Changes in *Paramecium caudatum* (Protozoa) near a switched-on GSM telephone

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Running title : *Paramecium caudatum* near a GSM telephone

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Abstract

The protozoan *Paramecium caudatum* was examined under normal conditions versus aside a switched-on GSM telephone (900 MHz; 2 Watts). Exposed individuals moved more slowly and more sinuously that usually. Their physiology was affected: they became broader, their cytopharynx appeared broader, their pulse vesicles had difficult in expelling their content outside the cell, their cilia less efficiently moved and trichocysts became more visible. All these effects might result from some bad functioning or damage of the cellular membrane. The first target of communication electromagnetic waves might thus be the cellular membrane.

Introduction

Many studies have been conducted on the possible negative impact of communication waves on living animals. These waves are increasingly recognized as severely perturbing their orientation, communication with congeners, and physiological functions (see web sites in the 'Discussion' section). Several studies are on line or published in scientific journals and/or orally presented in meetings. Most of them provide advice.

We already examined this issue using the ant *Myrmica sabuleti* Meinert as a model. During these observations, waves (900 MHz) were delivered in a continuous manner by a generator set to 10 dBm giving an electric field of about 1 V:m over the ants. Olfactory as well as visual conditioning was no more possible under the influence of electromagnetic waves. After such an influence, conditioning was of lower quality and electromagnetic waves destroyed acquired conditioning (Cammaerts et al., unpublished). The ants' response to their pheromones statistically decreased in the presence of electromagnetic waves and food recruitment then no more occurred (Cammaerts et al., unpublished). Deleterious physiological events also happened: ants exhibited locomotor ataxia, development of larvae and nymphs ceased. Moreover, many workers and a queen were found dead. This suggests that electromagnetic waves probably affect the nerve cells.

Other studies are in agreement with the latter hypothesis. Fragopoulou et al. (2010) showed that exposure to GSM 900 MHz affects spatial memory in mice. Adang et al. (2009) subjected, daily for 18 months (2 h/d), 124 rats to electromagnetic waves and recorded a mortality rate of 60% (vs 29% in a control group), pointing to a stress reaction. Benlaidi & Kharroussi (on line) reported that electromagnetic waves emitted by GSM telephones statistically reduce rats' ability to correctly travel through a maze. Mausset-Bonnefont et al. (2004 and references therein) exposed rats' brains to electromagnetic waves similar to those emitted by GSM (900 MHz) and observed disrupted neurotransmission due to cellular and molecular alterations. The works of Salford et al. (2003) and Xu et al. (2006) are in favor of an effect of electromagnetic waves on nerve cells. Orendaeova et al. (2009) exposed newborn and senescent rats either for 2 d (4 h/d) or for 3 d (8 h/d) and recorded changes in proliferating nervous cell numbers depending on the animal's age and the exposure dose.

It may thus confidently be presumed that electromagnetic waves act at the cellular level.

During our two previous experiments on ants (Cammaerts et al., unpublished), the electromagnetic waves were continuously delivered by a generator via two antennas located above the ant colonies. However, a switched on mobile phone does not continuously emit electromagnetic waves. It periodically emits packets of waves, with a period of a few seconds or a few minutes. It is difficult to program a generator so that it discontinuously emits electromagnetic waves. As a test, we thus placed an activated GSM on the foraging area of *M.sabuleti* colonies. Observation showed that the ants were obviously affected by such a presence: they presented difficulties in moving, failed in returning to their nest and no more recruited congeners Consequently, we designed an experiment to observe cells under normal conditions and under a weak electromagnetic field generated by an activated common mobile phone used in a typical manner, i.e. placed very near the cells. The protozoa *Paramecium caudatum* was used as a cell model because it is easy to maintain in the laboratory, is very large (250-300 µm) and, examined alive, presents cellular organelles (cilia, cytopharynx, pulse vesicle, trichocysts) that may reflect disruptive factors.

Using three different optical apparatus, we observed *Paramecium caudatum* under normal conditions as well as near a switched-on GSM telephone. We assessed several parameters characterizing their life. The result is that the cytological structures were affected by electromagnetic waves. This is an important step forward in determining how electromagnetic waves act on living individuals and how such effects can be avoided.

Material and methods

Maintenance of *Paramecium caudatum*

P. caudatum is a large protozoan (250µm - 300 µm) helicoidally swimming thanks to a sophisticated battling of cilia (Grassé, 1961)

This protozoan was obtained by placing hay in tap water. Bacteria, several protozoans, rotifers and finally many *P. caudatum* developed. The culture was controlled every three days and water added when necessary. Culture was maintained at 20 °C and experiments were performed at that temperature.

Used mobile phone

The used phone (Nokia 3120) emits electromagnetic waves at the exact frequency of 900 MHz. It has a battery of 3.7 Volt able to deliver electricity at 700 mAh. It emits the more when located the farthest from communication antennae. In such a case, the electromagnetic field around the used GSM amounts 3.7 Volt X 700 mAh, that is about 3.7 X 10 = 37 Watts. The present observations of *P. caudatum* were made at ca one hundred meters from a communication antenna, the power intensity near the GSM being then about 2 watts. This is a common value for the electromagnetic field surrounding mobile phones.

The kind of mobile phone here used (Nokia 3120) emits packets of waves about each 30 seconds. We placed the *P. caudatum* as near as possible from the activated GSM (at 1 to 2 cm from its antennae) during at least two minutes so that these cells, at least four times, received electromagnetic waves of about two watts, delivered by the activated mobile phone. During these two minutes, we could not detect any increase of the temperature of the

water containing *P. caudatum*. If any slight increase occurred, it was certainly far lower than that which occurred under a stereomicroscope and a light-transmission microscope. Only exposure to the mobile phone in stand-by position and no call phone was used, since the presence of a stand-by phone on ants' foraging area already caused serious perturbations (see the 'Introduction' section).

Quantitative observations under a stereomicroscope

About 3 ml of the *P. caudatum* culture (these 3 ml containing a few hundred individuals) were placed in a small glass Petri dish (diam.: 4 cm) and examined under a stereomicroscope (magnification: 23x). The trajectories of moving *P. caudatum* were pencil drawn, using a camera lucida, while listening to a metronome beating the second for assessing the running time in seconds (Fig. 1, A). The drawings were traced off on polyvinyl sheets which were then stuck onto the screen of a PC. The linear and angular speeds of the observed protozoans were quantified using specially designed software (Cammaerts et al., submitted). These parameters were quantified firstly, under normal conditions for 23 *P. caudatum* individuals, among those moving in the Petri dish and secondly, for 34 *P. caudatum*, also moving in the Petri dish, but set for two minutes at less than 1 cm from the activated GSM telephone (Fig. 1A). Each time, the protozoans were individually observed, the one after the other, their trajectories being recorded and individually analysed. After that, the mean values were established for the two conditions (Tab. 1. **A**).

Qualitative observations under a light-transmission microscope

Two kinds of microscopic preparations of live *P. caudatum* were observed under a microscope (magnification: 25 x, 100 x, 400 x): firstly, preparations maintained under normal conditions, and secondly, preparations set, for two minutes, on the activated GSM telephone (i.e. deposited onto the GSM). This allowed noting the morphological and physiological characteristics of 20 protozoans maintained under each of the two experimental conditions.

Quantitative observations under a light-transmission microscope provided with a webcam linked to a PC (Fig. 1, B)

To analyze the events occurring in *P. caudatum* exposed to electromagnetic waves, the protozoans were observed using an optical and numerical system similar to that used for studying the morphology of ant eyes (Rachidi et al., 2008). It consisted of a microscope (Zeiss Axioskop, 2.5 x, 40 x, 63 x lenses) to which a numerical camera (Sharpvision Co., Ltd., Guangzhou, China, chipset Omnivision 3.1 Mpixel, USB 2.0) was adapted. The final magnitude on the screen equaled 60 x, 912 x and 1,506 x according to the lens used. The acquisitions were made at 1,024 x 768 or 2,028 x 1,536 pixels. Grading allowed to precise that at 1,024 x 768 pixels, the ratio micron/pixel was 1.24, 0.63, 0.31, 0.22 for the 10 x, 20 x, 40x, 63x objective lens respectively while, at 2,028 x 1,536 pixels, the ratio micron/pixel was 0.62, 0.31, 0.16, 0,11for the 10 x, 20 x, 40x, 63x objective lens respectively. Twenty pictures being taken each second. The camera was coupled to a PC provided with software Amcap allowing filming (http://amcap.en.softonic.com/). The films were registered in avi.

Two studies were conducted:

1. Using the 2.5 x lens (final magnification: 60 x), six footages of moving *P. caudatum* were obtained under two different conditions: firstly, without an activated GSM, and secondly, with an activated GSM (Nokia, 3120) placed together with the preparation of live *P. caudatum* under the microscope, so that its antenna was at 1 to 2 cm from the microscopic preparation containing a few protozoans. In each of the two conditions, the trajectories of respectively 35 and 33 *P. caudatum* individuals were recorded and drawn, using a waterproof marker pen, on polyvinyl sheets stuck to the PC screen. These sheets were then stuck to the screen of another PC provided with specially designed software and the linear and angular speeds of each of the *P. caudatum*'s registered trajectories were quantified. The mean values were then calculated for the two conditions (Tab. 1, **B**).
2. Using the 40 x and 63 x lenses (final magnification: 912 x and 1,506 x), 11 footages were obtained of nearly motionless *P. caudatum* under two conditions: firstly, without any GSM and secondly, as above, with an activated GSM telephone (Nokia, 3120) placed, together with the preparation of live *P. caudatum,* under the microscope, so that its antenna was at 1 to 2 cm from the microscopic preparation containing a few protozoans. These two series of footages were viewed to assess the nine following variables (Fig. 2, Tab. 2): length of *P. caudatum* (L); width of anterior part (l 1); width of posterior part (l 2); cytopharynx length (L c); cytopharynx width (l c); time between two successive systoles of the pulse vesicles (v p); distance traveled by cytological structures in one second (m c); evaluation of cilia batting (cilia); trichocyst aspect. Each of these variables was measured for 11 individuals (giving 11 to 93 measures or assessments, see Tab. 2) and mean values were then calculated, except for cilia and trichocysts, the observations of which being only qualitative.

Statistical analysis

Let us note that while observing the protozoans under a stereomicroscope or a microscope, experimenters of course knew the situation, but they were blind to the situation during all the assessments and statistical analysis.

The distributions of the values obtained for a given variable for *P. caudatum* maintained under normal conditions versus next to or on an activated GSM telephone were compared using the non-parametric χ^2 test (Siegel & Castellan, 1988).

Results

The quantitative observations under a stereomicroscope (**obs 1**), the qualitative observations under a light-transmission microscope (**obs 2**) and the quantitative observations under a light-transmission microscope equipped with a web cam and a PC (**obs 3**) were entirely in agreement. They are thus related all together here below, the labels **obs 1, obs 2, obs 3** allowing to distinguish them.

<u>Movement</u>

obs 1 Under normal conditions, *P. caudatum* moved quickly and randomly. As usually, they turned helicoidally around their longitudinal axis, the individual trajectories being nearly linear. Mean values of linear and angular speeds (assessed considering the magnification used) were V = 0.63 mm/sec and S = 179 angular degrees/mm (Tab. 1, **A**). Since *P. caudatum* have a length of 300-500 µm, they progress about 1.5 times their length in one second. When positioned next to a GSM telephone, *P. caudatum* often changed their direction of movement. They also seemed to have difficulties swimming and their trajectories were more helicoids than under normal conditions. Analysis of their movement revealed that their average linear speed was about 0.50 mm/sec and their angular speed 465 angular degrees/mm, these two values differing statistically from those obtained under normal conditions (Tab. 1**A**.). The increase in angular speed was considerable. Such changes in *P. caudatum*'s movement were the inverse of those induced by an increase in temperature: when maintained under higher temperature (25-30°C instead of 20°C), *P. caudatum* moved more quickly and more linearly (personal observation).

obs 2 Under normal conditions, *P. caudatum* moved quickly, slightly helicoidally, turning as usual on their longitudinal axis. This yielded nearly linear trajectories intermittently interrupted by rapid changes in direction. After having been set for two minutes on a switched-on GSM telephone, they seemed to have difficulty swimming: the anterior part of the cells clearly oscillated, their path becoming more helicoids. This was combined with slower movement.

obs 3 The same differences as those previously observed occurred between the movement of *P. caudatum* under normal conditions and under GSM influence: under the latter condition, the protozoans' angular speed increased considerably while their linear speed decreased somewhat (statistically significantly) and difficulties in swimming appeared (Tab. 1, **B**).

Physiology

obs 2 Under normal conditions, *P. caudatum* had a narrow body, with a peristome slightly visible on the cell surface and a narrow cytopharynx. Their two or three pulse vesicles collapsed asynchronously about every four seconds, with a precise cellular rhythm. After having been set for two minutes on a switched-on GSM telephone, these cells became broader. The pulse vesicles apparently enlarged as usual, but their contraction to expel contents was delayed. The time between two successive systoles was evaluated using a metronome beating the second: it varied between 12 and 16 seconds.

obs 3 Eleven *P. caudatum* maintained under normal conditions and 11 ones subjected to GSM influence were observed and measured (Tab. 2 and Fig. 2). Under GSM influence, *P. caudatum* acquired a different appearance. Their length decreased while their width increased, particularly anteriorly. Their cytopharynx became twice as broad and often appeared as a broad part of a circle instead of a narrow crescent. Their pulse vesicles enlarged apparently as usual but came slowly into contact with the cell membrane and performed systole with difficulty. The time between two successive systoles nearly doubled.

P. caudatum's digestive vesicles and unidentified cellular material moved slower than usual: their mean displacement within a given period was reduced by half. The cilia were also affected by GSM waves: they no longer beat completely and their rhythm was somewhat slower than under normal conditions. On the other hand, the appearance of the trichocysts changed. These cellular elements are located just under the cellular membrane and are scarcely visible under normal conditions. Forming the defensive system of *P. caudatum,* they become more visible in chemically or mechanically attacked as well as in stressed or strained protozoans. In *P. caudatum* set under the GSM influence, the trichocysts became clearly discernible, just as if the protozoans were in some attack or stressing situation. Observation also showed that the cellular membrane seemed to be affected: it appeared to be broader at some places, potentially explaining the other GSM-related cytological perturbations.

The abnormalities observed in association with GSM never occurred when *P. caudatum* were maintained under normal conditions for about 10 min under the light of a microscope, and thus submitted to a temperature increase.

Recovering

obs 1 After a recovery period of five minutes during which most of the previously exposed protozoans remained motionless on the bottom of their cup, they moved again normally.

obs 2 After a recovery period of five minutes, the previously exposed protozoans recovered their usual form, aspect and functioning and moved then again normally.

Conclusion – Discussion

The present study was designed to determine the effect of a switched-on GSM telephone on a cell – the protozoan *P. caudatum*. Under this influence, *P. caudatum* moved more sinuously, apparently with difficulty. Body size and cytopharynx dimensions changed; the pulse vesicles less often contacted the cellular membrane; the cellular elements moved more slowly; the cilia beat less efficiently; the trichocysts became more visible. Let us recall that the latter organelles are those used by *P. caudatum* in unfavorable situations. All these observations are in agreement. The protozoan's difficulties in moving may result from a less efficient beating of the cilia. The different appearance of the cell including that of the cytopharynx may be a consequence of the slower functioning of the pulse vesicles. The GSM effects on the cytopharynx and the pulse vesicles may be due, among others, to damages in the cellular membrane. The exposure of *P. caudatum* to an activated mobile phone was stopped after 2 min because, if exposed during longer times (f.i. 15 min to 60 min), these protozoans were then either dying or in the process of encysting.

Two studies showed that ants exposed to electromagnetic waves (delivered in a continuous manner by a generator) could no longer associate food with encountered cues (Cammaerts et al., unpublished); nor efficiently respond to their own pheromones, nor collect food in the normal manner (Cammaerts et al., unpublished). Moreover, postnatal

development (which requires nervous cell secretions) was perturbed or stopped (personal observation). These two studies on ants point to an effect of electromagnetic waves on nervous cells. Cognitive impairment has also been observed in rats exposed to GSM 900 MHz radiation (Nittby et al., 2008). The present work on *P. caudatum* shows that electromagnetic waves intermittently delivered by a switched on mobile phone, act at the cellular level, prompting speculation that the cellular membrane is their first target. This may also be true for the above-mentioned impact on ants since the nervous influx requires a correct arrangement, operation and role of the cellular membrane and its ionic channels. Indeed, histological alterations in the hippocampus of mouse brain exposed to 835 MHz radiofrequency radiation might be due to changes in calcium permeability across cell membranes (Maskey et al., 2010). So, accordingly, the abnormalities observed in ants, in rodents and *P. caudatum* exposed to electromagnetic waves may reflect damage to cellular membranes. Future work should focus on that hypothesis.

It is not clearly known if intermittent emission of electromagnetic waves allows to decrease the possible toxic effect of such waves.. The present work incites to think that this toxic effect is maintained, even if somewhat decreased. It even might be that the discontinuity of electromagnetic waves emission has, by itself, some disastrous effects. Such a hypothetical effect might explain an observation not recorded in the present results: *P. caudatum* set next to the activated here used GSM regularly presented unpredictable abnormal contractions and cellular distortions never observed under normal conditions.

Scientific research on the impact of electromagnetic waves on living organisms is growing (Desai et al., 2009; www.who.int/mediacentre/factsheets/fs193/en/index.html; www.demain-conseils.com/progs/intro.php; Repacholi, 2001). Among the studies experimentally defining the effect of electromagnetic waves and in addition to those cited in the introduction section, Wang et al. (2009) demonstrated, in wistar rats exposed to microwave radiation, perturbation of the proteins associated with synaptic vesicles. Their study points again to an impact of electromagnetic waves on nervous cells and identifies that impact as being on the cell membrane. The exposure of male mice to electromagnetic waves from GSM stations caused about 40% sperm head abnormalities compared to 2% in control groups (Otitoloju et al., 2010). We suggest that these abnormalities also might result from disrupted membrane structure and function. Desai et al. (2009) identifies the plasma membrane as a target for electromagnetic waves; the authors explored, among others, the effect of electromagnetic waves on the membrane structure, calcium channels and NADH oxidase.

Cilia batting itself may be affected by the extreme low frequencies (ELFs) emitted by the mobile phone battery switching since (pulsed) GSM ELF values (2.1 - 8.3 Hz: Parentos et al., 2007) are in the order of human respiratory (10 - 14 Hz: Chilvers & O'Callaghan, 2000) and middle ear cilia beat frequency (11 Hz: Wake & Smallman, 1992). Let us also note that EEG rythms (ca 10 and 20 Hz: Babiloni et al., 2002) have similar frequencies.

Future work should thus focus on the hypothesis in accordance with the result of the present work: electromagnetic waves negatively act at the cellular level, probably targeting the cellular membrane. These waves might affect the properties, parts and functioning of the cellular membrane, perturbing the highly organized structure of this cellular element. One promising approach would be to examine a possible impact of electromagnetic waves on the molecular arrangement and ionization of the membrane phospholipids. Such an impact would imperil the properties and functioning of any unit membrane. It would also be interesting to examine effects of electromagnetic waves at different power density values in order to precise the limits of acceptable though efficient densities.

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Table 1. Mean linear and angular speeds of *Paramecium caudatum* under normal conditions (= control) and next to an activated GSM telephone (Nokia, 3120) (= + GSM on).
P. caudatum were observed under these two conditions: **A.** using a stereomicroscope provided with a camera lucida (Mag. = 23 X) (Fig. 1, A); **B.** using a microscope provided with a web cam coupled to a PC (final mag. on the screen = 60 X) (Fig. 1, B). Each time, **n** trajectories were recorded and their linear (V) as well as their angular (S) speed were quantified using specially designed software. P: level of probability, based on non parametric χ^2 tests between the 'control' and the '+ GSM on' distributions of variables.

Conditions	n	V (mm/sec)	P	S (ang. deg./mm)	P
A.					
Control	23	0.63 (0.57 – 0.67)		179 (138 – 200)	
+ GSM on	34	0.50 (0.39 – 0.58)	< 0.001	465 (340 – 534)	< 0.001
B.					
Control	35	0.56 (0.43 – 0.68)		151 (123 – 233)	
+ GSM on	33	0.46 (0.36 – 0.59)	< 0.001	459 (383 – 510)	< 0.001

Table 2. Cytological particularities of *Partamecium caudatum* set under normal conditions and next to an activated GSM telephone.

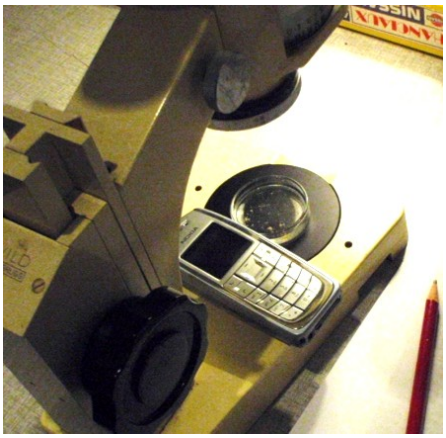
P. caudatum were observed in these two conditions under a microscope provided with a web cam coupled to a PC (final mag. on the screen: 912 x or 1,506 x) (Fig. 1, B). Each time, 11 registered footages allowed to assess the length of the cell (L), the width of its anterior part (l 1), the width of its posterior part (l 2), the length of its cytopharynx (L c), the width of its cytopharynx (l c), the time (sec) between two systoles of a pulse vesicle (v p), the displacement of cytological elements in one second (m c), the batting of the cilia (cilia), the visibility of the trichocysts (trichocysts). n = number of measures or assessments made for each variable (same number as that of observed *P. caudatum* cells for L, L 1, l 2, L c, l c, cilia, thrichocysts; more for v p and mc). P = level of probability, NS = not significant difference for P = 0.05 (non parametric χ^2 tests between the distributions of the same variable under the two experimental conditions).

	under normal conditions			next to an activated GSM	
particularities	n	mean (extremes)	statistics	(extremes) mean	n
L cm	11	15.8 (15.0 – 16.0)	P < 0.01	(12.5 – 16.0) 14.1	11
L 1 cm	11	2.98 (2.00 – 3.50)	P < 0.01	(2.50 – 4.00) 3.26	11
l 2 cm	11	4.41 (4.00 – 5.00)	P < 0.05	(4.00 – 5.70) 4.73	11
L c cm	11	2.33 (2.00 – 3.17)	NS	(1.50 – 4.00) 2.30	11
l c cm	11	0.28 (0.20 – 0.40)	P < 0.001	(0.40 – 1.00) 0.60	11
v p	88	4.29 (4.00 – 5.00)	P < 0.001	(6.00 – 16.0) 8.75	93
m c	88	0.46 (0.25 – 0.90)	P < 0.001	(0.09 – 0.35) 0.20	36
cilia	11	full, quick batting		partial, slow batting	11
trichocysts	11	slightly visible		clearly visible	11

Legend of the figures

Figure 1. Experimental designs used to observe *Paramecium caudatum* set normally or next to an activated GSM telephone: **A:** stereomicroscope provided with camera lucida; **B:** light-transmission microscope provided with web cam coupled to PC equipped with Amcap software.

Figure 2. Photographs (A) and drawings (B) of *Paramecium caudatum* set normally (on the left) or next to an activated GSM telephone (on the right). The photographs are snapshots taken from footages made with the equipment shown in Fig. 1, B. The drawings are made using the mean values given in Tab. 2 and the comments reported in the text. The arrows show the mean distance traveled by cellular elements in one second. The circles represent pulse vesicles, the indicated time being the mean laps of time between two systoles. C indicates the cell cytopharynx.



A **B**

Figure 1

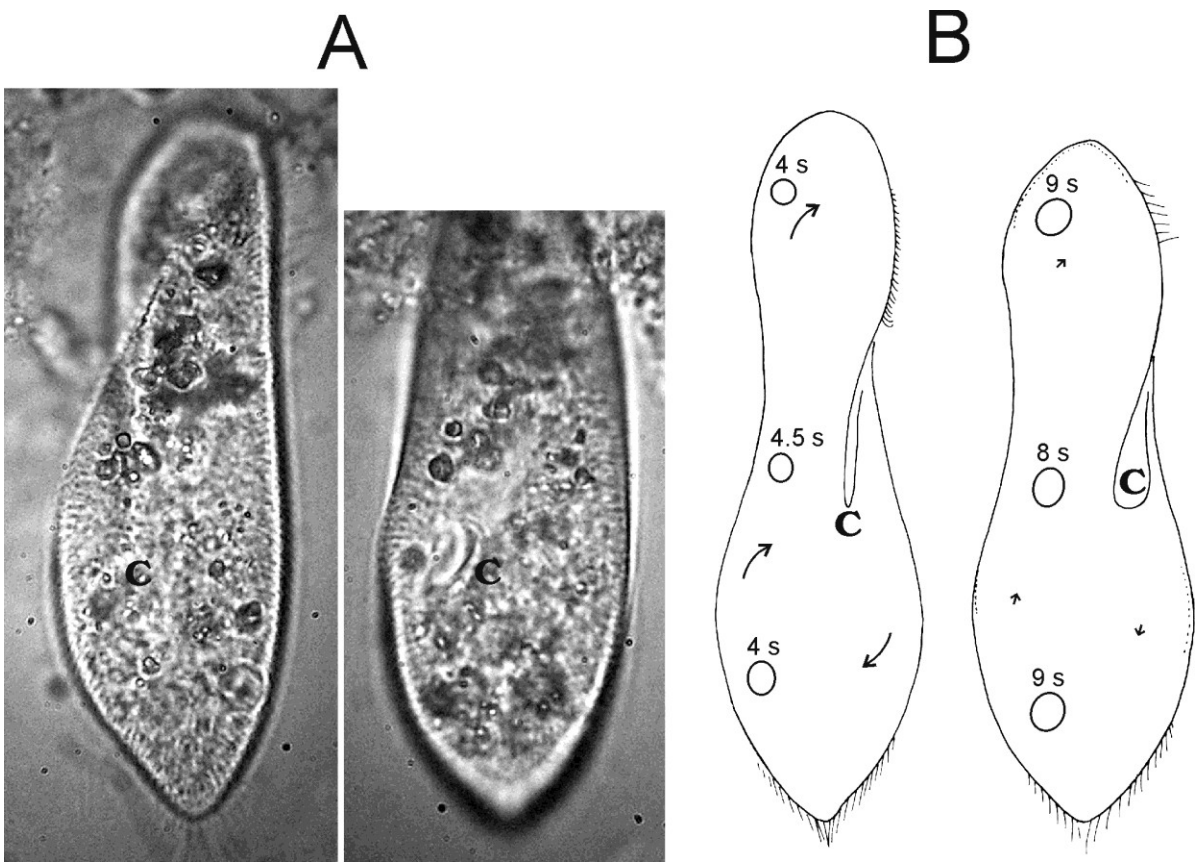


Figure 2