

# THE CYST OF *UROSTYLA GRANDIS* (HYPOTRICHIDA UROSTYLIDAE) : ULTRASTRUCTURE AND EVOLUTIONARY IMPLICATIONS

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## ABSTRACT

Ultrastructural study of *Urostyla grandis* cysts shows that the cyst wall is constituted of three layers : a fibrous ectocyst, a structurally homogeneous endocyst and a granular layer that exhibits a dispersed granular or fibrillar structure. The maintenance of only a few kinetosomes and scattered bundles of subpellicular microtubules, and other particularities distinguish the *Urostyla grandis* cyst, both KR and NKR cysts. It is proposed to amplify the WALKER and MAUGEL classification by an additional group. Based on comparative features, some of the phylogenetic relations are discussed.

Key words : cyst, ultrastructure, hypotrich, *Urostyla*. classification.

## RÉSUMÉ

L'examen, en microscopie électronique, du kyste d'*Urostyla grandis* a révélé que sa paroi se compose de trois couches : un exokyste fibrillaire, un endokyste à structure homogène et une couche granulaire avec une structure granulaire ou fibrillaire. Le maintien de quelques cinétozomes, microtubules subpelliculaires et d'autres particularités permettent de distinguer ces kystes des kystes KR et NKR. On propose d'ajouter un nouveau groupe à la classification de WALKER et MAUGEL. On commente quelques relations phylogéniques reposant sur des caractéristiques comparatives.

Mots-clés : kyste, ultrastructure, hypotriche, *Urostyla*. classification.

## INTRODUCTION

According to the ultrastructural characteristics displayed by the cysts of hypotrich ciliates, WALKER and MAUGEL in 1980, proposed a general classification for these of two groups :

KR cysts (kinetosome-resorbing cysts) : *Oxytricha fallax* (GRJMES, 1973), *Gastrostyla steinii* (WALKER *et al.*, 1980) and *Stylonichia mytilus* (WALKER *et al.*, 1975).

NKR cysts (no kinetosome-resorbing cysts) : *Dyophris scutum* (WALKER and MAUGEL, 1980).

Only a few species of the Oxytrichidae have been examined since 1980, (CALVO *et al.*, 1983; GUTJERREZ *et al.*, 1983; MATSUSAKA and HONGO, 1984; VERNI *et al.*, 1984), but all of them exhibit KR cysts; so apparently, KR cysts may be a feature of the Oxytrichidae. In the same way, WALKER and MAUGEL (1980) suggest that the NKR cysts seem to be a characteristic of the Euplotidae.

In this paper, we describe the ultrastructural topography and fine structure of the cyst of the hypotrich ciliate *Urostyla grandis*. Special emphasis has been placed on the fact that the *Urostyla grandis* cyst displays characteristics between KR and NKR cysts and we suggest the

possibility of the establishment of an additional group, including species showing similar resting cysts. These ultrastructural variations may be a new criterion offered to taxonomists to resolve both taxonomic and phylogenetic problems in the Order Hypotrichida.

## MATERIALS AND METHODS

*Urostyla grandis*. a hypotrich ciliate, was cultivated at  $20 \pm 1$  °C in mineral water and fed *Ch/orogonium* sp.

In cultures of *Urostyla grandis*. encystment occurs when food becomes a limiting factor. In order to induce this process, to obtain resting cysts, vegetative cells were transferred from the maintenance culture to mineral water without adding *C/orogonium* sp.

### Staining for light microscopy

The nuclear apparatus was stained by using the Feulgen procedure (TORRES, 1977).

### Electron microscopy

For scanning electron microscopy (SEM), the cysts were fixed for 30 minutes at 4 °C in cacodylate buffer O.OS M pH 7.2, containing a 1 :2 mixture of 1.S o/o glutaraldehyde and 2 o/o osmium tetroxide. The fixed material was dehydrated by increasing concentrations of acetone, was air-dried, then gold-coated and finally examined in a Jeol scanning electron microscope.

For transmission electron microscopy (TEM), the cysts were fixed for 40 minutes at 4 °C in a mixture 1 :2 of 1.S o/o glutaraldehyde and 2% osmium tetroxide in a O.OS M cacodylate buffer pH = 7.2. The fixed material was embedded in 2 o/o agar blocks and dehydrated by increasing concentrations of acetone and embedded in Spurr resin (SPURR, 1968). Ultrathin sections were cut with a Reichert-J ung ultramicrotome, double stained with uranyl-acetate and lead citrate and then examined with a Siemens Elmiscop 102 electron microscope.

Alternatively, the cysts were stained with ruthenium red (LUT, 1971) and then processed like those mentioned above.

## RESULTS

### Light microscopy

The *Urostyla grandis* cysts are spherical and average 22-24  $\mu$ m in diameter. The volume of the cyst is smaller than that of the vegetative cell. The cytoplasm exhibits an homogeneous aspect and in older cysts it is possible to observe a dark granule, in the center, that stands out clearly from the rest of the cytoplasm (Fig. 1). Cysts possess the normal vegetative nuclear complement consisting of a lot of macronuclei and a few micronuclei (Fig. 2).

### Electron microscopy

In electron micrographs obtained by SEM, the cyst surface appears irregular with wrinkles that give a rough

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FIG. 1. — Photomicrograph of a resting cyst of *Urostyla grandis* "in vivo". e, cytoplasm; g, central granule; w, cyst wall. (1400 x).

FIG. 2. — Resting cyst of *Urostyla grandis* showing a lot of macronuclear fragments. Stained by Feulgen. (1400 x).

FIG. 3. — Scanning electron micrograph of a resting cyst of *Urostyla grandis*. (1400 x).

FIG. 4. — Transmission electron micrograph of a cyst showing cyst wall, remaining subpellicular microtubules and mucocysts. ec, ectocyst; en, endocyst; gl, granular layer; mt, subpellicular microtubules; mu, mucocyst. (27 000 x).

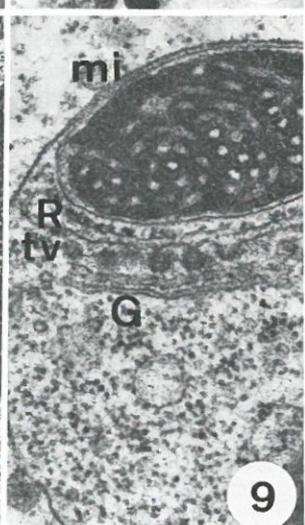
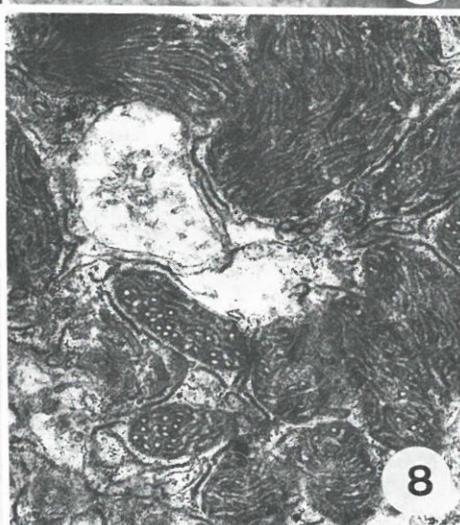
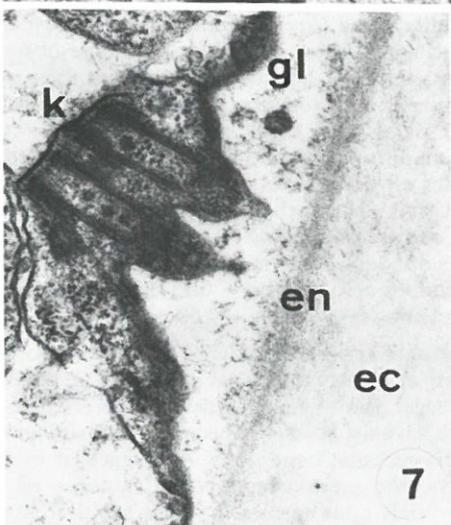
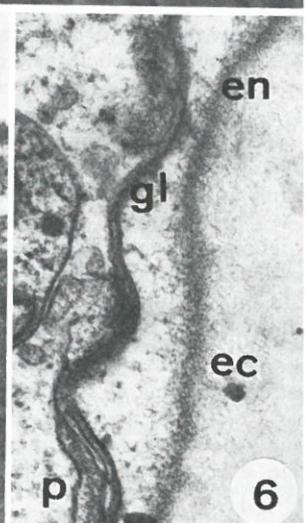
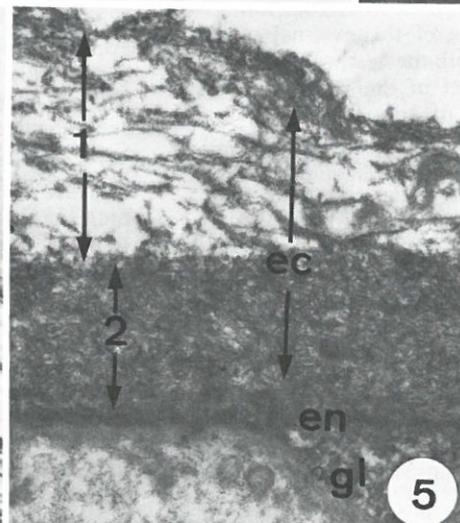
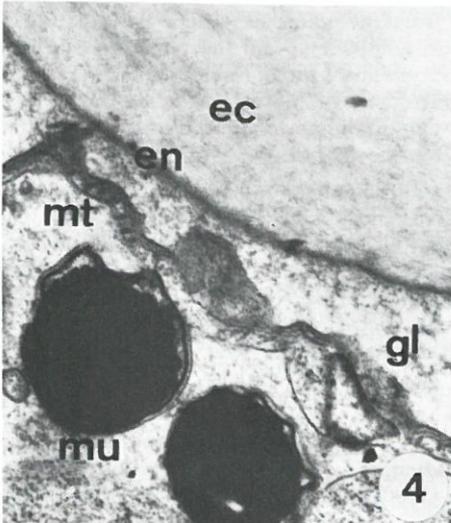
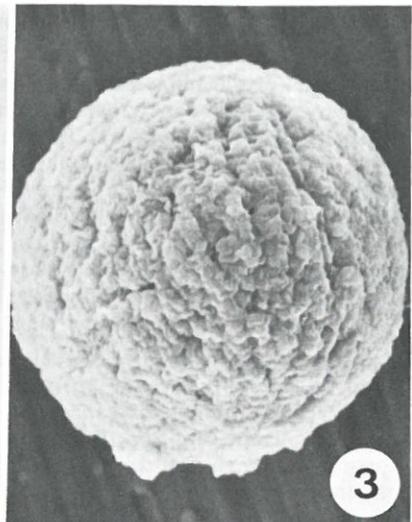
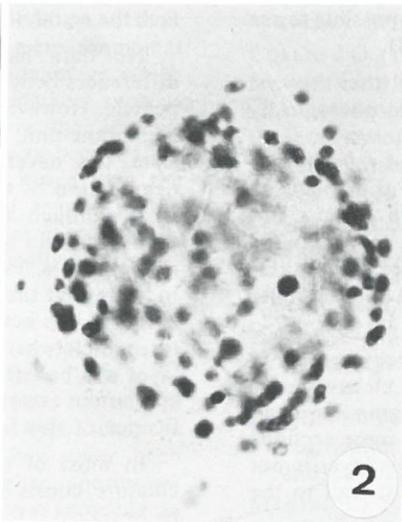
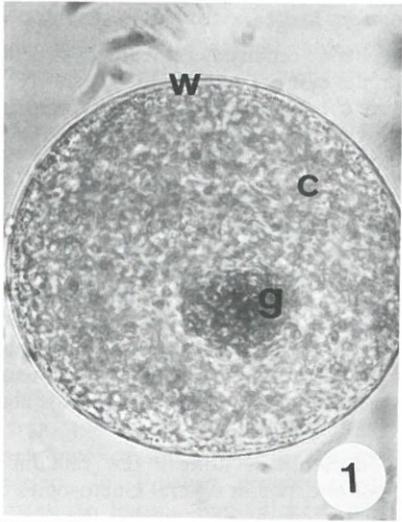
FIG. 5. — Transmission electron micrograph of the cyst wall stained with ruthenium red. ec, ectocyst; en, endocyst; gl, granular layer: (1. - outer ectocyst — 2. - inner ectocyst). (33 000 x).

FIG. 6. — Transmission electron micrograph. Cyst wall and cyst pellicle. ec, ectocyst; en, endocyst; gl, granular layer; p, pellicle. (45 000 x).

FIG. 7. — Transmission electron micrograph showing a pair of kinetosomes beneath the cyst wall. k, kinetosomes. (30 000 x).

FIG. 8. — Mitochondrial cluster. (16 000 x).

FIG. 9. — Cyst cytoplasm showing single mitochondria closely associated to RER and Golgi apparatus. Note transition vesicles. m, mitochondria; G, Golgi apparatus; tv, transition vesicles; R, rough endoplasmic reticulum. (50 000 x).



aspee!. Ostioles or pores have never been observed on the wrinkled mature cyst surface; although, it is possible to see some bacteria adhering to the folds (Fig. 3).

Observations of ultrathin sections reveal that the cyst wall of *Urostyla grandis* consists of three morphologically distinct layers (Figs. 4, 6 and 7). The outer most layer (ectocyst), the thickest one, is composed of fibrillar material which has a low electron density. For this reason, the structure of this layer is very difficult to observe, but in cysts stained with ruthenium red, two zones can be clearly recognized: the outer zone formed by fibrous material randomly oriented and less packed and the inner zone, also fibrillar and more packed (Fig. 5).

The endocyst is very homogeneous and regular both in shape and thickness. It is made up of an electro-dense, highly compact, amorphous substance. The granular layer, the most internal one, appears fibrillar in some sections and granular in others. The thickness of this layer is not uniform since it is uniting the spherical cyst wall to the grooved cell surface.

As a consequence of the loss of the external membrane-unit, the cyst pellicle, beneath the cyst wall, presents one membrane-unit less than that of the vegetative cell (Fig. 6). Likewise, the network of subpellicular microtubules, although visible in young cysts, disappears almost completely. In the mature cysts, it is possible to observe some remains at only a few points underneath the pellicle (Fig. 4). The function of these remainders of subpellicular microtubules in the cyst is unknown, but we think they might ensure the maintenance of the shape during the encystment. The only ciliature we could observe consists of a few isolated pairs of kinetosomes (Fig. 7).

Mitochondria can be seen to be formed in tight clusters or scattered throughout the cytoplasm (Fig. 8). When they appear isolated a closely association with rough endoplasmic reticulum and Golgi apparatus has been noted and, in every case, the transition vesicles are clearly observed (Fig. 9).

Both the vegetative cell and resting cyst present numerous mucocysts, but in the vegetative cell those organelles exhibit several different aspects, apparently representing different stages of development, while in the resting cyst all of them show the same appearance. They appear as highly osmiophilic-granules surrounded by a double membrane (Fig. 4). Unlike the vegetative cell, no fusion of mucocysts with the pellicle was noted in the resting cyst.

## DISCUSSION

Our observations obtained by TEM of *Urostyla grandis* cyst show that the wall consists of three layers, like the *Dyophris scutum* cyst (WALKER and MAUGEL, 1980). On the other hand, the morphology of these layers is basically similar to that of the three inner layers of Oxytrichid cysts (mesocyst, endocyst and granular layer). According to this

result, we could say that the *Urostyla grandis* cyst seems to lack the ectocyst present in all Oxytrichid cysts described.

No data have been reported indicating structural differences between the cyst pellicle and the vegetative cell pellicle. However, in *Urostyla grandis* cyst, the outer most membrane-unit, which always appears in the vegetative state, has never been observed. It may be due to the degradation of this structure during the encystment process; although another possibility also exists. This membrane-unit can remain intact but is masked by the endocyst. Studies in progress, in our laboratory, seem to indicate that the second option is the most probable one and that it is between this membrane-unit and the rest of the pellicle where the material that will form the granular layer will be left. This layer shows a variety of appearances, but, in every case, the cytoplasm matches it. The significance of this fact is as yet unknown.

In most of the ultrathin sections a few remains of ciliature, consisting of one, two or several kinetosomes can be observed. This characteristic distinguishes the *Urostyla grandis* cyst from the KR cyst, where the ciliature disappears almost completely, and the NKR cyst, in which the ciliature stays almost intact (including AZM). Another singular characteristic of these cysts is the fact that the subpellicular microtubules are reduced to several scattered bundles beneath the pellicle.

Since the numerous macronuclear fragments observed in the vegetative cell can be found in the cyst, it is possible to conclude that macronuclear fusion does not take place during the encystment process. No appreciable changes are noticeable in the macronuclear chromatin arrangement; although, occasionally, a small increase of the condensation degree could be seen.

In agreement with the classification proposed by WALKER and MAUGEL (1980) (KR and NKR cysts), all the species belonging to the Oxytrichidae family, whose cysts have been studied, exhibit KR cysts. In addition, WALKER and MAUGEL (1980) suggest that the NKR cysts may be a feature of the Euplotidae. However, this classification loses its validity in the *Urostyla grandis* cysts because they show mixed characteristics of both types. Some observations of other species of Urostylids reveal that their cysts exhibit the same characteristics described in this paper (unpublished data). For these reasons, we think it is necessary to amplify the classification with a third group, which includes all the species whose cysts: (1) exhibit a three-layered wall, (2) resorb all cilia but maintain a few kinetosomes and microtubules, (3) display mitochondrial clusters, (4) do not fuse macronuclei, (5) rate cyst volume/vegetative cell volume = 0.25-0.40 and (6) rate cyst wall thickness/cyst radius =  $0.08 \pm 0.02$ .

The encystment can be considered as a process of cell differentiation to defend itself against possible environmental challenges. Two general types of hypotrichs cysts (KR and NKR) exist as an adjustment for survival in different environmental conditions. As suggested by different authors, the main adaptative advantages of the formation of NKR cysts seem to be their rapid ability of encystment and their lower energy required for this

process, as a consequence of the conservation of all their cortical structures. With regard to the KR cysts, their higher resistance to extreme values of the environmental parameters (due to thicker wall) is an advantage which withstands the loss of valuable cortical markers such as cilia and kinetosomes. In respect to this, the *Urostyla grandis* cyst appears to be situated half-way between the two, since its resistance properties are lower than the KR cysts, but the ciliary structures that remain in the resting form (kinetosomes and subpellicular microtubules) may be useful indicators of morphogenetically determinative positions for the encystment process.

Up to the present time, the formation of the cyst wall in a few ciliates has been assumed to be the result of mucocysts discharge (CHEISSEN and MOSEVICH, 1962; HOLT and CHAPMAN, 1971; MeARDLE *et al.*, 1980; REPAK and PFISTER, 1967), at least partially. But, in hypotrichs, the appearance of mucocysts in the cyst has never been reported. In *Urostyla grandis* (and other species of Urostylidae family, unpublished data) it is possible to observe a lot of these organelles, both in the vegetative cell and the resting form, although their functional role in the encystment process still remains obscure. At this time, in our laboratory, we are studying the structure and function of mucocysts in encysting *Urostyla grandis*.

If we assume that the cyst described in this report is the general type of the Urostylidae cysts, in our opinion, sufficient data exist to establish a correlation between the morphogenetic division patterns and the cyst types. So, we suppose that the four-layered cyst present in the Oxytrichidae which exhibits a type of morphogenetic pattern characterizing evolved forms of hypotrichs is derived from the three-layered cyst present in the Urostylids whose morphogenetic pattern characterizes the more primitive hypotrichs. Therefore, the knowledge of the ultrastructure of the cysts support the idea of a lineal phylogeny of Stichotrichina with Sporadotrichina, as proposed by several authors (CORLISS, 1979; MARTIN *et al.*, 1983). However, we also think that the available data on the Euplotidae cysts are not sufficient to draw conclusions about their uncertain origin nor to establish phylogenetic relations although intuitive reasons exist to consider this family as divergent evolutionary line inside the Order Hypotrichida.

## REFERENCES

CALVO P., TORRES A., NAVAS P. and PEREZ-SILVA J. (1983). — Complex carbohydrates in the cyst wall of *Histiculus similis*. *J. Gen. Microbiol.*, 129, 829-832.  
CHEISSEN E.M. and MOSEVICH T.V. (1962). - An electron

microscope study of *Colpidium colpoda* (Ciliata, Holotricha). *Archiv. Protistenk.*, 106, 181-200.  
CORLISS J.O. (1979). — *The Ciliated Protozoa: Characterization, Classification and Guide to the Literature*, 2nd ed. Pergamon Press, London.  
GRIMES G.W. (1973). — Differentiation during encystment and excystment in *Oxytricha fallax*. *J. Protozool.*, 20, 92-104.  
GUTIERREZ J.C., TORRES A. and PEREZ-SILVA J. (1983). - Fine structure of the cyst wall of *Laurentia acuminata* (Hypotrichida : Oxytrichidae). *Trans. Amer. Microsc. Soc.*, 102, 55-59.  
HOLT P.A. and CHAPMAN G.B. (1971). — The fine structure of the cyst wall of the ciliated protozoan *Didinium nasutum*. *J. Protozool.*, 18, 604-614.  
LUF T.J. (1971). — Ruthenium red and violet. I. Chemistry, purification, methods of use for electron microscopy and mechanism of action. *Anal. Rec.*, 171, 347-368.  
MARTIN J., FEDRIANI C. and PEREZ-SILVA J. (1983). — Morphogenetic pattern in *Laurentia acuminata* (Ciliophora, Hypotrichida) : Its Significance for the Comprehension of Ontogeny and Phylogeny of Hypotrichous Ciliates. *J. Protozool.*, 30, 519-529.  
MATSUSAKA T. and HONGO F. (1984). - Cytochemical and electrophoretic studies on the cyst wall of a ciliate, *Histiculus muscorum* KAHL. *J. Protozool.*, 31, 471-475.  
MeARDLE E.W., BERGQUIST B.L. and EHRET Ch.F. (1980). — Structural changes in *Tetrahymena rostrata* during induced encystment. *J. Protozool.*, 27, 388-397.  
REPAK A.J. and PFISTER R.M. (1967). — Electron microscopical observations on the extracellular structures of the resting cyst of *Blepharisma stoltei*. *Trans. Amer. Microsc. Soc.*, 86, 417-421.  
SPURR A.R. (1968). — A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.*, 26, 31-43.  
TORRES A. (1977). — *El ciclo celular y procesos cariológicos relacionados con la regeneración en Laurentia acuminata*. Tesis Doctoral. Universidad de Sevilla.  
YERNI F., ROSATI G. and RICCI N. (1984). — The cyst of *Oxytricha bifaria* (Ciliata, Hypotrichida). 11. The Ultrastructure. *Protistologica*, 20, 87-95.  
WALKER G.K., MAUGEL T.K. and GOODE D. (1975). — Some ultrastructural observations on encystment in *Stylonichia mytilus* (Ciliophora, Hypotrichida). *Trans. Amer. Microsc. Soc.*, 94, 147-154.  
WALKER G.K., MAUGEL T.K. and GOODE D. (1980). — Encystment and excystment in hypotrich ciliates. I. *Gastrostyla steinii*. *Protistologica*, 16, 511-524.  
WALKER G.K. and MAUGEL T.K. (1980). — Encystment and excystment in hypotrich ciliates. 11. *Dyophris scutum* and remarks on comparative features. *Protistologica*, 16, 525-531.