Encystment and excystment in ciliated protists: multidimensional approach

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In unfavourable conditions, ciliated protists lower or shut down their metabolic activities and form resting cysts to survive. According to the present knowledge, most studies are based on one or two-dimensional approaches to understand the morphological, physiological and biochemical changes during encystment and excystment. However, in the last few years, research has been initiated to reveal proteins involved in encystment. This review provides an insight into the major findings related to the formation of resting cysts in ciliates using multidimensional approach and various limitations in the field.

Keywords: Ciliated protists, encystment, excystment, proteins.

Theories and schools, like microbes and globules, devour each other and by their struggle ensure the continuing of life. – Marcel Proust

LIVING systems adopt different survival strategies depending upon the variations in the habitat which they inhabit[1]. Protists are the earliest eukaryotes that appeared 2 billion years ago. A large number of variations occurred within the group to survive in different environmental conditions resulting in rich diversity of protists[2]. Some protists maintain a constant phenotype under similar environments, while others change their phenotype in obligatory stages of life as an adaptive strategy in response to alterations in the environment[2,3].

In most protists, the adaptive strategy against major stresses such as starvation and dehydration usually includes lowering the metabolic rate or completely shutting down all metabolic activities. This state is defined as cryptobiosis by Keilin[4]. Lowering of the metabolic rate is usually found in non-differentiating microbes and includes the production of degrading enzymes. There is an immediate emergence of vegetative cells in case of non-differentiating microbes upon the return of normal conditions. Shutting down the whole metabolic system in some protists requires a highly gene-regulated system[1,3].

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A resting cyst is the cryptobiotic form found in protists to survive in stress conditions[5]. Formation of a resting cyst during unfavourable condition is called ‘encystment’ and the emergence of the vegetative cell out of the cryptobiotic state is known as ‘excystment’. This constitutes a facultative encystment and excystment cycle (E–E cycle) which is genetically coded[6].

Among protists, E–E cycle is commonly found in free-living ciliates that are prone to unfavourable conditions such as starvation, high population density and salinity variation[6–8]. Ciliated protists are unicellular eukaryotes widely found in almost all habitats[9,10]. Presence of nuclear dualism, short generation time, species-specific ciliature and specialized oral apparatus to grasp food are some of the unique characteristics which distinguish ciliates from other protists[11,12]. Ciliates are heterotrophs; many of them feed on bacteria, diatoms and algae[13]. Furthermore, ciliates are found at various trophic levels in food webs, which make them important for the proper functioning of an ecosystem[6].

Resting cysts in ciliates have been understudied; less than 40 ciliate species have been studied with respect to their cyst morphology and physiology[14,15]. In parasitic ciliates, encystment is an obligatory phase of their life cycle, whereas in some species encystment occurs for the purpose of reproduction[3]. Most studies available delve upon the ultrastructure of the resting cysts in different species[2,16]. However, to understand the importance of the formation of resting cysts in ciliates, studies have been conducted in the last few years to explore the cellular process at the genetic and proteomic levels during E–E cycle[17,18].

This review highlights the morphological, physiological and molecular level changes (multidimensional approach) that usually occur during the process of E–E cycle to form a resting cyst in different species belonging to phylum Ciliophora, especially those belonging to the order Hypotrichida.

Material and methods

The research papers included in the present review have been downloaded from Google Scholar and PubMed. Keywords to determine relevant studies for the selected topic are as follows: encystment and hypotrichs, excystment and hypotrichs, morphological changes in excystment and hypotrichs, morphological changes in encystment and hypotrichs, genes involved during encystment in hypotrichs, genes involved during excystment in hypotrichs. Selected studies included morphological and physiological changes during encystment and excystment and genetic changes in the encystment and excystment process in members of the order Hypotrichia. Species with different cell size and habitat have been included.

Results

A total of 64 studies downloaded from Google Scholar and PubMed have been included in the present review; three studies have been taken from PubMed and the remaining from Google Scholar. Relevant chapters in books and journals, abstracts of 14 studies and 46 full papers were included in the study. Data of 38 ciliate species (mostly from Order Hypotrichia), proteomics of two ciliates species, *Euplotes encysticus* and *Pseudourostyla cristata* published from 2014 to 2018 have been included.

Discussion

Ecological conditions responsible for encystment and excystment (Figure 1)

Encystment is triggered mostly by a stimulus such as changes in environmental conditions, starvation, an excess/depletion of food, certain metabolites or crowding. The absence/depletion of nutrients[1] or water[6] in the medium causes starvation; a universal exogenous inducer[1]. In terrestrial ciliate species such as *Parentocirrus hortualis* (1997) desiccation is also an inducer of cyst formation[19]. In *Stylonychia pustulata*, depletion of oxygen has been reported as a cause of encystment[13]. Changes in pH, salinity and temperature are the most common environmental parameters that cause encystment[20–22]. Excretory substances of ciliates in the case of crowding of ciliates in a particular place causes encystment in some cells in a culture, while others remain in a vegetative state[23]. Some ciliates secrete specific metabolites that are harmful to other ciliates and act as inducers of encystment. Whenever the conditions become favourable, excystment begins[22]. Many factors cause encystment and excystment; however, the differential response of cells of the same species under similar conditions needs to be resolved in future studies.

Physiological changes during encystment and excystment

Resting cysts of hypotrichs are divided into three types– kinetosome resorbing (KR) in oxytrichids, non-kinetosomes resorbing (NKR) in euplotids[24] and partial kinetosomes resorbing cysts in urostylids[25]. KR cysts resorb all kinetosomes, cilia and microtubules[26–28]. In NKR cysts, cilia, microtubules and kinetosomes remain intact. In some *Urostylids*, there is partial resorption of the cilia and microtubules; these can be considered as ‘transition ciliates’ between sub orders Stichotrichina and Sporadotrichina. It has been found in *Urostyla* (renamed *Pseudourostyla*) sps.[29]. Several physiological and biochemical changes such as reduction of cell volume and condensation of chromatin in macronucleus occur during the process of encystation and excystation[12]. Most oxytrichids show similar salient features during encystment and excystment. During the initial stages of encystment, cell movement slows down and cytoplasm shrinks, thus reducing overall cell volume[19,20]. Dehydration of cytoplasm reduces fluidity and volume of cell compartments, therefore, causing crowding of organelles and macromolecules[6,30]. Reduction of cell volume is followed by the resorption of the ciliature[28]. In KR cysts, resorption of ciliature starts with the disassembly of undulating membranes, followed by the shortening of ciliary shafts, separation of associated microtubules and retraction of remaining ciliature in the cytoplasm[26]. Autophagocytosis in the cytoplasm is responsible for the resorption of ciliary shafts, kinetosomes, and cellular organelles. Autophagic vacuoles provide macromolecules and energy required for cellular differentiation and sustenance of resting cysts[6,12,27,31]. In the later stages of resting cyst formation, the cyst starts taking the shape of a sphere (young cyst) and macronuclear nodules begin to fuse together forming a round mass generally located at the centre[18]. In some species, macronuclear nodules do not fuse, as in *Australocirrus* cf. *australis*[27]. Cell dehydration and microtubules are responsible for the fusion of macronuclear nodules[6]. In partial and non-kinetosomes resorbing cysts, some ciliature is present on the outer surface of a mature cyst[3,5,28,].

Excystment in most oxytrichids begins either by rupturing the cyst wall[32] or by emerging from a pore with a removable plug[6]. In the case of emerging from a pore, ciliates actively rotate within the cyst till the plug disappears and then the vegetative cell escapes through the

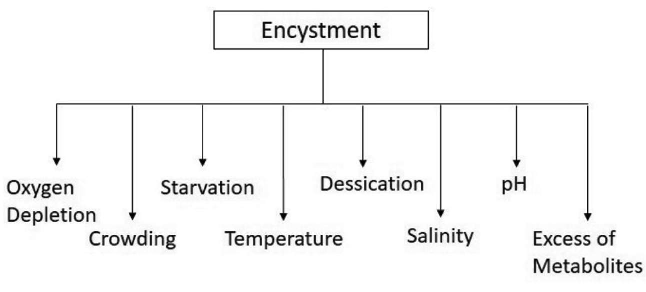

Figure 1. Environmental factors responsible for encystment in ciliates.

pore[33]. *Parentocirrus hortualis* and *Rigidohymena quadrinucleata* escape by breaking the cyst wall with the help of an excystation vacuole. Contractile vacuoles in these species stop pulsating and become excystation vacuoles during the excystment phase[16,19]. In rare cases, an excystation vacuole breaks only a thin membrane; the rest of the cyst wall is ruptured by the excysting cell. The structure and composition of the thin membrane enveloping the emerging cell during excystment needs to be explored[16]. In some species such as *Oxytricha fallax*, the excysting cell has fully differentiated ciliature before emerging out of the resting cyst, whereas some species such as *Parentocirrus hortualis* and C*oniculostomum monilata*, require some regeneration rounds before coming into a normal vegetative state[20,32]. All the cellular organelles resorbed during encystment form again before and/or after excystment. Certain abiotic factors affect excystment although there are few studies supporting this. Temperature affects the time period of excystment and increase in pH and bacteria retard the rate of excystment. In low concentrations of plant extracts, dilute solutions of certain plant acid mixtures neutralized with KOH and a number of organic substances such as bacto-yeast extract and bactopeptone, are also effective in inducing excystment[34].

Ultrastructure and biochemical characterization of mature cyst and cyst wall (Table 1)

The presence of partially permeable cyst wall layers is an important characteristic of resting cysts in ciliates. It protects the cell and helps cysts to adhere to the surface strongly. Many authors studied cyst wall structures of different species using scanning and transmission electron microscopy. Cyst walls are made up of three layers as found in *Urostyla grandis*[35] and *Gonostomum* sp.[36] or of four layers[37–39] as found in *Laurentiella acuminate*[40], *Stylonychia mytilus*[41], *Pleurotricha* sp.[42], *Oxytricha bifaria*[43], *Onychodromus acuminatus*[44], *Australocirrus* cf. *australis*[27] and *Rigidohymena quadrinucleata*[16]. The outermost layer is the ectocyst having finger-like protrusions externally and well-organized sub-spherical part internally. The second layer is a mesocyst which is fibrous and consists of concentric layers; it can sometimes be divided into two zones as in *Laurentiella acuminate*[40]. The third layer is the endocyst with regular shape and thickness, composed of some electron-lucent highly compact amorphous substance. The fourth layer is a granular layer which is the innermost and discontinuous layer that attaches cyst wall to the cell surface[41,45]. Certain non-hypotrichous ciliate species also have one or two layers in the cyst wall. Cysts in euplotids present an amorphic state[15]. KR cysts have four layered cyst walls and NKR cysts and *Urostyla*-type cysts have three layered cyst walls[29]. The cyst wall is mostly composed of polysaccharides and proteins in most hypotrichs[29,46]. The ectocyst and the endocyst consist of neutral and acidic mucopolysaccharides and proteins, whereas mesocyst and the granular layer are comprised of acidic mucous substances and neutral polysaccharides respectively[29].

Hypotrich cysts are generally simple and spherical, with rigid or spiny outer coats[2]. Cyst surface ornamentations are species-specific and can be categorized as spines (generated by the outer ectocyst lamellae) found in *R. quadrinucleata*[16], thorns (generated by the mesocyst) found in *Laurentiella strenua* and lepidosomes (produced in the cytoplasm content) found in *O. granulifera*[15]. Colour of the cyst also varies from colourless to yellow and red. This helps in identification of species-specific resting cysts[16].

Foissner *et al.*[47] compared resting cysts of hypotrichids and oligotrichids and concluded that although there is vast diversity of resting cysts due to morphological and ontogenic differences, it is difficult to separate ciliate species phylogenetically on the basis of resting cysts[15]. It has been stated that resting cysts play an important role in dispersal of ciliate species[47].

Encystment–excystment related proteins (Table 2)

Based on the presence of favourable and unfavourable environmental stimulus, special structural differences between resting cysts and vegetative cells show that there is a specific genetic program responsible for thesechanges[1,29]. The presence of transcripts in the resting cyst in *Sterkiella histriomuscorum* among ciliates and other protists indicate that mRNA storage is maintained in a dormant stage. Ciliate encystment and excystment are RNA and protein-dependent processes[1,48]. Forty two proteins were isolated from the *Euplotes encysticus* resting cyst wall. These proteins function in cell wall anchoring, cytoskeleton formation, energy metabolism, transport and catabolism, signal transduction, membrane-associated transportation, processing, sorting and degradation[18]. The cyst wall is mainly composed of carbohydrates and proteins as found in earlier studies using biochemical approach. Proteomic studies confirm that the cyst wall consists mainly of tubulin and actin proteins[5,18]. Most intracellular proteins degrade during encystment. This was confirmed by the identification of ubiquitin, ubiquitin carboxyl-terminal hydrolase family proteins and cullin family proteins in resting cyst. These proteins of the ubiquitin-proteasome pathway are involved in selective protein degradation[49,50]. This proteolytic activity might also be responsible for degradation of cyst wall during excystment. Non-proteolytic activities of ubiquitin systems help in other functions of resting cysts during encystment and excystment such as membrane transport, intracellular signal transduction[51,52]. Chen *et al.*[53] reported upregulation of 12 proteins and down regulation

Table 1. Characteristics of resting cyst of hypotrichous ciliates

Taxon	Family	Cyst type	Number of cyst wall layers	Cyst wall ornamentation
Euplotes muscicola[55]	Euplotidae	NKR	2 (EC, EN)	Crests
Urostyla grandis[25]	Urostylidae	PKR	3 (EC, MC, EN)	Smooth
Oxytricha granulifera[9]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Smooth with granules
Oxytricha fallax[26]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Irregularly serrated surface
Oxytricha bifaria[41]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Spines
Laurentiella strenua[26]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Thorns
Laurentiella acuminata[56,57]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Thorns
Gastrostyla steinii[24]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Thorns
Kahliella simplex[58]	Kahliellidae	KR	4 (EC, MC, EN, MT)	Cyst wall smooth
Australocirrus cf. *australis*[27]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Slightly wrinkled
Paraparentocirrus sibillinensis[59]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Reticulate with ridges
Rigidohymena quadrinucleata[16]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Spines
Parentocirrus hortualis[19]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Reticulate with ridges
Pleurotricha sp.[42]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Spines
Paraurostyla weissei[29]		KR	4 (EC, MC, EN, MT)	Spines
Coniculostomum monilata[32]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Hexagonal pattern
Steinia sphagnicola[15]	Oxytrichidae	KR	4 (EC, MC, EN, MT)	Spines

Table 2. Functions of different proteins isolated from resting cysts of hypotrichous ciliates[12,17]

Proteins	Functions
KR-multi-domain protein[17]	Oxidation–reduction reactions
Type II cytoskeletal 1[17] Keratin[17]	Mechanical properties of cyst wall and be involved in the stability of the cyst wall
Nop 16 domain-containing protein[17]	Cell-cycle progression and stress signalling, ribosome subunit biosynthesis
Protein arginine *n*-methyltransferase[17]	Post-translational modification in the regulation of protein signalling.
Epsilon-trimethyl lysine hydroxylase[17]	Energy metabolism
Calpain-like protein[17]	Cytoskeleton protein reorganization
Methylmalonyl-coenzyme A mutase[12]	Breakdown of the amino acids, cholesterol, and odd-chain fatty acids
ADP ribosylation factor[12]	Membrane trafficking and actin cytoskeleton remodelling
rab12[12]	Autophagy regulator
MAPK-related kinase[12]	Stress-signalling pathway

of 7 proteins during the process of encystment in *Euplotes encysticus*. These proteins play different roles in the process of encystment, thus pointing towards an association of morphological changes and molecular signals during the formation of the resting cyst[53]. Keratin, Nop 16 domain-containing protein, cytoskeletal 1, protein arginine *n*-methyltransferase, epsilon-trimethyl lysine hydroxylase and calpain-like proteins are six resting cyst-specific proteins in *Euplotes encysticus*[17]. Fibrillarin-like rRNA methylase, methylmalonyl-coenzyme A mutase, ADP ribosylation factor, rab12, MAPK-related kinase and KR-multi-domain proteins have been identified as specific proteins in resting cysts of *Pseudourostyla cristata*. These proteins are responsible for RNA methylation, protein degradation, dynamic actin remodelling, autophagy regulation and oxidation–reduction reactions during encystment[12]. This suggests that the peculiar proteins and differential proteins might play important roles in the transformation of the vegetative cells into resting cysts[18]. There is only one study that has shown an association of DNA methylation with encystment. Ma-DNA GCGC and CCGG sequences demethylated in resting cyst DNA, whereas these methylated in vegetative cell DNA. Inhibition of cysteine proteases in *Sterkiella histriomuscorum* inhibits encystation and is specifically required for excystation. *Cathepsin B* gene, a *cathepsin* L-like gene, and a *calpain*-like gene are involved in degradation of the cyst wall[54]. Some of the proteins are co-expressed in both vegetative and resting cysts[12,17].

Conclusion

Encystment and excystment play an important role in the survival of ciliated protist in varied ecological conditions. In the present review, ecological conditions responsible for encystment and excystment, ultrastructure of a resting cyst and the process of formation of a resting cyst with respect to physiological changes have been listed. Importantly, recent studies to understand the genomics and proteomics of encystation and excystation have shown the association of morphological changes with genetic changes. In the last few years, although work at an omic level has increased, studies to determine cellular mechanisms in the E–E cycle of ciliates are limited. Additionally, a number of genomic and protein databases are required to identify a large number of proteins that remain unidentified in the listed studies. This will help in understanding the intrinsic signalling pathways responsible for encystment and excystment. Combining morphological, physiological, biochemical and proteomic data (multidimensional approach), one can understand the whole mechanism of the formation of resting cyst from the vegetative cell.

1. Gutierrez, J. C., Callejas, S., Borniquel, S., Bénitez, L. and Martín-González, A., Ciliate cryptobiosis: a microbial strategy against environmental starvation. *Int. Microbiol.*, 2001, **4**, 151–157.
2. Corliss, J. O. and Esser, S. C., Comments on the role of the cyst in the life cycle and survival of free-living protozoa. *Trans. Am. Microsc. Soc.*, 1974, **93**, 578–593.
3. Ricci, N., Verni, F. and Rosati, G., The cyst of *Oxytricha bifaria* (Ciliata: Hypotrichida). I. Morphology and significance. *Trans. Am. Microsc. Soc.*, 1985, **104**, 70–78.
4. Keilin, D., The Leeuwenhoek lecture: the problem of anabiosis or latent life. History and current concept. *Proc. R. Soc. Lond, B, Biol. Sci.*, 1959, **150**, 149–191.
5. Gutiérrez, J. C., Martín-González, A. and Matsusaka, T., Towards a generalised model of encystment (cryptobiosis) in ciliates: a review and a hypothesis. *BioSystems*, 1990, **24**, 17–24.
6. Verni, F. and Rosati, G., Resting cysts: a survival strategy in Protozoa Ciliophora. *Ital. J. Zool.*, 2011, **78**, 134–145.
7. Gu, F., Ji, L., Ni, B. and Li, G., Observations on cellular ultrastructure during encystment of *Oxytricha platystoma*. *Acta Zool. Sin.*, 1996, **43**, 227–231.
8. Li, Q., Fan, X., Ni, B. and Gu, F., Scanning electron microscopic observations on the differentiation of cortex and cilia during the encystment of *Allotricha curdsi* (Ciliophora: Hypotrichida). *Chinese J. Cell Biol.*, 2012, **34**, 290–294.
9. Berger, H., Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). *Monographiae Biol*., 1999, **78**(i–xii), 1–1080.
10. Berger, H., Monograph of the Urostyloidea (Ciliophora, Hypotricha). *Monographiae Biol*., 2006, **85**(i–xv), 1–1303.
11. Corliss, J. O., *The Ciliated Protozoa*, Pergamon Press, Oxford, 1979, 2nd edn.
12. Fukang, G., Bing, N., Zhenyun, Y. and Baojian, D., Ultrastructure of the vegetative cell and resting cyst in *Pseudourostyla cristata* (Ciliophora, Hypotrichida). *Chinese J. Zool.*, 2002, **48**, 251–257.
13. Finlay, B. J., Corliss, J. O., Esteban, G. and Fenchel, T., Biodiversity at the microbial level: the number of free-living ciliates in the biosphere. *Q. Rev. Biol.*, 1996, **71**, 221–237.
14. Foissner, W. and Pichler, M., The unusual, lepidosome coated resting cyst of *Meseres corlissi* (Ciliophora: Oligotrichea): genesis of four complex types of wall precursors and assemblage of the cyst wall. *Acta Protozool*., 2006, **45**, 339–366.
15. Foissner, W., Müller, H. and Agatha, S., A comparative fine structural and phylogenetic analysis of resting cysts in oligotrich and hypotrich Spirotrichea (Ciliophora). *Eur. J. Protistol*., 2007, **43**, 295–314.
16. Benčaťová, S. and Tirjaková, E., A study on resting cysts of an oxytrichid soil ciliate, *Rigidohymena quadrinucleata* (Dragesco and Njine, 1971) Berger, 2011 (Ciliophora, Hypotrichia), including notes on its encystation and excystation Process. *Acta Protozool.*, 2017, **56**, 77–91.
17. Chen, J., Gao, X., Wang, B., Chen, F., Wu, N. and Zhang, Y., Proteomic approach to reveal the proteins associated with encystment of the ciliate *Euplotes encysticus*. *PLOS ONE*, 2014, **9**, e97362; doi:10.1371/journal.pone.0097362.
18. Wang, B., Niu, T., Bhatti, M. Z., Chen, F., Wu, L. and Chen, J., Identification of cyst wall proteins of the hypotrich ciliate *Euplotes encysticus* using a proteomics approach. *J. Microbiol.*, 2017, **55**, 545–553; doi:10.1007/s12275-017-6422-7.
19. Benčaťová, S., Tirjaková, E. and Vďačný, P., Resting cysts of *Parentocirrus hortualis* Voß, 1997 (Ciliophora, Hypotrichia), with preliminary notes on encystation and various types of excystation. *Eur. J. Protistol.*, 2016, **53**, 45–60.
20. Dallai, R., Miceli, C. and Luporini, P., *Euplotesrariseta* Curds *et al.* (Ciliophora Hypotrichida) from the Somalian coast: description and preliminary observations on cyst induction and ultrastructure. *Monit. Zool. Ital. (NS)*, 1987, **XXII**, 263–280.
21. Weisse, T., *Meserescorlissi*: rare oligotrich ciliate adapted to warm water and temporary habitats. *Aquat. Microb. Ecol.*, 2004, **37**, 75–83.
22. Weisse, T., Freshwater ciliates as ecophysiological model organism – lesson from *Daphnia*, major achievements and future perspectives. *Arch. Hydrobiol.*, 2006, **167**, 371–402.
23. Ekelund, F., Frederiksen, H. B. and Rønn, R., Population dynamics of active and total ciliate populations in arable soil amended with wheat. *Appl. Environ. Microbiol.*, 2002, **68**, 1096–1101.
24. Walker, G. K. and Maugel, T. K., Encystment and excystment in hypotrich ciliates 11. *Diophrys scutum* and remarks on comparative features. *Protistologica*, 1980, **16**, 525–531.
25. Zhao, L., Li, Y. S., Li, J. G. and Gu, F. K., Some ultrastructural observations of the vegetative, resting and excysting ciliate, *Urostyla grandis* (Urostylidae, Hypotrichida). *Biol. Res.*, 2009, **42**, 396–401.
26. Grimes, G. W. and Hammersmith, R. L., Analysis of the effects of encystment and excystment on incomplete doublets of *Oxytricha fallax*. *Development*, 1980, **59**, 19–26.
27. Li, Q., Sun, Q., Fan, X., Wu, N., Ni, B. and Gu, F., The differentiation of cellular structure during encystment in the soil hypotrichous ciliate *Australocirrus* cf. *australis* (Protista, Ciliophora). *Animal Cells Syst.*, 2017, **21**, 45–52; http://dx.doi.org/10.1080/19768354.2016.1262896).
28. Yun, M., Li, Q., Zhang, X., Sheng, C. and Gu, F., Ultrastructural observations on the resting cyst of the soil ciliate, *Territricha stramenticola* and the freshwater ciliate, *Urostyla grandis*. *Afr. J. Microbiol. Res.*, 2013, **7**, 853–859.
29. Delgado, P., Calvo, P. and Torres, A., Encystment in the hypotrichous ciliate *Paraurostyla weissei:* ultrastructure and cytochemistry. *J. Protozool.*, 1987, **34**, 104–110.
30. Gutiérrez, J. C., Martín-González, A. and Callejas, S., Nuclear changes, macronuclear chromatin reorganization and DNA modifications during ciliate encystment. *Eur. J. Protistol*., 1998, **34**, 97–103.
31. Gu, F. K. and Ni, B., An ultrastructural study on resting cyst of *Euplotes encysticus*. *Acta Biol. Exer. Sin.*, 1995, **28**, 163–171.
32. Kamra, K. and Sapra, G. R., Formation of a transient corticotype during excystment in *Coniculostomum monilata* (oxytrichidae, hypotrichida) and its modification by reorganizations. *Eur. J. Protistol.*, 1991, **27**, 331–342; doi:10.1016/S0932-4739(11)80249-X.
33. Montagnes, D. J. S., Lowe, C. D., Poulton, A. and Jonsson, P., Redescription of *Strombidium oculatum* Gruber 1884 (Ciliophora, Oligotrichia). *J. Eukaryot. Microbiol.*, 2002, **49**, 329–337.
34. Jeffries, W. B., Studies on excystment in the hypotrichous ciliate *Pleurotricha lanceolata*. *J. Protozool.*, 1956, **3**, 136–144.
35. Rios, R. M., Torres, A., Calvo, P. and Fedriani, C., The cyst of *Urostylagrandis* (Hypotrichida: Urostylidae): ultrastructure and evolutionary implications. *Protistologica*, 1985, **21**, 481–485.
36. Walker, G. K. and Hoffman, J. T., An ultrastructural examination of cyst structure in the hypotrich ciliate *Gonostomum* species. *Cytobios*, 1985, **44**, 153–161.

37. Calvo, P., Torres, A. and Perez-Silva, J., Ultrastructural and cytochemical study of the encystment in the hypotricous ciliate *Histriculus similis*. *Arch. Protistenkunde*, 1986, **132**, 201–211.
38. Gutierrez, J. C., Diaz, S., Ortega, R. and Martín-González, A., Ciliate resting cysts walls: a comparative review. *Recent Res. Devel. Microbiol.*, 2003, **7**, 361–379.
39. Rosati, G., Verni, F. and Ricci, N., The cyst of *Oxytricha bifaria* (Ciliata, Hypotrichida) IV. Cytochemical investigation. *Protistologica*, 1984, **20**, 197–204.
40. Gutierrez, J. C., Torres, A. and Perez-Silva, J., Fine structure of the cyst wall of *Laurentiella acuminata* (Hypotrichida: Oxytrichidae). *Trans. Am. Microsc. Soc.*, 1983, **102**, 55–59.
41. Walker, G. K., Maugel, T. K. and Goode, D., Some ultrastructural observations on encystment in *Stylonychia mytilus* (Ciliophora, Hypotrichida). *Trans. Am. Microsc. Soc.*, 1975, **94**, 147–154.
42. Matsusaka, T., An ultrastructural study of the encystment in the hypotrichous ciliate *Pleurotricha* sp. *Kumamoto J. Sci. Biol.*, 1976, **13**, 13–26.
43. Verni, F., Rosati, G. and Ricci, N., The cyst of *Oxytricha bifaria* (Ciliata, Hypotrichida) 11. The ultrastructure. *Protistologica*, 1984, **20**, 87–95.
44. Jarefio, M. A., Etude ultrastructurale de I'enkystement et du dtkystement chez *Onychodromus acuminatus* (Ciliata, Hypotrichida). *Protistologica*, 1985, **21**, 313–321.
45. Palacios, G., Martín-González, A. and Gutiérrez, J. C., Macronuclear DNA demethylation is involved in the encystment process of the ciliate *Colpodainflata*. *Cell Biol. Int.*, 1994, **18**, 223–228.
46. Rosati, G., Verni, F. and Ricci, N., The cyst of *Oxytricha bifaria* (Ciliata Hypotrichida). III Cytochemical investigation. *Protistologica*, 1984, **20**, 197–204.
47. Foissner, W., Agatha, S. and Berger, H., Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha Region and the Namib Desert *Denisia*, 2002, **5**, 1–1459.
48. Matsusaka, T. and Hongo, F., Cytochemical and electrophoretic studies on the cyst wall of a ciliate, *Histriculus muscorum* Kahl. *J. Protozool.*, 1984, **31**, 471–475.
49. Barin Tourancheau, A., Morin, L., Yang, T. and Perasso, R., Messenger RNA in dormant cells of *Sterkiella histriomuscorum* (Oxytrichidae): identification of putative regulatory gene transcripts. *Protist*, 1999, **150**, 137–147.
50. Chen, J. W., Wang, B. Z., Gao, X. X. and Zhang, Y. Y., Crosstalk between autophagy and proteasome systems. *Prog. Biochem. Biophys.,* 2013, **40**, 199–208.
51. Jung, T., Catalgol, B. and Grune, T., The proteasomal system. *Mol. Aspects Med.*, 2009, **30**, 191–296.
52. Mukhopadhyay, D. and Riezman, H., Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science*, 2007, **315**, 201–205.
53. Chen, J. W., Zheng, L. N., Wang, B. Z. and Xiao-Tao, L. I., The nonproteolytic functions mediated by ubiquitylation. *Prog. Biochem. Biophys.*, 2012, **39**, 613–621.
54. Villalobo, E., Moch, C., Fryd-Versavel, G., Fleury-Aubusson, A. and Morin, L., Cysteine proteases and cell differentiation: excystment of the ciliated protist *Sterkiella histriomuscorum*. *Euakryot. Cell*, 2003, **2**, 1234–1245; doi:10.1128/EC.2.6.1234–1245.2003.
55. Gutiérrez, J. C., Callejas, S., Borniquel, S. and Martín-González, A., DNA methylation in ciliates: implications in differentiation processes. *Int. Microbiol.*, 2000, **3**, 139–146.
56. Grimes, G. W., Differentiation during encystment and excystment in *Oxytricha fallax*. *J. Protozool.*, 1973, **20**, 92–104.
57. Gutierrez, J. C., Torres, A. and Perez-Silva, J., Excystment cortical morphogenesis and nuclear processes during encystment and excystment in *Laurentiella acuminate* (Hypotrichida, Oxytrichidae). *Acta Protozool.*, 1981, **20**, 145–152.
58. Foissner, I. and Foissner, W., The fine structure of the resting cysts of *Kahliella simplex* (Ciliata, Hypotrichida). *Zool. Anz.*, 1987, **218**, 65–74.
59. Kumar, S., Bharti, D., Marinsalti, S., Insom, E. and La Terza, A., Morphology, morphogenesis, and molecular phylogeny of *Paraparentocirrus sibillinensis*n. gen., n. sp., a 'Stylonychinae Oxytrichidae' (Ciliophora, Hypotrichida) without transverse cirri. *J. Eukaryot. Microbiol.*, 2014, **61**, 247–259.

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