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Morphologie et stomatogenèse chez deux Ciliés *Scuticociliatida* des genres *Philasterides* Kahl, 1926 et *Cyclidium* O. F. Müller; 1786

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Synopsis. Les structures de *Philasterides armata*, Kahl, 1926, sont détaillées. La morphogenèse buccale est du type *Uronema-Parauronema*, avec participation de cinétosomes issus de la parorale et de cinétosomes issus du scuticus. La morphologie de *Cyclidium bonneti* sp. n. est décrite. L'observation de la stomatogenèse de cette espèce montre la participation à la formation des organelles buccaux du scuticus représenté par deux parties distinctes. La séparation entre *Pleuronematina* et *Philasterina* n'apparaît plus nette en ce qui concerne les processus stomatogénétiques.

Depuis la création par Small de l'ordre des *Scuticociliatida* (1967), les processus de la stomatogenèse ont été observés chez les *Philasterina* des genres *Philaster* (Grolière 1974, Coats et Small 1976), *Parauronema* (Grolière 1974), *Paranophrys* (Didier et Wilbert 1976, Grolière et Léglise 1977), *Paralembus* (Grolière 1974), *Uronema* (Foissner 1972, de Puytorac et coll. 1974), *Dexiotricha* (Peck 1974), *Cinetochilum* (Grolière et Detcheva 1973, de Puytorac et coll. 1974), *Sathrophilus* (Grolière 1973, et 1974–1975), *Metanophrys* (de Puytorac et coll. 1974), *Urozona* (Grolière 1974), *Anophryoides* (de Puytorac et Grolière 1979) et *Cohnilembus* (Didier et Detcheva 1974), ainsi que chez des *Pleuronematina* des genres *Cyclidium* (Blacker et Evans 1969), *Histiobalantium* (Dragesco et Iftode 1972), *Pleuronema* (Grolière et Detcheva 1974) et *Ancistrum* (Hatzidimitriou et Berger 1977). Des types différents de stomatogenèse ont pu ainsi être mis en évidence (Grolière 1974 et 1974–1975). Ce-

pendant, nombreuses sont encore les formes dont on ne connaît avec exactitude ni la structure buccale ni les modalités de la stomatogenèse. Aussi, nous sommes-nous intéressé de ces points de vue à deux genres d'Infusoires récoltés dans les environs de Clermont-Ferrand, un *Philasterina*: *Philasterides armata* et un *Pleuronematina* du genre *Cyclidium*.

Materiel et Methodes

Philasterides armata récolté dans les plantes aquatiques bordant par place la rivière Allier, au niveau de la ville de Cournon (Puy-de-Dôme). est cultivé sur eau de Volvic additionnée de rate.

Cyclidium bonneti provient d'une source thermale, aux eaux très minéralisées, située sur la commune de Vic-le-Comte (Puy-de-Dôme). Il cohabite dans les plantes aquatiques avec différents Ciliés dont *Loxophyllum lanceolatum*, *Homalogastra setosa*, *Pseudocohnilembus persalinus*, *Radiophrya olivieri* (rencontré libre et présent aussi dans des *Enchytridae* du genre *Pachydrilus*), *Diophrys appendiculata* et différents autres Hypotriches. Des cultures de *C. bonneti* sont obtenues sur eau du milieu, filtrée, stérilisée et additionnée d'*Aerobacter aerogenes*; il se multiplie aussi toujours très rapidement dans des macérations de plantes aquatiques.

La morphologie générale et la stomatogenèse de ces Infusoires sont étudiées à l'aide d'imprégnations argentiques au protéinate d'argent selon la technique suivante:

— Les Ciliés sont fixés de 1 à 5 min par 4 ou 5 gouttes d'aldéhyde formique du commerce dilué à 10% et par 4 ou 5 gouttes de pyridine diluée à 5%; sans être lavés, les Infusoires sont mis sur lame et subissent une 1ère post-fixation de 20 s aux vapeurs d'acide osmique à 2%; une fine goutte d'albumine glycérinée est ajoutée au liquide contenant les cellules, puis l'ensemble est soigneusement homogénéisé. La préparation est laissée quelques heures à la température de la pièce (la lame à ce stade peut être gardée plusieurs jours), ou 15 min à 45°C.

— une 2ème post-fixation de 20 min est effectuée dans du Bouin alcoolique. Les Infusoires sont ensuite lavés 10 min à l'eau courante.

— La préparation est ensuite immergée 1 à 2 s dans du permenganate de potassium à 5% puis de 2 à 20 min dans de l'acide oxalique dilué à 5%, afin d'éclaircir les Ciliés. Le temps d'action de l'acide oxalique est fonction de la taille des cellules et des inclusions contenues, ainsi que de la quantité d'albumine glycérinée mise au préalable.

— La préparation est lavée 10 min à l'eau courante puis mise 25 min dans du protéinate d'argent dilué à 0.5%, la solution étant à une température de 45°C.

— Les structures argentophiles sont révélées par la passage de la préparation dans de l'hydroquinone à 1% puis fixée 10 min au minimum dans de l'hyposulfite de sodium dilué à 5%.

Cette technique permet une révélation très fine des cinétosomes et elle a l'avantage d'être relativement rapide.

Les Ciliés sont également étudiés sur imprégnations argentiques après fixation au Champy et au Da Fano, selon la technique de Chatton et Lwoff, afin de mettre en évidence plus particulièrement les pores des vacuoles contractiles, l'argyrome et les stries orales.

Toutes les mensurations sont faites sur des animaux fixés et après application des deux techniques d'imprégnation argentique.

Observations

Philasterides armata Kahl, 1926

Morphologie générale: Le Ciliés récolté dans les eaux de l'Allier est fusiforme, effilé au pôle antérieur, arrondi au pôle postérieur, porteur d'un long cil caudal. La plus grande largeur du corps (20–35 µm) est généralement voisine du tiers de la longueur (65–100 µm), les dimensions les plus couramment trouvées sont 80 × 27 µm.

Les cinéties somatiques, au nombre de 27 à 32, sont bipolaires, á l'exception des cinéties 1 et 2 qui n'atteignent pas le pôle antérieur. Les cinéties sont constituées dans leur région proximale par des cinétosomes appariés, des cinétosomes isolés pouvant également être présents très antérieurement, comme sur la cinétie n, par exemple, quelques éléments appariés pouvant par la suite s'intercaler. Les cinéties 1 et n s'écartent, d'une part dans le tiers antérieur de la cellule pour laisser place aux structures buccales, d'autre part, dans leur partie distale, où se trouve le cytoprocte (Fig. 1).

La vacuole pulsatile, située en dessous de l'équateur de la cellule, s'ouvre à l'extérieur, à ce niveau, par un pore disposé entre les cinéties 2 et 3 et très proche de cette dernière cinétie (Fig. 1). L'appareil nucléaire comprend un macronucleus ovoïde de 12–17 µm de plus grande dimension et un micronucleus sphérique proche du macronoyau.

Organisation buccale: La cavité buccale, antérieure, allongée, débute à 6–10 µm de l'apex de la cellule, elle occupe un territoire de 14–18 µm. Trois ensembles infraciliaires (M1, M2, M3) équidistants, sont disposés en file méridienne, une 4ème structure, coupée transversalement en 2 parties, est située à leur droite.

M1, de forme allongée, triangulaire, mesure 4 µm de longueur (3.2–5 µm). Les cinétosomes de cet organelle se répartissent selon 9 ou 10 niveaux. Les 3 cinétosomes antérieurs sont toujours isolés. Leur font suite, une paire cinétosomienne, un ou 2 groupes de 3 cinétosomes, puis 4 ou 5 groupes de 4 cinétosomes (Fig. 2). Tous les cinétosomes sont ciliés.

La base infraciliaire de M2, plus massive que celle de M1, mesure 5 µm de longueur (4.5–5.5 µm), 11 ou 12 niveaux cinétosomiens formés de 4, puis 6 et enfin 8–10 éléments constituent l'organelle. M3, de forme rectangulaire, à disposition générale oblique par rapport à l'axe de la

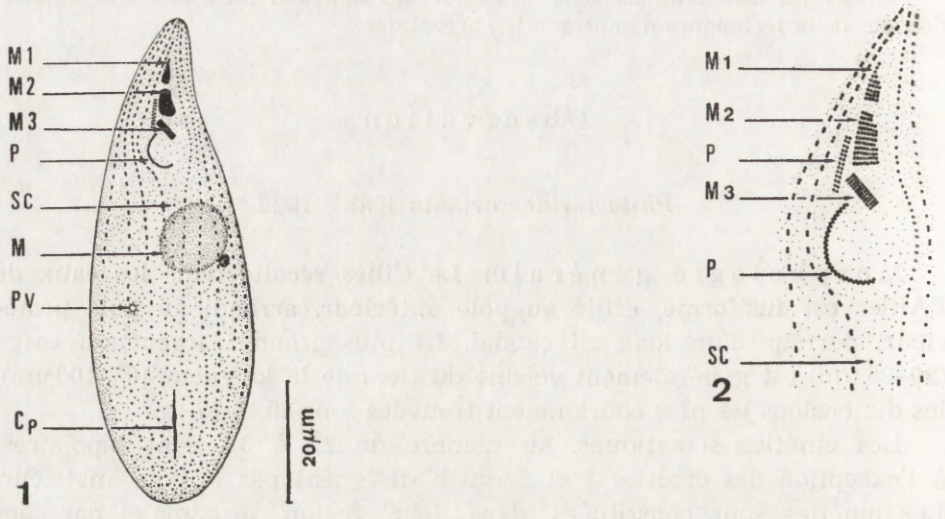


Fig. 1–2. Schémas de *Philasterides armata*. 1 — aspect général avec indication des 3 organelles adoraux (M1, M2, M3), de la parorale (P), du scuticus (SC), du macronoyau (M), du pore de la vacuole contractile (PV) et du cytoprocte (CP). 2 — détail de l'infraciliature buccale

cellule et aux organelles 1 et 2, mesure 3 à 4 µm de longueur. Quatre lignes cinétosomiennes d'une douzaine d'éléments chacune la constituent.

La parorale, en 2 parties distinctes, mesure 12 µm (10–15 µm). La partie proximale, débutant au niveau de la région moyenne de M2, est représentée par 2 lignes parallèles de cinétosomes juxtaposés. La 2ème partie, décalée sur la gauche par rapport à la lère, en forme ce C ouvert, est formé de cinétosomes disposés en zig-zag comme dans une parorale typique de *Scuticociliatida*.

En arrière du cytostome, entre les cinéties 1 et n, 9 à 12 cinétosomes alignés, non ciliés, représentent le scuticus.

Morphogenèse: Le premier indice de la mise en route des processus morphologiques de la stomatogenèse est la séparation des cinétosomes de la partie distale de la parorale. Ces éléments perdent leur disposition caractéristique en zig-zag pour s'ordonner en deux lignes infraciliaires parallèles, P'1 à gauche, P3 à droite. Cette séparation est corrélative de la multiplication des cinétosomes du scuticus (Fig. 3 a). Les cinétosomes de P3 se multiplient comme ceux de la ligne cinétosomienne antérieure droite de la parorale donnant le champ cinétosomien P2 (Fig. 3 b–c). Les cinétosomes de P'1 et de la zone gauche de la partie antérieure de la parorale forment une ligne infraciliaire P1 (Fig. 3 d).

Le champ cinétosomien P3 glisse en direction de la partie équatoriale de la cellule tout en se courbant vers la gauche, tandis que le

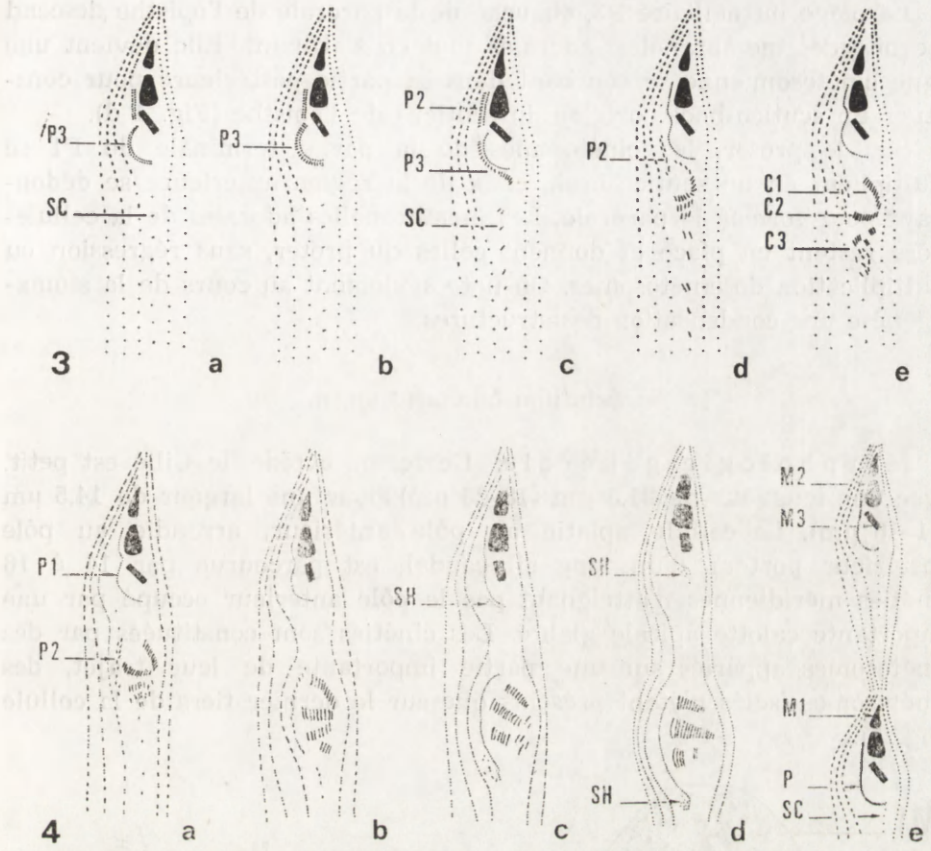


Fig. 3–4. Stades de morphogenèse de bipartition de *Philasterides armata*. Explication dans le texte

champ P2, ébauche de la parorale du futur opisthe, descend sur sa droite (Fig. 3 d). Les éléments du champ P3 se séparent en 2 plages, la plage antérieure constitue le champ C1 ébauche de la 1ère membranelle adorale de l'opisthe, la plage postérieure, C2, constituant l'ébauche de la 2ème membranelle adorale de ce même opisthe (Fig. 3 e et 4 a).

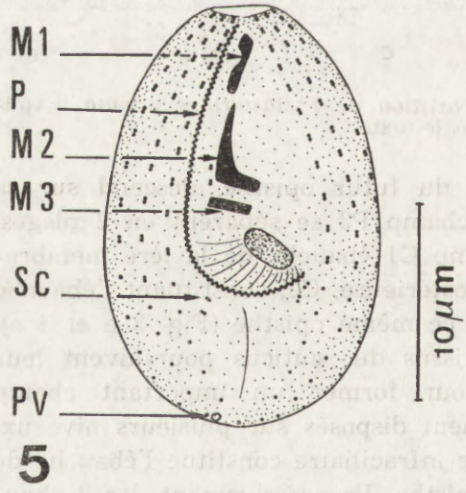
Simultanément, les cinétosomes issus du scuticus poursuivent leur multiplication et se regroupent pour former un important champ infraciliaire C3 à éléments initialement disposés sur plusieurs niveaux, puis seulement sur deux. Cette plage infraciliaire constitue l'ébauche de la 3ème membranelle adorale de l'opisthe. Progressivement, les 3 ébauches des membranelles s'individualisent. Il est probable que des éléments issus du champ C3, c'est-à-dire des cinétosomes du scuticus de la cellule mère viennent s'adjoindre au champ C2 pour constituer la 2ème membranelle adorale de l'opisthe (Fig. 4 c).

La plage infraciliaire P3, ébauche de la parorale de l'opisthe descend à droite des membranelles adorales tout en s'affinant. Elle devient une ligne cinétosomienne se courbant dans sa partie postérieure pour constituer un scutico-hook, origine du scuticus de l'opisthe (Fig. 4 d).

Sur le proter, les cinétosomes de la partie terminale de P1 se multiplient en un scutico-hook, ceux de la région antérieure se dédoublant pour former la parorale. Les membranelles adorales de la cellule-mère restent en place et donnent celles du proter, sans régression ou multiplication de cinétosomes. On note seulement au cours de la stomatogenèse une condensation des structures.

Cyclidium bonneti [1] sp. n.

Morphologie générale: De forme ovoïde, le Cilié est petit, avec une longueur de 21.5 µm (18–28 µm) pour une largeur de 14.5 µm (11–18 µm). La cellule, aplatie au pôle antérieur, arrondie au pôle postérieur, porteur d'un long cil caudal, est parcourue par 14 à 16 cinéties méridiennes n'atteignant pas le pôle antérieur occupé par une importante calotte apicale glabre. Les cinéties sont constituées par des cinétosomes appariés sur une partie importante de leur trajet, des cinétosomes isolés n'étant présents que sur le dernier tiers de la cellule (Fig. 5).



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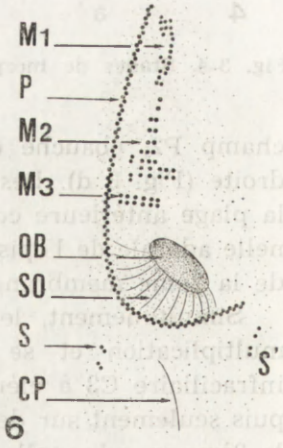

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Fig. 5–6. Schémas de *Cyclidium bonneti*. 5 — aspect général. 6 — détail de l'infraciliature buccale. Mêmes légendes que figures 1–2 et ouverture buccale (OB), stries orales (SO), partie droite du scuticus (S) et partie gauche du scuticus (S')

[1] Dédié à Monsieur Bonnet Louis, Professoeur à Touleuse.

La vacuole pulsatile, postérieure, possède un pore excréteur unique situé entre les cinéties 1 et 2, au niveau de leur cinétosome distal.

L'appareil nucléaire comprend 2 macronoyaux globulaires, plus ou moins accolés, de 4–5 μm de plus grande dimension, situés dans la partie antérieure de la cellule. Il n'a pas été décelé de micronucleus.

Organisation buccale: Débutant près de l'apex, la cavité buccale, très grande, occupe un territoire allongé de 14.5 μm (13–17 μm), c'est-à-dire dépassant les 2/3 de la longueur totale de la cellule, le rapport moyen longueur de la cavité buccale sur longueur de l'Infusoire étant de 70 (62–80). La parorale, très développée, débute près de l'apex; sa courbure postérieure entoure, en partie, le cytostome. Sur le plancher de la cavité buccale, 12 crêtes convergent vers le cytostome. Le scuticus est représenté par 2 groupes de cinétosomes, non ciliés, situés en arrière de la parorale. L'un, à droite (S), comprend 2 paires cinétosomiennes superposées, l'autre, à gauche (S′), comporte également 4 éléments mais disposés en ligne.

Le premier organelle adoral, M1, de forme allongé, légèrement courbé dans sa partie médiane, mesure 3.2 μm de longueur (3–3.5 μm). Ses cinétosomes ont une disposition constante, caractéristique (Fig. 6). Tous portent un cil. M2, plus important, a une longueur de 4.5 μm (4–5.5 μm), son infraciliature présente également une disposition constante. Les cinétosomes se répartissent sur une dizaine de niveaux transverses. Les 2 cinétosomes antérieurs sont constitués d'éléments isolés, leur font suite 3 paires cinétosomiennes, puis 2 groupes de 3 cinétosomes et enfin 2 groupes de 5 ou 6 cinétosomes. Dans la région distale de cette structure, sur sa droite, se trouvent 2 paires cinétosomiennes. M3, très courte, (1 μm) a sa plus grande longueur perpendiculaire à l'axe principal de la cellule. Deux rangées de 6 ou 7 cinétosomes constituent cette structure (Fig. 6).

Morphogenèse: Le premier stade que nous avons observé est la séparation des cinétosomes de la parorale à partir de la région proximale de cette dernière aboutissant à la formation de 2 lignes ciliaires, P1 à gauche, P2 à droite. Cette séparation est immédiatement suivie de la multiplication des cinétosomes de P2. Simultanément, les cinétosomes des 2 parties du scuticus S et S′ se multiplient donnant, à droite 4 groupes de cinétosomes (S1) situés sur 3 niveaux, à gauche 4 groupes de cinétosomes alignés (S2) (Fig. 7 b–c).

Les cinétosomes de la partie proximale de P2 s'écartent progressivement de P1 (Fig. 7d). Le champ cinétosomien P2 se coupe transversalement en 4 plages infraciliaires (P3, P4, P5, P6). Les champs P6, P5 et P4 glissent en direction de la partie équatoriale de la cellule. Tout en se superposant, ils constituent les ébauches des 3 organelles

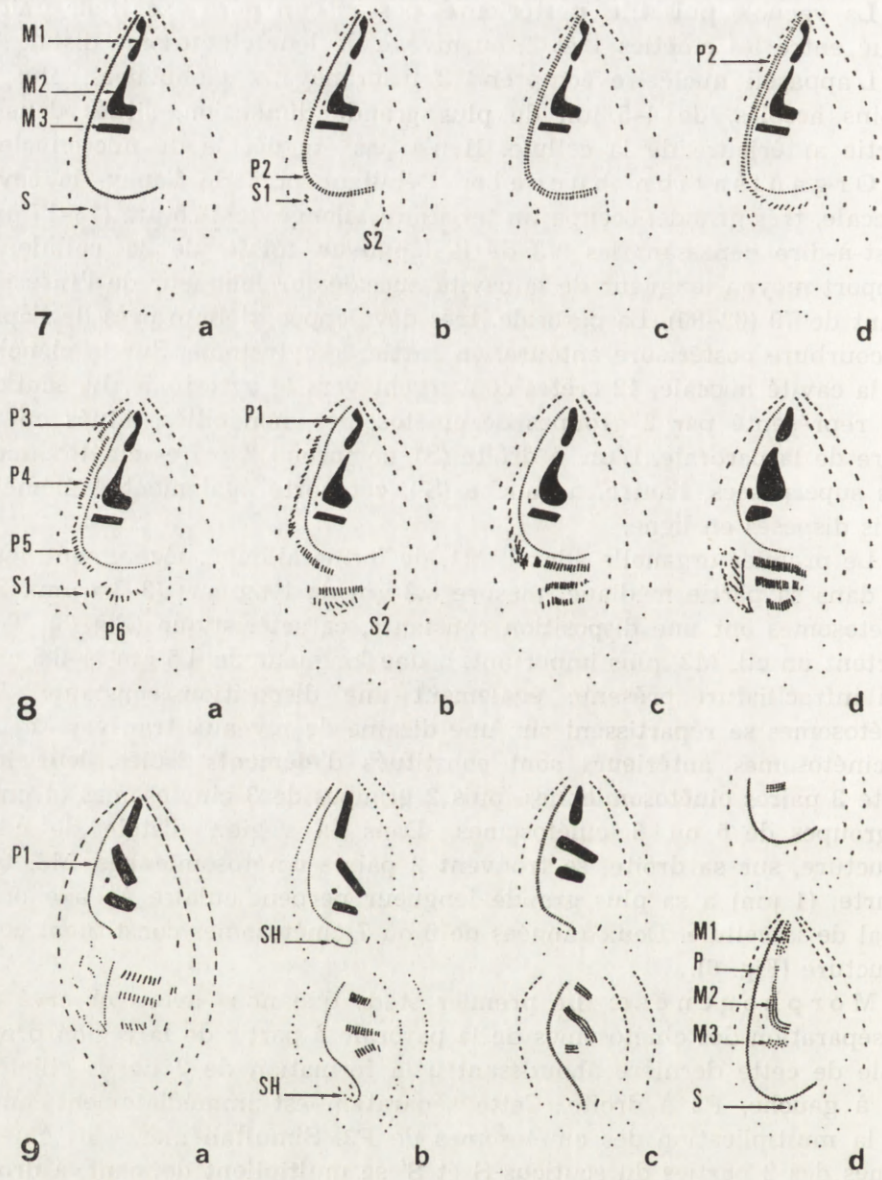

Fig. 7–8–9. Stades de morphogenèse de bipartition de *Cyclidium bonneti*. Explication dans le texte

adoraux du futur opisthe (respectivement M3, M2 et M1). Au champ cinétosomien P6 issus de la parorale de la cellule-mère sont venus s'ajouter les cinétosomes du champ S1 issus de la partie droite du scuticus (Fig. 8 a–c). Il est également possible qu'une partie de ces

cinétosomes participent à la formation du champ P5, c'est-à-dire à l'ébauche de M2.

Corrélativement à la mise en place des organelles adoraux, le champ infraciliaire P3 descend sur leur gauche. Il constitue l'ébauche de la parorale de l'opisthe (Fig. 8 b–d). Dans un stade ultérieur (Fig. 9 b), cette ébauche est formée par une ligne cinétosomienne terminée par une image caractéristique de scutico-hook. Les cinétosomes de cette ébauche se dédoublent pour former la parorale (Fig. 9 c).

Sur le proter les cinétosomes de P1, issus de la séparation des éléments de la parorale de la cellule-mère se multiplient; il y a formation d'un scutico-hook postérieur. Celui-ci constituera la partie droite (S) du scuticus du proter. Les autres structures buccales de la cellule mère redonneront celles du proter, sans régression ou multiplication de cinétosomes, mais après quelques modifications de forme et de disposition.

Simultanément le 1er groupe de cinétosomes du champ infraciliaire S2 issu de la partie S′ du scuticus de la cellule-mère (Fig. 6) se dispose à gauche de la partie distale de la parorale du proter, il formera la partie S′ du scuticus de cette cellule De même, le groupe distal de cinétosomes de S2 est à l'origine de la partie S′ du scuticus de l'opisthe, la partie S provenant du scutico-hook. Les 2 groupes moyens de cinétosomes de S2 s'intègrent aux ébauches des organelles adoraux de l'opisthe, vraissemblablement à l'ébauche de M2 (Fig. 9 a–b).

Discussion

Kahl (1931) a décrit *Philasterides armata* comme un petit Cilié vivant en eau douce, en forme de doigt, à vacuole pulsatile située juste en dessous de la partie médiane, appartenant à la famille des *Philasteridae* et voisin de *Philaster digitiformis*. Cet Infusoire revu par Fauré-Frémiet (1935) a été étudié par Mugard (1948) grâce à des imprégnations au nitrate d'argent. Cet auteur montre un champ deltoïde (M1) triangulaire, un champ trapézoïde (M2) simple et un champ falciforme (M3) rectangulaire. Le Cilié que nous avons récolté paraît pouvoir être assimilé avec *P. armata*, car il lui correspond par la forme générale, les dimensions, le mode alimentaire, les structures buccales et la position de la vacuole contractile.

Les structures buccales de *P. armata* sont assez proches de celles observées pour les espèces du genre *Paranophrys* (Didier et Wilbert 1976, Grolière et Léglise 1977), avec M1 et M2 y ayant la même allure générale. Cependant, elles en diffèrent par une M3 plus importante, rectangulaire, de position différente et par une parorale formée de deux parties distinctes. La situation sur la cellule du pore

de la vacuole contractile permet également de distinguer facilement les deux genres.

La stomatogenèse de *P. armata* telle que nous venons de la décrire est caractéristique des *Scuticociliatida* et plus particulièrement des *Philasterina*. En effet, les structures buccales tirent leur origine de champs cinétosomiens développés les uns à partir de la parorale, les autres à partir du scuticus. La stomatogenèse schématisée par

$$P \rightarrow P + M1 + M2 + \text{scuticus}$$
$$\text{scuticus} \rightarrow M3$$

correspond au type 3 que nous avons défini chez les *Philasterina* (1974) pour *Uronema* et *Parauronema* et retrouvé chez *Paranophrys carcini* (Grolière et Léglise 1977) et *Anophryoides salmacida* (de Puytorac et Grolière 1979).

Le Cilié récolté dans les eaux d'une source thermale, par sa forme générale, sa ciliature somatique, ses structures buccales, appartient au genre *Cyclidium*. Dans ce dernier, de très nombreuses espèces ont été décrites, mais, les structures buccales qui permettent, le plus souvent, de les différencier, n'ont pas été indiquées, pour nombre d'entre elles, avec précision. Nous ne les connaissons actuellement que pour quelques formes: *C. citrullus* (Czapik, 1963), *C. sphagnetorum* (Grolière 1973), *C. bergeri* (Agmaliev 1972), *C. plouneouri* (Dragesco 1963) et *C. glaucoma* (Berger et Thompson 1960, Grolière 1973). C'est avec cette dernière espèce que notre Cilié montre le plus de ressemblance; il en diffère cependant par un nombre plus important de cinéties (10 et 16), une cavité buccale plus développée (rapport longueur de la cavité buccale/longueur totale: 59 et 70) et une disposition des structures buccales différente. Il nous paraît difficile d'assimiler cet Infusoire à l'une des autres formes décrites, aucune diagnose ne correspondant à ses caractères. C'est pourquoi nous proposons d'en faire l'espèce *C. bonneti*.

C. bonneti possède un scuticus nettement individualisé, à éléments non ciliés, formé par deux parties distinctes. Un scuticus identique a été vu chez *C. glaucoma* par Blacker et Evans (1969), mais dans la description de la stomatogenèse donnée par ces auteurs pour une autre forme de *Cyclidium* (Blacker et Evans 1969), il n'intervient pas activement dans le processus de la formation des structures buccales de l'opisthe. Nous voyons très nettement que les cinétosomes du scuticus de *C. bonneti* participent à l'élaboration d'une partie des structures buccales. La stomatogenèse de *C. bonneti* pouvant être schématisée par

$$P \rightarrow P + S + M1 + M2\text{en partie} + M3 \text{ en partie}$$
$$S \rightarrow M2 \text{ en partie} + M3 \text{ en partie}$$
$$S' \rightarrow S' + M2 \text{ en partie}$$

Cette stomatogenèse est différente de celle observée chez *Pleuronema puytoraci* (Grolière et Detcheva 1974) où tous les nouveaux organelles ciliaires proviennent de la parorale de la cellule-mère. Elle met ici en jeu la multiplication de cinétosomes provenant du dédoublement de la parorale et la multiplication des cinétosomes du scuticus, ce qui est actuellement considéré comme typique des *Philasterina*. De même la présence de M1 à éléments ciliés et du scuticus à cinétosomes nus va dans le sens de l'appartenance de l'espèce à la famille des *Philasteridae*. Le seul élément rattachant le genre *Cyclidium*, ou peut être seulement certaines espèces de ce genre, aux *Pleuronematina* est la présence d'une membrane ondulante très développée. Ces observations concordent avec celles de Hatzidimitriou et Berger (1977) et de Puytorac et coll. (1978) mettant en évidence la présence d'un scuticus chez *Ancistrum* et chez *Proboveria* et sa participation à l'élaboration des structures buccales lors de la stomatogenèse d'*Ancistrum*. Une étude ultrastructurale de l'organisation corticale de représentants des *Pleuronematina*, des genres *Pleuronema* et *Cyclidium* en particulier apportera peut être des éléments nouveaux permettant de différencier plus aisément les deux sous ordres des *Scuticociliatida*.

SUMMARY

Structures of *Philasterides armata*, Kahl 1926, are shown in details. The stomatogenesis is of the *Uronema–Parauronema* type, with the participation of kinetosomes stemming from the paroral and others stemming from the scuticus. Infraciliary characteristics are described in *Cyclidium bonneti* sp. n. The observation of the stomatogenesis of this species shows that the scuticus, present in form of two separate parts, participates in the formation of mouth organelles. The distinction between *Pleuronematina* and *Philasterina* appears to be not clear, as far as their stomatogenesis is concerned.

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Trichodina ranae da Cunha, 1950 (*Ciliata, Peritrichida*), a Parasite of *Rana esculenta* s. l. and its Morphological Variability

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Synopsis. The trichodinas occurring in the urinary bladder of frogs, *Rana esculenta* s. l., are described. As a result of discussion of literature data the name *Trichodina ranae* da Cunha, 1950 is retained for them. Detail diagnosis of the species is given as well as the data on its distribution. The morphological variability of this species has been also studied and the variation among particular subpopulations has been found to be the main source of variation. The variation among local populations (groups of subpopulations from the same locality), host dependent variation as well as seasonal variation have appeared to be statistically insignificant.

The trichodinas parasitizing urinary bladder of frogs have been rarely reported up to now. They were noted by Bretschneider (1935) however without any information about their origin, as well as by Fauré-Fremiet and Mugard (1946) in France, by da Cunha (1950) in Portugal, Canella (1954) in Italy, Capuşe and Doncău (1957) in Roumania and by Haider (1964) in German Democratic Republic. Their ultrastructure was studied by Favard et al. (1963). Nevertheless there are some controversions not cleared up as far. First of all it is the problem of the proper name of the species, the problem of relations between trichodinas parasitizing frogs and those occurring in other amphibians, and the problem of distribution of these parasites in Europe. The author supposes that the material presented in this paper will elucidate at least some of these questions. The morphological variation of the examined species is also discussed.

Material and Methods

The trichodinas, being a subject of this paper, occur in the urinary bladder of edible frog, *Rana esculenta* sensu lato. This name embraces three closely re-

lated species: *R. lessonae* Camerano, *R. ridibunda* Pallas and *R. esculenta* L. In various periods of time the relations between these species were differently interpreted. In ancient literature *R. esculenta* was regarded as the main and the only valid species, the other two being considered as races or subspecies. Recently, Berger (1968, 1973, 1975) has proved that *R. esculenta* is a hybrid between *R. lessonae* and *R. ridibunda*. In the present paper most often the name *R. esculenta* s. l. is used (Table 1) because a part of the material had been collected before Berger's revision being published. However, great part of the material, the most interesting from the point of view of the incidence of trichodinas, originating from Konin lakes, was precisely determined (Table 2).

The frogs were collected and examined by Dr Bożena Grabda-Kazubska during many years of her investigations on parasites of amphibians. These studies have covered great part of the territory of Poland, the most numerous, however were the materials from the Mazurian Lakeland, environs of Warszawa and from the Konin lakes complex and the Goplo lake in central Poland. The frogs infested with trichodinas were found only in few localities (Table 1).

A number of frogs from France and Bulgaria was also examined and the trichodinas were found in the urinary bladder. Few frogs from the environs of Stralsund (GDR) were negative in this respect.

During dissection the content of the urinary bladder was used to make smears. Most smears were dried and silver impregnated preparations after Klein were made. A part of smears was fixed in Schaudinn's fixative, stained by Meyer's hematoxylin or the Feulgen's reaction was proceeded in order to reval the nuclear apparatus of ciliates.

Description of the adhesive disc and of the denticulate ring was made on the basis of silver impregnated preparations. From each subpopulation (a group of trichodinas from single host specimen) a random sample of about 30 individuals was measured. In cases of not numerous subpopulations, or not good preparations, smaller number of ciliates was measured. But only samples of 24–35 individuals measured were used for statistical purposes. In each case young and dividing specimens were excluded. In some trichodinas not all the features were sufficiently well visible to be measured. In such cases smaller numbers are marked at particular characters.

The following features were measured: (1) body diameter (DB), (2) diameter of the adhesive disc with border membrane (DDM), (3) diameter of the adhesive disc without border membrane (DAD), (4) diameter of the denticulate ring (DDR), (5) numer of denticles (ND), and (6) length of the denticle (LD). Moreover, for each subpopulation mean length of an arch of the denticulate ring corresponding to one denticle was counted according to the formula:

$$\frac{\text{denticulate ring diameter}}{\text{mean number of denticles}} \times \pi$$

The variation in trichodinas was studied with the aid of statistical methods, mainly the analysis of variance was performed using two and multilevel nested ANOVA with unequal sample sizes (Sokal and Rohlf 1969, Box 10.4 and 10.5). This analysis was made for three features: (1) diameter of the adhesive disc without border membrane, (2) denticulate ring diameter, and (3) the number of denticles. Choice of these characters regarded as representative for trichodinas was argued in previous papper (Kazubski 1979 b).

In some cases the correlation between particular characters was examined.

Table 1

A list of examined frogs *Rana esculenta* s. l. harbouring trichodinas in urinary bladder

Locality	Period of examination	No. of frogs	
		examined	infected
Poland			
Coastal region of Baltic Sea:			
Świdwie Lake near Szczecin	1977	35	—
Hel Peninsula and Żarnowieckie Lake	1959	8	—
Drużno Lake near Elbląg	1961	3	—
Mazurian Lakeland:			
Arklity Lake	1962—63	42	5
Gołdapiwo Lake	1954—56	213	—
Mamry, Święcajty and Stręgiel Lakes	1956—57	73	—
	1966—76	109	—
Gaudy Lake	1959, 1962	8	—
environs of Olsztyn	1956—57	53	—
Central Poland:			
Gopło Lake	1970—75	70	1
artifically heated Konin lakes[a]:			
Ślesińskie		51	20
Pątnowskie	1970—75	20	3
Gosławskie		53	12
Licheńskie		163	59
environs of Warsaw, Łomna	1954—79	238	2
environs of Puławy	1961	2	—
Subcarpathian region:			
Porąbka at Soła river	1960	4	1
France			
Camargue — Lac Vaccarès and Tour de Valat, dep. Bouches-du-Rhone	10.10.1974	18[b]	4
Balazuk at Ardeche river, dep. Ardeche	17.8.1976	5[c]	—
Pont du Chateau at Allier river, dep. Puy de Dome	9.9.1976	1[d]	—
Bulgaria			
Environs of Sofia	14.5.1970	8[d]	
Arkutino Reserve at Ropotamo river	17.5.1970	19[d]	3
German Democratic Republic			
Coastal region of Baltic Sea, environs of Straslund	14.8.1977	4[c]	—

[a] see also Table 2, [b] determined as *R. ridibunda perezi*, [c] determined as *R. esculenta*, [d] determined as *R. ridibunda*

The correlation was computed with the aid of TI-SR-51-II calculator for mean values of particular samples.

An influence of geographical distribution and variation among particular local groups of subpopulations was examined. In the case of fairly abundant material from the Konin lakes also the influence of the host species was studied as well as seasonal changes.

Table 2

Incidence of trichodinas in particular species of water frogs in artificially heated Konin lake

Locality	*R. esculenta*		*R. lessone*		*R. ridibunda*	
	examined	infected	examined	infected	examined	infected
Ślesińskie lake	27	11	21	7	3	2
Pątnowskie lake	12	3	6	—	2	—
Goslawskie lake	46	9	4	2	3	1
Lichenskie lake	107	40	55	19	1	—
Total	192	63	86	28	9	3
per cent of infection		32.81		32.56		33.33

Results and Discussion

Description of Species and its Taxonomy

The trichodinas found in the urinary bladder of frogs *Rana esculenta* s. l. are characterized by proportionally great height of the body — the height being almost equal to cell diameter. The apex of the cell is somewhat shifted from the body axis. The dimensions of the adoral spiral, describing 1 1/6 turns, are equal to 40.5 × 35.7 μm at the average (10 specimen measured at random). Dimensions of the body, of the adhesive disc, of the denticulate ring and the number of denticles of eleven local populations of trichodinas are given in Table 3. Morphological variation of these characters, examined with statistical methods, is discussed in further part of this paper.

The adhesive disc has the structure characteristic of ciliates of the genus *Trichodina* (Fig. 1 a). The structure of denticles is also typical; both parts of the denticle, the outer blade and the inner ray, are almost of the same length. The blade is sickle-shaped clearly tapering in the distal part from about 1/3 of its length. The inner ray has not the same thickness at the whole length, it is finger-like, sometimes slightly bent. Spaces between particular blades are fairly large (Fig. 1 b–d).

The nuclear apparatus is composed of a horseshoe-shaped macronucleus, measuring about 43 μm in the diameter and 5.5 μm wide, and of an elongate spindle-shaped micronucleus measuring 5 × 1 μm. The micronucleus tightly adheres to one end of the macronucleus being difficult to perceive.

The trichodinas were found in all three species of the complex *R. esculenta* s. l. Their incidence was very similar in all these hosts (Table 2).

Table 3

Metric and meristic data of trichodinas occurring in water frogs in different localities

Locality	Diameter (μm)								No. of denticles		Length of denticle (μm)	
	body		adhesive disc with border membrane		adhesive disc		denticulate ring					
Arklity Lake	49–88.5[a]	67.74[b]	31–55	45.15	29.5–44	36.96	18.5–29.5	24.40	21–33	28.34	10–15	12.30
	8.79[c]	116[d]	3.84	120	3.14	120	2.50	120	1.94	120	1.09	120
Gopło Lake	55–74	65.57	34.5–44	39.98	28–37	32.50	18–25.5	21.45	27–31	29.03	10–12	11.00
	6.56	30	2.48	30	2.17	30	2.06	30	1.30	30	0.63	30
Ślesińskie Lake	44–94	67.08	29.5–54	42.45	24–45	34.89	16–29.5	22.93	23–35	29.36	9–14.5	11.54
	10.52	307	3.91	372	3.63	372	2.75	372	2.04	372	0.95	372
Pątnowskie Lake	66.5–87	75.37	41.5–48	43.90	36–39	38.10	22.5–25	24.30	28–31	29.20	12–13	12.60
	8.52	4	2.56	5	1.24	5	1.30	5	1.30	5	0.55	5
Gosławskie Lake	47–94	68.56	33–53	41.47	25–44	34.22	17–32	22.01	24–33	28.74	9–14	11.71
	9.31	186	3.53	201	3.17	201	2.58	201	1.83	201	0.925	201
Licheńskie Lake	44–100	68.88	33–53	42.47	25–44	35.07	17–31	22.67	23–35	29.05	9–15	11.84
	10.29	626	3.68	666	3.30	666	2.71	666	1.83	666	0.92	666
Environs of Warsow, Łomna	52–88.5	70.12	35–50	42.37	26.5–44	34.38	16–29.5	22.56	23–32	28.09	10–14.5	11.95
	10.54	24	3.89	34	3.70	34	3.11	34	1.83	34	0.86	34
Porąbka at Soła river	49–82	66.17	40–53.5	45.27	31–41	36.03	21–29.5	24.43	25–30	28.40	11–14	12.35
	7.96	30	3.54	30	2.55	30	2.34	30	1.25	30	0.75	30
Poland — summarized data	44–100	68.23	29.5–55	42.56	24–45	35.02	16–32	22.80	21–35	28.99	9–15	11.78
	10.26	1323	3.83	1458	3.42	1458	2.74	1458	1.90	1458	0.96	1458
France — Camargue	53–94	71.14	36.5–50	43.42	29.5–44	36.71	18–31	23.72	26–36	30.03	10–14	12.30
	9.83	66	3.57	72	3.15	72	2.85	72	2.18	72	0.70	72
Environs of Sofia	48–77	61.81	35–50.5	41.75	27–41	33.83	17–26	22.23	26–33	30.23	9.5–13	11.30
	10.13	29	3.82	30	3.73	30	2.45	30	2.13	30	0.93	30
Arkutino Reserve	54–96	70.67	32.5–49	40.97	25–40.5	33.28	17–28	22.04	22–36	29.51	9–13	11.08
	10.23	81	3.68	90	3.91	90	2.81	90	3.87	90	0.90	90
Bulgaria — summarized data	48–96	68.34	32.5–50.5	41.16	25–41	33.42	17–28	22.09	25–36	29.69	9–13	11.14
	10.89	110	3.72	120	3.86	120	2.72	120	3.52	120	0.91	120
Total — summarized data	44–100	68.36	29.5–55	42.49	24–45	34.98	16–32	22.79	21–36	29.09	9–15	11.76
	10.30	1499	3.83	1650	3.48	1650	2.75	1650	2.09	1650	0.97	1650

[a]range, [b]mean, [c]standard deviation, [d]sample numerosity

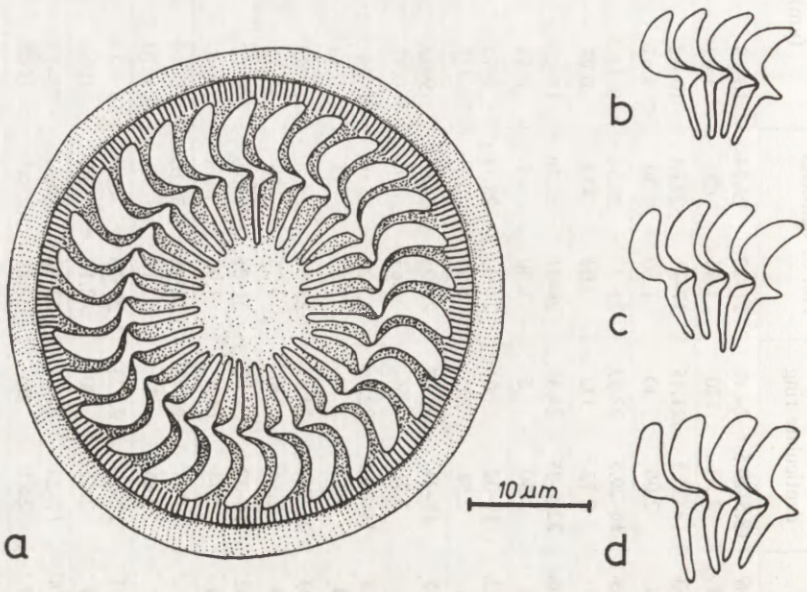

Fig. 1. *Trichodina ranae* da Cunha, a — adhesive disc with border membrane, b–d — denticles of different specimens from the Gosławskie lake (Konin lakes)

The trichodinas considered in the present paper are undoubtedly identical with those described by Fauré-Fremiet and Mugard (1946), da Cunha (1950), Canella (1954), Favard et all. (1963) and Haider (1964) (Table 4). By the body dimensions they clearly differ from protozoans described by Bretschneider (1935). The drawing given by this author (Bretschneider 1935, Abb. 1, p. 363) represents a protozoan of a great body height but differing in shape from trichodinas found by present author in *Rana esculenta* s. l.

A supposition seems to be justified that in the urinary bladder of frogs in Europe two different species of trichodinas occur: *T. entzii* Bretschneider, 1935 and the trichodina described in the present paper and recorded by Fauré-Fremiet and Mugard, da Cunha and other authors. The first mentioned species was found only once and its description needs to be completed. The second species widely distributed in Europe, is well known with regard to the morphology, but its nomenclature needs some comments. First of all this species was described by Fauré-Fremiet and Mugard (1946) under the name *T. urinicola* Fulton, 1923 — the name used for trichodinas parasitizing the urinary bladder of toads of the genus *Bufo* in North America (Fulton 1923). The American species differs from the European one by the body shape. Other characters, such as dimensions

Table 4

A review of trichodinids occurring in urinary bladder of water frogs in Europe

Name used and references	Diameter (μm)			No. of denticles	Length of denticles (μm)	Body height (μm)	Country	Host
	body	adhesive disc	denticulate ring					
Trichodina enzli Breischneider, 1935	60–130	–	–	–	12–15	75–112	?	*R. esculenta*
T. urinicola: Fauré-Fremiet et Mugard, 1946	–	–	16–23	22–31 26–28	–	–	France	*R. esculenta*
T. ranae de Cunha, 1950	40–50	–	–	23–31	10–12	30–50	Portugal	*R ridibunda perezi*
T. ranae de Cunha: Canella, 1954	–	–	–	28, 30 31, 34[a]	–	–	Italy	*R. esculenta*
Trichodina sp.: Capuşe et Doncau, 1957	60–75	–	–	–	–	75–85	Roumania	*R.ridibunda*
T. urinicola: Favard et al., 1963	–	–	–	25[a]	–	–	France	*R. esculenta*
T. ranae de Cunha: Haider, 1964	47.7–57.3	38.2–51.5	19.1–24.8	28–33	10–12	28.6–49.6	GDR	*R.esculenta*

[a] from figures

of the adhesive disc and of the denticulate ring can be compared since they were not described in the American species. This and other points of view, considered in my previous paper (Kazubski 1979 a) justify retention of the name *T. urinicola* for trichodinas occurring in American toads. Taking all this into account, the only valid name for trichodinas under consideration is the name *Trichodina ranae* proposed by da Cunha (1950).

Within this species the trichodinas found by Capuşe and Donaau (1957) in Roumania (Table 4) ought to be placed. Despite incompleteness and lack of details the discription given by these authors is not in disagreement with the description of *T. ranae*. Slight differences may be the result of deformation of ciliates during fixation.

There is one more species of trichodina from the urinary bladder of frogs in the holarctic region, namely *T. vesicola* Suzuki, 1950 occurring in *R. rugosa*. These trichodinas are however, larger and have greater number of denticles than *T. ranae*, being clearly distinct from the latter.

It seems also that *Trichodina* sp. recorded by Golemansky and Miceva (1975) from the urinary bladder of *Bombina variegata* in Bulgaria ought to be assigned to *T. ranae*. On account of photographs given by mentioned authors great similarity of both trichodinas may be ascertained. Also the body dimensions are similar except the dimensions of the denticulate ring — it may be the result of another method of measuring of this element. However, to be finally elucidated this problem needs reexamination of trichodinas collected by Bulgarian authors.

Another problem to be discussed is the distinctness of trichodinas from frogs and newts. Fauré-Fremiet and Mugard, (1946) Favard et al. (1963) supposed that the trichodinas from the urinary bladder of frogs and those from newts represent the same species. This opinion has been shared by Lom (1958). Now, on the base of the material given in this paper as well as on earlier papers (Kazubski 1979 a, b) it may be ascertained that these trichodinas represent different species. *T. ranae* and *T. vesicularum*, inspite of similar dimensions of the adhesive disc and of the denticulate ring and similar number of denticles, significantly differ by the body dimensions, the dimensions of the macronucleus and, first of all, by the shape of denticles. Greater differences embracing the dimensions of the adhesive disc and of the denticuate ring exist between *T. ranae* and *T. faure-fremieti*. Moreover, the considered species are highly specific towards their hosts.

Summing up the above discussion it may be ascertained that in the urinary bladder of frogs in Europe two species of trichodina occur,

namely *T. entzii* Bretschneider, 1935, the description of which should be completed, and *T. ranae* da Cunha, 1950 with the following diagnosis:

Trichodina ranae da Cunha, 1950

(Synonyms: *T. urinicola* Fulton, 1923: Fauré-Fremiet et Mugard, 1946 and Favard et al. 1963).

The body is in form of a fairly high, slightly leaned cone with rounded top. The adoral spiral describes about 1 1/6 turn measuring 40.5 × 35.7 μm. Dimensions of trichodinas from various countries in Europe are given in Tables 3 and 4. The structure of denticles is as follows: the anterior blade and the inner ray are of the same length. The blade is sickle-shaped tapering centrifugally from about 1/3 of its length. The ray is finger-like, sometimes slightly bent. Its width is not equal at the whole length. The nuclear apparatus is composed of a horseshoe-like macronucleus measuring about 45 μm in the diameter and 5.5 μm wide, and of an elongate micronucleus measuring 5 × 1 μm. The micronucleus tightly adheres to one end of the macronucleus and is difficult to be noticed.

The hosts of *T. ranae* are *Rana esculenta* s. l., *R. lessonae*, *R. ridibunda* and its west-European subspecies *R. ridibunda perezi*.

Distribution

T. ranae was noted in Portugal, Italy, France, Roumania and in southern part of GDR. The present study has ascertained the widespread distribution of the species in southern region of France, in the delta of Rodan, and frequent occurrence in Bulgaria. Its distribution in Poland is interesting: the trichodina occurs in the whole country but is very rare. The only locality in which more frequent occurrence of the parasite was noted are Konin lakes. This is an artificially heated complex of lakes with greatly changed microclimat of its environs (Pojmańska et al., in press). So, it may be concluded that *T. ranae* occurs in frogs mainly in warmer, southern parts of Europe. Poland is at the border of its distribution area — the ciliates are greatly dispersed being more concentrated only in warmer localities securing favourable conditions of the environment.

Variation in *T. ranae*

The study on variation of *T. ranae* was made on 47 subpopulations originating from various parts of Poland, mainly from the Konin lakes, from France and Bulgaria. Mean values, standard deviation and numerosity of samples for all examined subpopulations are given in Table 5.

Table 5

Mean values (M) and standard deviations (SD) of main characters in samples of 47 subpopulations of *Trichodina rana*e (n-sample numerosity)

Locality	Date	No. of subpopulations[a]	Diameter (μm)												No. of denticles			Length of denticle (μm)			Width of denticle[b] (μm)
			body			adhesive disc with border membrane			adhesive disc			denticulate ring									
			M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	
Arklity Lake	18.08.1962	18	70.10	7.94	30	48.85	2.97	30	39.57	2.57	30	26.28	1.91	30	28.57	1.92	30	13.25	0.88	30	2.89
	18.08.1962	21	69.32	7.30	28	44.73	3.74	30	36.18	3.01	30	23.65	2.35	30	27.67	1.54	30	12.60	0.82	30	2.69
	29.07.1964	590	62.86	9.87	29	42.45	2.62	30	35.00	2.47	30	22.98	2.02	30	28.63	2.08	30	11.32	0.86	30	2.52
	29.07.1964	596	68.65	8.34	29	44.57	2.95	30	37.08	2.64	30	24.67	2.43	30	28.50	2.10	30	12.03	0.80	30	2.72
Gopło Lake	20.10.1971	210 R.e.	65.57	6.56	30	39.98	2.48	30	32.50	2.17	30	21.45	2.06	30	29.03	1.30	30	11.00	0.63	30	2.32
Ślesińskie Lake	8.10.1971	191 R.l.	72.74	7.65	25	42.43	2.73	30	34.77	2.83	30	23.68	2.01	30	29.80	1.52	30	11.23	0.85	30	2.50
	8.10.1971	195 R.e.	63.00	8.86	27	42.00	2.62	30	33.22	2.56	30	21.68	1.83	30	31.23	1.61	30	11.50	0.69	30	2.18
	19.10.1971	197 R.r.	73.05	9.26	29	40.67	2.74	30	33.85	2.84	30	22.25	2.19	30	29.40	2.88	30	11.27	0.76	30	2.38
	19.10.1971	198 R.r.	73.79	11.46	29	38.63	3.69	30	32.03	3.00	30	20.10	2.29	30	28.70	2.02	30	11.52	0.64	30	2.20
	22.05.1972	254 R.e.	58.98	7.97	27	39.78	4.51	32	32.19	3.28	32	20.95	2.23	32	29.75	1.68	32	11.05	0.76	32	2.21
	22.05.1972	257 R.l.	58.26	8.05	29	41.48	3.36	30	34.20	3.38	30	23.05	2.42	30	27.77	1.96	30	10.57	0.97	30	2.61
	22.05.1972	258 R.e.	61.93	5.97	30	41.90	2.99	30	33.92	2.46	30	22.28	1.94	30	28.07	1.95	30	11.33	0.61	30	2.49
	9.05.1974	432 R.l.	63.06	7.21	26	44.80	2.60	30	36.18	2.71	30	24.03	3.53	30	28.97	1.73	30	11.90	0.92	30	2.61
	9.05.1974	434 R.e.	66.96	8.18	13	43.37	3.12	30	34.78	2.69	30	22.67	1.89	30	28.93	1.72	30	11.32	0.85	30	2.46
	13.05.1074	444 R.l.	67.11	6.62	18	44.69	3.50	26	37.40	3.10	26	24.50	2.11	26	29.62	1.88	26	12.02	0.79	26	2.60
	13.05.1974	449 R.e.	71.56	9.17	24	45.62	3.39	30	38.53	3.36	30	25.15	2.66	30	30.40	1.30	30	12.43	0.80	30	2.60
Gosławskie Lake	10.09.1971	187 R.e.	65.50	7.38	30	38.60	2.93	30	32.72	2.62	30	21.02	2.07	30	28.27	1.64	30	11.43	0.95	30	2.34
	10.09.1971	190 R.r.	66.06	6.93	25	43.08	2.40	26	35.77	2.64	26	23.11	1.74	26	27.96	1.56	26	12.04	0.89	26	2.60
	19.10.1971	196 R.l.	76.78	7.60	30	41.02	2.49	30	34.20	2.07	30	21.47	1.79	30	28.80	1.56	30	11.73	0.78	30	2.34
	24.06.1972	268 R.e.	65.03	7.76	31	38.69	3.34	31	31.56	2.74	31	19.69	2.15	31	28.97	1.52	31	11.06	0.93	31	2.13
	11.07.1972	284 R.e.	71.03	9.76	20	44.52	3.50	28	36.61	3.44	28	24.79	2.48	28	29.39	1.47	28	12.04	0.87	28	2.65
	25.06.1973	359 R.e.	66.84	9.94	35	45.57	2.40	35	34.53	2.75	35	21.94	1.98	35	28.86	1.82	35	12.09	0.70	35	2.39

Licheńskie Lake	25.09.1970	28	R.e.	65.07	7.87	27	40.12	3.17	30	33.22	2.82	30	20.72	2.17	30	29.23	1.41	30	11.57	0.70	30	2.23
	7.05.1971	83	R.e.	67.93	6.25	28	42.82	2.66	30	34.88	2.96	30	22.30	1.98	30	29.53	1.79	30	11.23	0.57	30	2.37
	7.05.1971	84	R.e.	64.84	6.98	28	41.80	2.85	30	34.17	2.41	30	21.77	1.71	30	28.53	1.38	30	11.30	0.79	30	2.40
	7.05.1971	87	R.e.	62.02	8.33	28	41.17	3.68	30	33.65	3.12	30	22.02	2.42	30	29.77	1.43	30	11.33	0.55	30	2.32
	7.05.1971	88	R.e.	72.02	8.50	29	42.63	3.06	30	35.45	2.93	30	23.17	2.73	30	29.13	1.22	30	11.58	0.60	30	2.50
	7.05.1971	91	R.e.	68.64	8.63	25	39.02	1.91	30	32.55	2.14	30	19.75	1.19	30	26.87	1.33	30	11.70	0.66	30	2.31
	7.08.1971	136	R.l.	74.71	9.69	29	41.78	2.15	30	35.32	2.26	30	22.22	1.86	30	27.40	1.38	30	12.17	0.66	30	2.55
	21.10.1971	209	R.e.	77.12	8.90	29	42.78	3.16	30	34.73	2.58	30	22.45	2.03	30	29.00	1.76	30	11.87	0.89	30	2.43
	22.05.1972	243	R.e.	62.13	8.53	30	39.68	3.83	30	31.92	3.31	30	20.33	2.49	30	28.27	1.78	30	11.17	0.92	30	2.26
	28.10.1972	317	R.e.	78.19	11.63	29	46.27	3.62	30	37.73	3.28	30	24.57	2.82	30	30.07	1.28	30	12.63	0.96	30	2.57
	12.05.1973	333	R.e.	67.15	8.29	30	43.22	3.42	30	35.80	3.01	30	23.02	2.86	30	28.73	1.60	30	12.20	0.95	30	2.52
	12.05.1973	342	R.e.	65.07	10.85	30	43.15	3.56	30	36.07	2.89	30	23.82	2.34	30	29.87	2.06	30	11.75	0.83	30	2.51
	12.05.1973	345	R.e.	67.64	8.77	29	45.92	3.14	30	37.85	2.48	30	24.47	1.96	30	28.50	1.38	30	12.50	0.69	30	2.70
	11.10.1973	406	R.l.	72.50	9.44	29	44.92	3.69	30	37.00	3.82	30	24.68	2.97	30	28.60	1.83	30	12.52	0.89	30	2.71
	16.10.1973	416	R.l.	72.86	11.93	29	43.33	2.74	30	36.32	2.76	30	24.08	1.94	30	29.30	1.12	30	12.63	0.82	30	2.58
	8.05.1974	427	R.e.	63.27	9.14	24	40.23	3.23	30	32.62	2.38	30	20.98	1.96	30	29.60	1.69	30	11.57	0.84	30	2.23
	15.08.1974	463	R.l.	68.37	8.03	20	42.22	2.56	30	35.15	2.88	30	22.07	2.14	30	30.40	1.91	30	12.13	0.52	30	2.28
Environs of Warsow, Łomna	20.03.1975	813	R.e.	67.81	10.35	16	42.56	3.36	24	34.48	3.48	24	22.77	3.06	24	27.96	1.52	24	12.10	0.83	24	2.56
Porąbka at Soła river	21.05.1960	393		66.17	7.97	30	45.27	3.54	30	36.03	2.95	30	24.43	2.34	30	28.40	1.25	30	12.35	0.75	30	2.70
France — Camargue	10.10.1974	F.	4	77.05	8.07	29	45.15	3.55	30	38.50	2.79	30	25.55	2.34	30	31.73	1.78	30	12.35	0.71	30	2.53
	10.10.1974	F.	17	65.41	8.37	28	42.38	3.04	30	35.57	3.04	30	22.52	2.50	30	28.63	1.50	30	12.23	0.74	30	2.47
Bulgaria, environs of Sofia	14.05.1970	B.	2	61.81	10.13	29	41.75	3.83	30	33.83	3.73	30	22.23	2.45	30	30.23	2.13	30	11.30	0.93	30	2.31
Arkutino Reserve	17.05.1970	B.	5	63.72	5.13	30	37.95	2.96	30	29.23	2.22	30	19.27	1.59	30	24.57	1.19	30	10.32	0.82	30	2.46
	17.05.1970	B.	8	75.78	10.06	27	43.40	2.53	30	36.10	2.51	30	23.80	2.07	30	31.83	1.55	30	11.53	0.59	30	2.35
	17.05.1970	B.	11	73.63	10.67	24	41.55	3.24	30	34.52	2.98	30	23.05	2.29	30	32.13	2.01	30	11.40	0.75	30	2.25

[a] Explanations: R.e. — *Rana esculenta*, R.l. — *Rana lessonae*, R.r. — *Rana ridibunda*.
[b] Mean length of arch of the denticulate ring according to the formula from page 208.

Table 6

Correlation coefficient and regression line parameters between mean values of characters in examined *Trichodina ranae* subpopulations

Characters	Correlation coefficient	Regression coefficient	Intercept
DB:DDR	0.36875	1.13150	42.3679
DDM:DDR	0.91051	1.31030	12.7626
DAD:DDR	0.95077	1.20152	7.5944
ND:DDR	0.34719	0.27430	22.825
LD:DDR	0.68787	0.24731	6.131
LD:ND	0.13301	0.60530	9.989

Table 7

Mean values (M) and standard deviation (SD) of three examined characters in *Trichodina ranae* from different localites and countries

Locality	Diameter (μm)				No. of denticles		Numerosity in groups, and (...) number of subpopulations
	adhesive disc		denticulate ring				
	M	SD	M	SD	M	SD	
Arklity Lake	36.96	3.14	24.40	2.50	28.34	1.94	120 (4)
Gopło Lake	32.50	2.17	21.45	2.06	29.03	1.30	30 (1)
Ślesińskie Lake	34.59	3.48	22.73	2.72	29.33	2.08	328 (11)
Goslawskie Lake	34.16	3.18	21.93	2.57	28.72	1.65	180 (6)
Licheńskie Lake	34.97	3.30	22.49	2.66	28.99	1.82	510 (17)
environs of Warsaw, Łomna	34.48	3.48	22.77	3.06	27.96	1.52	24 (1)
Porąbka	36.03	2.95	24.43	2.34	28.40	1.25	30 (1)
Poland — summarized data	34.90	3.39	22.69	2.72	28.94	1.88	1222 (41)
France — Camarague	37.03	3.25	24.03	2.85	30.18	2.26	60 (2)
environs of Sofia	33.83	3.73	22.23	2.45	30.23	2.13	30 (1)
Arkutino Reserve	33.28	3.91	22.04	2.81	29.51	3.87	90 (3)
Bulgaria — summarized data	33.42	3.86	22.09	2.72	29.69	3.52	120 (4)

The correlation between particular characters has been counted also (Table 6). High correlation was found between the denticulate ring diameter and all other metrical features except the body diameter. Low correlation occurred between the denticulate ring diameter and the number of denticles. Similar dependence between metrical features of the adhesive disc and the number of denticles has been observed in *T. vesicularum* (Kazubski 1979 b). Such correlation seems to be

characteristic of this group of ciliates. Proportionally low correlation between the dimensions of the adhesive disc and its elements with the body diameter may be the result of allometric growth of the cell and of the adhesive disc as well as the result of deformation of the body shape and dimensions during preparation.

The nested analysis of variance has been made for three chosen characters — diameter of the adhesive disc without border membrane, diameter of the denticulate ring and the number of denticles. The possible influence of the following agents has been verified: (1) geographical distribution (the country of the origin of ciliates), and (2) belonging to a defined local population, and (3) belonging to a particular subpopulations (i.e., individuals from single host specimen). The data for analysis (means, standard deviation, sample size) are given for various group levels in Tables 5 and 7.

Some differences have been noted in the dimensions and the number of the denticles between trichodinas from Poland and Bulgaria and those from France. However, the analysis of variance has shown that these differences, similarly as the differences between local groups, are statistically insignificant. Instead, highly significant are the differences between subpopulations. The counted values of F_0 overpass several times the critical values even at 1% level of error (Table 8).

Slight differences in the structure of denticles, concerning mainly the outer blade, its bending as well as thickness, and the direction of the inner ray, have been also observed. These differences seem to exhibit

Table 8

Three-level nested ANOVA table for three examined characters of *Trichodina ranae* from different localities and countries

Source of variation	Degree of freedom	F_0 — value			Critical value	
		diameter of adhesive disc	diameter of denticulate ring	number of denticles	$F_{0.05}$	$F_{00.1}$
Among groups from various countries	3–1 = 2	2.170 ns	0.870 ns	3.362 ns	4.74	9.55
Among groups from various localities	10–3 = 7	1.014 ns	1.104 ns	0.385 ns	2.28	3.18
Among particular subpopulations	47–10 = 37	14.872 s	15.548 s	18.688 s	1.44	1.66
Within subpopulations	1402–47 = 1355	—	—	—		

the individual variation. However in general, the outer blade in trichodinas from Bulgaria and France are wider than that in specimens from Poland (Pl. I).

The statement that the examined characters in *T. ranae* do not show significant differences among local groups allows to consider eventual influence of other factors if subpopulations would be grouped in another order. The analysis has been made in order to recognize the influence of the host species and the influence of seasonal changes of temperature. In order to eliminate eventual influence of geographical factor the analysis was based on 34 subpopulations from the Konin lakes.

In the analysis of the influence of the host species three species of frogs were considered: *R. esculenta*, *R. lessonae* and *R. ridibunda*. It has been found (Tables 9 and 10) that the differences between mean

Table 9

Mean values (M) and standard deviation (SD) of three examined characters in *Trichodina ranae* from different hosts in the Konin lakes

Host	Diameter (μm)				No. of denticles		Numerosity in groups and (...) number of subpopulations
	adhesive disc		denticulate ring				
	M	SD	M	SD	M	SD	
Rana esculenta	34.47	3.41	22.23	2.67	29.13	1.83	666 (22)
Rana lessone	35.59	3.06	23.29	2.58	28.95	1.88	266 (9)
Rana ridibunda	33.80	3.18	21.76	2.44	28.72	2.30	86 (3)

Table 10

Two-level nested ANOVA table for three examined characters of *Trichodina ranae* from different hosts in the Konin lakes

Source of variation	Degree of freedom	F_0 — value			Critical value	
		diameter of adhesive disc	diameter of denticulate ring	number of denticles	$F_{0.05}$	$F_{0.01}$
Among groups from various hosts	3–1 = 2	1.620 ns	1.958 ns	0.329 ns	3.32	5.39
Among particular subpopulations	34–3 = 31	11.883 s	13.099 s	8.611 s	1.44	1.72
Within subpopulations	1018–34 = 984	—	—	—		

values of characters in trichodinas originating from various host species are small and statistically insignificant.

The same analysis of variance, on the same material, was made in order to recognize the seasonal changes, i.e., the changes dependent on the temperature. On account of specific climatic circumstances at the Konin lakes (Pojmańska et al., in press) the months May and October were regarded as cool and June to September as warm period of the year. The mean values of examined characters in *T. ranae* are represented in Tables 11 and 12. A slight decrease of the adhesive disc dimensions and of the number of denticles in trichodinas during warmer months in comparison with these data in cool months has been observed, this tendency being in agreement with earlier observations by Kazubski (1975). However, the analysis of variance, taking into account

Table 11

Mean values (M) and standard deviation (SD) of three examined characters in *Trichodina ranae* from the Konin lakes in different seasons of year

Seasons	Diameter (μm)				No. of denticles		Numerosity in groups, and (...) number of sub-populations
	adhesive disc		denticulate ring				
	M	SD	M	SD	M	SD	
Spring: May	34.80	3.43	22.58	2.72	28.95	1.88	508 (17)
Summer: June–September	34.31	3.17	21.89	2.51	28.82	1.80	240 (8)
Autumn: October	34.87	3.34	22.77	2.66	29.43	1.94	270 (9)

Table 12

Two-level nested ANOVA table for three examined characters of *Trichodina ranae* from the Konin lakes in different seasons of year

Source of variation	Degree of freedom	F_0 — value			Critical value	
		diameter of adhesive disc	diameter of denticulate ring	number of denticles	$F_{0.05}$	$F_{0.01}$
Among groups from various seasons	3–1 = 2	0.237 ns	0.784 ns	1.204 ns	3.32	5.39
Among particular subpopulations	34–3 = 31	12.926 s	14.112 s	8.159 s	1.47	1.71
Within subpopulations	1018–34 = 984	—	—	—		

the variation among subpopulations, has shown that the observed differences are statistically insignificant (Table 12).

The material presented above shows that the main source of variation in *T. ranae* from the urinary bladder of frogs *R. esculenta* s. l. is the variation among subpopulations. It seems to be the result of endoparasitic way of life of these ciliates (in the urinary bladder) and difficult contacts with outer environment. Infection of frogs and subsequent exchange of parasites is possible only through a narrow isthmus leading from the urinary bladder to cloaca. So it may be suggested that particular populations of *T. ranae* correspond to single clones or to few mixed clones.

High variability among subpopulations occurring in a small area, frequently in the same habitat, shows the limited influence of the outer environment on the morphology of examined trichodinas.

In conclusion *T. ranae* show similar character of variability as it has been observed in *T. vesicularum* and *T. faurefremieti* occurring in newts. In all these species the main source of variability is the variation among subpopulations, while other sources play only a secondary role. May be, it is connected with the occurrence of these trichodinas in an internal organ of the host. Some differences observed at lower values of F_θ characterizing the differences among subpopulations of *T. ranae,* in comparison with the differences among subpopulations of *T. vesicularum* from newts (K a z u b s k i 1979 b), beside genetic condition (norma of reaction of the genotype) may be the result of differences in ecology of parasites and their hosts (facility in penetration of hosts, frequency of contacts among hosts etc.).

The variability in other species of ciliates being conditioned by other factors has been already discussed (K a z u b s k i 1979 b). The present study gives an additional account supporting earlier conclusions.

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RÉSUME

On a décrit les trichodines présentes dans la vésie urinaire de grenouille *Rana esculenta* s. l. et on leur préserve, après une discussion des données de la

littérature, le nom de *Trichodina ranae* da Cunha, 1950. On donne une diagnose détaillée de cette espèce et on discute sa distribution géographique. L'étude de la variation morphologique de cette espèce aboutit à la conclusion que'lle est basée en premier lieu sur la variation entre les differentes sous-populations. La variation entre les populations locales (c'est-à-dire entre des groupes des sous-populations provenenat de la même localité), la variation liée à l'espèce hôte, ainsi que le variation dépendante de la saison, sont dépourvues de signification statistique.

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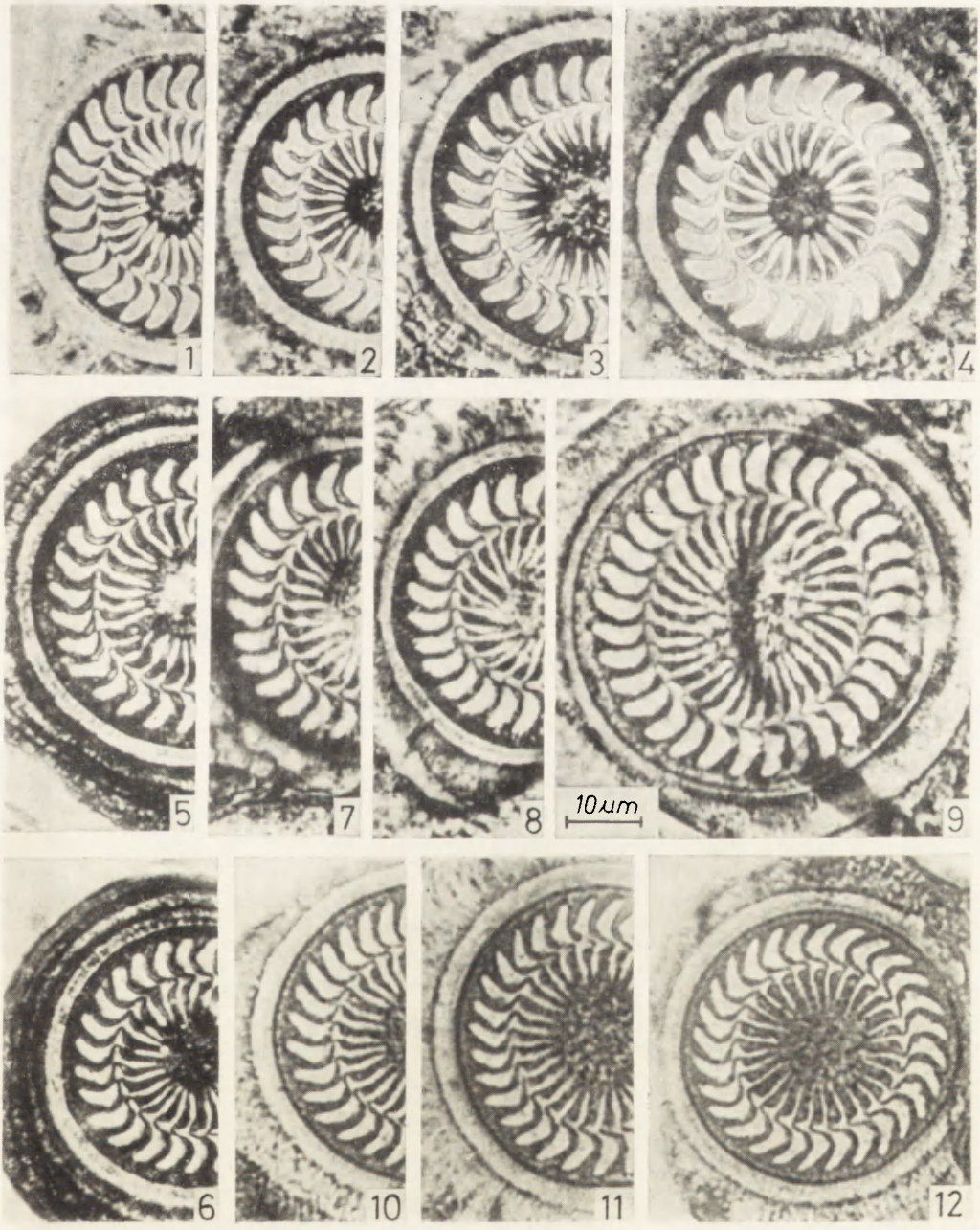
EXPLANATION OF PLATE I

Trichodina ranae da Cunha. Adhesive disc

1–6 — specimens from Poland, 1, 2 — Gosławskie, 3 — Ślesińskie, 4 — Licheńskie lakes, 5, 6 — Porąbka

7–9 — specimens from France, Camargue

10–12 — specimens from Bulgaria, environs of Sofia



S. L. Kazubski auctor phot.

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Cellular Cycle in *Gastrostyla steinii*

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Synopsis. By using cytophotometric techniques the cellular cycle of *Gastrostyla steinii* has been studied. Macronuclear S phase occupies 35% of the cellular cycle; G_1 phase taking 55% and G_2 period is very short or lacking. The micronuclear S phase (15% of the cellular cycle) occurs immediately before mitosis; G_2 phase is also lacking; micronuclear G_1 period occupies 75% of the cellular cycle. The macronuclear S period precedes the micronuclear one. The total macronuclear DNA content in G_1 cells is 80.6 fold more than the DNA content in G_1 micronuclei.

One of the most outstanding characteristics of the ciliates is the nuclear dualism, that is, the presence in a cell of two types of nuclei, which show sharp differences in both structural and physiological terms, including their behaviour during the cellular cycle.

As we are aware, cellular cycle has been studied in a few hypotrichous ciliates, such as: *Urostyla weissei* (Jerka-Dziadosz and Frankel 1970), *Keronopsis rubra* (Ruthman 1972), *Stylonychia mytilus* (Ammermann 1970), *Euplotes eurystomus* (Prescott 1962), *Euplotes crassus* (Salvano 1974), *Diophrys* and *Oxytricha* (Dini et al. 1975), *Laurentia acuminata* (Torres et al. 1979) and *Aspidisca aculeata* (Dini and Bracchi 1976).

In most ciliates of this group a tendency to the reduction of the G_2 period can be observed for both macro- and micronuclei.

In the present paper, attention is focused on the behaviour of macro- and micronuclei during the cellular cycle of *Gastrostyla steinii.*

Material and Methods

Gastrostyla steinii, a hypotrichous ciliate, was isolated from a sample of water collected at Puebla del Rio (Sevilla). Cultures were maintained at 20±1°C in Pringsheim's solution and fed on *Chlorogonium sp.*

In order to determine the DNA contents of macro- and micronuclei, cells were stained with Feulgen procedure as previously reported (Torres et al. 1979). Measurements were carried out using a scanning microdensitometer and interferometer Vickers M-85, at wavelength of 550 nm. Micronuclear DNA contents was expressed as an arbitrary unit (a.u.), and the macronuclear ones as the ratio ma/mi, where mi represents the G_1 average value of all the micronuclei on the same slide.

Results

In order to determine the duration of the cellular cycle of *Gastrostyla steinii* 40 living just newly divided cells were transferred to individual cultures where the behaviour of such cells was observed and timed under stereoscopic microscope until next division was completed. These observations on living cells revealed that the generation time was 9.85 h ($\sigma = 0.82$). Cytokinesis took about one hour.

Macronuclear Cycle. To establish the duration of the macronuclear S phase, the percentage of individuals with banded macronuclei was determined on slides previously stained with Feulgen. Table 1 shows the variation of such a percentage along the cellular cycle. As it can be seen in that Table, during the first three hours no individuals with banded macronuclei were observed, and after this time, the percentage of such cells increased raising a 100% in 7 h. After the 8 h, the percentage was gradually decreasing. Immediately after completion of the courses of the reorganization bands, the four macronuclear fragments fused into a single macronuclear body, and such fusion was followed by three successive macronuclear fragmentations. Those fusion and fragmentation processes took about one hour, and they resulted in eight macronuclear fragments which were distributed between the two daugther cells.

Micronuclear Cycle. To determine the micronuclear cycle, measurements of micronuclear DNA contents were carried out on Feulgen stained cells at three times of their cellular cycle:

(1) In 40 micronuclei belonging to cells without macronuclear replication bands (Fig. 1).

(2) In 25 micronuclei belonging to cells with macronuclear replication bands at the first half of their travel (Fig. 2).

(3) In 35 micronuclei belonging to cells with macronuclear replication bands in the second half of their travel (Fig. 3).

(4) In 20 metaphase micronuclei (Fig. 4).

When the macronuclear reorganization bands were in the second half of their runs (Fig. 3), the micronuclei showed a great variability in their absorbance (note the value 10.23 of the standard deviation). In

Table 1

Percentage of cells containing macronuclei with replication bands, throughout the cellular cycle

Time (hours) after last division	Number of individuals observed	Percentage of cells containing replication bands
1.0	48	0
2.0	43	0
2.5	39	0
3.0	47	0
3.5	23	15
4.0	28	21
4.5	31	25
5.0	32	43
5.5	36	50
6.0	83	60
6.5	68	80
7.0	80	100
7.5	56	100
8.0	34	100
8.5	25	75
9.0	29	46
9.5	34	16

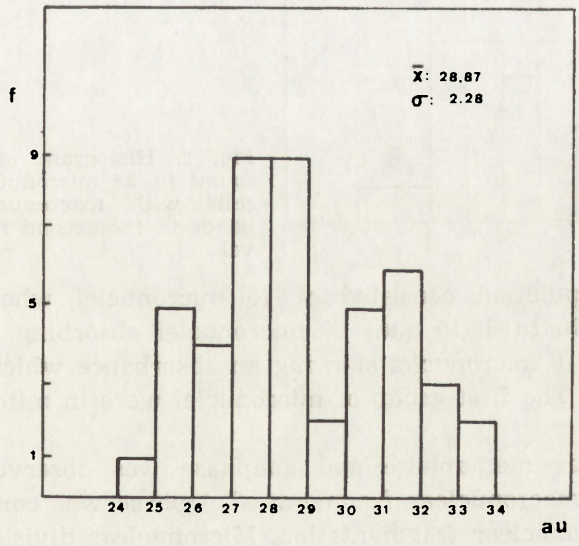

Fig. 1. Histogram of DNA contents found in 40 micronuclei belonging to cells without macronuclear replication bands

Figs. 1-4. *Abcissa* — Content of DNA, as arbitrary units. *Ordinate* — Number of micronuclei

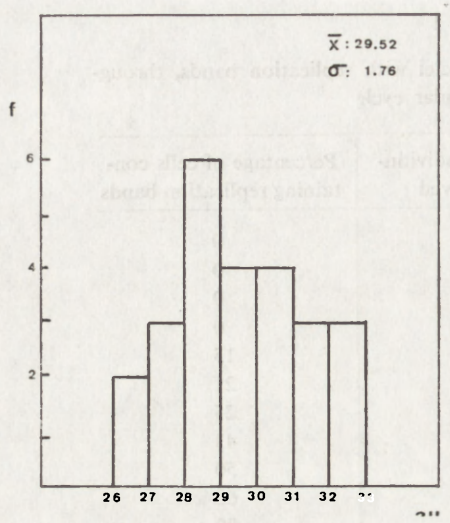

Fig. 2. Histogram of DNA contents found in 25 micronuclei belonging to cells with macronuclear replication bands at the first half of their travel

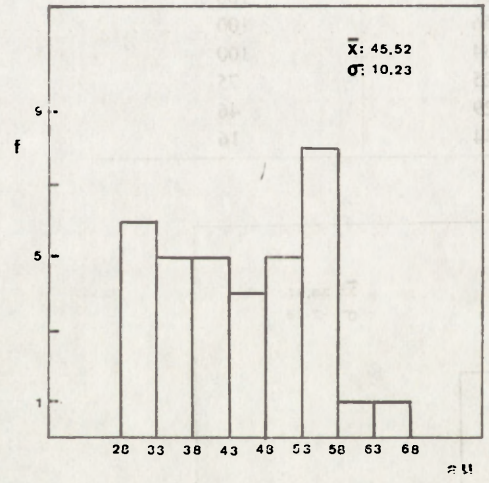

Fig. 3. Histogram of DNA contents found in 35 micronuclei belonging to cells with macronuclear replication bands in the second half of their travel

fact, such population consisted of 15 micronuclei whose absorbance ranged from 50 to 62.15 a.u.; 14 micronuclei absorbing between 34.32 and 47.13, and 6 micronuclei showing an absorbance which ranged from 28.96 to 31.42. The first group of micronuclei were in mitosis (prophase–metaphase).

Micronuclear methaphase and anaphase was observed during the fusion of the macronuclear fragments. Telophase was concomitant with the first macronuclear fragmentation. Micronuclear division was always completed when the third macronuclear fragmentation took place.

Macronuclear DNA Content. The relative DNA content of macronuclei was estimated by the ratio $\overline{ma}/\overline{mi}$, as described in the

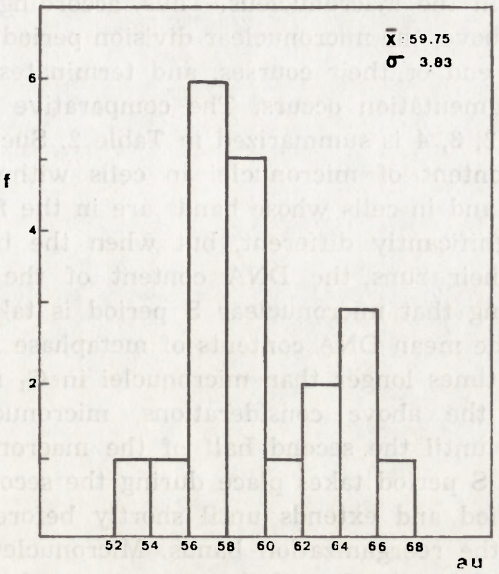

Fig. 4. Histogram of DNA contents found in 20 metaphasic micronuclei

methods. Measurements carried out on 312 fragments in 78 G_1 cells (each of them having 4 macronuclear fragments) revealed that the absorbance value of each fragment ranged between 8.92 and 35.45 ma/mi, the mean value being 20.15 ma/mi, and the standard deviation 5.02. The total DNA content of the whole macronuclei of each of that 78 cells ranged between 54.11 and 112.57, with an average value of 80.60 ma/mi and a standard deviation of 11.35.

Discussion

As in a previous paper (Torres et al. 1979), we have considered that the S macronuclear period begins when 50% of individuals in the population exhibits reorganization bands (5.5 h after division) and ends when the bands have completed their courses in the 50% of the cells (about 9 h after division). As cytokinesis takes about 1 h, the following durations for the different periods of the macronuclear cycle can be estimated: G_1, 5.5 h; S, 3.5 h; fusion and fragmentations, 1 h. Because the macronuclear fusion occurs just after the end of travel of the reorganization bands, it can be considered that the macronuclear cycle lacks of G_2 period, or that this period is represented by the time during which the macronucleus is fused.

In order to time the micronuclear cycle, observations and DNA measurements on micronuclei were referred to concomitant morphological

events occurring in the macronucleus. Thus, according to the observations mentioned above, the micronuclear division period begins when the bands are at the end or their courses, and terminates when the third macronuclear fragmentation occurs. The comparative analysis of data plotted in Fig. 1, 2, 3, 4 is summarized in Table 2. Such analysis shows that the DNA content of micronuclei in cells without macronuclear replication bands and in cells whose bands are in the first half of their travels is not significantly different, but when the bands are in the second half of their runs, the DNA content of the micronuclei has increased indicating that micronuclear S period is taking place.

As expected, the mean DNA contents of metaphase micronuclei (Fig. 4) are about two times longer than micronuclei in G_1 in Fig. 1.

According to the above considerations, micronuclear G_1 period extends, at least, until the second half of the macronuclear S period. The micronuclear S period takes place during the second half of the S macronuclear period and extends until shortly before the completion of the travel of the reorganization bands. Micronucleus also lacks G_2 period, as indicated by the fact that some micronuclear prophases-metaphases occur before such a completion of the travel of the bands.

Figure 5 shows the summary of our results. As can be seen: (a) no synchronism exists between analogous periods in both cycles; (b) except for the division periods, the relative durations of each period are also different in both cycles.

Similarly as in most of the studied hypotrichous ciliates G_2 period is lacking in both micro- and macronuclear cycles, although in the later, G_2 period can be represented by the macronuclear fusion.

On the other hand, *Gastrostyla steinii* has a cellular cycle quite similar to those reported for *Stylonychia* (A m m e r m a n n 1970), *Uro-*

Table 2

Statistical analysis of the data plotted in Figs. 1, 2, 3 and 4

Fig. 1	Fig. 2	Fig. 3	Fig. 4
$\bar{x}_1 = 28.87$	$\bar{x}_2 = 29.52$	$\bar{x}_3 = 45.52$	$\bar{x}_4 = 59.75$
$\sigma_1 = 2.28$	$\sigma_2 = 1.76$	$\sigma_3 = 10.23$	$\sigma_4 = 3.83$
$2\bar{x}_1 = 57.74$			$t_{2\bar{x}_1, \bar{x}_4} = 1.23^a$
$2\sigma_1 = 4.56$			
	$t_{\bar{x}_1, \bar{x}_2} = 1.2^a$	$t_{\bar{x}_1, \bar{x}_3} = 9.85^b$	$F_{\sigma_1, \sigma_4} = 1.46^a$
	$F_{\sigma_1, \sigma_2} = 1.65^a$	$F_{\sigma_1, \sigma_2} = 20.21^b$	

a — Significant at 95%

b — Not significant at 95%

For the comparison between G_1 micronuclei (Fig. 1) and metaphasic micronuclei (Fig. 4), both mean and standard deviation of G_1 micronuclei has been multiplied by 2

styla (Jerka-Dziadosz and Frankel 1970), *Oxytricha* (Dini et al. 1975) and *Laurentia* (Torres et al. 1979).

With regard to the DNA contents, the macronuclear apparatus of *Gastrotyla steinii* has 80.6 times more DNA than the micronuclear one in G_1 period. This figure is between those reported for *Laurentia acuminata* (Torres et al. 1979) and those for *Stylonychia mytilus* (Ammermann 1970).

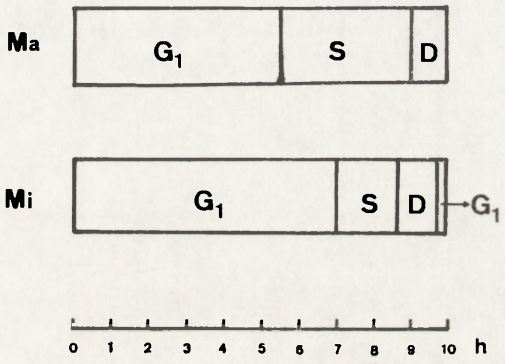

Fig. 5. Schematic representation of macro- and micronuclear cycles in *Gastrostyla steinii*

RÉSUMÉ

Le cycle de division a été déterminé chez *Gastrostyla steinii* à l'aide de la cytophotométrie après coloration de Feulgen. La phase S du macronoyau représente 35% du cycle cellulaire; la phase G_1 représente 55% et la période G_2 est très courte ou manque. La phase S du micronoyau (15% du cycle cellulaire) intervient juste avant la mitose, il n'y a pas de phase G_2 micronucléaire; la période G_1 micronucléaire représente le 75% du cycle cellulaire. La phase S du macronoyau précède celle du micronoyau. La teneur totale en ADN macronucléaire chez cellules en phase G_1 est 80.6 fois supérieure à la teneur en ADN des micronoyaux à la même phase.

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Quelques données sur l'infraciliature et les systèmes myonèmique-fibrillaires chez *Spirostomum ambiguum*

Received on 5 February 1980

Synopsis. L'emploi des techniques du Protargol (Tuffrau 1967) et du carbonate d'argent amoniacal piridiné (Fernández-Galiano 1976), a permis d'obtenir des images nouvelles qui complétent la connaissance de la morphologie infraciliaire et myonémique-fibrillaire de *Spirostomum ambiguum.*

Il existe de nombreux travaux sur le genre *Spirostomum* et surtout sur l'espèce la plus connue, *Spirostomum ambiguum.* La systématique la plus actuelle de ce genre est celle de Repak et Isquith (1974). Pour quelques études sur les myonèmes on a utilisé plusieurs fois ce cilié par sa capacité de contraction et par la forme de cette contraction (Sleigh 1970; Etienne 1970; Legrand 1972; Legrand et Prensier 1976).

La morphologie de l'infraciliature et des systèmes fibrillaires a été étudiée avec l'aide du microscope électronique (Randall 1957; Finley et al. 1964; Daniel et Mattern 1965; Grain 1968). Cependant, les descriptions générales avec la microscopie optique sur les structures qui dépendent de l'infraciliature sont rares (Tuffrau 1967 b). Ce fait est dû, surtout, au masquerement des cinéties somatiques et des formations cinétosomiques orales par le système myonèmique, très développé dans ce cilié. Ces descriptions sont importantes, parce qu'elles montrent la situation de l'infraciliature orale et somatique, mais aussi les systèmes myonèmique-fibrillaires, dans l'ensemble de l'organisme. Ce fait est fondamental pour comprendre non seulement la systématique des ciliés, mais encore leur physiologie.

L'utilisation des techniques du Protargol (Tuffrau 1967) et de Fernández-Galiano (1976) nous a permis de montrer quelques données sur les structures infraciliaires et myonèmiques de *Spirostomum ambiguum*.

Materiel et méthodes

Nous utilisons des cultures qui proviennent d'échantillons du barrage de Santillana (Manzanares El Real, Madrid, Espagne) qui ont été traitées avec les techniques du Protargol (Tuffrau 1967a) et de Fernández-Galiano (1976). Avec les préparations nous avons obtenu des microphotographies.

Observations

La morphologie pénérale

Les exemplaires de cette espèce mesurent de 1 à 4 mm de long (Kahl 1932). Cependant, dans nos cultures, les organismes ne dépassaient pas les 2 mm. Les exemplaires contractés ont une taille de 390–430 × 197–215 µm. La région orale surpasse l'équateur du cilié. De même que d'autres ciliés hétérotriches, la vacuole contractile est terminale et située dans le pôle postérieur en donnant à cette zone un aspect plus clair, où on y voit, *in vivo*, les rangées formées par les cinéties somatiques. D'accord avec Boggs (1965) le nombre de cinéties somatiques est aproximativement 46. La macronoyau est composé par des nodes relationnés par de minces connexions. Le nombre de nodes est 10–25 et la taille de chaque node: 28–53 × 16–24 µm (Pl. I 1).

Les cinéties somatiques de *S. ambiguum* sont composées de cinétosomes qui possèdent, le même que d'autres hétérotriches, des fibres Km (Haller 1977) ou microtubules postciliaires (mp), qui se situent à gauche (vu le cilié dehors) de la rangée de cinétosomes, comme dans *Stentor* (Fernández-Leborans 1979 a). Ces fibres ont été interprétées par Finley et al. (1964) comme ectomyonèmes latérales et périphériques. Cet auteur se base pour cette interprétation sur les études réalisées par Fauré-Fremiet et Rouiller (1958) sur l'ultrastructure du *Stentor*. D'accord avec Sleigh (1973) et de Haller (1977) et aussi Grain (1968), les ectomyonèmes correspondent à des microtubules postciliaires des cinéties somatiques du cilié (Pl. I 2).

La région orale de ce cilié est constituée par deux structures: la zone adorale de membranelles (ZAM) et la cinétie parorale (CP) (Pl. I 3).

La ZAM est située sur le côté gauche de la région orale. Selon

Daniel et Mattern (1965) elle est composée de membranelles, chacune formée par trois rangées de 10 cils pour chaque rangée. Selon Tuffrau (1967 b) il existe deux rangées de cinétosomes dans presque toute la ZAM et trois rangées à la partie de la ZAM la plus proche du cytostome.

Dans nos exemplaires la ZAM se présente avec deux parties: (a) une partie antérieure, située entre le pôle apical du cilié et les alentours du cytostome. Elle a une longueur de 287–328 μm et est formée par des membranelles, dont chacune possède deux cinéties avec 7–8 cinétosomes. La membranelle a une longueur de 4–5.6 μm (Pl. I 4), (b) une partie postérieure qui entoure la région la plus voisine du cytostome et délimite cette région avec la cinétie parorale. Elle a une longueur de 50–57 μm et elle est formée par des membranelles, plus allongées et avec plus de cinétosomes que les membranelles de la partie antérieure. Ces membranelles possèdent deux cinéties chacune; il y a 16–20 cinétosomes par cinétie. La membranelle dans cette partie a une longueur de 9.7–12 μm (Pl. II 5).

La cinétie parorale se rencontre sur le côté droit de la région orale de l'organisme; elle est située entre le pôle apical et la région du cytostome avec un trajet parallèle à la ZAM; entre les deux structures reste une zone plus claire, où il n'y a pas de cinétosomes; cette zone est le péristome du cilié. La cinétie parorale est formée par une seule rangée de cinétosomes; cependant, dans un trajet très court, près du cytostome, il semble qu'il s'y trouve une rangée double de cinétosomes (probablement une plus courte haplocinétie comme dans *Blepharisma* (Fernàndez-Leborans 1979 b) (Pl. II 5).

Les systèmes fibrillaires

Dans les préparations realisées avec la technique de Fernández-Galiano (1976) on observe, dans plusieurs cas, la disposition du système myonèmique de cette espèce (Pl. II 6) La surface somatique du cilié montre un grillage myonèmique qui se localise entre la région orale et le pôle postérieur du cilié; il est probable que ce système soit l'ensemble d'endomyonèmes décrits dans ce cilié par Finley (1964) et dont la configuration a été expliquée par Legrand et Prensier (1976). Dans ce grillage et en tenant compte de la torsion due à la contraction de l'organisme, il est possible de distinguer des myonèmes primaires somatiques (Pmys) parallèles aux cinéties somatiques et des ramifications transversales somatiques (CBs, entre les Pmys), semblables à des Pmys et CBs du *Stentor* (Newman 1974, Fernàndez-Leborans 1979 a). Nous avons observé que ce grillage arrive à une fibre commune que nous appelons fibre submembranellaire (FSM) (Pl. III 7). Cette fibre submembranellaire connecte avec les membranelles de la

ZAM et avec une "fibre" semblable qu'on rencontre au dessous de la cinétie parorale et que nous appelons fibre subparorale (FSP). Dans les préparations réalisées au Protargol (Tuffrau 1967 a) on observe que de chaque membranelle de la ZAM, sort un faisceau de fibrilles (Pl. III 7 a); ces faisceaux s'étendent perpendiculairement à l'axe de la ZAM par la surface du peristome (Pl. III 7 b) et arrivent à une zone située à gauche et parallèle à la cinétie parorale (Pl. III 7 c) dans cette zone les faisceaux qui proviennent de chacune des membranelles se courbent approximativement 180° et cette torsion donne l'image d'une fibre continue (fibre subparorale) dans les préparations réalisées avec la technique de Fernández-Galiano (1976). Ces faisceaux de fibrilles (fibrilles péristomales FP, observées aussi mais non photographiées par Tuffrau 1967 b), après la torsion prennent un sens opposé au sens qu'ils avaient et arrivent aux cinétosomes de la cinétie parorale (Pl. III 7 d). Dans un plan plus superficiel on observe l'aspect de la cinétie parorale (CP) (Pl. III 7 e, f) et ses cils, ainsi que les cinéties somatiques adjacentes.

SUMMARY

Staining with protargol (Tuffrau 1967a) and with pyridinated silver carbonate method (Fernández-Galiano 1976) produces new images which complete the picture of morphology of the infraciliature and of the fibrillar myonemes system in *Spirostomum ambiguum*.

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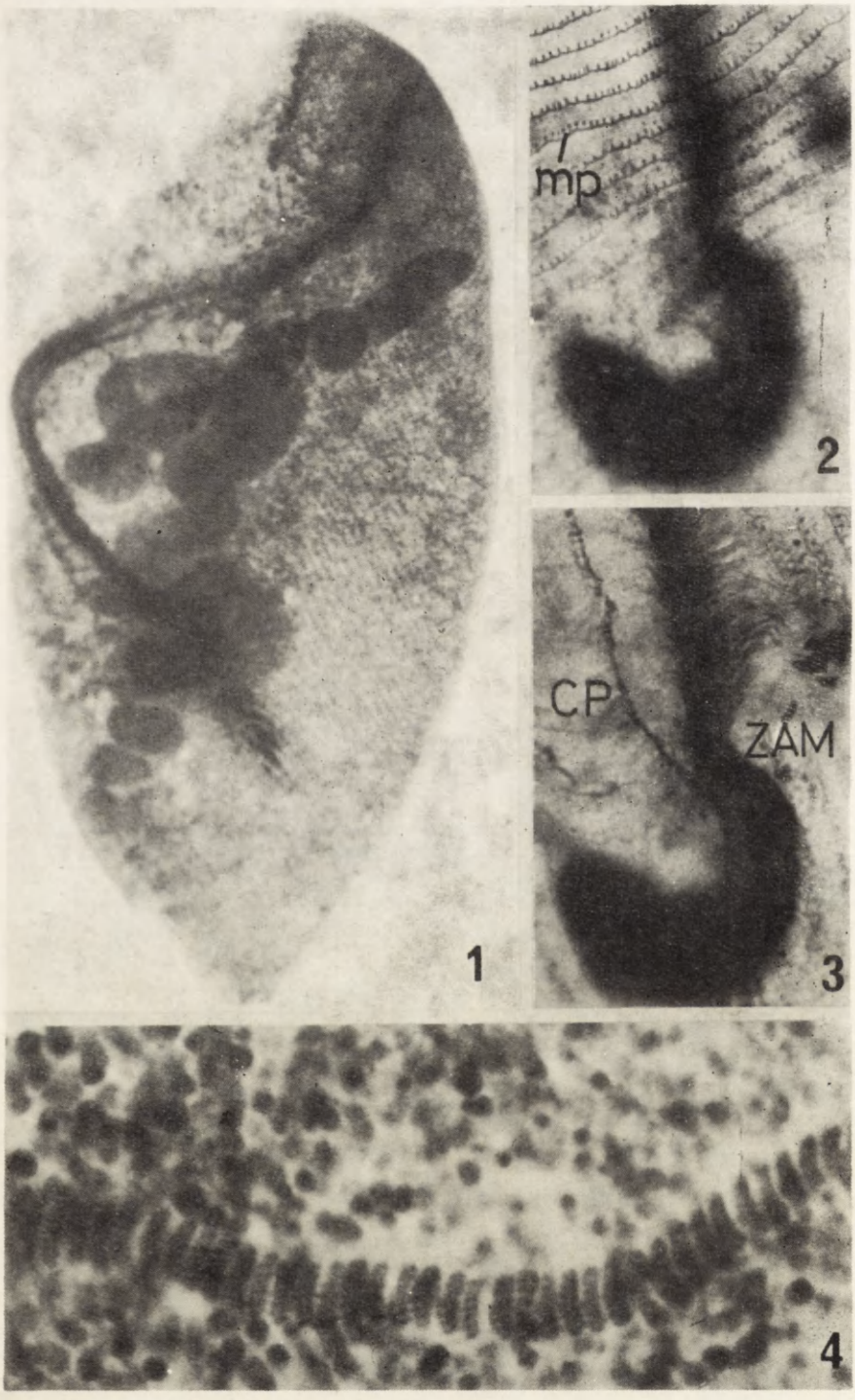
EXPLICATION DE PLANCHES I–III

1: *Spirostomum ambiguum.* L'aspect général (Protargol × 332)
2: *Spirostomum ambiguum.* Détail des cinéties somatiques; on observe les microtubules postciliaires (mp) (Protargol × 747)
3: *S. ambiguum.* Un autre plan de la figure antérieure en montrant la zone orale, la zone adorale de membranelles (ZAM) et ses cils et la cinétie parorale (CP) (Protargol × 747)
4: *S. ambiquum.* Les membranelles de la zone antérieure de la ZAM (Fernández-Galiano × 1800)
5: *S. ambiguum.* L'aspect des membranelles à la zone postérieure de la ZAM. Aussi on observe la cinétie parorale (CP) (Fernández-Galiano × 1330)
6: *S. ambiguum.* Plan général du système myonèmique du cilié (Fernández-Galiano × 350)
7: *S. ambiguum.* Détail du système myonèmique à niveau de la région orale. On observe la fibre submembranellaire (FSM) parallèle à la ZAM et la "fibre" subparorale (FSP) (Fernández-Galiano × 1050)
7: a–f: *S. ambiguum.* Plans successifs qui montrent les fibres péristomales qui se disposent dès les membranelles (a), en s'étendant sur la surface du peristome (b) et en secourbant à un niveau parallèle à la cinétie parorale ("fibre" subparorale) pour atteindre les proximités des cinétosomes de la cinétie parorale (d); a e et f on observe les cinétosomes et les cils de la cinétie parorale et les cils, les cinetosomes et les microtubules postciliaires des cinéties somatiques adjacentes (Protargol × 2.240).



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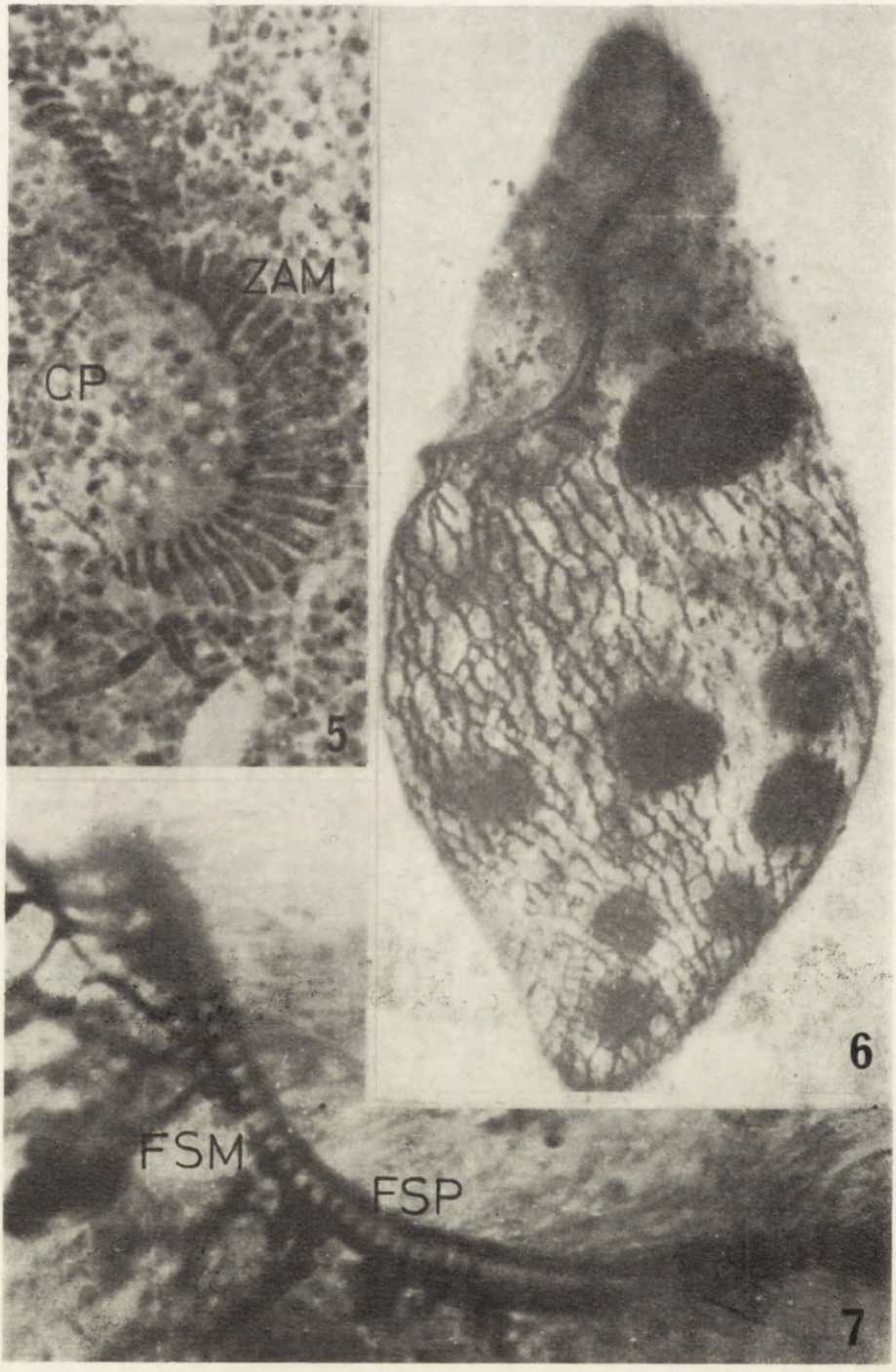

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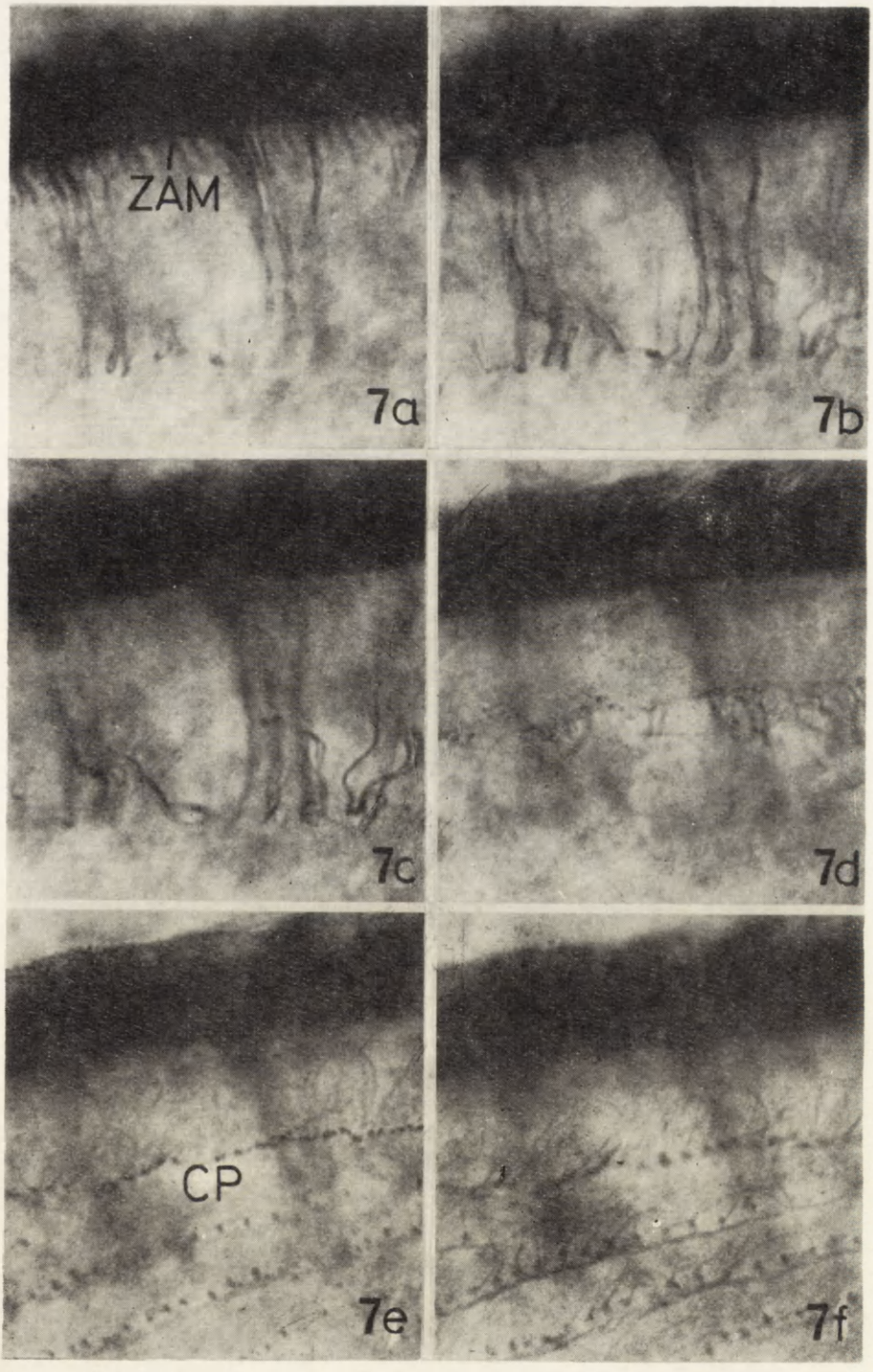

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ACTA PROTOZOOLOGICA

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A Freeze-etch Study of the Ultrastructure of *Colpoda cucullus* Protective Cysts

Received on 27 March 1979 and *revised on* 4 March 1980

Synopsis. Protective cysts of the infusorian *Colpoda cucullus* were studied using the freeze-etch technique. The cyst coat consisted of three components — the upper mucous layer, 0.2–1.4 μm thick, the middle lamellar layer, 0.2–0.4 μm thick, and the inner homogenous layer, 0.2–0.4 μm thick. The inner layer was attached to the pellicle enveloping the cyst contents. The pellicle had numerous impressions which divided its surface into irregular polygons. Under the pellicle there were many membranes of subpellicular alveoli. The cytoplasm contained the macronucleus, nuclear fragments made up of chromatin extruded during macronuclear reorganization, the micronucleus, membranes of the endoplasmic reticulum frequently found around mitochondria, lipid droplets, small vacuoles and small bodies.

The freeze-etch technique provided an insight into the ultrastructure of protective cysts in *Colpoda cucullus*, particularly with respect to the distribution and the shape of cellular structures, avoiding artifacts resulting from chemical fixation. Surface views of membrane structures documented in our replicas contributed to a better understanding of the structure and formation of cyst coast and intracellular membranes.

The infusorian *Colpoda cucullus* belongs to the group of protozoans that possesses the ability to survive unfavourable conditions in the form of a protective cyst. As environmental conditions improve, the vegetative form of the infusorian is released from its coats in the process called excystment. In the cyst stage, the infusorian is characterized by a low metabolic rate and a morphology, especially with respect to ultrastructure, which differs considerably from the vegetative form. The morphology of *Colpoda cucullus* cysts has been documented by a number of studies using either light microscopy (Kidder and Claff 1938,

Goodey 1913, Hashimoto 1966) or electron microscopy in ultrathin sections (Kawakami and Yagiu 1963 c, Tibbs 1968). The latter, in particular, revealed details of the structure of both the pellicle and the coats of protective cysts. However, a new insight into the ultrastructure of protective cysts has been provided by the freeze-etch technique, which has the advantage of deep-freeze fixation that maintains cellular structure almost intact. Freeze-etched replicas then allow to view the surface of membrane structures. This study reports some new data on the ultrastructure of protective cysts of *Colpoda cucullus* obtained by the freeze-etch technique.

Material and Methods

The clone of *Colpoda cucullus* used in our experiments was isolated from hay infusion. Infusorians were cultured in Petri dishes in the medium with wheat grain according to Villeneuve-Brachon (1940) and fed on *Aerobacter aerogenes*. Protective cysts were seen with a stereomicroscope as early as at 3 or 4 days after inoculation. The amount of cysts required for mass processing was achived at 10 to 14 days of cultivation.

The cyst were removed from the dishes, sedimented by centrifugation and transferred into distilled water with 20% glycerol. Subsequently, they were frozen in liquid Freon 22 and processed in a Balzers BA 360 M freeze-etch apparatus to produce platinum carbon replicas of freeze-fractured surfaces (Moor et al 1961). After cleaning with 40% chromic acid, 70% sulphuric acid and sodium hydroxide, the replicas were observed in a Tesla BS 500 electron microscope. All measurements of cyst coat thickness were made on replicas where the fracture plane ran through the cyst centre.

Results

The appearance of cysts on freeze-etched replicas varied with the fracture plane. Convex surface of cyst envelopes were displayed when the upper layer of the frozen medium was split off. Concave imprints of different layers of cyst coats were produced when parts of cysts were torn out from the frozen medium. Cross-fractured cysts were also found. These gave a clear picture of all intercellular structures (Fig. 1).

The cyst wall consisted of three components (Pl. I 1, 2). The outer layer showed a sponge-like structure essentially composed of hydrated mucous material. Its thickness was 0.2 to 1.4 μm. The sponge-like pattern seen on replicas was, in fact, a mere artificial structure produced by deep freezing of hydrated material. After the specimens were treated with glycerol, the sponge-like structure became finer and more

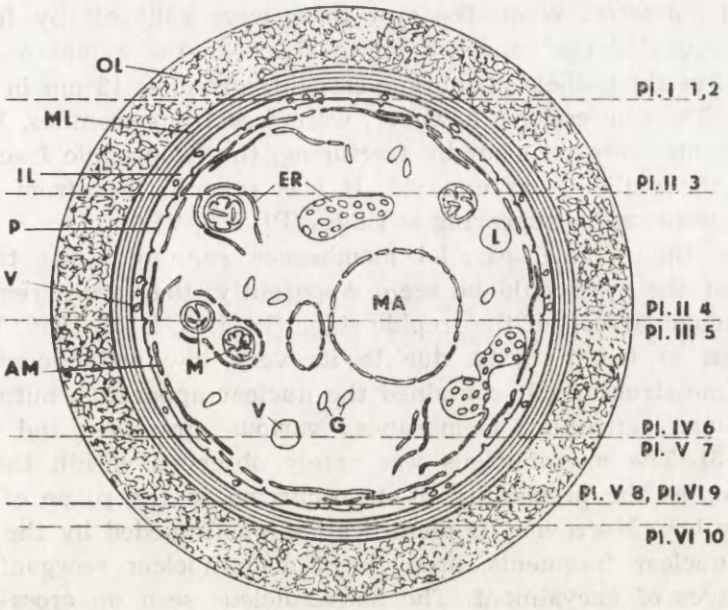

Fig. 1. General view of cell structures and their arrangement in a protective cyst of *Colpoda cucullus*. Dashed lines indicated fracture planes, the replicas of which are presented on the Plates I–VI

OL — outer mucous layer of the cyst wall, ML — medium layered part of the cyst wall, IL — inner homogenous layer of the cyst wall, P — pellicle, AM — alveolar membranes, M — mitochondria, ER — endoplasmic reticulum, G — cytoplasmic grains, MA — macronucleus, L — lipid droplets, VC — vacuoles in the cytoplasm, V — small vacuoles in the inner layer of the cyst wall

like the other eutectic of the surrounding frozen medium (Pl. I 1, VI 10). Occasionally this layer lacked a clear margination separating it from the surrounding environment (Pl. IV 6). Below this envelope a rigid lamellar layer, 0.2 to 0.4 μm thick, was found. This was the true cyst comprising 8 to 18 clearly discernible layers (Pl. I 1, 2). The thickness of layers, as seen on replicas, depended on the distance of the fracture plane from the cyst center. In cases where the fracture plane ran tangentially, the layers appeared much broader; occasionally the inner layer occupied the whole centre of the cyst replica (Pl. IV 10). In such replicas the lamellar nature of the true cyst coat was most obvious. The third, innermost part of the cyst wall was formed by a very fine granulated layer of varying thickness (0.2 to 0.4 μm), which filled the space between the lamellar part and the pellicle. Occasionally, it contained minute vesicle about 150 nm in diameter (Pl. I 2).

The pellicle, a layer covering the contents, appeared as a membrane wrinkled with a net of impressions 0.1 to 0.2 μm deep and 0.05 to 0.1 μm wide dividing the surface into irregular polygons approximately 0.3 to

1 μm in diameter. When the cyst coats were split off by fracturing, replicas revealed the convex surface; this was the cytoplasm-adjacent (PF) half of the pellicle membrane carrying granules 12 nm in diameter (Pl. I 2). The impressions, however, were free from granules. When the cyst contents were torn out by fracturing, the exoplasmic fracture face (EF) of the pellicle was exposed. It was smooth, free from granules, with the impressions appearing as ridges (Pl. V 8, VI 9).

Below the pellicle, parallel membranes running along the whole surface of the cyst could be seen. Apparently, they were remnants of sub-pellicular alveoli of the trophic stage (Pl. II 3, III 5, IV 6, V 7). The cytoplasm of frozen cysts, due to its very low water content, had a very fine structure. It contained the nuclear apparatus, mitochondria, endoplasmic reticulum membranes, various granules and vacuoles (Pl. III 5). The micronucleus was rarely observed within the nuclear apparatus, as the probability of its occurring in the plane of fracture was very low. Moreover, its identification was impeded by the presence of tiny nuclear fragments, products of macronuclear reorganization in early stages of encystment. The macronucleus seen on cross-fractured faces appears structurally similar to the cytoplasma. Macronuclear membranes possessed pores about 0.1 μm in diameter (Pl. III 5). Mitochondria were spherical in shape, about 1 μm in diameter, with cristae seen on cross-fractured faces (Pl. II 3, 4, III 5, IV 6, V 7). Invariably they were surrounded by endoplasmic reticulum membranes which had perforation resembling nuclear pores.

Small round vacuoles, 1 μm in diameter, had contents similar to those of cytoplasm (Pl. II 3, 4, III 5, IV 6). Each of them was covered with a membrane showing numerous granules on PF face. Round lipid droplets, also found in the cytoplasm, were 1.0–1.5 μm in diameter. On cross-fractured faces they revealed very fine and smooth surfaces typical of lipoid substances (Pl. II 3, 4, V 7). In addition, there were minute grains densely distributed in the cytoplasm. They were mostly egg-shaped, 0.4–0.7 μm in size (Pl. II 4, III 5, IV 6, V 7). Their convex surfaces or imprints is the cytoplasm, both free from granules, could be seen on replicas. The grains were never observed cross-fractured. Presumably, they were analougous to refractile bodies seen in the light microscope. Another small round bodies (0.1–0.2 μm) occasionally found in the cytoplasm were most likely the dense bodies observed by Kawakami and Yagiu (1963 c) in ultrathin sections.

Discussion

The ultrastructure of *Colpoda cucullus* protective cysts and the changes occurring during encystment and excystment, as seen in

ultrathin sections, have been reported by Kawakami and Yagiu in a series of papers (1963 a, b, c, 1964 a, b, c). The outer mucous coat in our replicas was analogous to the net-like fibrillar and amorphous structures around the cyst reported by these authors. Presumably, this mucous coat plays a role in attachment of the cyst to a solid substrate. In ultrathin sections, the true coat was differentiated into 3 separate layers presumably due to the fixation technique used. In freeze-etched replicas, on the other hand, the cross-fractured rigid part of the cyst wall revealed only two layers- the outer one with stratified structure and the inner one with occasional vesicles. Association of these vesicles with secretion of cyst coat material could not be determined from this study. The stratified structure of the cyst coat was also noted by Tibbs (1968) in protective cysts of *Colpoda steinii*. He reported extensive folding of the pellicle in the cyst, which was due to shrinkage of the cell on encystment. The shrinkage was especially marked on comparison of trophic forms with the cysts.

The structure of the cytoplasm depends mainly on the water contents. Generally, the occurrence of artificial structures in the freezing process can be avoided by infiltrating the cells with 10 to 30% glycerol. Protective cysts of *Colpoda cucullus* have a very low water content which ensures that their ultrastructure is not affected by deep freezing. This makes the use of cryoprotective substances redundant. On the contrary, addition of glycerol (at higher concentrations) results in a considerable shriking of the cysts volume.

Cilia were not observed in the cyst coat of our specimens. This finding is consistent with the earlier notion that cilia are resorbed during encystment and newly formed from kinetosomes during excystment (Kawakami and Yagiu 1963 c, Tibbs 1969, Hashimoto 1966).

Of interest are our findings of the endoplasmic reticulum surrounding mitochondria. In the cyst of *Colpoda cucullus*, Kawakami and Yagiu (1963 c) reported transformation of mitochondria into lipid bodies or into structures enveloped in two membranes. In ultrathin sections of *Colpoda steinii* cysts, Tibbs (1968) did not find any decrease in the number of mitochondria as compared with trophic forms. In accordance with his findings, our results showed the mitochondria surrounded by membranes of endoplasmic reticulum. On replicas showing cross-fractured cysts, the frequently occurring membranes of endoplasmic reticulum had surfaces with perforations resembling nuclear pores (Pl. II 3). They are probably remnants of nuclear envelope fragments arising in the process of macronuclear reorganization during encystment, which is a common feature in the protective cysts of the

family "*Colpodiae*" (Kidder and Claff 1938, Burt et al. 1944, Kawakami and Yagiu 1963 c). The nature of both the egg-shaped bodies and the small spherical bodies remains to be explained.

Although there are many data on the ultrastructure of various infusorian cells after freeze-etching (Speth and Wunderlich 1972, Janisch 1972, Satir et al. 1973, Sattler and Staehelin 1974, Batz and Wunderlich 1976, Plattner et al. 1977) none of them has been concerned with freeze-etched cysts of infusoria. This paper is a contribution to the understanding of the ultrastructure of infusorian cysts. It is suggested that the freeze-etching technique employed may, in future, aid the discovery of the process of encystment and excystment in infusoria.

RÉSUMÉ

L'ultrastructure des kystes protecteurs des infusoires *Colpoda cucullus* a été étudiée à l'aide de la méthode de cryodécapage. La membrane cystique se compose de trois couches — couche externe muquese dont l'épaisseur est de 0.2–1.4 μm, couche moyenne lamelaire de 0.2–0.4 μm et couche interne homogène de 0.2–0.4 μm s'attachant à la pellicule qui couvre le contenu propre du kyste. La pellicule apparaît rugueuse à cause de nombreuses impressions divisant sa superficie en polygones irréguliers. Sous la superficie du kyste on trouve les membranes des alvéoles infrapelliculaires. C'est dans le cytoplasme que l'on trouve le macronucléus éventuellement ses fragments qui sont le résultat de l'extrusion de la chromatine pendant la réorganisation, le micronucléus, les membranes du reticulum endoplasmique qui entourent souvent des mitochondries, les gouttes lipoides, les petites vacuoles et les granules.

L'application du procédé du cryodécapage pour létude de l'ultrastructure des kystes protecteurs de *Colpoda cucullus* a permis de donned des informations concernant surtout les structures cellulaires singulières et leur forme sans l'influence de la fixation chimique. Les possibilités d'examiner les superficies des structures membraneuses ont contribué à gagner de nouvelles connaissances sur la structure et la formation des superficies même des membranes intracellulaire des kystes protecteurs.

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EXPLANATIONS OF PLATES I–VI

Fine structure of freeze-etched cysts of *Colpoda cucullus* in the fracture planes shown on the schematic representation (Fig. 1).

OL — outer mucous layer of the cyst wall, ML — medium layered part of the cyst wall, IL — inner homogenous layer of the cyst wall, P — pellicle, AM — alveolar membranes, M — mitochondria, ER — endoplasmic reticulum, G — cytoplasmic grains, MA — macronucleus, MI — micronucleus, L — lipid droplets, VC — vacuoles in the cytoplasm, V — small vacuoles in the inner layer of the cyst wall

1: A tangential section through the cyst at low magnification. In the center there is the convex surface of the pellicle (P) wrinkled with a net of impressions. All the other parts of the cyst coat, the inner, medium and outer layers (IL, ML, OL), are relatively wide due to the tangential plane of section. 7000 ×

2: A tangential section through the cyst at a high magnification. All three layers of the cyst coat (OL, ML, IL) are cross-fracture and the convex surface of the pellicle (P) can be seen. Its protoplasmic fracture face is exposed revealing 12 nm granules. The impressions, however, are free of them. There are minute vesicles (V) in the inner part of the cyst coat. The medium part of the cyst wall is layered. 60 000 ×

3: A cross fracture plane through the wall and the content of a cyst. There are alveolar membranes below the pellicle. Remnants of nuclear envelope fragments (MA) show rounded pores and are continuous with endoplasmic reticulum membranes (ER) that often surround mitochondria (M). There are also small vacuoles (VC) and lipid droplets (L) in the cytoplasm. 21 000 ×

4: The cross fractured central part of a cyst. It reveals the fractured macronucleus (MA), mitochondria (M), surrounded by endoplasmic reticulum membranes (ER), lipid droplets (L), vacuoles (VC) and egg-shaped minute grains (G). 25 000 ×

5: A cross fractured cyst. 12 000 ×

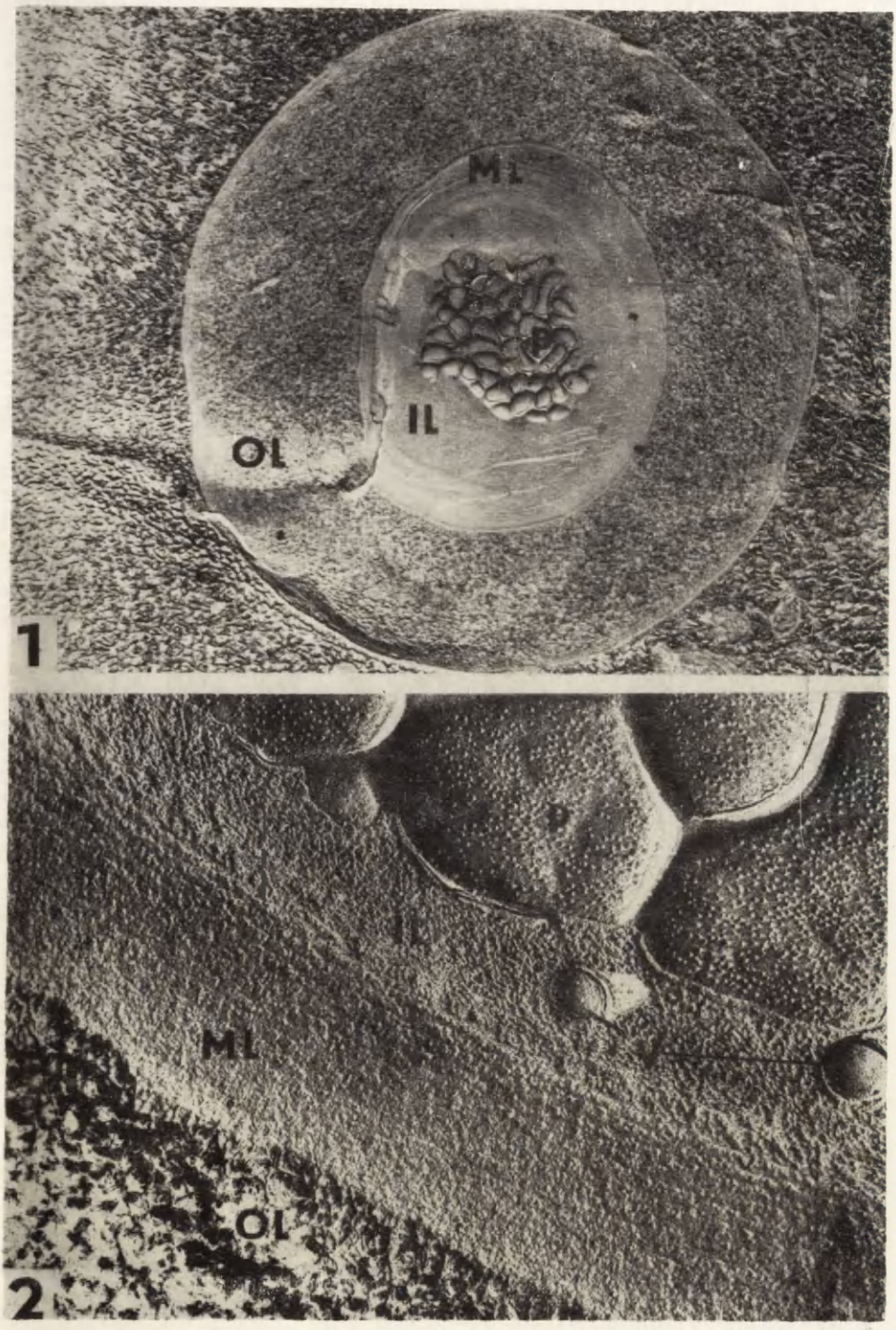
6: A semitangential section exposing the concave exoplasmic fracture face of the pellicle (P) after a part of cytoplasmic content has been torn out. Pellicle impressions look like ridges. Below the pellicle there are many alveolar membranes. 22 000 ×

7: A cross fracture view showing the position of the endoplasmic reticulum membrane complex (ER) with many perforations resembling nuclear pores. 22 000 ×

8: A semitangential section showing the concave exoplasmic fracture face of the pellicle (P) without granules. This is wrinkled with impressions appearing as ridges. 28 000 ×

9: A tangential fracture plane through the cyst when all the cyst content has been torn out exposing a large area of the exoplasmic fracture face of the pellicle (P) in the central part of the replica. 10 000 ×

10: A tangential section running through the inner layer of the cyst coat. All cyst content together with the pellicle is torn out. 8000 ×



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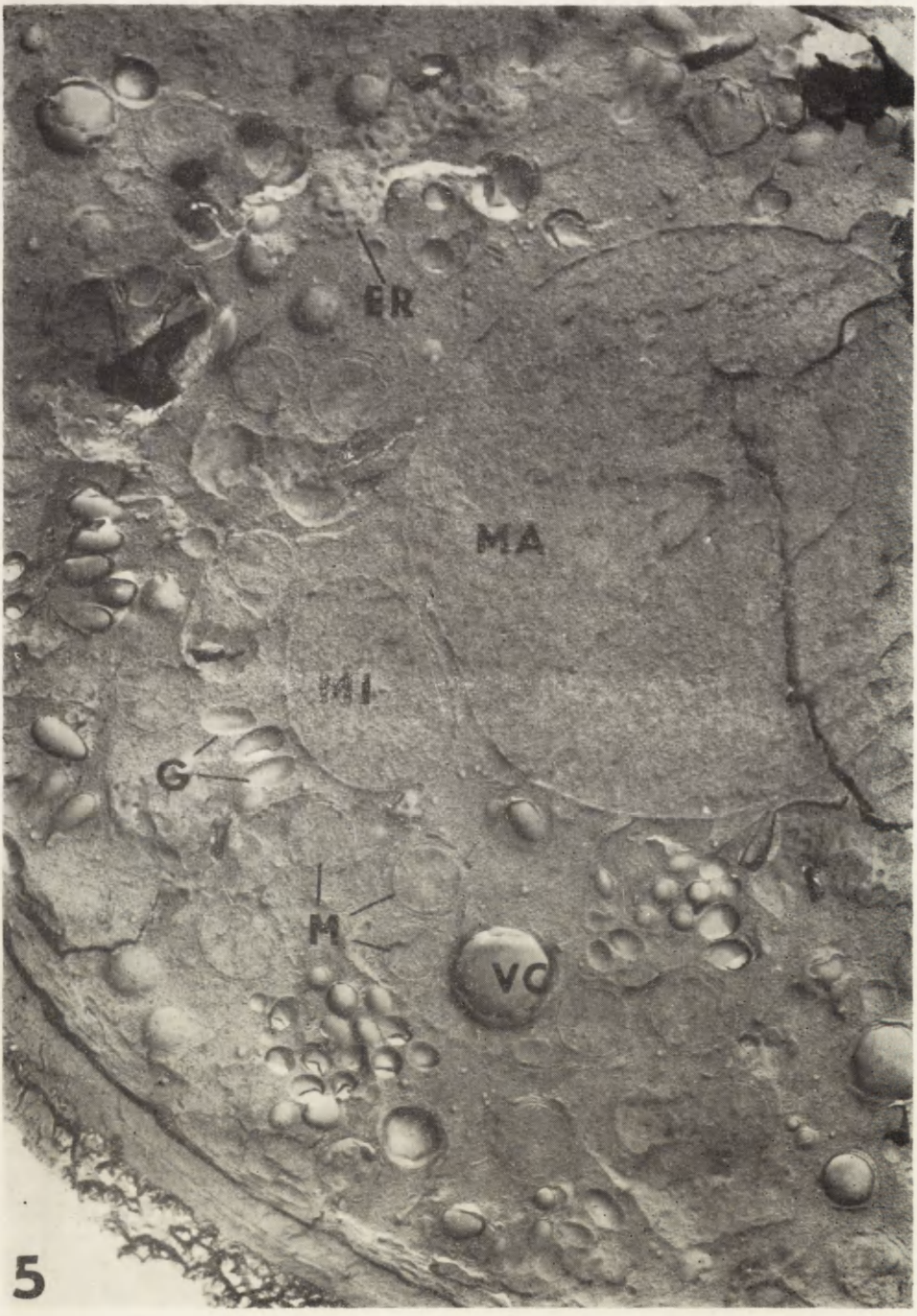

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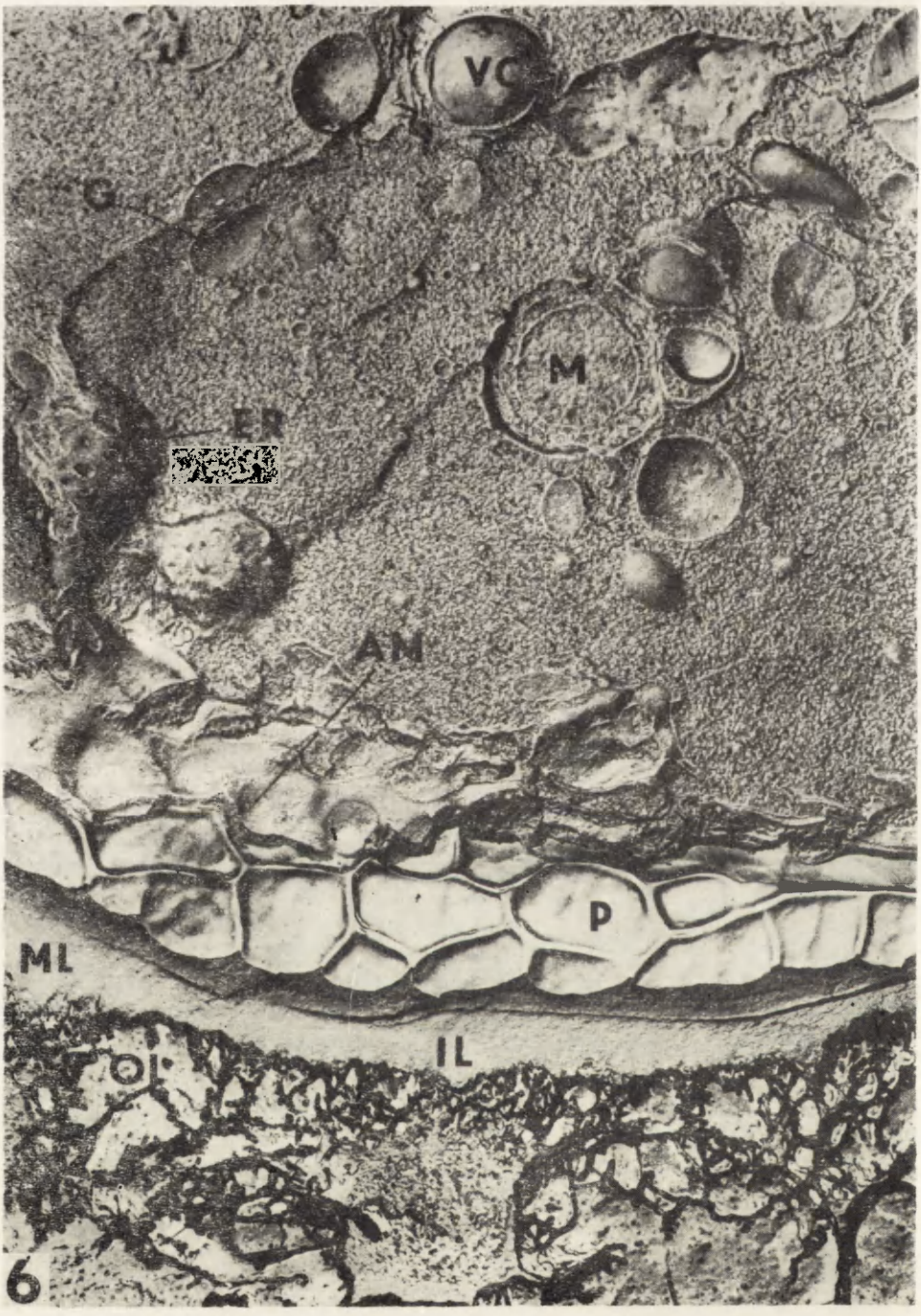

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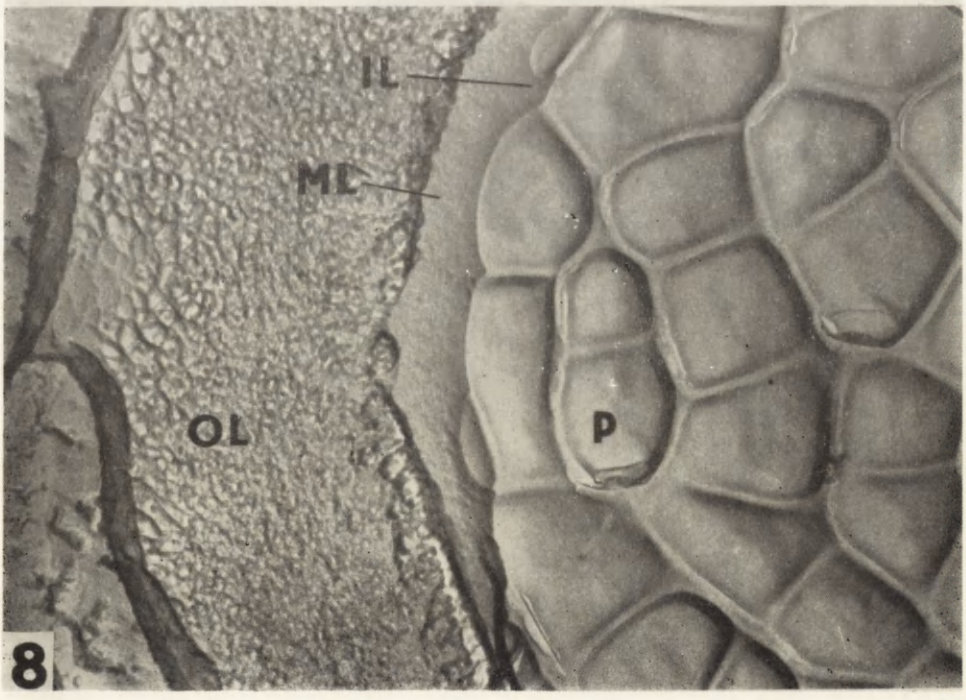

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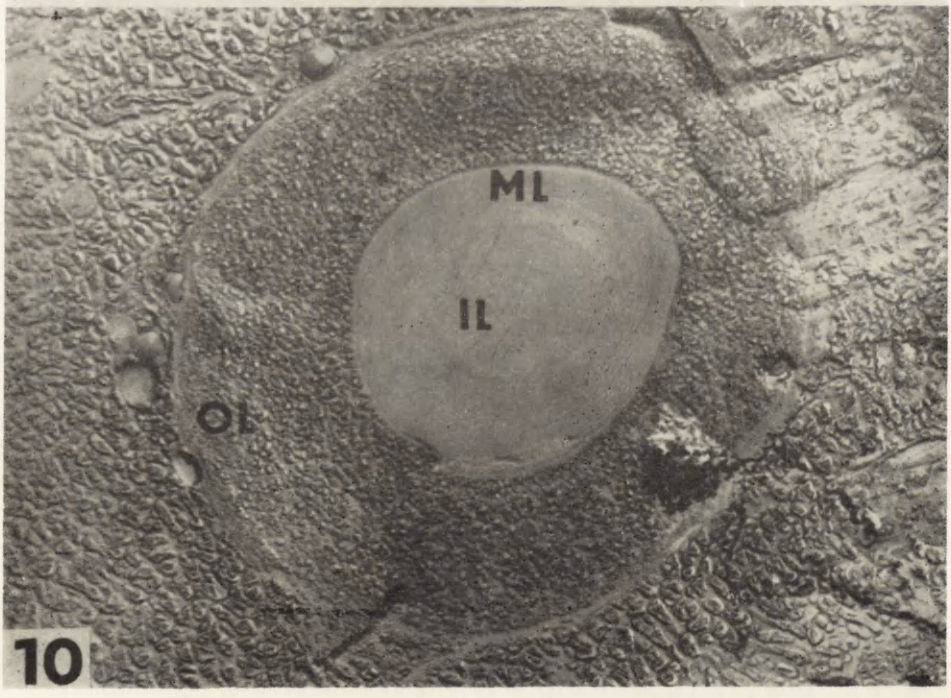

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Nuclear Behavior and RNA Synthesis During Regeneration of the Anterior Fragment of a Transected *Blepharisma*

Received on 2 January 1980

Synopsis. Regeneration is slower in the anterior than in the posterior fragment of a *Blepharisma japonicum* transected just behind the peristome. Reorganization of the macronucleus is similar but somewhat slower in the anterior than in the posterior fragment. The rate of labelling of the RNA in the macronucleus and cytoplasm with ^{14}C-uridine (presumably mRNA formation) is about the same in both fragments. It is likely that regeneration is delayed in the anterior fragment primarily because its peristome must first be removed before it is replaced.

Whereas the postperistomial fragment of a transected *Blepharisma* must reconstitute a new peristome, the anterior fragment lacks only a contractile vacuole, which regenerates within 30 to 60 min (Suzuki 1957). The anterior fragment retains about half the macronucleus and many micronuclei. If the old peristome remained functional, one might expect the anterior fragment to regain its original size and to grow and divide before the postperistomial fragment. Observations indicated that this was not the case — regeneration was slower in the anterior than in the posterior fragment. The peristome is known to be replaced in the anterior fragment (Suzuki 1957). Experiments here reported were performed to determine whether or not the delay in regeneration of the anterior fragment resulted from retarded reorganization of the macronucleus and slower RNA synthesis.

Materials and Methods

The same methods were employed as in preceding papers. Cells from a logarithmic phase culture of axenic *Blepharisma japonicum* var. *intermedium* (Smith and Giese 1967) were fixed in Nissenbaum's fluid and Feulgen-stained to

determine the distribution of DNA. Sketches of 270 cells were made under oil immersion (A. O. Microstar, 100 × 15), with a camera lucida to indicate the state of the macronucleus and the micronuclei at various stages in regeneration. The micronuclei lie at many focal planes of the cell, making necessary visual rather than photographic determinations. With positions marked on a calibrated stage vernier it was possible to return to verify their location and number (Parker and Giese 1966).

The tracer ^{14}C-thymidine (17.1 μC/mM) was used at 20 μC/ml for 5 h and ^{14}C-uridine (24.4 μC/mM) was used at 20 μC/ml for 3 h (0–3 and 3–6 h after transection). The cells were then rinsed free of tracer and placed on a slide subbed with gelatin and dried, after which they were fixed in 95% ethanol-glacial acetic acid (1 : 1) for 15 min at room temperature. They were then rinsed and coated with Eastman Kodak NTB-3 emulsion. The slides were dried and kept in a light-tight box with Drierite for 3–4 weeks; they were developed in Kodak D-19 developer for 2 min, rinsed in tap water and fixed (Giese 1970).

Results

Macronuclear Behavior

The macronucleus of the anterior fragment of a transected *Blepharisma* coiled singly, then doubly, and subsequently condensed into a solid mass that later elongated to reform the vegetative macronucleus in the same manner as in the posterior fragment (Parker and Giese 1966). Macronuclear reorganization was complete in 6 to 7 h (Table 1, Fig. 1). Condensation reached a peak in 6.5 h instead of the 5.5 h required for the posterior fragment.

Micronuclear Behavior

Some metaphase figures were observed in the anterior fragment about 3 h after cutting (Fig. 1), suggesting multiplication of micronuclei, much as in the posterior fragment. However, the extreme variability in the number of micronuclei (Table 1), made a definitive conclusion impossible.

Nucleic Acid Synthesis

Although the macronuclear changes are slower in the anterior than in the posterior fragment of a transected *Blepharisma*, incorporation of uridine tracer in the macronuclear RNA of the anterior fragment was comparable to that in the posterior fragment (Table 2). For instance, every anterior fragment exposed to ^{14}C-uridine for 3 h after transection was strongly labeled. The label was strongest in the macronucleus where the number of grains accumulated in three hours was often too great to count. Tracer labeling appeared in the cytoplasm around the

Table 1

Nuclear Events During the Regeneration of *Blepharisma Japonicum* (Anterior Fragment)

	Whole Cells	Regenerating Cells					
		h 0	h 1	h 2	h 3	h 4	h 5
Number of cells	8	17	56	49	49	47	44
Total number of micronuclei (mn)	114	310	903	776	543	562	557
Mean number mn/cell	14.2	18.2 ± 6.9	16.1 ±10.8	15.8 ±11.5	11.1 ±8.5	12.0 ±8.8	12.7 ±10.0
% Pre-metaphase	0	9.7	21.7	20.0	42.2	22.6	20.1
% Metaphase figures	0	1.0	1.9	1.9	1.5	14.6	26.0
% Primary coiling of macronucleus	27.0	76.5	85.7	81.6	81.6	57.4	27.3
% Secondary coiling of macronucleus	12.0	23.5	46.4	38.8	49.0	44.7	25.0
% Condensation of macronucleus	5.0	0.0	5.4	12.2	14.3	8.5	36.4

macronucleus and still less at a distance, in both cases increasing with exposure time in the tracer solution. Almost equal labeling occurred 0–3 h and 3–6 h after transection. Observations were made on a total of 460 cells.

In three trials with ^{14}C-thymidine on anterior fragments 0–3 h and 3–6 h after transection, only minor scattered labeling of macronucleus and cytoplasm was observed in 279 cells, much as found for post-peristomial pieces (Giese 1970).

Discussion

The data presented in this paper indicate similarities in regeneration of the anterior and posterior fragments of a transected *Blepharisma japonicum*, var. *intermedium*. The macronucleus shows primary coiling, then a secondary coiling followed by condensation into a solid ovoid mass. The ovoid mass subsequently reforms the elongate vegetative macronucleus. Some micronuclei divide. In the postperistomial piece the micronuclei appear to duplicate the original number present in a vegetative individual (Parker and Giese 1966), but micronuclear doubling was not demonstratable in anterior pieces because of the extreme variability in number of micronuclei. It is possible that

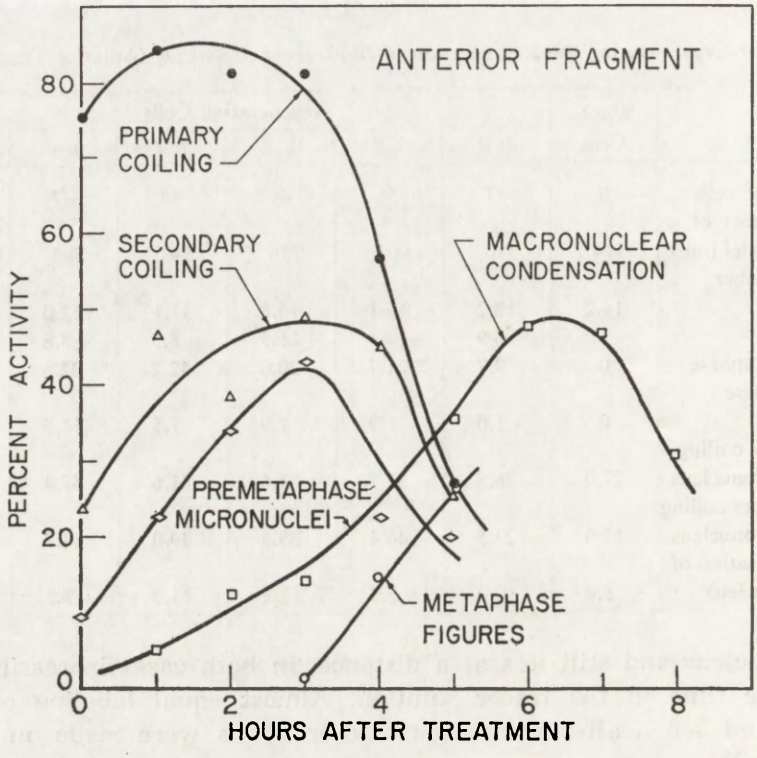

Fig. 1. Changes in macronucleus and micronuclei during the regeneration of anterior fragments of *Blepharisma japonicum*

Table 2

Incorporation of ^{14}C-uridine[a] into RNA of the Anterior Fragment of *Blepharisma*

Experiment	Over MN[b]	Near MN	Far from MN	No. cells
	Exposed 0 to 3 h after transection			
1	>100	83	71	71
2	90	71	61	40
3	100	86	60	53
	Exposed 3 to 6 h after transection			
1	>100	86	66	78
2	94	75	59	31
3	>100	85	75	44

a 20 µc/ml.

b MN = macronucleus.

micronuclear multiplication occurs just before cell division (ca. 36 h) during the prolonged division interval.

In both anterior and posterior fragments RNA synthesis, as measured by ^{14}C-uridine incorporation, occurred in about the same manner, mainly in and around the macronucleus and in both fragments cytoplasmic labeling increased with time of exposure in tracer solution. Pulse-labeling in the postperistomial fragment showed that the label is first dense in the macronucleus, then spreads out into the cytoplasm until it becomes almost equal in macronucleus and cytoplasm. This has been interpreted as the passage of mRNA from the macronucleus into the cytoplasm carrying the message (in mRNA) for the construction of a new peristome (Giese 1970). In the anterior fragment messages for both the removal of the peristome, which is out of proportion to the cell, and the reconstruction of a new peristome may be required.

Because the macronuclear reorganization and what is presumed to be mRNA synthesis are only slightly delayed, the slow division of an anterior as compared to a posterior fragment previously reported (Giese 1973, p. 244) must be due to other factors. The additional delay in division of the anterior fragment may be the need to increase the cytoplasmic mass, which is smaller in the anterior fragment, as well as the need to remove the old peristome and replace it with a new one proportional to the size of the regenerated fragment.

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RÉSUMÉ

Chez *Blepharisma japonicum* sectionnée juste derrière le péristome la régénaration du fragment antérieur est plus lente que celle du fragment postérieur. La réorganisation du macronoyau est pareille dans les deux fragments mais également un peu plus lente dans l'anterieur. La vitesse de l'incorporation de ^{14}C-uridine dans l'ARN macronucléaire et cytoplasmique (donc probablement la formation du mARN) est à peu près égale dans les deux fragments. Il paraît que la régéneration est rétardée dans le fragment antérieur principalement à cause de son péristome qui doit être résorbé avant d'être remplacé.

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Localization of Ribonucleoproteins in the Macronucleus of *Paramecium aurelia*

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Synopsis. In the vegetative macronucleus of *Paramecium aurelia*, numerous ribonucleoprotein structures are revealed in the nucleoplasm as well as in the nucleoli using Bernhard's staining. In the nucleoplasm both perichromatin and interchromatin granules are found. The RNP material accumulated on the surface of bleached small bodies is considered to be perichromatin fibrils. In the nucleoli, RNP fibrils penetrating a large ring of DNP material are observed. Externally to this ring, in close vicinity of RNP fibrils, a great quantity of nucleolar granules is noted.

The meaning of these structures as morphological markers of transcriptional activity is discussed.

Visualization of ribonucleoproteins (RNPs) according to Bernhard's procedure (1969), although it is rather qualitative than quantitative method, allows a general evaluation of RNA content. Some suggestions concerning the intensity of transcription are also possible, since the newly synthesized highly molecular RNAs and their partially mature forms may be distinguished by their morphological appearance. Moreover, the localization of these RNP structures makes it possible to ascertain the character of the synthesized RNAs.

The studies on RNP metabolism in the macronucleus of Ciliates seem to be of special interest because these nuclei are characterized by a peculiar organization and a high degree of polyploidy. Bernhard's reaction was applied for macronuclei of *Lacrymaria* (Bohatier 1975) and *Tetrahymena* (Nilsson 1976). However, the authors report only the presence of RNP material in the macronuclei without any detailed analysis of its character.

The present paper deals with localization of different ribonucle-

oprotein structures in a vegetative macronucleus of *Paramecium aurelia*. Some suggestions as to the transcriptional activity of this nucleus will be also discussed.

Material and Methods

Paramecium aurelia, stock 299 S (Kappa free) grown axenically after Soldo et al. (1966) were used.

Cells collected by gentle centrifugation at 250 g were fixed in 2% glutaraldehyde pH 7.4 in 0.1 M cacodylate buffer, for 1 h at 0 — +4°C. Then they were dehydrated in an ethanol gradient, processed through propylenoxide and embedded in Epon 812. Ultrathin sections cut on a LKB III ultramicrotome were stained according to Bernhard's procedure, preferential for ribonucleoproteins (Bernhard 1969). Sections contrasted with 4.5% uranyl acetate in 70% ethanol, for 15 min, well dried, were treated with freshly prepared 0.2 M EDTA neutralized with NaOH, for 30–60 min, followed by Reynolds (1963) lead citrate, for 5 min.

For comparison, some cells were processed according to the conventional electron microscope technique. They were fixed as before and then postfixed in 1% osmium tetroxide during 30 min, on ice. Sections were contrasted as mentioned above, but without EDTA treatment. All sections were examined with a JEM 100 B electron microscope.

Results

The ultrastructural organization of the vegetative macronucleus of *Paramecium aurelia* contrasted by a conventional method, is presented in Pl. I. The characteristic small bodies are randomly distributed throughout the whole macronucleus. Between them the larger ones designated as nucleoli are observed. The small and large bodies are embedded in a granulo-fibrillar matrix (nucleoplasm and euchromatin) where coiled, very short, thick filaments have been observed (Pl. I circle). Besides these known characteristics (Jurand et al. 1962, Stevenson and Lloyd 1971, Ehret and McArdle 1974), some new details of macronuclear ultrastructural organization have been noted. These are the fibrillar attachment of nucleoli to the inner membrane of the nuclear envelope (Pl. I a, 1), and helical forms of some fibrils in the matrix (Pl. I a, 3). Among the variety of granules, the perichromatin ones are easily seen owing to the presence of an electron-transparent halo around the granule of 30–40 nm in diameter (Pl. I a, 2). Moreover, in the perinuclear space, very delicate fibrils joining the two membranes of the nuclear envelope are observed (Pl. I a, 7).

Preferential staining of ribonucleoproteins reveals many more details of macronuclear organization. The small bodies remain unconstrasted confirming thus their deoxyribonucleoprotein (DNP) nature. Ribonucleoprotein material noted at the surface of small bodies delineates them from the surrounding nucleoplasm (Pl. II a, 5). In some places this RNP layer is sufficiently wide to be clearly seen, but in the others, even at the surface of the same body, it is hardly observable (Pl. II a, dashed circle). The high content of RNP structures is also seen both in the nucleoplasm as well as in the nucleoli. In the latter structures the ribonucleoproteins are localized at the periphery of the material with unexpected high content of DNP (Pl. II a). RNP fibrils of about 7 nm in width and about 60 nm in length penetrate into the uncontrasted ring (Pl. II c). The RNP granules from 18–22 nm in diameter, are visible externally to the nucleolar RNP fibrils (Pl. II c). Sometimes these granules seem to be stuck together and dangle in the nucleoplasm (Pl. II a, c).

In nucleoplasm, on the other hand, some granules, most frequently those of about 30–40 nm diameter, can be considered as the central part of the perichromatin ones. These particles join by an occasionally seen stalk to the remaining RNP material (Pl. II d, 6). The others, single granules dispersed through the macronucleus seem to be interchromatin ones (Pl. II a, 4). The coiled, very short thick filaments seen in Pl. I, are revealed now to contain RNP material (Pl. I b). Some helices seen in the nucleoplasm in macronucleus contrasted by conventional procedure are show to be also of ribonucleoprotein nature (Pl. II d). Moreover, in Pl. II d zig-zag configuration of RNP material is noted as well. Besides, the ribonucleoproteins are observed in the nuclear envelope, in pores (Pl. II d).

Discussion

In nucleoplasm of rat liver nuclei, the most frequently noted RNP structures are: perichromatin fibrils, perichromatin and interchromatin granules (Monneron and Bernhard 1969).

Perichromatin fibrils usually found at the surface of compact chromatin (Monneron and Bernhard 1969) may be also found in the nucleoplasm (Puvion and Moyne 1978). Under modified physiological conditions, as after injection of cortisone to the animal, or refeeding of starved rats, the number of perichromatin fibrils increases (Petrov and Bernhard 1971). Since the incorporation of

^{3}H-uridine over the perichromatin fibrils was observed shortly after 2 min of isotope administration, it was found that these fibrils contain rapidly labelling RNA (Fakan and Bernhard 1971). Moreover, the results of fractionation of RNA from isolated mammalian cell nuclei suggest that the fraction containing perichromatin fibrils, at least partially, corresponds to the high molecular weight RNA (Bachellerie et al. 1975). Thus the perichromatin fibrils observed in rat liver nuclei consist of newly transcribed, heterodispersed high molecular weight nuclear RNA, complexed with proteins.

The RNP material observed on the surface of small bodies in the macronucleus of *Paramecium aurelia* resembles the perichromatin fibrils found in mammalian cell nuclei. Therefore, it seems probable that they also represent heterogenous nuclear RNA. Different accumulation of this RNP material at the surface of small bodies suggests a varying intensity of this RNA transcription.

The perichromatin granules of 35–45 nm in diameter, are observed in rat liver nuclei in the vicinity of compact chromatin and close to the perichromatin fibrils (Monneron and Bernhard 1969, Petrov and Bernhard 1971). At high magnification it was revealed that they consist of fibrils, which are 30 and 15 nm thick in rat liver nuclei and in nuclei of dipterous salivary gland cells, respectively (Monneron and Bernhard 1969, Vazques-Nin and Bernhard 1971). It was supposed that these are perichromatin fibrils (Devilliers et al. 1977), which together with nucleosomes form a perichromatin granule (Daskal et al. 1978). Perichromatin granules of liver nuclei resemble morphologically the granules observed in the Balbiani ring of *Diptera* salivary glands. The latter granules were known to contain specific messenger RNA and it was therefore suggested by analogy that perichromatin granules in mammalian nuclei consist of some classes of messenger RNA as well (Bernhard 1977). The presence of the fibrils, which were believed to be perichromatin ones containing heterogenous nuclear RNA, seem to support the preceeding suggestion. Since the perichromatin granules were revealed near the nuclear pores, they are considered to represent a storage and/or transport form of messenger RNA (Monneron and Bernhard 1969). According to Herman et al. (1978), at least some of perichromatin granules might serve as attachment points of RNP material to the nuclear skeleton, playing therefore not only a physiological role but also a structural one.

Perichromatin granules are also found in the macronucleus of *Paramecium aurelia*. They resemble the perichromatin granules described in rat liver cell nuclei and it is not excluded that they also contain

some types of messenger RNAs, having thus a similar role in nuclear RNA metabolism.

The interchromatin granules observed in mammalian cell nuclei are organized in clusters covering an extensive nuclear area (Monneron and Bernhard 1969). In the monkey kidney cell line BSC_1 the interchromatin granules were very weakly labelled, usually only at the periphery of clusters even after a long time of incubation with ^{3}H-uridine, thus indicating that they contain slowly labelled RNA (Fakan and Bernhard 1973). It was suggested that they may participate in transfer of nucleolar RNA into the cytoplasm (Monneron and Bernhard 1969). However, the role of interchromatin granules in nuclear RNA metabolism is considered to be rather unclear, up to now.

In *Paramecium* we never observed granules appearing in clusters but many single granules of 20 nm are found to be dispersed throughout the nucleoplasm. It therefore remains to elucidate, whether these may be regarded as interchromatin granules similar to those of rat liver nuclei.

In nucleoplasm of *Paramecium aurelia* macronuclei, besides described above RNP structures, we observed flexuous and helical RNP fibrils which may be regarded as part of the ribonucleoprotein network. Such configuration of RNP network was found recently in rat liver nuclei (Miller et al. 1978).

In the nucleolus, the procedure of Bernhard revealed also ribonucleoproteins organized in fibrils and granules. The former are localized within the fibrillar part of nucleolus, whereas the latter are the only structural component of its granular part (Bernhard 1977). It is known that in the fibrillar part of the nucleolus a preribosomal RNA is synthesized, while its granular part contains partially processed r-RNA (Busch and Smetana 1970).

In the macronucleus of *Paramecium aurelia* both the numerous nucleoli which in a sections cover about 15% of the macronuclear area (Krawczyńska et al. 1978), as well as the abundance of RNP fibrils and granules noted in these nucleoli, suggest the high activity in transcription and processing of ribosomal RNA. In closely related protozoan cell — *Tetrahymena,* it was found that the transcription of nucleolar DNA is very rapid, indeed (Eckert and Franke 1975, Gocke et al. 1978). In the macronucleus of this organism the r-RNA content is nearly 85% of the total macronuclear RNA (Engberg and Pearlman 1972), although the template for ribosomal RNA amounts to as little as 0.5–2.0% of the macronuclear DNA (Andersen 1977, Gocke et al. 1978, Yao and Gorovsky 1974).

In conclusion, the results presented in this paper show that the macronucleus of *Paramecium aurelia* is very rich in various ribonucleoprotein structures, some of which resemble those described in liver cell nuclei. These are regarded as morphological counterparts of definite classes of RNA and suggest a rather high transcriptional activity of *Paramecium aurelia* macronucleus.

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RÉSUMÉ

La coloration d'apres Bernhard revèle des nombreuses structures ribonucléoproteiques dans le nucléoplasme et dans les nucléoles du macronoyau végétatif du *Paramecium aurelia*. Dans le nucléoplasme on trouve des granules périchromatiniens ainsi que interchromatiniens. Le matériel ribonucléoprotéique accumulé à la surface des petit cors blanchissés est considéré comme représentant les fibrilles de périchromatine. Dans les nucléoles on trouve les fibrilles ribonucléoprotéiques pénétrant à travers un large anneau formé du matériel déoxyribonucléoproteique. A l'extérieur de cet anneau les granules nucléolaires sont observées en grande quantité, strictement voisins des fibrilles ribonucleoprotéiques. On discute la signification de ces structures en tant que marqueurs morphologiques du processus de transcription.

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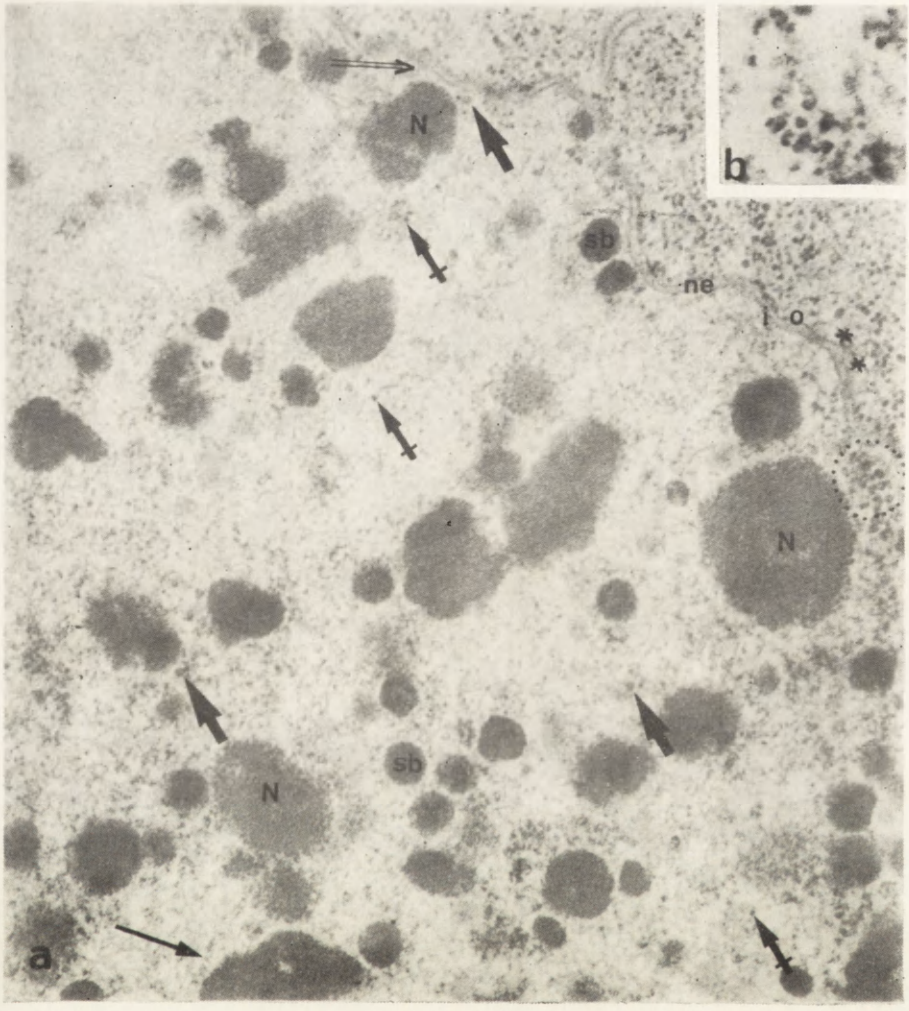
EXPLANATION OF PLATES I–II

Pl. I. Macronucleus of *Paramecium aurelia*
a: general pattern of ultrastructural organization, conventional procedure. × 35 000.
b: coiled, short filaments, Bernhard's staining. × 54 000.
Pl. II. Ribonucleoproteins in the macronucleus of *Paramecium aurelia*, Bernhard's staining.
a: general pattern. × 35 000.
b: nuclear envelope. × 36 000.
c: fragment of nucleolus × 91 000.
d: RNP structures in the nucleoplasm. × 60 000.
Abbreviations:
ne — nuclear envelope, i — inner and o — outer membrane of nuclear envelope,
sb — small body, N — nucleolus, f — nucleolar fibrils, g — nucleolar granules.
1 — attachment of nucleolus to the inner membrane of nuclear envelope,
2 — perichromatin granule,
3 — helical form,
4 — interchromatin granule,
5 — RNP layer at the surface of small body,
6 — stalk of perichromatin granule,
7 — fibrils of perinuclear space,
8 — nuclear pore,
9 — zig-zag configuration
dotted circle — coiled, short filaments,
dashed circle — RNP layer hardly visible.
dashed line — row of nucleolar granules.



Legends

1 4 7
2 5 8
3 6 9

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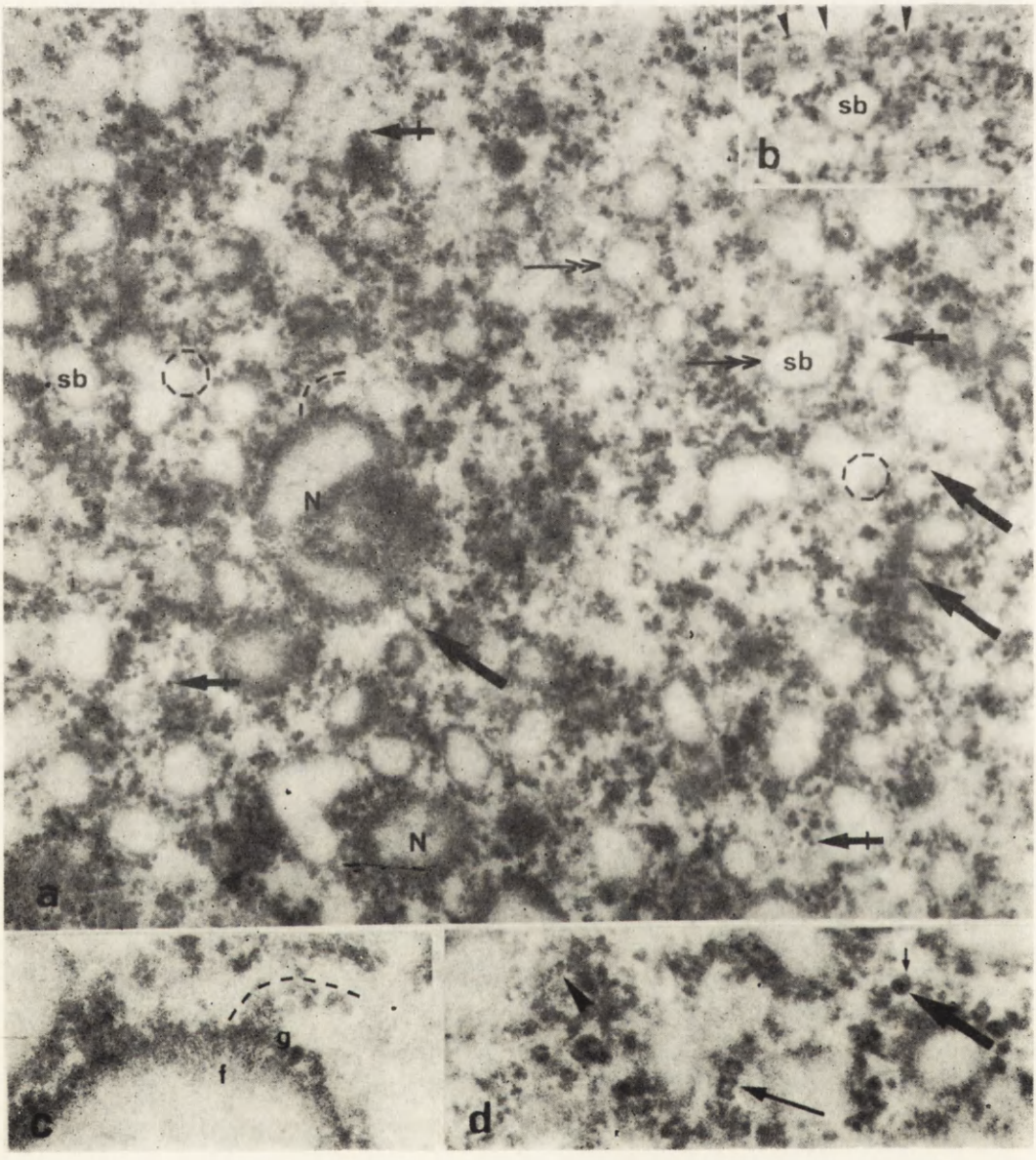

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Dependence of Toxic Effects of Detergents in *Paramecium caudatum* on Ionic Composition of External Medium

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Synopsis. **Increase of concentration of NaCl, KCl, $CaCl_2$, $MgCl_2$, $BaCl_2$ in external medium caused elongation of survival rate of *Paramecium caudatum* exposed to cationic detergent CTAB, while in the case of anionic detergent SDS the same factors caused a marked decrease of survival rate. Toxic effects of non-ionic detergent Triton X-100 did not show any dependence on concentrations of applied KCl and $CaCl_2$ solutions.**

Information about effects of surface active substances on protozoa is rather scarce and fragmentary, although recently much more attention is paid to the possible mechanism of their action on various physiological system in unicellular organisms and this undoubtedly may lead in future to better understanding of cytopathological action of detergents. Chaix and Baud (1947) found that lysis in *Tetrahymena pyriformis* is usually preceded by decreased cell motility and characteristic rounding of cell shape. Bailenger and Troadec (1953) and Bailenger et al. (1953) reported occurrence of immobilization, inhibition of fission and cell lysis in *Tetrahymena pyriformis, Euglena gracilis, Balantidium coli* and *Entamoeba invadens* caused by ionic detergents. They attributed the stronger toxic effects of cationic detergents to their positive charge and increased adsorption constant of applied substance.

Interesting findings on physiological effects of ionic detergents on protozoa were reported recently by Dryl and Bujwid-Ćwik (1972 a, b) and Bujwid-Ćwik and Dryl (1971, 1975). These authors were able to show that cationic detergent cetyl trimethyl am-

monium bromide (CTAB) inhibits potassium-induced ciliary reversal (CR) in *Paramecium*, while anionic detergent sodium dodecyl sulphate (SDS) exerts an opposite effect, i.e., prolongation of potassium-induced CR.

Dryl and Mehr (1976) showed in extensive study on *Paramecium caudatum* that increase of lethal-toxic effects of detergents is associated with their higher liophilic balance and critical micelle concentration. Except Tween 80, all other detergents tested (CTAB, Triton X-100, SDS, Tween 40, Tween 60) induced contraction within ectoplasm expressed by characteristic pear-shaped or lemon-shaped deformation of cell body with a marked shortening of longitudinal axis of the initial length of *Paramecium*. It was proved that the above mentioned ectoplasmic contraction results from influx of external calcium throughout the damaged cell membrane by detergent.

The preliminary observations revealed that changes of ionic composition of external medium may influence in esential way toxic action of detergents on *Paramecium*. This enhanced the authors of present report to perform more detailed studies in this respect.

Material and Methods

All observations and experiments were carried out on *Paramecium caudatum* strain from Nencki Institute of Experimental Biology in Warsaw (isolated near Warsaw in 1965), which was grown in the lettuce medium inoculated with *Aerobacter aerogenes* according to method of Sonneborn (1950).

18–20 h before starting experiments, paramecia were collected and washed in 1mM $CaCl_2$ + 1mM Tris/HCl solution (pH 7.3) by application of geotactic technique described in detail elsewhere (Dryl 1963).

The stock solutions of applied detergents (CTAB, SDS, Triton X-100) were prepared as 0.5% solution on bidistilled water. Solution of desired concentration of detergent was prepared by dilution of stock solution with Tris/HCl solution of appropriate ionic composition in given series of experiments.

The 50% survival rate (LD_{50}) was calculated as 1:1 ratio between still alive and killed animals. The criterion of cell death was the occurrence of non-reversible immobilization of ciliates with complete lack of any visible activity of cilia, food vacuoles formation and contractile vacuoles rhythmic action. The symptoms of cell death were usually preceded by the characteristic pear-shaped, cigar-shaped or lemon-shaped deformation of cell body (Dryl and Mehr 1976).

Observations were performed at room temperature (20–22°C) on approximately 50–60 animals in 0.4 ml of experimental medium. Mixing in proportion 1:1 of ciliates suspension in salt solution and experimental detergent solution was carried out in depression slides which allowed observation of behavior and other visible properties of protozoan cell under binocular microscope at low magnification.

Before adding of detergent solution for the final testing paramecia were preincubated for 10 min in the medium with desired ionic composition.

All calculations necessary for establishing LD_{50} were based on 15 repetitions of experiments peformed under the same conditions. The results were presented in diagramms as arithmetic means and standard deviations of experimentally checked survival time.

It should be pointed out that control paramecia (not exposed to detergent solution) showed always the survival rate higher than 99% and their cell fission rate was lower than 0.01 fissions per day, so that the authors could exclude any significant error associated with cell growth.

For technical reasons the applied detergent concentrations were in the range which rendered possible checking of LD_{50} doses during 10–60 min of exposure of ciliates to tested detergent solutions. The applied concentrations of tested detergents were as follows:

2.7×10^{-4} M SDS
5.4×10^{-4} M SDS
2.7×10^{-5} M CTAB
2.2×10^{-4} Triton X-100

Results and Discussion

Series I. The Dependence of Toxic Action of CTAB and SDS on pH of External Medium

In this series of experiments paramecia were exposed to detergents in 1 mM $CaCl_2$ + 1 mM Tris/HCl solutions of different pH (in the range of pH between 6.5–8.8). The obtained results are shown in diagramme (Fig. 1), they indicate for 2.7×10^{-5} M CTAB very slight increase (ca. 10 min) of survival time at pH 6.5 and slight decrease of survival time (ca. 8–10 min) when related to average values of survival time at other pH values approximately 30–40 min. The survival time of paramecia exposed to 5.4×10^{-4} M SDS was in the range 13–18 min and did not show any significant differences with regard to pH value.

Series II. The Dependence of Toxic Action of CTAB, SDS and Triton X-100 on the Presence and Concentration of Cations

The final concentrations of various salts ($CaCl_2$, $MgCl_2$, $BaCl_2$, NaCl, KCl) were prepared on the basis of the same solution: 1 mM $CaCl_2$ + 1 mM Tris/HCl (pH 7.4) with addition of one of the following detergents in concentrations:

2.7×10^{-5} M CTAB
or 2.7×10^{-4} M SDS
or 2.2×10^{-4} M Triton X-100

The salt solutions were applied in 1–2.5 mM final concentration. As shown in Fig. 2 A the toxic action of CTAB decreases parallel to increase of all tested cations in concentrations from 1–15 mM of their chloride solutions. In the case of $CaCl_2$ the toxic effect of CTAB in-

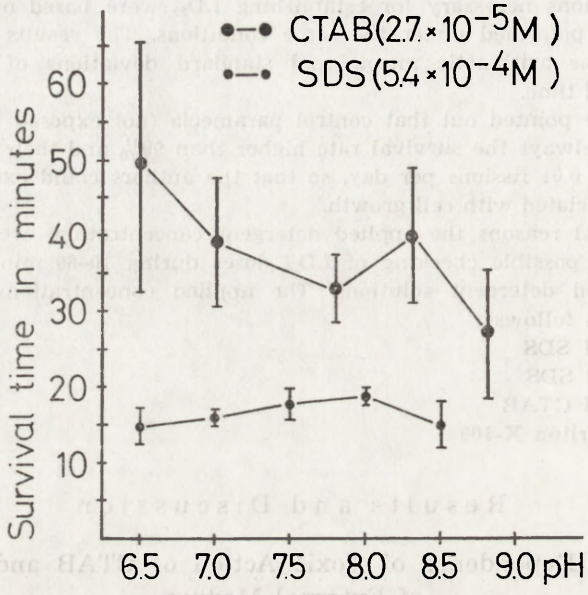

Fig. 1. Toxic effects of CTAB and SDS at various level of pH. Each point of the diagramme represents arithmetic mean (with standard deviation) of experimentally established 15 measurements of survival time (based on LD_{50})

creases abruptly at 20 mM and higher concentrations of $CaCl_2$ as indicated by shortening of survival time. Other cations, except potassium, showed similar but less marked toxic effect at higher concentrations of their chloride solutions.

The opposite effect of cations is evident in the case of toxic action of SDS. As indicated in the Fig. 2 B the survival time of paramecia exposed to SDS decreases (from average 30 min to 10–20 min) parallel to increase of concentrations of all applied chloride solutions. However, no significant effect of KCl and $CaCl_2$ solutions could be detected in the case of exposure to Triton X-100 (Fig. 2 C).

Lethal effects of tested detergents on paramecia brought evidence that CTAB exerts stronger toxic effects than SDS and Triton X-100. This finding is in a good agreement with data reported by other authors on paramecia (Dryl and Bujwid-Ćwik 1972 a, Bujwid-Cwik and Dryl 1975, Dryl and Mehr 1976) and other protozoa (Bailenger and Troadec 1953, Bailenger et al. 1953).

The elongation of survival time of paramecia exposed to CTAB and SDS at low pH (pH 6.5) and shortening of survival time at higher pH values (pH 8.8) suggest that positively charged H^1 ions decrease toxic

[1] The dramatic decrease of survival rate of paramecia exposed to 15–25 mM concentrations of $CaCl_2$ (Fig. 2 A) can be explained by synergic action of CTAB and relatively high concentration of $CaCl_2$.

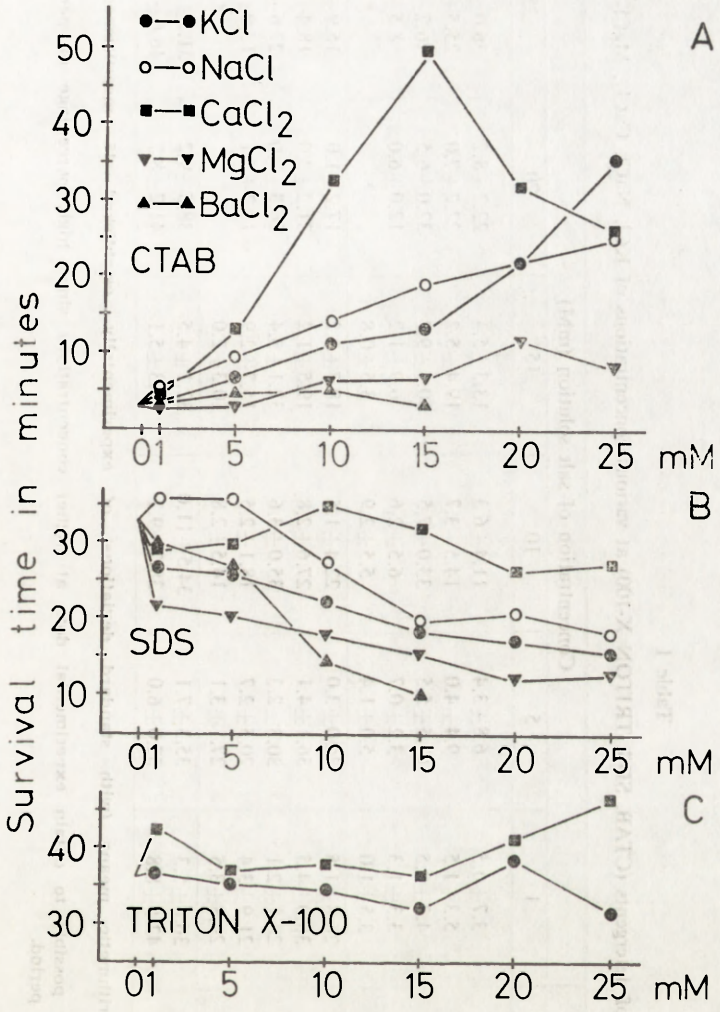

Fig. 2. Toxic effects of detergents at various concentrations of NaCl, KCl, $CaCl_2$, $MgCl_2$, $BaCl_2$. A — Toxic effects of CTAB, B — Toxic efects of SDS, C — Toxic effects of Triton X-100

Each point of the diagramme represents arithmetic mean of experimentally established 15 measurements of survival time (based on $LD_{.0}$)

action of applied surface-active substances. The role of positive charge of cations in modifying toxic effects of detergents seems to be confirmed by more extensive series of experiments performed at various concentrations of NaCl, KCl, $CaCl_2$, $MgCl_2$, $BaCl_2$. The obtained results (Fig. 2, Table 1) indicate that toxic action of cationic detergent CTAB decreases parallel to increase of concentration in the range 1–25 mM

Table 1

Cumulative data concerning toxic effects of detergents (CTAB, SDS, TRITON X-100) at various concentrations of KCl, NaCl, $CaCl_2$, $MgCl_2$, $BaCl_2$

Detergent	Salt solution	Concentration of salt solution (mM)						
		0	1	5	10	15	20	25
CTAB	KCl	3.0±0.6	3.7±0.5	6.8±3.4	11.4±6.3	13.3±5.4	22.2±8.2	36.0±10.0
	NaCl	3.0±0.6	5.3±1.5	9.4±4.0	14.5±3.7	19.4±5.2	22.2±7.0	25.5±6.9
	$CaCl_2$	3.0±0.6	4.3±0.5	13.5±5.5	33.0±7.5	50.3±9.7	32.0±6.5	26.2±7.9
	$MgCl_2$	3.0±0.6	3.3±1.3	3.2±0.7	6.5±2.6	6.9±1.2	12.0±6.0	8.5±4.4
	$BaCl_2$	3.0±0.6	3.5±1.0	5.0±1.4	5.5±2.9	3.5±0.8	*	*
SDS	KCl	33.2±3.0	26.8±1.5	26.0±3.0	22.4±1.5	18.7±3.2	17.2±1.0	15.9±2.1
	NaCl	33.2±5.0	36.0±4.5	36.1±4.1	27.6±2.8	19.5±1.2	21.2±2.0	18.4±3.2
	$CaCl_2$	33.2±3.0	28.1±2.1	30.3±2.3	35.0±3.6	32.1±3.4	26.3±3.0	27.6±3.2
	$MgCl_2$	33.2±1.0	21.9±3.4	20.5±2.7	18.1±2.5	15.7±2.9	12.3±4.1	13.0±3.0
	$BaCl_2$	33.2±2.0	29.8±3.5	27.1±3.1	14.5±2.8	10.3±2.0	*	*
TRITON X-100	KCl	36.2±4.6	36.8±5.3	35.3±7.1	34.5±11.0	32.1±4.5	38.5±9.2	31.3±12.0
	$CaCl_2$	36.2±4.6	42.5±8.8	37.0±6.0	38.2±9.2	36.3±5.1	41.2±8.7	46.6±13.0

In vertical columns are given arithmetic means (with standard deviations) of experimentally established 15 measurements of survival time (in min).

* In the case of $BaCl_2$ it was not possible to obtain experimental data at higher concentrations since high percentage of paramecia perished during 10 min of pre-incubation period.

of applied electrolite solutions, while in the case of anionic detergent SDS the same factors show opposite effect, i.e., slight shortening of survival time parallel to increase of salt concentration (Fig. 2 B). It should added that toxicity of SDS was on more or less the same level at all tested pH levels (Fig. 1). It is clear from data on Fig. 2 C that toxic action of neutral detergent Triton X-100 was not much influenced by $CaCl_2$ and KCl solutions of various concentrations.

Although the reported changes of survival rate of paramecia at various external ionic conditions are rather clear, it is still not easy to give for them an appropriate theoretical explanation. The authors suggest that protective role of cations against toxic effect of CTAB depends on competitive action of cations and positively charged detergent molecules on the negatively charged anionic binding sites within cell membrane of *Paramecium*. This view is closely related to hypothesis of Dryl and Mehr (1976) that disruption of the cell membrane of *Paramecium* caused by cationic detergents results from interaction of anionic groups of membrane with positively charged detergent molecule.

RÉSUMÉ

L'augmentation de la concentration de NaCl, $CaCl_2$, $MgCl_2$ et $BaCl_2$ dans le milieu ambiant prolonge la survie de *Paramecium caudatum* exposé à l'action de détérgent cationique CTAB. Les mêmes facteurs suppressent la survie dans les solutions du détérgent anionique SDS. Les effects toxiques du détérgent non-ionique Triton X-100 ne dépendent pas de la concentration du KCl ni du $CaCl_2$.

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Enerthecoma dissimilis a new Rhynchodid Ciliate from Freshwater Gastropod *Viviparus dissimilis* (Müller)

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Synopsis. A new ciliate *Enerthecoma dissimilis* sp. n., is described from the freshwater gastropod *Viviparus dissimilis* (Müller) in India. It is characterized by not possessing a cytostome and instead, have a suctorial tentacle. The ciliate is elongated, symmetrical, banana-shaped, 27–49 × 9–19 μm with a mean of 37.61 ± 0.51 × 13.58 ± 0.27 μm. This is the first record of a thigmotrich ciliate from freshwater gastropod in India and also in Asia.

Among the thigmotrich ciliates, the rhynchodine group are characterized by not possessing a cytostome and instead have a suctorial tentacle. They belong to the order *Rhynchodida* with the families *Ancistrocomidae* and *Sphenophryidae* (Corliss 1975). These families show a clear cut parasitic adaptation which has reached its maximum in the species belonging to the family *Sphenophryidae* retaining only strongly reduced ciliature. Raabe (1970) has discussed in detail the systematic position of many of the species of the sub-family *Ancistrocomidae* and finally classified them under nine genera comparising 26 species. Except for the two genera *Enerthecoma* and *Hypocomina* in which the reduced ciliature of thigmotactic zone is even and symmetrical, all the other genera are characterized by having reduced but uneven or asymmetrical thigmotactic ciliature.

The genus *Enerthecoma* was created by Jarocki (1935) for the individualization of *E. properans* from the gastropod *Viviparus fasciatus* among other genera of *Ancistrocomidae* which he found in the mantle cavity and on the gill surface of the freshwater snails in the region of Warszawa. Although the description of this species was adequate, it was not supplemented by any illustration.

Kozloff in 1946 described with illustrations another ciliate from *Viviparus malleatus* in North America as *Enerthecoma properans* and confirmed all characters similar to *Enerthecoma properans* which Jarocki had indicated in 1935. Chatton and Lwoff (1950) individualized the form of Kozloff (1946) as a new species *E. kozloffi* but later Raabe (1970) has considered it as a synonym to the *Enerthecoma properans* Jarocki, 1935.

Raabe (1970) proposed that the species *Hypocomina tegularum* Kozloff, 1946 should be brought to the *Enerthecoma*, for it has a more or less symmetrical thigmotactic arrangement which deviates from the distinctly asymmetric arrangement found in the representatives of the genus *Hypocomella* and other genera of the sub-family *Hypocomallinae*, thus, it was finally classified as *Enerthecoma tegularum.*

Presently there are only two species in this genus viz., *E. properans* Jarocki and *E. tegularum* Kozloff. The new species described here constitutes the third species under this genus. This is the first record of a thigmotrichid ciliate from freshwater gastropod in India and also in Asia.

Materials and Methods

One thousand and seventy specimens of *Viviparus dissimilis* (Müller) were collected from various ponds, lakes and Moosi river in and around the city of Hyderabad, Andhra Pradesh, India, during July 1974 to June 1976. Approximately 71% of the snails were found to be infested with a thigmotrich ciliate.

Shells of the snails were broken with the help of a scalpel and the mantle cavity was washed with its content in a cavity block (40 mm × 40 mm) and examined under a binocular microscope at a magnification of 50 ×. The thigmotrich ciliate could easily be recognized by its characteristic spiral swimming movement which was quite different from the movements of the other ciliates present in the contents of the mantle cavity. They were picked up with a fine pipette on a glass slide. Digestive tract of the snalis was also excised routinely and examined for the parasitic ciliates. Scrapings from the mantle tissue, foot and ctenidia were screened for the location of ciliates.

Live ciliates were observed under phase contrast optics where cilia and their movements could be easily distinguished. For the demonstration of cilia, vital stains such as Toluidine blue and Nigrosin were used whereas neutral red, Methyl green, Methylene blue and Lugol's iodine were used for the observation of cytoplasmic details in the temporary preparations. Osmium tetroxide vapour and da Fano's fixative were used for silver impregnation staining by the methods of Klein (1928, 1956) and Chatton and Lwoff (revised by Corliss 1953).

Air-dried specimens were fixed in Nissenbaun's fixative (1953), Schaudin's and Cornoy's fixatives prior to staining with Heidenhain's iron haematoxyline (Kirby 1950) and Feulgen's nuclear reaction (Mac Kinnon and Hawes 1961).

Microphotographs were taken on Leitz Wetzlar microscope with a Leica M-3 camera and the drawings were made with the help of a camera lucida.

The slides of the type material of the species described have been deposited in the Protozoology Section, Department of Zoology, Osmania University, Hyderabad-500 007, India.

Morphology

The body of the ciliate is elongated, symmetrical and banana-shaped. The anterior end is tapered sharply, curved in an arc when viewed laterally while the posterior end is rounded. The ciliary system is disposed on a relatively flat area occupying the anterior two thirds of the ventral surface, the dorsal surface is convex and devoid of any ciliature. The body is widest at a distance of the two third of its length from anterior end (Fig. 1).

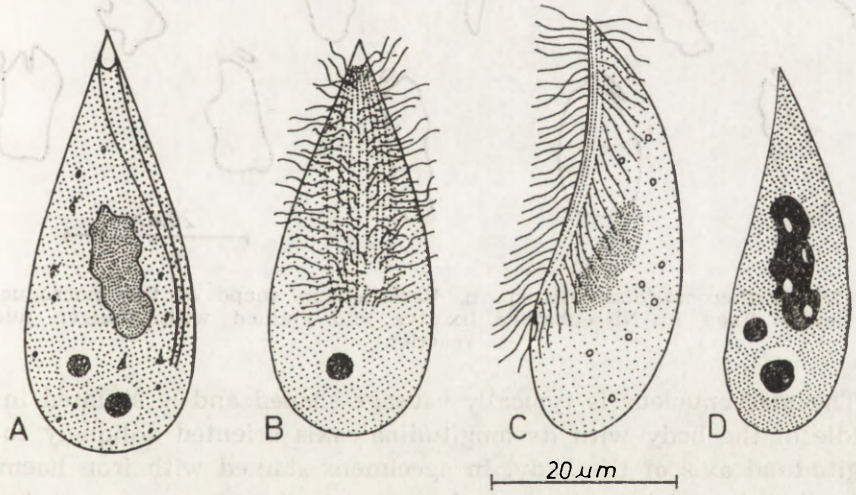

Fig. 1. *Enerthecoma dissimilis* sp. n. Diagrammatic representation of living organism as seen from dorsal (A), ventral (B) and lateral (C) aspects and stained in Heidenhain's haematoxylin (D)

The anterior end is provided with a suctorial tentacle continuous with an internal tubular canal. The nature of the canal is very similar to that of other members of the genus. It is directed at first dorsally and then ventrally and obliquely towards the right side of the body. It can be traced in most fixed specimens stained with iron haematoxylin for about one-half to two-third of the length of the body.

The ciliary system is composed of eight approximately equal rows extending two third the length of the body from its anterior end.

These rows originate close to the base of the suctorial tentacle. The first five rows from the right side are usually a little more widely spaced than the last three rows. This was also noted by Jarocki (1935) and Kozloff (1946) in *E. properans*.

The cytoplasm is colourless and contains numerous small refractile granules in addition to food inclusions. One or more larger food vacuoles surrounded by few smaller ones are usually present in the posterior part of the body. The contractile vacuole is situated in the middle, slightly towards the left side of the body and opens up to the exterior on the ventral surface. Sometimes two contractile vacuoles of equal size were seen.

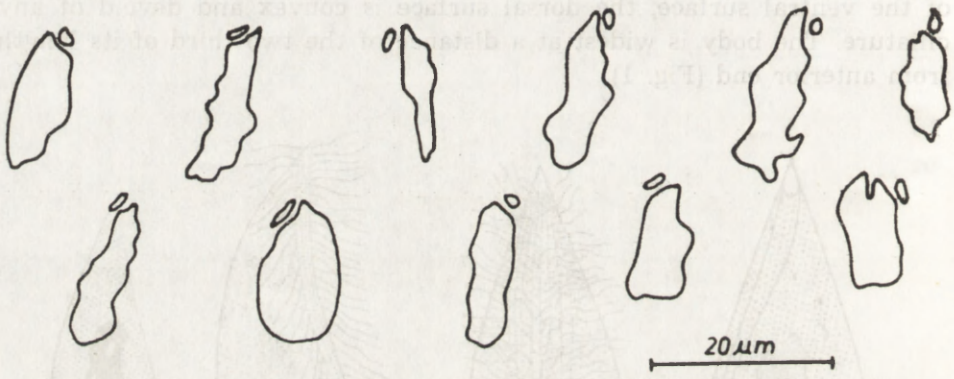

Fig. 2. *Enerthecoma dissimilis* sp. n. Variation in shape of the macronucleus. (Specimens fixed in Nissenbaun's fixative and stained with Feulgen nuclear reaction)

The macronucleus is typically sausage-shaped and is situated in the middle of the body with its longitudinal axis oriented obliquely to the longitudinal axis of the body. In specimens stained with iron haematoxylin the chromatin appears to be more or less homogeneous, but in preparations stained by the Feulgen nuclear reaction it appears to be organized into a dense reticulum enclosing clear spaces of varying size. The shape of the macronucleus is variable (Fig. 2). The micronucleus is situated anterior to or at one side of the macronucleus. In most of the individuals it is elongated and more or less fusiform. Very few specimens show a round micronucleus. The micronucleus does not stain readily with iron haematoxylin as has been reported earlier by Kozloff (1946) in *E. properans* (syn. *E. kozloffi* according to Raabe, 1970).

The dimensions of the organism based on measurements of 100 individuals are shown in Table 1. Specimens were fixed in Nissenbaun's fixative and stained with Feulgen nuclear reaction, counter-

Table 1

Morphological characteristics	Range	Mean	S.D.
Length of the organism	27–49	37.61	5.10
Width of the organism	9–19	13.58	2.68
Length/Width ratio	1.63–4.77	2.83	0.50
Length of the macronucleus	9–18	12.97	2.39
Width of the macronucleus	2–8	3.88	0.96
Length of the micronucleus	1.1–3.24	2.29	0.12
Width of the micronucleus	1.1–2.16	1.35	0.32

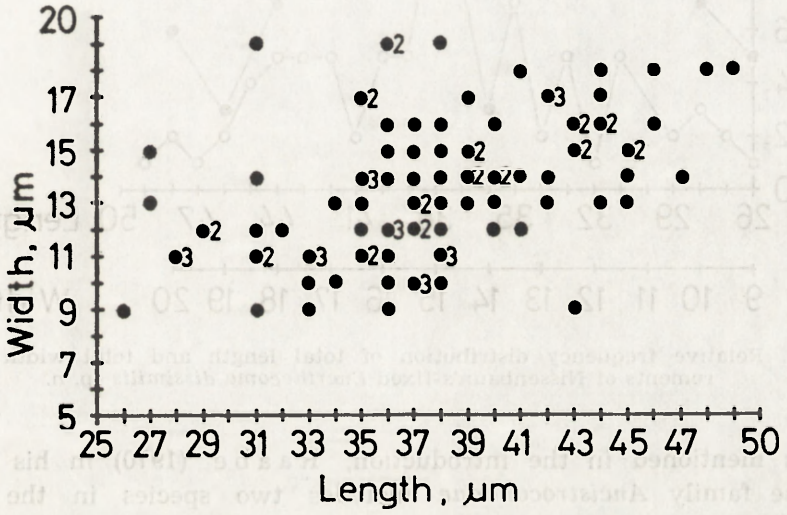

Fig. 3. Scatter diagram of the bivariate distribution of length and width of 100 individuals of *Enerthecoma dissimilis* sp. n.

stained with 1% aqueous light green. (All measurements are in µm).

The scatter diagram of the bivariate distribution of length and width of 100 individuals in µm (Fig. 3) show positive correlation ($r = 0.5133$). The shape index (length divided by width) is 1.63–4.77 µm with a mean of 2.83 µm. A relatively wide variation in length and width was observed. There are two peaks in width, 12 µm and 15 µm, and one peak in length, 36 µm (Fig. 4).

Discussion

The general features of the new ciliate which can be regarded as characteristic of the genus *Enerthecoma* Jarocki are as follows: the

strongly elongated body with a flattened thigmotactic area, kineties of equal length on flat ventral thigmotactic surface with a longitudinal elevation which runs along the thigmotactic field.

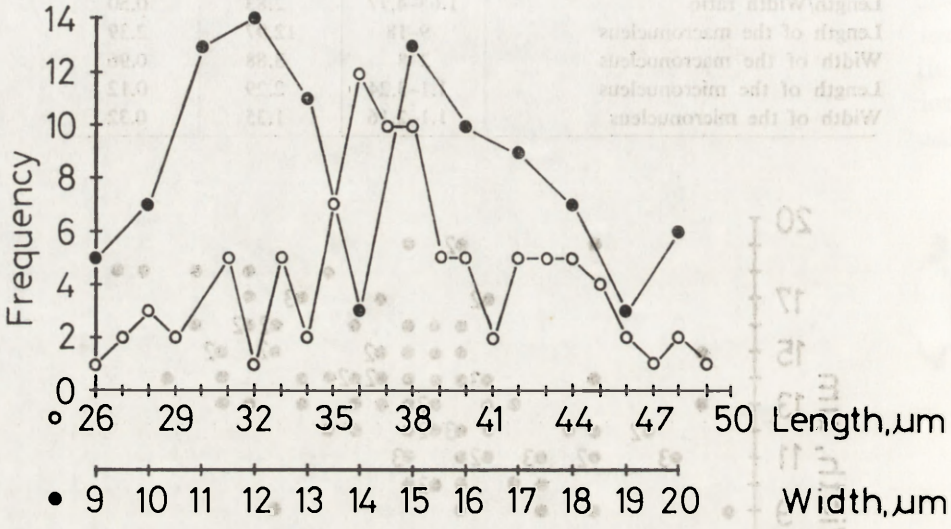

Fig. 4. Relative frequency distribution of total length and total width measurements of Nissenbaun's-fixed *Enerthecoma dissimilis* sp. n.

As mentioned in the introduction, R a a b e (1970) in his review on the family *Ancistrocomidae* includes two species in the genus *Enerthecoma* namely *E. properans* Jarocki, 1935 and *E. tegularum* Kozloff, 1946. The observed ciliary system consisting of eight symmetrical kineties of equal length forming a narrow thigmotactic area occupying about two third of the body length on the ventral surface brings new species closer to *Enerthecoma properans* Jarocki and distinguishes it from the other species of this genus i.e., *E. tegularum* Kozloff by differences in body shape and its dimensions, number of kineties and their unequal length, shape and size of the macronucleus. A comparative account of new species with the other existing species is given in Table 2.

The characteristics of the new species are the elongated symmetrical body with relatively smaller dimensions, pulsating contractile vacuole, sausage-shaped macronucleus and its variability in shape which distinguishes it from *E. properans* Jarocki, whereas the smaller size of this new ciliate recognises it as an intermediate form being smaller than *E. properans* Jarocki and larger than the *E. tegularum* Kozloff.

Table 2

Name of the species with author's name	Body shape	Length width and thickness	Number of kineties	Host	Locality
E. properans Jarocki, 1935	symmetrical, lanceolate	33–60 33–60 10–13	8	*Viviparus fasciatus*	Warszawa
E. properans Kozloff, 1946	elongated symmetrical	32–56 13–21 10–13	8	*Viviparus malleatus*	California
E. tegularum Kozloff, 1946	pyriform	26–36 12–17 9–11	9	*Tegula brunnea*	California
New species	elongated symmetrical	27–49 9–19	8	*Viviparus dissimilia*	Hyderabad

Besides the differences in the body dimensions, the variability in the shape of the macronucleus and pulsating contractile vacuole also form the characteristic feature of this ciliate. Further, the locality and host of the present species is also new. In view of these facts this ciliate is considered as new to science and since it is constantly associated with the freshwater gastropod *Viviparus dissimilis* (Müller) in Hyderabad, India, it seemed appropriate to name it *Enerthecoma dissimilis* sp. n.

Diagnosis

Body elongated, banana shaped, anterior end tapered sharply and curved in an arc posterior end its rounded. Dorsal margin convex; ventral margin slightly concave, measuring 27–49 μm × 9–19 μm; anterior end provided with a suctorial tentacle with an internal tubular canal, ciliary system is disposed on flat area occupying the anterior two third of the ventral surface. Kineties are 8 in number, equal in length, originate close to the base of the tentacle, the first five rows from the right side are usually a little more widely spaced than the next three rows; contractile vacuole in the middle, slightly towards the left side of the body and opens to the exterior on ventral surface; macronucleus typically sausage-shaped situated in the middle and placed obliquely to the longitudinal axis of the body. Size 9–19 × 2–8 μm. Semi-parasite of the mantle cavity of *Viviparus dissimilis* (Müller) from the freshwater ponds and lakes of Hyderabad, India.

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RÉSUMÉ

Un nouveau Cilié *Enerthecoma dissimilis* sp. n. est décrit chez un *Gastropode* d'eau douce *Viviparus dissimilis* (Müller) aux Indes. Il est caractérisé par l'absence de cytostome et par le developpement à sa place d'un tentacule suctorien. Le corps est oblong symétrique, en forme de banane, mésurant 27–49 × 9–19 μm (en moyenne: 37.61±0.51 × 13.58±0.27 μm). C'est la premiere découverte d'un Cilié tigmotriche chez un Gastropode d'eau douce aux Inde, et même en Asie.

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Thecamoebiens du Mesopsammon des Plages de Lacanau et Leporge-Ocean (Gironde, France)

(*Protozoa, Rhizopoda testacea*)

Received on 5 February 1980

Synopsis. Ce travail donne la description de 19 espèces de Thécamoebiens récoltés sur les plages de Lacanau et Leporge-Ocean (Gironde, France). Les auteurs décrivent aussi 6 sp. nov. et 1 gen. nov.

Les Thécamoebiens jusqu'ici considérés comme dulçaquicoles ont d'assez nombreux représentants dans les eaux salées et saumâtres (estuaires des fleuves, prés salés, zone marginales des mers et des océans). Ce n'est que depuis une dizaine d'année à peine, qu'on décèle une faune particulière vivant dans la zone littorale des côtes où s'effectue le mélange des eaux douces souterraines continentales à l'eau de mer, zone que l'on peut définir comme un substrat lacunaire et poreux, constitué par du sable baigné d'eau plus ou moins salée, ce biotope révèle une faune psammophile comprenant jusqu'a présent environ 4% des espèces connues de Thécamoebiens (Golemansky 1978).

C'est grâce aux travaux de Valkanov (1970), Golemansky (1970), Chardez (1971), Decloitre (1972) et Sudzuki (1976), que l'attention à été attirée sur ces biotopes particuliers.

L'ensemble de la faune thécamoebienne du mésopsammon comporte des espèces strictes, dont les plus nombreuses sont rassemblées dans la Famille des *Psammonobiotidae* crée par Golemansky et aux Familles: *Centropyxidae, Cyphoderiidae, Difflugiidae* et *Cryptodifflugiidae,* à ces espèces strictes, peuvent s'ajouter quelques espèces adaptées venues du continent par les eaux douces.

Matériel et méthode

La technique de prélèvement consiste a creuser un trou à une certaine distance (de 1 à 100 m) du bord de l'eau et a prélever l'eau, qui ne tarde pas a le combler.

Les échantillons sont conservés non fixés dans des flacons non remplis et bouchés, afin d'éviter l'évaporation, ce qui permet une longue conservation des microorganismes vivants.

Les études sont faites sur le vivant en contraste de phase. Une partie des prélèvements est fixé au Boin aqueux et au formol neutre en vue de conservation.

Le montage des préparations se fait après lavage à l'eau distillée, par décantations successives et centrifugation.

Des préparations types numérotées: H64, H66, H67, H68 et H69 sont déposées dans les collections de la Faculté des Sciences Agronomiques de l'Etat à Gembloux, Belgique. Des paratypes sont conservés dans les collections des auteurs.

Prélèvements

(1) Plage de Lacanau-Ocean (Gironde, France).

A — (6.8.78) maré basse à 100 m du bord de l'eau et 1 m 20 de profondeur. Quatre espèces présentes les nº 16, 6, 15 et 19.

B — (13.9.78) marée basse à 10 m du bord de l'eau et á 0.50 m de profondeur. Les pH oscillent aux environs de 7,5. Espèces présentes les nº 2, 3, 4, 5, 8, 7, 9, 10, 14, 15, 16, 17 et 19.

(1) Plage Leporge-Ocean (Gironde, France).

C — (22.7.79) marée haute à 7 m du bord de l'eau et 65 cm de profondeur. Espèces présentes les n° 1, 13, 12 et 11.

L'etude de ces prèlévements, nous a révélé 19 espèces dont 6 nouvelles pour la Science et 1 Genre nouveau.

Les symboles utilisés sont: L = longuer, l = largeur, H = hauteur, ép. = épaisseur, col. = collerette, RB = Répartition biogéographique.

Résultats

(1) *Pseudocorythion undulacollis* sp. nov. Fig 1 *1*

Diagnose: La thèque est formée de deux parties distinctes:

(1) Une collerette importante infundibuliforme aboutissant à un pseudostome circulaire.

(2) Le corps même de la thèque, allongé, terminé ou non par une pointe caudale. Section elliptique. Le revêtement est constitué d'idiosomes arrondis régulièrement rangés.

La collerette est finement ondulée sur ses bords; les dentelures arrondies dessinent un périmètre régulièrement sinueux; elles se prolongent sur la surface du col par des canelures fines, radiales

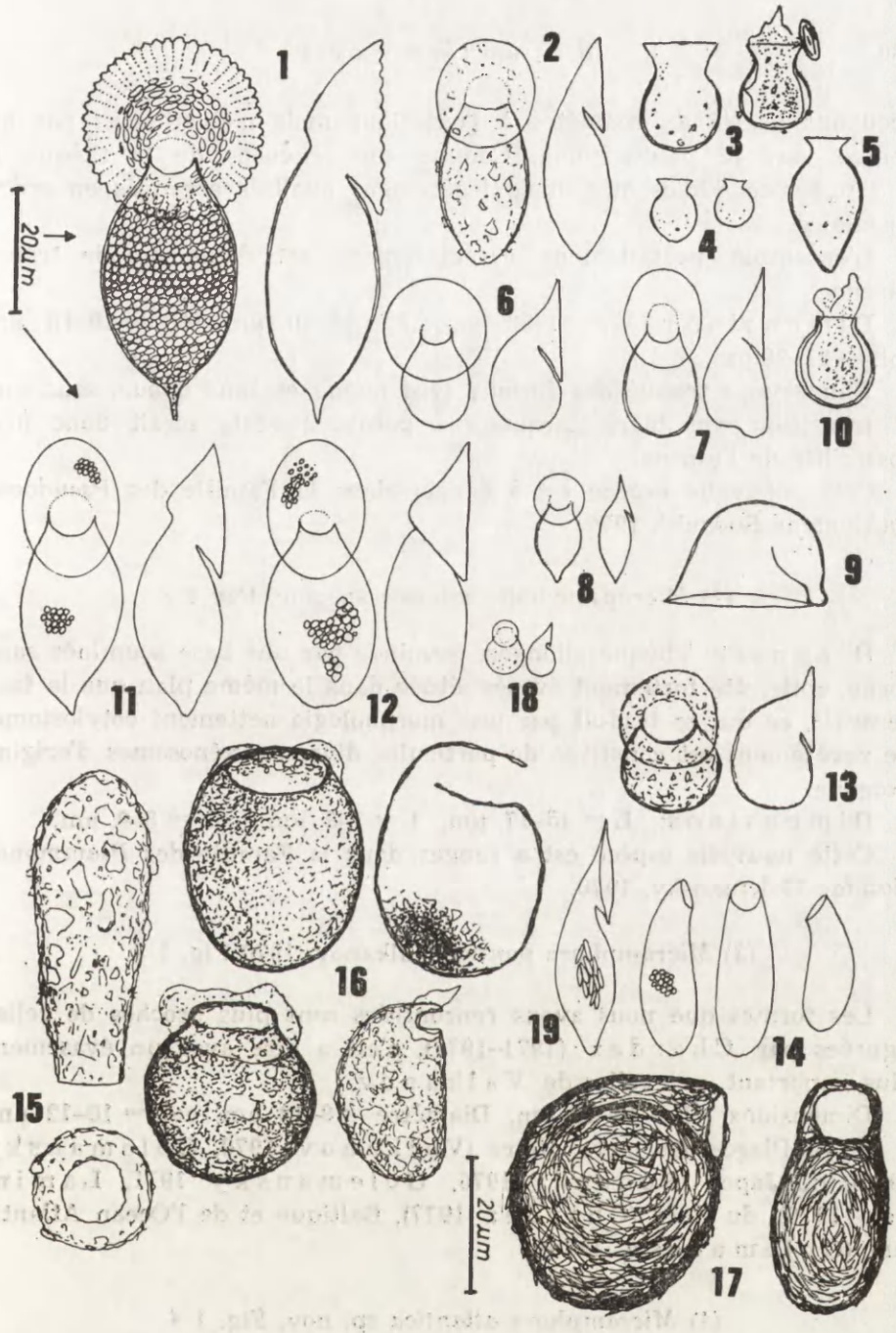

Fig. *1* — *Pseudocorythion undulacollis* sp. nov., *2* — *Micropsammella minima* sp. nov., *3* — *Micramphora pontica* Valkanov, 1970, *4* — *Micramphora atlantica* sp. nov., *5* — *Micramphora amphoriformis* sp. nov., *6* — *Psammonobiotus minutus* Golemansky, 1970, *7* — *Psammonobiotus golemanskyi* Chardez, 1977, *8* — *Psammonobiotus linearis* Golemansky, 1970, *9* — *Psammonobiotus balticus* Golemansky, 1970, *10* — *Difflugiella collum* Chardez, 1971, *11* — *Pseudocorythion wailesi* Golemansky, 1970, *12* — *Pseudocorythion wailesi* Golemansky, 1970, *13* — *Centropyxiella gibbulina* sp. nov., *14* — *Cyphoderia littoralis* Golemansky, 1973, *15* — *Difflugia submarina* nom. nov., *16* — *Rhumbleriella filosa* Golemansky, 1970, *17* — *Lesquerella mesopsammophila* sp. nov., *18* — *Psammonobiotus septentrionalis* Chardez, 1977, *19* — *Corythionella minima* Golemansky, 1970

occupant le 1/3 du diamètre à l'extérieur mais n'aboutissant pas au centre vers le pseudostome. Comme sur le corps de la thèque il y a présence d'idiosomes, mais légèrement ovalisés arrangés en ordre circulaire.

L'ensemble parfaitement caractéristique est d'une grande transparence.

Dimensions: L = 41–52 µm, l = 15–20 µm. ép. = 10–18 µm col. = 21–28 µm.

Nous avons trouvè des formes avec queue et sans queue, sans que la transition soit bien marquée, la pointe candale serait donc une possibilité de l'espèce.

Cette nouvelle espèce est à ranger dans la Famille des *Pseudocorythionidae* Sudzuki, 1979.

(2) *Micropsammella minima* sp. nov. Fig. 1 *2*

Diagnose: Thèque allongée, terminée par une base acuminée sans corne, collerette fortement évasée située dans le même plan que la face ventrale, ce qui se traduit par une morphologie nettement cotylostome. Le revêtement est constitué de particules diverses, xénosomes d'origine exogène.

Dimensions: L = 15–17 µm. l = 7–8 µm. col. = 8–9 µm.

Cette nouvelle espèce est à ranger dans la Famille des *Psammonobiotidae* Golemansky, 1970.

(3) *Micramphora pontica* Valkanov, 1970 Fig. 1 *3*

Les formes que nous avons rencontrées sont plus proches de celles figurées par Chardez (1971–1977), c'est a dire avec un évasement plus important, que celles de Valkanov.

Dimensions: H = 15–20 µm, Diamètre = 8–12 µm, col. = 10–12 µm.

R. B. Plages des mers: Noire (Valkanov 1970, Golemansky 1970); du Japon (Sudzuki 1976, Golemansky 1971, Laminger 1976), du Nord (Chardez 1977), Baltique et de l'Ocean Atlantique (Golemansky 1976).

(4) *Micramphora atlantica* sp. nov. Fig. 1 *4*

Les *Micramphora* sont relativement communes dans le psammon marin. La morphologie de la thèque est conforme aux Thécamoebiens bien que prudement Golemansky (1974) ne fait pas entrer ce Genre dans la Famille des *Psammonobiotidae*.

Il nous apparait cependant util de signaler la morphologie de ces thèques fréquentes dans nos prélèvements.

Nous avons trouvé deux formes qui ne correspondent pas aux espèces connues.

M. atlantica prend la forme d'une amphore courte, revêtement entièrement chitinoide. Cette espèce diffère de *M. pontica* par trois points: (1) plus petite, (2) pseudostome au bout d'un col étranglé, (3) fond légèrement acuminé.

Dimensions: H = 9–10 µm, Diamètre = 7–9 µm.

(5) *Micramphora amphoriformis* sp. nov. Fig 1 5

Diagnose: Thèque très allongée, la partie postérieure est fortement acuminée, structure entièrement chitinoide avec quelquefois quelques rares et fines incrustations de particules minérales.

Dimensions: H = 15–18 µm, Diamètre = 5–6 µm.

(6) *Amphorellopsis elegans* Golemansky, 1970

Les individus rencontrés dans nos récoltes correspondent bien aux descriptions données par Golemansky (1970).

Dimensions: L = 30–35 µm, l = 12–25 µm, ép. = 9–13 µm. col. = = 12–26 µm.

Chez certains spécimens, l'arrière est comprimé et se termine par une membrane mince flexible et tronquée irrégulièrement.

Comme Golemansky, nous pensons que l'animal peut vivre fixé par cette membrane sur la surface des grains de sable.

R. B. Plage des mers: Noire, du Japon, du Golf de Gdańsk (Golemansky 1970), du Nord (Chardez 1977).

(7) *Psammonobiotus golemanskyi* Chardez, 1971 Fig. 1 7

La face ventrale très bombée de la thèque, s'accorde avec une collerette placée dans le même plan, ouverture large et elliptique.

Dimensions: L = 20–30 µm, H = 10–15 µm.

R. B. Plages des mers: du Nord (Chardez 1977), du Japon (Sudzuki 1976)

(8) *Psammonobiotus minutus* Golemansky, 1970 Fig. 1 *6*

Dimensions: L = 22–30 µm, l = 10–13 µm, col. = 12–14 µm.
R. B. Plage des mers: Noire (Golemansky 1973) du Japon (Sudzuki 1976) du Nord et Méditéranée (Chardez 1971, 1977).

(9) *Psammonobiotus linearis* Golemansky, 1970 Fig. 1 *8*

Assez rare dans nos récoltes.
Dimensions: L = 16–17 µm, au lieu de 21–24 µm, signalé pour le type.
R. B. Plages des mers: Noire et Baltique (Golemansky, 1970, 1973).

(10) *Psammonobiotus balticus* Golemansky, 1973 Fig. 1 *9*

Dimensions: H = 18–20 µm, l = 20–22 µm.
R. B. Plages des mers: Baltique (Golemansky 1973) du Nord (Chardez 1977).

(11) *Psammonobiotus septentrionalis* Chardez, 1972 Fig. 1 *18*

Espèce abondante dans la récolte 2C.
Dimensions: L = 14–16 µm, l = 12–13 µm.
R. B. Plage de la mer du Nord (Chardez 1977).

(12) *Pseudocorythion wailesi* Golemansky, 1971 Fig. 1 *11* et *12*

Les nombreux exemplaires des plages de Lacanau montrent des formes très fortement acuminées mais dont la queue manque parfois. Nous pensons qu'il faut voir là une variation intraspécifique.

(13) *Centropyxiella gibbulina* sp. nov. Fig. 1 *13*

Diagnose: Cette nouvelle espèce d'allure très proche de *C. gibbula* Valkanov, 1969, a comme elle, une thèque fortement renflée. Le revêtement est constitué de fines particules pierreuse noyées dans une chitine transparente, la collerette est incrustée également de fines particules minérales.
Ce qui diffère vraiment ce sont les dimensions qui sont réduites de moitié de *C. gibbula*.

Dimensions: L = 23–24 µm, l = 17–18 µm, pseudostome = 8; 5–9 µm, col. = 19–20 µm, ép. = 14–15 µm.

Cette nouvelle espèce est à ranger dans la famille des *Centropyxidae* Deflandre, 1928.

(14) *Difflugiella collum* Chardez, 1971 Fig. 1 *10*

C'est avec un point de doute que nous rattachons à cette espèce de petites thèques d'allure semblale a la description faite par l'une de nous d'une *Difflugiella* rencontrée dans la zone marginale des eaux douces, ici le biotope diffère car il est de forte salinité. Sa caractéristique est d'être très hyaline et dépouvue de revêtement.

Dimensions: H = 10–12 µm, Diamètre = 7–8 µm, ps. = 2,5–3 µm.

(15) *Difflugia submarina* nom. nov. Fig. 1 *15*

= *Difflugia subterranea* f. *circularis* Chardez, 1971

Cette espèce ressemble bien à *D. subterranea*, mais elle n'est jamais comprimée, sa section est circulaire. Le facies général la fait ressembler à *D. lanceolata et* à *D. linearis*, mais ces espèces sont plus grandes et vivent dans le sapropèle d'eau douce. H = 50–60 µm.

(16) *Cyphoderia littoralis* Golemansky, 1973 Fig. 1 *14*

Cette espèce du psammon est très comparable à *C. ampulla*, mais avec des dimensions beaucoup plus modestes.

Dimensions: L = 40–48 µm, l = 13–18 µm, pseudostome = = 8–10 µm.

R. B. Page des mers: du Nord (Chardez 1977), du Japon (Sudzuki 1976) Baltique (Golemansky 1973).

(17) *Rhumbleriella filosa* Golemansky, 1970 Fig. 1 *16*

Cette espèce commune dans notre récolte correspond aux caractéristiques du type décrit. La vue latérale donne quelques variations: panse plus plate, pseudostome plus grand, variations mineures pour une population trouvée loin de son lieu de première découverte.

Dimensions: L = 30–38 µm, l = 25–28 µm, ép. = 30–32 µm, pseudostome = 4–13/8–18 µm.

R. B. Plages des mers: Noire (Golemansky 1970), du Nord (Chardez 1977) de l'Océan Atlantique (Decloitre 1976).

(18) *Corythionella minima* Golemansky, 1970 Fig. 1 *19*

Thèque très transparente, acuminée, légèrement plus petite que le type.

Dimensions: L = 38–42 µm, l = 15–17 µm, col. = 15–20 µm.

Nous avons observé des kystes de 10 µm de diamètre.

R. B. Plages des mers: du Japon, Caraïbe, Baltique (Golemansky 1971, 1973).

Lesquerella gen. nov.

Diagnose: Thèque enroulée en spirale, comprimée latéralement, pseudostome plus ou moins circulaire.

Ce nouveau genre proche des *Lesquereusia* par sa mophologie générale se justifie par son écologie particulière et ses pseudopodes de type "filosa". Il est à classer dans la famille des *Psammonobiotidae* Golemansky, 1973.

(19) *Lesquerella mesopsammophila* sp. nov. Fig. 1 *17*

Diagnose: Thèque comprimée latéralement, enroulée en spirale simple, le col ne dépasse pas la face ventrale, l'enroulement thécal très prononcé laisse peu de place à l'animal pour l'émission pseudopodique. Le revêtement est constitué par des vermicules très fins, enchevètrés rappelant celui de *Lesquereusia spiralis stenolepis.*

Dimensions: L = 85–90 µm, l = 71–80 µm, ép. = 40 µm, pseudostome = 18–20 µm.

Nous avons vu peu de formes de cette intéressante espèce à Lacanau, mais l'un de nous l'avait entrevue dans les plages d'Oostende en Belgique, ce qui laisse présager que cette espèce est rare, mais serait dispersée dans d'autres points géographiques.

SUMMARY

The results of investigations are presented concerning the fauna of Thecamoeba from the beach of Lacanau and Leporge-Ocean (Gironde, France). The authors described 19 species from which 6 sp. nov. and 1 gen. nov. are new.

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Eugregarine Parasites of Coleoptera from North-East Region of Karnataka. III. *Cystocephalus rhytinotus* sp. n. Found in the Gut of *Rhytinota tristis* (Kr.)

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Synopsis. Tenebrionid beetles, *Rhytinota tristis* (Kr.) and *Himatismus fasciculatus* (F.) were examined for their gut protozoa. The former revealed the presence of *Leidyana gnyanagangai* Patil and Amoji, 1979 and a new species of *Cystocephalus*. *H. fasciculatus* has been found to be a new host for *Cystocephalus devdharii* Patil and Amoji, 1979. This paper deals with morphology and biology of a new cephaline gregarine, *C. rhytinotus* sp. n.

Since the creation of the genus *Cystocephalus* (Schneider 1886) only eight species have originally been reported. These are *C. algerianus* (Schneider 1886); *Cystocephalus* sp. (Théodoridès 1961); *C. albrechti* (Théodoridès and Jolivet 1963), *C. hollandei*, *C. aethiopicus* and C. *gabei* (Théodoridès et al. 1964), *Cystocephalus* sp. (Théodoridès et al. 1965) and *C. devdharii* (Patil and Amoji 1979 a). According to Corbel (1971), *C. aethiopicus* and *C. gabei* are synonymous. In addition to these, *Cystocephalus leidyi* (Watson, 1917) Théodoridès (1954), C. *madagascariensis* (Théodoridès 1954) Corbel (1971) and *C. dromii* (Geus 1969) Corbel (1971) are transpositioned from different genera on the basis of structure of epimerite in cephalins.

Among ten cystocephalan species, the complete life-cycle is known only in three species (*C. algerianus* Schneider, *C. aethiopicus* Théodoridès et al. and *C. devdharii* Patil and Amoji).

During exploratory studies on eugregarine parasites of coleoptera from North-East Region of Karnataka we have found some gregarines infesting Tenebrionid beetles. Patil and Amoji (1979 a, b) have

reported *Cystocephalus devdharii* and *Leidyana gnyanagangai* from *Rhytinota impolita* Fairm. It was also revealed that *C. devdharii* and *Leidyana gnyanagangai* are also natural parasites in new hosts, *Himatismus fasciculatus* (F.) and *Rhytinota tristis* (Kr.) respectively. *L. gnyanagangai* cohabits in the intestine of *R. tristis* with an unknown *Cystocephalus species*. The morphology and biology of it is described in this communication. For the reasons mentioned elsewhere in this paper we have considered this gregarine as a new species.

Materials and Methods

Host specimens (*Rhytinota tristis* (Kr.) were collected from January to December 1978 around Gulbarga city. The methodology adopted for examining hosts for their gregarine parasites, for studying intracellular developmental stages and for development of gametocysts is that of Patil and Amoji (1979 a). Permanent slides of different stages of gregarine development were prepared by prefixing air dried slides either in Carnoy's fluid or in absolute alcohol and staining with iron alum haematoxylin or Delafield's haematoxylin. India ink illustrations in this paper are camera lucida drawings of unfixed fresh specimens.

Observations

Our observations over one year period has disclosed that *Rhytinota tristis* (Kr.) harbours *Leidyana gnyanagangai* Patil et Amoji from November to July with maximum infection during April to June. On the contrary, *Cystocephalus* infection persists throughout the year and maximum number of sporadins were found during October to December. However, both the gregarine species coexist in the midgut of the beetles when *Leidyana* infection prevails.

Smear preparations of different regions of the gut has revealed that cephalins occupy anterior region of the midgut followed by sporadins in the posterior region. Gametocysts are present in the posterior part of midgut and also in the hindgut region. All the stages in the life-cycle of the gregarine are completed in lumen of the beetle and there is no intracellular development at any stage. Below given is the morphology of various stages of the gregarine in development.

Cystocephalus rhytinotus sp. n.

Young cephalins (30 µm long) are oval in shape (Fig. 1 *1*, *2*). They have rounded deutomerite and hemispherical protomerite. Epimerite in the early stages is globular in shape with minute collar. Later it

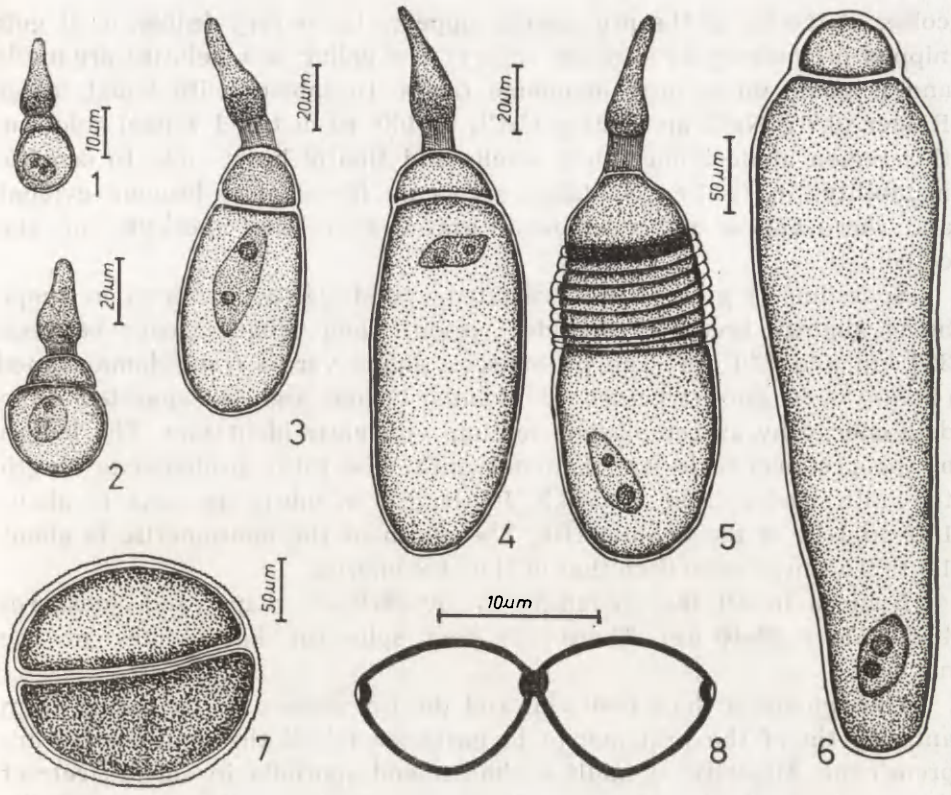

Fig. 1. *1–8* — *Cystocephalus rhytinotus* sp. n. are camera lucida drawings. *1–2* — Early cephalins, *3* — Young cephalin. Note the change in the deutomerite and nucleus, *4–5* — Adult cephalins. In Fig. 5. note retraction of protomerite and folded deutomerite, *6* — Sporont, *7* — Early stage of gametocyst, *8* — Sporocysts in chain

becomes elongated (12 µm × 8 µm) cone-shaped structure. The collar, on which the epimerite is situated, is short (3 µm long and 6 µm wide) and cylindrical in shape. Nucleus is oval (8 µm × 5 µm).

As the development progresses cephalins undergo marked change in general morphology. In young cephalins the deutomerite becomes elongated (Fig. 1 *3*) oval and the protomerite assumes globular shape. Epimerite in these forms has retained the same shape as that of early cephalins, but without increase in volume. Nucleus is elongated oval (35 µm × 15 µm).

Adult cephalins (Fig. 1 *4*, *5*) are stout bodied and measure up to 300 µm in length. The maximum length of the epimerite collar is 9 µm and the breadth 12 µm, while the conical part of the epimerite measures about 36 µm long and 15 µm broad. The attachment of the

collar at the tip of the protomerite appears to be very feable, as it gets nipped off quickly at slightest injury. The collar is a delicate organelle and is made up of only myoneme fibres. In smears with usual insect Ringer (0.7 g NaCl and 0.02 g $CaCl_2$ in 100 ml distilled water) solution, this region alone immediately swells and finally bursts due to osmotic instability. In swollen condition myoneme fibres of it become evident and there appear no endoplasmic granules in the endocyte of the collar.

Sporadins (Fig. 1 *6*) are elongated cylindrical or fusiform in shape being slightly broad at shoulder region. Their length varies between 320 μm and 540 μm. The protomerite shape varies from dome-shaped (40 μm × 60 μm) structure to globular shape and is separated from deutomerite by an ectoplasmic septum with clear identation. The length of the protomerite is less than its width. The ratio: protomerite length to width varies from 1:1.5–3.5. Its length in adult sporonts is about 1/12 to 1/18 of the deutomerite. The width of the deutomerite is about 1.5 to 2.5 times more than that of the protomerite.

Nucleus in all the sporadins is ovoidal in shape and measures 40–50 μm × 30–40 μm. There are two spherical karyosomes in the nucleus.

The epicyte is thick (5–6 μm) and the myonemes in it running from anterior tip of the protomerite to posterior tip of the deutomerite are prominent. Majority of adult cephalins and sporonts in smears retract their protomerite into the anterior region of the deutomerite and the deutomerite also contracts thus changing the body from cylindrical to oval shape. In such forms the epicyte in deutomerite is thrown into wrinkles (Fig. 1 *5*) due to the contraction of myoneme fibres.

Gametocysts (Fig. 1 *7*) are spherical in shape (200–210 μm in diameter) with tuberculated cyst wall. In moist chamber they sporulate on 4th day by simple dehiscence with the formation of oval pseudocyst. Sporocysts (Fig. 1 *8*) are hat-shaped and are released in chain while attached to each other at tips in their long axis. Mature sporocysts measure 11.25 μm × 8.75 μm.

Taxonomic Position

On the basis of characters like, simple epimerite beset on short cylindrical collar, gametocyst with tuberculated cyst wall developing with the formation of pseudocyst and hat-shaped sporocysts in chain, this gregarine is placed under the genus *Cystocephalus* Schneider (Family *Stylocephalidae* Ellis, 1912, Subfamily *Stylocephalinae* Corbel, 1971).

Among thus far known cystocephalan species, the presently described gregarine resembles, to some extent, *C. algerianus* Schneider, *C. aethiopicus* Théodoridès et al. and *C. devdharii* Patil et Amoji in possessing conical shaped epimerite. Sporonts of this species share common features with *C. aethiopicus* like being cylindrical stout bodied forms and the epicyte being thrown into folds by contraction of myonemes. Since shape and size of sporocysts are considered as most important and unchangable characters in the life of gregarine, we have compared (Table 1) with sporocysts of known species. The spores of new gregarine are longer than others. Since this gregarine possesses unique characters which are not found in other known species, we consider it a new one and propose to name *Cystocephalus rhytinotus* sp. n. The specific name signifies the occurrence of *Cystocephalus* gregarine in Tenebrionid beetles belonging to the genus *Rhytinotus*.

Table 1

Species studied	Epimerite	Sporocyst in μm
C. algerianus Schneider	Papillated sphere or enlarged conical structure	10×10.5
C. aethiopicus = *C. gabei* Théodoridès et al.	"Bonnet de nuit" or "Chapeau Japanais"	8×6
C. devdharii Patil and Amoji	Lance shaped	10×7.5
C. rhytinotus sp. n.	Elongated conical structure	11.25×8.75

Parasites: *Cystocephalus rhytinotus* sp. n.
Host: *Rhytinota tristis* (Kr.)
Locality: Gulbarga (Karnataka, India)
Site of infection: Mid-intestine
Repository: The permanent slides of type specimens of this parasite are deposited in the Microbiology Department, Post-Graduate Centre, Gulbarga

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RÉSUMÉ

On a examiné les protozoaires du contenu de l'intestin des Coléoptères *Rhytinota tristis* (Kr.) et *Himatismus fasciculatus* (F.). On a révélé chez la première espéce la présence de *Leidyana gnyanagangai* Patil et Amoji, 1979 et d'une nouvelle

espèce de *Cystocephalus*. *H. fasciculatus* s'est avéré être l'hôte nouveau pour le *Cystocephalus devdharii* Patil et Amoji, 1979. Ce travail est consacré à la morphologie et biologie de la nouvelle grégarine céphaline, *C. rhytinotus* sp. n.

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Eimeria darjeelingensis sp. n. from a House-shrew, *Suncus murinus soccatus* (Hodgson)

Received on 1 December 1979

Synopsis. Eimeria darjeelingensis sp. n. (*Sporozoa: Eimeriidae*) is described from the faecal sample of a house-shrew, *Suncus murinus soccatus* (Hodgson) collected at Darjeeling (altitude 2000 m). Trophozoites, schizonts, macro and microgamonts are found in the sections of small intestine. Oocysts are subspherical with mean dimensions of 16.7 μm by 14.9 μm. Sporocysts are ovoid with mean dimensions of 8 μm by 5.6 μm.

In course of investigation of eimeriosis of some North-Eastern Himalayan insectivores, a new species of *Eimeria* from the faeces of a house shrew, *Suncus murinus soccatus* (Hodgson) was found. Pellérdy (1974) listed eighteen valid species of *Eimeria* from insectivoran hosts and Mirza (1975) has described one more species form a hedge-hog. A preliminary note on the occurrence of *Eimeria* in *S. m. soccatus* has been published by Sinha and Sinha (1979).

Materials and Methods

Out of four shrews, trapped in Darjeeling, one was positive for coccidian oocyst in its faeces. Oocysts were kept in 2.5% potassium dichromate solution for sporulation at room temperature (13°C–16°C). Infected shrew was killed and small pieces of intestine were fixed in Carnoy's fluid, sectioned and stained with Heidenhain's iron-haematoxylin as well as with haematoxylin-eosin. Drawings were made under camera lucida.

Results

Eimeria darjeelingensis sp. n.

Type Host: *Suncus murinus soccatus* (Hodgson)
Type Locality: Darjeeling (altitude 2000 m), West Bengal, India.

Localization: Intestine.
Sporulation Time: 38–52 h.

Trophozoites (Fig. 1 *1–2*) were found in a large number in the epithelial and sub-epithelial cells of the mucosa. They are subspherical to ovoid, measuring 5.5 to 7.7 μm (mean 6.4 μm) by 5.2 to 6.3 μm (mean 5.8 μm). Cytoplasm is homogeneous and contains a sub central nucleus with a distinct nuclear membrane. Binucleated schizont (Fig. 1 *3*) measures 9.3 μm by 6.3 μm and the multinucleated one with twelve nuclei (Fig. 1 *4*) is 13.9 μm long by 11.9 μm wide.

Young macrogamont with centrally placed nucleus (Fig. 1 *5*) is ovoid, measuring 12.6 μm by 9.3 μm. A fully developed macrogamont with a sub central nucleus (Fig. 1 *6*) attains 14.8 μm by 11 μm. The young microgamont (Fig. 1 *7*) is spherical, 6.3 μm in diameter and contains several small nuclei arranged at the periphery. Mature microgamonts (Fig. 1 *8–9*) are subspherical and measure 5.6 to 11 μm (mean 8.3 μm) by 5.6 to 9 μm (mean 7 μm). They contain small comma shaped microgametes.

Oocysts (Fig. 1 *10–12*) are subspherical, measurements thirty of them are 15 to 18.7 μm (mean 16.7 μm) by 13.2 to 17.4 (mean 14.9 μm). Oocyst wall is smooth, bilayered with an uniform thickness of 0.3 μm. The distance between the two walls is 0.5 μm. Cytoplasm of unsporulated oocyst (Fig. 1 *10*) contains fine granules and at about 20 h it divides to form four sporoblasts (Fig. 1 *11*) which measure 6.6 to 7.1 μm (mean 6.9 μm) by 4.4 to 5.5 μm (mean 4.8 μm). An oocystic residuum, a micropyle and a polar granule are absent. Four sporocysts (Fig. 1 *12*) are ovoid, single layered, 0.16 μm in thickness and measure 7.1 to 8.9 μm (mean 8 μm) by 5.5 to 7 μm (mean 5.6 μm). A small stieda body and sporocystic residue in the form of refractile granules, 0.5 μm in diameter, are present. Each sporocysts develops two sporozoites measuring 4 to 5.5 μm (mean 4.4 μm) by 1.4 to 1.8 μm (mean 1.6 μm).

Diagnosis of Eimeria darjeelingensis sp. n.

Oocysts subspherical, 15.4 to 18.7 μm (mean 16.7 μm), by 13.2 to 17.8 μm (mean 14.9 μm), oocyst wall smooth, bilayered, 0.3 μm in thickness, oocystic residuum, micropyle, micropylar cap, polar body

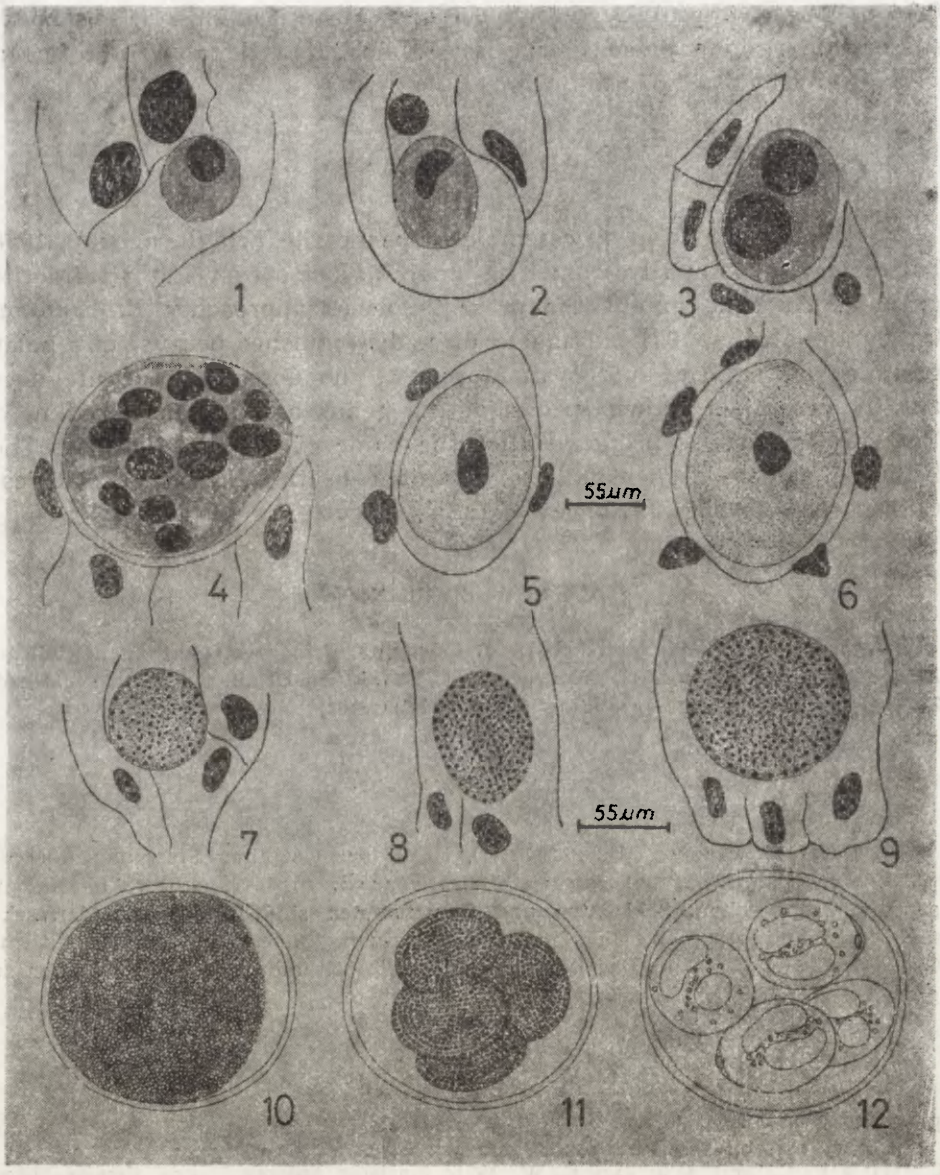

Fig. 1. *1–12* Camera lucida drawings of *Eimeria darjeelingensis* sp. n. *1–2.* Trophozoites, *3* — Binucleated schizont, *4* — Multinucleated schizont, *5* — Young macrogamont, *6* — Mature macrogamont, *7* — Young microgamont, *8–9.* Mature microgamonts, *10* — Unsporulated oocyst, *11* — Oocyst with four sporoblasts, *12* — Sporulated oocyst with four sporocysts each containing two sporozoites

(Fig. 1. 1-9. In the section of small intestine. 10-12. In the faecal contents).

absent, sporocysts ovoid, single layered, 0.16 μm in thickness, 6.9 to 8.8 μm (mean 8 μm) by 5.6 to 6.5 μm (mean 5.6 μm), stieda body, sporocystic residue present; sporozoites elongated, 4 to 5.5 μm (mean 4.45 μm) by 1.43 to 1.87 μm (mean 1.6 μm).

Discussion

E. darjeelingensis sp. n. resembles superficially *E. milleri* Bray, 1958 but differs substantially from it in small size of oocyst and a refractile granule. To some extent the present species approaches *E. bentongi* Colley and Mullin, 1971 but again can be distinguished because of a polar granule and a large sporocyst. Moreover, the described parasite does not fit any other known species of the genus and is considered new. The species name is proposed after the name of the type locality. The holotype and paratypes will be deposited to the National Zoological Collection of Zoological Survey of India, Calcutta.

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ZUSAMMENFASSUNG

In Darjeeling, Indien (Höhe: 2000 M), wurde der Kot einer Spitzmaus (*Suncus murinus soccatus* (*Hodgson*), *Eimeria darjeelingenesis* sp. n. (*Sporoza: Eimeriidae*), geprüft. Trophozoiten, Schizonten und Gametozysten sind in der Dünndarmsektion fostgestellt worden. Kugelformögen Oozysten haben die durchschnitliche Grösse von 16.7 × 14.9 μm, Sporozysten sind eiförmig und etwa 8 × 5.6 μm gross. gross.

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