphyletic.

In the classification of Small & Lynn (29) the Polyhymenophoria has been reduced to the level of a subclass and 'tentatively' attributed, together with the Hypostomia, to a new class the Nassophorea. Only the heterotrichs and odontostomes are retained within the Spirotrichea, now raised to the level of a class. The hypotrichs and oligotrichs are included together in the Polyhymenophoria, but it was pointed out that they may well be divided in the future and in part be allocated to the same subphylum as the Spirotrichea. This survey of cyst forms has served to confirm and strengthen most of the observations of Small & Lynn. We propose that, within their classification, the Order Oligotrichida should be included in the Spirotrichea with the Heterotrichida, and that the Hypotrichida should be divided with the creation of a new taxonomic group (order or suborder) to include *Euplotes* and related genera.

Ciliate cysts have been largely ignored in the past and have only been described for a small number of species, despite the fact that their formation is a common feature of the phylum (2, 3). Encystment appears to be a primitive feature of the class Polyhymenophorea and possibly also of the class Vestibulifera. Hashimoto (14) has demonstrated, for instance, that the kineties within the cysts of Colpodidae assume what he considers to be a 'primitive' pattern during reorganization on excystment. Maintenance of conservative morphological characteristics in cysts may be a consequence of a slowing down of selection pressures within cysts because of their reduced metabolism and protection from the variable environmental stresses to which the trophic stage is subject. This work has served to emphasize the possible importance of cysts as a new tool for determining phylogenetic relationships and demonstrates the need for a greater emphasis to be placed on the study of encystment in the biology of ciliates.

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