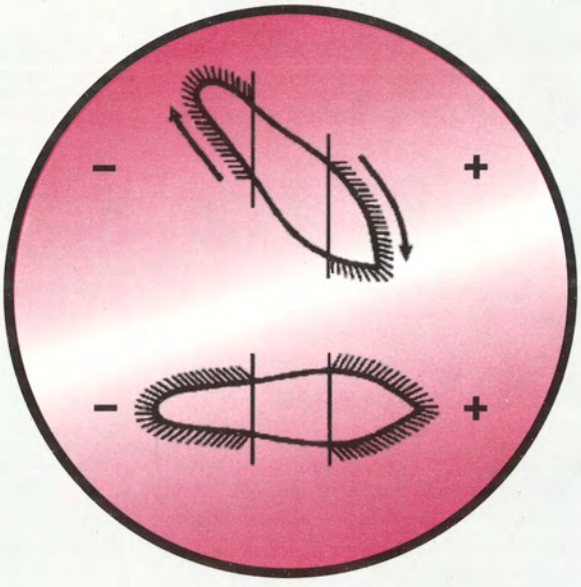
ACTA PROTOZOOLOGICA



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Effects of Tropical Solar Radiation on the Velocity and Photophobic Behavior of Filamentous Gliding Cyanobacteria

Victoria A. DONKOR*, Damian H. A. K. AMEWOWOR and Donat-P. HÄDER***

*Institut für Botanik und Pharmazeutische Biologie, Friedrich-Alexander Universität, Erlangen, Germany; **Department of Botany, University of Cape Coast, Cape Coast, Ghana

Summary. The effects of tropical solar radiation (Ghana, 4.30°N) on several filamentous gliding cyanobacteria (*Anabaena variabilis*, two strains of *Phormidium uncinatum* and *Oscillatoria tenuis*) were studied. The linear velocities were drastically impaired when the organisms were exposed to unfiltered solar radiation. Reducing the ultraviolet radiation to different extents by covering the organisms with various long pass or band pass filters (WG320, GG395 and UG5) decreased the inhibitory effect of solar radiation. The results indicate that both UV and visible radiation of the solar spectrum impair the velocities of the organisms. Only partial recovery was observed and only after shorter exposure times. Step-up and step-down photophobic responses of the organisms were also strongly impaired by unfiltered solar irradiation, with the most effective radiation in the UV region of the solar spectrum.

Key words. *Anabaena variabilis*, cyanobacteria, *Oscillatoria tenuis*, *Phormidium uncinatum*, photophobic responses, solar radiation, ultraviolet radiation (UV), velocity.

INTRODUCTION

Cyanobacteria are of great importance in many terrestrial and aquatic ecosystems both because of their large biomass production and their ability to fix atmospheric nitrogen. It has been estimated that cyanobacteria fix over 35 million tons of nitrogen annually (Häder et al. 1989) which is thus made available for use by higher plants. Filamentous cyanobacteria, such as the Oscillatoriaceae *Phormidium uncinatum* and *Oscillatoria tenuis* and the Nostocaceae *Anabaena variabilis* glide on a substratum using a mechanism not yet fully understood (Walsby 1968; Häder 1986, 1987, 1988). Recent investigations suggest that slime secretion (Kiermayer and Staehelin 1972, Häder 1984), helical rotation around their long axis (Thomas 1970; Halfen and Castenholz 1970, 1971) or surface undulations (Jarosch 1963, Halfen 1973) are possibly involved.

Many cyanobacteria are typical shade organisms; they use effective orientation strategies to move to areas of suitable light conditions for growth and survival of the population (Nultsch et al. 1979; Nelson and Castenholz 1982; Häder 1987, 1988). Probably, the most important external factor for orientation is light to which they respond using three different photoresponses - photokinesis, photophobic responses (Nultsch 1962a, b) and a primitive phototaxis mechanism (Drews 1959).

Dedicated to Prof. W. Rüdiger on the occasion of his 60th birthday.

Address for correspondence: D.-P. Häder, Institut für Botanik und Pharmazeutische Biologie, Friedrich-Alexander Universität, Staudtstr. 5, D-8520 Erlangen, Germany.

Any factor affecting their ability to move or orient in their environment would greatly affect the survival of these organisms and consequently, the entire ecosystem.

Recent increases in solar UV-B radiation as a result of the gradual depletion of the ozone layer due to the emission of anthropogenic trace gases have been suspected to pose a serious stress to higher plants as well as microorganisms (Biggs et al. 1981, Tevini et al. 1981, Teramura 1983, Iwanzik et al. 1983, Tevini and Iwanzik 1983, Ascenzi and Jagger 1979, Nozu et al. 1980, Ohnishi et al. 1982a,b). The aim of this paper was to investigate the effects of strong solar radiation on the velocity and photophobic behavior of cyanobacteria; since the UV stress is strongest near the Equator, the exposure experiments were conducted in Ghana.

MATERIAL AND METHODS

Organisms and culture conditions

Two strains of the filamentous cyanobacterium *Phormidium uncinatum*, isolated in Tübingen (Nultsch, 1962a) and from Lake Baikal (Glagoleva et al. 1980), *Anabaena variabilis* (strain number B1403) and *Oscillatoria tenuis*, obtained from the Göttingen algal culture collection (Schlösser 1982) were used. The organisms were grown on agar (0.35%) mineral medium in 100-mm Petri dishes (Nultsch and Häder 1974) covered with 200-nm pore membrane filters (SM11307, Sartorius, Göttingen, F.R.G.). The cultures were kept under constant white light from mixed fluorescent lamps at 600 lx in a temperature controlled room (20°C).

Irradiation procedures

When the populations had grown and covered the filter surface, the organisms were harvested, the long filaments cut into shorter segments and suspended in a molten 0.3% agar at a temperature of 40°C. When the agar had solidified the samples were exposed to solar radiation between 11 a.m. and 2 p.m. local time on clear days between 9 September and 27 October, 1991 at the University of Cape Coast in Ghana, West Africa. During that time the white light component of solar radiation averaged 134 klx. The organisms were either exposed to unfiltered solar radiation or covered with WG320 or GG395 long pass filters or with a UV passing UG5 bandpass filter (Schott and Gen., Mainz, F.R.G.). Excessive heating was prevented by exposing the organisms on ice chips separated by layers of parafilm; due to this measure the temperature was kept in the range between 22-23°C.

Determination of motility

Samples of the organism suspensions were used to prepare microscope slides sealed with vaseline. These slides were placed on the stage of a light microscope (Zeiss Standard, 2.5x objective, Oberkochen, F.R.G.), equiped with a CCD camera (LHD 0600, Philips, The Netherlands) and the movements of the filaments were recorded on a time-lapse VHS video recorder (HS-3600, Mitsubishi, Japan) which allowed time compression by a factor of up to 120. The recordings were analyzed frame by frame on a large video screen (FM100-20 CE, Blaupunkt, F.R.G.) to determine the velocities of the organisms using the interactive image analysis system (Häder and Vogel 1991).

RESULTS

The speed of movement of the organisms was measured before and after exposure to solar radiation covered without or with the optical filters described above. The organisms were exposed to solar radiation for 5, 10, 15 or 30 min and the velocities plotted against time. When filaments of *Phormidium uncinatum* (Tübingen) were exposed to unfiltered radiation the velocity was drastically reduced to zero within 10 min (Fig. 1a). When the organisms were exposed covered with the WG320 long pass filter, the reduction in the velocity was slower and reached zero after 15 min of exposure. When exposed under the GG395 filter, the reduction in velocity after 5 min exposure was still less drastic, but was comparable to the previous filter later on. With the UV transmitting filter, UG5, the reduction in velocity was more gradual and reached zero only after 30 min irradiation.

In the Baikal strain of *Phormidium uncinatum* unfiltered solar irradiation also resulted in a drastic reduction in velocity, and also the tolerance under the WG320 filter was comparable to that of the Tübingen strain (Fig. 1b); irradiation under the GG395 and UG5 filters was even better tolerated by the Baikal strain. In contrast, *Oscillatoria* tolerated the unfiltered radiation and that filtered

Table 1

Percentages of organisms of *Phormidium uncinatum* (Baikal strain) and *Oscillatoria tenuis* showing step-up and step-down photophobic behavior after exposure to 5 min solar radiation under the various conditions. Values are means of 3 experiments ±.S.D.

Organism	Treatment	Step-up %	Step-down %
Phormidium uncinatum	control	4.5	44
	unfiltered solar radiation	11.1	36
	WG320 filter	7.8	47
	GG395 filter	4.8	43
Oscillatoria tenuis	control	11	50
	unfiltered solar radiation	35	18
	WG320 filter	21	29
	GG395 filter	8	50

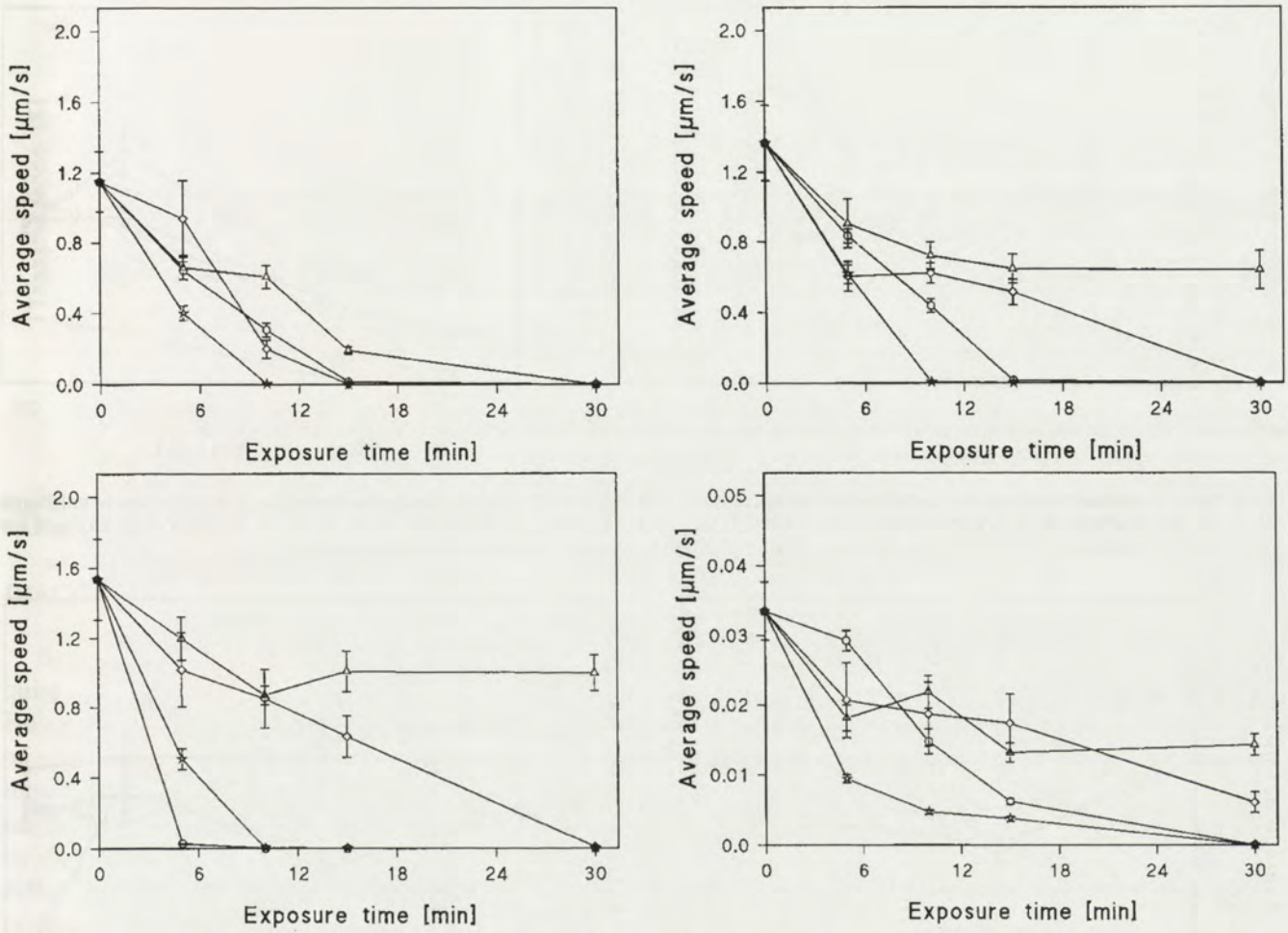

Fig. 1. Speed of (a) *Phormidium uncinatum* (Tübingen strain), (b) *Phormidium uncinatum* (Baikal strain), (c) *Oscillatoria tenuis* and *Anabaena variabilis* exposed to solar radiation (ordinate, speed, µm/s) under different conditions for increasing time intervals (abscissa, exposure time, min). Samples were exposed to unfiltered solar radiation (stars) or covered with GG395 (diamonds), WG320 (circles) or UG5 (triangles) filters. Values are means of 5 experiments ± S.D.

by the WG320 filter less well while the behavior under the GG395 and UG5 filters was similar to that of *Phormidium* (Baikal strain) (Fig. 1c). Effects of solar irradiation on *Anabaena variabilis* were significantly less drastic as compared to the other three organisms. When exposed to unfiltered radiation, the velocity reached zero only after 30 min exposure (Fig. 1d). With the WG320 filter the reduction was more gradual even though it also reached zero by 30 min irradiation. With the GG395 and UG5 filters the organisms continued to move even after 30 min irradiation.

After irradiation the organisms were kept in dim light (ca. 500 lx, 22°C) in the laboratory and the velocities were measured at various time intervals to determine whether they recover from the effect of solar irradiation. *Phormidium uncinatum* (Tübingen) exposed to solar irradiation for 15 or 30 min showed no movement at all throughout the recovery period (Fig. 2a). Organisms irradiated for 10 min showed an insignificant increase in velocity up to 2 h after irradiation, which subsequently decreased again up to 24 h. In organisms irradiated for 5 min the velocity increased significantly up to 2 h and then decreased again afterwards. In *A. variabilis,* irradiated organisms recovered from exposure to unfiltered radiation (Fig. 2b). When exposed under the UG5 UV transmitting filter for up to 15 min, velocities of *Phormidium uncinatum* were almost stable with a small decrease toward the end of the recovery period (Fig. 3a). Organisms irradiated for 30 min did not show any appreciable signs of recovery. Similar effects were observed for *A. variabilis* irradiated under the UG5 filter (Fig. 3b).

Photophobic responses

Organisms were exposed to solar radiation for 5 min without or with the various filters and the photophobic

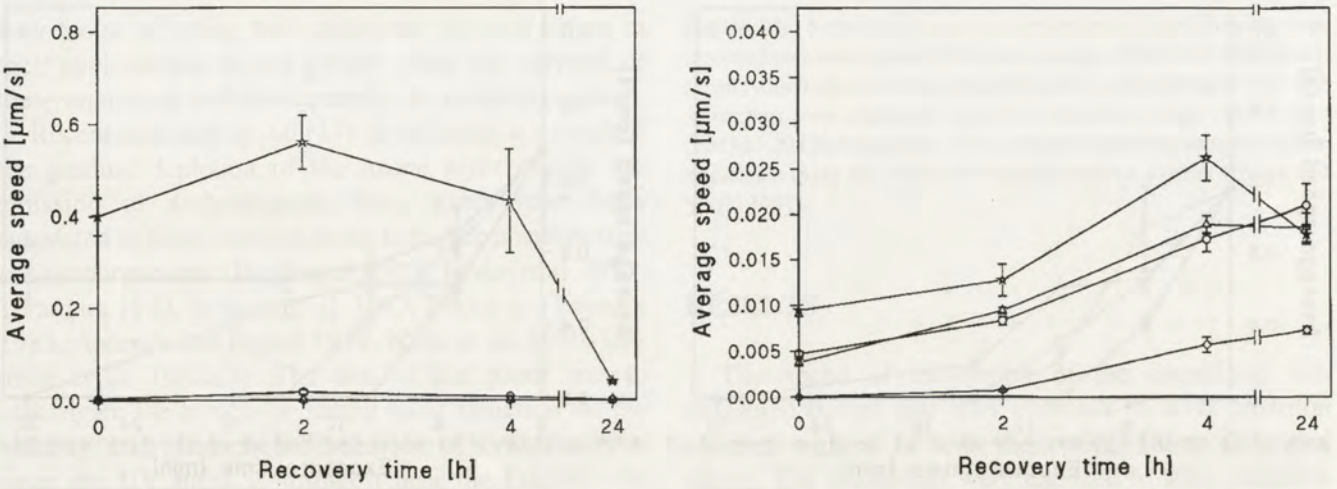

Fig. 2. Rate of recovery of samples of (a) *Phormidium uncinatum* (Tübingen strain) and (b) *Anabaena variabilis* after exposure to unfiltered solar radiation (ordinate, speed of movement, µm/s) for different time intervals (abscissa, time of recovery, h). Samples were exposed for 5 min (stars), 10 min (circles), 15 min (triangles) or 30 min (diamonds). Values are means of 5 experiments ± S.D.

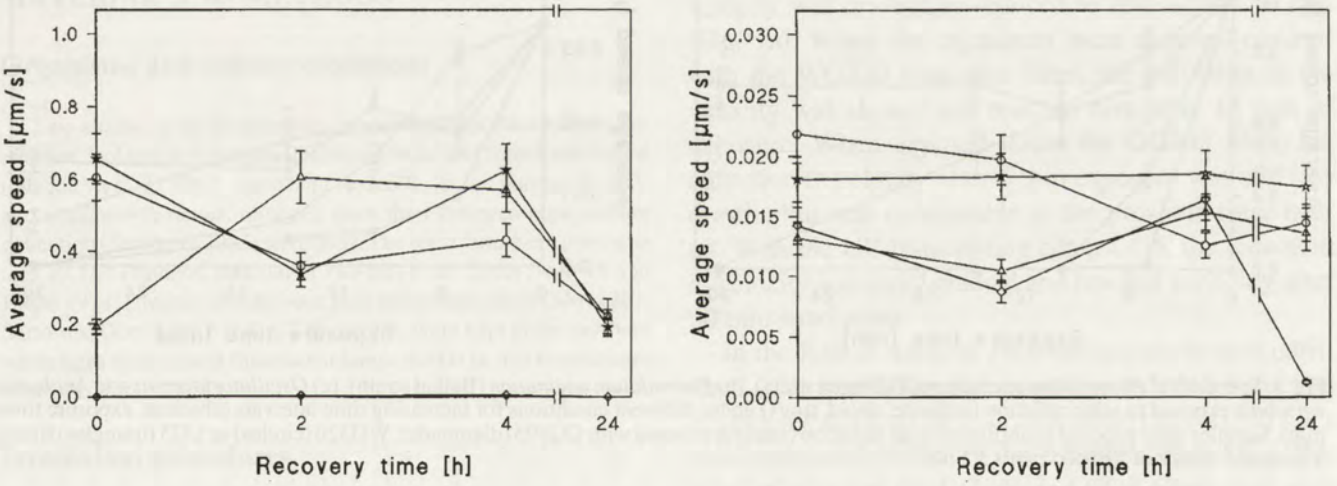

Fig. 3. Rate of recovery of samples of (a) *Phormidium uncinatum* (Tübingen strain) and (b) *Anabaena variabilis* after exposure to solar radiation filtered through a UV passing UG5 filter (ordinate, speed of movement, µm/s) for different time intervals (abscissa, time of recovery, h). Samples were exposed for 5 min (stars), 10 min (circles), 15 min (triangles) or 30 min (diamonds). Values are means of 5 experiments ± S.D.

responses at a light-dark border, projected into the microscope field of view, were observed. Some of the organisms moved from the light into the dark field, and *vice versa*, without showing any phobic response (reversal of movement) but others stopped and returned into the light field (step-down response) or the dark field (step-up response) after sensing the change in the fluence rate.

Phormidium uncinatum (Baikal strain) irradiated for 5 min without a filter showed an increase in step-up responses (Table 1). This increment was lower when the WG320 filter was used, and about the same as the control when the GG395 filter was used. The percentage of organisms showing step-down responses decreased when the organisms were irradiated for 5 min without filter. With the WG320 and GG395 filters the percentage of organisms showing step-down responses was similar to the control value.

Oscillatoria tenuis showed a large increase in step-up photophobic reactions when irradiated for 5 min without a filter. Using the WG320 or GG395 filters during exposure resulted in a less drastic increase. On the other hand, the step-down responses were drastically reduced when irradiated for 5 min without a filter. With the WG320 filter, the reduction was less while under the GG395 filter there was about the same percentage of step-down responses as in the control.

As the transmission curves and the amount of radiation passed by the various filters used in this study have a marked effect on the responses by the organisms the transmission curves are shown in Fig. 4.

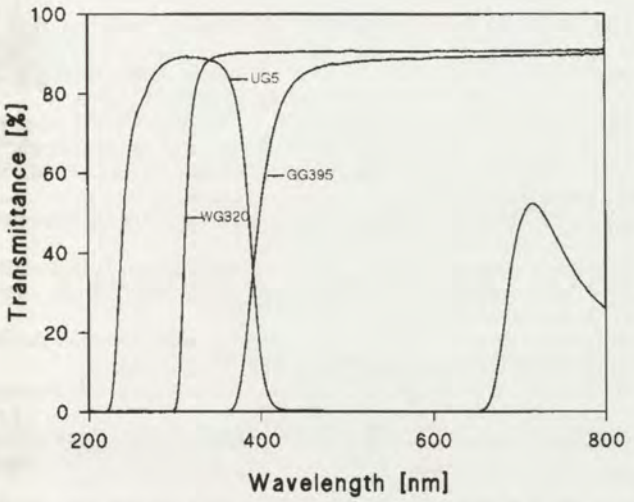

Fig. 4. Transmission in percent of the incident irradiation of the optical filters used in this study. All filters were 3 mm thick

DISCUSSION

Being photosynthetic organisms cyanobacteria require adequate intensities of light for energetic reasons. However, many of these organisms have been found to be adapted to rather low irradiances and are damaged or impaired by fluence rates exceeding a few percent of unfiltered solar radiation (Walsby, 1968). As indicated by the results all tested organisms, though they slightly differ in their sensitivity to solar radiation, are impaired in their movement velocity even by short exposure to unfiltered solar radiation. Ultraviolet-B radiation (280 - 315 nm) is responsible for part of the inhibition as shown by the exposure under the WG320 filter which removes this wavelength band. This is also documented by the fact that exclusive UV radiation is also effective, even though to a smaller extent than the other irradiation regimes which may be partially due to the limited transmittance of the UG5 bandpass filter. In addition, also UV-A (315 - 400 nm) is partially responsible for the inhibition of velocity. In addition to UV, also visible radiation at fluence rates emitted by the tropical sun is capable of affecting the movement velocity in cyanobacteria. These results confirm earlier reports using exposure to mediterranean solar radiation and artificial monochromatic ultraviolet radiation in *Phormidium uncinatum* (Häder 1984, Häder et al. 1986, Häder and Häder 1990, Donkor and Häder 1991).

It has been demonstrated that filaments of *Phormidium uncinatum* avoid gliding into shaded areas by means of step-down photophobic responses (Nultsch 1962a, Häder 1979); likewise, the filaments avoid very bright areas using step-up photophobic responses (Häder and Burkart 1982 a,b). Thus, the organisms accumulate in areas of suitable irradiation by means of a balance between these responses. These photophobic responses in *Phormidium uncinatum* (Baikal) and *Oscillatoria tenuis* are greatly impaired by UV radiation of the solar spectrum. Solar ultraviolet radiation seems to affect the step-up photophobic behavior more strongly than the step-down behavior. Impairing their movement velocity and their phobic behavior at light-dark boundaries disables the organisms to respond to the external cues in their environment. Some cyanobacteria are easily photobleached by bright visible light: exposure to white light of a few thousand lux (less than 10% of the value measured on a clear summer day in central Europe) drastically bleached a *Phormidium* population within 24 h (Häder 1984). This might also be the reason why cyanobacterial blooms disappear from shallow ponds or puddles during summer as a result of being photokilled by solar radiation. Being left in a too dark area is also detrimental because of the energetically required solar radiation.

The experiments studying the potential to restore the velocity of the organisms indicate that this capability is very limited and at best restricted to a few hours. In contrast, the values after 24 h indicate even larger damage than immediately after exposure to solar radiation. The molecular target of the radiation is not known. As the organisms were left in dim light there was obviously no photorepair mechanism as described for the repair of DNA damage by the photolyase system (Yasui and Chevallier 1983, Sancar and Sancar 1984) which confirms earlier results (Häder et al. 1986). Another potential target of excessive irradiation are membranes which generate electrical potential changes which are known to be involved in the reversal of movement (Milotic and Solic, 1983).

In conclusion, unfiltered solar radiation is detrimental to the studied organisms of *Phormidium uncinatum* (both Tübingen and Baikal strains), *Oscillatoria tenuis* and *Anabaena variabilis* since their motility and photoorientation are drastically affected. UV radiation is mainly responsible for the inhibition of motility and the inhibition of step-up and step-down photophobic responses. Thus, the role of UV radiation as a stress factor in the environment cannot be ignored. Even at ambient levels solar UV-B and UV-A radiations affect motility and photoorientation especially in tropical ecosystems with high UV irradiances. However, also visible radiation must be considered as stress factor when not filtered by a layer of water.

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Step-up Photophobic Response of *Euglena gracilis* at Different Irradiances

Michael J. DOUGHTY

University of Waterloo, School of Optometry, Waterloo, Ontario, Canada

Summary. *Euglena gracilis*, suspended in an inorganic salt medium, were red light adapted and then subjected to a sudden and sustained increase in white light. The resultant step-up photophobic response showed two phases; an initial continuous cell turning followed by periodic cell turning interrupted by brief episodes of forward swimming. The number of continuous 360° cell turns increased from 5 at 105 W m^{-2} to 23 at 1100 W m^{-2} while the overall duration of the complete response increased from 60 s at 15 W m^{-2} to 110 s at 1100 W m^{-2}. The step-up photophobic response is thus graded with respect to fluence rate. The cell, however, adapts to sustained white light illumination in that forward swimming behavior is resumed.

Key words. *Euglena*, flagella, light, step-up, photophobic response.

INTRODUCTION

The free-swimming flagellated alga, *Euglena gracilis*, can respond to a sudden increase in light intensity with a step-up photophobic response (Doughty and Diehn 1980). Numerous published descriptions indicate that this response is a light-induced, end-over-end cell tumbling mediated by flagellar reorientation. The these end-over-end tumbles appear to executed in a single plane and are therefore best considered as 360° turns. As such, a partial turn of less than 360° can be observed (Doughty 1991). It has been noted that a cell can execute as many as 30 consecutive tumbles following a step-up stimulus (Mikołajczyk and Diehn 1975). The cells eventually return to normal swimming motion after a step-up photophobic response. However, it has not been reported how long cells take to complete the cell tumbling episodes and, for example, whether the number of continuous tumbles is related to the magnitude of the stimulus. Using criteria for assessment of the step-up response established from a detailed study of cells in culture medium (Doughty 1991), the present study provides some documentation of the responses of cells, in a mixed salts solution (after Mikołajczyk and Diehn 1975), to different intensity white light stimuli.

Address for correspondence: M.J. Doughty, University of Waterloo, School of Optometry, Waterloo, Ontario N2L 3G1, Canada.

MATERIAL AND METHODS

A culture of *Euglena gracilis* Z was obtained from the University of Toronto Culture Collection (Department of Botany). The cells were cultured under a 14 : 10 h light : dark cycle (starting at 06.00 h) in 12 mM acetate-supplemented, bactotryptone-beef extract (Bloomington medium) as previously detailed (Doughty 1991). Cells in mid-logarithmic phase of growth (circa 10^5 cells/ml) were harvested between 13.30 and 14.30 h by low speed centrifugation (350 x g for 3 min), resuspended in an inorganic salt medium,

recentrifuged and finally resuspended to a density of circa 10^4 cells/ml. The inorganic salt solution contained 4.65 mM Ca $(NO_3)_2$, 4 mM KH_2PO_4, 1 mM K_2HPO_4 and 2 mM $MgSO_4$ (after Mainx 1927/28, Diehn 1969, Mikołajczyk and Diehn 1975). The cell suspension was transferred to dim red lighting (650 nm, circa 150 mW m^{-2}) and used for experiments 0.5 to 2.5 h after being transferred to the red background light (starting at 13.30 to 14.30 h). All experiments were carried out at room temperature (23-25°C).

Aliquots of cell suspensions, in covered glass depression slides (1.5 mm deep), were stimulated with a halogen source placed 7 cm from the microscope stage that provided a broad spectral output peaking at 606 nm with an overall output of 1100 W/m^{-2} over the spectral range of 300-1100 nm (as assessed at 7 cm from the source). The incident fluence rate on the cells was attenuated by use of calibrated neutral density filters placed after a 5 mm heat filter. The cell suspensions were observed through a binocular objective of a dissecting microscope (at 40 times magnification) with illumination being provided from below (red, 650 nm; 200 W m^{-2}). Individual samples of cells were evaluated over periods of up to 120 s after switching on the stimulus light and then discarded and a fresh sample prepared. The total period of cell responses was assessed by visual observation with an event marker being used to provide a printed record of the sequence of cell turning episodes. From these records, the total number of cell turning episodes can be counted (to allow calculation of the relative frequency cell turning episodes) and estimates made of the actual time spent in such turning episodes (by adding up all the time periods spent in cell turning as opposed to forward swimming; Doughty 1991). Where cells showed periods of continuous cell turning (see results), the number of consecutive 360° turns was simply counted. The event marker traces from the response sequences of 18 to 25 different cells were used to assess the prevalence of cell turning in a sample of cells as previously detailed (Doughty 1991).

RESULTS AND DISCUSSION

Cell motion under continuous red illumination

In all experiments, at least 85% of the cells were continuously motile in the inorganic salt solution and exhibited forward swimming that was only occasionally interrupted by transient stops or partial turns (but not complete 360° turns). The frequency of these spontaneous directional changes was estimated to be 8.5 ± 0.6 changes min^{-1} (n = 25, 5 separate culture samples tested).

Overall characteristics of the step-up photophobic response for cells in inorganic salt solution

For light intensity increases of 100 W m^{-2}, all cells started turning almost immediately (i.e. within no more than 1 s after the stimulus light was switched on). At much lower fluence rates, obvious delays between actinic illumination and response were readily apparent, e.g. 5 ± 1 s at 15 W m^{-2}. For light intensity changes of 100 W m^{-2}, showed a series of consecutive full turns of 360°. The cell motion appeared to be restricted to the same plane throughout the continuous turning phase. After completing several such full turns, all cells then showed a series of briefer periods of turning episodes followed by forward swimming; these latter episodes could be repeated up to 28 times before the cell eventually returned to forward swimming (with only occasional directional changes). The step-up response thus can have an initial continuous cell turning phase and a second phase of periodic cell turning.

Quantitative analysis of the step-up response for cells in inorganic salt solution

With moderate to high intensity (≥ 100 W m^{-2}) light stimuli, all cells evaluated showed an initial phase of continuous cell turning. The duration of this phase of continuous turning ranged from very brief (e.g. 1.5 ± 1.1 s at 105 W m^{-2}) to prolonged (e.g. 24.5 ± 1.8 s at 1100 W m^{-2}) and was clearly related to the magnitude of the fluence rate change (Fig. 1a). For cells responding to 105 W m^{-2} white light, one to three 360° turns were observed. However, as the stimulus intensity increased, the number of full turns executed in this initial phase also increased. An average of 23 ± 3 full turns were observed in response to 1100 W m^{-2} (Fig. 1b).

At both low (70 W m^{-2}) and moderate to high (100 W m^{-2}) stimuli, all cells respond with repeated episodes of periodic cell turning. The total time spent in either such episodes or continuous cell turning followed by periodic turning was also assessed. At less than 70 W m^{-2}, the cells could show from 30 to 60 s of partial turns or the occasional full turn; the overall duration increases slightly in relation to the light intensity change (Fig. 2a). At fluence rates of > 105 W m^{-2}, the overall response duration clearly increases as light intensity difference increases (Fig. 2a). The cells spend proportionately more time in actual cell turning during the overall response period as opposed to intermittent forward swimming (e.g. 17 s in a total of 57 ± 7 s at 15 W m^{-2}; 25 s in a total of 64 ± 3 s at 70 W m^{-2}). Overall, as the stimulus intensity increases, the proportion of the total response time actually spent in turning episodes increases; this is presented as the actual time spent turning (expressed as a percentage of the total response time) in Figure 2.

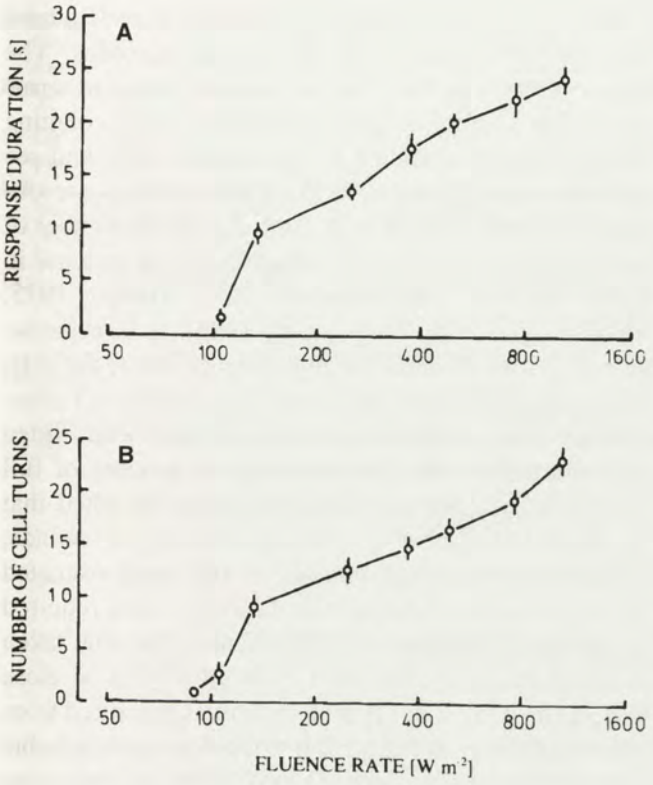

Fig. 1. Magnitude of the initial phase of the step-up response in *Euglena gracilis* in relation to fluence rate as evaluated by assessments of the overall response duration (A) or by counting the number of 360° cell turns (B) that occur after actinic illumination. Data points are averages of 20-30 determinations (± SEM)

The progressive increase in the actual cell turning time during the step up response is achieved by a lengthening of the individual episodes of cell turning that make up the overall response. That this lengthening of individual turning episodes does occur was clearly apparent from assessments of the durations of these episodes or from calculation of the frequency of individual cell turning episodes during the response period (unpublished results). For example, the relative frequency of turning episodes was around 14 min^{-1} at 105 W m^{-2} but only 6 at 1100 W m^{-2}.

Evaluation of the prevalence of cell tumbling in a cell population after step-up light stimuli

Time-response plots (Doughty 1991) were used to estimate the prevalence of cell tumbling in a cell population, i.e. to estimate how many cells were undergoing a turning response (as opposed to forward swimming) in any 1-s interval. At stimulus intensities just below threshold (i.e. 7.5 W m^{-2}) the time-response plots (Fig. 3a) show that there was an overall prevalence of cell turning, in any 1-s period, of around 10%, i.e. an average 2 of 20 cells were tumbling in any successive analysis interval (range 0 to 4). At 15 W m^{-2} stimulus intensity, an average of 5 of the 20 cells analyzed were found to be turning in any one second interval (i.e. 25%). However, at this stimulus intensity the response was variable with 3 to 8 cells being observed to be turning in any 1-s interval so that the time-response plot (Fig. 3b) was irregular. After 30 s the prevalence of turning events slowly declined so that by 65-70 s, there was again only a 10% prevalence of cell turning. With moderate stimuli (105 W m^{-2}), at least 16 of the 20 cells analyzed were found to be turning continuously for 10 to 12 s (so the overall prevalence of turning was high at 80%). The response was not sustained so that, by 70 to 75 s, the prevalence of turning was again approx. 10% (Fig. 3c). For larger magnitude step-up stimuli, the prevalence of turning was 100% for 20 to 30 s before the cells also slowly returned to predominantly forward swimming, e.g. by 90 s at 275 W m^{-2} (Fig. 3d) and 110-115 s at 1100 W m^{-2} (Fig. 3d).

Qualitative comparisons between the step-up and the step-down response

The present observations and measures on *Euglena* clearly show that the step-up photophobic response is graded. As also observed at a single stimulus level (Doughty 1991), the present studies confirm that step-up response is actually a sequence that lasts up to tens of

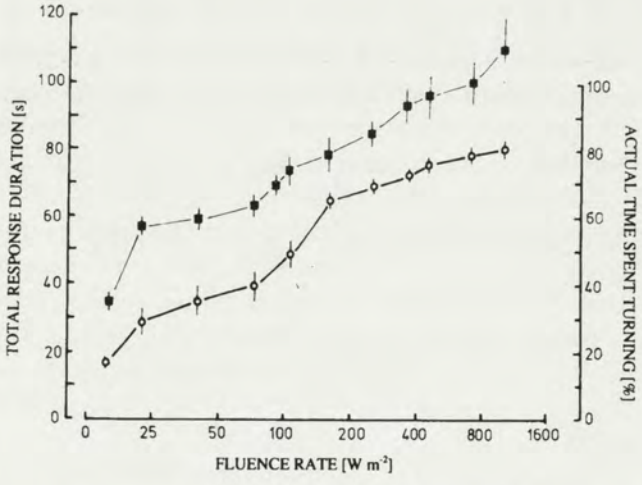

Fig. 2. Magnitude of the step-up response in *Euglena gracilis* in relation to fluence rate as evaluated by assessments of the total response duration (■) or the actual time spent in cell turning (○) during the entire response sequence (expressed as a percentage of the total response time). Other details as Fig. 1

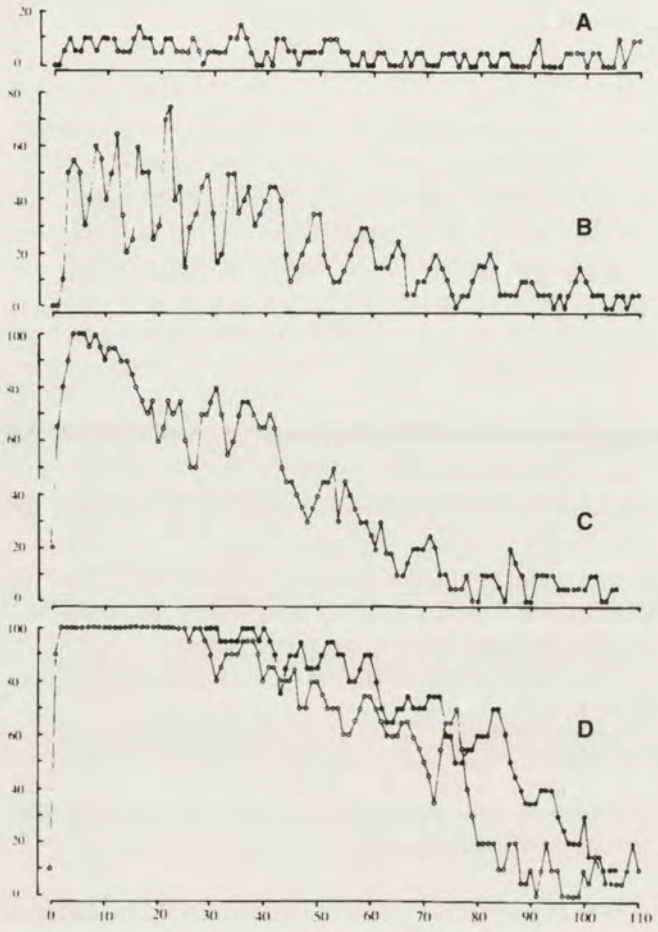

Fig. 3. Prevalence of step-up photophobic (cell tumbling) responses of *Euglena gracilis* assessed by averaging the swimming tracks of 18 to 25 cells. The percentage of these cells that were undergoing turning responses (as opposed to forward swimming) was assessed in each consecutive 1-s period. (A) 7.5 W m^{-2}, (B) 15 W m^{-2}, (C) 105 W m^{-2}, (D) 275 (○) and 1100 (●) W m^{-2}

seconds during which the cells periodically switch between forward swimming and cell turning episodes. This periodic cell turning results from periodic flagellar reorientation. At moderate to high stimulus levels, there is an additional initial phase to the response in which the cells execute continuous end-over-end turning (360° turns) mediated by continuous flagellar reorientation. After such an initial continuous turning phase, there follows a secondary phase of periodic cell turning. The step-up response is therefore qualitatively similar to the step-down photophobic response (Doughty and Diehn 1979).

The sequence of repeated cell turns, observed for the step-up photophobic response over a wide range of light intensities, is clearly long enough to permit simple evaluation with a stop-watch (as previously done for the step-down response; Doughty et al. 1980) but is easier to quantify if an event marker is used to provide a hard copy of the sequence of cell turning episodes. The step-response can therefore be assessed either in terms of its overall duration (as in this study) or by counting the number of completed full turns (this study and see Mikołajczyk and Diehn 1975). Such methods are obviously clearly distinct from estimates of the number of cells in sample that initially responded to an increase in light intensity (Mikołajczyk and Diehn 1975, Mikołajczyk 1984, Walne et al. 1984). It is acknowledged that at moderate to high fluence rates, the cells are turning rather fast and it would be useful to further evaluate the continuous turning phase with video analyses (rather than just counting the number of full 360° turns completed). However, it can be noted that the overall time spent in continuous turning, in seconds, closely resembles that number of full turns executed during this period. An average of 24.5 s were required to execute 23 tumbles (at 1100 W m^{-2}); the time taken for each successive full turn is thus likely to be close to 1-s. This time is consistent with times measured from video recordings of full turns for step-down photophobic responses (Doughty and Diehn 1979) or following ionophore treatment (Doughty and Diehn 1982). However the cells have been reported to be able to turn at much faster rates. Acuna and Bovee (1979) report that, on stimulation with light, *Euglena* could complete nearly 10 turns in 1-s for they report an average turning time of 117 ms. Their experimental solution (Chalkey's solution) was not the same as that used in the present study and was reported to support an average cell swimming speed of 140 μm s^{-1} which is nearly three times higher than that reported by Doughty and Diehn (1979). The very fast cell turning was observed with the addition of 0.1 mM K_2ATP to the solution and this addition was reported to also increase the swimming speed to 280 μm s^{-1}. It will be useful to confirm these observations.

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Chemosensitivity of a Chloroquine-resistant *Plasmodium falciparum* Isolate to Quinine - Type Compounds in Vitro

Lathika NAIR and Virendra K. BHASIN

Department of Zoology, University of Delhi, Delhi, India

Summary. In vitro relative chemosensitivity of erythrocytic stages of a chloroquine and antifol resistant *P. falciparum* isolate to quinine-type compounds was evaluated. Apart from natural cinchona alkaloids, quinine and quinidine, their hydrogen derivatives proved to be more potent erythrocytic schizontocidal compounds. Some drug combinations also show extremely good antimalarial property in vitro. Going by the IC_{50} values of the eight preparations tested the compounds can be arranged in the following order with respect to their schizontocidal activity: Quinidine/Hydroquinidine ≥ Hydroquinidine > Quinine/Hydroquinine ≥ Quinidine > Hydroquinine > Quinine > Apoquinine > Epiquinine.

Clearly in depth investigations are required for exploiting the antimalarial potentials of these cinchona derivatives or mixtures and other combinations.

Key words. *Plasmodium falciparum*, quinine, derivatives, chemosensitivity.

INTRODUCTION

Emergence of resistant *Plasmodium falciparum* strains to commonly used synthetic drugs in different endemic areas of the world (Wernsdorfer and Payne 1991, Payne 1987) has reestablished quinine as an indispensable therapeutic antimalarial in acute cases (Wernsdorfer 1987). Quinine is not the only cinchona alkaloid to possess antimalarial properties; quinidine for instance is known to be more potent antiplasmodial agent (Hofheinz and Merkli 1984, Phillips et al.1985). However, sporadic reports of quinine failure to cure falciparum malaria have emanated from various places (Bastien 1987, Isaacs and Ellis-Pegler 1987, Neiva 1910) and decline in quinine sensitivity confirmed in Thailand through in vitro investigations (Suebsaeng et al. 1986, Bunnag et al. 1987). These observations guided us to evaluate in vitro the relative toxicity of some natural cinchona products and their derivatives or mixtures to asexual erythrocytic stages of a drug resistant *P. falciparum* isolate of Indian origin with an aim to identify quinine-type preparations possessing better erythrocytic schizontocidal activity.

Address for correspondence: V. K. Bhasin, Department of Zoology, University of Delhi, Delhi-110007, India.

MATERIAL AND METHODS

Quinine-type compounds. Coded preparations of cinchona alkaloids and their derivatives or mixtures were kindly provided by Dr. P.I. Trigg of World Health Organization. Detail chemical structure of these compounds has been given elsewhere (Bhasin and Trager 1987). Identity of the eight preparations used in this investigation is as follows:

	Name	Abbreviations
1.	Quinine (base)	Q
2.	Quinidine (base)	Qn
3.	Apoquinine (base)	AQ
4.	Hydroquinine (base)	HQ
5.	Hydroquinidine (base)	HQn
6.	Quinine/Hydroquinine 60 : 40 mixture (bases)	Q/HQ
7.	Quinidine/Hydroquinidine 60 : 40 mixture (bases)	Qn/HQn
8.	Epiquinine/epiquinidine sulphate 60 : 40 mixture	EQ/EQn

Stock solution of each compound was made by dissolving 1000 mg of the substance in 61.6 ml of O.1N sulfuric acid and diluted with glass distilled water to 100 ml. Further dilutions of the stock solutions were made with HEPES-buffered RPMI-1640 medium.

Parasites. Locally collected *P. falciparum* isolate FCD-4, being routinely maintained in vitro in our laboratory using candle-jar method of Trager and Jensen (Trager and Jensen 1976) was employed in the present study. The parasites were grown in A^+ erythrocytes in HEPES-buffered RPMI-1640 medium supplemented with 10% AB^+ human serum. Isolate FCD-4 has been demonstrated in vitro to be insensitive to commonly used antimalarial drugs like chloroquine and pyrimethamine.

Blood schizontocidal activity. Erythrocytic schizontocidal activity of each compound at graded concentrations was evaluated in vitro using the modified 48 hr test method (Nguyen-Dinh and Payne 1980) in 24-well tissue culture plates. The parasites for this purpose were obtained from stock cultures, diluted with uninfected erythrocytes so as to obtain parasitemia of about 1%. This seed material (zero hour) was made to 50% cell suspension and 20 µl aliquot of the suspension added to each of the series of wells holding 480 µl of complete medium with or without drug. The multiwell plates were incubated at 37°C in a candle jar. After 24 hr of incubation, medium in each well was replaced, drug being included at same concentration as initially and plates returned to the candle jar. Blood smears were made at the end of 48 hr and stained with Giemsa. Minimum of 5000 erythrocytes were enumerated for determination of percent parasitemia. Percentage reduction of parasitemia in relation to control was calculated. Inhibitory concentrations (IC_{50} and IC_{90}) were determined from semilog plot of varying concentrations against percentage control of inhibition.

RESULTS

Sensitivity of isolate FCD-4 was initially evaluated with each compound in duplicate at 1, 3, 6, 10, 30, 60, 100, 300, 600, 1000 ng/ml concentrations. Minimum inhibitory concentration (MIC) required to completely eliminate parasites with EQ/EQn mixture, AQ, Q, HQ was found to be 600, 100, 60, 30 ng/ml respectively. Whereas other preparations effectively eliminated all parasites at concentration of 10 ng/ml or less. HQn was the most toxic with 100% killing at 6 ng/ml. Parasites were subjected to at least four or more drug concentrations between the MIC and maximum drug concentration causing no parasite mortality for determination of IC_{50} and IC_{90} values which are presented in the Table. These results clearly depict that many of the quinine-type preparations evaluated were more potent in their erythrocytic schizontocidal action than quinine. Hydroderivatives of quinidine and quinine were more active than parent compounds. HQn and combination containing HQn were the most effective preparations.

DISCUSSION

Increasing failure rate of synthetic drugs to cure malaria has brought quinine back into the focus. It is generally recognized that drug-resistant strains of *P. falciparum* are still sensitive to quinine, although to varying degree, and today use of quinine as an emergency medicament is undisputed (Wernsdorfer 1987). A local FCD-4 isolate was found to be extremely sensitive to quinine in vitro at doses much lower than the mean plasma level of 1.5 µg/ml attainable in a person 4 h after consuming a single dose of 300 mg of quinine (Saggers et al. 1970). Results presented here are well in accordance with the observations of other workers that quinidine is more potent blood schizontocidal than quinine (Hofheinz and Merkli 1984, Phillips et al. 1985). Quinidine was found to be three fold more active than quinine at IC_{50} level. Amount of quinidine needed to completely eliminate parasites in vitro was far below the non-toxic peak quinidine levels achievable in plasma of human subjects (White 1987). Information on the relationship between in vitro antimalarial sensitivity and in vivo response to treatment with quinidine is lacking. Apart from natural cinchona alkaloids the hydroderivative of quinidine, quinine and their combinations show

Table 1

IC_{50}, IC_{90} and MIC values of Quinine and Quinine-type compounds assayed in vitro against a chloroquine resistant isolate of *P. falciparum*

Compound	Concentrations are in ng/ml of culture		
	IC_{50}	IC_{90}	MIC
Quinine	11	48	60
Quinidine	4	5.8	10
Apoquinine	13	78.0	100
Hydroquinine	5.6	9.4	30
Hydroquinidine	2.5	5	6
Quinine/Hydroquinine	3.9	8.4	10
Quinidine/Hydroquinidine	2.2	7	10
Epiquinine/Epiquinidine sulphate	140	270	600

extremely good erythrocytic schizontocidal activity. Some of these compounds also have been demonstrated to possess gametocytocidal activity in vitro (Bhasin and Trager 1987). Advantages of hydroquinine over quinine were also observed by Giemsa and Werner (Giemsa and Werner 1914). In depth investigations are urgently needed to explore antimalarial activity of these and other lead compounds in order to replenish the fast depleting arsenals of effective antimalarials. Large scale deployment of quinine monotherapy as antiprotozoal drug has inevitably resulted in selection of drug resistance (Peters 1990) and therefore utility of other quinine-type compounds in combination with other drugs should be explored further.

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Nuclear Transplantation Induces Heritable Variations in Phenotypes of *Amoeba proteus*

Violetta A. SOPINA and Alexander L. YUDIN

Institute of Cytology, Russian Academy of Sciences, St.Petersburg, Russia

Summary. The present work has found that most clones grown from amoebae with nuclei transplanted from cells of the same or another strain exibit a disappearance of the fast-moving electromorphs of glucose-6-phosphate dehydrogenase (G6PD) at the first testing for this characteristic one or two months after the transplantation. The three original ("parental") *Amoeba proteus* strains used for the transplantations, have such electromorphs permanently and invariably. Subsequently, upon repeated testing of the transplant clones over long periods of time, such electromorphs appeared and disappeared within a clone at irregular intervals, i.e. a kind of phenotypic instability of the clone with time was observed. It seems most likely that each of the phenotypes of a transplant clone can be inherited for more or less prolonged periods of time (many scores of cell generations) before its reversion occurs. Moreover, the instability itself (i.e. the clone's ability to revert) appears to be a novel hereditary trait of the clone. This is the first example of inheritable variations in amoebae caused by the nuclear transfer itself. The nature of the treatment inducing the hereditary instability in the fast-moving electromorphs of G6PD (a micrurgical operation) and the very high frequency of its induction suggest that this hereditary variation is epigenetic (in the sense proposed by Nanney 1958) rather than mutational. Phenomenologically at least, it is comparable with the inducible heritable instability of some hereditary characters previously found in amoebae (reviews: Yudin 1973, 1982).

Key words. *Amoeba proteus*, glucose-6-phosphate dehydrogenase, PAGE, hereditary variation.

INTRODUCTION

A technique of micrurgical nuclear transplantation from one cell into another, as applied to large uninucleate amoebae of the *Amoeba proteus* type, was developed more than 50 years ago (Comandon et de Fonbrune 1939). Shortly thereafter, determination of the respective roles of the cell nucleus and cytoplasm in genetic control of various characters became one of the most important applications of the technique to these agamous protozoans in a number of laboratories (Comandon et de Fonbrune 1942, Danielli et al. 1950, and others - see for review Jeon and Danielli 1971; Yudin 1979, 1982). A typical experiment involved obtaining nucleocytoplasmic "hybrids", i.e. chimaeric cells which combined the nucleus of one amoeba with the cytoplasm of another. That no hereditary changes arise in the transplanted nucleus under the influence of either the operation itself or the cytoplasm of the recipient cell, are essential assumptions for valid conclusions based on experiments of this sort. In fact, non-fulfilment of either of these assumptions makes the whole situation very uncertain and the conclusions reached rather ambiguous.

Address for correspondence: V.A. Sopina, Institute of Cytology, Russian Academy of Sciences, 4 Tikhoretsky Avenue, 194064 St.Petersburg, Russia.

Before long, it was shown that, in certain situations, the cytoplasm of the recipient cell can influence the implanted nucleus of the donor cell in a way that alters its phenogenetic activity in subsequent cell generations (reviews: Yudin 1973, 1982).

As to the possibility that the procedure of nuclear transfer itself could affect the heredity of the operated cell, the progeny of intrastrain nuclear transplants, used as controls, have up to the present been indistinguishable from their original, "parental" forms (Yudin and Sopina 1970, Sopina 1976a, Gorjunova and Kalinina 1977, and others).

This paper presents for the first time experimental evidence that this postulate, which is fundamental to the method of evaluation of the respective role of the cell nucleus and cytoplasm in amoeban heredity with the aid of nucleocytoplasmic "hybrids", can also fail in some cases. We show here that with respect to at least one marker - fast-moving electromorphs (FEMs) of glucose-6-phosphate dehydrogenase (G6PD) - the nuclear-transfer operation itself can induce heritable alterations in transplanted cells.

MATERIAL AND METHODS

Used as experimental material were 3 strains (recloned many times) of uninucleate amoebae *Amoeba proteus*: C, B and L. The amoebae were cultured according to Prescott and Carrier (1964) at 25°C. G6PD was detected by disc electrophoresis in polyacrylamide gel in a standard Tris-HCl, Tris-glycine buffer system with the addition of $NADP^+$ (10 mg/l) to the cathode buffer (Sopina 1989). Stained gels (Sopina 1989) were fixed with 7.5% acetic acid and scanned on a MD-100 microdensitometer ("Carl Zeiss", Germany) in a specially made microcuvette. Peaks on the densitograms were numbered in decreasing order of their electrophoretic mobilities. The relative activity of electromorphs was assessed by the height of the corresponding peaks indicative of light absorption by these fractions, and was expressed as a percentage of the total one. In this paper, the activity will be indicated in arbitrary units (arb. un.).

Transplantation of nuclei was performed with the aid of a MM-1 micromanipulator (USSR) in three ways: (a) in an oil chamber according to de Fonbrune (1949) in a drop of 0.05 M NaCl, the latter adding much to the success of such operations (Yudin 1962); (b) in an oil chamber in a drop of common cultural medium; and (c) on a slide covered with a layer of semi-liquid agar (Jeon and Lorch 1968). The percentage of viable clones obtained using the two latter methods was rather small. From one to six intrastrain transplants or nucleocytoplasmic "hybrids" produced by each method were used for further investigation (Table 1).

The testing of clones grown from the transplanted amoebae was intentionally started at least 2 to 2.5 months after the transplantations to avoid the possible influence of phenomic lag and reveal only true heritable alterations. It was not possible to determine directly the number of cell generations which occurred in these mass cultures during this period of time. But given that at 25°C the generation time of amoebae from original strains ranges from 1.6 to 2.6 days (Sopina 1976b), it may be assumed that cells of the 20th or 30th generation were the first to be tested. Subsequently, most of the tranaplant clones were tested repeatedly, although irregularly, with time, some of them over a period of two years, i.e. even more remote progeny were tested. Thus, any difference between a transplant clone and corresponding "parental" strais, all of them having been cultured under identical conditions, must be considered as hereditary.

RESULTS

A previous comparison of electrophoretic patterns of G6PD in *Amoeba proteus* (Sopina 1989) has shown that in the C and L strains this enzyme is usually represented by three bands (electromorphs), whereas in the B strain it is represented by four, since in these amoebae there are two FEMs (major and minor) instead of only one as in the C and L strains (Fig. 1).

In the C strain, G6PD FEM was observed in all 9 tests carried out with these amoebae; its relative activity varied from 2 to 9 arb.un. In the B strain, activity of the major FEM was from 20 to 50 arb.un. in 9 tests performed at different time. In L amoebae, activity of their single FEM was from 16 to 42 arb.un. In L amoebae the range of the variation of FEM activity was also checked in subclones of the initial mass culture. With a two-time testing of 6 subclones, whose age from the start of subcloning was from 2.5 to 4.5 months, the presence of a single FEM was registered in all the subclones, activities ranging from 16 to 55 arb.un.

Table 1

Clones of descendants from intrastrain transplants and nucleocytoplasmic "hybrids" tested for a marker character - number and activity of G6PD FEMs

Types of clones	Types of operation*					
	1		2		3	
	no. of clones	no. of tests	no. of clones	no. of tests	no. of clones	no. of tests
B_nB_c**	2	1–3				
C_nC_c	2					
L_nL_c	5		2	3–4	6	1
B_nL_c	3	10–12[a]				
L_nB_c	2					
C_nL_c	3		1	3–4	1	1
L_nC_c	2		1		1	1

*See "Materials and Methods"; ** n - nucleus, c - cytoplasm;
[a] in this experiment, one B_nL_c clone and one C_nL_c were tested only three times each.

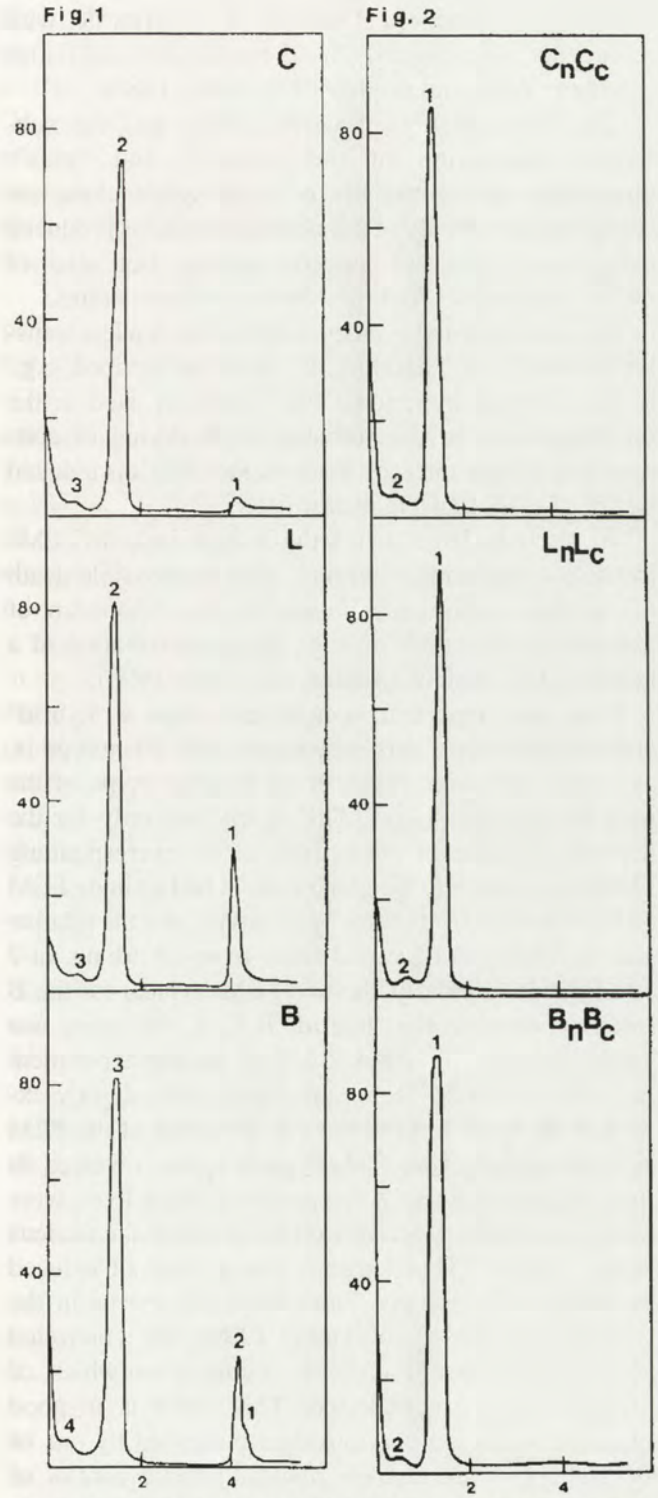

Fig. 1. Densitograms of the G6PD electromorphs in three strains of *Amoeba proteus*. Abscissa - distance from origin, arbitrary units, ordinate - optical density of the bands (percent of total absorption, arb.un.). 1-4 - numbers of bands in decreasing order of their electrophoretic mobilities

Fig. 2. Densitograms of the G6PD electromorphs in three *Amoeba proteus* clones - L_nL_c, B_nB_c and C_nC_c (n - nucleus, c - cytoplasm) expressing the "minus"-phenotype. Designations and axes as in Fig. 1

Thus, all of the strains retain their specific G6PD FEMs (this state will be referred to as the "plus"-phenotype) under standard conditions of cultivation, and reproduce them in subclones, these interstrain differences being undoubtedly hereditary.

Quite unexpectedly, when clones grown from intrastrain transplants were tested, 16 of them showed a complete absence of any G6PD FEMs. This novel phenotype is dramatically different from the original one; it will be referred to as the "minus"-phenotype (Fig. 2). Only one such clone (L_nL_c-4; n -nucleus; c - cytoplasm) had an electromorph and its activity was the same as that of the FEM of the original L amoebae.

The disappearance of G6PD FEMs was found not only in the progeny of intrastrain transplants of the three different types, but also in 14 clones grown from interstrain nucleocytoplasmic "hybrids" (i.e. "hybrids" of L and B amoebae, on one hand, and L and C amoebae, on the other hand). Eight of these "hybrids" have been studied in detail for two years (Table 2).

It was found that most of the "hybrid" clones (except B_nL_c-3 and C_nL_c-2) could have either the "plus"- or the "minus"-phenotype at different periods of the clone's life. Some quantitative variations of the "plus"-phenotype can be noted in different tests; however, the difference between "plus"- and "minus"-phenotypes remains qualitative rather than quantitative.

In all likelihood, either the "plus"- or the "minus"-phenotype may be maintained for many scores of cell generations under identical culture conditions; this means that they are inheritable. But, at the same time, the "plus"- or "minus"-phenotype can change during a transplant clone's life. The cause of such change remains unknown.

Also for unknown reasons, a great majority or even all cells of a culture seem to change their phenotypes more or less simultaneously because we always observed either the "plus"- or the "minus"-phenotype when testing the marker character on a random sample of amoebae from a mass culture.

When a "hybrid" clone exhibits the "plus"-phenotype, the latter is, as a rule, indistinguishable from the phenotype of the "parental" clone which was the donor of the nucleus to the "hybrid". Among "hybrids" shown in Table 2, there was only one exception to the above rule. The clone C_nL_c-4 which was tested 14 times over two years, had the "minus"-phenotype in the first 4 tests. In the subsequent 10 tests, it exhibited the "plus"-phenotype, but in 8 of these tests this phenotype was not in agreement with the original donor nucleus: namely,

Table 2

Phenotypes of "hybrid" clones of *Amoeba proteus* as revealed by repeated testings over a period of two years ("-" "minus"-phenotype; L, B, C designate L-, B-, and C-like "plus"-phenotypes, respectively)

L_nB_c-4	L_nB_c-5	B_nL_c-1	B_nL_c-3	L_nC_c-1	L_nC_c-3	C_nL_c-2	C_nL_c-4
1989							
May-Sep.	Jul.-Aug.	May-Aug.	May-Aug.	Apr.-Jul.	Apr.-Jul.	Apr.-Jul.	Apr.-Aug.
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
L	-	-	-	-	-	-	-
L	-	-	-	-	-	-	B
L	-	-	-	-	-	-	B
L							B
L							B*
1990							
Apr.	Mar.-Apr.	Apr.	Apr.-Aug.	Apr.	Mar.-Apr.	Apr.-Jul.	Apr.
L	L	B**	-	L	L	-	B
L	L	B	-	L	L	-	B
	L		-		L	-	
			-			-	
			-			-	
			-			-	
1991							
Feb.-May	Feb.-Mar.	Mar.	Feb.-Mar.	Feb.-May	Apr.-May	Feb.-Mar.	Mar.-May
-	L	B	-	-	-	-	B
-	L	B	-	-	-	-	C
-				-	-		C
-				-	-		B

*In this experiment, indistinct separation into two FEMs was observed.

**In this experiment, the activity of the major FEM exceeded that in two FEMs were revealed on its densitograms - just as in the B strain - although the latter was not used for producing the "hybrid"! It might be suggested that the "hybrid" clone was identified erroneously, but in two experiments it had the C-like "plus"-phenotype. It seems that the B-like phenotype of the clone is a realization of latent, previously unexpressed genotypic potentialities of its nucleus.

DISCUSSION

This paper presents the first instance in "proteus" type amoebae where the operation of nuclear transplantation by itself induced inheritable variations in the majority of transplants. Such effect was not observed earlier with respect to the previously used marker characteristics (reviews: Yudin and Sopina 1970, Yudin 1982).

The disappearance of G6PD FEMs and the subsequent alternation of the "minus"- and "plus"-phenotypes during the life of a transplant clone are characteristic not only of intrastrain transplants produced using three different amoeba strains, but also of nucleocytoplasmic "hybrids" between these strains.

This phenomenon is independent of the nuclear transfer method used. Therefore, it cannot be ascribed, e.g., to the effect of hypertonic NaCl solution used in the oil chamber or to the probable slight drying of cells operated on agar surfaces. We conclude that it is induced by the nuclear transplantation itself.

We also failed to associate the disappearance of FEMs in nucleocytoplasmic "hybrids" with the possible death of bacterial endobionts present in the cytoplasm of amoebae as the result of, e.g., the transplantation of a heterological nucleus (Sopina and Yudin 1993).

It is very important to note that when a "hybrid" clone exhibits the "plus"-phenotype, this phenotype is, as a rule, indistinguishable from the phenotype of the nuclear "parental" clone. This is true not only for the number but also for the activity of the corresponding FEMs. For instance, the clone L_nB_c-4 had a single FEM after restoration of its "plus"-phenotype, and the relative activity of this FEM varied from 16 to 27 arb.un. in 7 experiments. Similarly, the two FEMs typical for the B strain appeared in the "hybrid" B_nL_c-1, the major one having activity from 20 to 46 arb.un. (in one experiment the activity was 55-58 arb.un. which only slightly exceeded the upper limit of the activity range of the FEM in B amoebae). Thus, "plus"-phenotypes, revealed in "hybrid" clones during some periods of their lives, were L-, C- and B-like depending on the origin of the nucleus in the "hybrid". This suggests that in spite of induced instability of phenotype, interstrain differences in the number and activity of G6PD FEMs are controlled genetically by the cell nucleus - a conclusion which, of course, is *a priori* expected. This result is in good agreement with the previous data published by one of the authors on exclusively nuclear genetic control of the water-soluble esterase pattern in nucleocytoplsmic "hybrids" between various *Amoeba proteus* strain (Sopina 1975, 1978), but it is at variance with the published statement that electrophoretic mobilities of malate dehydrogenase and esterases in "hybrids" between *A. proteus* and *A. indica* are independent of the origin of their nuclei (Chatterjee and Rao 1974). Quite

recently, Friz (1992) reported similar data for a "hybrid" with the *A. indica* nucleus and *A. proteus* cytoplasm tested for a large set of enzymes as a markers. The "hybrid" differred from its nuclear "parent" in 26 of 37 enzymes studied by the author! In our opinion, of real importance at the present time is the cause of this discrepancy rather than a more or less obvious conclusion about nuclear control of the majority of enzymes.

The possibility should not be ruled out that some peculiar properties of the marker under study account for the observed phenomenon. In fact, this enzyme (G6PD) showed unusual variation in some other organisms. We mean irreversible inhibition of G6PD in yeast by penicillin G (Han et al. 1981), or by free fatty acids and thioethers of acyl-CoA in *Trypanosoma cruzi* (Racagni et al. 1989) or reversible changes in electrophoretic G6PD mobility induced by glucose and other sugars in *Daphnia pulex* (Ruvinsky et al. 1983).

To summarize the specific features of those changes in the G6PD electrophoretic patterns of amoebae which are induced by the nucleus transfer operation, we can point out that: (1) the operation induces the disappearance of G6PD FEMs in the progeny of transplanted cells ("minus"-phenotype expression), these FEMs being characteristic of the original amoebae strains, (2) the "minus"-phenotype occurs with a very high frequency - namely, in the overwhelming majority of clones grown from transplanated cells, (3) in all probability, the "minus"-phenotype can be inherited for many scores of cell generations, but (4) in most cases it appears reversible, i.e. the "plus"-phenotype characteristic of the nuclear "parental" clone reappears, and (5) in some cases the "minus"-phenotype and the "plus"-phenotype alternate repeatedly.

The above listed characteristics are in agreement with those described previously as heritable phenotypic instability for some other hereditary characters in amoebae (reviews: Yudin 1973, 1982; Kalinina 1993). Such instability can be induced with high frequency for many characters controlled by the nucleus by various (including *a priori* non-mutagenic) physical or chemical effects on amoebae, and can also be the result of the mutual influence of interphase nuclei in experimentally produced heterokaryotic cells.

Taking into account the characteristic features of this variation (very high frequency, the nature of inducers, etc.), it is assumed that it differs from classical mutations and, therefore, can be considered as epigenetic (Nanney 1958), i.e. as a change in genic activity inherited for many cell generations. Rather substantial material on the phenomenology of this type of variation in amoebae has been accumulated (reviews: Yudin 1973, 1982), but its molecular mechanisms remain totally unknown.

One of the authors of this paper has earlier put forward a hypothesis on the mechanisms of genetic determination and inheritance of destabilizable characters in amoebae (cf. Jeon and Danielli 1971); this hypothesis permits generalizing the bulk of experimental material accumulated from studies on inheritance and variation in these protozoans (Yudin 1979, 1982). The hypothesis is based essentially on the idea of a two-operon genetic trigger (Monod and Jacob 1961). The hypothesis attaches particular importance to continuous shuttling of regulatory molecules between the cell nucleus and cytoplasm as a basis of maintaining stable phenogenetic activity of the nucleus. In terms of this model, "plus"- and "minus"-phenotype, with respect to the electrophoretic pattern of G6PD, represent a realization of alternative potentialities of one and the same genotype, i.e. different reversible phenogenetic differentiations of the amoeba nucleus. For instance, the "plus"-phenotype may be due to the active state of a corresponding gene(s), the "minus"-phenotype - to the inactive one, and the instability of the character with time - to an oscillatory regime of a given genetic system. At present it is difficult to tell to what extent this earlier suggested model is applicable to the data obtained in this study. To answer this question, it is necessary to examine a much wider range of effects for their ability to induce inheritable changes in G6PD patterns as well as to analyze the role of nucleocytoplasmic relationships in these changes.

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Changes of Cellular cAMP Level in *Tetrahymena* during Starvation and Conjugation

Yuhe H. YU, Xin XIANG, Dechang WU and Yuezeng CHEN

Department of Biology, Peking University, Beijing, China

Summary. Changes of cellular cAMP level in *Tetrahymena shanghaiensis*, a selfer species collected in Shanghai, and in *Tetrahymena thermophila* during starvation and conjugation, and in *Tetrahymena pyriformis* GL, an amicronucleate and asexual species, during starvation were studied with the aim to see if dropping of cAMP level is a prerequisite for conjugation. In *T. shanghaiensis* and in mixed cell suspension of mating types I x VII of *Tetrahymena thermophila* during starvation, the cellular cAMP level rose to a peak and then dropped to minimum, when conjugation occurred. When the cellular cAMP level was experimentally prevented from dropping by treating the starved cells with adrenaline or theophylline, conjugation occurred as well in these two species. Starved cells of *T. pyriformis* GL, as well as of *T. thermophila* mating types I and VII in separate suspensions gave the same pattern of cellular cAMP changes although no conjugation occurred. It seems that it is the starvation that results in the fluctuation of the cellular cAMP level, while the change of the cellular cAMP level seems not to be directly connected with the occurrence of conjugation in *T. shanghaiensis* and *T. thermophila*.

Key words. cAMP, *Tetrahymena* spp., starvation, conjugation.

INTRODUCTION

In a previous paper (Ding et al. 1987), we have reported dropping of cellular cAMP level of the selfer species of *Tetrahymena shanghaiensis* (formerly T. S1, Feng et al. 1987) during starvation and conjugation. Further experiments were carried out to see if dropping of cellular cAMP is a prerequisite for conjugation in *Tetrahymena*. In addition to *T. shanghaiensis*, *T. pyriformis* GL, an amicronucleate and asexual species, and *T. thermophila* mating types I and VII were also used for the study. The changes of cellular cAMP level in *T. shanghaiensis* and *T. thermophila* during starvation and conjugation and in *T. pyriformis* GL during starvation were examined and the results compared.

Address for correspondence: Y. H. Yu, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, P.R. China.

MATERIAL AND METHODS

Tetrahymena shanghaiensis was collected in Shanghai and has been maintained axenically in the laboratory for nearly 10 years (Chen et al. 1982). *T. pyriformis* was obtained from W.J. Pan who brought it back from E. Blackborn's laboratory at Berkeley, USA, and *T. thermophila* from E.M. Simon of Illinois University, USA.

Induction of conjugation by starvation

The medium used for starvation was 10 mM Tris-HCl buffer (pH7.4) or a phosphate-citrate solution ($NaH_2PO_4 \cdot 2H_2O$, 0.21g; $Na_2HPO_4 \cdot 12H_2O$, 0.35g; Na citrate $\cdot 5H_2O$, 0.67g, in 1000 ml of

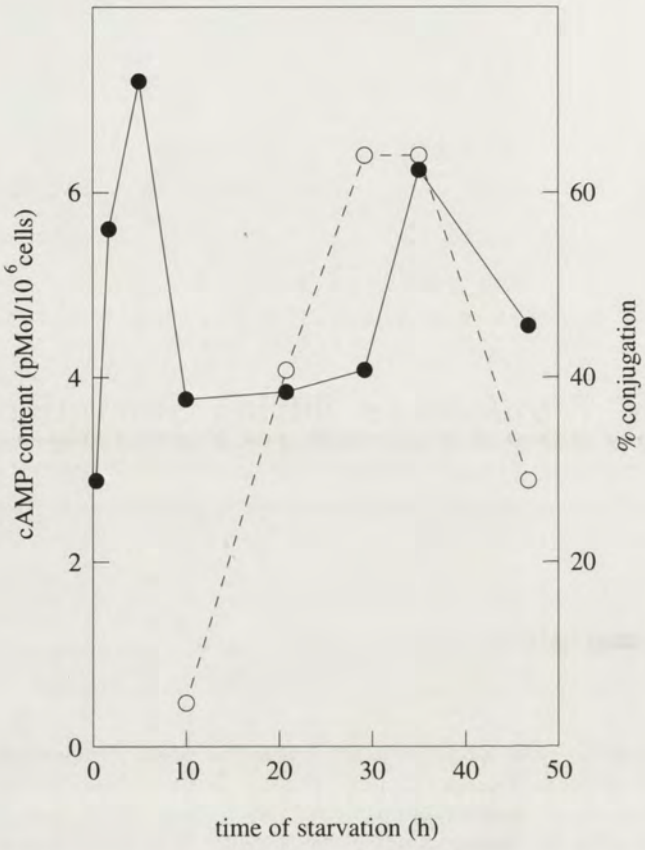

Fig. 1. *Tetrahymena shanghaiensis* in 10 mM Tris-HCl. (—) cAMP content; (– –) %conjugation

dist. water). Cells at late logarithmic growth were collected by centrifugation and, after a brief washing in 10 mM Tris-HCl buffer or in the phosphate-citrate solution, were resuspended in fresh starvation medium at the density of approximately $1x10^5$ cells/ml and left in a 25°C incubator for examination. Starved cell suspensions were examined at frequent intervals and the number of the mating pairs appeared counted under a stereomicroscope. The time from the beginning of starvation to the appearance of mating pairs varied considerably at different time of the year. Under the experimental conditions in this work, it fluctuated between 5 hours and 17 hours and was mostly about 10 hours.

Measurement of cellular cAMP

Cellular cAMP content was measured by radioimmunoassay following the methods given by Steiner et al. (1969) and Brooker et al. (1979), with modifications by Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. The radioimmunological kits were purchased from the Institute.

10 ml of the cell cultures each were collected for assay and, after washing in the Tris-HCl buffer or in the phosphate-citrate solution, were concentrated to about 1ml by centrifugation. To each sample, an equal volume of 12% trichloracetic acid was added. The supernatant obtained was extracted with water-saturated diethyl ether to remove the lipids and was then vapor-dried or freeze-dried for cAMP measurement.

A series of standard cAMP solutions of 8, 4, 2, 1, 0.5 and 0.25 pMol respectively was prepared by dilution of the stock solution with T E buffer (50 mM Tris pH 7.4, 4 mM EDTA) or 50 mM Na acetate buffer(pH 4.5). The radioactive tracer (^{3}H-cAMP) solution was made by immersing ^{3}H-cAMP-bound-paper in the T E buffer or in the Na acetate buffer. The radioactivity of the solution was adjusted to 4000 cpm per 50 µl. Antiserum solution used in the assay was prepared by dilution of the stock to a concentration when about 40-60% of the radioactive tracer could be bound by the antiserum solution added.

The measurement was carried out as follows: the dried cAMP samples were redisolved in the T E buffer or in the Na acetate buffer. To each test tube, 50 µl of standard cAMP solution or sample for cAMP measurement, 50 µl of ^{3}H-cAMP solution and 100 µl of antiserum solution were added successively. The test tubes were shaken mildly and left for 3 hours in an ice bath. Binding reaction was stopped by addition of 100 µl of charcoal suspension (50 mg/ml in the T E buffer, containing 20 mg/ml bovine serum albumin). The reacting fluid was centrifugated and 200 µl of the resulting supernatant was collected into a scintillation vial and the radioactivity measured by means of a liquid scintillation counter (LKB, Wallace).

An alternative method used is to pass the reacting fluid through a milliporous filter (0.45 µm) and reactivity of the filter measured.

By comparison with the standard cAMP curve, the cAMP content of the sample could be obtained which was converted to concentration in pMol/10^6 cells by the following equation:

$$cAMP\ (pMol/10^6\ cells) = \frac{sample\ cAMP\ (pMol/ml)}{No.cells\ in\ sample/ml} \times 10^6$$

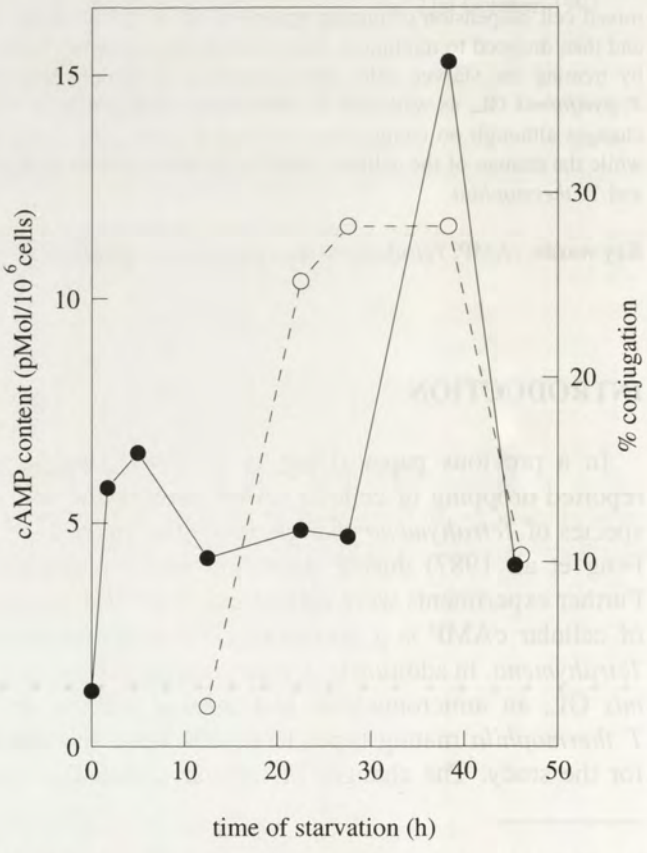

Fig. 2. *Tetrahymena thermophila* mating types I x VII in 10 mM Tris-HCl. (—) cAMP content; (– –) %conjugation

RESULTS AND DISCUSSION

1. Change of cellular cAMP in species capable of conjugation

1.1 *Tetrahymena shanghaiensis* in 10 mM Tris-HCl buffer

Cellular cAMP level rose to a peak (~7 pMol/10^6 cells) in the first 4 or 5 hours of starvation and then dropped to minimum (3.6 pMol/10^6 cells) at about the 10th hour, when mating pairs began to appear (Fig.1). It remained low until the number of the mating pairs increased to maximum (64%), when it rose abruptly to about 6 pMol/10^6 cells. Then it dropped and, at the same time, the mating pairs began to separate.

1.2 *Tetrahymena thermophila* mating types I x VII in 10 mM Tris-HCl buffer

Measured volumes of late logarithmically growing cultures of mating types I and VII of *T. thermophila* were mixed and, after centrifugation and washing, were resuspended in the Tris-HCl buffer. The numbers of the cells of the two mating types were maintained at 1:1 approximately. Cellular cAMP level of the mixed cell suspension was measured at intervals of 5 or 10 hours. The curve obtained (Fig.2) was similar to that from *T. shanghaiensis* (Fig.1). Cellular cAMP level increased in the first 5 hours of starvation to a peak value (~7 pMol/10^6 cells), then dropped to minimum at about the 10th hour, and remained low for about 15 hours.

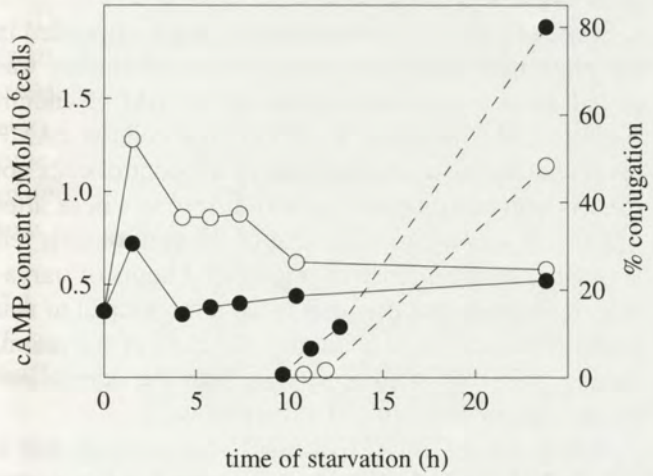

Fig. 3. *Tetrahymena shanghaiensis* in phosphate-citrate solution containing theophylline (10 μM) (– –) %conjugation; (—) cAMP content; O theophylline; ● no theophylline

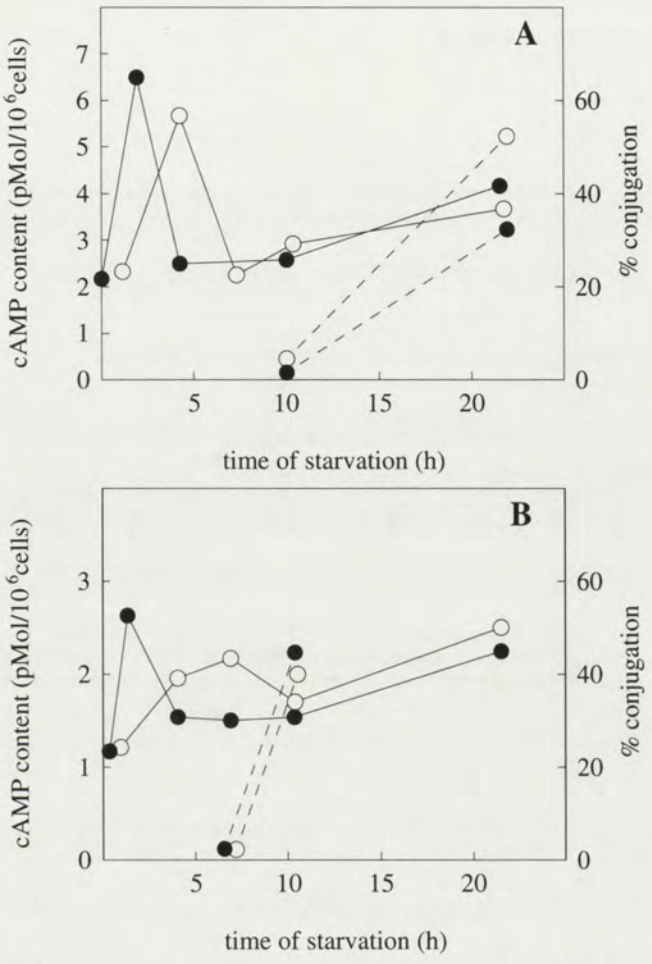

Fig. 4. *Tetrahymena shanghaiensis* in phosphate-citrate solution containing adrenaline (10 μM) A. one dose; B. three doses; each equal to that in A; (– –) %conjugation; (—) cAMP content; O adrenaline; ● no adrenaline

Mating began to occur after 10 hours of starvation, and when the number of the mating pairs increased to maximum, cellular cAMP level rose abruptly to a second climax (15.6 pMol/10^6 cells). Then it fell and the mating pairs began to separate simultaneously.

Similar results were obtained in cells of *T. shanghaiensis* and *T. thermophila* I x VII in the phosphate-citrate solution.

2. Effect of theophylline and adrenaline

In the experiments described above, conjugation occurred only when cellular cAMP level dropped. Is dropping of cellular cAMP level a prerequisite for conjugation? We tried to answer this question by experimentally preventing dropping of cellular cAMP level and to see if conjugation could occur. It has been noted that in vegetative population of *Tetrahymena*,

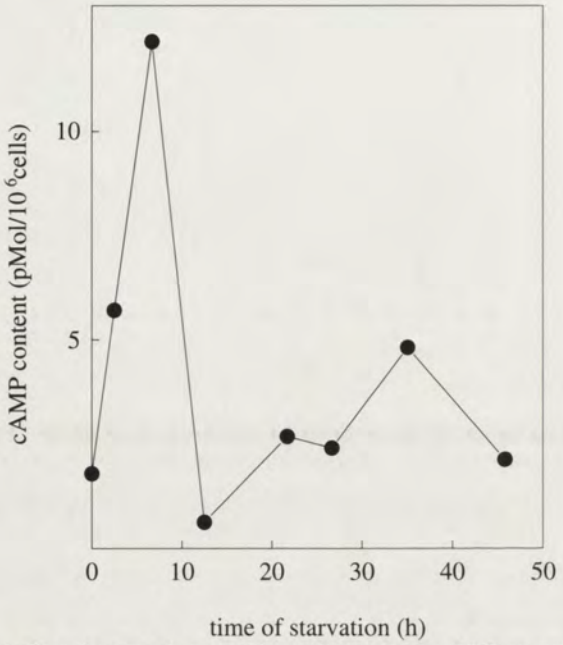

Fig. 5. *Tetrahymena thermophila* mating types I and VII suspended separately in 10 mM Tris-HCI, and equal volumes of the two suspensions were mixed for measurements, no conjugation

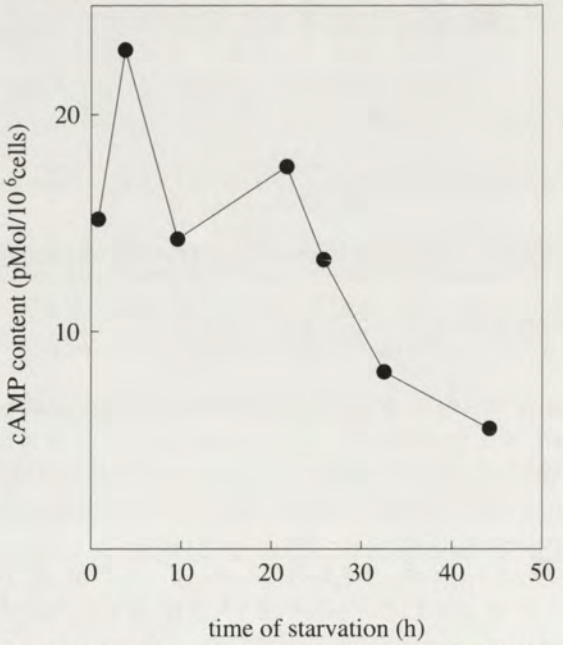

Fig. 6. *Tetrahymena pyriformis* GL in 10 mM Tris-HCI, no conjugation

dropping of cellular cAMP level is correlated with rising of phosphodiesterase activity and dropping of adenylate cyclase activity (Voichick 1973). Therefore, we treated the starved cells with theophylline or adrenaline, of which the former has the effect of inhibiting the activity of phosphodiesterase and the latter stimulating that of adenylate cyclase, to see whether conjugation did occur or not.

2.1 Theophylline

Washed *T. shanghaiensis* cells were suspended in the phosphate-citrate solution to which theophylline (Ramanathan and Chou 1973) was added to a final concentration of 10 μM. Another sample of washed cells was suspended in the phosphate-citrate solution without theophylline as control.

The cellular cAMP levels in both the experimental and the control cell suspensions rose to the highest at about the first hour of starvation, but the peak value of the cell suspension treated with theophylline was higher than that of the cell suspension not treated with theophylline (Fig. 3). At the 4th hour of starvation, the cAMP level of the control suspension fell to minimum (0.36 pMol/106 cells), and after another 5 hours, mating pairs began to appear. The cAMP level of the cell suspension in medium with theophylline also fell at the 4th hour of starvation, but it remained at a level even higher than the peak value of the control. Only after 10 hours of starvation did it drop to minimum still slightly higher than that of the control, and cells began to pair.

A comparison of the two curves in Fig. 3 gives us the impression that, either treated or not treated with theophylline, cells began to mate in nearly the same time of starvation. The length of the time of the lowest cellular cAMP level seemed not to be important to conjugation, although conjugation only occurred, in this experiment, when cellular cAMP level dropped to minimum.

2.2 Adrenaline

2.2.1 One dose of adrenaline given at the beginning of starvation

Washed cells of *T. shanghaiensis* were suspended in the phosphate-citrate solution to which adrenaline was added to a final concentration of 10 μM (Nandini-Kishore and Thompson Jr. 1979). The cellular cAMP level rose more slowly than that of the control cells not treated with adrenaline (Fig. 4A). It rose to a peak after 4 hours of starvation, when that of the control cells fell from peak to minimum. After another 3 hours of starvation, it dropped to a minimal value almost equal to that of the control cells. At about the 10th hour of starvation, mating pairs began to appear in both the adrenaline-treated and the control cell suspensions.

The result of this experiment also showed that a certain time of starvation was required for the occurrence of conjugation, but the length of the time when the lowest cAMP level of the cells was maintained

seemed not to be critical for conjugation. This led us to think that dropping of cellular cAMP level to minimum may not be a prerequisite for conjugation. This was proved by the next experiment.

2.2.2 Three doses of adrenaline given successively

At the beginning of starvation, one dose of adrenaline was added to the cell suspension to a final concentration of 10 μM. Another two doses each equal to that of the first one were added at the 4th and the 7th hour of starvation respectively, with the aim to see if mating could occur when the cellular cAMP was maintained at a high level (Fig. 4B). Mating pairs began to appear after the 7th hour of starvation in both the cell suspensions with and without adrenaline, but then the cellular cAMP level of the former was at the peak while that of the latter had fallen to minimum.

3. Changes of cellular cAMP of starved cells without conjugation

Dropping of cellular cAMP level to minimum may not be a prerequisite for conjugation, so it is reasonable to examine the changes of cellular cAMP of starved cells without conjugation to see if the pattern of cellular cAMP change of the cells shown above is caused merely by starvation without the intervention of conjugation.

3.1 Cells of *T. thermophila* mating types I and VII suspended separately in 10 mM Tris-HCl buffer

The curve obtained (Fig. 5) was comparable to that from mixed I x VII cell suspensions (Fig. 2), i.e., the cAMP level rose to its climax in the first 5 hours of starvation, and after another 20 or 30 hours (at the same time when in mixed I x VII cell suspension, the number of mating pairs increased to maximum), it rose again to a second peak.

3.2 Cells of non-conjugating amicronucleate species *T. pyriformis* GL suspended in 10 mM Tris-HCl buffer

The fluctuation of cellular cAMP (Fig. 6) level was similar to that in *T. shanghaiensis* and in *T. thermophila* (mating types), i.e., it dropped sharply after the first rising, then rose to a second climax.

As the pattern of the fluctuation of cellular cAMP level in starved cells without the occurrence of conjugation was similar to that in starved cells of mixed mating types of *T. thermophila* and in selfer *T. shanghaiensis* in which conjugation occurred, it can be concluded that it is the starvation which causes the fluctuation of the cellular cAMP level.

The results of the experiments described above can be summarized as follows: (1) Dropping of cellular cAMP level during starvation occurs not only in *T. thermophila* and in the selfer species *T. shanghaiensis*, but also in an amicronucleate and asexual species, *T. pyriformis* GL; (2) The patterns of the change of cellular cAMP level in all the three species of *Tetrahymena* during starvation are similar and are not necessarily correlated with the occurrence of conjugation; (3) Conjugation occurs as well if the cellular cAMP of the starved cells of *T. shanghaiensis* maintains at a high level during starvation; (4) Therefore, starvation induces conjugation and also affects the change of cellular cAMP level, but change of cAMP level seems not to be a prerequisite for the occurrence of conjugation.

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The Life Cycle of *Tetrahymena rostrata* (Ciliata) in the Laboratory

Leszek SZABLEWSKI

Department of General Biology and Parasitology, Institute of Biostructure, Medical Academy, Warsaw, Poland

Summary. The processes of encystment and excystment in *Tetrahymena rostrata* were investigated. Bacteria were important, but not necessary, for the production of cysts. The excystment depends on the temperature and the composition of the nutrient medium affects the duration of this process. The duration of the postexcystment stages depends on the presence of bacteria and composition of medium. These factors have little effect on the morphological parameters of the trophozoites of *T. rostrata.*

Key words. *Tetrahymena rostrata*, encystment, excystment, morphological stages

INTRODUCTION

Tetrahymena rostrata (Ciliata) occurs as a free-living protists in nature and as a facultative parasite in widely diverse hosts (Corliss 1960, 1973; Kazubski and Szablewski 1978). Although there are few studies on morpho-physiological variability of *T. rostrata* produced by the environmental conditions, this species has considerable potential as an experimental organism in several areas of cellular research.

Production of cysts (especially **resting** cysts) is necessary in life cycle of *T. rostrata*. During the cyst stage the process of autogamy takes place. If this process does not occur the cells die (Corliss 1965, Dobra et al. 1980, McArdle et al. 1980). In a previous paper on this subject these observations were confirmed (Szablewski and Oleszczak 1992). Here I investigate the question: what affects the processes of encystation and excystation in *T. rostrata*?

Address for correspondence: L. Szablewski, Department of General Biology and Parasitology, Institute of Biostructure, Medical Academy, 5 Chałubinskiego Str., 02-004 Warsaw, Poland

There are few stages in life cycle of *T. rostrata* (Corliss 1973). It is possible to distinguish four types recognized by Stout (1954) and Corliss (1973), viz., **theront**, **trophont**, **tomont** and **tomite**. All these forms are specific in morphology and physiology. The theront is an almost cylindrical, narrow, slightly compressed form. The trophont is larger, more "pyriform" with a more acutely pointed anterior end. The tomont is the quiescent division stage which produces the tomites. This form can metamorphose into the theront stage. The theront is attracted for example by peptone or by histolyzed tissue. Feeding transforms it into a trophont. Theronts and trophonts encyst in the absence of food.

During investigations in the natural environment (Szablewski unpublished) and in the laboratory (Szablewski and Oleszczak 1992), the theront stage was not found. This indicates that the theront stage is short.

Which environmental conditions have an effect on the duration of theront stage? Do environmental conditions affect the other stages? These questions are related to investigations on natural infection of hosts by *T. rostrata*. In *Zonitoides nitidus* (Mollusca, Gastropoda)

trophont and sometimes tomont stages were found (Szablewski unpublished). If the process of excystment depends on the environmental conditions, which effects have these conditions on morphology of *T. rostrata*?

The investigations were aimed especially at the life cycle of *Tetrahymena rostrata* in the laboratory compared to natural environmental conditions.

MATERIAL AND METHODS

Cultivation of ciliates

The experiments were carried out on a laboratory strain of *Tetrahymena rostrata*, established from parasitic populations (Oleszczak and Szablewski 1992). The ciliates were cultivated in tubes containing 5 ml of medium (1.5% proteose peptone + 0.1% yeast extract + salts) PPYS. The salt containing medium was prepared according to the method of Plesner et al. (1964). The cultures were placed in an incubator at 20°C. The sterilization of PPYS was made by autoclaving at 121°C for 30 min.

Encystment and excystment

Tetrahymenas were cultivated in Erlenmeyer flasks containing 50 ml of PPYS and were subcultivated every day. Twenty four hours after the last inoculation, *Lactobacillus bifidus* was added as food. In this medium the ciliates remained for 24 hours and were then washed free of bacteria several times with Ringer solution (4 min., 1000 rpm.). After this procedure tetrahymenas were transferred into Petri dishes with nonnutrient medium (1.5% agar (Difco)) (McArdle et al. 1980). The cells remained in this medium for about 1 month. During this time, the cultures dried up and trophozoites were not observed. For investigation of excystment, the following procedures were used: culture A: 0.4% NaCl solution was added and culture was kept at 20°C; culture B: 0.4% NaCl solution, 4°C; culture C: 0.4% NaCl solution + *Lactobacillus bifidus,* 20°C; culture D: 0.4% NaCl solution + *Lactobacillus bifidus,* 4°C; culture E: PPYS, 20°C; culture F: PPYS, 4°C; culture G: PPYS + *Lactobacillus bifidus*, 20°C and culture H: PPYS + *Lactobacillus bifidus*, 4°C.

The time of medium addition is referred to as 0 h. Cultures were investigated at preselected time points (Fig. 1). The number of ciliates and their distribution stages at each time point were observed by stereoscope microscopy.

Morphology

The observations were performed on *T. rostrata* incubated for 50.5 h in one of the above mentioned media. Specimens for observations were prepared using a Chatton-Lwoff procedure modified by Frankel and Heckmann (1968). The measurements were taken in accordance with the system and nomenclature proposed by Taylor et al. (1976):

AM1 - from anterior end of cell to the cytostome
AUM - from anterior end of cell to the posterior end of cytostome
WLEN - from anterior end of cell to maximal width
LENGTH - maximal length between anterior and posterior ends of *Tetrahymena*
WIDTH - maximal width of cell
BDUM - width of cell on the level of posterior end of cytostome
BDM1 - width of cell on the level of anterior end of cytostome
CYT - maximal length of cytostome
%AWD-%BDM1/BDUM %PWI-%WIDTH/BDUM %AM1-%AM1/LENGTH
%AUM-%AUM/LENGTH %WLN-%WLEN/LENGTH %BRD-%WIDTH/LENGTH
%BUC-%CYT./LENGTH %BDM-%BDUM/LENGTH %BD1-%BDM1/LENGTH
The investigated sample contained ca. 50 specimens.

RESULTS

After transfer of the tetrahymenas to nonnutrient medium, the ciliates ceased their random swimming and began to aggregate. The aggregation was generally observed within about 12 hours, then the ciliates bècame rounded, and after few days free swimming trophozoites were no longer observed.

At different times after 0 h the ciliates began excystment. The duration of the excystment period depended on the conditions of cultivation. This process was first completed in culture C (2.5 h after 0 h), then in culture E (3.5 h) and in cultures A and G (7.5h) (Fig. 1). In cultures remaining at 4°C, the process of excystation was not observed.

The increase in number of free swimming ciliates was also different in particular cultures. The highest increase was observed in culture C, lower in cultures A, E and G, in that order (Fig. 1).

The results of experiments on duration of theront stage are presented in Fig. 2. In all cultures, at first only theronts were observed. In cultures C and E this stage was observed 2.5 h, and in cultures A and G 7.5 h after 0 h. The investigations at the next time points indicated a decrease in number of theronts and an increase in number of other stages. The duration of the theront stage was different in the investigated cultures. This time was comparatively long in cultures C and E (about 29 h) and shorter in cultures A and G (about 24 h). In this case, the moment at which early theront stages occurred was different in particular cultures, but the moment of the completion of this stage was the same (about 32 h after 0 time). Theronts were, however, seen just as long (about 29 h), but the duration of large numbers of this stage was in all cases

about 2 h. After this time, the fraction of theronts in the cultures rapidly decreased.

The conditions of incubation of *T. rostrata* after excystment had little effect on morphological parameters (Tables 1 and 2). Only in culture E were the ciliates bigger than in other cultures (compare LENGTH, WIDTH and WLEN). In other cultures the observed differences were not significant. Note that the number of cells in particular stages (without theronts) was the same in all cultures.

Table 1

Values of morphological data in accordance with system and nomenclature proposed by Taylor et al. (1976) in investigated cultures of *T. rostrata*. Length - maximal length between anterior and posterior ends of *Tetrahymena*. Width - maximal width of cell. Cytostome - maximal length of cytostome. A-M1 - from anterior end of cell to cytostome. A-UM - from anterior end of cell to posterior end of cytostome. BDM1 - width of the cell at the level of anterior end of cytostome. BDUM - width of the cell at the level of posterior end of cytostome. WLEN - from anterior end of cell to maximal width of cultures A, C, E and G as in Fig. 1

		CULTURE			
		A	C	E	G
Length	Mean	51.44	52.61	56.48	54.52
	Min.	40.60	32.80	43.70	43.70
	Max.	62.40	62.50	71.90	68.70
	SD.	4.33	6.18	6.75	5.62
Width	Mean	32.53	31.06	38.49	33.32
	Min.	25.00	21.80	26.50	25.00
	Max.	43.70	53.10	57.80	40.60
	SD.	4.67	4.78	5.71	3.77
Cytostome	Mean	10.29	10.38	10.42	9.92
	Min.	7.80	7.80	9.40	7.80
	Max.	14.00	12.50	11.00	12.50
	SD.	1.33	0.95	0.77	0.88
A-M1	Mean	3.14	3.58	3.32	2.67
	Min.	1.60	1.60	1.60	1.60
	Max.	4.70	6.20	6.20	4.70
	SD.	0.66	0.85	0.86	0.75
A-UM	Mean	13.42	13.74	13.68	12.62
	Min.	9.40	7.80	11.00	11.00
	Max.	17.10	17.10	17.30	15.60
	SD.	1.59	1.45	1.18	1.05
BDM1	Mean	7.85	10.09	11.19	8.87
	Min.	3.10	4.30	4.90	4.60
	Max.	12.40	14.00	34.30	12.50
	SD.	2.05	1.83	4.98	1.93
BDUM	Mean	20.24	20.96	25.16	21.55
	Min.	15.60	15.60	15.60	17.10
	Max.	31.20	28.10	46.90	28.10
	SD.	3.06	2.40	5.52	2.80
WLEN	Mean	29.87	29.97	31.19	30.19
	Min.	23.40	15.60	21.80	23.40
	Max.	37.50	39.00	40.60	37.50
	SD.	2.76	4.32	4.35	4.14

DISCUSSION

These results indicate that the conditions of the environment have an effect on encystment and excystment of *Tetrahymena rostrata*. Corliss (1965) suggested that the presence of bacterial food in the culture is helpful for excystment. The results presented here confirm this suggestion. Ciliates grown in peptone broth sometimes formed temporary cysts (Stout 1954, Szablewski and Oleszczak 1992). If bacterial food was added to the medium, the process of encystment was successful, despite the methods of encystation (McArdle et al. 1980, Szablewski unpublished). Excystment takes place quite readily. This process is seen in cultures at different conditions of cultivation, but temperature plays a role. If cysts remained at 4°C., this process was not observed, although the other factors (composition of medium) were appropriate for excystment.

The composition of medium has an effect on completion of the cyst stage. The results suggest that the presence of bacteria in the medium accelerates the excystment. Comparison of cultures A and E in Fig. 1 confirms this observation. On the other hand there were no observed differences between cultures E and G (Fig. 1). In this case, the presence of bacteria was not important. For excystment 0.4% NaCl solution fortified with bacteria is better than nutrient medium (PPYS or PPYS with bacteria).

Other correlations were made in experiments on the duration of the theront stage. In this case the presence of bacteria was not important. In culture C (0.4% NaCl solution + bacteria) the duration of this stage was longer than in culture A (only 0.4% NaCl solution). On the other hand, if we compare cultures E and G, the presence of bacteria makes the duration of this stage shorter. It is possible that other factors play a role in this case.

Is it possible to compare of life cycle of *T. rostrata* in the laboratory with the life cycle in the natural environment? During investigations of infection of natural hosts (*Zonitoides nitidus*) in nature, only trophont stages were observed (Szablewski unpublished). The observations were performed only from late spring to early autumn. Probably cyst stages appear during winter (in snail or in soil?). The excystment takes place at the appropriate environmental temperature. After this process comes the very short theront stage (in snails or in soil?) and then the trophont stage in snails. The presence of ciliates in snails probably greatly prolongs the duration of trophont stage. The tomont stage may be present in the host, until maximum density of parasites

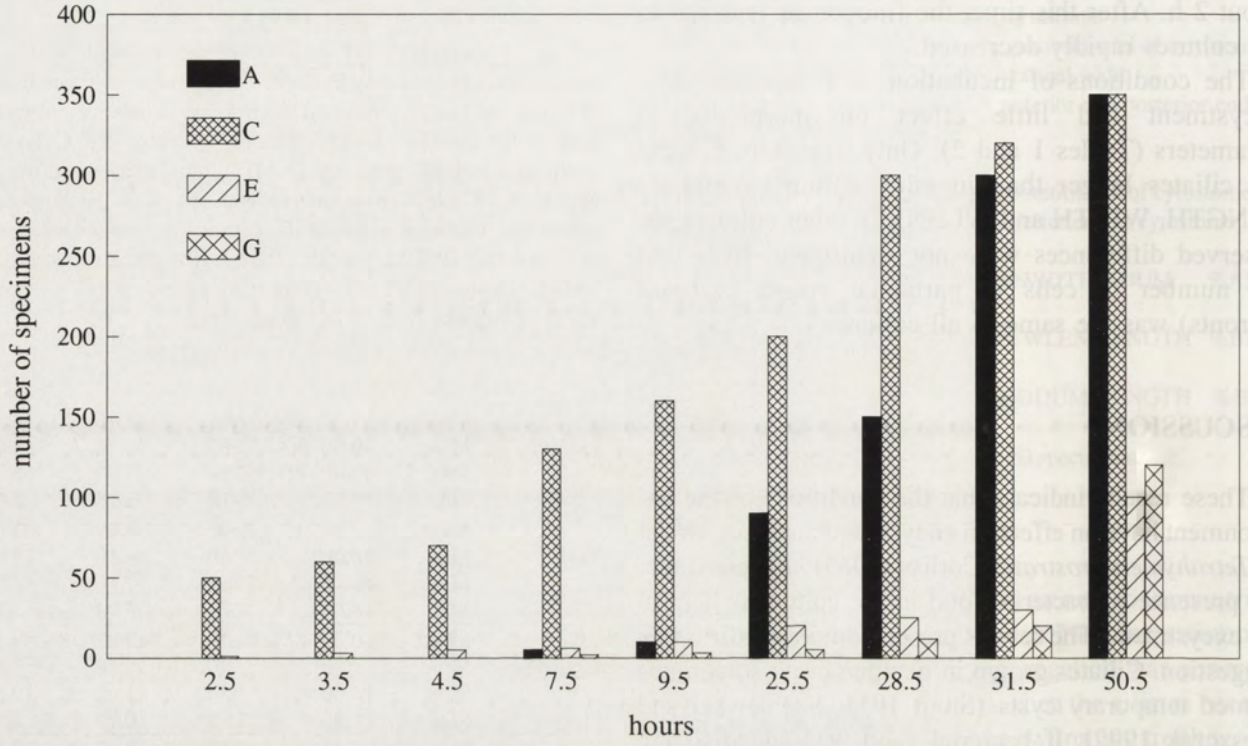

Fig. 1. Number of free swimming specimens as a function of conditions of excystment and cultivation. Culture A - 0.4% NaCl solution was added and culture was kept at 20°C; culture C - 0.4% NaCl solution + *Lactobacillus bifidus*, 20°C; culture E - PPYS, 20°C; culture G - PPYS + *Lactobacillus bifidus*, 20°C

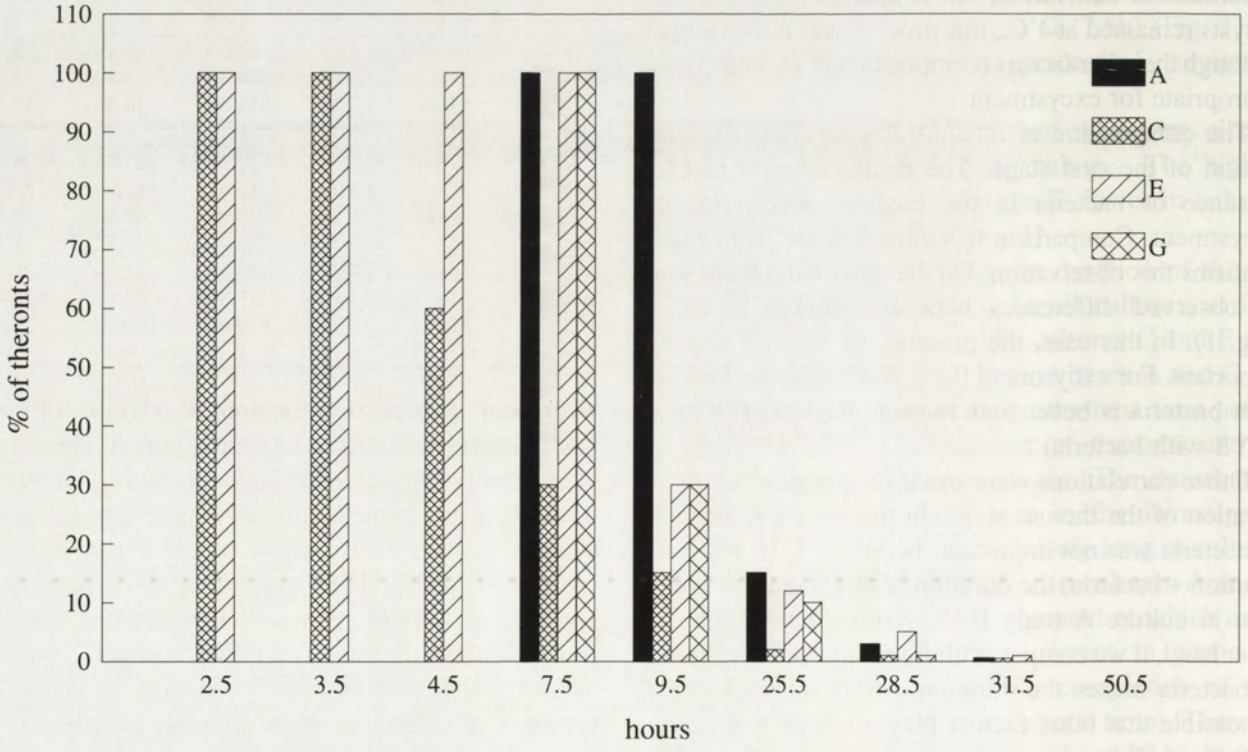

Fig. 2. Percentage of theronts in particular cultures (A, C, E and G as in Fig. 1) as a function of conditions of excystment and cultivation

Table 2

Values of morphological data in accordance with system and nomenclature proposed by Taylor et al. (1976) in investigated cultures of *T. rostrata*. % AWD – % BDM1/BDUM, % PWI – % WIDTH /BDUM, % AM1 – % AM1/LENGTH, % AUM – % AUM/LENGTH, % WLN – % WLEN/LENGTH, % BRD – % WIDTH/LENGTH. Cultures A, C, E and G as in Fig. 1

		CULTURE			
		A	C	E	G
% AWD	Mean	38.74	48.43	43.71	41.49
	Min.	19.87	19.72	22.47	19.65
	Max.	54.18	72.67	73.58	61.57
	SD.	8.12	8.85	11.71	8.82
% PWI	Mean	162.15	148.37	156.48	156.09
	Min.	129.43	123.15	112.89	123.15
	Max.	239.74	212.40	229.48	200.58
	SD.	21.65	15.91	25.38	19.74
% AM1	Mean	6.11	6.80	5.95	4.88
	Min.	3.01	4.87	3.20	2.92
	Max.	9.40	10.37	13.68	7.16
	SD.	1.34	1.50	1.77	1.20
% AUM	Mean	26.18	26.26	24.55	23.41
	Min.	20.52	17.21	18.19	18.19
	Max.	34.20	33.33	38.18	28.60
	SD.	3.20	3.03	3.63	2.48
% WLN	Mean	58.16	56.55	55.29	55.22
	Min.	50.00	47.56	42.24	37.18
	Max.	66.38	64.03	65.56	68.55
	SD.	3.96	3.57	5.04	5.00
% BUC	Mean	20.07	19.91	18.81	18.45
	Min.	14.68	15.04	13.98	13.68
	Max.	28.00	25.61	25.17	25.17
	SD.	2.63	2.48	2.48	2.53
% BRD	Mean	62.94	59.16	68.34	61.62
	Min.	48.44	45.84	51.35	48.62
	Max.	82.29	87.19	84.60	75.58
	SD	6.43	6.68	7.84	6.71
BDM	Mean	39.44	40.18	45.01	40.10
	Min.	29.37	29.75	30.31	27.36
	Max.	57.03	61.89	70.86	54.45
	SD.	5.51	5.70	10.48	6.85
% BD1	Mean	15.23	19.25	19.93	16.33
	Min.	7.63	8.60	8.47	8.66
	Max.	23.40	28.60	51.65	23.54
	SD.	3.69	3.45	8.75	3.27

is attained when they transform into trophonts. This conclusion is compatible with Stout's (1954) investigations. He observed rapid division of ciliates within the enchytraeids after infection by *T. rostrata*.

The result that only trophonts were observed in snails may be due to the fact that the trophont stage is dominant in life cycle of *Tetrahymena rostrata*.

How can we explain so small morphological differences between ciliates from different cultures? Probably these differences could be greater but only a during longer time of cultivation of *T. rostrata* at different conditions. Maybe it is important that the ciliates were in the same conditions before encystment? This is one of the many questions awaiting further research.

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First Record of the European Trichodinids (Ciliophora: Peritrichida), *Trichodina acuta* Lom, 1961 and *T. reticulata* Hirschmann et Partsch, 1955 in South Africa

Linda BASSON and Jo G. VAN AS

Department of Zoology-Entomology, University of the Orange Free State, Bloemfontein, South Africa

Summary. At least 20 species of freshwater fish have so far been introduced into southern Africa. These have been responsible for the introduction of at least two parasite species; now widespread in southern Africa, i.e., *Argulus japonicus* Thiele, 1900 and *Bothriocephalus acheilognathi* Yamaguti, 1934. No introductions of ectoparasitic trichodinids were reported from the subcontinent until now. In the present paper confirmed cases of two introductions of trichodinids, i.e., *Trichodina acuta* Lom, 1961 from the rainbow trout, *Oncorhynchus mykiss* (Walbaum) and *T. reticulata* Hirschmann et Partsch, 1955 from the goldfish, *Carassius auratus* Linnaeus are presented and taxonomic and morphometric data is provided.

Key words. Introduced trichodinids, *Trichodina acuta*, *T. reticulata*, South Africa.

INTRODUCTION

The southern African freshwater ichthyofauna comprises some 200 species of which 20 are alien, introduced to this region for various purposes (Bruton and Van As 1986). These introductions include the goldfish, *Carassius auratus* Linnaeus and the rainbow trout, *Oncorhynchus mykiss* (Walbaum). It is inevitable that at least some fish parasites were introduced with this large number of introduced fish species. Well documented cases of such introductions are those of *Argulus japonicus* Thiele, 1900 and *Bothriocephalus acheilognathi* Yamaguti, 1934, both originally from the Far East (Brandt et al. 1981, Kruger et al. 1983). The only records to date of trichodinid introductions to southern Africa were that reported earlier by us (Basson et al. 1983, Basson and Van As 1987), where it was reported that *Trichodina acuta* Lom, 1961, *T. pediculus* Ehrenberg, 1838, *T. nigra* Lom, 1961, *T. mutabilis* Kazubski et Migała, 1968 and *Trichodinella epizootica* (Raabe, 1950) Sramek-Husek, 1953 were found on fish populations in Transvaal. A subsequent evaluation showed that some of these identifications were incorrect and that the parasites thought to be *T. acuta* was a new species, i.e., *T. compacta* Van As et Basson, 1989. Alleged populations of *T. pediculus* found in southern Africa proved to be a new species, i.e., *T. magna* Van As et Basson, 1989. Trichodinids identified as *T. mutabilis* was subsequently described as two species, i.e., *T. uniforma* Van As et Basson, 1989 and *T. kazubskii* Van As et Basson, 1989. This left only *T. epizootica* and *T. nigra* as possible

Address for correspondence: J.G. Van As, Department of Zoology-Entomology, University of the Orange Free State, Bloemfontein, 9300, South Africa.

Table 1

Biometrical data (in µm) of *Trichodina acuta* Lom, 1961 and *T. reticulata* Hirschmann et Partsch, 1955 collected from South Africa

Trichodinid species	*Trichodina acuta* Lom,1961	*T. reticulata* Hirschmann et Partsch, 1955
Host	*Oncorhynchus mykiss*	*Carassius auratus*
Locality	Metsi-matsho Reservoir	Fish dealer, Bloemfontein
Body diameter	50.0-67.0(59.0 ± 4.1, 22)	40.0-55.0(47.9 ± 4.4, 18)
Adhesive disc diameter	39.0-57.0(49.4 ± 4.2, 22)	31.0-46.0(39.3 ± 4.6, 18)
Border membrane width	4.0-5.0(4.8 ± 0.3, 22)	3.0-5.0(3.9 ± 0.6, 18)
Denticle ring diameter	23.0-36.0(29.7 ± 3.4, 22)	20.5-29.0(24.7 ± 2.7, 18)
Central circle /structures	8.0-13.0(11.0 ± 1.3, 22)	8-14(10, 18)
Denticle number	18-22 (19, 22)	22-27 (24, 18)
Radial pins per denticle	7-11 (9, 16)	8-10 (9, 18)
Denticle length	6.0-9.0 (7.7 ± 0.7, 22)	4.0-6.0(5.2 ± 0.6, 18)
Blade length	4.5-6.0 (5.4 ± 0.5, 22)	4.5-6.5(5.2 ± 0.5, 18)
Central part width	2.0-3.5 (2.8 ± 0.4, 22)	1.0-2.0(1.8 ± 0.4, 18)
Ray length	5.0-8.0 (6.3 ± 0.7, 22)	3.5-6.0(4.9 ± 0.7, 18)
Denticle span	13.0-17.0 (14.31.1, 22)	10.0-14.5(12.1 ± 1.3, 18)
Adoral spiral	410°	370°

introduced trichodinids. The identification of both these species is, however, under suspicion as we have evidence to believe that both species require a reevaluation and are most likely not representatives of these species.

After realizing our initial erroneous identifications of European trichodinids in Africa and taking heed of the caution expressed by Kazubski (1986), we have been very careful in equating African species to those found in Europe. During the last 10 years we have examined numerous specimens of introduced cyprinids, including goldfish and trout, and have so far not yet positively identified any introduced trichodinids.

In a recent examination of goldfish from an ornamental fish dealer, trichodinids were obtained which conform to an Asian and European species, i.e., *T. reticulata* Hirschmann et Partsch, 1955. In another case specimens of rainbow trout originating from a trout farm in Qua-Qua, Eastern Orange Free State, trichodinids were recovered conforming to the description of another European species, *T. acuta*. These are now the first confirmed records of introduced trichodinids in southern Africa.

MATERIAL AND METHODS

During the last winter, goldfish were brought to us by a local distributor of ornamental fish. Upon examination these fish were found to be infested by a trichodinid of which the center of the adhesive disc displayed distinct round structures visible in live specimens. Skin and gill smears were prepared and stained as described below.

Skin and gill smears of the rainbow trout *O. mykiss* were sent to us by Dr. Lew Taylor collected from a cage culture on a trout farm in the Metsi-matsho Reservoir in Qua-Qua (Eastern Orange Free State). These smears, together with those of the goldfish, were impregnated with silver nitrate in order to study details of the adhesive disc. All measurements are presented in micrometers and follow the uniform specific characteristic system proposed by Lom (1958). A detailed description of the denticles of *T. reticulata* is presented in accordance with the method proposed by Van As and Basson (1989). Minimum and maximum values are given, followed in parentheses by the arithmetic mean, standard deviation, and number of specimens measured. In the case of the number of cell like structures in *T. reticulata*, as well as the denticles and radial pins, the mode is given instead of the arithmetic mean. Body diameter is measured as the adhesive disc plus the border membrane. Reference material is in the collection of the authors.

RESULTS AND DISCUSSION

The morphometric data of the two trichodinids, *T. reticulata* and *T. acuta* is presented in Table 1.

Trichodina acuta Lom, 1961

Reference material: 87/07/22-03 in the collection of the authors.

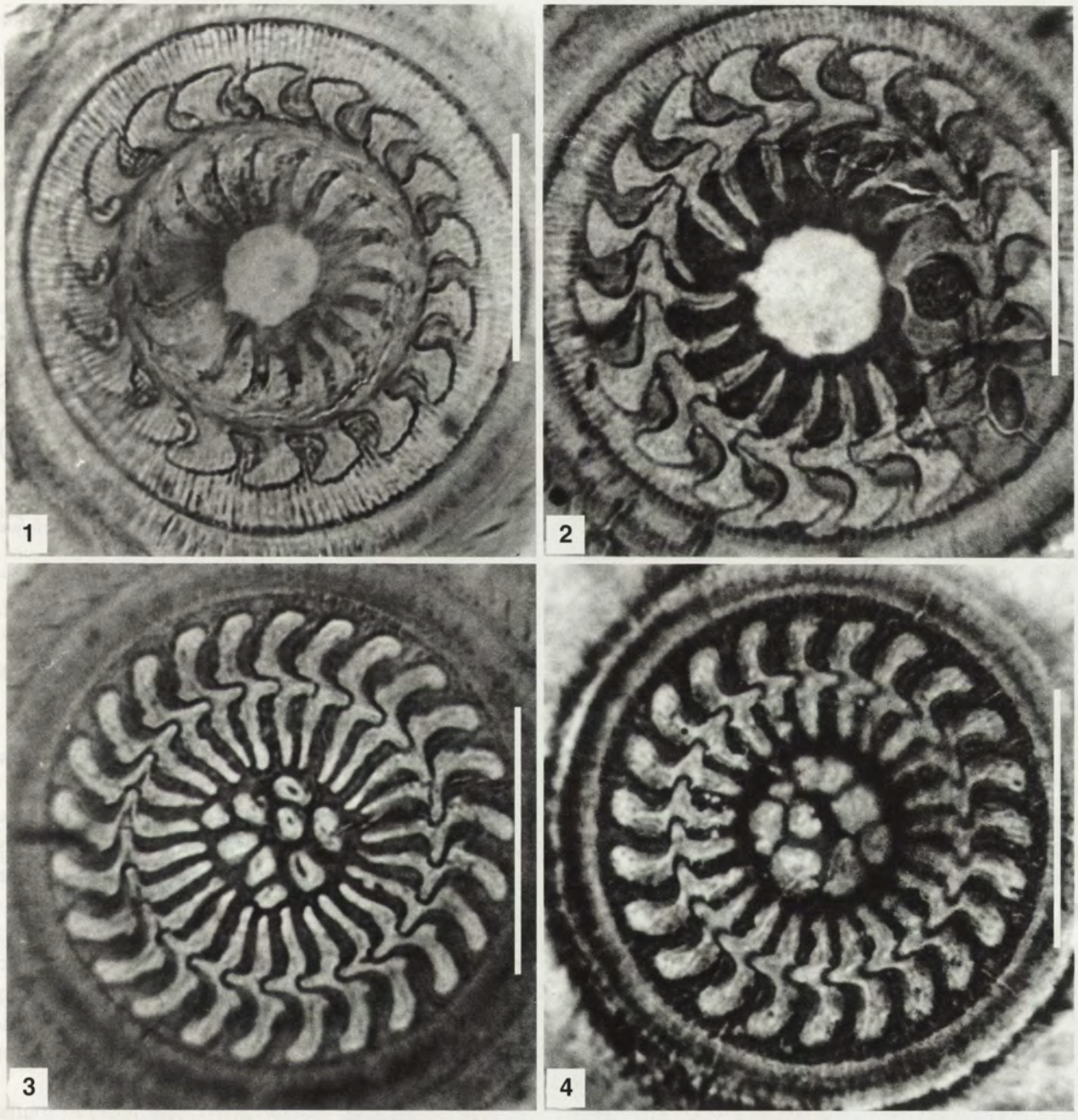
The denticle morphology of trichodinids from the trout conforms exactly to that described for this species from Israel by Van As and Basson (1989), as well as the original description by Lom (1961) from Europe.

In the present population we observed the same constant characteristics that we showed earlier for *T. acuta* (Van As and Basson 1989). The overall body dimensions of the present population falls within the range provided by Lom (1970), but is slightly larger than the population we described from Israel.

Since Van As and Basson (1989) presented the denticle morphology of this species, it is therefore not repeated in this paper.

T. reticulata Hirschmann et Partsch, 1955

Reference material: 91/06/26-03 in the collection of the authors. Denticle drawings and the description based

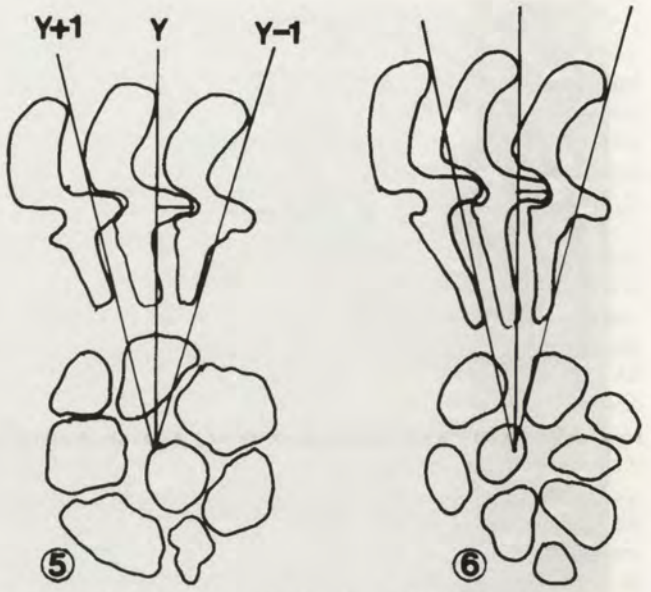


Figs. 1-4. Photomicrographs of silver impregnated specimens of trichodinid species. 1 and 2 - *Trichodina acuta* Lom, 1961 from rainbow trout. 3 and 4 - *T. reticulata* Hirschmann et Partsch, 1955 from goldfish. Scale, 20μm

on the method proposed by Van As and Basson (1989) is presented below.

Blade broad, angular. Distal surface rounded, truncated, parallel to border membrane. Tangent point distinctly round, head of blade almost bulbous at this point. Anterior blade surface parallel to curve of posterior surface. Apex angular, extending somewhat beyond y + 1 axis. Blade apophysis sharp, resting on central part of following denticle. Posterior blade surface with deep curve, forming almost L-shape. Deepest point low, slightly above apex. Blade connection thin and short. Posterior projection not clearly visible on central part. Central part well developed, short and stout, of same thickness throughout. Central part extending only slightly beyond y axis, fitting tightly into preceding denticle with blunt rounded point. Section of central part above and below x axis similar in shape. Indentation in section below x axis visible in some denticles. Ray connection very short. Rays projecting almost directly from central part. Ray apophysis not clearly visible. Rays short, stout,

of equal thickness throughout with blunt rounded points. Denticles straight, parallel to y axes. Section of denticle above to section below x axis slightly more than one (1.1). Denticles fit neatly into space between y axes with only short tip of central part extending beyond y axes. A distinct feature of the denticles is a longitudinal ridge extending from the ray, across the central part to the blade. This ridge is reminiscent of a similar ridge found in *T. centrostrigata* Basson, Van As et Paperna, 1983. Center of adhesive disc with circular cell-like structures varying in number from eight to 14. These structures are mostly round, but vary in shape from specimen to specimen.



Figs. 5 and 6. Diagrammatic drawings of denticles of *Trichodina reticulata* Hirschmann et Partsch, 1955 from goldfish

Remarks

T. reticulata has been described from different hosts from various localities, mostly cyprinids and in the majority of cases from the genus *Carassius*. It was originally described from the USA by Hirschmann and Partsch (1955), but it is recognized as an Asian species that has distributed widely via the introduction of *Carassius* species (Lom and Hoffman 1964). *T. reticulata* has been reported from the USSR, Eastern Europe, Iran, North Korea, Japan, China, Indonesia, Israel and the USA (Stein 1954, 1968, 1984; Lom 1960; Chen 1963; Lom and Hoffman 1964; Wellborn 1967; Ahmed 1976; Kazubski 1982, 1988; Basson et al. 1983; Albaladejo and Arthur 1989).

The different populations of *T. reticulata* described by the above-mentioned authors, show some variation in body dimensions, but this species is still clearly recognizable based on the distinct shape of the denticles, as well as the constant occurrence of cell-like structures in the center of the adhesive disc.

T. reticulata is a medium-sized trichodinid. The largest populations have so far been recorded by Ahmed (1976), Kazubski (1982) and Stein (1984). The present material appears to be the smallest population so far reported for this species.

Concluding remarks

T. acuta was originally described from Czechoslovakia by Lom (1961), but has since been reported from the USA (Lom 1970), the USSR (Ivanova 1966, 1967, 1970), Eastern Europe (Lom 1961, 1970; Kazubski and Migała 1968; Migała 1970, 1976), the Philippines (Duncan 1977), North Korea (Stein 1984), Israel (Basson et al. 1983) and Indonesia (Albaladejo and Arthur 1989) from various fish species, mostly cyprinids. *T. reticulata* has an even wider distribution occurring mainly on cyprinids with a clear preference for the genus *Carassius* (see discussion under *T. reticulata*).

It is most likely that both these trichodinids are parasites of European and Asian cyprinids, which have spread via the translocation of their hosts.

Despite comprehensive surveys of indigenous as well as introduced species of fish in southern Africa (Basson et al. 1983; Van As and Basson 1989, 1992), we have so far not yet recorded *T. acuta* or *T. reticulata* in the African subcontinent. Maslin-Leny (1988) reported the occurrence of *T. acuta* and *T. reticulata* from a brackish environment in South Benin (West Africa). It is, however, impossible to determine whether these identifications were correct as no description is provided and only a single photomicrograph of each is presented.

The absence of *T. acuta* and *T. reticulata* in southern Africa in the past suggests that we are either dealing with a recent introduction or if these introductions have occurred earlier, they remained localized, not spreading. The latter is unlikely as these parasites have successfully established in various parts of the world. We must therefore come to the conclusion that we are dealing with recent introductions of these two trichodinid species. It is therefore not unlikely that the two trichodinids will in the future also become part of the fish parasite fauna of southern Africa as has been the case with some other fish parasites like *A. japonicus* and *B. acheilognathi*.

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Morphometric and Morphogenetic Comparisons between *Onychodromus indica* sp. n. and *O. quadricornutus* Foissner, Schlegel et Prescott, 1987; Phylogenetic Note on *Onychodromus* and Related Genera

Komal KAMRA* **and Gulshan Rai SAPRA**

Department of Zoology, University of Delhi, Delhi; * SGTB Khalsa College, University of Delhi, India

Summary. Morphometric and morphogenetic comparisons between *Onychodromus indica* sp. n. and *O. quadricornutus* Foissner, Schlegel and Prescott, 1987, showed that the two species are similar in their morphology, in the presence of dorsal horns and in the morphogenetic pattern. However, the two species differ significantly in morphometry and nuclear characteristics. A morphogenetic comparison of *Onychodromus* and related genera shows distinctive features such as non-participation of right ventral rows and marginal cirri in primordia formation.

Key words. *Onychodromus indica, O.quadricornutus,* morphogenetic pattern, morphometry.

INTRODUCTION

An exceptionally large hypotrichous ciliate *Onychodromus quadricornutus* was isolated by Prescott (Lin and Prescott 1985) from a freshwater aquarium, and its morphogenesis was described by Foissner et al. (1987). The presence of four prominent dorsal horns makes the species very distinctive. Recently we have isolated a similar ciliate, *Onychodromus indica* sp. n., from a fresh water body in Delhi, India. Morphometry and cytology of this cell (results of which are being presented here) showed that, despite the presence of the distinctive dorsal horns, it is sufficiently different from Prescott's isolate.

The morphogenetic pattern of *O. indica* is very similar to that of *O. quadricornutus* (Foissner et al. 1987) and the differences are largely due to biometric differences between the two species. The present study also highlights some additional features including those regarding the formation and final placement of the right ventral rows and the dorsal kineties during division and reorganization morphogenesis. In addition, phylogenetic implications of these observations are also discussed.

MATERIAL AND METHODS

Onychodromus indica was isolated from the river Yamuna in Delhi (28°34'N, 76°07'E) at a point where a large amount of fly-ash was being discharged by a thermal power station. The cells were cultured in the laboratory at 23 ±1°C with the food organism *Chlorogonium elongatum* which was grown axenically (Ammermann et al. 1974). Starving cultures were used for the study of reorganization morphogenesis.

For biometric characterization, enumeration of cortical structures was done on randomly selected non-dividers, while for dimensional measurements, cells with very early oral primordium were selected.

Address for correspondence: G. R. Sapra, Department of Zoology, University of Delhi, Delhi-110 007, India.

Morphometric comparison of proter and opisthe was performed on late cytokinetic cells.

Cortical staining was done by the modified protargol technique (Kamra and Sapra 1990) of Jerka-Dziadosz (1985). Feulgen staining was used for cytological observations.

OBSERVATIONS

Characteristics of the living cell

Chlorogonium fed cells of *Onychodromus indica* measure 205.3 x 136.0 µm (n=10 cells), and are flat, with a blunt anterior end, slightly pointed near the left anterior margin. They exhibit slow movements, grazing along the substratum and leave the substratum with great difficulty following vigorous shaking. They are voracious feeders. Under optimal conditions the generation time is 9 ± 0.5h. The dorsal surface has three prominent horns - two postero-lateral and one posterior. There is also a slight elevation in the mid-anterior region (Fig. 2).

Cortical morphology of the non-dividing cell

Ciliature on the ventral surface (Figs. 1,15). A prominent adoral zone of membranelles (AZM) with 66-98 adoral membranelles (AMs) borders the anterior and left margins of the peristome. Two parallel undulating membranes (UMs) are present on its right wall. The frontal ciliature consists of 10-12 longitudinal rows of frontal-ventral-transverse (FVT) cirri occupying about 2/3 of the ventral surface to the right of the peristome. Left 7-8 FVT rows have hypertrophied anterior cirri and are designated here as the crowned FVT rows (C_1-$C_{7/8}$). C_1 consists of a single cirrus - the buccal cirrus. C_2 lies close to the UMs and is termed as the buccal row (after Foissner et al. 1987). Next to the crowned FVT rows are 2/3 FVT rows which do not have hypertrophied anterior cirri and start successively posteriad from the left to the right. These are designated as the non-crowned FVT rows (NC_1-$NC_{2/3}$). The last two FVT rows on the right are called the right-ventral rows (RV_1 & RV_2). RV_1 starts at the equatorial region while RV_2 begins well near the anterior right corner of the cell. The last ventral cirrus in each of the RV rows is larger than other ventral cirri and is displaced leftwards. The two displaced cirri are not in alignment with the parental rows. Besides the complete FVT rows, there may often be 1-2 short rows of cirri present in between. Marginal cirri are present in 2 rows, one each on the left margin (LMC row) and on the right margin (RMC row).

Ciliature on the dorsal surface (Figs. 2, 22). There are 19-25 linear dorsal kineties (DKs), each a row of paired kinetosomes. The infraciliature of each pair forms a well defined rhomboidal structure which is diagonally oriented. Most of the DKs are distinguishable in three linear groups. At the left of each group, there are 2-3 kineties whose infraciliature points in the antero-posterior direction. The two right-most rows are termed dorsomarginal (DM) rows (after Martin 1982). There are 3 caudal cirri, one associated with each group of DKs, at the posterior end of the cell.

A biometric characterization of *O. indica* is given in Table 1. The differences between *O. indica* and *O. quadricornutus* (description from Foissner et al. 1987) are highlighted in the discussion.

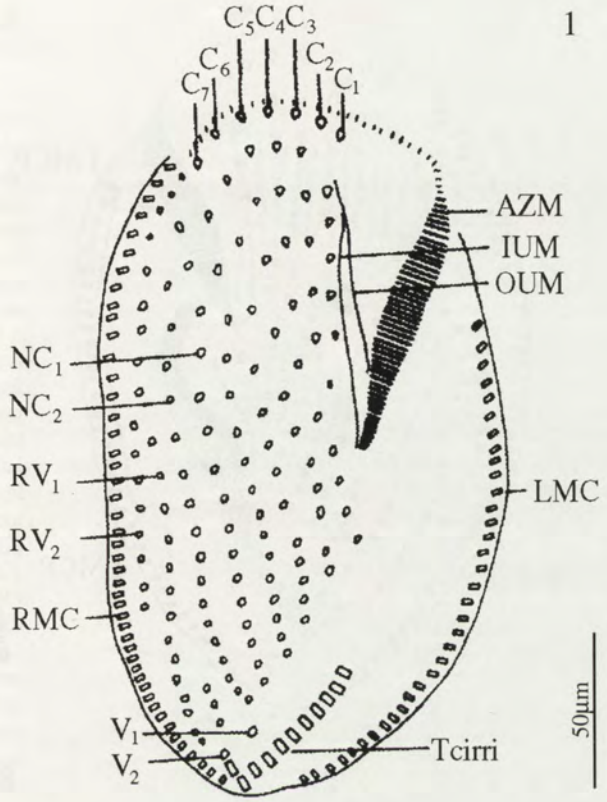
Cortical development during cell division

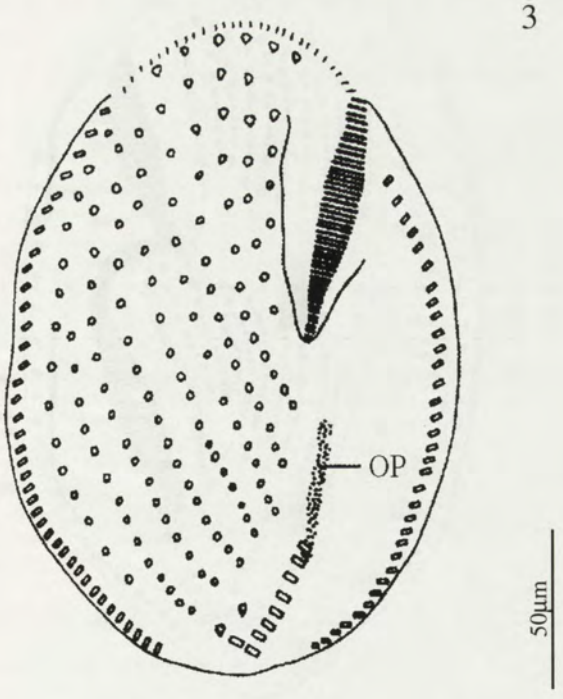
In general, the morphogenetic events during cell division of *O. indica* are quite similar to those described by Foissner et al. for *O. quadricornutus*. For the purpose of morphometric comparison and additional morphogenetic features, a description of the cortical events related to cell division in *O. indica* is provided here.

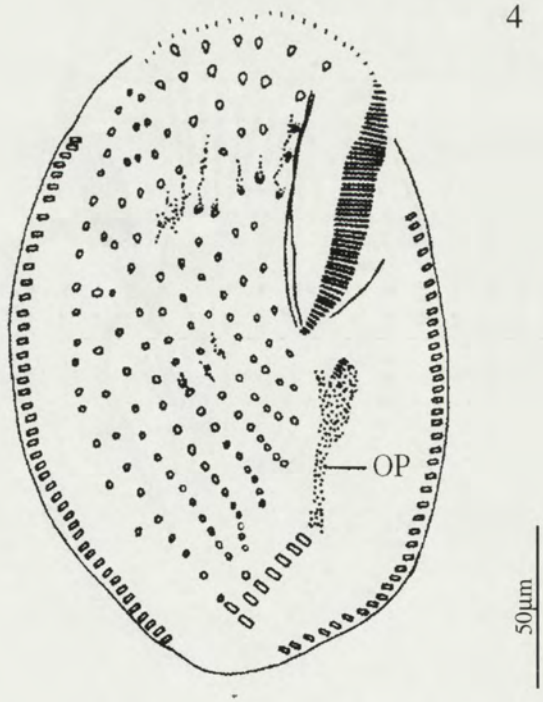
Buccal ciliature (Figs. 3-8, 16-18). While the parental AZM is retained for the proter, a new one develops for the opisthe from an oral primordium (OP). The opisthe UMs and buccal cirrus are formed anew from the first FVT primordium (see below), while the parental UMs reorganize to form the UMs and buccal cirrus for the proter.

FVT ciliature (Figs. 3-11, 17-21). Primordia formation for the FVT rows (except C1, see above) is signalled by disaggregation of the 4th cirrus in rows C6, C7 and C8 and the 3rd cirrus in NC1. Later disaggregation occurs in these four parental rows at the level of the 8th/9th/10th cirrus. The disaggregation wave spreads at the above-mentioned two levels forming linear streaks in C2-8 and NC1 for the proter, and C_{4-8} and NC_{1-2} for the opisthe. The streaks elongate involving kinetosomes from 2 cirri per parental FVT row to form distinct FVT primordia. For the opisthe the first three primordia are formed from the OP.

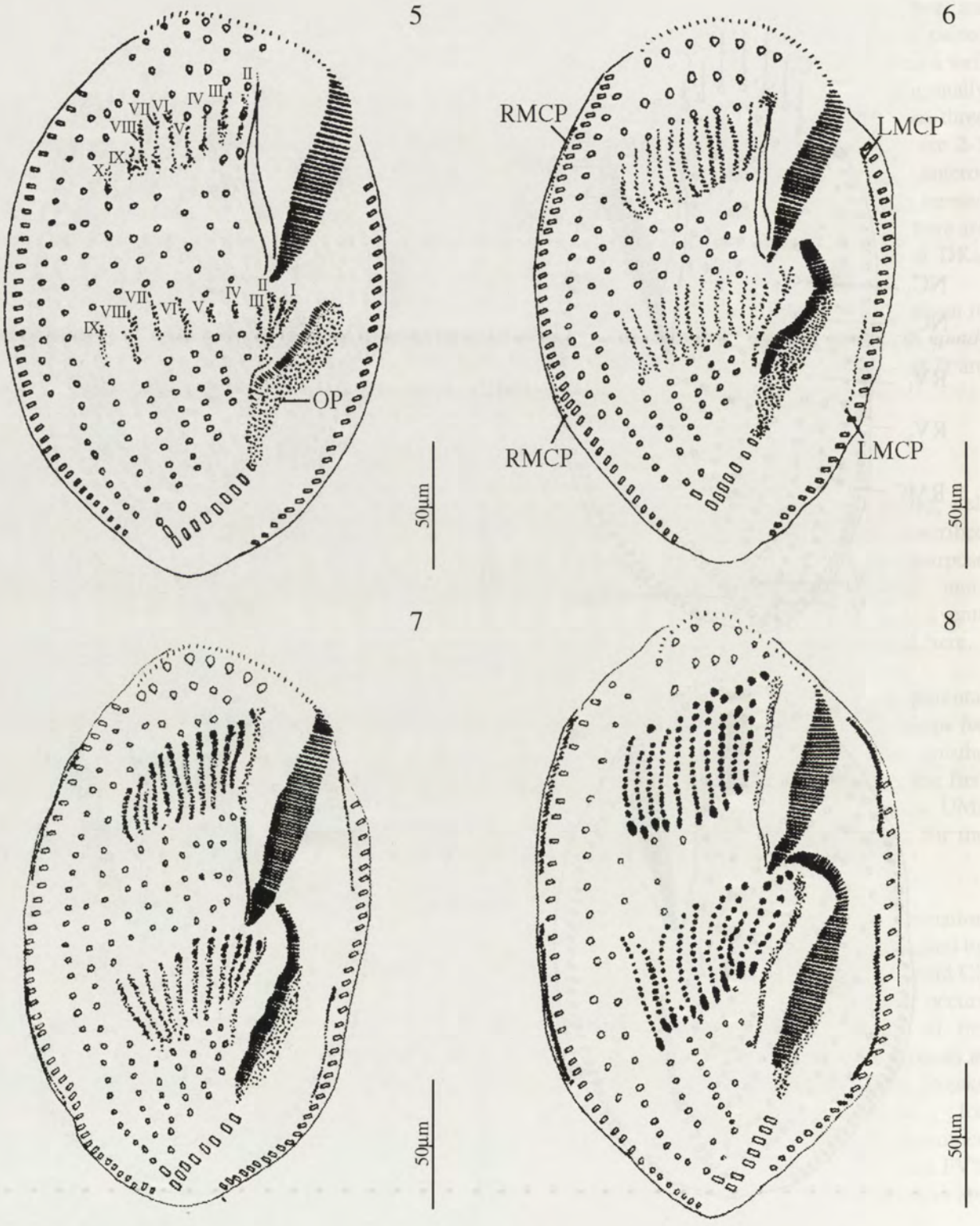
The two RV rows take no part in streak formation. Yet the number of FVT primordia is the same or sometimes more (10-13 in proter, 10-14 in opisthe) than the number of parental rows (10-12). This is due to the formation of extra primordia in between parental







Figs. 1-4. Line diagrams of protargol impregnated non-dividing cells (Figs. 1, 2) and dividing cells (Figs. 3, 4) of *Onychodromus indica*. 1 - ventral surface; AZM- adoral zone of membranelles; IUM and OUM- inner and outer undulating membranes; C_1-C_7 - crowned rows of FVT cirri (C_1represents the buccal cirrus and C_2 is the buccal row); NC_{1-2} - non-crowned rows of FVT cirri; RV_{1-2} - right ventral rows of FVT cirri; V_1, V_2 - enlarged last ventral cirri of RV rows; T cirri - transverse cirri; LMC and RMC- left and right marginal cirri. 2 - dorsal surface; dorsal kineties are present in rows and the dorsomarginal rows are not distinguishable from them; arrows mark the areas where the infraciliature points antero-posteriorly arrowheads point to the dorsal horns; CC_{1-3} - Caudal cirri 1-3. 3 - formation of oral primordium (OP). 4 - formation of cirral streaks



Figs. 5-8. Line diagrams of protargol impregnated cells of *O. indica* (ventral surface) in early and mid stages of morphogenesis. 5 - formation of streaks I-IX/X for frontal ciliature. Note the involvement of parental cirri in streak formation, excepting for streaks I-III for opisthe which are formed in conjunction with the OP. Some streaks (e.g. streak IX in proter) may form within old rows. RV rows do not take part in streak formation. Membranelle differentiation begins. 6 - formation of primordia for an LMC row (LMCP) and an RMC row (RMCP) for each daughter cell. Note that no parental marginal cirri are involved in primordia formation. 7 - cirri begin to develop within the frontal and marginal primordia. 8 - as membranellar and cirral differentiation is completed, parental cirri begin to be resorbed

Table 1

Biometric characterization of *Onychodromus indica* (*Chlorogonium* fed). All data are based on protargol impregnated specimens. See text for abbreviations

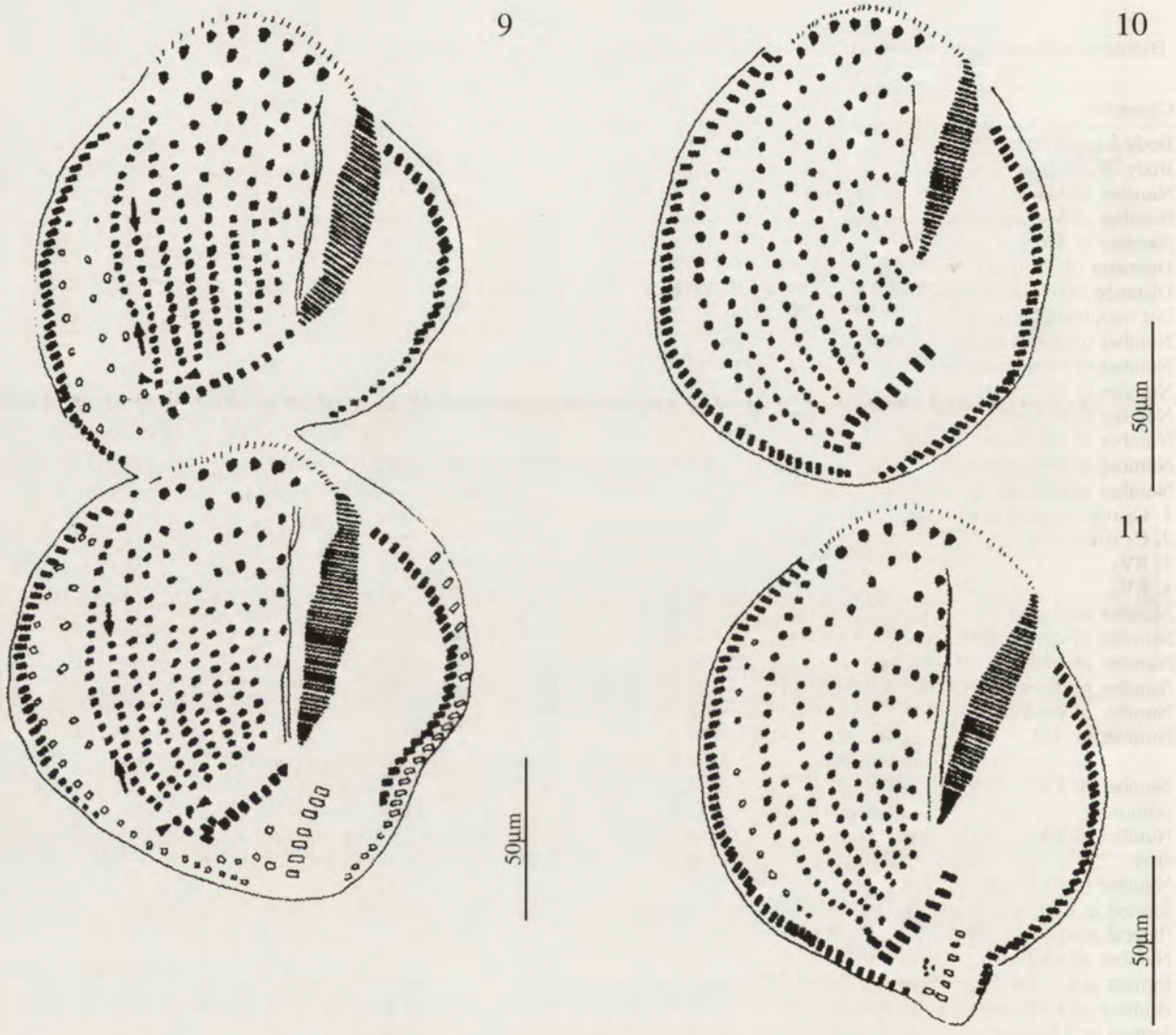
Character		Mean	SD	CV	Min.	Max.	No
Body Length (μm)		205.3	24.32	11.84	180.0	225.6	10
Body Width (μm)		136.0	15.45	11.36	118.8	166.3	10
Number of Ma		1	0	0	1	1	10
Number of Ma segments		11.4	1.27	11.10	10	13	10
Number of Mi		3.3	0.48	14.60	3	4	10
Diameter of Mi (μm)		6.4	0.53	8.29	5.5	7.3	20
Distance from anterior end to last membranelle (μm)		100.4	12.19	12.13	88.6	123.8	10
Number of dorsal horns		3	0	0	3	3	10
Number of membranelles		82.0	8.08	9.80	66	98	25
Number of FVT rows		11.1	0.53	4.73	10	12	25
Number of C rows		7.0	0.47	6.73	6	8	10
Number of NC rows		1.9	0.32	16.60	1	2	10
Number of FVT cirri		147.8	7.19	4.87	136	159	10
Number of FV cirri in							
1. C_2 row (Buccal row)		5.0	0.68	13.40	4	6	25
2. C_3 row		7.1	0.70	9.92	6	8	25
3. RV_1		15.4	1.35	8.77	13	18	10
4. RV_2		18.8	1.62	8.61	16	21	10
Number of T cirri		10.0	0.74	7.32	9	12	25
Number of cirri in RMC row		47.2	3.33	7.05	43	53	10
Number of cirri in LMC row		35.4	2.22	6.27	32	39	10
Number of dorsal rows (DKs & DMs)		21.8	1.99	9.12	19	25	10
Number of caudals		3	0	0	3	3	10
Number of AM	in Proter	77.9	10.65	13.68	67	98	7
	in Opisthe	78.0	10.44	13.39	68	98	7
Number of FVT primordia	in Proter	11.5	0.85	7.39	10	13	10
	in Opisthe	11.5	1.27	11.04	10	14	10
Number of FVT cirri	in Proter	144.8	17.38	12.01	129	173	5
	in Opisthe	150.6	19.24	12.78	125	177	5
Number of FVT cirri formed in C_2 row (buccal row)	in Proter	6.1	0.69	11.23	5	6	6
	in Opisthe	6.3	0.82	12.89	6	8	6
Number of FVT cirri formed in C_3 row	in Proter	8.3	0.52	6.20	8	9	6
	in Opisthe	8.3	1.51	18.07	7	11	6
Number of FVT cirri formed in RV_1 row	in Proter	15.0	1.27	8.43	13	16	6
	in Opisthe	15.5	1.38	8.89	13	17	6
Number of FVT cirri formed in RV_2 row	in Proter	16.8	0.75	4.47	16	18	6
	in Opisthe	17.8	2.64	14.80	14	20	6
Number of T cirri formed	in Proter	10.0	0.82	8.17	9	11	10
	in Opisthe	10.0	1.49	14.91	8	13	10

rows $C_{6,7,8}$ and NC_1. Table 2 shows the relationship between parental rows and the primordia formed within or between them.

Kinetosome proliferation occurs within the FVT streaks. There are on an average 3-5 cirri left in between the streaks in each of the parental rows $C_{3\text{-}7/8}$, NC_1 and NC_2. There are 2-3 parental cirri left anterior to, and 2-5 cirri posterior to the streaks in each parental FVT row. FVT streaks begin to differentiate into individual cirri in the antero-posterior direction. The new $NC_{1\text{-}2}$ as well as RV_1 show a net posterior displacement. The new RV_2 is displaced anteriorly leaving behind the last two cirri developed within it. The last ventral cirri in the RV rows are displaced leftwards. The transverse cirri together form a characteristic shape due to these displacements.

After the new cirri have differentiated, all old FVT cirri are resorbed. Interestingly, there is resorption of some new cirri, especially those in the posterior part of the new buccal row and those in the small primordia formed within parental rows. Sometimes the first transverse cirrus (posterior most product of the buccal row) is also resorbed

Marginal ciliature (Figs. 6-8, 18-21). As the FVT primordia take shape, 4 marginal cirral primordia develop. Primordia for LMC (LMCPs) arise to the right of the parental LMC row at the level of the 1st cirrus



Figs. 9-11. Line diagrams of the ventral surface of protargol impregnated cell of *O. indica* in cytokinesis (Fig. 9) and newly separated daughter cells, proter (Fig. 10) and opisthe (Fig. 11). New cirri are shown filled in. Parental cirri left in the opisthe are fated for resorption. Note the net posteriad movement of RV_2 (downward arrow) and the anteriad movement of RV1 (upward arrow). The last two ventral cirri in RV_1 and RV_2 (arrowheads) show a net leftward displacement

expanding to the level of the 7th cirrus, and at the level of the 11th/12th/13th cirrus expanding upto about the 21st cirrus. The RMCPs also arise at about the same levels, to the right of the parental RMC row. Finally they extend from the level of the 2nd to the 12th cirrus and from the level of the 17th to the 28th cirrus.

Significantly, the formation of marginal primordia involves no participation of parental cirri; the latter remain intact until their resorption much later in development.

Splitting occurs in these primordia in the antero-posterior direction leading to the formation of RMC and LMC rows for proter and opisthe.

Dorsal ciliature (Figs. 12-14, 22-24). Three dorsal primordia (DPs) arise for each daughter cell, within the region where the rhomboidal infraciliature of the parental kineties point antero-posteriorly, i.e. one DP to the left of each set of DKs. Each DP splits into a set of 4-7 partially overlapping linear rows of kinetosomes. The right-most in each set develops a posterior thickening which later cuts off to form a caudal cirrus. Each linear row spreads out to form a dorsal kinety for a daughter cell. Lateral displacement of kineties continues even as the cell grows.

In each daughter cell two rows of kinetosomes form to the right of the RMCP which later develop into

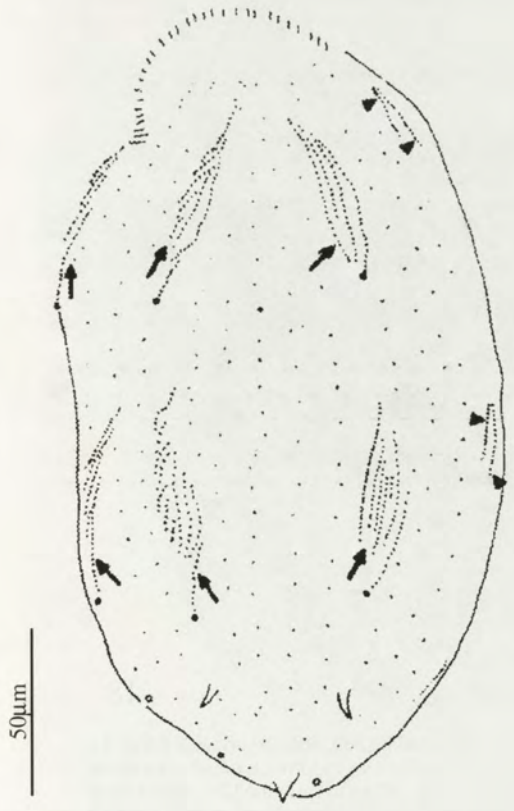
dorsomarginal rows. Subsequently, lateral splitting of these rows may be occurring.

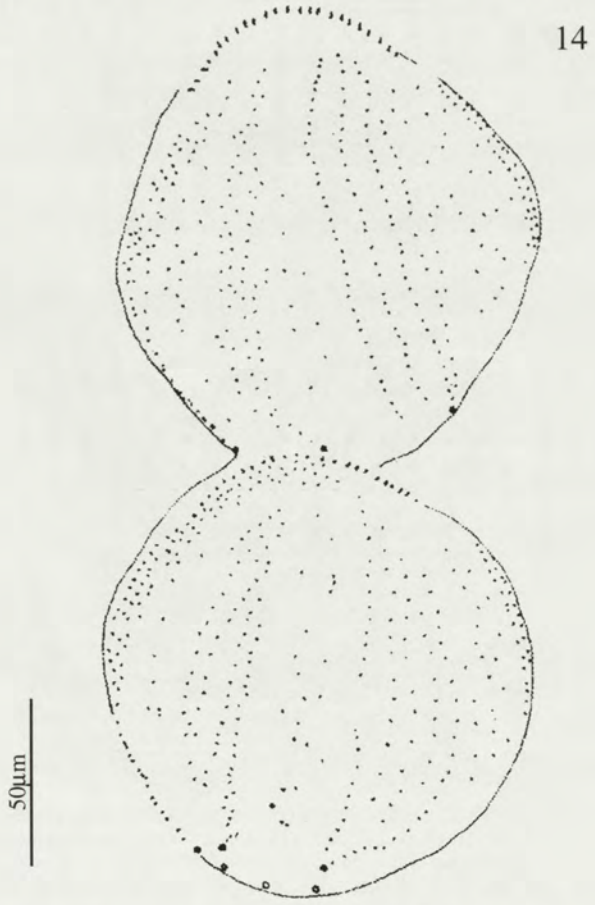
Cortical development during reorganization

The sequence of morphogenetic events during reorganization is similar to that in division, except that only one set of primordia is formed. The oral primordium gives rise to a variable number of membranelles which merge with the partially resorbed posterior end of the parental AZM. The rest of the morphogenetic events are similar to those for formation of proter ciliature. Figs. 26-29 highlight the displacement of new RV rows which is similar to that in division.

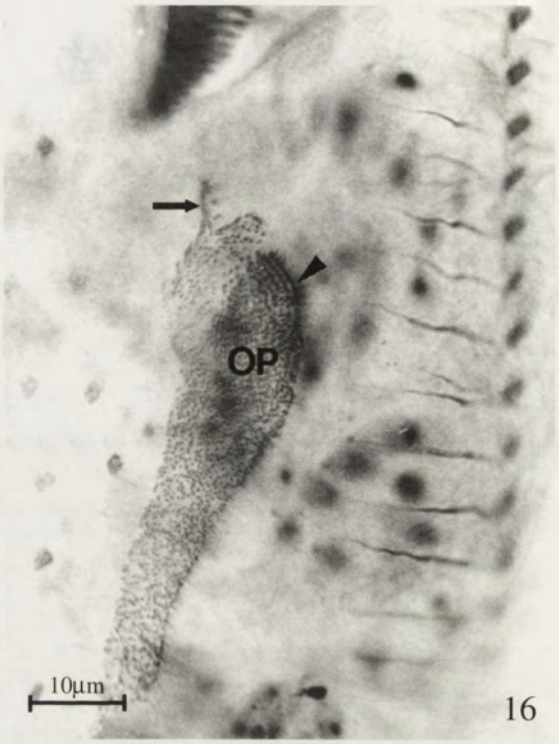
Nuclear cytology and cell cycle

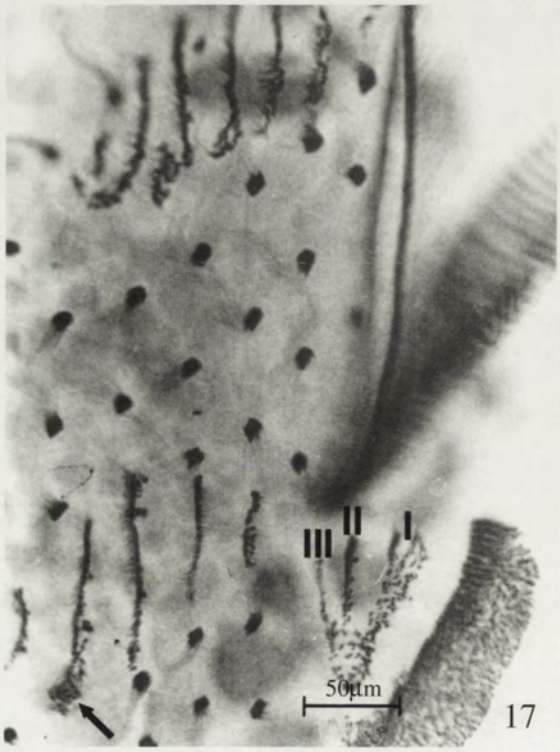
The cell has a moniliform macronucleus with 8-10 distinct well separated beads of variable sizes and shapes (Fig. 25). During the S phase of cell cycle, a distinct replication band (RB) traverses each bead. The direction

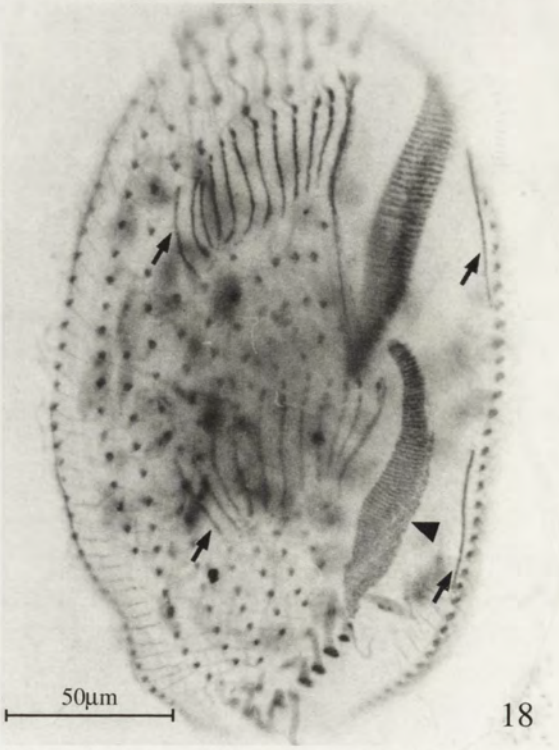




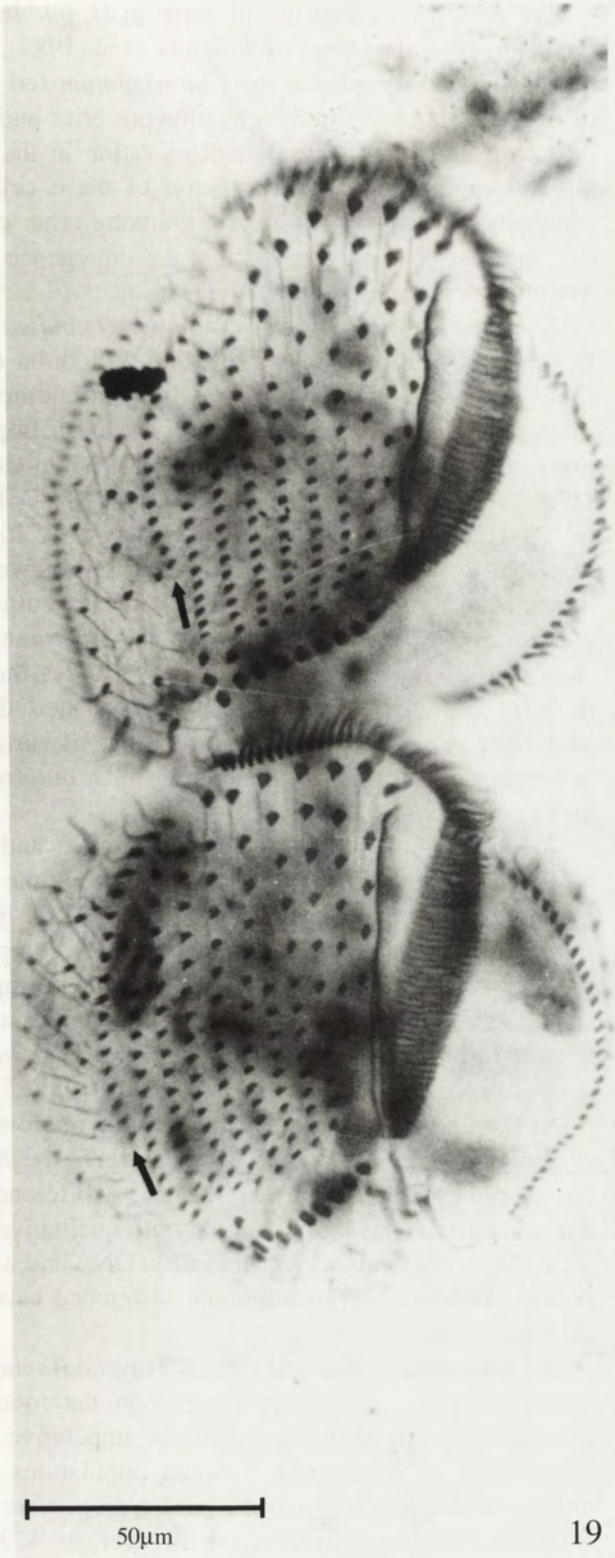
Figs. 12-14. Line diagrams of protargol impregnated cells of *O. indica* showing morphogenetic events on the dorsal surface. 12 - development of 3 DPs (arrows) for each daughter cell. 13 - fragmentation of each DP (arrows) to yield 4-7 primordia each. The right-most DP in each group cuts off a caudal cirrus (filled in circle) at its posterior tip. Two dorsomarginal rows (arrowheads) are formed for each daughter cell from the anterior end of the new RMC row on the ventral surface. 14 - a cell in cytokinesis. New dorsal rows replace the parental rows. New caudal cirri are shown filled in. The parental caudal cirri are fated for resorption

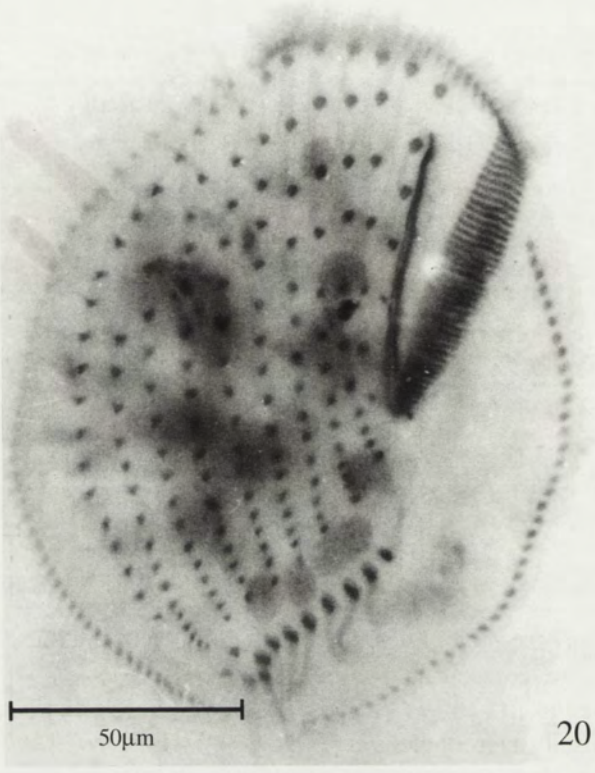


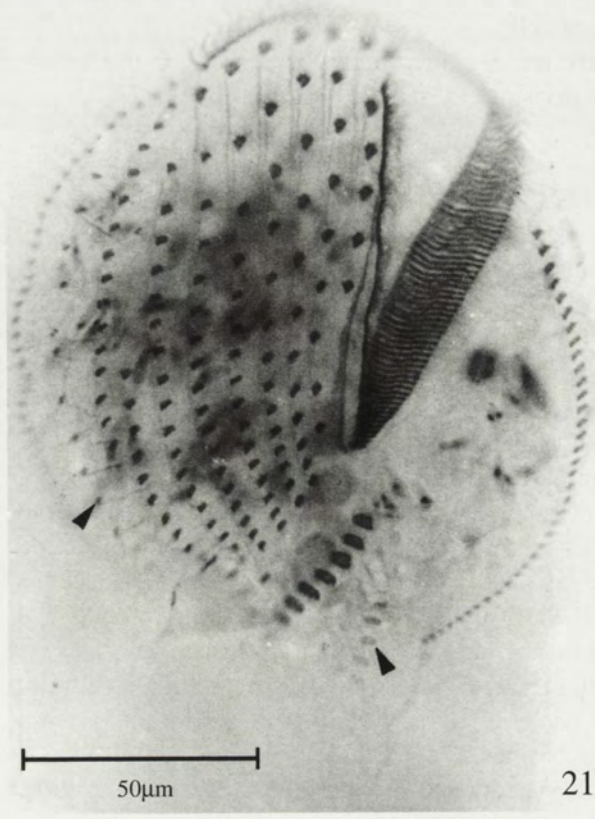




Figs. 15-18. Photomicrographs of protargol impregnated cells of *O. indica*. 15 - non dividing cell, ventral surface (cf. Fig. 1). 16 - high magnification photomicrograph showing the alignment (arrowhead) of kinetosomes to form membranelles in the anarchic oral primordium (OP). Note that some kinetosomes at the right anterior margin align to form the UM primordium and FVT primordia II and III (arrow). 17 - formation of FVT primordia by disaggregation of parental cirri. Arrow marks a cirrus that is about to be incorporated into a streak. The UM primordium (I) and the next two FVT primordia (II and III) that were formed in conjunction with the OP (cf. Fig. 16) are now separated from it to its right. The UM primordium is beginning to split longitudinally and the anterior end of the right row is thickened to form the buccal cirrus for the opisthe. 18 - early stages of primordia split. The alignment of the new AZM is nearly complete (arrowhead). FVT primordia and LMC primordia (arrows) are split linearly into cirri. The RMC primordia are not in view. Note that the parental marginal rows are completely intact and no contribution has been made to the marginal primordia







Figs. 19-21. Photomicrographs of protargol stained divider (Fig. 19) and newly divided cells - proter (Fig. 20) and opisthe (Fig. 21) of *O. indica*. Arrows show the anteriad movement of RV_2 row. Some parental cirri in the opisthe (arrowheads) are fated for resorption

Table 2

Parental structures associated with origin of FVT primordia I-IX/XII of the proter and the opisthe in *Onychodromus indica*. Parental cirral rows are named as given in the text

	FVT Primordium number	Parental structure associated with origin of primordium
For Proter	I	UM
	II	C_2 (buccal row)
	III	C_3
	IV	C_4
	V	C_5
	VI-IX/XII	From or between C_{6-8} NC_1 sometimes NC_2
For Opisthe	I	OP
	II	OP
	III	OP
	IV	C_4
	V	C_5
	VI-IX/XII	From or between C_{6-8} NC_1 and often NC_2

of RB progression varies in the different beads and thus shows no correlation to the cell's axes. The beads coalasce at the end of S phase to form a single mass. The fused macronucleus undergoes 3-4 divisions. The macronuclear products are later distributed to the two daughter cells.

There are 3-4 micronuclei which divide mitotically during division.

DISCUSSION

Comparison between *Onychodromus indica* and *O. quadricornutus*

1. Comparative morphology and ecology. *O. indica* is a tropical species. *O. quadricornutus* was isolated by Prescott (Lin and Prescott 1985) from a fresh water aquarium of tropical fishes in Colorado, USA. Foissner et al. (1987) state that the hypotrich is probably from some tropical region. Wicklow (1988) mentions that a similar organism has been isolated from Shanghai, China by Y.-B Pang and J.O. Corliss. However, no published details on the latter population are available.

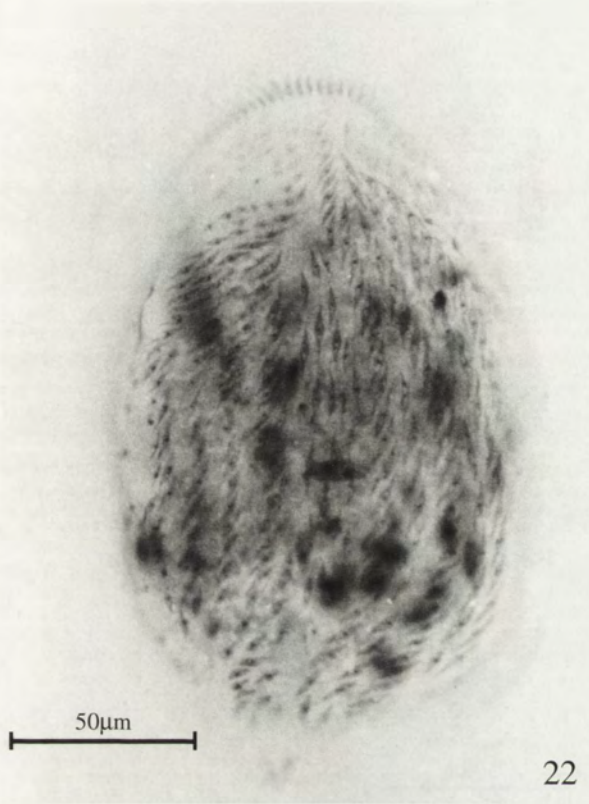
Cultures of *O. indica* were grown successfully on mixed bacteria, *Tetrahymena thermophila, Stylonychia lemnae* besides *Chlorogonium*. Foissner et al. (1987) have also mentioned that *O. quadricornutus* can grow on a variety of food organisms. Following starvation some cells of *O. indica* turn cannibalistic while others in the culture become lanceolate. Cannibalism has also been reported in *O. quadricornutus* (Wicklow 1988).

The presence of four dorsal horns in *O. quadricornutus* has been described by Foissner et al.(1987). The present study showed that the *Chlorogonium* fed cells of *O. indica* possess three horns (one posterior and two postero-lateral) and have a small elevation in the mid anterior region. The transformation of these cells to cannibals and lanceolates involves, among other changes, increase in horn lengths, and the emergence of a fourth horn at the mid-anterior elevation.

2. Comparative nuclear cytology. *O. indica* and *O. quadricornutus* possess a single multinodular macronucleus (Ma) but the average no. and numerical range of Ma beads differ significantly in natural populations of the two species. The number of Ma nodes in *O. quadricornutus* ranges between 11 and 28 ($\bar{y}$=19.6) (Foissner et al. 1987), which is significantly higher than that in *O. indica* (range 10-13). It is noteworthy that this difference is certainly not due to culturing conditions. *O. indica* grown on various food organisms shows little variation in number of Ma nodes though the size of nodes does change (unpublished data). Moreover as pointed out earlier, the range of variation in number of Ma nodes is far greater in *O. quadricornutus* cells.

A comparison of the micronuclear number and size also shows rather significant differences in the two species. The number of micronuclei (Mi) in *O. quadricornutus* ranges between 4 and 26 ($\bar{y}$=9.6) as compared to the 3-4 Mi in *O. indica*. However, the most impressive, notable and important difference is with regard to the size of Mi - the average diameter of Mi in *O. indica* is 6.4 µm while in *O. quardricornutus* it is only 5 µm. Mi in *O. indica* are perhaps the largest micronuclei encountered in the hypotrich ciliates. Since the Mi is the genomic repository of the species, a difference of this nature must have a bearing on both qualitative and quantitative implications on Mi and Ma DNA and would perhaps stand out as an important difference between the two species.

3. Comparative biometry. Since *Onychodromus* acquires different sizes depending upon the food organisms (unpublished results), it is imperative that comparison be made only between populations kept under similar feeding regimens. *Chlorogonium* fed *O. quadricornutus* measures on an average 423.3 x 235.6 µm (Foissner et al. 1987) while *O. indica* measures 205.0 x 136.0 µm. The cannibals are the largest morphs and in *O. indica* their length varies between 288.0-372.2 µm while the cannibals in *O. quadricornutus* can be as large as 810 µm (Wicklow 1988).



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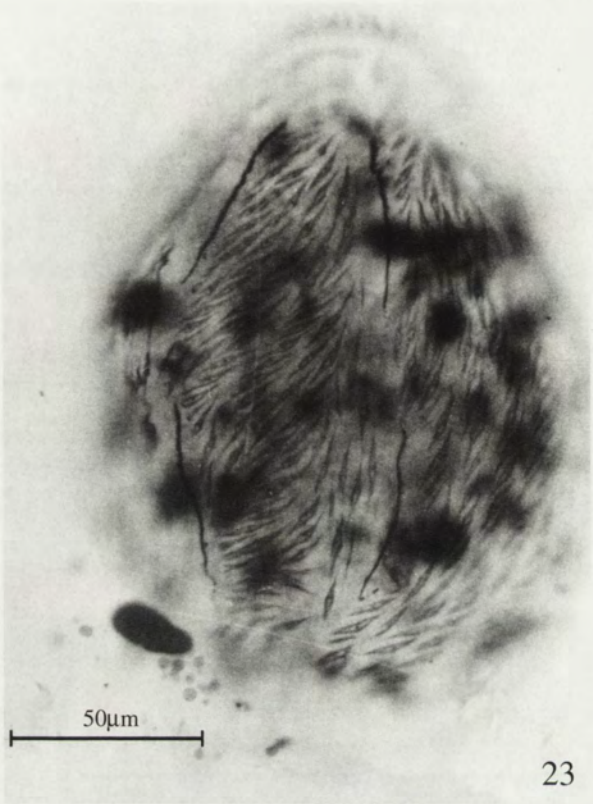

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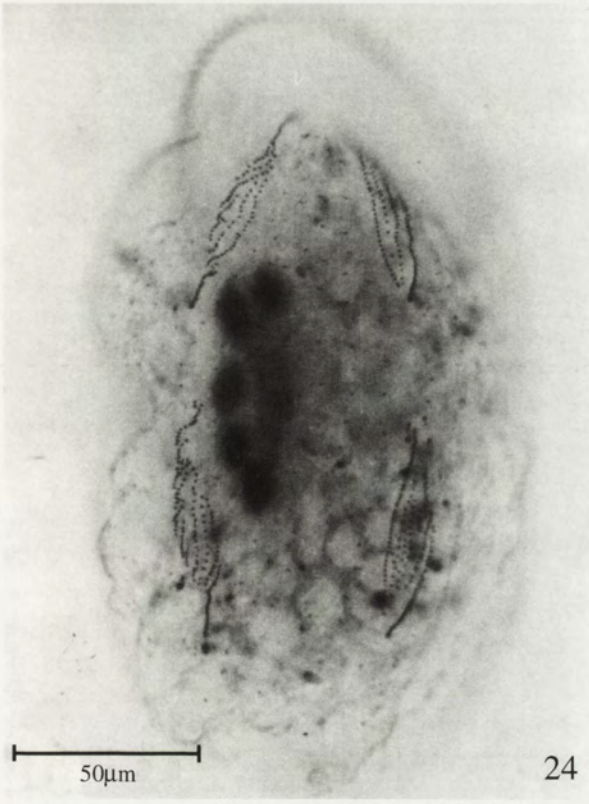

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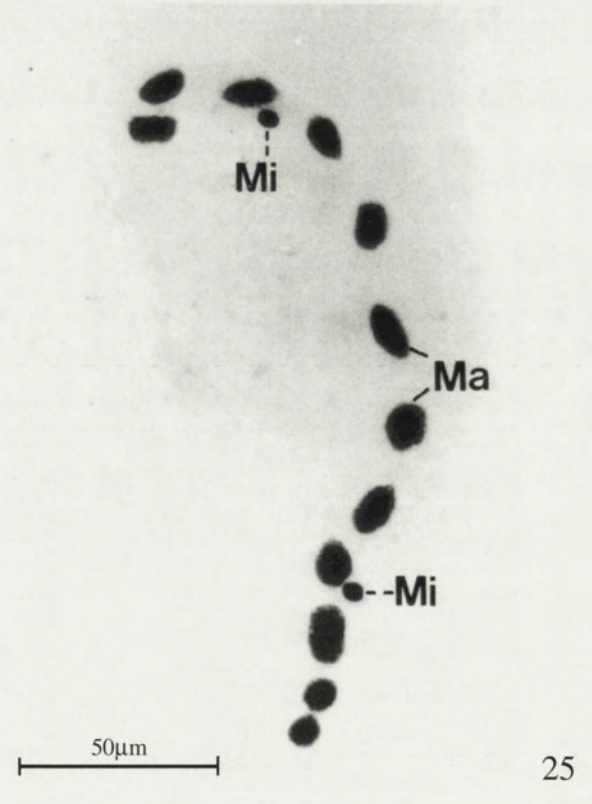

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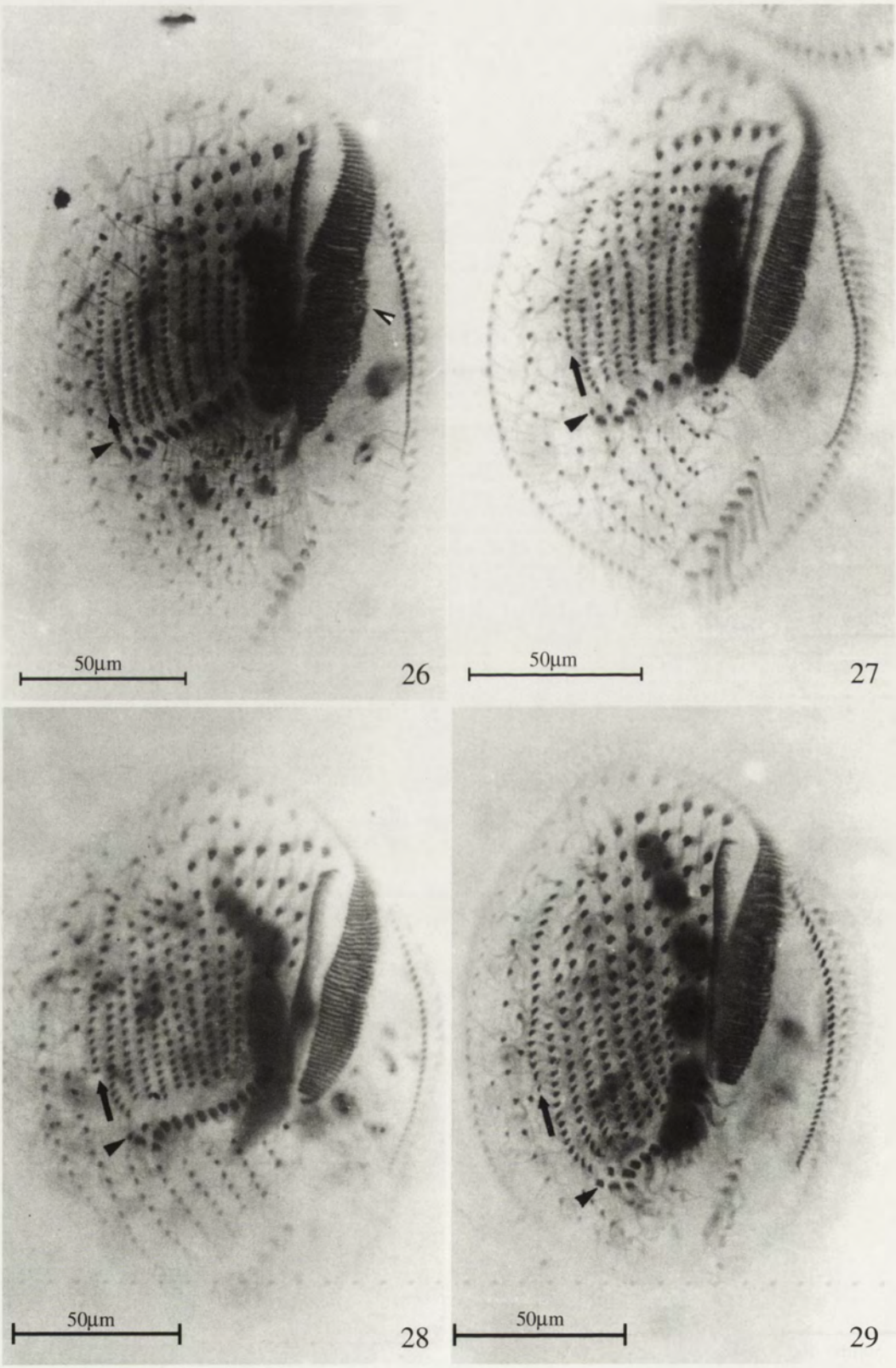
Figs. 22-24. Photomicrographs of protargol impregnated cells of *O. indica* showing dorsal ciliature (Fig. 22; cf Fig. 2) and the morphogenetic events on the dorsal surface (Figs. 23, 24; cf Figs. 12, 13)
Fig. 25. Photomicrograph of a Feulgen stained G_1 cell of *O. indica* showing the nuclear apparatus, Ma - macronucleus (beaded), Mi - micronuclei (n = 2)



Figs. 26-29. Photomicrographs of protargol impregnated cells of *O. indica* in late stages of reorganization. A small number of membranelles are formed from the OP and these fuse with the partially resorbed old AZM (open arrowhead). One set of FVT cirri formed replaces the old set. Note the anteriad movement of RV_2 row (arrows) leaving behind the last ventral cirrus and the leftward movement of the last ventral cirri of rows RV_1 and RV_2 (arrowheads)

Our studies have also shown that cells of *O. indica* when fed on different sized food organisms not only vary in size but also show corresponding differences with regard to the number of repeated ciliary structures such as adoral membranelles, marginal cirri, FVT rows and FVT cirri. These differences are proportional to cell sizes (unpublished observations). A direct comparison of quantitative data other than size is not possible since the biometric values for ciliature given for *O. quadricornutus* are for a population raised in spring water containing mixed food organisms. These conditions are not exactly reproducible. This may also be one of the reasons for the wide ranges of values for biometric features reported for *O. quadricornutus* by Foissner et al. (1987).

4. Comparison of morphogenetic events. The present study is a more detailed account of the morphogenetic process and has revealed some additional features, such as the exact origin, development and relative movements of the new RV rows and the origin of DPs in specific regions of the dorsal surface. The morphogenetic events during division essentially follow the same pattern as described by Foissner et al. (1987) for *O. quadricornutus*. The differences are largely due to the morphometric separation between the two species. For example, the number of FVT primordia is more in *O. quadricornutus*; the number of unincorporated parental FVT cirri between the two primordial zones in *O. quadricornutus* is much more than in *O. indica* (cf Fig. 16, Foissner et al. 1987, and Figs. 6, 7, 18, present report).

In *O. quadricornutus* the origin of proter buccal row is stated to be to the right of the parental buccal row without participation of pre-existing cirri in this row. The present work revealed the origin of the buccal row in *O. indica* to be within the parental buccal row utilizing pre-existing ciliature (Figs. 4, 17). The fact that the primordium soon shifts to the right and that the rest of the cirri in the parental buccal row are resorbed much later, may account for this having been overlooked in *O. quadricornutus*.

Comparative study among related genera

1. Right ventral rows. A comparison of the two extreme right FVT rows (termed as the right ventral rows in this report) amongst the oxytrichids *sensu lato* reveals interesting similarities and differences in their origin and deployment. In *Paraurostyla weissei* (Wirnsberger et al. 1985) these rows originate in the right-most parental row which is of composite origin (i.e. formed from 2 streaks). In this manner, the right ventral rows are self-replicating. In the American strain of *P. weissei* (Jerka-Dziadosz and Frankel 1969) two primordia form in the right-most rows but exact relationship between the old and the new rows has not been indicated by the authors. Grimes and L'Hernault (1978) state that the two right ventral rows are self replicating in *P. hymenophora*. In *Laurentiella acuminata* (Martin et al. 1983), *Onychodromus quadricornutus* (Foissner et al. 1987) and in *O. indica* (present study), the right ventral rows do not contribute towards primordia formation. In *Gastrostyla steinii* (pers. obs.), the two primordia for right ventral rows arise from a cirrus that forms a part of the inner of the two right rows. Incidently this is also true for oxytrichids *sensu stricto* (Winsberger et al. 1986, Kamra and Sapra 1990). One may conclude from the above discussion that the right ventral rows (the two right most FVT rows) in the various oxytrichids *sensu lato* are not homologous.

Interestingly, once developed, the right ventral rows exhibit similar morphogenetic "movements", i.e. displacements with respect to other cortical features, in most oxytrichids. The one on the left shifts posteriad and the one on the right shifts anteriad leaving behind the last two cirri. In addition the last ventral cirrus in each of the two rows shifts leftwards in most of the cases, losing their alignment with the parent rows (depicted in *O. indica* in Figs. 9-11, 19-21, 26-29).

2. Extra FVT primordia, subsequent resorptions. Variability in the number of primordia, the number of cirri formed therein and subsequent resorption of some new cirri is a common phenomenon in the lower oxytrichids (Jerka-Dziadosz and Frankel 1969, Grimes and L'Hernault 1978, Martin et al. 1983, Wirnsberger et al. 1985, Foissner et al. 1987), and leads to polymorphism in the population. It is possible that this is a strategy by which the cell can make finer numerical adjustments in relation to the final cell size. On the other hand the oxytrichids *sensu stricto* possess a constant number of FVT primordia and FVT cirri and their adjustment with respect to cell size can occur only by adjusting the number of kinetosomes in each cirrus.

3. Fragmentation of dorsal primordia. In *O. quadricornutus* (Foissner et al. 1987) and in *O. indica* (present study) all the dorsal primordia undergo multiple fragmentation. In contrast, only the first dorsal primordium from the left undergoes multiple fragmentation in *Laurentiella acuminata* and *O. grandis* (Foissner et al. 1987). In *Paraurostyla weissei* (Jerka-Dziadosz and Frankel 1969, Wirnsberger et al. 1985), *Gastrostyla steinii* (deduction from observations in Walker and Grim

1973) and most oxytrichids *sensu stricto* (Grimes and Adler 1976, Martin 1982, Wirnsberger et al. 1986), the dorsal primordium on the right fragments into two. With the limited information available, one can conclude that multiple fragmentation of DPs is an oxytrichid *sensu lato* characteristic expressing in the large sized cells of the group.

4. Origin of OP. The formation of oral primordium in *Onychodromus* and *Laurentiella* close to T_2 (50% cells) and rarely even T_3 in addition to the usual T_1 is significant especially in the light of the fact that these cirri are products of different primordial streaks. Amongst the hypotrichs only Urostylines, where OP develops next to products of different streaks, may be considered in this category. This may indicate a phylogenetic linkage of the oxytrichids *sensu lato* with the Urostylines.

5. Origin of marginals. A review of various hypotrichs shows that the marginal primordia appear in association with certain parental marginal cirri ("within row" development). In the oxytrichids one or two parental cirri disaggregate and contribute kinetosomes to form a primordium. This early primordium later moves right of the parental rows. The marginal primordia in *Onychodromus indica* and *O. quadricornutus* develop without participation of any parental cirri; this is a unique feature amongst the hypotrichs thus far investigated. Even in the closely related *Laurentiella acuminata* parental marginal cirri participate in primordia formation. This aspect must be borne in mind before an attempt is made to combine these genera as suggested by Hemberger (1982).

In view of the above characteristics, a linear relationship amongst the oxytrichids, as suggested by Martin (1982) based on reduction of FVT cirri, is a remote possibility. The phylogeny amongst the oxytrichids *sensu lato* appears to be far more complex.

Diagnosis of *Onychodromus indica* sp. n.

1. Large, epibenthic, slow moving hypotrichous ciliate, polymorphic with respect to food organisms; bacteria fed cells average 165.7 x 69.5 µm while the large cannibals average 320.0 x 178.3 µm.

2. Three prominent dorsal horns, one postero-medial and two posterolateral. A hump on the mid anterior region may develop a horn in some morphs.

3. Prominent AZM extends to about half the cell length. The frontal ciliature is in 9-13 linear rows (number varies with type of food) each ending posteriorly with a prominent transverse cirrus. Most rows on the left have large anterior cirri. The last two rows on the right (the right ventral rows) typically displaced, one posteriad and the other anteriad.

4. One RMC row and one LMC row.

5. Dorsal ciliature consists of 19-25 DB rows (two on the right are DM rows).

6. Cirral primordia (except the first three rows for opisthe) utilize parental cirri. RMC and LMC primordia formed *de novo*.

7. Except for the two DM rows, all other dorsal kineties arise by lateral splitting of 3 dorsal primordia.

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Parvicapsula hoffmani sp.n. (Myxozoa: Parvicapsulidae) from the Mullet, *Liza macrolepis* (Smith)

Kurapaty PADMA DOROTHY and Chaganti KALAVATI

Department of Zoology, Andhra University, Visakhapatnam, India

Summary. A new myxosporean parasite, *Parvicapsula hoffmani* sp.n. is described. It infects, the intestinal muscles of *Liza macrolepis*, a common mullet, inhabiting the backwater regions of Visakhapatnam harbour on the east coast of India. The systematic position of the parasite is discussed.

Key words. *Liza macrolepis*, *Parvicapsula hoffmani* sp.n.

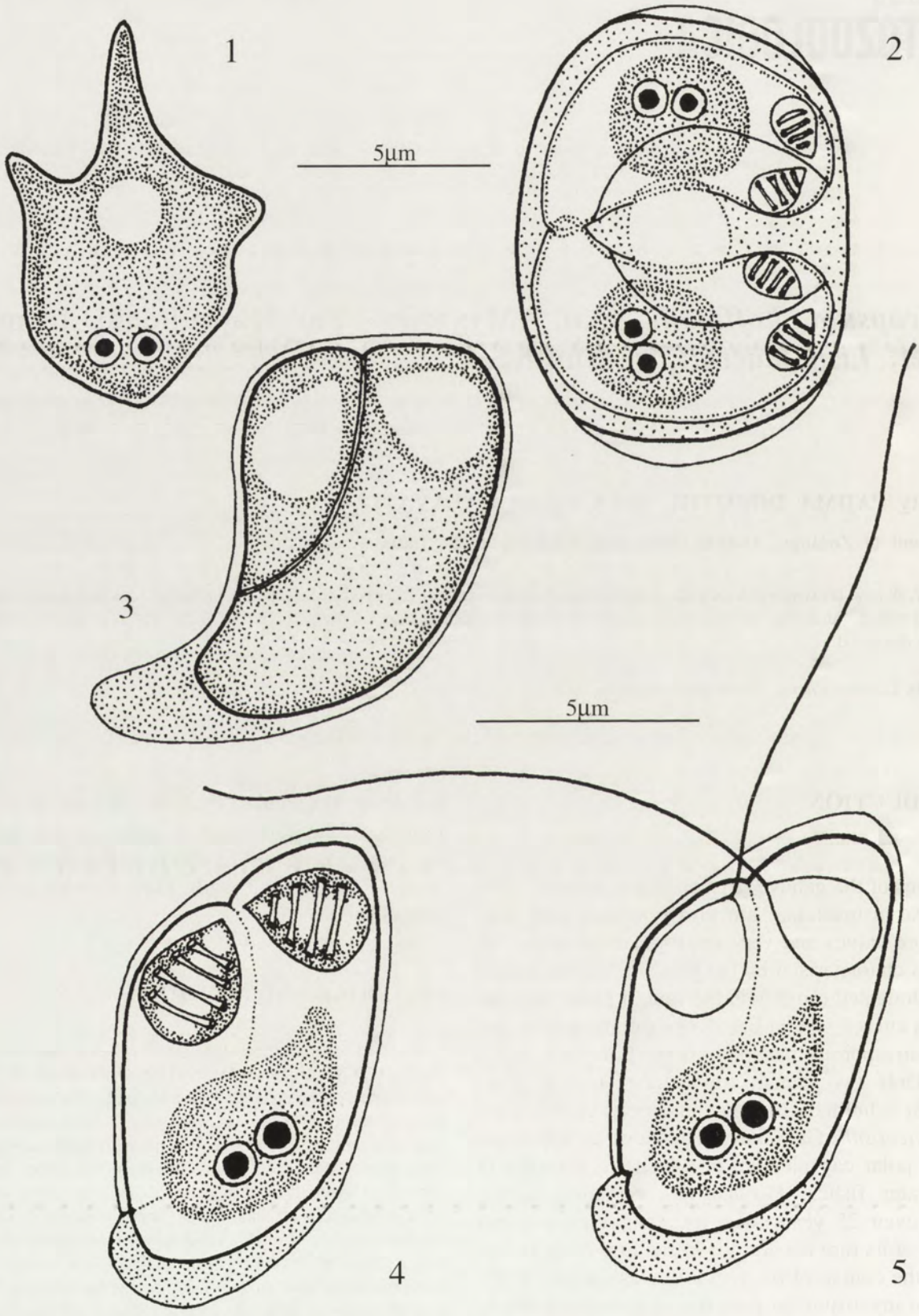
INTRODUCTION

Species of the genus *Parvicapsula* Schulman, 1953 (Fam: Parvicapsulidae) are myxosporeans with unequal spore valves and very small polar capsules. The family is characterised by the presence of thin walled spores elongated roughly in the sutural plane, unequal valves, a curved sutural line, 2 or 4 polar capsules and di- or tetrasporous pansporoblasts (Lom and Noble 1984). Only two genera are included namely, *Parvicapsula* Schulman, 1953 having 2 polar capsules and *Neoparvicapsula* Gaevskaya, Kovaleva et Schulman, 1982, 4 polar capsules, all of which are parasites of deep water fishes. *Parvicapsula* remained poorly studied even 25 years after its recognition possibly because of its rare occurrence in shallow water fishes. During the course of the present investigation (1985-1988) on myxosporean parasites of mullets of Visakhapatnam Harbour (17°41'34" N Lat: 83°17'35" E Long.) on the east coast of India, a new species of the genus *Parvicapsula* was recorded in the intestinal muscles of *Liza macrolepis*. The following is a description of the species.

Address for correspondence: K. Padma Dorothy, Department of Zoology, Andhra University, Visakhapatnam-530 003, India.

MATERIAL AND METHODS

The host fish, *Liza macrolepis*, were collected from the backwater regions of Visakhapatnam harbour. In the laboratory, the fish were autopsied and different organs examined under low magnification for possible infections. The cysts, when present, were carefully isolated from adhering tissues and smears prepared from the contents. Fresh observations were made with a drop of normal saline. The smears were either air-dried, fixed in methyl alcohol and stained with Giemsa or wet-fixed in Schaudinn's or Carnoy's fluid and stained with Heidenhain's haematoxylin. Fresh spores were treated with Lugol's iodine for the identification of the iodinophilous vacuole and with India ink (Lom and Vavra 1963) for mucus envelops. H_2O_2 and saturated aqueous urea were used to induce extrusion of polar filaments. Illustrations were made with the aid of camera lucida and measurements taken with an ocular micrometer and expressed in microns (μm).



Figs. 1-5. 1 –developing plasmodia of *Parvicapsula hoffmani* sp.n., 2 –sporogonic plasmodium, 3 –fresh spore showing sutural line and posterior lappet, 4 –spore stained with Giemsa, 5 – spore with extruded polar filaments. Upper scale bar of Figs. 1 and 2, lower: of Figs. 3-5

RESULTS AND DISCUSSION

Morphology

Trophozoites opaque white, oval, 125-156 x 107-130 µm deeply embedded in the intestinal epithelium. Plasmodia irregular, broadly oval 9.6 x 6.4 µm with 1-6 nuclei and 1 or 2 pseudopodia (Fig. 1). Sporogonic plasmodia disporous, oval, elongated 11.2-14 x 8.0-9.6 µm. Sporoblasts arranged with their concave valves facing each other with a large vacuole in between (Fig. 2). Fresh spores broadly oval, anterior end flat, posterior end narrow. Shell valves smooth, thin, unequal and curved. Larger valve convex dorsally and concave ventrally, extending into a blunt lappet-like projection posteriorly. Smaller valve fits into the concavity of large valve (Fig. 3). Sutural line thin, curved; polar capsules relatively small, pyriform, convergent, situated at right angles to each other Single capsular foramen. Sporoplasm generally binucleate, oval to comma-shaped, posterior to polar capsules; polar filament forms 4-5 coils while inside the capsule, thin and lightly stained when extended (Figs. 4, 5). Iodinophilous vacuole absent.

Spore dimensions in µm, mean in parentheses of n = 50:

Length of spore 8.5-10.32 (9.4); width 5.6-6.46 (5.89); polar capsule 3.0-3.8(3.46) x 1.7-2.6 (2.47); polar filament 13.7-18.9 (15.65).

Systematic Position

So far, only 5 species of *Parvicapsula* namely, *P. asymmetrica* found in *Cyclopterus lumpus* (Schulman, 1953); *P. unicornis* from *Callionymus lyra* (Kabata, 1962); *P. lobata* from *Austroatherina incisa* (Evidokimova, 1977); *P. schulmani* from *Beryx splendens* (Kovaleva et Gaevskaya, 1981) and *Parvicapsula* sp., from *Oncorhynchus kisutch* (Hoffman, 1981) have been reported. Lester and Sewell (1989) listed *Parvicapsula* sp., from the kidney of *Chaetodon aureofasciatus* and *Diodon hystrix*. Species *Parvicapsula* are identified on the basis of certain specific morphological characters in addition to the general characters meant for the genus. For example diagnostic features include a prominent process measuring 4 µm at capsular end of concave valve as in the case of *P. unicornis*; ellipsoidal spores with a small pinlike process at the posterior pole in *P. schulmani* and an unilaterally raised head organ in *Parvicapsula* sp., all of which characters are absent in the present species. *P. lobata* was incompletely described and synonymised with *Ceratomyxa lobata* by Gaevskaya, Kovaleva et Schulman, 1982. The only other species, *P. asymmetrica* has larger spores (10.5-17.0 x 6-9 µm) than in the present form (8.5-10.32 x 5.16-6.46 µm). Further, *P. asymmetrica* also differed in shape of the spore which is curved and narrow at the anterior end; while in the present form the spores are oval with flat anterior end, a bluntly pointed posterior end having a lappet-like extension. Pyriform polar capsules arranged at right angles to each other opening through a common foramen appeared unique to this species. In addition while all the other species of *Parvicapsula* are coelozoic in the urinary bladder or in renal tubules, the present species formed cysts in the intestinal epithelium. In view of these salient features and its occurrence in a brackishwater fish *Liza macrolepis*, the present species is considered new. We propose the name *Parvicapsula hoffmani* sp.n. in honour of Professor G.L. Hoffman, of the U.S. Fish and Wildlife Service, U.S.A.

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Dr Donald W. Duszynski
Department of Biology
The University of New Mexico
Albuquerque, New Mexico 87131
U.S.A.

Dr Steve J. Upton
Division of Biological Sciences
Ackert Hall
Kansas State University
Manhattan, Kansas 66506
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