

SOME SECRETS ABOUT THE METHODS USED INTO THE STUDY OF AQUATIC CILIATES

GINA-RALUCA KERKMANN*

Abstract: Ciliates are unicellular organisms, important indicators of the pollution degree of the aquatic ecosystems. At the beginning of protistological studies, the observations of ciliates were made *in vivo* for all the species. Despite the increasing scientific progress – especially during the first half of the twentieth century – the protistologists' desire to understand the cell infrastructure required the intervention of different types of reagents on various cell structures. However cell treatment protocol is laborious and sometimes it is extremely difficult to obtain results – even for experienced researchers. – for some special ciliate species like geleides.

Keywords: ciliates, cell infrastructure, cell treatment protocol.

Ciliates are unicellular organisms which register a high abundance in most living environments. They can be found into the aquatic sediments with particles of various size into coastal sands, wet soils, moss and lichens and transported long distances as inactive forms of life (cysts) by different animal and vegetal vectors. In the aquatic ecosystems they are free living ciliates (planktonic), which have an active displacement in the volume of water, as well as benthic ciliates; their life is highly dependent on aquatic sediments.

They are important indicators of the pollution degree of the aquatic ecosystems.

A brief examination of a water and sediment drop under the microscope introduces the observer into a special micro-universe dominated by different fragile forms, all of them having as common denominator the movements of ciliary rows with various degrees of evolution.

At the beginning of protistological studies, the observations of ciliates were made *in vivo* for all the species. After countless hours of study under the microscope, there were discovered new species. Moreover, based on accurate drawings, there were published very useful protistological books; some of them are still valid and they represent the first source used to establish the diagnosis of ciliates species [4].

Anyway, as information concerning the taxonomy of ciliates increased and there was investigated ciliate fauna of other ecosystems, several important issues aroused. The collection of benthic ciliate samples does not represent a problem because even the simple scraping of the sediment surface provides abundant evidence of ciliates; for planktonic forms it is necessary a planktonic net. The adherence of ciliates to the surface of sediments – thigmotaxis (wall hugging) [15] –

* Dr., Research Assistant, Ağrı İbrahim Çeçen University, Faculty of Arts and Sciences, Department of Biology, 04100-AGRI/TÜRKİYE (e-mail: ginaraluca@yahoo.com).

put the researcher in difficulty and made the observations more difficult. Thus it appeared the necessity of separating the ciliates from the related sediments in order to perform more accurate observations and to establish the diagnosis.

Uhlig [12] [13] [14] has devised a simple technique for extracting ciliates; the method consists of ciliates migration to the bottom sediment when ice is added on its surface. Ciliates are collected in Petri dishes. Afterwards, they are individually collected using micropipettes and analysed under the microscope. Although beneficial in terms of working time (only two hours effective extraction!), the method is aggressive.

Most of the ciliate species (geleides), as these fragile forms suffer cytolysis¹, the degeneration or dissolution of cell caused by the disruption of cell membrane [16].

Webb [10] is the discoverer of a simpler and not so aggressive extraction method such as Uhlig method. Essentially the mechanism of migration of ciliates from bottom sediments on slides under the surface of the sediment takes two days.

Based on the same characteristic of ciliates – thigmotaxis – Fauré – Fremiet (Faure-Fremiet, 1951) and Deroux (Deroux, 1978 in [1]) tried to collect aquatic ciliates from the origin ecosystem using slides fixed in a wooden frame. This method is non-aggressive for ciliates but it takes time. All presented methods are successfully used today by researchers depending on the intended purpose and the degree of fragility of the species [3].

Another problem that needs to be solved concerns the *decrease of the ciliate speed*. The researcher cannot establish the diagnosis of species as long as they are constantly moving. So, the Romanian protistologists started with shy tests – some drops of a smoker's saliva or quince seed mucilage – and then chose individual collecting of ciliates using micropipettes and their inclusion in various fixing solutions such as mercuric chloride, Bouin solution, Champy solution, etc. [1].

Manufacturing micropipettes represents itself a delicate operation, as from fine glass tubes, with the help of Bunsen burner, there result pipettes the diameter of which is adapted to the micrometre dimensions of ciliates species. Chatton used to say jokingly “Protistology is the art of pipette manufacturing” [1].

Despite the increasing scientific progress – especially during the first half of the twentieth century – the protistologists' desire to understand the cell infrastructure required the intervention of different types of reagents on various cell structures.

Thus, there appeared new vital staining techniques (with blue cresil and neutral red to highlight mucocysts² [16] and gasterioles³) [1] [7] or postvital staining in solution of mercury bichloride (for emphasizing infraciliary network), methyl green (for macro and micronucleus) or nigrosine (for emphasizing infraciliary network and various details of the pellicle⁴ [18]) [1].

¹ *Cytolysis* – the degeneration or dissolution of cell caused by the disruption of cell membrane [16].

² *Mucocyst* – Small membrane-bounded vesicular organelle in pellicle of ciliate protozoans that will discharge a mucus-like secretion [17].

³ *Gasteriole* – food vacuoles; follow a special motion inside of cell while the food is digested inside their by digestive enzymes [1] [7].

⁴ *Pellicle* – Thin protective membrane in some protozoa [1] [18].

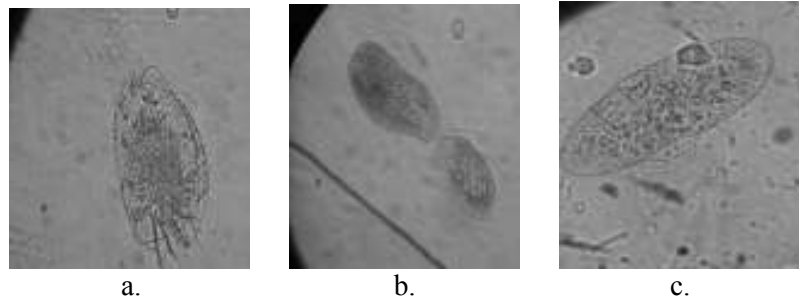


Fig. 1. Some living ciliates from Dobrogea (a. *Euplotes* sp., 97 μ m; b. *Oxytricha* sp., 73 μ m; c. *Paramecium* sp., 120 μ m – microscopic (original).

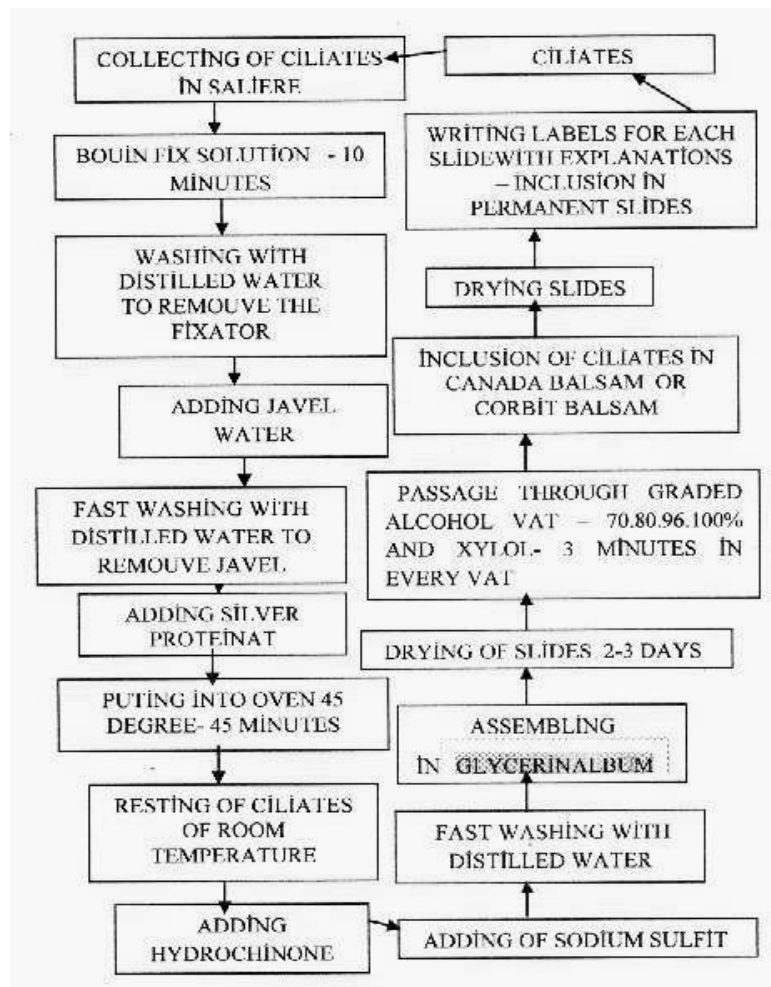


Fig. 2. Protocol of Bodian method (Wilbert variant) [1] [11].

‘The body’ of these delicate creatures – represented by a single cell, miracle of miniature body function, has much to offer to researchers in terms of abundance of infrastructure details. In order to enhance them it was necessary to discover complex methods-based on complex combinations of reagents.

The crucible of these chemical interventions upon the cell structure is represented by the ordinary melting pot; it can keep in various fixation solutions a variable number of ciliates – from several tens to several hundreds of individuals, depending on the size of the species. The chemical interventions of reagents consist in attacking the cell wall from outside to inside; the removal of each reagent is achieved by washing with distilled water.

Some protistologists proposed several stages of evolution of protistology science. So, Lynn [7] proposed five periods of important discoveries in protistology: discovery period of protistology as a science (1880–1930); period of its exploitation (1930–1950); infraciliature period and sophistication period.

Specific methods for highlighting the infrastructural details of ciliates used especially silver nitrate and proteinate.

Acknowledgments:

All my gratitude to Professor Dr. Telat Yanik, Vice-Rector of İbrahim Çeçen University Ağrı, for his useful advice and kind support of my scientific activity.

REFERENCES

1. Dragesco, J. & Dragesco-Kerneis A. (1986), *Écologie des Ciliés. Ciliés libres de l'Afrique intertropicale (introduction à la connaissance et à l'étude des Ciliés)*, Faune Tropical, ORSTOM, Paris, 26, pp. 77–114.
2. Dumitrache-Kerkmann, G.-R. (2004), *Istoricul cercetărilor protistologice în România*, in: „Studii asupra ciliatelor (Protozoa-Ciliophora) psamofile din Dobrogea” – teză doctorat, pp. 3–53.
3. Dumitrache-Kerkmann, G.-R. (2005), *WEBB – metodă eficientă de separarea a ciliatelor din sedimentele marine și lacustre*, in: “Argesis – studies and communications”, series Natural Sciences, Pitesti, XIII, pp. 145–150.
4. Kahl, A. (1933), *Die Tierwelt der Nord – und Ostsee*, Leipzig, Teil II, C3, pp. 1–133.
5. Kerkmann, G.-R. (2011), *Contribuții ale cercetătorilor austrieci la identificarea tehnicilor de impregnare argentică utilizate în protistologie*, in Noema, X, pp. 479–487.
6. Lepși, I. (1960), *Fauna R.P.R., Protozoa*, (1) pp. 1–319.
7. Lynn, D. (2010), Chapter 1 – „Introduction and Progress in the Last Half Century”, in: „The Ciliate Protozoa: characterization, classification and guide to the Literature”.
8. Petran, A. (1976), *Contribuții la cunoașterea faunei de ciliate psamofile din Marea Neagră – litoralul românesc*, in: „Studii și cercetări de biologie”, Edit. Acad., București (15), pp. 187–197.
9. Țuculescu, I. (1965), *Biodinamica lacului Techirghiol*, Editura Științifică și Enciclopedică, București, pp. 1–525.
10. Webb, M., G. (1956), *An ecological study of brackish water ciliates*, in: „J. Anim. Ecol.”, (25), pp. 148–175.
11. Wilbert, N. (1975), *Eine verbesserte Technik der Protargolimprägung für Ciliaten*, in: “Mikrokosmos”, (6), pp. 171–179.

12. Uhlig, G. (1964), *Eine einfache Methode zur Extraction der vagilen, mesopsammeln Mikrofauna*, in: "Helgol. Wiss. Meeresunters", 11, pp. 178–185.
13. Uhlig, G. (1966), *Untersuchungen zur Extraction der vagilen Mikrofauna aus marinen Sedimenten*, in: "Zool. Anz. Suppl.", 29, pp. 151–157.
14. Uhlig, G. (1968), *Methoden der Meeresbiologischen Forschung*, Gustav Fischer Verlag, Jena, pp. 119–129.
15. Harris, A. P., D'Eath R. B. & Healy S. D. (2009), *Environmental enrichment enhances spatial cognition in rats by reducing thigmotaxis (wall hugging) during testing*, in: "Animal Behaviour", 77, 1459–1464.

http://books.google.com.tr/books?id=Hd3jKGDBR48C&pg=PA474&lpg=PA474&dq=denis+Lynn+ciliates&source=bl&ots=EqxTCuSpaA&sig=P7Iz4vU0ubMB45UowoCDjTs48KI&hl=tr&ei=AYx5ToaqOIfMswbpvtTNDw&sa=X&oi=book_result&ct=result&resnum=10&ved=0CGkQ6AEwCQ#v=onepage&q=denis%20Lynn%20ciliates&f=false

<http://www.sciencedirect.com/science/article/pii/S0003347209001183>

<http://www.biology-online.org/dictionary/Cytolysis>

<http://www.encyclo.co.uk/define/mucocyst>

<http://www.thefreedictionary.com/pellicle>