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L. Kuźnicki.

Integration of Oral Structures in cdaA1 Mutant of *Tetrahymena thermophila*

Janina KACZANOWSKA

Institute of Zoology, Warsaw University, 00-927 Warszawa, Poland

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Synopsis. At restrictive temperature the cdaA1 (cell division arrest) mutant of *Tetrahymena thermophila* performs cyclic initiations of stomatogenesis despite of a mutational block to cytokinesis. After the first round of the cell cycle, two oral apparatuses (OA1 and OA2) are completed, and after the second cycle, either one or two oral sets are added. Some of the old and new oral sets are clustered, which offers an opportunity for studying the interaction of two oral sets — formed during different rounds of cell-cycles. Two clustered anterior (A) oral sets, or two or three clustered posterior (P) oral sets, in which at least one OA was produced during an earlier round of cell—cycle, eventually fuse. The fused sets at the A and P locations represent the common territory filled with variably oriented remnants of membranelles of apparently different oral sets. This type of fused oral apparatus may even arise when one constituting set of oral structures has been rotated in situ upside down prior to the fusion. These results are consistent with the idea of extrinsic factor delineating of the common territory where oral structures derived from different sets are pushed into and stacked against the outline boundary surrounding this territory. The fused A and P oral structures appear transiently followed by the appearance of a new type of enlarged oral territory probably replacing A and P fused oral sets.

The origin of variability of defective patterns of stomatogenesis in cdaA1, versus the invariant course of this process in wild type *Tetrahymena* is briefly discussed.

The organization of the oral apparatus (OA) in *Tetrahymena thermophila* and the course of development of oral structures (stomatogenesis) are remarkably complex and strictly invariant in wild type cells (Williams and Bakowska 1982. Bakowska et al. 1982 a) and provide a good reference for genetically provoked variation. The mature OA is usually relatively stable, although it partially regresses and re-

forms in situ during each cell division (Bakowska et al. 1982 b).

Oral development can occur in *Tetrahymena* without cell division and is known as an oral replacement. During oral replacement, the new complete OA is formed at the site occupied by the preceding OA, which is gradually resorbed. The oral primordium (OP) is formed in part near the anterior end of the right postoral ciliary row (the same ciliary row as in division) and in part from the disaggregation of some part of the old OA (Frankel 1969, 1970, Kaczanowski 1976, Nelsen 1981). A similar, but not identical succession of OAs is also observed during conjugation (Tsunemoto et al. 1988).

The processes of interaction and fusion of oral structures concurently formed during cell division have been observed in bcd mutant of *Tetrahymena thermophila*. In this mutant prior to cell division many oral primordia (OPs) may simultaneously appear in a number of adjacent ciliary rows. At the later stage of development, extra OPs are either resorbed, or multiple adjacent OPs may be completely or partially integrated into a single enlarged OA (Cole et al. 1987, 1988).

The cdaA1 mutant of *Tetrahymena thermophila* (Frankel et al. 1976) offers an opportunity to follow how preformed OAs may interact with newly produced OPs. Thermosensitive cdaA1 mutant at the restrictive temperature above 35°C develop new OPs at the normal time and position, but fission line and cytokinesis are prevented. Despite the prevention of cell division, a second generation of OPs is later initiated in sites anterior and/or posterior to the now completed OA of the first arrested generation (Frankel et al. 1977). In undivided specimens the micronuclear mitoses, macronuclear sDNA and related initiation of stomatogenesis may be repeated three to five times (Frankel et al. 1976) but the number of separate oral structures rarely increases above four. Thus secondary regression, fusion and in situ reformation of oral structures take place on the ventral surface of specimens, creating an array of morphologic variants with increasingly accumulated abnormalities of patterns. Among these different cdaA1 specimens two spatial configurations of oral structures within one specimen are informative in respect to instances of interaction of preformed OA (i.e. OA that have been formed in previous morphogenesis of undivided specimen) and oral structures of the subsequent generations. The temporal sequence of appearance of abnormal patterns of oral apparatuses strongly suggests that individual oral apparatuses formed during different cell cycles are able to fuse altogether. These fused oral sets appear transiently followed by the appearance of the new type of enlarged oral territory with the single (although also usually abnormal) oral set.

Material and Methods

Tetrahymena thermophila CU 399 Chx/Chx (cy sens. mt VI) as a wild type, and strain B cdaA1 IA 104 (mt II) homozygous for cdaA1 and cdaA1 WU 60 line (mit IV) heterozygote with assorted cdaA1 (Gaertig et al. 1987) were routinely grown in PPY (Nelsen et al. 1981).

The following protocol was applied for observation of cortical development during expression of the cdaA mutation: Erhlenmeyer flasks 300 ml with 50 ml of 1% PPY supplemented with iron and antibiotics (Nelsen et al. 1981) were inoculated with the mutant strain (IA 104, or WU 60) grown at permissive temp (30°C) and incubated overnight at this temperature to yield densities of about 1000 cells per ml. Then one set of flasks was shifted to nonpermissive temp (36°C) while another one served as control of normal cell growth at 30°C. Experimental flasks were temporarily sampled for protargol staining at the moment of transfer to high temperature and then at successive hourly intervals during heating. This experiment was repeated twice with IA 104 for 5 h, and twice with WU 60 cells for 12 h.

Protargol staining followed Ng and Nelsen (1977) protocol with a bleaching modification (Kaczanowska et al. in prep). Cells were counted and photographed with a Zeiss NFPK microscope with the varioautomat camera.

Results

Stomatogenesis of wild type and cdaA1 at permissive temperature (30°C) and at restrictive temperature (36°C)

cdaA1 and wild type cells kept at the permissive temperature display typical morphology of OA and an invariant course of stomatogenesis (Frankel et al. 1976; Kaczanowska et al., in prep). The parental oral apparatus (OA1) is situated near the anterior and of the cell. Two ciliary rows terminate at its posterior margin and are designated as postoral ciliary rows. This OA is composed of four compound ciliary structures (UM and M1, M2 and M3 as designed in parental OA1 in the specimen presented in Fig. 1 a). The crescent-shaped undulating membrane (UM) outlines the right and posterior margins of oval OA territory. There are three diagonally oriented nearly parallel membranelles: the longest anterior one (M 1) defines the anterior left outline of the oral territory. Posterior to it and roughly parallel is M2, and the shortest and most posterior is M3. All M1, M2 and M3 are composed of three rows of basal bodies and all of them display some characteristic sculpture due to partial ciliary and basal body regression and displacement at the right ends designated as the A-ends (Frankel et al. 1984, Frankel 1989). This specificity is usually discernible after protargol staining. This complex of UM and M1, M2 and M3,

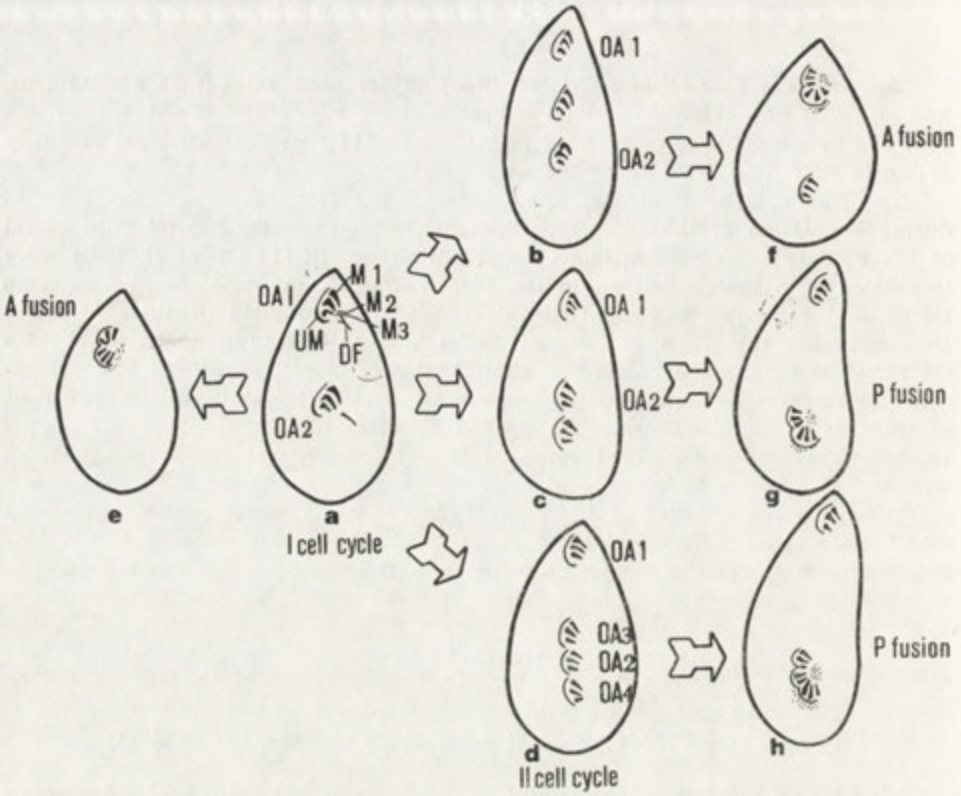

Fig. 1. Scheme of developmental pathways of morphological variants of specimens of cdaA1 *Tetrahymena thermophila* grown at restrictive temperature (36°C) (a) Specimen after first stomatogenesis: in anterior oral apparatus (OA1) elements of tetrahymenium: UM and M1, M2 and M3, and deep fibre (DF) are marked, the posterior OA2 manifests all normally patterned oral elements, (b) and (c) after second cell cycle round only one new oral apparatus may be added either anterior or posterior to OA2 yielding specimens with three oral sets, (d) after second cell cycle round also two oral apparatus may be added yielding specimens with four oral sets whose numbering may be precisely specified as an axial sequence of oral sets: OA1, OA3, OA2 and OA4. (e) specimen performing anterior fusion (A fusion) of OA1 and OA2 after first cycle performed at restrictive temperature, (f) specimen performing anterior fusion (A fusion) after second cell cycle round, (g, h) specimens performing posterior fusion (P fusion) after second cell cycle originating either from specimen with three oral sets (g) or with the four oral sets (h).

All remaining figures represent protargol stained specimens. Magn. 1450. Left-right orientation of structures was alway deduced from somatic ciliature markers: longitudinal bundles of microtubules (lms) always located to the right to adjacent ciliary rows, and tranversal fibres to the left from them (even if they are often out of focus at photomicrographs). Left-right convention is observed assuming a viewer being inside the specimen and looking out (Frankel 1989)

known as tetrahymenium forms a buccal cavity with posteriorly and leftward directed deep fibre bundles (DFs in Fig 1a).

Prior to cell division, a new oral apparatus develops posterior to the presumptive fission zone, and to the left of the right post-oral ciliary row. Oral primordium (OP) develops by a local process of basal body proliferation, forming an anarchic field of basal bodies. Within this field, in the antero-posterior direction, the membranelles become organized by ordering and addition of basal bodies. The UM is organized later starting from the anterior right border of anarchic field and extending to the posterior margin of it. The secondary shortening of M1—3, resorption of some extra basal bodies within the membranelles and within the oral field, sculpturing of right A-ends of membranelles, formation of buccal cavity provided with deep fibre and resorption of surplus basal bodies at the posterior end of UM are the final set of events completing formation of OA2 for posterior daughter (B a k o w s k a et al. 1982 b). Whereas this late set of events of stomatogenesis starts in OP, the parental oral apparatus (OA1) manifests the loss of its deep fibre and the buccal cavity of OA1 becomes shallow. These partial structural regression of OA1 is transient. During cytokinesis, OA1 and forming OA2 parallely finish formation of buccal cavities and deep fibres at the same time (B a k o w s k a et al. 1982 b).

The same normal process of stomatogenesis was also found in cdaA1 cells kept at restrictive temperature 36°C and their first unsuccessful cell division yielded specimens with two OAs (respectively OA1 and OA2) provided with two deep fibres (Fig. 1 a). During the subsequent second round of cell cycle performed at restrictive temperature either one or two new OPs are added yielding specimens with a total number of three or four oral sets (Tables 1 and 2, respective columns indicating 3 and 4 oral sets, Fig. 1 b, c, d and Pl. I 2, 3, 4). OAs produced during the second cell cycle at restrictive temperature may be either provided with shortened deep fibre (Pl. I 2; DF in "OA3") or this fibre may be absent. During this second cell cycle, the old and newly produced OAs very often manifest the shallowing of buccal cavity provided with the flatly exposed membranelles (Pl. I 3).

In specimens cdaA1 grown at restrictive temperature during a period corresponding to two rounds of stomatogeneses (i.e. no more than 5h), the apical OA1 is definitely the parental one, whereas in specimens with three oral sets it is uncertain which set corresponds to the OA2 and which to OA3. This doubt is diagramatically represented in alternative between Fig. 1b and Fig 1c. Therefore the OA2 and OA3 legends in Pl. I 2 are provided with quotation marks, and numbering of OAs in Pl. I 3 is omitted.

In specimens with four sets of oral structures the sequence of their

Table 1

Frequency distribution of fractions (in %) of IA 104 specimens with: indicated total number of the separated oral sets, the anterior (A) and posterior (P) fused oral structures, the enlarged oral territory (EOT), monsters (M), phenotypes manifesting regression of the oral structures (REGR) tested in samples kept at permissive temperature (30°) (control) and after sequent hours of incubation at restrictive temperature (36°C). n — 200-300 specimens. * — phenotypes of abnormal morphology

Period of incubation	None 0 oral set	1 oral set	2 oral sets	A fused oral set	3 oral sets	P fused oral set	4 oral sets	More than 4 oral sets	EOT	M	REGR
Control 0		66.5	33.5								
1		40.0	55.0	3.0	1.5						0.5
2		25.0	71.5	1.0	2.5						
3		5.5	47.0	5.0	13.5	4.5	15.5	1	0.5	7.5	
4	0.5	4.0	20.0	8.5	18.0	5.0	14.5	1	3.5	16.5	8.5
5	1.0	4.5*	9.0	4.5	21.0	3.5	16.5	2	5.5	25.0	7.5

Table 2

Frequency distribution of fractions (in %) of WU 60 specimens with: indicated total number of the separated oral sets, the anterior (A) and posterior (P) fused oral structures, the enlarged oral territory (EOT), monsters (M), phenotypes manifesting regression of the oral structures (REGR) tested in samples kept at permissive temperature (30°C) (control) and after sequent hours of incubation at restrictive temperature (36°C). n — 200–300 specimens. * — phenotypes of abnormal morphology

Period of incubation	None 0 oral set	1 oral set	2 oral sets	A fused oral set	3 oral sets	P fused oral set	4 oral sets	More than 4 oral sets	EOT	M	REGR
Control 0		76.0	24.0								
1		71.0	28.0								1.0
2		56.0	40.5	1.0	2.5						
3		42.5	52.5	1.0	3.0		1.0				
4	1.0	18.5	74.5	1.0	2.0	1.0	2.0				
5	0.5	9.5	74.0	1.0	7.5	1.0	5.0			1.5	
6	2.0	7.0	47.5	2.0	28.5	2.0	2.0			9.0	
7	3.0	9.0	40.0	3.0	8.5	3.5	10.0			23.0	
8	1.0	8.0*	36.0	1.0	7.0	7.0	3.0	4.0	3.0	30.0	
10	2.5	10.0*	24.0	3.5	6.0	3.5	5.0		3.0	42.5	
12	1.0	1.0	10.0		7.0		0.5		13.0	62.5	5.0

formations may be clearly deduced: the apical OA1 is the oldest structure, then the third oral set corresponds to OA2 and the second and fourth oral sets are oral structures of the newest generation (Fig. 1 d, and Pl. I 4; OP and OA4). These oral sets very often reveal some abnormalities, such as increased numbers of membranelles (for instance in OP on Pl. I 4), their variable sizes (for instance the small size of OP on Pl. I 4)), tendency for persistence of remnant basal bodies within the oral territories (Pl. I 4; OP arrowhead), or posterior to UM (Pl. I 4; arrow), and again shallowing of buccal cavities and shortening or absence of deep fibres. Hence it is concluded that in cdaA1 grown at restrictive temperature stomatogenesis may appear repeatedly but controls of the number of new OPs and controls over final patterning of oral apparatuses are greatly relaxed.

It has been observed that spatial deployment of oral sets also becomes also irregular. While usually OA2 appears in about mid distance between OA1 and posterior pole of the cell, in some instances OA2 develops in the vicinity of OA1 (Pl. I 5). Appearance of OP very close behind OA1 was also disclosed in some specimens. Therefore it is assumed that the pattern of OA2 in Pl. I 5 results rather from interaction between the old OA1 and nearby forming OA2 than from their secondary displacement. In specimen presented in Pl. I 5 OA1 demonstrates the normal pattern of oral structures; the OA2 also keeps the normal left-right organization of UM and membranelles, but the OA2 oral territory apposed to OA1 becomes more slender at its anterior part. This change of shape is correlated with the changes of lengths of membranelles: the M1 is the shortest and M3 the longest membranelles, with M2 of the intermediate length.

Much more frequent irregularity of deployment of oral structures is observed during and/or after the second round of stomatogenesis. In specimens with three or four oral sets, the new OPs and subsequently new OAs appear just anterior, and/or just posterior to the OA2 (Figs 1 c, d; Pl. II 6). Such clustering of oral sets one after another in the area of "would be" fission line in cdaA1 has been described by Frankel et al. (1977). Pl. II 6 represents the cdaA1 specimen with three clustered oral apparatuses. Individuality of each of the sets is marked by three UMs (arrows). Fusion of M3 from the first oral set with M3 of the second set (i.e. a derivative of OA2) and upward orientation of membranelles of the third set are the major disturbances in patterning of these fusing oral sets. Most intriguing is the appearance of an additional structure (marked with ?) at the extreme left border of this field. Directions and stacking of remaining membranelles also suggest that all three sets tend to fuse.

Generally speaking there are two spatial configurations of oral sets

where interaction of previously formed oral apparatus with the newly formed one(s) may be discerned: this is in the case of interactions of OA1 with the oral set located just after it, and in the case of clustered OA2 with oral sets located in its vicinity. If specimens presented respectively in Pl. I 5 for first configuration and Pl. II 6 for the latter represent only slight disturbances within individual oral sets in many other instances such distinction of individuality of oral components is not possible. Such patterns may only be described as the fused oral sets with no detailed specifications of origin of particular components. The fused oral set appearing in the anterior part of specimen will be classified to the A (anterior) fusion; the fused oral sets located within the posterior part of specimen then corresponds to the P (posterior) fusion (respectively Fig. 1 e, f and 1 g, h).

The anterior and posterior fusion of oral apparatuses in cdaA1 specimens grown at 35°C

The fusion of oral sets may be localized either in the anterior part of the cell (the A fusion) or in its posterior part (the P fusion). The anterior fusion may appear either after the first stomatogenesis performed at a restrictive temperature (Fig. 1 e; Pl. II 7) or after the second cycle (Fig. 1 f). Posterior fusion appears later i.e. after second cycle (Pl. II 8). In both cases presented in Pl. II 7 and 8 the fused oral territory takes the shape of a round shallow pocket where the fragmented membranelles are radially stacked and many dispersed basal bodies persist within and outside this pocket. The UMs might be only partially differentiated and then they mark the edge of this pocket. The posterior fusion in Pl. II 8 involves short the apparently three-rowed membranelles stacked on the margin of the united roughly round oral territory. These membranelles do not display sculpturing indicating their specificity. Anteriorly located UM is short and the anarchic field of basal bodies persists on the right part of this territory, whereas UM is very short and uncompleted. Such fused oral apparatus, although greatly abnormal, is able to complete stomatogenesis as evidenced by cases of appearance of fused oral apparatuses with two or three deep fibres radiating from the bottom of the single shallow pocket (Pl. II 9). Appearance of the fused oral set may precede general deformation of the shape and pattern of ciliary rows, nevertheless gradually with the elapse of time more and more specimens become monsters. In mosters fused oral sets are also sometimes observed; but it cannot be established whether they originate from the primary clustering of old and new oral sets, or from their secondary displacements.

Fusion of oral structures of different orientations in respect to main body axis

Nelsen et al. (1989) have analyzed stomatogenesis in left-handed (mirror-image of left-right symmetry), non-genically produced phenotypes of *Tetrahymena thermophila*. They found that most of the oral apparatuses produced during division of such phenotypes tend to be rotational permutations of the normal OAs.

Such permutations were also observed in cdaA1 specimens undergoing fusion. In Pl. III 10 the cell with four oral structures demonstrates the third oral structure provided with normally oriented tetrahymenium. Nevertheless the three—rowed membranelles are significantly shortened, and oral territory is flattened and diminished in width. This oral apparatus is the derivative of the partially regressed OA2. The fourth oral apparatus again represents a normal pattern of tetrahymenium but rotated through approximately 180 degrees. This oral apparatus is also provided with the shortened membranelles, the flat buccal cavity and manifests decreased width of oral territory. Both oral apparatuses that are in reverse antero-posterior orientations (marked with arrows) display UM- to -UM reverse apposition of structures.

In Pl. III 11 the normally oriented tetrahymenium is fused with another oral set apposed to its UM. The latter set is provided with its own very short and partially regressed UM, whereas the shortened membranelles are shifted in such a way that their sculptured A-ends are apposed to UM of the former set (thus A-ends of membranelles of both sets are finally arranged in roughly mirror image symmetry in respect to UM). Membranelles of both sets are shortened at their B ends (i.e. ends opposite to A-ends).

In Pl. III 12 the posterior fused oral set also involves elements of different orientations in respect to the main body axis. The left margin of the oral territory is occupied by two shortened membranelles normally oriented. Posterior to them there are four membranelles with their sculptured ends directed to the left margin of the oral territory. The right part of this territory is filled with basal bodies, some of which are ciliated. Posterior to this fusing oral apparatus there is another normally oriented set of oral structures.

All presented cases of the fusion either of parallely aligned oral sets (Pl. II 6—9), or the fused oral sets marking variable orientations of A ends of individual membranelles (Pl. III 10—12) share some common features: they tend to occupy a single oval territory and membranelles are trimmed and more or less radially oriented within this territory.

Appearance of the single oral primodia differentiating subsets of membranelles (EOTs)

New type of oral apparatus may appear after the second and next rounds of cell cycles. This oral apparatus starts with the enlarged anarchic field and is usually observed in more or less deformed cells. Within this enlarged oral primordium the simultaneuos differentiation of subsets of short aligned membranelles is observed. The subsets may demonstrate different orientations of their A-ends one to others but they are flanked with the single UM (Pl. III 13). The appearance of single UM and the initial concurrent differentiation of subsets of membranelles proves that this type of oral apparatus represents rather the primary subsegmentation within the original primordial anarchic field than the secondary fusion of preexisting and newly produced oral sets. Such oral apparatuses are referred to here as an oral apparatus with the enlarged oral territory or EOT. Instances of the appearance of the enlarged OP developing into normal pattern of tetrahymenium are noted even during second cell cycle (ex. of the enlarged OA3 in Pl. III 11) and in some monsters (not shown). Thus within the enlarged oral primordium either enlarged tetrahymenium or fragmented subsets of membranelles may differentiate.

Kinetics of appearance of classes of specimens with different number and organization of oral apparatuses

The frequency distribution of fractions of cdaA1 specimens with indicated number of separate sites of oral structures during subsequent hours of heating is presented in Table 1 for IA 104 and Table 2 for WU 60. The Tables also separately indicate fractions of specimens with A and P fused oral structures, and monsters in which recognition of sequence of appearance of oral sets become uncertain. Irregular monsters appeared more readily in IA 104 and they predominated after 5 h of heating. WU 60 cells less readily transformed into monsters; therefore frequency distribution of fractions was followed during 12 h (Table 2). The specimens with anterior or posterior fused oral sets completely disappeared after 10 h of heating (in WU 60, Table 2). Specimens with EOT appeared after 3 h of heating of IA 104 and after 8 h of heating of WU 60. Such enlarged oral fields, filled with many fragmented membranelles, may appear at anterior and posterior locations within specimens. A fraction of specimens with the enlarged oral territories (EOTs) was observed in monsters of both strains even after 24 h of incubation (result not shown). In Tables 1 and 2 at least two other types of va-

riants were also detected that did not fit into other classes. These are specimens with regressing oral structures (REGR) and specimens totally mouthless. Their origin is analyzed elsewhere (Kaczanowska et al. in prep).

Frequency distribution of fractions indicated in Tables 1 and 2 are consistent with the assumption that fusion of oral sets appears either after the first or the second round of stomatogenesis. Although similar groups of morphologic variants appear in WU 60 and IA 104 strains, the specimens with EOTs, or specimens demonstrating regressing oral structures (REGR) and irregular monsters appear more readily in IA 104 than in WU 60 strains.

Discussion

Data presented here confirm that cdaA1 kept at restrictive temperature performs repeatedly sequences of stomatogenesis (Frankel et al. 1976). During the first and second round of cell cycle performed at restrictive temperature, the preformed OAs and actually formed OPs may sometimes reciprocally interact with one another if they are clustered either at anterior or at posterior locations within cells. In this experimental condition the fusion of oral apparatus is followed. In specimens with more than 4 oral sets (Tables 1 and 2) the appearance of the secondary subdivision of some single enlarged oral primordium cannot be excluded. Therefore only within limits of two cell cycles and in specimens manifesting two specific spatial configurations of oral sets such an eventuality is not expected to occur. Integration and fusion of separate oral sets in cdaA1 cells are realized by partial dedifferentiation of preformed OAs and development of new OPs. It is important to note that the partial dedifferentiation of old OA is a part of normal developmental program of divisional morphogenesis and is followed by a final its re-differentiation and stabilization. In cdaA1 phenotypes the dedifferentiation of old OAs is normal but it is not accomplished by a proper run of re-differentiation. Then it is suggested that oral structures of different origins are pushed forcibly into a single oval territory, even violating the orientation and stacking of particular components. Membranelles are usually trimmed and radially oriented to the centre of the united oral territory.

The manifested variability of patterns of fused oral sets and appearance of specimens with the enlarged oral territories in cdaA1 specimens performing stomatogenesis at restrictive temperature suggest that

their final morphologies are incidental results of some mutation-dependant more general developmental defect in addition to the suppression of fission line formation and proper deposition of some specific protein (Ohba et al. 1986). The nature of this defect remains unknown. It may only be speculated that in *Tetrahymena* some extrinsic structural factor operating within the cortex may be involved in recruitment and scaffolding of oral territories during late stomatogenesis in addition to mechanism involved in fission line formation. If in cdaA1 mutant pattern of scaffolding operating at the end of stomatogenesis is relaxed it may either happen that newly recruited oral territory may involve a previously formed oral set and newly produced oral primordia, creating per se mechanisms of their fusion, and/or this newly recruited oral territory is not focused enough to maintain integral development of a single tetrahymenium (instances of EOTs). Such an assumption is consistent with the model of pattern formation proposed for mp mutant of *Tetrahymena thermophila* (Kaczanowski 1976) and is compatible with other suggestions of pattern regulation during stomatogenesis in *Tetrahymena* (Cole et al. 1988, Frankel 1989).

In both strains of cdaA1, the morphologies of fusing oral structures and appearance of the enlarged oral territory were observed; nevertheless with the background of homozygosity of cdaA1 alleles in IA 104 these transformations were produced much more readily than at the heterozygous background of WU 60.

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EXPLANATION OF PLATES I—III

2: Specimen with three oral apparatuses (OAs) provided with deep fibres (DF). Apical parental OA1 is followed with next OAs. The sequence of appearance of next OAs in this specimen remains unknown and their numbers therefore are marked in with OAs in inverted commas. The "OA3" is provided with a shortened deep fibra. IA 104 cell after 4 h at 36°C

3: Specimen with three oral structures. Both posterior oral sets are at the same developmental stage, despite one of them being previously completed OA2. Note the supplementary basal bodies posterior to UMs (arrows) in the second and third OAs. IA 104 after 4 h at 36°C

4: Specimen with four separate oral sets. The third one is an OA2 with shallowed buccal cavity and supplementary basal bodies located posterior to UM (arrowhead). The second oral set of diminished size is irregular with four membranelles and with disorganized field of basal bodies at the place of UM. The posterior oral set is located just posterior to OA2. WU 60 h at 36°C

5: Specimen with two anteriorly clustered OAs after first cell cycle performed at restrictive temperature. Respective OA1 and OA2 show the proper left-right orientation of tetrahymenia. The pattern of OA2 becomes a mirror image of OA1 pattern if considered in respect to the horizontal plane of symmetry. IA 104 cell after 3 h at 36°C

6: Specimen with three clustered OAs marked with three separate UMs (arrows). Note the fusion of two M3 from different sets and an abnormal orientation of membranelles of posterior set. The ? marks an additional structure of unknown origin. WU 60 6 h at 36°C

7: Specimen with (A) anterior configuration of fusing oral structures. IA 104 after 4 h at 36°C

8: Specimen with (P) posterior fused oral apparatus with abnormally oriented membranelles. Shortened remnant of UM and field of basal bodies occupy the right part of the oral territory, whereas the multiple membranelles are stacked at the left margin of it IA 104 5 h at 36°C

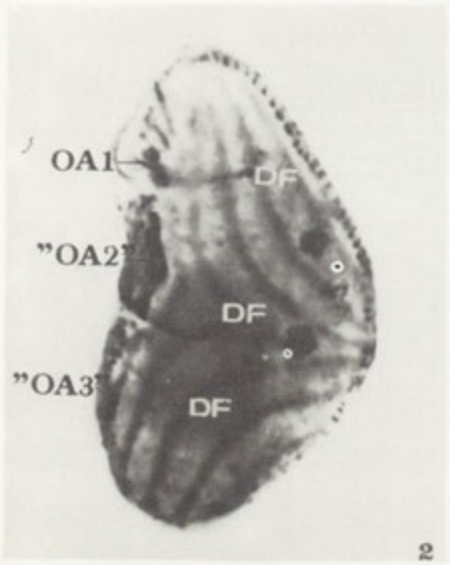
9: Specimen with (P) posterior fusion of oral apparatuses. Identities of separate oral components marked with two independent deep fibres (DFs). WU 60 8 h at 36°C

10: Specimen with four separate OAs. The third and fourth OAs display UMs and shortened membranelles. Their antero-posterior orientations are marked with ciliated). IA 104 4 h at 36°C

11: Specimen with four oral structures. Note the increased size of the second OA. The third and fourth sets are fusing. The third set (OA2) is a normally antero-posteriorly oriented OA with somewhat regressing UM. Another oral set is located just to the right to this tetrahymenium and apposed to its UM. In this highly disorganized set the UM is paralleled with differently oriented and short membranelles and all these elements are together stacked against the UM of the another set. IA 4 h at 36°C

12: Posterior (P) fused oral territory of the OA2 fused with anteriorly located oral primordium (OP) of next cell cycle. Membranelles of these two sets manifest different orientations. All these elements are apposed to the left margin of this territory. Right part of this territory occupied with basal bodies (some of them ciliated). IA 104 4 h at 36°C

13: Specimen with a posteriorly located enlarged oral territory (EOT). Single UM flanks the enlarged single territory composed of subsets of differently oriented groups of short membranelles. These groups are stacked at the right angle each to other, with no sign of tendency to radial rearrangement. WU 60 12 h at 36°C



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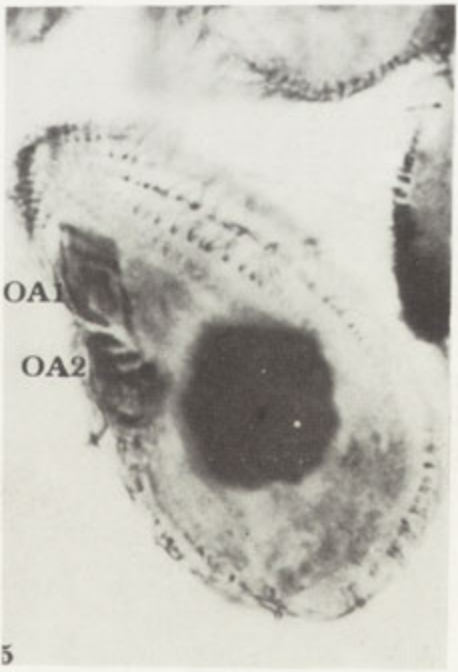

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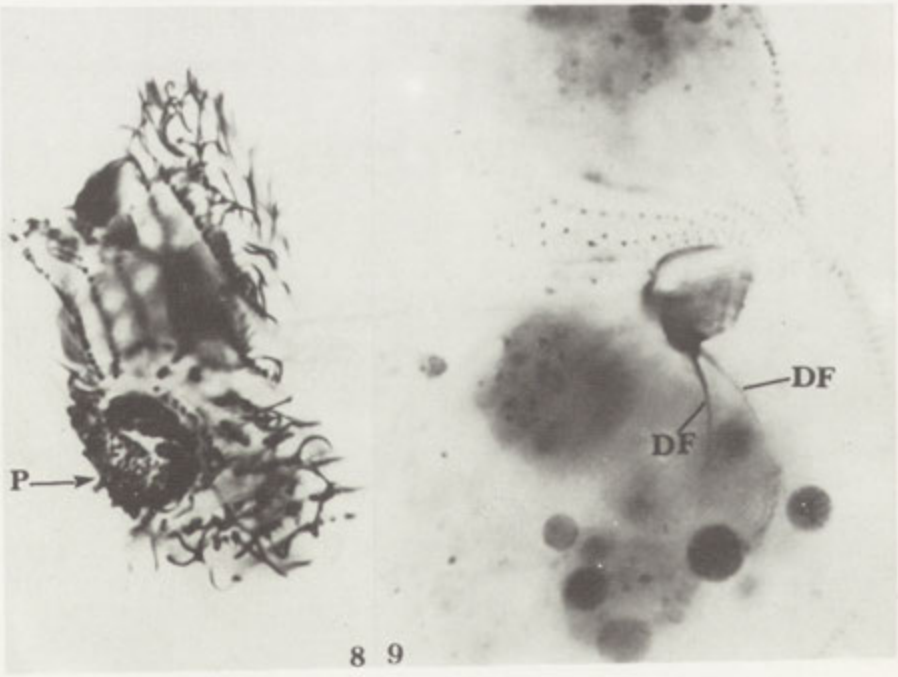

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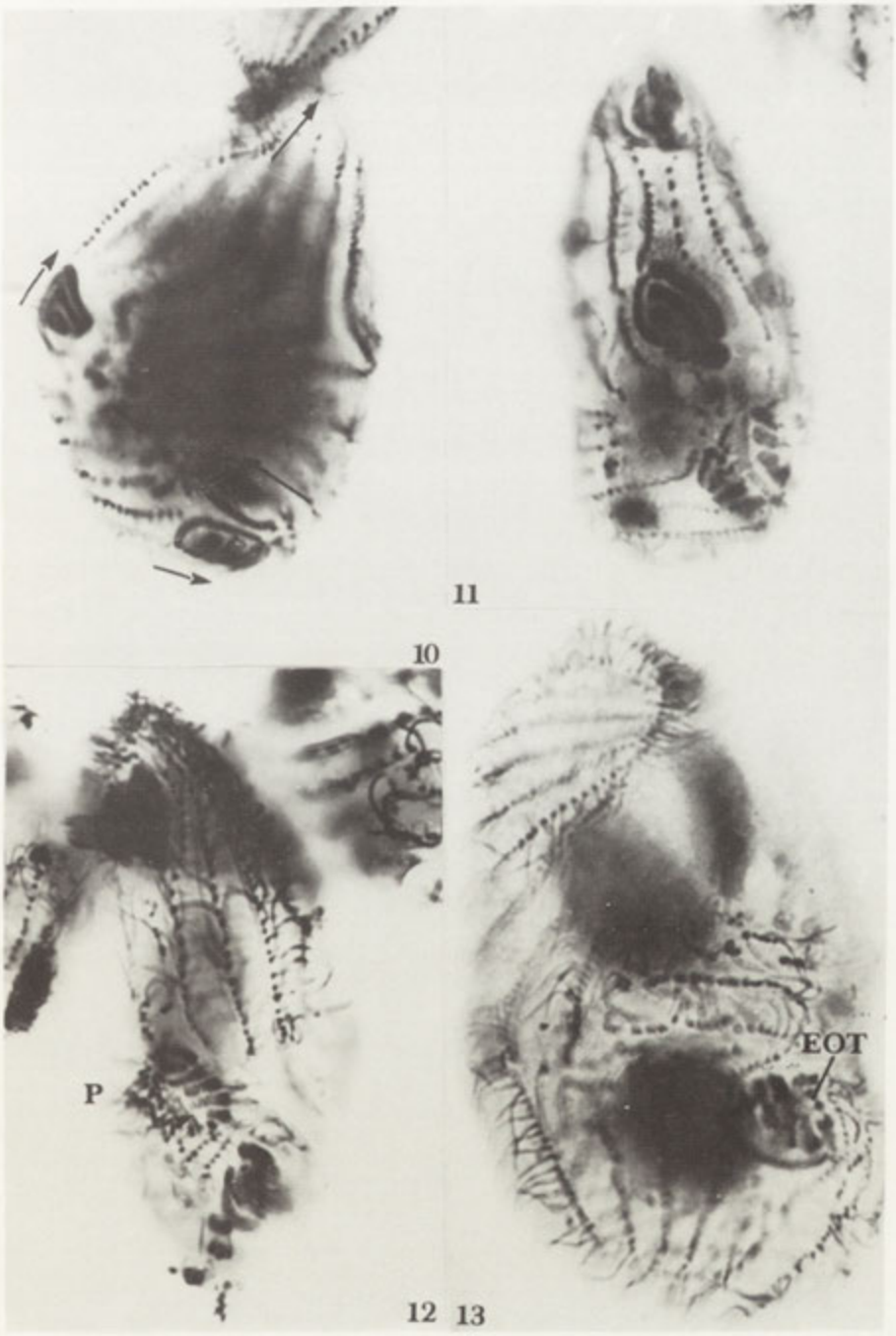

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Effects of Solar Radiation on Motility, Photomovements and Pigmentation in Two Strains of the Cyanobacterium, *Phormidium uncinatum*

Donat-P. HÄDER and Maria HÄDER

Institut für Botanik und Pharmazeutische Biologie, Friedrich-Alexander Universität, Staudtstr. 5, D-8520 Erlangen, Federal Republic of Germany

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Synopsis. The effects of solar radiation and artificial UV on motility, photomovement and pigmentation in two strains of the filamentous cyanobacterium, *Phormidium uncinatum*, have been studied. After exposure to solar radiation the filaments failed to accumulate in light fields due to a drastically reduced motility, while photophobic responses were not impaired. In addition, in vivo absorption spectra show a rapid bleaching of the photosynthetic pigments during exposure to solar radiation. Pigment bleaching is not due to thermal effects. While the organisms can be bleached by visible radiation, UV radiation alone can induce the same effect as shown by artificial UV radiation. Kinectics of bleaching at specific wavelengths, indicative for the absorption of the major photosynthetic pigments, show that the accessory phycobilin, C-phycoerythrin, is bleached first, followed by carotenoids while chlorophyll a is most resistant to UV bleaching.

The filamentous cyanobacterium, *Phormidium uncinatum*, moves when in contact with a substratum using a mechanism not yet fully understood (Walsby 1968, Häder 1987a, 1987b, 1988a). The organisms use a set of strategies to orient in their environment utilizing light as a key source of information (Nultsch and Häder 1988, Häder 1988b). Phototaxis is not a very prominent response, since the organisms are not capable of active steering but rather orient in lateral light using a trial-and-error mechanism (Nultsch 1975, Burkart and Häder 1980). Photokinesis (dependence of the linear velocity on the ambient irradiance) has been shown to be due to the amount of

energy equivalents (ATP or proton motive force, pmf) available to the motor apparatus (Nultsch 1962a, Häder 1988b). A strain isolated from lake Baikal has been reported to completely stop after a few minutes in darkness (Glagoleva et al. 1980).

Though both phototaxis and photokinesis can result in accumulation patterns with respect to light, they may not play the major role in photoorientation of these organisms. Rather, cyanobacteria seem to rely heavily on photophobic responses, which are transient responses to sudden changes in the fluence rate. The filaments reverse the direction of movement when they enter a dark area (step-down photophobic response), thus avoiding shaded areas and also when they move into a too bright area (step-up photophobic response) which prevents the organisms from being exposed to detrimental radiation which may photobleach and eventually kill the organisms (Häder 1986a, 1987a, 1987b).

Artificial UV-B radiation has been found to drastically impair photoorientation and motility in *Phormidium* at low fluence rates (Häder 1984). Accumulations in light traps were effectively inhibited by 2 h exposure to 280 nm monochromatic radiation at 0.2 mW m^{-2}. This effect was due to a decrease in the number of motile organisms in the population and also to an almost quantitative inhibition of step-down photophobic responses. In addition, phototaxis was also impaired at comparable UV-B irradiances. Also, when exposed to unfiltered solar radiation, the number of motile filaments in a population decreased within 35 min to zero.

Experiments performed in the Large Spectrograph at Okazaki, Japan, indicated that specifically UV-B wavelengths below 300 nm were effective (Häder et al. 1986). At 4 W m^{-2} the duration of motility decreased to 10 min at 270 nm, 30 min at 280 nm and 55 min at 290 nm, respectively.

Inhibition by UV-B radiation could not be photorepaired in subsequent dim UV-A or visible radiation indicating that DNA may not be the primary UV-B target Jorns et al. 1984. Likewise, neither specific diagnostic reagents for photodynamic reactions of type I (free radical formation) or type II (singlet oxygen production) had any effect on UV-B inhibition indicating that probably photosensitization can be ruled out as a possible mechanism for UV-B inhibition.

The aim of the work reported here is to demonstrate the effectivity of solar radiation on motility, photoorientation and pigmentation in two strains of *Phormidium uncinatum* to confirm the extreme UV sensitivity of these cyanobacteria and to prove that solar UV radiation has a strong effect on the survival of these organisms in their habitat.

Material and Methods

Organisms and culture conditions. All experiments were carried out using two strains of the filamentous Oscillatoriaceae, *Phormidium uncinatum*, isolated in Tübingen (Nultsch 1962b) and from Lake Baikal (Glagoleva et al. 1980). The organisms differ in pigmentation and their physiological response (Häder 1975, 1976, 1981, Häder and Poff 1982, Murvanidze and Glagolev 1981, Murvanidze et al. 1982a, 1982b). Both strains were grown on agar (0.3%) mineral medium in 100 mm glass plates) (Nultsch and Häder 1974) covered with 200 nm pore membrane filters (SM 11307, Sartorius Göttingen FRG) under constant white light from mixed fluorescence lamps at 500 lx. No Fe was added to the medium in order not to attenuate the UV radiation.

Determination of motility. Well grown cultures were harvested and the long filaments were cut into shorter segments using a homogenizer (60 s at 15 000 rpm, HO4 Bühler Tübingen FRG) in the laboratory or a razor blade in field experiments. The organisms were suspended in a 0.5% water agar at 40°C, which was then poured into plastic microtiter plates (25 wells of 18 × × 18 × 18 mm inner dimensions) and kept at room temperature until the agar had solidified.

The organisms in plates were exposed to solar radiation at Caparica, south of Lisbon (Portugal, 38°N) on sunny days between September, 2. to 14., 1988 starting each day at 11 a.m. local time. In order to avoid overheating the plates were placed in a custom-made growth chamber (Weiss Gießen, FRG) temperature-controlled at 21°C for predefined periods of time (Häder and Häder 1988a, 1988b). The top of the growth chambers was made from a plexiglass cuvette which could be filled with air or with ozone at a concentration of 45 μg ml^{-1} produced on site (ozone generator Technomed, FRG). The spectral distribution of solar radiation was constantly monitored with a computer-controlled double monochromator spectroradiometer (model 742 Optronic, Orlando Fla) equipped with a flexible quartz fiber to follow the solar azimuth and zenith angle by to Prof. Tevini and his coworkers (Karlsruhe, FRG) and will be published elsewhere. Maximal irradiance (UV, visible, plus infrared) outside the growth chamber was about 1050 W m^{-2} during the experimental period and total irradiation in the UV-B range amounted to about 1.6 W m^{-2} at solar noon. Exposure time was adjusted by covering the individual samples at the appropriate times with aluminium foil.

Samples of the suspension of organisms in agar were taken to prepare microscope slides, which were sealed with vaseline. These slides were placed on the stage of a transmission light microscope (Labovert Leitz Wetzlar FRG) and the movements of the filaments were recorded using a CCD camera (Philips LHD 0600) and a time-lapse VHS video recorder (HS-3600 Mitsubishi Japan) which allowed compressing the time by a factor of 120. The recordings were subjected to single frame analysis and the percentage of motile organisms as well as the velocity were analyzed on a large video screen (FM 100—20 CE Blaupunkt FRG).

Measurement of photoresponses. The organisms were harvested and treated as described before. During time-lapse video recording an infrared transmitting cut-off filter (RG 705, Schott and Gen., Mainz, FRG) was partially inserted into the microscope beam, so that the edge was focussed into the lower third of the image. Thus, the behavior at a light/dark boundary could be obser-

ved and quantified during playback by evaluating the percentage of photophobically responding organisms, which reversed direction of movement upon crossing the light/dark boundary. In order to determine photoaccumulations in light fields, microtiter plates were prepared as described above. After solar irradiation, aluminum foil masks were placed under the microtiter plates with holes (6 mm in diameter) in the center of each cell in the plate. The plates were suspended on a glass plate and irradiated from below via a front surface glass mirror by a white light projector (100 W Pradix, Leitz Wetzlar) at 2000 lx for 12 h. The organisms accumulated within the light fields defined by the circular holes and were immobilized by drying the plates with open lids using a fan. The accumulations were quantified by running the plates over a densitometer (Quickscan, Desaga Heidelberg, FRG). The graphical output was analyzed with a planimeter (Haff, Pfronten, FRG).

Absorption spectra. Cell suspensions were prepared as described above and pipetted into two quartz spectrophotometer cuvettes (optical path length 10 mm, 2 mm thick). The cuvettes were filled free of bubbles, closed with lids and additionally sealed with vasiline and parafilm. The two cuvettes were placed in the sample and reference compartments, respectively, of a double beam spectrophotometer (Shimadzu, model UV-210) and a calibration performed by which all absorption and scatter differences between the sample and reference were determined and stored at all wavelengths measured. The sample cuvette was then exposed to solar radiation and spectra were measured at regular time intervals. The decrease in the absorption spectra at the respective wavelength band(s) reflects the bleaching of individual pigments.

Pigment bleaching under artificial UV irradiation was determined using a single beam spectrophotometer (DU-70, Beckman). Samples were prepared as described above, filled in one quartz cuvette and spectra of the suspension were measured before and at regular intervals during UV treatment. The radiation source was a transilluminator (Il-350-M, Bachofen, Reutlingen, FRG). This UV source produces higher irradiances below 320 nm than in the solar spectrum but lower irradiances above and hardly emits any visible radiation. To evaluate progressive bleaching, the reference spectrum before UV radiation was substracted from the subsequent spectra. Plotting, smoothing and adjusting routines were written in Pascal (Turbo Pascal, Borland Int.).

Results

Exposure of *Phormidium uncinatum* to solar radiation decreased the density of photoaccumulations in light fields drastically even after short exposure times (Fig. 1) While the strain isolated from Lake Baikal showed measurable accumulations after a solar exposure of 280 min the strain isolated in Tübingen was almost completely impaired. A decrease in the accumulation density may be caused by a number of factors: the velocity of movement may be impaired by the UV irradiation or the photophobic mechanism by which the organisms detect the light/dark boundary may be disturbet. In addition, phototactic responses induced

by light scattered from particles or organisms already in the light field have been shown to be involved in the formation of photoaccumulations in cyanobacteria (Burkart and Häder 1980). Step-down photophobic responses were not impaired by solar radiation in both strains even when exposed for 240 min (Fig. 2). About 70% of the organisms in the Tübingen strain show step-down phobic responses when leaving the light field while the value is almost 90% in the Baikal strain. In

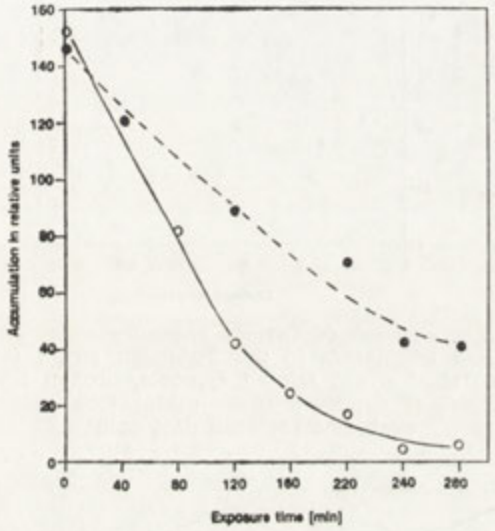

Fig. 1. Effects of solar radiation on photoaccumulations of *Phormidium uncinatum* in light fields depending on exposure time in the Tübingen strain (open symbols, continuous line) and the Baikal strain (closed symbols, broken line). Ordinate: accumulation density, relative units. Abscissa: exposure time, min.

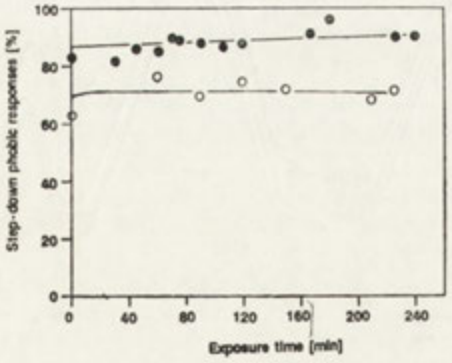

Fig. 2. Effects of solar radiation on step-up photophobic responses of *Phormidium uncinatum* at light (2000 lx)/dark boundaries in the Tübingen strain (open symbols) and the Baikal strain (closed symbols). Ordinate: percent of responding organisms. Abscissa: exposure time to solar radiation, min.

contrast, motility is strongly affected by solar radiation (Fig. 3). Under control conditions before solar exposure, the Tübingen strain of *Phormidium uncinatum* shows an average velocity of about 100 μm min^{-1} which drastically decreases to almost 10% of the initial value after

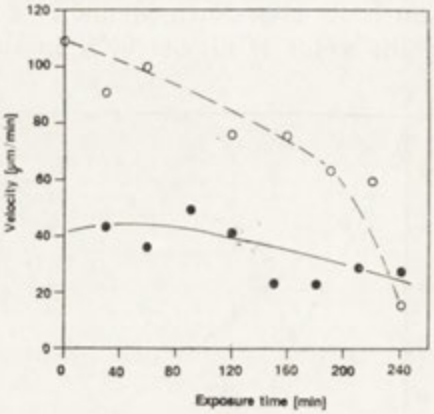

Fig. 3. Effects of solar radiation of various exposure times on the average linear velocity of *Phormidium uncinatum* in the Tübingen strain (open symbols, continuous line) and the Baikal strain (closed symbols, broken line). Ordinate: linear velocity, μm/min. Abscissa: exposure time, min. More than 50 filaments were evaluated for each data point

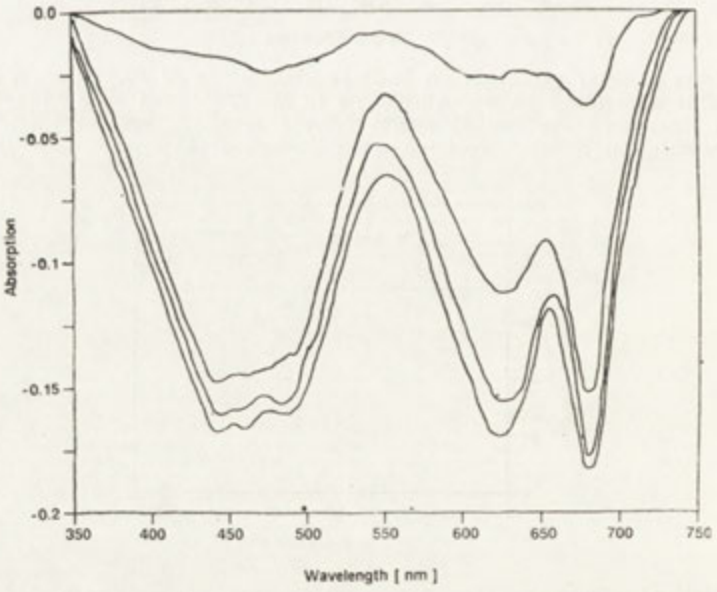

Fig. 4. Absorption spectra of *Phormidium uncinatum* (Baikal strain) after increasing exposure times to solar radiation (15, 60, 120 and 180 min, from top to bottom, respectively) measured with a double beam spectrophotometer

240 min of solar radiation. The Baikal strain has a lower initial velocity of about 40 μm min^{-1} which is not as much affected by solar radiation as in the Tübingen strain.

In order to determine whether the effects on motility and photoorientation are related to a possible bleaching of the photosynthetic pigments, *in vivo* absorption spectra were measured before and after regular time intervals of solar exposure. In both strains obvious color

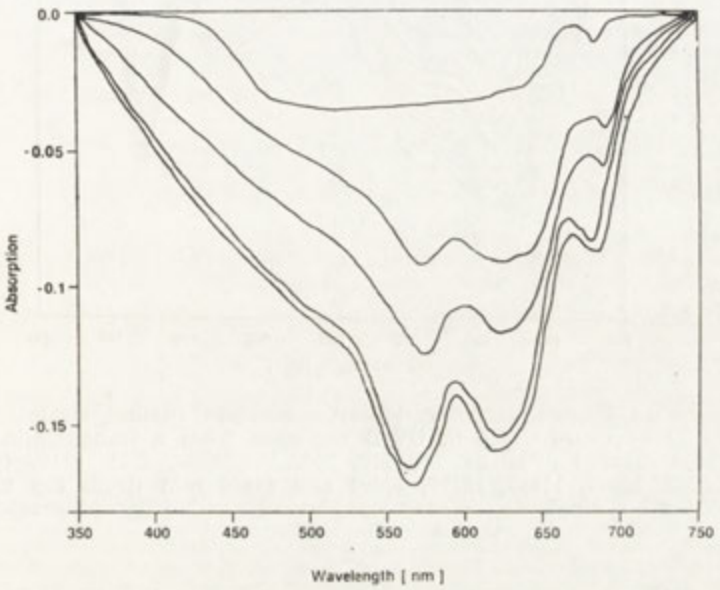

Fig. 5. Absorption spectra of *Phormidium uncinatum* (Tübingen strain) after increasing exposure times to solar radiation (30, 60, 90, 150 and 210 min, from top to bottom, respectively) measured with a double beam spectrophotometer

changes could be observed even by visual inspection which started within the first 15 min of solar exposure. In the Baikal strain there is a pronounced absorbance decrease at 620 nm indicating a bleaching of the accessory pigment C-phycocyanin (Fig. 4). The bleaching at 680 nm involves chlorophyll a and in the blue region the Soret band of chlorophyll a and some carotenoids overlap. In addition to the pigments discussed above, the Tübingen strain has a large amount of C-phycoerythrin which gives it a greyish color in contrast to the blue-green Baikal strain and which is also effectively bleached by solar radiation (Fig. 5).

In order to determine whether pigment bleaching is due to the visible or the UV component of solar radiation, samples were exposed to artificial UV radiation produced from a transilluminator. Also under this treatment there was a prominent photobleaching in both strains.

The Baikal strain showed a strong bleaching in the 620 nm region and an even stronger absorbance decrease in the chlorophyll and carotenoid absorption maxima (Fig. 6). In order to determine the kinetics of ble-

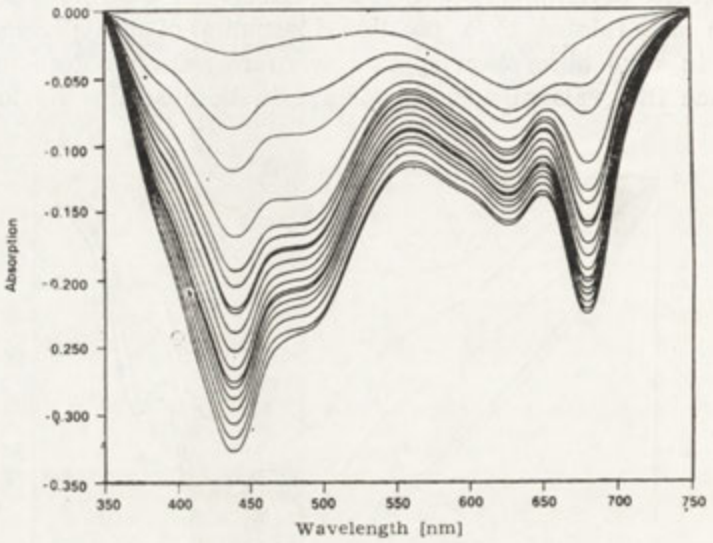

Fig. 6. Absorption spectra of *Phormidium uncinatum* (Baikal strain) measured after increasing exposure times to UV-B radiation from a transilluminator. The spectra were measured after 25, 35, 145, 350, 755, 1560, 2915, 3840, 4380, 5615, 6355, 7760, 9205, 10605, 11685, 12775, 13960 and 14630 min (from top to bottom, respectively) with a single beam spectrophotometer and differences calculated to the unirradiated control

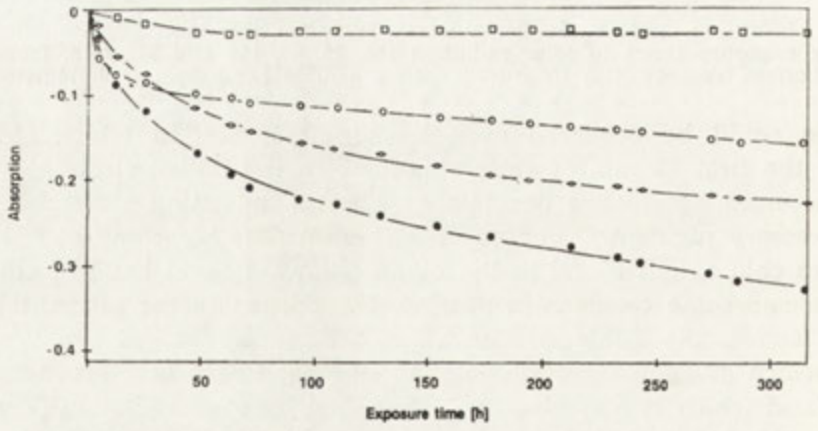

Fig. 7. Kinetics of bleaching of the major photosynthetic pigments in *Phormidium uncinatum* (Baikal strain) exposed to UV radiation. Ordinate: absorption units. Abscissa: exposure time, min. Measurements at wavelengths: 680 nm — chlorophyll a, rhomboids; 620 nm — C-phycocyanin, open circles; 480 nm — carotenoids, squares; 440 nm — Soret band of chlorophyll a plus carotenoids, closed circles

aching the irradiation was continued until the absorbance changes had almost reached a constant value. (This long term constant irradiation has not been performed because of its ecological significance; however, it has been used to obtain an almost complete bleaching.) As an aside, this method is an effective way to obtain true in vivo absorption spectra, excluding the disturbing scattering components which are filtered out by calculating the absorbance differences. The rate of bleaching was determined by calculating the absorbance changes during exposure at prominent absorption maxima indicating the involvement of the major photosynthetic pigments (Fig. 7). The half lifetime for C-phycocyanin was 17.1 h and for the carotenoid maximum at 480 nm 28.9 h. The red peak of chlorophyll a had a half life of about 46.1 h and the Soret band at 440 nm, which should have the same lifetime as the red peak, showed a value of 47.4 h.

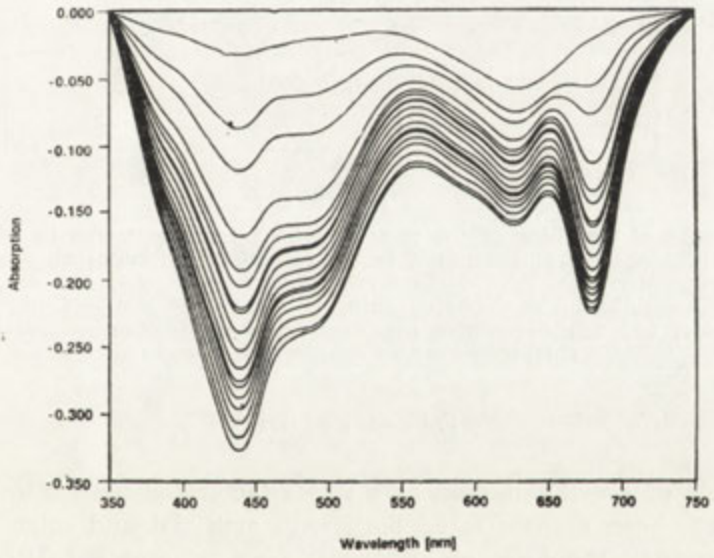

Fig. 8. Absorption spectra of *Phormidium uncinatum* (Tübingen strain) measured after increasing exposure times to UV-B radiation from a transilluminator. The spectra were measured after 25, 35, 50, 145, 350, 755, 1560, 2915, 3840, 4380, 5615, 6355, 7760, 9205, 10605, 11685, 12775, and 13960 min (from top to bottom, respectively) with a single beam spectrophotometer and differences calculated to the unirradiated control

Also the Tübingen strain was subjected to long term continuous exposure to artificial UV radiation (Fig. 8). The first obvious bleaching effects were found in the C-phycoerythrin region, later the chlorophylls and carotenoids and finally C-phycocyanin were bleached. The kinetic analysis (Fig. 9) indicated a lifetime of 11.8 h for C-phycoerythrin, 75 h for C-phycocyanin, 43.4 h for the carotenoid. Chlorophyll a measur-

ed at 680 nm had a lifetime of 57.8 h and the maximum at 440 nm had a lifetime of 50 h. The difference between the 680 nm and 440 nm maxima may be due to the fact that at 440 nm in addition to the Soret band of chlorophyll a carotenoid absorption is involved.

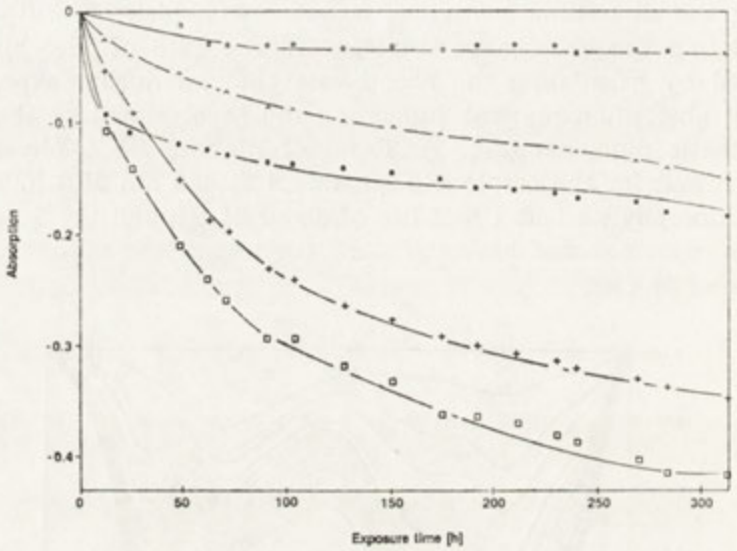

Fig. 9. Kinetics of bleaching of the major photosynthetic pigments in *Phormidium uncinatum* (Tübingen strain) exposed to UV radiation. Ordinate: absorption units. Abscissa: exposure time, min. Measurements at wavelengths: 680 nm — chlorophyll a, crosses; 620 nm — C-phycocyanin, up-arrows; 560 nm — C-phycoerythrin, closed circles; 500 nm — carotenoids, open circles; 440 nm — Soret band of chlorophyll a plus carotenoids, squares

Discussion

The role of UV-B radiation as a stress factor for filamentous cyanobacteria has been demonstrated both with artificial and solar radiation (Häder 1984, Häder et al. 1986). Even at ambient UV-B levels unfiltered solar radiation would be detrimental for the survival of the organisms when they are not protected by attenuation in the water column or by actively moving to habitats with only dim irradiation. Similar light restrictions have been found in the cyanobacteria *Oscillatoria agardhii* (Post et al. 1986) and *Microcystis aeruginosa* (Zevenboom and Mur 1984). UV-B irradiation seems to be an important factor for cyanobacteria in their habitat and may be responsible for the observed disappearence of cyanobacteria blooms during summer when the UV component in solar radiation increases. Similar dramatic effects of UV-B irradiation have been found in other motile microorganisms

as diverse as slime molds (Häder 1983, Häder and Häder 1989), flagellates Häder 1986b, Häder and Häder 1988a, 1988b) and marine phytoplankton (Worrest et al. 1981). In contrast to artificial monochromatic UV-B radiation, solar radiation does not seem to drastically impair photoorientation, while motility and photoaccumulation are dramatically inhibited.

Previous experiments have ruled out photodynamic responses as the major mechanism by which UV-B radiation damages motility and affects photoorientation (Häder et al. 1986), since neither diagnostic reagents for the involvement of free radials (Ito 1983, Spikes and Straight 1981) nor specific quenchers for singlet oxygen (Merkel et al. 1972, Maurette et al. 1983) had any effect on UV-B inhibition. Equally, DNA does not seem to be the major target for UV-B inhibition since first of all the effect is too fast to involve the whole chain of events leading to protein biosynthesis. In addition, no photorepair (Jorns et al. 1984, Eker 1983) has been detected following damage by UV-B radiation. Therefore, it was assumed that UV-B inhibition may be due to a specific damage of an intrinsic component of the photoreceptor and motor apparatus.

One of the possible targets for UV-B radiation could be the photosynthetic pigments since they have been identified to play a dual role in photoperception and in energy provision for the motor apparatus. Bleaching of the photosynthetic pigments can be due to a number of factors. Thermal effects of the solar infrared radiation can be excluded for the experiments described here since the samples were exposed in a temperature-controlled growth chamber. While photobleaching can be induced by visible light in cyanobacteria (Nultsch and Agel 1986) bleaching by transilluminator radiation indicates that it can be induced exclusively by UV radiation. In solar radiation both UV and visible radiation seem to exert collaborative but different effects as seen by comparing the bleaching patterns in UV and solar radiation (Figs. 5 vs 7 and Figs. 6 vs 8).

The kinetic analysis of bleaching shows that the accessory phycobilin, C-phycoerythrin is the first pigments affected by the radiation in the Tübingen strain. This effect is also obvious by visual inspection: the grayish colored filaments look green after a short irradiation time. It is interesting to note, that the carotenoids which serve the dual function of photoprotection and energy transduction to the chlorophyll reaction centers are damaged before the chlorophylls are bleached, which are the last pigment lost after both solar and artificial UV-B radiation. The only exception is the long lifetime of C-phycocyanin in the Tübingen strain, which cannot be accounted for.

In summary, solar radiation is a major factor for the survival of cyanobacteria in their habitat, since it affects motility and thus the capability to adjust to the constantly changing conditions in the environment. One of the direct targets for UV damage may be the photosynthetic pigments, which are bleached starting with the accessory pigments followed by carotenoids, while the essential chlorophylls are more resistant to radiation.

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Contributions of Fast and Slow Potassium Outward Currents to Repolarization of the Action Potential in *Euplotes vannus*

Ralph WESTERMANN, Thomas KRÜPPEL
and Wolfgang LUEKEN

Zoophysiologie, FB Biologie/Chemie, Universität Osnabrück, D-4500 Osnabrück, Federal Republic of Germany

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Synopsis. In the marine hypotrichous *Euplotes vannus*, fast and slowly activating potassium outward currents ($I_{K\,fast}$, $I_{K\,slow}$) were studied in current- and in voltage-clamp, by use of a single-microelectrode equipment. Their contributions to regulation of the membrane potential were isolated by i) blocking Ca^{2+}-dependent $I_{K\,slow}$ by TEA or EGTA and ii) inactivation of $I_{K\,fast}$ via a two-pulse protocol. Current activation was tested in voltage ramps that mimicked the membrane response to current injection. $I_{K\,fast}$ limits the action potential amplitude and causes small initial repolarization. $I_{K\,slow}$ continues repolarization and is responsible, due to delayed activation and deactivation, for damped potential oscillation.

In spite of tremendous knowledge concerning ion currents in ciliates (Andrivon 1988, Machemer 1988a, b, 1989, Machemer and Deitmer 1987, Machemer and Sugino 1989, Pernberg and Machemer 1989, Ramanathan et al. 1988), "our understanding of ionic mechanisms at the single-cell level is fragmentary" (Machemer 1989). The present work is focussed on how, in the marine hypotrichous ciliate *Euplotes vannus/crassus*, two potassium outward currents, $I_{K\,fast}$ and $I_{K\,slow}$, which formerly have been studied (Krüppel and Lueken 1988), interact with each other and contribute to regulation of the membrane potential. It is intended as a step in understanding behaviour of that ciliate (Ricci et al. 1987a, b). Cooperation of ion currents is expected to become especially important in respect to conjugation, where locomotion changes drastically after cells have communicated by means of mechanical or chemical signals (Kusch and Heckmann 1988, Raffioni et al. 1988).

Material and Methods

Clone D35 was derived from a syngen originally collected at *Naples* (Italy). It belongs to the *vannus* morphospecies of the *E. vannus/minuta* species complex (Fernandez-Leborans 1986, Génermont et al. 1985, Valbonesi et al. 1988, Voss 1989). Composition of artificial sea water (ASW), and techniques for cultivation and for breeding have been reported elsewhere (Lueken et al. 1981, 1983, 1987).

Standard electrophysiological medium, EASW, the mechanical set-up, and general techniques were as described (Krüppel and Lueken 1988, Lueken et al. 1987). Microelectrodes were filled with 1 M KCl. They had resistances of 15—22 Mohm. Recordings were performed by use of a single-electrode current- and voltage-clamp system (npi SEC 1L, H.-R. Polder, D-7146 Tamm, FRG). Switching frequency was 10 kHz with a duty cycle of 50%. Data were acquired with a Labmaster analog-digital interface board (Tecmar Inc., Scientific Solutions Division, Solon, Ohio, USA) installed into a personal computer (Tandon AT). The computer generated the command signals and simultaneously recorded data by use of the pCLAMP program (Axon, Foster City, California, USA). Records were on-line filtered at 1 kHz with an 8-pole Bessel filter (48 dB/octave).

Specimens were exposed to TEA by changing the bath medium to EASW with 20 mM tetraethylammonium chloride added. EGTA (Ethylene glycol-0,0′-bis(2-aminoethyl)-N,N,N′,N′-tetraacetic acid) was introduced into the cells via the microelectrode, which contained 200 mM in addition to the standard electrolyte.

Holding potential in voltage clamp was -25 mV. Special use of the pCLAMP software is described together with the results. Current traces have been electronically purified from capacitive transients. Experiments were performed at room temperature (19—21°C).

Reliability was ascertained as follows: Test programs were repeated 4—6 times on each cell. Random spot tests revealed variability coefficients (standard error of the mean as percentage of the mean) in the range of < 1 to 3%. Only recordings that met these requirements of consistency were electronically averaged. Analysis was performed on these averaged traces. For mean values that compile data from several cells — the actual number will be indicated together with results — the variability coefficients do not exceed 15%. Where non-averaged original recordings are shown, identical results of at least 4 cells have been obtained.

Results

The aim was to understand the cells' response to depolarizing current stimuli in the light of known characteristics of two constituents of repolarizing current, $I_{K\ fast}$ and $I_{K\ slow}$ (Krüppel and Lueken 1988). The typical pattern of V_m oscillation (Fig. 1A) started with a rapid regenerative depolarization. After 3 phases of repolarization, r_l, r_r, r_l, a new depolarization, d_p, developed from the potential minimum. It was followed by further cycles of repolarization and depolarization (the

first one is included in Fig. 1A). When the pulse terminated, after-pulse repolarization drove V_m below the resting potential to an after hyperpolarization (AHP). It was followed by a delayed depolarization (DD). The outward currents in question are demonstrated in Fig. 1B. Their

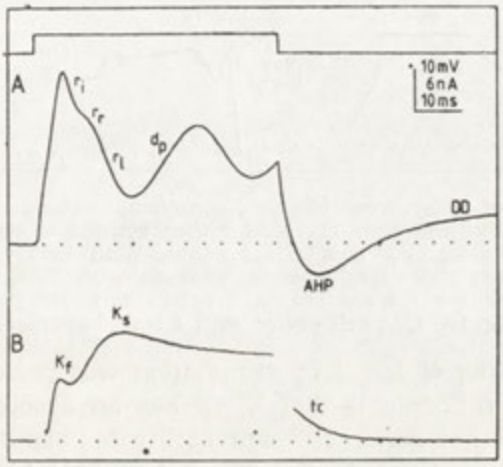

Fig. 1A, B: Membrane potential, induced by constant-current injection, and current, elicited by depolarization. A. Peak amplitude of the action potential is 45 mV; r_i — initial repolarization; r_r — retarded repolarization; r_l — late repolarization; d_p — new depolarization; AHP — after hyperpolarization; DD — delayed depolarization. B. The sum current trace reveals rapid activation of $I_{K\ fast}$ (K_f), slow activation of $I_{K\ slow}$ (K_s), both separatet by a slight depression of current strength. The tail current (tc) belongs to $I_{K\ slow}$. The pulse protocol refers to both parts: current strength of +3 nA, voltage amplitude of +45 mV, 50 ms duration either. Resting potential — -24.5 mV; holding potential — -25 mV; n — 6 cells

characteristics suggest the following relationships: stop of potential upstroke as well as initial repolarization can be attributed to development of $I_{K\ fast}$, retard in repolarization to decrease of $I_{K\ fast}$, and late repolarization to increase of $I_{K\ slow}$. The AHP, finally, should be due to the tail current of $I_{K\ slow}$, the deactivation kinetics of which becomes manifest in Fig. 1B. The DD, however, is not directly concerned with the outward currents.

The validity of these derivations was tested by application of compounds that differentially suppressed $I_{K\ slow}$ (Krüppel and Lueken 1988) and a stimulation protocol that specifically ruled out $I_{K\ fast}$. Effects of each of the drugs, EGTA or TEA, respectively, are demonstrated in Fig. 2. Either of them, leaving initial repolarization unchanged, nearly completely inhibited late repolarization. Also the fact that, in the presence of EGTA, an AHP was not developed, is in agreement

with suppression of Ca^{2+}-dependent $I_{K\ slow}$. Maintained depolarization in the presence of TEA, on the contrary, could be due to Ca^{2+}-dependent sodium influx (K r ü p p e l and L u e k e n 1990), which is suppressed by EGTA but not by TEA. Responsibility of $I_{K\ slow}$ for r_i thus is ascertained.

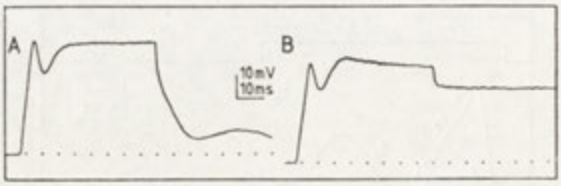

Fig. 2A, B: Effects of K^+-current blocking compounds on repolarization. A. Intracellular EGTA. B. Extracellular TEA. At either condition, only initial repolarization is left. Thereafter, V_m depolarizes again. With EGTA, it returns to the resting potential when the stimulus ends, whereas with TEA it maintains a depolarized state, after some reduction in amplitude. Same pulse protocol as in Fig. 1A. One cell either with 4 traces averaged

Selective blocking of $I_{K\ fast}$ by stimulation was performed by a two-pulse regime. Its rationale is that $I_{K\ fast}$ has an absolute refractoriness of 12 ms and does not regain full activability for about 50 ms (K r ü p p e l and L u e k e n 1988). In Fig 3, couples of double pulses with increasing interval lengths are combined, which were performed both in

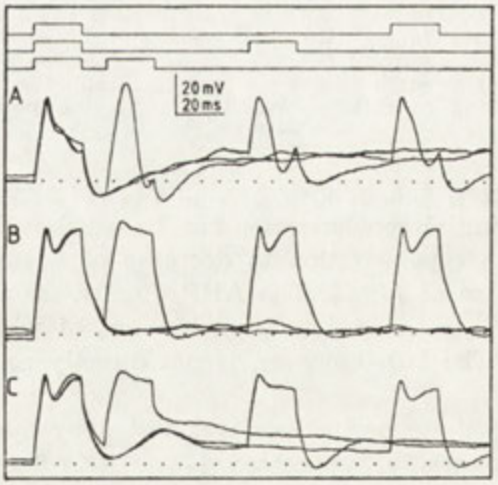

Fig. 3A—C: Responses to two subsequent current injections (+3 nA, 20 ms, either). A. Standard EASW. B. Standard EASW, but cell containing EGTA. C. EASW with TEA added. For each experimental condition, 3 traces with increasing intervals between stimuli, as indicated in the pulse protocol, are superimposed on one time axis. The 1st pulses always coincide. Note disappearance of initial repolarization after short intervals between pulses. A, C one cell, B another cell

absence and in presence of the K^+-current blocking agents. Initial repolarization was expressed, irrespective of experimental conditions, in all first pulses. Consistently, it was no more elicited in the 2nd pulses after 10 ms. In standard EASW, where $I_{K\ slow}$ was left, repolarization was strongly effective; it ran farther than before, although it started later, such that the peak developed broader. With EGTA, as well as with TEA, there was (nearly) no repolarization at all. In all circumstances, with increasing interval duration the traits developed towards those of the 1st reaction. These experiments confirm the assumption that r_i is due to $I_{K\ fast}$. With EGTA, the potential traces were less complex, possibly because EGTA, by trapping intracellular Ca^{2+} ions, blocked several Ca^{2+}-dependent currents, I_{Na} included.

Oscillation of V_m during current injection was suppressed when $I_{K\ slow}$ was blocked by EGTA or TEA, respectively (cp. Figs. 1, 2). The role of $I_{K\ slow}$ activation-stages in V_m oscillation was determined in two kinds of experiments: A continuous current stimulus was terminated

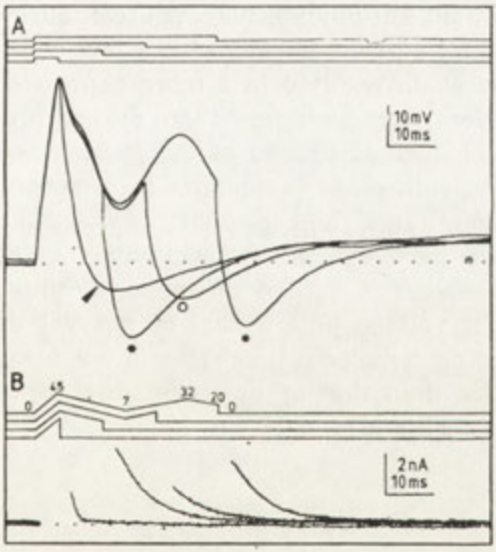

Fig. 4A, B: Correlation of after-pulse repolarization and tail current. From either experimental procedure, 4 traces are superimposed on one time axis. A. Constant-current injection (+3 nA) was terminated at times as indicated in the pulse protocol: before onset of repolarization (arrowhead); during repolarization (*); during depolarization (o). After repolarization drove V_m to different levels. B. Voltage in downstroke or upstroke ramps was stepped back to the holding potential at different times; breaktimes and sequence of ramps were in correspondence with the potential pattern of A. Numbers in the pulse protocol indicate mV depolarization. The 1st break was at the peak voltage, subsequent ones at -5 mV. From current traces obtained, only tail currents are plotted. n — 5 cells

at various points of the oscillating potential trace, in order to observe the course of after-pulse repolarization (Fig. 4A). And, a sequence of voltage ramps was performed which mimicked that potential oscillation, in that it continuously depolarized and repolarized to same extents and with same slopes as V_m did during current injection. Ramps were stopped (Fig. 4B) at points that corresponded to breaks in membrane oscillation and tail currents were recorded. Results from both kinds of experiments were in full correspondence. If current incjection was stopped at the action-potential peak, after-pulse repolarization moved V_m only slightly below the resting potential, and there was only a small, quickly deactivating tail current, which belonged to $I_{K\ fast}$, as judged from deactivation speed. Further results, obtained from the three later stops, do only concern $I_{K\ slow}$. Whenever breaks were set, in downstroke as well as in upstroke of the oscillation curve or the corresponding ramp, respectively, V_m was at the same potential level, and the ramps ended at the same voltage. However, extents of after-pulse repolarization or tail currents, respectively, were different. If the break occurred during r_I, decrease of the potential continued to a deep AHP, and the corresponding tail current had a high initial amplitude. A break during d_p led to a smaller AHP, and the tail current started with a low amplitude. Stop during new repolarization again resulted in a more expressed AHP and a more extended tail current, although less than during preceding repolarization. Both, expression of AHPs and of tail currents confirm the conclusion that at a given voltage, activation of $I_{K\ slow}$ depends on the direction of a preceding ramp. It is higher during downstroke than during upstroke. This should be due to slow deactivation of the $I_{K\ slow}$ tail current: At each breakpoint, $I_{K\ slow}$ conductance is composed by an amount according to actual voltage plus a part of not yet deactivated conductance from foregoing depolarization. The latter term is higher during voltage downstroke than during upstroke, due to voltage dependency of $I_{K\ solw}$ (Krüppel and Lueken 1988).

Discussion

By means of a two-pulse protocol, together with K^+-current blocking agents EGTA and TEA (Figs. 2, 3), and complex series of voltage ramps (Fig. 4), a lot of traits of a cell's response to a current stimulus (Fig. 1) could be explained: $I_{K\ fast}$ causes the 1st step of repolarization during the action potential. It obviously limits the potential upstroke and thus is responsible for the gradedness of the action potential in *E. vannus*,

together with inactivation of I_{Ca} (Krüppel and Lueken 1988). After inactivation of $I_{K\ fast}$, repolarization is taken upon by $I_{K\ slow}$, which not inactivate.

The $I_{K\ slow}$ induced repolarization leads to deactivation of $I_{K\ slow}$ channels, because of their voltage dependence, and, parallelly, to reduction of the electromotive force for K^+ ions. These cooperative events limit progressive downstroke of the repolarization. Since $I_{K\ slow}$ is deactivated, the persistently injected current moves V_m in depolarizing direction. Both slow deactivation and activation kinetics of $I_{K\ slow}$ (Figs. 1 4B) lead to overshooting membrane responses in repolarization and depolarization. This supports a damped oscillation leading to a steady-state at 15—20 mV below the peak (Krüppel and Lueken 1988). Slow deactivation of $I_{K\ slow}$ is also assumed to be solely the cause for development of AHPs. That is also valid for the slight AHP after the 5-ms break in Fig. 4A. At that time, only $I_{K\ fast}$ should be elicited. We suppose activation of $I_{K\ slow}$ while V_m ran from the peak towards the resting potential. In TEA solution, *E. vannus* responds to the injected current with prolonged depolarization (Fig. 2), unlike *Paramecium*, but like *Fabrea salina* (Kubalski 1987). This points to activation of long lasting I_{Na} (Krüppel and Lueken 1990), which could also occur in the marine *F. salina*.

The unexpected increase in repolarization amplitude in Fig 3A is due to voltage dependence of $I_{K\ slow}$ activation. If $I_{K\ fast}$ is lacking because of refractoriness, $I_{K\ slow}$ is activated at a higher potential level. This enhanced conductance meets a stronger electromotive force for K^+ ions. The resulting, higher current amplitude repolarizes the membrane more effectively than usual.

Spontaneous depolarization in *E. vannus* runs only to an upper limit of -5 mV (data not shown). This seems to be surprising, since the activation threshold for $I_{K\ fast}$ and $I_{K\ slow}$ was found at about -10 mV (Krüppel and Lueken 1988). In recent experiments, however, even at -20mV substantial current amplitudes were obtained (data not shown). Therefore the two K^+ currents can be very effective in preventing the membrane from depolarization above -5 mV. $I_{K\ fast}$ activation starts only 1 ms after onset of $I_{Ca\ fast}$, and its amplitude by far exceeds that of $I_{Ca\ fast}$. Its decay, at around 10 ms after start of a pulse, coincides with inactivation of $I_{Ca\ fast}$. We assume that $I_{K\ fast}$ is a specialized antagonist of $I_{Ca\ fast}$ that shortcircuits Ca^{2+} spikes. As for the possible role(s) of $I_{K\ slow}$, several possibilities remain: (i) support for $I_{K\ fast}$ in continuation of repolarization after a brief depolarization; (ii) shut off of a voltage-dependent depolarizing current, as $I_{Ca\ slow}$ (not yet demonstrated in *Euplotes*), or I_{Na}; (iii) reduction of excitation during prolonged

depolarizations which are due to mechanosensitive inward currents. This functional partition resembles the type I and II excitations in *Paramecium* and their shut off voltage- and Ca^{2+}-dependent K^+ current (Saimi et al. 1983). But, in *E. vannus*, the events are faster. $I_{K\ fast}$ which shuts off the action potential in *E. vannus* seems to be more specialized for this role than I_K in *Paramecium*. The A current of *Stylonychia* shows similar fast activation kinetics, low amplitude and fast inactivation as $I_{K\ fast}$ of *E. vannus* does, but its role in repolarization is still unknown. The combination in *E. vannus* of a fast, small, and quickly inactivating K^+ current with a large, slow and persistent K^+ current perhaps enables a better controlled repolarization than one component alone could achieve.

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Impact of the Length of Exposure to Peptides of Different Molecular Mass on the Establishment of Imprinting in *Tetrahymena*

László KŐHIDAI and György CSABA

Department of Biology, Semmelweis University of Medicine, H-1445 Budapest, Hungary

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Synopsis. Treatment of *Tetrahymena pyriformis* cells with oxytocin, insulin, thyrotropic hormone (TSH) or bovine serum albumin (BSA) for different times resulted in negative imprinting by oxytocin, whereas in positive imprinting by the other three molecules. The optimal time period for positive imprinting was 60 min, and prolongation of exposure beyond 60 min accounted for down-regulation in the case of oxytocin and insulin, whereas the imprinting potential of the non-hormone peptide BSA was still present at 240 min.

The collection and processing of environmental information is vitally important for the survival of unicellular organisms and species. Communication with the environment is furnished mainly by certain, in all probability randomly formed, patterns in the membrane of the unicellular, which are able to receive information by interaction with environmental signal molecules (Koch et al. 1979). The signal molecules participate in the amplification of the receiver structures by transforming the originally non-specific binding site to a specific receptor (Lenhoff 1968, 1974, Csaba 1980, 1984). The primary interaction of a binding structure with the signal molecule gives rise to imprinting, which accounts for amplification of the receptor and for a consequent alteration of the binding capacity and response of the cell to the signal molecule on subsequent exposure (Csaba 1985, 1986). Thus the cell acquires a receptor-level "memory" of primary interaction with the

signal molecule and transmits it to several hundreds of offspring generations (Csaba et al. 1982).

Many signal molecules possess an imprinting potential, but the interrelatedness of imprinting with the size and quality of the molecule, and/or with the duration of contract between cell and signal molecule is still obscure. The present experiments were performed to throw more light on this problem.

Material and Methods

Tetrahymena pyriformis GL cells, maintained in 0.1 per cent yeast extract containing 1 per cent Bacto tryptone medium (Difco, Michigan, U.S.A.) were used in the logarithmic phase of growth.

The cells were treated with 10^{-6}M oxytocin (Gedeon Richter Ltd. Budapest), insulin (Semilente, MC, Novo, Copenhagen, Denmark), thyrotropin (TSH, Ambinon-Organon Oss, Holland) or bovine serum albumin (BSA, HUMAN, Budapest) for 10, 30, 60 or 240 min. after which they were returned to plain medium for 24 h. Subsequently the daughter cells of the pretreated cells were fixed in 0.4 per cent formaline containing phosphate buffer solution (PBS, pH 7.2), washed in two changes of PBS, and incubated for 1 h in presence of fluorescein-isothiocyanate-labeled (FITC, BDH, London, England) peptide, the same as used for pretreatment. After incubation, the cell suspensions were washed in several changes of PBS, spread on slides, and dried.

The binding of the FITC-labeled peptide was assessed by cytofluorimetry, using a Zeiss Fluoval cytofluorimeter, which was connected with a HP-42CX calculator for statistical analysis of inter-group variation by Student's t-test and analysis of variance. Twenty cells were assayed for fluorescence in each group and each assay was performed in flour replicates. Thus each column diagram in Fig. 1 represents the mean value for 80 cells.

Results

Of the four peptides tested in the present study three, insulin, TSH and BSA, induced a positive imprinting (Fig. 1) after exposure for 60 min. No positive imprinting took place on shorter exposures for 10 and 30 min, and only imprinting by BSA was still positive after 240 min. Oxytocin induced consistently a significant negative imprinting. Insulin accounted after 10 min for a negative imprinting, after 30 min for binding comparable to the control, after 60 min for a positive imprinting and after 240 min the value was comparable to the control.

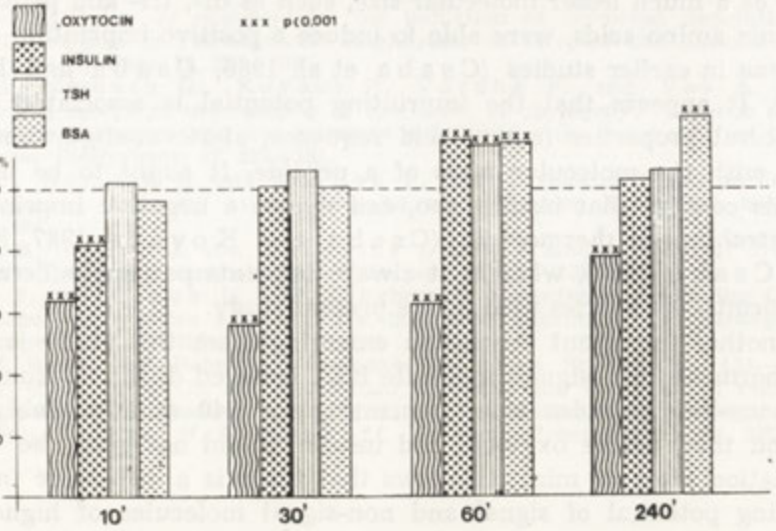

Fig. 1. Impact of treatment (imprinting) with 10^{-6}M concentration of different peptides for different times on the FITC-peptide binding of *Tetrahymena pyriformis* 24 h after exposure, related to the control as 100

Discussion

The peptide concentration used (10^{-6} M) was high enough for induction of imprinting, and — as demonstrated earlier (Csaba and Kőhidai, 1986) — also sufficient for initiation of down-regulation. The trend of imprinting may as well be positive as negative, and account for a greater or lesser binding (or cellular response), depending on the nature of the imprinter, or on the species of *Tetrahymena* used (Csaba and Kovács 1987, Kovács and Csaba 1987). Imprinting was usually positive with *Tetrahymena pyriformis*.

Taking into consideration the molecular mass of the imprinters (insulin 6000, TSH 28 000; BSA 67 000 dalton), it appears that the imprinting potential of peptides (and of the protein BSA) did take effect in that molecular mass range, without appreciable differences between the levels. It is also obvious that such peptides or BSA fail to induce positive imprinting within 30 min or less time.

It also deserves mention that oxytocin, whose molecular mass is about 1000 dalton, induced a negative imprinting in a relatively short time in practically all conditions of the experiment. This effect was, however, in all probability unrelated to the molecular mass, since pep-

tides of a much lesser molecular size, such as di-, tri- and pentapeptides an even amino acids, were able to induce a positive imprinting in *Tetrahymena* in earlier studies (Csaba et al. 1986, Csaba and Darvas 1987). It appears that the imprinting potential is associated with the structural properties (amino acid sequence, steric configuration) rather than with the molecular mass of a peptide. It ought to be mentioned in this context that insulin, too, can induce a negative imprinting, e.g. in *Tetrahymena thermophila* (Csaba and Kovács 1987, Kovács and Csaba 1987), whereas it always imprints positively *Tetrahymena pyriformis*, the species used in the present study.

Another important conclusion emerging from this study is that the non-hormone (non-signal) molecule BSA behaved different from the two hormone-like peptides after imprinting for 240 min, probably on the ground that, unlike oxytocin and insulin, it did not give rise to down-regulation after 60 min. It follows that there is a difference in the imprinting potential of signal and non-signal molecules of higher vertebrates in *Tetrahymena*. This does not, of course, exclude that non-hormone polypeptides, too, may possess an imprinting potential (Csaba et al. 1985).

The experimental results uncequivocally indicate that the time factor plays an important role in the issue of imprinting by a given concentration of peptide. The molecular mass of the hormone (peptide molecule) seems to play a lesser role than its chemical nature. The imprinting potential differs between peptide molecules of signal and non-signal nature, and certain signal molecules may even account for down-regulation on lasting exposure.

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Catalogue des espèces et taxons infraspécifiques de Dinoflagellés marins actuels publiés depuis la révision de J. Schiller. V (Complément)

Catalogue of the species and infraspecific taxa of the recent marine Dinoflagellates (*Dinophyceae*) published since the revision by J. Schiller. V (Supplement)

Alain SOURNIA

(Station Biologique de Roscoff) c/o Laboratoire de Géologie, Muséum National d'Histoire Naturelle; 43, rue de Buffon, 75005 Paris, France

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Synopsis. On énumère ici, dans l'ordre alphabétique des genres et des espèces, les innovations introduites dans la littérature mondiale au cours de la période 1981—82 à 1989; ce "Complément" ne contient lui-même aucune innovation taxinomique. Le cas échéant, les manquements aux conditions de légalité selon les règles de la nomenclature botanique sont signalés.

Ce complément, comme les précédents (Sournia et al. 1975, Sournia 1978, 1982), vise à mettre à jour le "Catalogue" de Sournia (1973) dont le but reste inchangé: rassembler les innovations taxinomiques introduites dans la littérature depuis la première -et, à ce jour, unique- monographie mondiale des genres et taxons infraspécifiques (Schiller 1931—37). Bien que les dispositions pratiques soient rigoureusement maintenues, rappelons les principales.

Ce catalogue n'est pas critique, en ce sens qu'il n'inclut pas d'appréciation ou d'interprétation personnelle sur les caractères morphologiques ou biologiques des Protistes considérés. Les seuls commentaires portent sur les conditions objectives de légalité selon les règles de la nomenclature botanique (Greuter et al. 1988), en particulier:

— description sans diagnose latine d'un organisme nouveau;

— publication d'un nom nouveau ou d'une combinaison nouvelle sans référence détaillée du basionyme;
— innovation résultant d'une erreur ou innovation apparemment involontaire;
— simple erreur orthographique (ici rappelée entre guillements et corrigée).

Sur ces différents points, sur les exigences respectives des codes de nomenclature botanique et zoologique et sur leur interprétation, on peut se reporter aux deux premières parties (Sournia 1973, Sournia et al. 1975) ou à l'ouvrage plus général de Jeffrey (1977).

Les taxons sont énumérés dans l'ordre alphabétique des noms de genres, d'espèces et d'épithètes infraspécifiques. Les espèces indéterminées mais ayant fait l'objet d'une description ou d'une illustration sont mentionnées à la fin du genre concerné; les Dinoflagellés indéterminés, sous la même réserve, le sont à la fin de ce "Complément".

Dans le cas de combinaison ou nom nouveau, le basionyme est rappelé entre parenthèses (et précédé du signe =). Ce basionyme fait lui-même l:objet d'un appel dans le texte à sa place alphabétique.

Rappelons que Lentin et Williams (1989) tiennent à jour un catalogue identique pour les Dinoflagellés fossiles.

Genre *Adenoides* Balech

A. kofoidii (Herdman)Dodge, 1982, p. 241, fig. 32C (= *Amphidinium kofoidii*).

Genre *Alexandrium* Halim emend. Balech

A. acatenella (Whedon et Kofoid) Balech, 1985, p. 37, fig. 19 (= *Gonyaulax acatenella*).

A. affine: Balech, 1985, p. 38. Combinaison invalide: basionyme non indiqué (probablement *Protogonyaulax affinis* Inouye et Fukuyo in Fukuyo et al. 1985).

A. balechii: Balech, 1985, fig. 13. Combinaison invalide: basionyme non indiqué (probablement *Gonyaulax balechii* Steidinger).

A. catenella (Whedon et Kofoid) Balech, 1985, p. 37, fig. 2 (= *Gonyaulax* catenella).

A. cohorticula (Balech) Balech, 1985, p. 37, fig. 5 (= *Gonyaulax cohorticula*).

A. concavum (Gaarder) Balech, 1985, p. 38, fig. 17 (= *Goniodoma concavum*).

A. depressum (Gaarder) Balech et Tangen, 1985, p. 342 (= *Goniodoma depressum*).

A. excavatum (Braarud) Balech et Tangen, 1985, p. 334, fig. 1A—L, 2, 5E—F (= *Gonyaulax tamarensis* var. *excavata*).

A. fraterculus (Balech) Balech, 1985, p. 37, fig. 4 (= *Gonyaulax fraterculus*).

A. fukuoi Balech, 1985, p. 38, fig. 66. Sans diagnose latine. Invalide: non accepté par son auteur car donné comme synonyme postérieur d'*A. affine* (lui aussi invalide).

A. fundyense Balech, 1985, p. 37, fig. 18. Sans diagnose latine.

A. hiranoi Kita et Fukuyo, 1988, p. 2, fig. 1, pl. 1, fig. a—k.

A. ibericum Balech, 1985, p. 37, fig. 15. Sans diagnose latine.

A. insuetum Balech, 1985, p. 37, fig. 1. Sans diagnose latine.

A. kutnerae (Balech) Balech, 1985, p. 37, fig. 8 (= *Gonyaulax kutnerae*).

A. leei Balech, 1985, p. 37, fig. 9. Sans diagnose latine.

A. lusitanicum Balech, 1985, p. 37, fig. 16. Sans diagnose latine.

A. minutum Halim emend. Balech, 1989, p. 207, fig. 1—26. Sans diagnose latine (qui manquait également dans la description de Halim).

A. monilatum: Balech 1985, p. 34, fig. 3. Combinaison invalide: basionyme non indiqué (probablement *Gonyaulax monilata* Howell).

A. ostenfeldii (Paulsen) Balech et Tangen, 1985, p. 338, fig. 3, 4, 5A—D, G (= *Goniodoma ostenfeldii*).

A. peruvianum (Balech et Mendiola) Balech et Tangen, 1985, p. 342 (= *Gonyaulax peruviana*).

A. pseudogonyaulax: taxon de statut indéfini, utilisé dans une thèse de T. Horiguchi citée par Kita et Fukuyo 1988, p. 2.

A. tamarense (Lebour) Balech, 1985, p. 38, fig. 20 (= *Gonyaulax tamarensis*).

A. tropicale Balech, 1985, p. 37, fig. 7. Sans diagnose latine.

Alexandrium sp. Taylor: désignation para-taxinomique employée par Balech 1985, p. 36, fig. 14.

Genre *Amphidiniopsis* Wolosyńska

A. hirsuta (Balech) Dodge, 1982, p. 248, fig. 33A ("*hirsutum*") (= *Thecadinium hirsutum*).

A. swedmarkii (Balech) Dodge, 1982, p. 248, fig. 33D—E.

Genre *Amphidinium* Claparède et Lachmann

A. kofoidii: voir *Adenoides*.

A. poecilochroum Larsen, 1985, p. 25, fig. 38—45, 94.

A. psittacus Larsen, 1985, p. 26, fig. 46—51, 95.

A. semilunatum Herdman: voir *Thecadinium*.

Genre *Amphidoma* Stein

A. biconicum Kofoid: voir *Oxytoxum*.
Amphidoma sp.: B a l e c h 1988b, p. 174, pl. 73, fig. 9—10.

Genre *Amphisolenia* Stein

Amphisolenia sp.: B a l e c h 1988b, p. 70, pl. 18, fig. 7.

Genre *Amylax* Meunier

A. buxus (Balech) Dodge, 1989, p. 291, fig. 1L, 29 (= *Gonyaulax buxus*). Sans diagnose latine (qui manquait également dans la description de Balech).
A. triacantha (Jörgensen) Sournia, 1984, p. 350 (= *Gonyaulax? triacantha*).

Genre *Apsteinia* Abé

Nom proposé par A b é (1981, p. 49) en remplacement *d'Entzia* Lebour. Cependant, l'existence d'un homonyme antérieur dans le règne animal (*Entzia* Daday: Foraminifère) ne porte pas atteinte à la légitimité *d'Entzia* Lebour tant que ce nom est réputé végétal. *Entzia* Lebour n'est illégitime que dans le règne animal mais le nom proposé par Abé ne peut alors le remplacer, du fait de l'existence de deux homonymes antérieurs (*Apsteinia* Schmeil: Crustacé et *Apsteinia* Metcalf: Tunicier).

Genre *Blepharocysta* Ehrenberg

Blepharocysta sp.: M u r o b u s e 1937, p. 379, fig. 15 (4) ("*Blephalocysta*"). (Omis dans le "Catalogue... I".)

Genre *Boreadinium* Dodge et Hermes

B. breve (Abé) Sournia, 1984, p. 348 (= *Diplopsalis brevis*).
B. pisiforme Dodge et Hermes, 1981, p. 22, fig. 9—14 ("*pisiformis*").

Genre *Brigantedinium* Reid

B. asymmetricum Matsuoka, 1987, p. 56, fig. 4B, pl. 5, fig. 10—12.
B. grande Matsuoka, 1987, p. 55, fig. 4D, pl. 5, fig. 1—2.
B. irregulare Matsuoka, 1987, p. 56, fig. 4C, pl. 5, fig. 13—16.

Genre *Cachonina* A. R. Loeblich III

C. hallii (Freudenthal et Lee) Dodge, 1982, p. 146, fig. 17M—N (= *Glenodinium hallii*). Invalide: sans la référence de l'auteur du basionyme. Invalide: basionyme invalide (sans désignation de type et sans diagnose latine; cf. A. R. Loeblich III et al. 1981, J. Plankton Res. 3 (1) p. 77).

C. pygmaea (A. R. Loeblich III et al.) Sournia, 1984, p. 347 (= *Heterocapsa pygmaea*).

Genre *Ceratium* Schrank

C. egyptiacum f. *libanum* Abboud-Abi Saab, 1985, p. 96, fig. 1a—b. Sans diagnose latine.

C. pavillardii var. *hundhausenii* (Schröder) Guo et Ye in Guo et al., 1983, p. 89, fig. 20, pl. 2, fig. 18 pl. 3, fig. 21. Invalide: sans les références du basionyme.

C. tenue var. *buceros*: Balech 1988b, p. 149, pl. 66, fig. 2. Combinaison illégitime: publiée antérieurement par Pavillard (1916, Recherches sur les Péridiniens du golfe du Lion, Trav. Inst. Bot. Univ. Montpellier, 4, p. 18).

C. tripos neglectum: Balech 1988b, p. 139, pl. 59, fig. 5—6, attribue par erreur ce nom à "(Ostenfeld) Paulsen". Si nouveau, invalide: rang taxinomique non précisé.

C. tripos porrectum: Balech 1988b, p. 140, pl. 59, fig. 1—2, attribue ce nom à Karsten. Si nouveau, invalide: rang taxinomique non précisé.

C. tripos var. *schmidtii*: Balech 1988b, p. 197, pl. 58, fig. 7. Invalide: combinaison inédite, attribuée par erreur à Sournia.

C. vultur f. *recurvum*: Balech 1988b, p. 198, pl. 68, fig. 2—4. Invalide: combinaison inédite, attribuée par erreur à Schiller.

Genre *Cladopyxis* Stein

C. claytonii Holmes: voir *Micracanthodinium*.

Genre *Cochlodinium* Schütt

Cochlodinium sp.: Balech 1988b, p. 22, pl. 1, fig. 5.

Cochlodinium sp.: Yoki et Yoshimatsu 1989, p. 452, fig. 2.

Genre *Congruentidium* Abé

C. compressum Abé: voir *Peridinium*.

Genre *Corythodinium* A. R. Loeblich Jr et A. R. Loeblich III

Corythodinium sp. cf. *milneri*: Balech 1988b, p. 179, pl. 81, fig. 7—10 (désignation para-taxinomique). Remarque: la combinaison *C. milneri* reste inédite.

Genre *Crepidodinium* Lom et Lawler in Lom

C. cyprinodontum (Lawler) Lom, 1981, p. 7, fig. 3 (=*Oodinium cyprinodontum*).

Genre *Dinophysis* Ehrenberg

D. acuminata var. *lachmannii*: Balech 1988b, p. 40. Invalide: combinaison inédite attribuée par erreur à Paulsen.

Genre *Diplopelta* Stein ex Jörgensen

D. excentrica (Nie) Balech: Balech et al. 1984, p. 13. Si nouvelle combinaison, invalide: sans les références du basionyme (probablement *Diplopsalis excentrica*). Remarque: ces auteurs se réfèrent à une monographie de Balech "en prensa" qui, publiée en 1988, ne traite pas de ce taxon.

D. parva (Abé) Matsuoka, 1988, p. 100, fig. 2A—B, pl. 1, fig. A—K (= *Dissodium parvum*).

D. pusilla Balech et Akselman, 1988, p. 28, fig. 1—17. Sans diagnose latine.

D. steinii (Abé) Balech, 1988b, p. 187, pl. 19, fig. 14—17 (= *Diplopsalopsis steinii*).

Genre *Diplopsalis* Bergh

D. brevis Abé, 1981, p. 31, fig. 3 (12—16). Voir *Boreadinium*.

D. excentrica Nie: voir *Diplopelta* et *Gotoius*.

D. lebourae Abé, 1981, p. 28, fig. 3 (7—11). Illégitime: combinaison publiée antérieurement par Abé puis par Balech (voir Catalogue..., I).

D. lenticula f. *minor* Paulsen: voir *Diplopsalopsis minor*.

D. minuta (Abé) Abé, 1981, p. 31 (= *Lebouraia minuta*).

D. parva (Abé) Abé, 1981, p. 32 ("*parvum*") (= *Dissodium parvum*).

D. torta Abé: voir *Dissodium*.

Genre *Diplopsalopsis* Meunier

D. globulus Abé: voir *Dissodium*.

D. imitatio (Balech) Balech, 1988b, p. 80, pl. 20, fig. 3—5 (= *Protope-*

ridinium imitatio). Remarque: le basionyme a été publié sans diagnose latine.

D. minor (Paulsen) Abé, 1981, p. 38, fig 5 (23—28), 6 (29—38) = *Diplopsalis lenticula* f. *minor*.

D. orbicularis var. *orbicularis*: Abé 1981, p. 45, fig 7 (39—43). Ne constitue pas, contrairement à l'indication "n. nom.", une innovation taxinomique, mais représente la variété-type à désigner comme *D. orbicularis* (Paulsen) Meunier) var. *orbicularis*.

D. steinii Abé: voir *Diplopelta* et *Dissodium*.

Genre *Dissodium* Abé

D. globulus (Abé) Dodge et Hermes, 1981, p. 22 ("*globula*") = *Diplopsalopsis globulus*.

D. parvum Abé: voir *Diplopelta* et *Diplopsalis*.

D. sphaericum (Mangin) Dodge et Hermes, 1981, p. 22 ("*sphaerica*") = *Peridiniopsis asymmetrica* var. *sphaerica*. Remarque: ces auteurs citent par erreur le basionyme comme *P. asymmetrica* f. *sphaerica*.

D. steinii (Abé) Dodge et Hermes, 1981, p. 22 (= *Diplopsalopsis steinii*).

D. tortum (Abé) Dodge et Hermes, 1981, p. 22 ("*torta*") = *Diplopsalis torta*.

Genre *Ensiculifera* Balech

E. angulata, p. 202, pl. 83, fig. 11—14. Sans diagnose latine.

E. mexicana: voir *Scrippsiella*.

Genre *Entzia* Lebour: voir *Apsteinia*

Genre *Exuviaella* Cienkowski

E. perminuta Hada, 1942, p. 9, fig. 25. Invalide: sans description. Sans diagnose latine.

Genre *Fragilidium* Balech ex A. R. Loeblich III

F. lacustre (Lindemann) Balech, 1988, p. 483 ("*lacustris*") = *Goniodoma lacustre*.

F. mexicanum 1988a, p. 480, fig. 1—9. Sans diagnose latine.

Genre *Glenodinium* Ehrenberg

G. hallii Freudenthal et Lee: voir *Cachonina*.
G. trochoideum Stein: voir *Scrippsiella*.

Genre *Goniodoma* Stein

G. concavum Gaarder: voir *Alexandrium*.
G. depressum Gaarder: voir *Alexandrium*.
G. lacustre Lindemann: voir *Fragilidium*.
G. milneri Murray et Whitting: voir *Lingulodinium*.
G. ostenfeldii Paulsen: voir *Alexandrium* et *Protogonyaulax*.

Genre *Gonyaulax* Diesing

G. acatenella Whedon et Kofoid: voir *Alexandrium*.
G. buxus Balech: voir *Amylax*.
G. catenella Whedon et Kofoid: voir *Alexandrium*.
G. cohorticula Balech: voir *Alexandrium*.
G. fraterculus Balech: voir *Alexandrium*.
G. kutnerae Balech: voir *Alexandrium* et *Protogonyaulax*.
G. lebourae Balech, 1980, p. 102. (Omis dans Catalogue..., IV.) = *Gonyaulax orientalis* partim.
G. milneri (Murray et Whitting) Kofoid: voir *Lingulodinium*.
G. nigricans (Schiller) Balech, 1988b, p. 171, pl. 76, fig. 8—14 (= *Melanodinium nigricans*).
G. orientalis Lindemann: voir *G. lebourae*.
G. peruviana Balech et Mendiola: voir *Alexandrium*.
G. polyedra Stein: voir *Lingulodinium*.
G. tamarensis Lebour: voir Alexandrium.
G. tamarensis var. *excavata* Braarud: voir *Alexandrium excavatum*.
G. ?triacantha Jörgensen: voir *Amylax*.
Gonyaulax (*Alexandria*) sp. 1: Balech 1988b, p. 173, pl. 76, fig. 7.
Gonyaulax (*Alexandria*) sp. 2: Balech 1988b, p. 173, pl. 73, fig. 11—13.
Gonyaulax sp.: Murobuse 1937, p. 291, fig. 13 (4). (Omis dans le "Catalogue..., I").
Gonyaulax sp. A: Balech 1988b, p. 172, pl. 75, fig. 11—12.
Gonyaulax sp. B: Balech 1988b, p. 173, pl. 75, fig. 13—15.

Genre *Gotoius* Abé ex Matsuoka

G. abei Matsuoka, 1988, p. 111, fig. 6—7, pl. 5, fig. A—L.

G. excentricus (Nie) Sournia, 1984, p. 350 (= *Diplopsalis excentrica*).
G. mutsuensis Abé ex Matsuoka, 1988, p. 100. Cet auteur fournit ici la diagnose latine qui manquait à la description d'Abé 1981, p. 33, fig. 4 (17—22). Remarque: Matsuoka mentionne par erreur comme espèce-type *G. excentricus* au lieu de *G. mutsuensis*.

Genre *Gymnodinium* Stein

G. angulosum Borcakli, 1981, p. 44, pl. 7, fig. 3—6. Invalide: publication non effective (thèse). Sans diagnose latine.
G. bei Spero, 1987, p. 315, fig. 7 ("*béii*").
G. blax Harris: espèce continentale signalée pour la première fois en milieu marin par Konovalova et al. (1989).
G. bonaerense Akselman, 1985, p. 40, fig. 5—7, 12—13.
G. cyanophilum Borcakli, 1981, p. 49, pl. 4, fig. 3—4. Invalide: publication non effective (thèse). Sans diagnose latine.
G. falcatum: Yamaji 1966, p.? [ouvrage non consulté]. Erreur pour *Gyrodinium falcatum* Kofoid et Swezy.
G. fungiforme Anisimova: voir *Katodinium*.
G. nagasakiense Takayama et Adachi, 1984, p. 8, fig. 1—2, pl. 1, fig. 1—6, pl. 2, fig. 1—5 (= *Gymnodinium* type 65).
Gymnodinium cf. *nagasakiense*: Partensky et al. 1988, p. 414.
Gymnodinium sp.: Balech 1988b, p. 21, pl. 3, fig. 2.
Gymnodinium sp.: Balech 1988b, p. 21, pl. 1, fig. 7.
Gymnodinium sp.: Balech 1988b, p. 38, pl. 5, fig. 1—2.
Gymnodinium sp. A: Throndsen 1983, p. 18, fig. 70—71.
Gymnodinium sp. B: Throndsen 1983, p. 18, fig. 72—73.
Gymnodinium sp. 1: Fraga et Sanchez 1985, p. 54, fig. 7—9.
Gymnodinium sp. 1: Takayama 1985, pl. 1, fig. 1.
Gymnodinium sp. 2: Takayama 1985, pl. 1, fig. 3.
Gymnodinium type 65: voir *G. nagasakiense*.
Gymnodinium type 84K: Onoue et al. 1985, fig. 1 ("will soon be published elsewhere").

Genre *Gyrodinium* Kofoid et Swezy

G. albae-petrae Borcakli, 1981, p. 52, pl. 3, fig. 1 ("albi-petri"). Invalide: publication non effective (thèse). Sans diagnose latine.
G. falcatum Kofoid et Swezy: voir *Gymnodinium*.
G. frontignanense Borcakli, 1981, p. 53, pl. 8, fig. 5. Invalide: publication non effective (thèse). Sans diagnose latine.
G. fusiforme Kofoid et Swezy: voir *G. fusus*.

G. fusus (Meunier) Akselman, 1985, p. 45, fig. 8, 15—16 (= *Spirodinium fusus*). Remarque: ce taxon doit remplacer *G. fusiforme* Kofoid et Swezy, illégitime.
Gyrodinium sp.: Konovalova et al. 1989, p. 99, fig. 38 (3).
Gyrodinium sp.: Larsen 1985, p. 31, fig. 83.
Gyrodinium sp. A: Throndsen 1983, p. 19, fig. 78—79.
Gyrodinium sp. B: Throndsen 1983, p. 19, fig. 80—81.
Gyrodinium sp. 1: Takayama 1985, pl. 2, fig. 13.
Gyrodinium sp. 2: Takayama 1985, pl. 2, fig. 16.

Genre *Heterocapsa* Stein

H. minima Pomroy, 1989, p. 131, fig. 1.
H. pygmaea A. R. Loeblich III et al.: voir *Cachonina*.

Genre *Impagidinium* Stover et Evitt

I. aculeatum (Wall) Lentin et Williams, 1981, p. 153 (= *Leptodinium aculeatum*). Kyste attribué par Harland (1983) au "groupe" *Gonyaulax spinifera*.
I. aculeatum (Wall) Harland, 1983, p. 347, fig. 21, pl. 46, fig. 1—3 (= *Leptodinium aculeatum*). Illégitime: combinaison publiée antérieurement par Lentin et Williams (1981). Kyste attribué au "groupe" *Gonyaulax spinifera*.
I. paradoxum (Wall) Stover et Evitt, 1978, p. 166 (= *Leptodinium paradoxum*). (Omis dans les précédentes parties du "Catalogue".) Kyste attribué par Harland (1983) au "groupe" *Gonyaulax spinifera*.
I. patulum (Wall) Stover et Evitt, 1978, p. 166 (= *Leptodinium patulum*). (Omis dans les précédentes parties du "Catalogue".) Kyste attribué par Harland (1983) au "groupe" *Gonyaulax spinifera*.
I. sphaericum (Wall) Lentin et Williams, 1981, p. 154 (= *Leptodinium sphaericum*). Kyste attribué par Harland (1983) au "groupe" *Gonyaulax spinifera*.
I. sphaericum (Wall) Harland, 1983, p. 352, fig. 24, pl. 46, fig. 8—9 (= *Leptodinium sphaericum*). Illégitime: combinaison publiée antérieurement par Lentin et Williams (1981). Kyste attribué au "groupe" *Gonyaulax spinifera*.
I. strialatum (Wall) Stover et Evitt, 1978, p. 166 (= *Leptodinium strialatum*). (Omis dans les précédentes parties du "Catalogue". Kyste attribué par Harland (1983) au "groupe" *Gonyaulax spinifera*.

Genre *Katodinium* Fott

K. fungiforme (Anisimova) A. R. Loeblich III, 1965, p. 16. (Omis dans les précédentes parties du "Catalogue".) Organisme récolté originellement en milieu continental mais mentionné par la suite en milieu saumâtre ou marin (par exemple: Dodge 1982).

Genre *Lebouraia* Abé

L. minuta Abé: voir *Diplopsalis.*

Genre *Lejeunecysta* Artzner et Dörhöfer

L. concreta (Reid) Matsuoka, 1987, p. 58, fig. 1—4, pl. 7, fig. 9—10, pl. 8, fig. 1—9, pl. 9, fig. 1—4 = *Trinovantedinium sabrinum* ("*subrinum*"). Basionyme non désigné formellement, mais sans confusion possible.
L. ?epidoma Matsuoka, 1987, p. 59, pl. 9, fig. 5—6.
L. psuchra Matsuoka, 1987, p. 60, pl. 9, fig. 1—8, pl. 14, fig. 11—12.

Genre *Lejeunia* Gerlach emend. Kjellström

L. applanata Bradford, 1977, p. 47, fig. 2 (1—8). Sans diagnose latine.
L. diversiforma Bradford, 1977, p. 49, fig. 4 (1—4, 6—7). Sans diagnose latine.
L. diversiforma subsp. *muscatensis* Bradford, 1977, p. 55, fig. 6 (1—3) ("*muscatense*"). Sans diagnose latine.
Lejeunia sp. A: Bradford 1977, p. 56, fig. 4 (8).
Lejeunia sp. B: Bradford 1977, p. 56, fig. 6 (6—8).

Genre *Leptodinium* Klement

Plusieurs espèces de ce genre, initialement réputées fossiles, ont maintenant été signalées dans les sédiments actuels (Morzadec-Kerfourn 1977, Harland 1983, etc.). Elles ont toutes été transférées dans le genre Impagidinium.

L. aculeatum: voir *Impagidinium.*
L. paradoxum: voir *Impagidinium.*
L. patulum: voir *Impagidinium.*
L. sphaericum: voir *Impagidinium.*
L. strialatum: voir *Impagidinium.*

Genre *Lingulodinium* Wall

L. milneri (Murray et Whitting) Dodge, 1989, p. 294 (= *Goniodoma milneri* = *Gonyaulax milneri*).
L. polyedra (Stein) Dodge, 1989, p. 291, fig. 1H, 34—38 (= *Gonyaulax polyedra*).

Genre *Melanodinium* Schiller

M. *nigricans*: voir *Gonyaulax*.

Genre *Micracanthodinium* Deflandre

M. claytoni (Holmes) Dodge, 1982, p. 250, fig. 32L (= *Cladopyxis claytonii*). Invalide: sans la référence de l'auteur du basionyme.

Genre *Multispinula* Bradford

M. quanta Bradford: voir *Selenopemphix*.

Genre *Nematodinium* Kofoid et Swezy

Nematodinium sp. 1: Takayama 1985, pl. 3, fig. 28.
Nematodinium sp. 2: Takayama 1985, pl. 3, fig. 26—27.

Genre *Omanodinium* Bradford

O. alticinctum Bradford: voir *Selenopemphix*.
O. tholus Bradford: voir *Protoperidinium*.

Genre *Oodinium* Chatton

O. cyprinodontum Lawler: voir *Crepidodinium*.

Genre *Ostreopsis* Schmidt

O. heptagona D. R. Norris et al., 1985, p. 40, fig. 1—15.
O. lenticularis Fukuyo, 1981a, p. 970, fig. 30—34, 52—53.
O. ovata Fukuyo, 1981a, p. 971, fig. 35—38, 54—55.

Genre *Oxytoxum* Stein

O. biconicum (Kofoid) Dodge et Saunders, 1985, p. 117, fig. 70—73, 77 (F) (= *Amphidoma biconicum*). Invalide: la référence indiquée de l'auteur du basionyme est fausse.
O. pyramidale Dodge et Saunders, 1985, p. 108, fig. 30—32, 76 (B).
O. strophalatum Dodge et Saunders, 1985, p. 108, fig. 26—27, 76 (I).

Genre *Paulsenella* Chatton

P. kornmannii Drebes et Schnepf, 1988, p. 569, fig. 17—28.
P. vonstoschii Drebes et Schnepf, 1988, p. 571, fig. 29—41.

Genre *Pentadinium* Gerlach

P. membranaceum (Eisenack) Stover et Evitt, 1978, p. 180 (= *Planinosphaeridium membranaceum*). (Omis dans les précédentes parties du "Catalogue".)

Genre *Pentapharsodinium* Indelicato et A. R. Loeblich III

P. dalei Indelicato et A. R. Loeblich III, 1986, p. 158, fig. 5.
P. trachodium Indelicato et A. R. Loeblich III, 1986, p. 159.

Genre *Peridiniopsis* Lemmermann

P. asymmetrica var. *sphaerica* Mangin: voir *Dissodium sphaericum*.

Genre *Peridinium* Ehrenberg

Presque toutes les espèces marines de ce genre ont maintenant été transférées dans *Protoperidinium*. Les renvois spécifiques de *Peridinium* à *Protoperidinium* sont ici omis, sauf dans les cas où le nom de l'espèce a changé lors du transfert.

P. acutum (Fauré-Frémiet) Fauré-Frémiet ex Abé, 1981, p. 220, fig. 18 (110—118) (= *P. pellucidum* var. acutum).
P. aequilimbus Abé, 1981, p. 302 (= *P. levanderi* Abé, non *P. levanderi Lemmermann*). Illégitime: synnyme postérieur de *P. nux* Schiller.
P. angusticollum Abé, 1981, p. 204, fig. 16 (95—99) ("angusticollis"). Sans diagnose latine.
P. bidentatum Abé, 1981, p. 392, fig. 61 (414—418). Sans diagnose latine.
P. bifurca Abé, 1981, p. 346. Nomen nudum (peut-être décrit dans le même travail, p. 368, sous le nom de "*Peridinium* sp.").

P. brachypus Abé, 1981, p. 224, fig. 20 (125—131). Sans diagnose latine. Illégitime: homonyme postérieur de *P. brachypus* Schiller.

P. brasilianum: Balech 1964, p. 184. Invalide: combinaison proposée au conditionnel et sans les références du basionyme. (Omis dans le Catalogue..., I).

P. carum Abé, 1981, p. 326, fig. 46 (303—306) ("*carus*"). Sans diagnose latine.

P. cerasiforme Abé, 1981, p. 240, fig. 27 (176—179) ("*cerassiformis*"). Sans diagnose latine.

P. compressum: Abé 1981, p. 308: Illégitime: cet auteur semble vouloir introduire là une nouvelle combinaison (=*Congruentidium compressum*) mais celle-ci a été établie antérieurement par Nie.

P. consimile Abé, 1981, p. 316, fig. 42 (276—281) ("*consimilis*"). Sans diagnose latine.

P. decens Balech: voir *Protoperidinium cassum* var. *decens*.

P. expansum Abé, 1981, p. 394, fig. 62 (419—422). Sans diagnose latine.

P. furcatum Abé, 1981, p. 363, fig. 53 (360—367). Sans diagnose latine.

P. fusiforme Abé, 1981, p. 305, fig. 41 ("*fusiformis*"). Sans diagnose latine.

P. gibberum Abé, 1981, p. 254 (= *P. okamurai* Abé, non *P. okamurai* Marukawa).

P. globiferum Abé ex Abé, 1981, p. 4, fig. 13a (72—77) ("*globifera*"). Cet auteur complète ici la description très sommaire qu'il avait donnée antérieurement. Sans diagnose latine.

P. globosum (Gourret) Abé, 1981, p. 254, fig. 32 (209—214) (= *Ceratium globosum*). Illégitime: homonyme postérieur de *P. globosum* Dangeard.

P. gregarium Lombard et Capon: voir *Scrippsiella caponii*.

P. inlandicum Hada, 1972, p. 15, fig. 52 ("*inlandica*"). (Omis dans les précédentes parties du "Catalogue".) Sans diagnose latine.

P. isthmus Abé, 1981, p. 250, fig. 31 (202—208). Sans diagnose latine.

P. levanderi Abé: voir *P. aequilimbus*.

P. microangusticolle Abé, 1981, p. 206 ("*microangusticollis*"). Invalide: sans illustration. Sans diagnose latine.

P. okamurai Abé: voir *P. gibberum*.

P. parvum Abé, 1981, p. 222, fig. 19 (119—124). Sans diagnose latine.

P. paulsenii Abé, 1981, p. 359, fig. 51 (349—354). Illégitime: homonyme postérieur de *P. paulsenii* Mangin et de *P. paulsenii* Pavillard. Sans diagnose latine.

P. pellucidum var. *acutum* Fauré-Frémiet: voir *P. acutum*.

P. petersii Abé, 1981, p. 361, fig. 52 (355—359). Illégitime: homonyme postérieur de *P. petersii* Balech. Sans diagnose latine.

P. punctatum (Cleve) Abé, 1981, p. 303 (= *Steiniella punctata*).
P. rhombiforme Abé, 1981, p. 354, fig. 49 (331—335) ("*rhombiformis*"). Sans diagnose latine.
P. rhomboidale Abé, 1981, p. 235, fig. 25 (161—168 ("*rhomboidalis*"). Sans diagnose latine.
P. schilleri f. *complexum* Abé, 1981, p. 244, fig. 29 (186—191), 30 (192—201). Sans diagnose latine.
P. spinulosum Schiller: Abé 1981, p. 262, fig. 33 (215—222). Erreur pour *P. spinosum* Schiller (qui, par ailleurs, est devenu *P. spiniferum* Schiller).
P. steinii f. *breve* Paulsen: voir *Protoperidinium pyriforme* subsp. *breve*.
P. thorianum var. *planiceps* Abé, 1981, p. 301, fig. 40b (273—275). Sans diagnose latine. Voir *Protoperidinium planiceps*.
P. valgum Abé, 1981, p. 318, fig. 43 (282—284) ("*valgus*"). Sans diagnose latine.
P. yonedai Abé, 1981, p. 206, fig. 17 (103—109). Sans diagnose latine.
Peridinium sp.: Abé 1981, p. 368, fig. 9 (52) (?). Voir à propos de *P. bifurca*.
Peridinium sp.: Endo et Nagata 1984, pl. 1, fig. A—B. Remarque: il s'agit probablement d'une *Scrippsiella*.
Peridinium sp.: Hermosilla 1977, p. 23, pl. 4, fig. 1—5. (Omis dans les précédentes parties du "Catalogue".)
Peridinium sp. (Cyst-form A): Matsuoka 1976, p. 362, pl. 3, fig. 7. (Omis avec les 3 taxons suivants dans les précédentes parties du "Catalogue".)
Peridinium sp. (Cyst-form B): Matsuoka 1976 p. 363, pl. 3, fig. 5.
Peridinium sp. (Cyst-form C): Matsuoka 1976 p. 363, pl. 3, fig. 13.
Peridinium sp. (Cyst-form D): Matsuoka 1976 p. 364, pl. 3, fig. 6.

Genre *Phalacroma* Stein

P. kofoidii: voir *Thecadinium*.
Phalacroma sp. (1): Murobuse 1937, p. 290, fig. 12 (9). (Omis, comme le taxon suivant, dans le "Catalogue..., I".)
Phalacroma sp. (2): Murobuse 1937, p. 290, fig. 13 (10).

Genre *Pheopolykrikos* Chatton emend. Matsuoka et Fukuyo

P. beauchampii Chatton: voir *Polykrikos*.
P. hartmannii (Zimmermann) Matsuoka et Fukuyo, 1986, p. 811, fig. 1—22. Remarque: basionyme indiqué implicitement et sans confusion possible.

Genre *Phtanoperidinium* Drugg et A. R. Loeblich Jr

Cf. *Phtanoperidinium* sp. A: Bradford 1977, p. 57, fig. 6 (5).
Cf. *Phtanoperidinium* sp. B: Bradford 1977, p. 58, fig. 6 (4).

Genre *Planinosphaeridium* Eisenack

P. membranaceum Eisenack: voir *Pentadinium*.

Genre *Planodinium* Saunders et Dodge

P. striatum Saunders et Dodge, 1984, p. 278, 282, fig. 5—6, 24—30.

Genre *Plectodinium* Biecheler

P. nucleovelatum: Balech 1988b, p. 199, pl. 82, fig. 25—27. Erreur orthographique pour *P. nucleovolvatum*.

Genre *Polykrikos* Bütschli

P. beauchampii (Chatton) Dodge, 1982, p. 117, fig. 14E—F (= *Pheopolykrikos beauchampii*). Invalide: Combinaison publiée antérieurement par A. R. Loeblich III (voir "Catalogue..., IV").

Genre *Prorocentrum* Ehrenberg

P. concavum Fukuyo, 1981a, p. 968, fig. 13—19, 49.
P. emarginatum Fukuyo, 1981a, p. 968, fig. 8—12, 48.
P. foveolatum Croome et Tyler, 1987, p. 71, fig. 3—4, 12—16 ("*foveolata*").
P. perminutum: Fukuyo in Iwasaki et al. 1981, p. 21. Erreur pour *Exuviaella perminuta* Hada ou nouvelle combinaison, en ce cas invalide: sans les références du basionyme.
P. playfairii Croome et Tyler, 1987, p. 68, fig. 2, 5—11.
P. venetum Tolomio et Cavolo, 1985, p. 348, fig. 1—8.

Genre *Protoceratium* Bergh

Protoceratium sp.: Balech 1988b, p. 162, pl. 73, fig. 7—8.

Genre *Protogonyaulax* F. J. R. Taylor

P. affinis Inoue et Fukuyo in Fukuyo et al., 1985, p. 30, fig. 1E, 3A—C, 24—29.

P. compressa Fukuyo et al., 1985, p. 30, fig. 3D—F, 30—33.

P. kutnerae (Balech) Sournia, 1984, p. 349 (= *Gonyaulax kutnerae*).

P. leei (Balech) Fukuyo et al., 1988, p. 13, pl. 2, fig. 1—5 (= *Alexandrium leei*).

P. ostenfeldii (Paulsen) Fraga et Sanchez, 1985, p. 52, fig. 5—6 (= *Goniodoma ostenfeldii*).

Protogonyaulax sp. (HIR) SR-749: Fukuyo 1981b, p. 3, fig. 1—6.

Protogonyaulax sp. (HIR) SR-779: Fukuyo 1981b, p. 4, fig. 1—4.

Protogonyaulax sp. (HIR) SRRC-807: Fukuyo 1981b, p. 5, fig. 1—6.

Protogonyaulax sp. (HIR) AM-798-2: Fukuyo 1981b, p. 14, fig. 1—5.

Protogonyaulax sp. (ISH) OK-7910: Fukuyo 1981b, p. 35, fig. 1—5.

Genre *Protoperidinium* Bergh

Voir note à propos du genre *Peridinium*. Dans le cas des transferts de *Peridinium* à *Protoperidinium*, on omet ici de rappeler le basionyme, sauf dans les cas où le nom de l'espèce a changé au cours du transfert.

P. balechii (Akselman) Balech, 1988b, p. 90, pl. 29, fig. 1—3.

P. capurroi subsp. *subpellucidum* (Balech) Balech, 1988b, p. 118, pl. 50, fig. 1—5. Remarque: le basionyme a été publié sans diagnose latine.

P. cassum var. *decens* (Balech) Balech, 1988b, p. 96, pl. 34, fig. 11—13 (= *Peridinium decens*). Remarque: le basionyme a été publié sans diagnose latine.

P. divaricatum (Meunier) Balech, 1988b, p. 91, pl. 28, fig. 11—13, pl. 30, fig. 6—9. Illégitime: combinaison publiée antérieurement par Parke et Dodge (voir Catalogue..., III).

P. dolichoporum Borgese, 1987, p. 333, fig. 1—2, 7, etc. Sans diagnose latine.

P. hangoei (Schiller) Lewis et al., 1984, p. 28, fig. 2i—j, pl. 1, fig. 7.

P. hemisphaericum (Abé) Balech, 1988b, p. 84, pl. 22, fig. 8, 10.

P. imitatio Balech: voir *Diplopsalopsis*.

P. joergensenii var. *luculentum* Balech, 1988b, p. 95, pl. 32, fig. 13—17. Sans diagnose latine.

P. *levanderi*: Balech, 1988b, p. 84. Erreur pour *Peridinium levanderi*.

P. maranense Tolomio, 1982, p. 383, fig. 1—13.

P. obtusum (Karsten) Balech, 1988b, p. 88, pl. 28, fig. 3—6. Illégitime: combinaison publiée antérieurement par Parke et Dodge (voir Catalogue..., III).

P. ovatum subsp. *asymmetricum* (Dangeard) Balech, 1988b, p. 100, pl. 36, fig. 14—18 (= *Peridinium ovatum* var. *asymmetricum*).

P. pacificum (Kofoid et Michener) F. J. R. Taylor et Balech ex Balech, 1988b, p. 107, 203, pl. 40, fig. 13—18.

P. parthenopes Zingone et Montresor, 1988, p. 119, fig. 3—6.

P. planiceps (Abé) Balech, 1988b, p. 203 (= *Peridinium thorianum* var. *planiceps*). Basionyme cité incorrectement par Balech, mais sans confusion possible. Remarque: le basionyme a été publié sans diagnose latine.

P. pouchetii (Kofoid et Michener) F. J. R. Taylor et Balech ex Balech, 1988b, p. 190, pl. 86, fig. 1—4.

P. pyriforme subsp. *breve* (Paulsen) Balech, 1988b, p. 94, pl. 31, fig. 20—21 (= *Peridinium steinii* f. *breve*).

P. subcrassipes Balech, 1988b, p. 111, pl. 43, fig. 8—11. Sans diagnose latine.

P. tholus (Bradford) Bradford et Wall, 1984, p. 49, pl. 2, fig. 15—16 (= *Omanodinium tholus*).

P. tumidum (Okamura) Balech, 1988b, p. 191, pl. 86, fig. 5—7.

P. venustum var. *facetum* Balech, 1988b, p. 189, pl. 84, fig. 13—16. Sans diagnose latine.

Protoperidinium (*Archaeperidinium*) sp. 1: Balech 1988b, p. 85, pl. 20, fig. 9—11.

Protoperidinium (*Archaeperidinium*) sp. II: Balech 1988b, p. 85, pl. 20, fig. 12—14.

Protoperidinium cyst, *Lejeunia paratenella* Benedek: Harland 1982, pl. 40, fig. 1—8.

Protoperidinium sp. A: Balech 1988b, p. 113, pl. 35, fig. 5—7.

Protoperidinium sp. B: Balech 1988b, p. 114, pl. 46, fig. 4.

Protoperidinium sp. C: Balech 1988b, p. 114, pl. 46, fig. 5—6.

Protoperidinium sp. (cf. *globifera* Abé?): Balech 1988b, p. 189, pl. 85, fig. 8—10.

Protoperidinium sp. D: Balech 1988b, p. 114, pl. 46, fig. 7—9.

Protoperidinium sp. E: Balech 1988b, p. 114, pl. 46, fig. 10—11.

Protoperidinium sp. F: Balech 1988b, p. 114, pl. 46, fig. 12—13.

Protoperidinium sp. G: Balech 1988b, p. 114, pl. 46, fig. 14—16.

Protoperidinium sp. H: Balech 1988b, p. 115, pl. 46, fig. 17—20.

Protoperidinium sp. I: Balech 1988b, p. 122, pl. 52, fig. 6—9.

Protoperidinium sp. J: Balech 1988b, p. 122, pl. 52, fig. 10—12.

Protoperidinium sp. K: Balech 1988b, p. 122, pl. 52, fig. 13—15.

Indet. *Protoperidinium* cyst, specimen MPK 2950: Harland 1982, pl. 42, fig. 2.

Indet. *Protoperidinium* cyst, specimen MPK 2957: Harland 1982, pl. 42, fig. 9.

Indet.? *Protoperidinium* cyst, *Xandarodinium xanthum* Reid: Harland 1982, pl. 39, fig. 4—5.
?Indet. *Protoperidinium* cyst: Harland 1982, pl. 38, fig. 10—12.

Genre *Sabulodinium* Saunders et Dodge

S. inclinatum (Balech) Saunders et Dodge, 1984, p. 280 (= *Thecadinium inclinatum*).
S. undulatum Saunders et Dodge, 1984, p. 278, 282, fig. 7, 31—35.

Genre *Schizochytriodinium* Elbrächter

S. calani Elbrächter, 1988, p. 598, fig. 1—5.

Genre *Schuettiella* Balech

S. mitra (Schütt) Balech, 1988b, p. 174, pl. 78, fig. 1—17 (= *Steiniella mitra*). Sans diagnose latine.

Genre *Scrippsiella* Balech ex A. R. Loeblich III

S. arenicola Horiguchi et Pienaar, 1988b, p. 426, fig. 1—37.
S. caponii Horiguchi et Pienaar, 1988a, p. 38 (= *Peridinium gregarium*, non *Scrippsiella gregaria*).
S. hexapraecingula Horiguchi et Chihara, 1983b, p. 357, fig. 1—17.
S. mexicana (Balech) Indelicato et A. R. Loeblich III, 1986, p. 159, fig. 11 (= *Ensiculifera mexicana*). Ces auteurs fournissent ici la diagnose latine que Balech avait omise.
S. precaria Montresor et Zingone, 1988, p. 388, fig. 1—10.
S. tinctoria Indelicato et A. R. Loeblich III, 1985, p. 128, fig. 1.
S. trochoidea (Stein) Bujak et Davies, 1983, p. 163 (= *Glenodinium trochoideum*). Illégitime: combinaison publiée antérieurement par A. R. Loeblich III (voir Catalogue..., III).
Scrippsiella sp.: Gao et al. 1989, p. 155, fig. 1—23.

Genre *Selenopemphix* Benedek

S. alticincta (Bradford) Matsuoka, 1985, p. 52, pl. 15, fig. 6—10 (= *Omanodinum alticinctum*). Kyste de *Protoperidinium subinerme*.

S. quanta (Bradford) Matsuoka, 1985, p. 51, pl. 11, fig. 1—9 (= *Multispinula quanta*). Kyste de *Protoperidinum conicum*.

S. nephroides Benedek: Matsuoka, 1987, p. 61, pl. 10, fig. 1—9. Kyste initialement décrit comme fossile, mentionné ici dans les sédiments actuels et supposé attribuable à un *Protoperidinium*.

Genre *Spiniferites* Mantell

S. asperulus Matsuoka: Matsuoka 1985, p. 28, pl. 4, fig. 7—8. Kyste initialement décrit comme fossile, mentionné ici dans les sédiments actuels et attribué à un *Gonyaulax*.

Genre *Spiniferodinium* Horiguchi et Chihara

S. galeiforme Horiguchi et Chihara, 1987, p. 479, fig. 4—5.

Genre *Spirodinium* Schütt

S. fusus Meunier: voir *Gyrodinium*.

Genre *Steiniella* Schütt

S. mitra Schütt: voir *Schuettiella*.

S. punctata Cleve: voir *Peridinium*.

Genre *Stelladinium* Bradford

S. abei Matsuoka, 1985, p. 59, pl. 14, fig. 7—10. Kyste de *Protoperidinium*?

Genre *Stylodinium* Klebs

S. litorale Horiguchi et Chihara, 1983a, p. 23, fig. 1—10 ("*littorale*").

Genre *Syltodinium* Drebes

S. listii Drebes, 1988, p. 584, fig. 1—11.

Genre *Symbiodinium* Freudenthal emend. Trench et Blank

S. goreaui Trench et Blank, 1987, p. 479, fig. 3a—f ("*goreauii*").

S. kawagutii Trench et Blank, 1987, p. 479, fig. 4a—b.

S. pilosum Trench et Blank, 1987, p. 479, fig. 5a—c.
Symbiodinium sp.: Blank 1986, p. 278, fig. 1d, 2.

Genre *Thecadinium* Kofoid et Skogsberg

T. hirsutum Balech: voir *Amphidiniopsis.*
T. inclinatum Balech: voir *Sabulodinium.*
T. kofoidii (Herdman) Larsen, 1985, p. 17, fig. 4—6 (= *Phalacroma kofoidii*).
T. neopetasatum Saunders et Dodge, 1984, p. 275, 282, fig. 19—23.
T. semilunatum (Herdman) Dodge, 1982, p. 58, fig. 5D (= *Amphidinium semilunatum*).
T. swedmarkii Balech: voir *Amphidiniopsis.*

Genre *Thoracosphaera* Komptner

Considéré jusque-là comme un coccolithophoride, ce genre a été transféré dans les Dinoflagellés par Fütterer (1976), suivi par Tangen et al. (1982).

Genre *Trinovantedinium* Reid emend. Harland

T. concretum Reid: voir *Lejeunecysta.*
T. pallidifulvum Matsuoka, 1987, p. 63, pl. 13, fig. 1—9.

Genre *Warnowia* Lindemann

Warnowia sp. 1: Takayama 1985, pl. 3, fig. 20—21.
Warnowia sp. 2: Takayama 1985, pl. 3, fig. 22—23.
Warnowia sp. 3: Takayama 1985, pl. 3, fig. 24—25.

Dinoflagellés et kystes de Dinoflagellés indéterminés

?Cyst form A: Bradford et Wall 1984, p. 50, pl. 6, fig. 14.
?Cyst form B: Bradford et Wall 1984, p. 51, pl. 6, fig. 18.
?Cyst form C: Bradford et Wall 1984, p. 51, pl. 6, fig. 15—17.
Dinoflagellate cyst type A: Matsuoka 1985, p. 65, pl. 15, fig. 11. Kyste de Diplopsalinae?
Dinoflagellate cyst type A: Matsuoka 1987, p. 66, pl. 3, fig. 9—11, etc.
Dinoflagellate cyst type B: Matsuoka 1985, p. 65, pl. 14, fig. 11—12. Kyste de *Protoperidinium*?

Dinoflagellate cyst type B: Matsuoka 1987, p. 68, pl. 18, fig. 3—11, pl. 19, fig. 11—14.
Dinoflagellate cyst type C: Matsuoka 1985, p. 66, pl. 17, fig. 7—12. Probablement un kyste de *Protoperidinium*.
Dinoflagellate cyst type C: Matsuoka 1987, p. 69, pl. 19, fig. 1—2, 7—10.
Dinoflagellate cyst type D: Matsuoka 1985, p. 66, pl. 17, fig. 5—6. Kyste de Peridiniaceae.
Dinoflagellate cyst type D: Matsuoka 1987, p. 50, pl. 19, fig. 3—6, 11—12.
Dinoflagellate cyst type E: Matsuoka 1985, p. 67, pl. 16, fig. 13—14. Kyste de *Peridinium*.
Dinoflagellate cyst type E: Matsuoka 1987, p. 69, pl. 14, fig. 8—9.
Quelques dinoflagellés nus [...]: Grall et Jacques 1982, p. 119, fig. 1 (1—15).
Quelques dinoflagellés à thèque [...]: Grall et Jacques 1982, p. 119, fig. 2 (16—29).
Strain Y-100: Watanabe et al. 1987, p. 383, fig. 1—6.

SUMMARY

The new taxonomic entries published between 1981—82 and 1989 are listed here in the alphabetical order of the generic and specific names. This "Supplement" does not contain itself any new taxonomic entry. The general purpose of the "Catalogue" is kept unchanged, that is: to provide a first-aid index that should help to update the first (and last) world monograph by Schiller (1931—1937). This "Catalogue" is not a critical one in the sense that no consideration is paid to the subjective significance of the morphological or biological features of the protists considered. However, the taxonomic status of the names as regards the rules of botanical nomenclature is mentioned where failings occur.

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Contribution á la connaissance des Thecamoebiens aquatiques de Laponie suédoise (*Rhizopoda, Testacea*)

Didier CHARDEZ

Unité de Zoologie Générale et de Faunistique (Prof. Ch. Gaspar), Faculté des Sciences Agronomiques de l'Etat, Gembloux, Belgique

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Synopsis. Dans un sédiment lacustre, constitué principalement de Diatomées, provenant de Laponie duédoise, nous avons relevé la présence de vingt neuf espèces de Thecamoebiens, dont certaines sont rares ou peu connues.

Quelques travaux traitent déjà de la faune rhizopodiques de Laponie, Harnisch (1938), Thieneman (1938), Grospietsch (1954), ces auteurs ont principalement étudié la faune thecamoebienne des Mousses et des Sphaignes. Decloitre (1956), a publié une liste de 67 taxons présents dans différentes régions de Laponie.

Schönborn (1966), recense 134 espèces et variétés observées dans ces régions et il en donne d'importantes informations écologiques.

Dans une courte note (Chardez 1979), nous avions signalé la présence de quelques espèces aquatiques.

Dans le présent travail, nous nous proposons d'étudier les espèces observées dans une série de prélèvements d'un sédiment aquatique particulier.

Cette étude essenciellement faunistique se bornera a compléter les listes déjà émises et a révéler quelques espèces intéressantes, rares ou peu connues, ainsi que celles qui jusqu'à présent, n'ont été observées que dans ces régions.

Matériel et méthode

Prélèvements de sédiments aquatiques près d'une source de la rivière STUOR JERTA, située dans le Parc National SAREK, altitude 1400 m.

Ces prélèvements sont contitués d'un substrat formé presqu'exclusivement de Diatomées.

Les Diatomées formant l'essenciel du sédiment lacustre, constitue une niche écologique pour une population importante de Thecamoebiens.

Ces Diatomées sont représentées par de nombreuses espèces des genres: *Achnantes, Eunotia, Navicula, Fragillaria, Frustulia, Pinnularia, Stauroneis* et *Caloneis.*

Les échantillons ont été fixés au formol neutre, les Thecamoebiens triés à la micropipettes au microscope inversé ont été montés en préparations dans l'Euparal.

Liste des espèces

Les espèces marquées *, sont nouvelles pour la faune de Laponie.

Centropyxis sylvatica (Deflandre) Thomas
Centropyxis aerophila Deflandre
* *Centropyxis pontigulasiformis* Beyens et Chardez
Difflugia bacillifera Penard
* *Difflugia septentrionalis* Awerintzew
Lesquereusia spiralis (Ehrenberg) Bütschli
Lesquereusia epistomium Penard
Nebela militaris Penard
Nebela lageniformis Penard
* *Nebela retorta* (Leidy) Stepanek
Nebela penardiana Deflandre
Nebela dentistoma Penard
Nebela dentistoma v. *major* Grospietsch
Nebela wailesi Deflandre
* *Nebela nobilis* (Cash) Deflandre
Nebela collaris (Ehrenberg) Leidy
Nebela tubulata Brown
Nebela minor Penard
* *Hyalosphenia humicola* Decloitre
Euglypha cristata Leidy
* *Euglypha bryophila* Brown
Euglypha loevis Perty
Euglypha rotunda Wailes
Trinema enchelys (Ehrenberg) Leidy
Trinema lineare Penard
Trinema complanatum Penard
Sphenoderia lenta Schlumberger
Phryganella acropodia (Hertwig et Lesser) Hopkinson

Remarques sur quelques espèces

Les dimensions relevées sont données en μm. (n) — nombre d'individus mesurés.

Nebel aspeciosa Deflandre (Pl. I 1; Pl. II 15A)

Cette grande espèce se distingue de *N. penardiana* (Pl. I 3; Pl. II 15B) par sa grande taille et par les lèvres du pseudostome peu développées ou absentes.

Les longueurs de la thèque varient ici de 215 à 275 avec quelques individus atteignant 310 µm. (n) — 10.

Nebela retorta (Leidy) Stepanek (Pl. I 4)

Longueur — 125—150, diamètre — 56—70, pseudostome — 20—25. (n) — 15.

Nebela tubulata Brown (Pl. I 7)

Longueur — 50—74, largeur — 24—40, pseudostome — 8—14. (n) — 10.

Nebela dentistoma v. *major* Grospietsch (Pl. I 5)

Longueur — 180—240, largeur — 90—112, pseudostome — 40—48. (n) — 5.

Nebela nobilis (Cash) Deflandre (Pl. I 2)

Syn.: *Hyalosphenia nobilis* Cash, 1908.

Cette espèce était connue de Grande Bretagne. Playfair (1917), cite une variété comprimée d'Australie. Nous l'avons observée (1986) dans quelques échantillons de Mousses aquatiques du Spitsberg et de l'Ile Edgeoya (Arctic).

Longueur — 211—216, diamètre — 100—112, pseudostome — 38—40; col — 60—70. (n) — 5.

Hyalosphenia humicola Decloitre (Pl. I 6)

Les quelques spécimens observés, correstondent parfaitement à la description qu'en donne Decloitre (1973), cet auteur l'a observée dans l'humus d'une pinède en France. C'est une des plus petites espèces du genre Hyalosphenia.

Centropyxis pontigulasiformis Beyens et Chardez (Pl. II 11—13)

La thèque est parfaitement sphérique, le pseudostome simple perforation circulaire, il est surmonté d'une large visière hémisphérique de protection.

Cet ensemble censtitue une thèque faite de deux parties distinctes.

La structure de la thèque est constituée d'un ciment organique incorporant de très fines particules minérales et des petites Diatomées remaniées.

Longueur — 76—86, diamètre — 26—30, pseudostome — 8—10, visière — 26—30. (n) — 6.

Nous avons décrit cette espèce de l'Ile Edgeøya (Arctic), (1986) où elle était abondante dans des Mousses épigées et humides.

Difflugia septentrionalis Awerintzew (Pl. II 8, 9)

Hauteur totale — 135—150, diamètre du corps — 70—85; col — 55—65. (n) — 10

Difflugia bacillifera Penard (Pl. II 10)

C'est à cette espèce que nous assimilons les spécimens observés, malgré une morphologie générale qui ne correspond pas au type.

Longueur — 113—146, diamètre — 67—80, pseudostome — 28—33. (n) — 7.

Conclusion

La population thecamoebienne de ce milieu particulier, représenté ici par un véritable tapis de Diatomées sédimentées (Pl. II 14), donne une idée des associations et des microbiocénoses possibles chez les Thecamoebiens.

L'importance de ces Diatomées, présente un certain intéret biologique, non seulement par leur nombre d'espèce et de genres, mais également par les variantes également nombreuses.

Plusieurs de ces Diatomées sont souvent présentes dans les tourbières et les sources et ont généralement une répartition assez large.

Nous avons relevé 29 espèces, dont quantitativement les *Nebela* représentent plus de 70% du nombre total d'individus.

Centropyxis sylvatica, C. potigulasiformis, Euglypha cristata, E. bryo-

phila et *Trinema enchelys* sont fréquentes dans les Mousses, mais également dans les Sphaignes.

Le nombre important de *Nebela* habituellement sphagnicoles, indique assez clairement qu'il s'agit ici d'une eau katharobe riche en oxygène.

Dans l'ensemble, il sagit d'espèces acidophiles.

Difflugia bacillifera et *septentrionalis* sont des espèces aquatiques qui utilisent normalement des Diatomées pour consolider leur theque, éléments exogènes le plus abondant dans cette station biotope.

Cette population d'espèces acidophiles est caractéristique des eaux pures.

SUMMARY

The results about the Testate Amoebae, from Lacustrian sediment of Diatom frustules from Lapland produced the list containing twenty nine species, Six new species are cited for Lapland.

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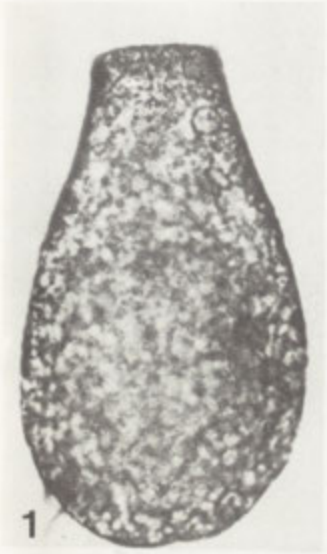
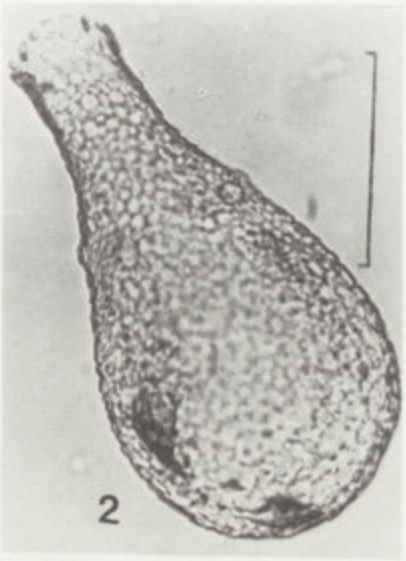
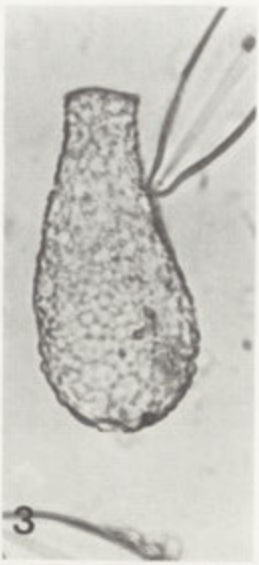
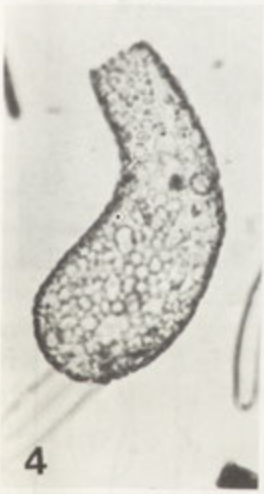
EXPLICATION DE PLANCHE I—II

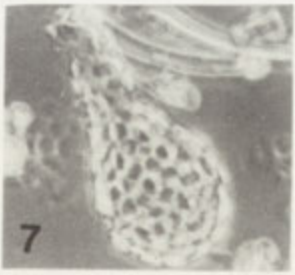
1: *Nebela speciosa* (× 400)
2: *N. nobilis* (× 400)
3: *N. penardiana* (× 400)
4: *N. retorta* (× 400)
5: *N. dentistoma* v. *major* (× 400)
6: *Hyalosphenia humicola* (× 400)
7: *Nebela tubulata* (× 400)
8—9: *Difflugia setrentrionalis* (× 400)
10: *D. bacillifera* (× 400)
11: *Centropyxis pontigulasiformis* (× 400)
12: *C. pontigulasiformis*, vue ventrale (× 600)
13: *C. pontigulasiformis*, vue de profil (× 600)
14: Diatomée sédimentée (× 100)
15: Types de silhouttes de *Nebela*, A — *N. speciosa*, B — *N. penardiana*, C — *N. lageniformis*, D — *N. wailesi*
Bar — 100 μm











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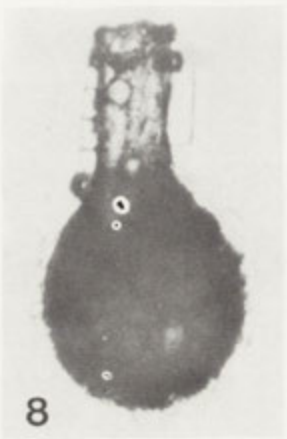
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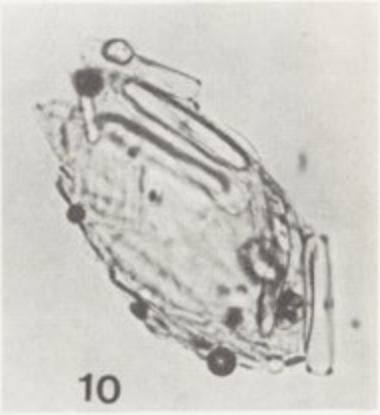
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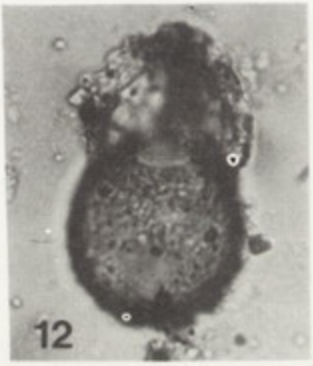
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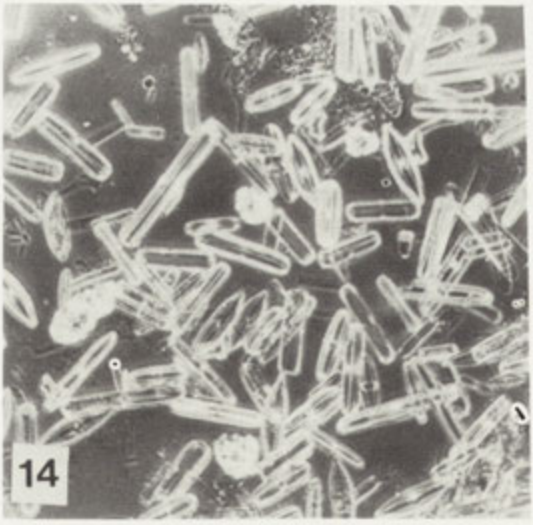
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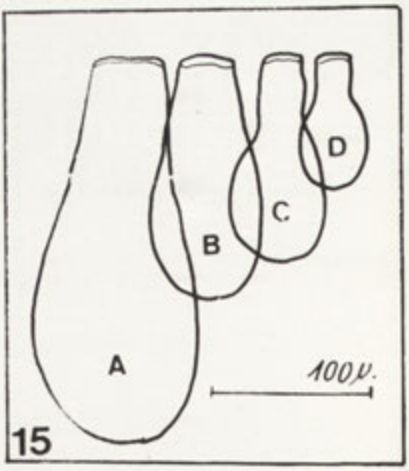

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The Occurrence of Monocystid Gregarines in Some Polish Earthworms

Václav PIŽL

Institute of Soil Biology, Czechoslovak Academy of Sciences, Na sádkách 7, CS-370 05 České Budějovice, Czechoslovakia

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Synopsis. Nineteen monocystid species, thirteen new to Poland, are reported from 11 species of earthworms. Diagnosis, hosts and data from literature about its distribution are given for each species.

Monocystid gregarines (*Protozoa, Apicomplexa, Monocystidae*) have been studied by many authors (see Levine 1977), especially from taxonomic point of view. Over 190 species of these parasites, belonging to twenty six genera, have been described from invertebrates all over the world, 108 of which from lumbricid earthworms. Some of the species are widely distributed and occur in a number of hosts, while others are more restricted in their range. However, our present knowledge of geographical distribution of this group is very uncomplete. For instance, 46 species have been indentified from British (Segun 1971 a, b), 30 from Czechoslovak (Pižl 1989 a, b) and 24 from Bulgarian (Duhlinska 1977 a, b) earthworms, but no records are known from many other countries. Up to now, one paper only has been published reporting Polish records of monocystids. This was Marek (1967), who investigated monocystid gregarines in seminal vesicles of two earthworm species, *Lumbricus terrestris* L., 1758 and *L. rubellus* Hoffm., 1843, collected from Wrocław and the neighbourhood. He listed ten gregarines with short notes on their status.

During the course of my stay in Poland in May—June 1988 aimed at the investigation of earthworm populations in various forest eco-

systems and urban green areas and the incidence level of their infection by parasites, nineteen monocystid gregarines were found. The present contribution is a review of these species.

Material and Methods

In all, 357 adult specimens of lumbricid earthworms were collected at 13 localities in Poland during May 24th — June 8th 1988. The main sampling areas are shown in Fig. 1. The earthworms examined belong to following eleven species: *Allolobophora chlorotica* (Savigny, 1826), *Aporrectodea caliginosa* (Savigny, 1826), *A. rosea* (Savigny, 1926), *Dendrobaena octaedra* (Savigny, 1826), *Dendrodrilus rubidus* (Savigny, 1826), *Eiseniella tetraedra* (Savigny, 1826), *Helodrilus antipae tuberculatus* (Černosvitov, 1935), *Lumbricus castaneus* (Savigny, 1826), *L. rubellus Hoffmeister*, 1843, *L. terrestris* Linnaeus, 1758 and *Octolasion lacteum* (Örley, 1881).

After the earthworms were identified, each was opened by a median dorsal incision and the body cavity, nephridia, blood vessels, spermathecae and seminal vesicles were examined for monocystid gregarines. Subsequently, smears were prepared from the lobes of seminal vesicles. The majority of worms was studied alive immediately after they were collected, the remainder (from localities Puszcza Białowieska and Białowieża National Park) was studied fixed in 2—4% formalin.

Isolated trophozoites were observed with the aid of a Amplival or Jenamed Variant microscope and their dimensions recorded. Occasionally made permanent smears, used in the study of morphological details, were fixed in sublimat-alcohol, stained in Ehrlich's hematoxylin, counterstained with eosin and mounted in Canada balsam.

Fig. 1. Poland, study areas: *1* — Warsaw, *2* — Klembów, *3* — Bory Tucholskie, *4* — Puszcza Białowieska and Białowieża National Park

List of recorded gregarines

Monocystis acuta Berlin, 1924 (Fig. 2)

Host: *Lumbricus rubellus* Hoffm., 1843
Habitat: seminal vesicles
Locality: Klembów 3.VI.1988

Trophozoites elongated, worm-like shaped, without any foot-like body ending. Body length 270—296 μm, breadth 28—32 μm, nucleus oval, 23—31 μm × 9—12 μm, nucleolus 4.5—6 μm, paraglycogen granules ovoid, 4.2 × 2.7 μm. Body dimensions of observed specimens were somewhat smaller than those recorded by Marek (1967), all other characteristics correspond to both Berlin's (Berlin 1924) and Marek's (Marek 1967) descriptions.

Distribution: Sweden (Berlin 1924); previously found in Poland by Marek (1967)

Monocystis agilis Stein, 1848 (Fig. 3)

Host: *Lumbricus castaneus* (Sav., 1826), *L. rubellus Hoffm.*, 1943, *L. terrestris* L., 1758
Habitat: seminal vesicles
Locality: Bory Tucholskie, Łoboda 25.V.1988, Krąg, 26.V.1988; Warsaw, Cemetery of Soviet Soldiers 30.V.1988, Łazienki Park 31.V.1988, Saski Park 1.VI.1988; Białołęka Dworska 1.VI.1988; Klembów 3.VI.1988; Puszcza Białowieska, 6.VI.1988; Białowieża National Park, 7.VI.1988

Elongate body of trophozoites possessing characteristic "foot" on the anterior and varied from 140 to 300 μm in length, maximum breadth 52 μm. Length of nucleus 12 μm, breadth 6—10 μm, nucleolus 5—6 μm in diameter, paraglycogen granules oval, 2—3 × 5 μm.

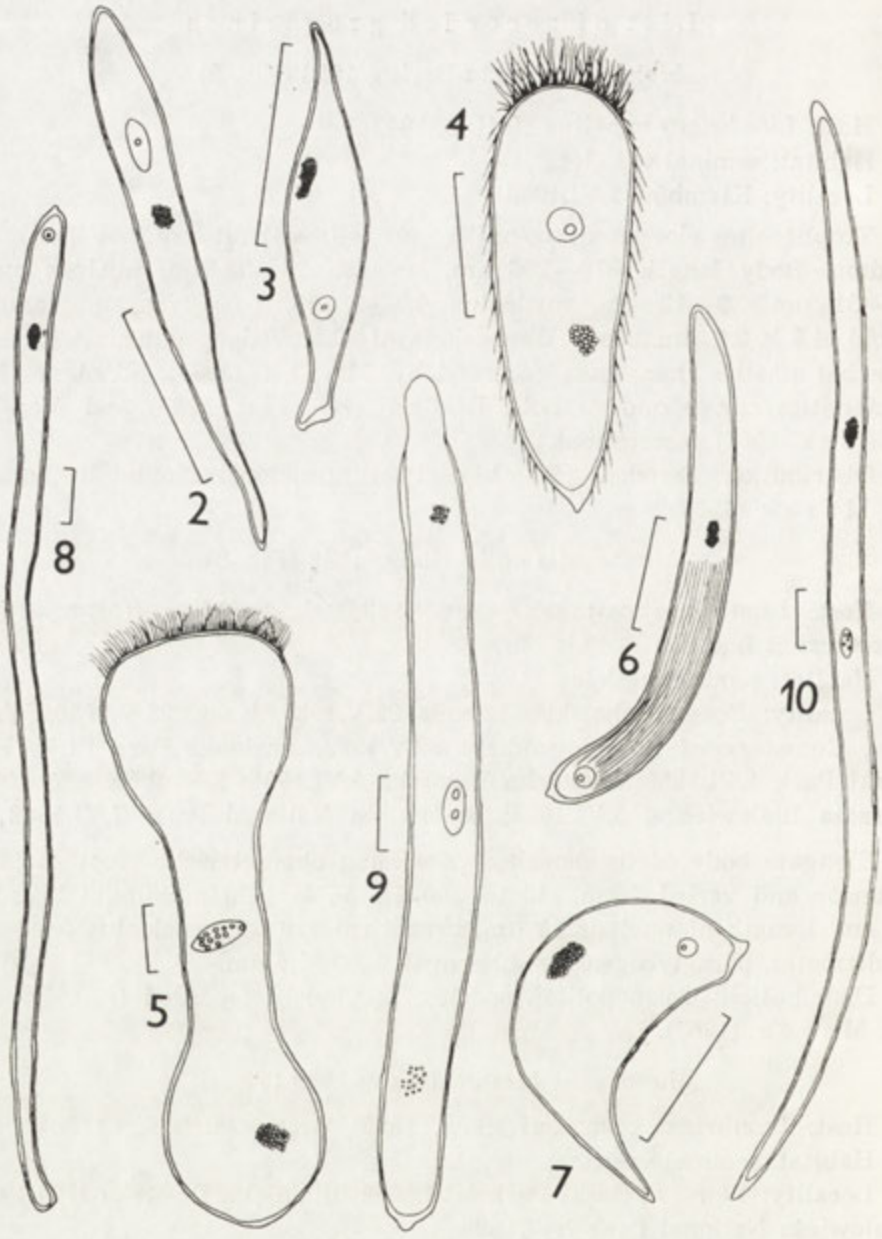
Distribution: Cosmopolitan species; previously recorded from Poland by Marek (1967).

Monocystis hirsuta Hesse, 1909 (Fig. 4)

Host: *Lumbricus castaneus* (Sav., 1826), *L. terrestris* L., 1758
Habitat: seminal vesicles
Locality: Warsaw, Saski Park 1.VI.1988, Białołęka Dworska 1.VI.1988, Białowieża National Park 7.VI.1988

Trophozoites elipsoidal or elongated oval-shaped covered with dense ectoplasmic processes. Body length 120—310 μm, breadth 40—160 μm. Spherical nucleus 20—30 μm in diameter with large elipsoidal nucleus, 7—8 μm. Paraglycogen granules oval, 2—3 × 4—6 μm.

Distribution: Czechoslovakia (Pižl 1989a), France (Hesse 1909), Germany (Meier 1956); for the first time recorded in Poland.



Figs. 2—10: 2 — *Monocystis acuta*, 3 — *M. agilis*, 4 — *M. hirsuta*, 5 — *M. lumbrici*, 6 — *M. striata*, 7 — *M. ventrosa*, 8 — *Nematocystis elmassiani*, 9 — *N. magna*, 10 — *N. vermicularis* (scale — 0.1 mm)

Monocystis lumbrici (Henle, 1845) (Fig. 5)

Host: *Lumbricus terrestris* L., 1758
Habitat: seminal vesicles
Locality: Białołęka Dworska 1.VI.1988, Kraków 3.VI.1988

Trophozoites oval and cylindrical in shape with sets of hairlike processes terminally. Body size: length 720—1210 μm, breadth 90—295 μm. Length of nucleus 42—95 μm, breadth of nucleus 28—50 μm. Paraglycogen granules 2 × 3 μm. Several small nucleoli.

Distribution: Cosmopolitan species; previously found in Poland by Marek (1967).

Monocystis striata Hesse, 1909 (Fig. 6)

Host: *Lumbricus rubellus*, Hoffm., 1843
Habitat: seminal vesicles
Locality: Bory Tucholskie, Łoboda 26.V.1988, Krąg 26.V.1988

Trophozoites long and narrow, with longitudinal ectoplasmic striations. Length 360—470 μm, breadth 30—40 μm, length of nucleus 24 μm, paraglycogen granules oval, 5 × 2.5 μm.

Distribution: Czechoslovakia (Pižl 1989a), Bulgaria (Duhlinska 1977b), England (Miles 1963), France (Hesse 1909), Germany (Meier 1956); for the first time recorded in Poland.

Monocystis ventrosa Berlin, 1924 (Fig. 7)

Host: *Lumbricus castaneus* (Sav., 1836), *L. rubellus* Hoffm., 1843, *L. terrestris* L., 1758
Habitat: seminal vesicles
Locality: Bory Tucholskie, Łoboda 25.V.1988, Warsaw, Cemetery of Soviet Soldiers 30.V.1988, Plac Zbawiciela 30.V.1988, Saski Park 1.VI. 1988, Bielany Park 1.VI.1988, Wierzbno 1.VI.1988, Białołęka Dworska 1.VI.1988, Klembów 3.VI.1988, Białowieża National Park 7.VI.1988

Body of trophozoites thick and broad, always bulging at one of its site, with characteristic foot-shaped ending. Length 110—190 μm, breadth 55—110 μm, nucleus 16—17 μm, nucleolus 5 μm, paraglycogen granules 5—6 μm. Validity of this species is unclear, however, the evidences supporting the opinion that *M. ventrosa* is a moribund *M. agilis* are insufficient.

Distribution: Cosmopolitan species; previously recorded from Poland by Marek (1967).

Nematocystis elmassiani (Hesse, 1909) (Fig. 8)

Host: *Aporrectodea caliginosa* (Sav., 1826), *Lumbricus terrestris* L., 1758

Habitat: seminal vesicles and coelom

Locality: Warsaw, Łazienki Park 31.V.1988, Saski Park 1.VI.1988, Wierzbno 1.VI.1988

Trophozoites elongated, with mucron at anterior end. Body 500—1750 μm long and 35—55 μm wide. Nucleus 40 × 35 μm, paraglycogen granules 3—4 μm in diameter. Some of the coelomic specimens infecting *L. terrestris* were observed attached to the coelomic epithelium overlying body wall and intersegmental septum of the host. This type of behaviour was previously reported only by Miles (1963).

Distribution: Czechoslovakia (Pižl 1989a), England (Miles 1963), France (Hesse 1909), Germany (Meier 1956), Sweden (Berlin 1924), USA (Vávra and Small 1969); for the first time found in Poland.

Nematocystis magna (Schmidt, 1854) (Fig. 9)

Host: *Lumbricus terrestris* L., 1758

Habitat: testis

Locality: Klembów, 3.VI.1988

Trophozoites large, length 850—4100 μm, breadth 50—105 μm. Nucleus ovoid, 70 × 40 μm in largest specimens. Paraglycogen granules 6—8 μm.

Distribution: Czechoslovakia (Pižl 1989a), Bulgaria (Duhlinska 1977b), England (Miles 1963), France (Hesse 1909), Germany (Meier 1956), Scotland (MacMillan 1964), Sweden (Berlin 1924); for the first time recorded in Poland.

Nematocystis vermicularis Hesse, 1909 (Fig. 10)

Host: *Lumbricus rubellus* Hoffm., 1843, *L. terrestris* L., 1758

Habitat: seminal vesicles

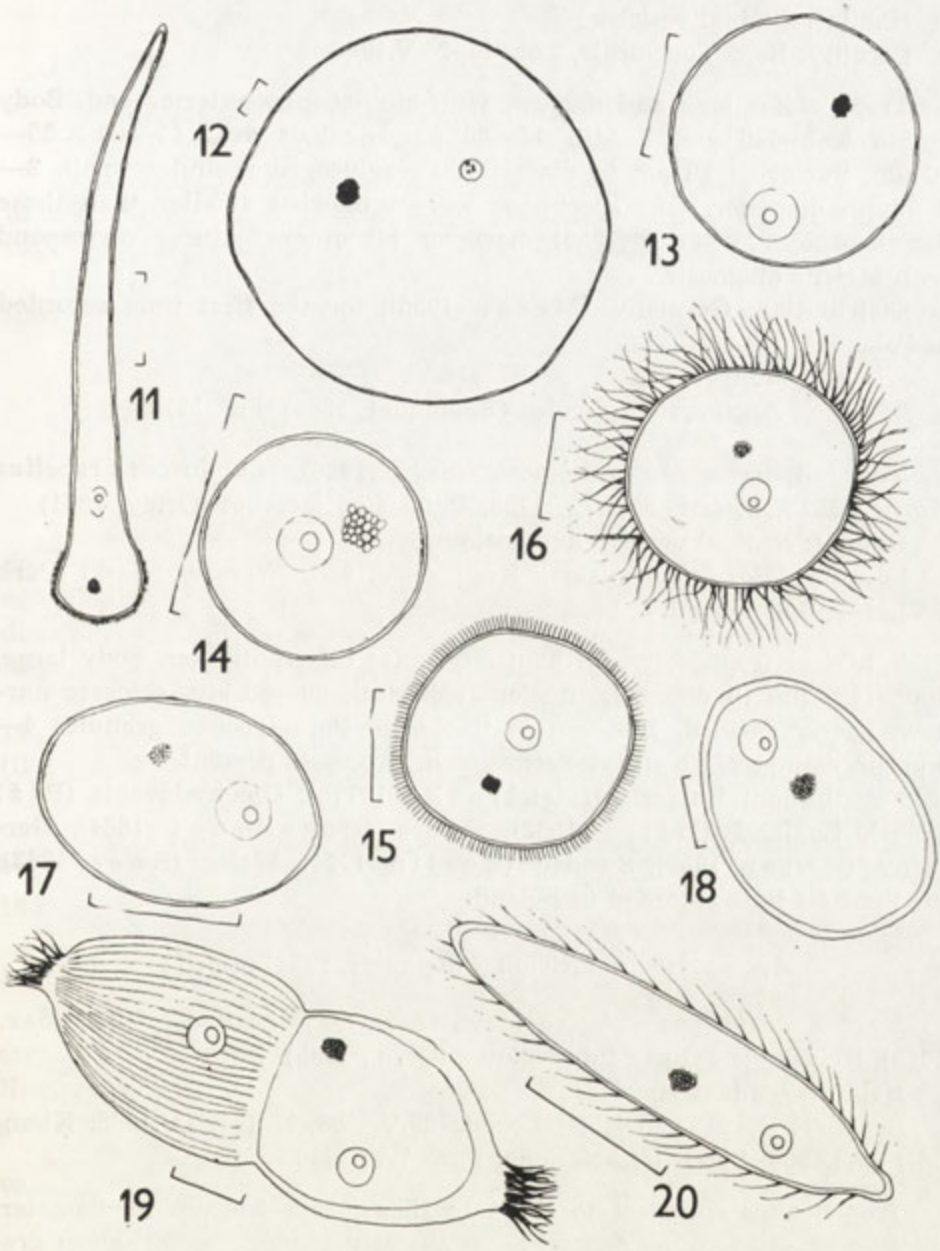
Locality: Bory Tucholskie, Krąg 26.V.1988

Trophozoites elongated, very slim, without mucron. The measurements vary from 1800 to 2400 μm long and 35 to 75 μm wide. Nucleus ovoid, 45—50 × 20 μm, with several small nucleoli. Paraglycogen granules 4 μm.

Distribution: Cosmopolitan species; for the first time found in Poland.

Rhabdocystis pilosa Meier, 1956 (Fig. 11)

Host: *Lumbricus rubellus* Hoffm., 1843



Figs. 11—20: 11 — *Rhabdocystis pilosa*, 12 — *Apolocystis herculea*, 13 — *A. lumbriciolidi*, 14 — *A. minuta*, 15 — *A. pilosa*, 16 — *A. spinosa*, 17 — *A. vivax*, 18 — *Apolocystis* sp., 19 — *Zygocystis cometa*, 20 — *Rhynchocystis cuneiformis* (scale — 0.1 mm, for *A. minuta* — 0.05 mm)

Habitat: seminal vesicles
Locality: Bory Tucholskie, Łoboda, 26.V.1988

Trophozoites long and narrow, with club-shaped anterior end. Body length 650—830 μm, beadth 15—65 μm, nucleus oval 45—60 × 25—30 μm, nucleolus 20 μm in diameter. Paraglycogen granules small, 2—3 μm in diameter. Our specimens were somewhat smaller than those described by Meier (1956), however all other features correspond with Meier's diagnosis.

Distribution: Germany (Meier 1956); for the first time recorded in Poland.

Apolocystis herculea (Bosanquet, 1894) (Fig. 12)

Host: *Aporrectodea caliginosa* (Sav., 1826), *Lumbricus rubellus* Hoffm., 1843, *L. terrestris* L., 1758, *Octolasion lacteum* (Örley, 1881)
Habitat: seminal vesicles and coelom
Locality: Bory Tucholskie, Krąg 26.V.1988, Warsaw, Saski Park 1.VI.1988, Klembów 3.VI.1988

Spherical trophozoites without any polar differentiation. Body large, 700—1400 μm in diameter, nucleus spherical, 60—80 μm, ectosarc narrow, poorly defined, 2—4 μm in thickness. Paraglycogen granules 4—8 μm in diameter. No epicytic striation or processes present.

Distribution: Bulgaria (Duhlinska 1977b), Czechoslovakia (Pižl 1989a), England (Miles 1963), France (Bosanquet 1884), Germany (Meier 1956), Sweden (Berlin 1924), Wales (Rees 1963); for the first time recorded in Poland.

Apolocystis lumbriciolidi (Schmidt, 1854) (Fig. 13)

Host: *Dendrobaena octaedra* (Sav., 1826), *Dendrodrilus rubidus* (Sav., 1826), *Helodrilus antipae tuberculatus* (Čern., 1935)
Habitat: seminal vesicles
Locality: Bory Tucholskie, Łoboda 25.V.1988, Krąg 26.V.1988; Klembów 3.VI.1988, Białowieża National Park 7.VI.1988

Trophozoites spherical to ovoid in shape, 150—200 μm in diameter, nucleus 25—50 μm, nucleolus 17—20 μm in diameter. Paraglycogen granules spherical, fine, 2.5—4.5 μm. Without epicytic striation and/or processes.

Distribution: Czechoslovakia (Pižl 1989a), England (Miles 1963), France (Hesse 1909), Germany (Meier 1956); previously found in Poland by Marek (1967).

Apolocysis minuta Troisi, 1933 (Fig. 14)

Host: *Lumbricus castaneus* (Sav., 1826), *L. terrestris* L., 1758
Habitat: seminal vesicles
Locality: Warsaw, Cemetery of Soviet Soldiers 30.V.1988

Small spherical trophozoites without polar differentiations, 45—50 μm in diameter. Nucleus 10—12 μm, nucleolus 5 μm in diameter. Paraglycogen granules ovoid, 4—6 μm. No epicytic striation present.

Distribution: England (Miles 1963), USA (Troisi 1933); for the first time recorded in Poland.

Apolocystis pilosa Meier, 1956 (Fig. 15)

Host: *Lumbricus rubellus* Hoffm., 1843
Habitat: seminal vesicles
Locality: Klembów 3.VI.1988, Białowieża National Park 7.VI.1988

Trophozoites hairy and spherical, often found in irregular ovoid forms due to their movements. Body diameter varies from 110 to 265 μm, ectoplasmic processes uniform, 3—7 μm long. Nucleus spherical with diameter of 12—30 μm, nucleolus 4—12 μm. Paraglycogen granules densely packed, 3—8 μm.

Distribution: Bulgaria (Duhlinska 1977b), Czechoslovakia (Pižl 1989a), England (Miles 1963), Germany (Meier 1956), Hungary (Bereczky 1967); for the first time recorded in Poland.

Apolocystis spinosa Rees, 1963 (Fig. 16)

Host: *Allolobophora chlorotica* (Sav., 1826), *Eiseniella tetraedra* (Sav., 1826)
Locality: Bory Tucholskie, Krąg 26.V.1988, Warsaw, Saski Park 1.VI.1988

Trophozoites spherical without any polarity, 120—250 μm in diameter. Body with dense cover of ectoplasmic processes, 60—80 μm long. Nucleus spherical, 26 μm, nucleolus 8.5—15 μm in diameter. Paraglycogen granules 3—5 μm.

Distribution: Czechoslovakia (Pižl 1989a), England (Segun 1972), Wales (Rees 1963); for the first time recorded in Poland.

Apolocystis vivax (Berlin, 1924) (Fig. 17)

Host: *Eiseniella tetraedra* (Sav., 1826)
Habitat: seminal vesicles
Locality: Bory Tucholskie, Krąg 26.V.1988

Trophozoites ovoid, length 115—190 μm, breadth 90—125 μm, nu-

cleus 25—39 μm in diameter, nucleolus 8—9 μm. Paraglycogen granules very fine, 1.5—3 μm.

Distribution: Czechoslovakia (P i ž l 1989a), Germany (M e i e r 1956), Sweden (B e r l i n 1924); for the first time recorded in Poland.

Apolocystis sp. (Fig. 18)

Host: *Allolobophora chlorotica* (Sav., 1826), *Aporrectodea caliginosa* (Sav., 1826)

Habitat: seminal vesicles

Locality: Klembów 3.VI.1988

Trophozoites ovoid in shape, body length 280—285 μm, breadth 160—180 μm, without any ectoplasmic processes. Nucleus 25—40 μm in diameter, excentrically located nucleolus 10 μm in diameter. Paraglycogen granules 2—4 μm. Observed trophozoites resemble those of *A. pertusa* Loubatiéres, 1955, however, despite of some differences in body shape and measurements, they differ in possesing of thick ectosarc, 5—7 μm. Further studies are needed to clarify the systematic position of this species.

Distribution: Poland.

Zygocystis cometa Stein, 1848 (Fig. 19)

Host: *Allolobophora caliginosa* (Sav., 1826), *Aporrectodea caliginosa* (Sav., 1826)

Habitat: seminal vesicles

Locality: Bory Tucholskie, Krąg 26.V.1988, Warsaw, Cemetery of Soviet Soldiers 30.V.1988

Trophozoites piriform, always in frontal syzygy. Body with longitudinal ectoplasmic striations and with ectoplasmic processes at the end. Length of body 150—890 μm. Breadth of body 45—250 μm. Nucleus 50—65 μm in diameter, nucleolus 20—25 μm. Paraglycogen granules 3.5—6 μm.

Distribution: Cosmopolitan species; for the first time recorded in Poland.

Rhynchocystis cuneiformis (Ruschaupt, 1885) (Fig. 20)

Host: *Lumbricus terrestris* L., 1758

Habitat: seminal vesicles

Locality: Warsaw, Łazienki Park 31.V.1988

Oval trophozoites terminated with cylindroconical rostrum, covered thinly with ectoplasmic processes. Body length 156—270 μm, breadth

30—50 μm, nucleus 10—15 μm in diameter, nucleolus 7—10 μm in diameter. Spherical paraglycogen granules 3—4 μm.

Distribution: Cosmopolitan species; previously recorded in Poland by Marek (1967).

Conclusions

During investigations of the earthworm infection by various parasites at 13 localities in Poland, nineteen monocystid species were found, thirteen new for the Polish fauna. As new hosts, *Aporrectodea caliginosa* for *N. elmassiani*, *Eiseniella tetraedra* for *A. spinosa* and *Helodrilus antipae tuberculatus* for *A. lumbriciolidi*, were established. Together with the records presented here, the list of Polish Monocystidae contains 23 species now.

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Two New Species of Cephaline Gregarines (*Apicomplexa, Sporozoa*) from the Marine Prawn *Penaeus indicus* H. Milne Edwards

S. PREMA and K. P. JANARDANAN

Parasitology Laboratory, Department of Zoology, Calicut University, 673 635 Kerala, India

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Synopsis. Two new species of septate gregarines, *Nematopsis indicus* and *Cephalolobus indicus*, have been described from the marine prawn, *Penaeus indicus* H. Milne Edwards, in Kerala, India and compared with related species reported from prawns.

An explorative study undertaken at our laboratory on gregarines infecting the marine prawn, *Penaeus indicus*, in Calicut district revealed 2 species of gregarines, one assigned to *Nematopsis* and the other to *Cephalolobus*. The genus *Nematopsis* was created by Schneider (1892) for the monozoic, thick-walled spores in the bivalve *Solen*. Léger (1903) found identical spores in *Mytilus* and other lamellibranchs and proposed the name *Nematopsis schneideri*. Léger and Duboscq (1911) demonstrated that the spores hatched in the crab *Portunus* and developed into the gregarine, *Porospora portunidarum* Frenzel, 1885. Since this gregarine has resistant, monozoic spores in molluscs, Hatt (1931) shifted the species to *Nematopsis* and renamed it *Nematopsis portunidarum* (Frenzel, 1885). Setna and Bhatia (1934) reported *Hirmocystis parapeneopsisi* and *Protomagalhaensia attenuata* from *Parapeneopsis sculptilis*. Sprague and Couch (1971), with due justification, assigned them to *Nematopsis*, combined and named the combined species *Nematopsis parapeneopsisi* (Setna and Bhatia). Théodoridès (1964, 1965) described *Porospora mizoulei* and *P. soyeri* from the shrimps *Solenocera membranacea* and *Aristeus antennatus* respectively. Since their vegetative stages are similar to those of

confirmed species of *Nematopsis* but strikingly different from those of *Porospora*, Sprague and Couch (1971) shifted them to *Nematopsis* and called *N. mizoulei* and *N. soyeri*. The other named species of *Nematopsis* from prawns were reported by Sprague (1954), Kruse (1966), Feigenbaum (1975) and Shanavas et al. (1989). Kruse (1959) created the genus *Cephalolobus* with its type species *C. penaeus* from *Penaeus aztecus* and *P. duorarum*. Later, two species were added to it by Théodoridès (1964) and Feigenbaum (1975).

The present paper records our observations on the different stages of the 2 gregarines from the prawn host. The two species have been compared with related species and are found to be new. We report them as *Nematopsis indicus* sp.n. and *Cephalolobus indicus* sp.n. Our reasons are discussed elsewhere.

Material and Methods

The prawns, *Penaeus indicus*, collected from Chaliyar river in Feroke and a rivulet in Ramanattukara in Calicut district were brought alive to the laboratory. The specimens were cut along their sides, tergites were removed and the intestine, along with pyloric stomach, were individually transferred on to slides. Various development stages of live gregarines were studied by staining them supravitally with neutral red or methylene blue. For permanent preparation, smears were fixed in Schaudinn's fluid and subsequently stained with Heidenhain's hematoxylin. Gametocysts collected from the rectum were maintained in moist chamber for further development.

Sketches were made with the aid of a camera lucida; descriptions are based on the measurements of a minimum of 20 specimens.

Abbreviations used in this paper are:

Primite: DL — Deutomerite length, DW — Deutomerite width, PL — Protomerite length, PW — Protomerite width, TL — Total length.

Satellite: dl — Deutomerite length, dw — Deutomerite width, pl — Protomerite length, pw — Protomerite width, tl — Total length.

Results

Nematopsis indicus sp. n.

Host: *Penaeus indicus* H. Milne Edwards.
Location in host: Intestine.
Type locality: Feroke and Ramanattukara, Calicut District, Karela (India).
Date of collection: May to July 1985, 1986 and 1987.
Holotype: To be deposited in the parasite collections, Parasitology Laboratory, Dept. of Zoology, University of Calicut, Kerala (India).

Description

Sporadins (Fig. 1 *1*): Sporadins biassociative, linear, elongate, cylindrical, opaque, milky-white; epimerite absent. Syzygy early, occasionally 3 — 4 sporadins in a linear chain or Y-shaped (Fig. 1 *2*) with two primites in syzygy with a satellite. Length of association from 494 to 1047.2 μm (:mean, 804.2 μm).

Primites: Primites elongate, narrow behind the septum, gradually broadens to a flat caudad; protomerite hemispherical, broader than long; apical papilla and apical pore absent; protomerite epicyte uniformly thick, longitudinally striated, striations continuous with those on deutomerite; septum circular, flat; constriction at septum conspicuous. Deutomerite narrow behind septum, gradually broadens to a flat caudad; epicyte hyaline, uniformly thick; endocyte granular. Nucleus spherical to ovoid, feebly visible in fresh sporonts, varying in position; endosome 1 or 2, round; extra-endosomal region clear. The nucleus in a 385 μm long primite measured 30.8 μm in diameter.

Satellites: Satellites elongate, broader at the anterior region, gradually tapering to a round caudad; protomerite absent; interlocking device between primite and satellite well developed; epicyte hyaline, uniformly thick; endocyte granular; nucleus spherical to ovoid, feebly visible, variable in position; endosomes 1 or 2, round; extraendosomal region clear. The nucleus in a 539 μm long satellite measured 30.8 μm in diameter.

Measurements (in micrometers): Measurements of sporadins (with mean in parentheses) are noted below:

Primites:

TL = 190—415.8 (301.1); DW = 30.4—92.4 (52.5)
PL = 15.2—30.8 (24.1); PW = 15.2—46.2 (31)

Satellites:

tl = 304—646.8 (503.1); dw = 30.4—77 (49.4)

Ratios (of primites):

PL : TL = 1 : 12.5; PW : DW = 1 : 1.7

Gametocysts (Fig. 1 *3*): Gametocysts spherical, opaque, milky-white; cyst wall thin, hyaline. Fresh gametocysts measured 138 to 215.6 μm (:mean, 174.5 μm).

Gametes: Anisogamous; macrogametes non-motile, spherical, measuring 3.7 to 4.5 μm; microgametes motile, with a spindleshaped body measuring 2.2 to 3 μm long and a slender tail measuring 5.2 to 6.7 μm.

Gymnospores: (Fig. 1 *4*): The gymnospores spherical; uninucleated bodies arranged radially in a rosette-like manner around a central, hyaline, cytoplasm. Fresh gymnospores measured 4.5 to 6 μm in diameter.

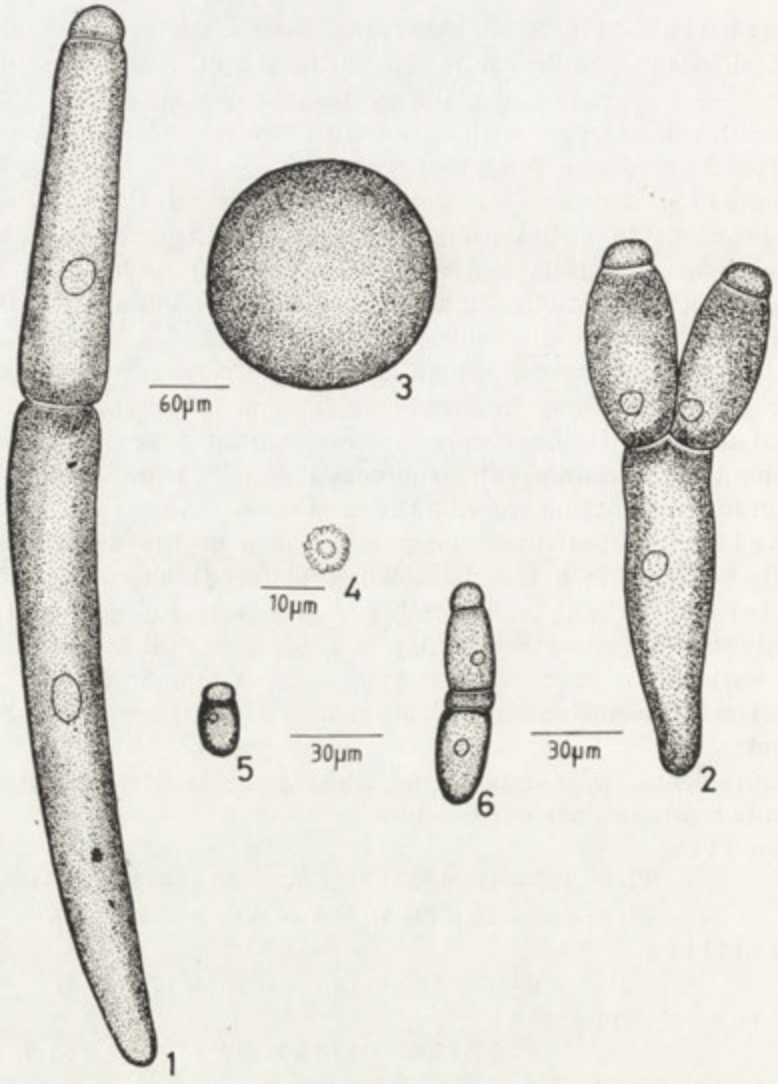

Fig. 1. *Nematopsis indicus* sp. n. 1 — Biassociative sporadins, 2 — Y-shaped sporadins, 3 — Gametocyst, 4 — Gymnospore, 5 — Early trophozoite, 6 — Early association

Life-cycle stages

The gametocysts, in various stages of development, were attached to the rectal wall of the hosts with their hyaline cyst walls; a few completed development, ruptured and released the gymnospores into the

rectal lumen. Even after making consistent attempts, the molluscan host could not be discovered; hence the subsequent development of the gymnospores could not be observed.

The smallest observed trophozoite (Fig. 1 *5*) was ovoid, translucent, measuring 24.7 by 13.2 μm. The protomerite measured 7.6 μm long and deutomerite 19 μm. Its nucleus was spherical, 3.8 μm in diameter. The largest observed trophozoite measured 144.4 μm long; its hemispherical protomerite measured 22.8 μm, elongated deutomerite 121.6 μm and spherical nucleus 15.2 μm in diameter.

The smallest observed association (Fig. 1 *6*) measured 79.2 μm long with a 39.6 μm long primite and 39.6 μm long satellite. Occasionally linear association of 3—4 sporadins were observed in the prawn's intestinal lumen.

Association of three sporadins: The association measured 615.6 μm long with a 190 μm long primite, 159.6 μm long satellite I and 266 μm long satellite II.

Association of four sporadins: The association measured 909.6 μm long; its primite measured 220.4 μm, satellite I 157.2 μm, satellite II 182.4 μm and satellite III 349.6 μm in length.

Cephalolobus indicus sp. n.

Host: *Penaeus indicus* M. Milne Edwards.
Location in host: Trophozoites and sporadins attached to the filter of the pyloric stomach and gametocysts attached to the rectal wall.
Type locality: Feroke and Ramanattukara, Calicut district, Kerala (India).
Date of collection: May to July of 1985, 1986 and 1987.
Holotypes: To be deposited in the parasite collections, Parasitology Laboratory, Dept. of Zoology, University of Calicut, Kerala (India).

Description

Sporadins (Fig. 2 *1*): Biassociative, linear, opaque, milky-white, laterally curved; occasionally two to five satellites in syzygy with primite; true epimerite absent; anterior end of primite modified into a hyaline, sub-cylindrical protoepimerite. Length of association from 320 to 623.2 μm (:mean, 466.7 μm).

Primite: Short and stumpy, occasionally elongate; protoepimerite hyaline, sub-cylindrical (Fig. 2 *2*); protomerite rectangular, broader than long; epicyte uniformly thick, longitudinally striated. Septum circular, flat or slightly convex towards deutomerite; constriction at septum inconspicuous. Deutomerite curved laterally; epicyte hyaline, uniformly thick, longitudinally striated; endocyte granular. Nucleus spherical, feebly visible, varying in position, enclosing 10—15 round endosomes. The nucleus in a 228 μm long primite measured 32 μm in diameter.

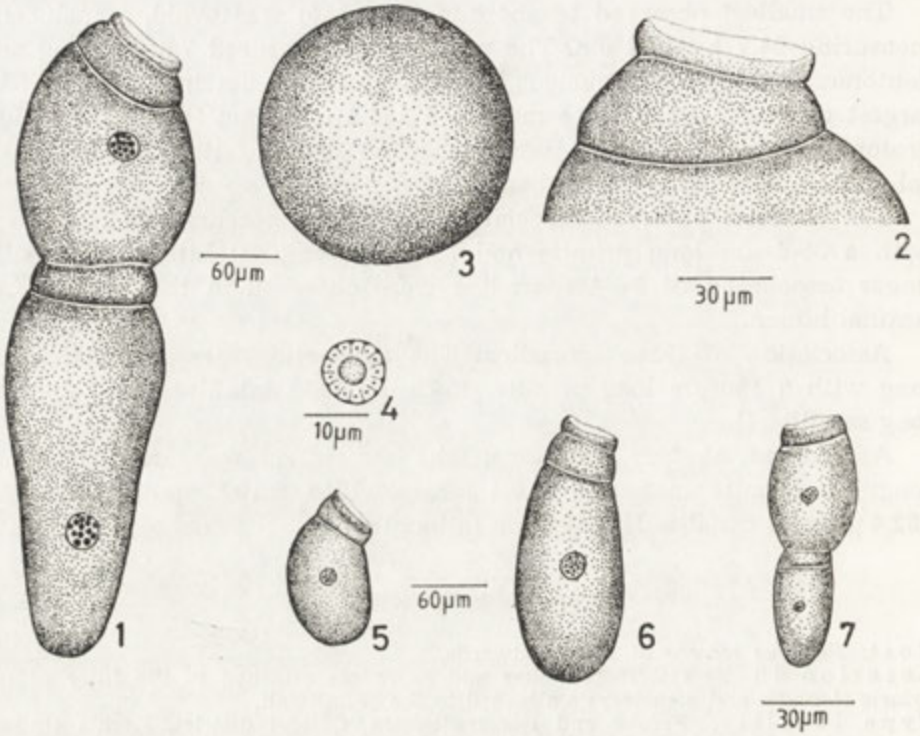

Fig. 2. *Cephalolobus indicus* sp. n. 1 — Sporadins, 2 — Anterior region of primite showing proto-epimerite, 3 — Gametocyst, 4 — Gymnospore, 5, 6 — Trophozoites, 7 — Smallest association

Satellite: Elongate, anteriorly broad, gradually tapers to a round caudad. Protomerite rectangular, broader than long; its apex flat or slightly concave. Septum circular, flat or slightly convex towards deutomerite; constriction at septum inconspicuous. Deutomerite elongate, broader at the anterior region and gradually tapering to a round caudad; epicyte hyaline, uniformly thick, endocyte granular. Nucleus as in primites. The nucleus in a 395.2 µm long satellite measured 32 µm in diameter.

Measurements (in micrometers): Measurements of sporadins with mean in parenthesis) are noted below:

Primites:

TL = 112—264 (207); DW = 76—144 (109.2)
PL = 24—48 (36.8); PW = 56—96 (75.7)

Satellites:

tl = 152—395.2 (259.7); dw = 64—120 (81.7)
pl = 24—40 (27.6); pw = 32—88 (62.4)

Ratios:

Primites: PL : TL = 1 : 5.6; PW : DW = 1 : 1.4
Satellites: pl : tl = 1 : 9.1; pw : dw = 1 : 1.4

Gametocysts (Fig. 2 *3*): Spherical, milky-white, opaque. Fresh gametocysts measured 196 to 215.6 μm (:mean, 202.3 μm).

Gymnospores (Fig. 2 *4*): The gymnospores spherical; uninucleated bodies arranged radially in a rosette-like manner around a central, hyaline cytoplasm. Fresh gymnospore measured 10 μm in diameter.

Life-cycle stages

The gametocysts were attached to the rectal wall of the hosts with their hyaline cyst wall; a few completed development, ruptured and released the gymnospores into the rectal lumen. Molluscan hosts could not be discovered even after consistent attempts. Hence further development of the gymnospores could not be observed.

Trophozoites develop attached to the filter of the stomach. The smallest observed trophozoite was globular, translucent, measured 15.4 μm. The largest observed trophozoite (Fig. 2 *6*) was 74.2 μm long; its protoepimerite measured 8 μm, rectangular protomerite 11.5 μm and elongate deutomerite 54.7 μm long.

The smallest observed association (Fig. 2 *7*) measured 89 μm with a 44.5 μm long primite and 44.5 μm long satellite. With further growth and differentiation, the associating sporadins get transformed into gametocysts.

Mixed infection with *Cephalolobus indicus* sp.n. and *Nematopsis indicus* sp.n. was frequently observed in *Penaeus indicus*.

Discussion

Nematopsis indicus sp.n.

Caridohabitans indicus Janardanan and Ramachandran, 1980 is the only gregarine known from *Penaeus indicus*. The present gregarine from *P. indicus* is distinct from *C. indicus* in size, shape of sporadins and in having (1) extracellular development, (2) vesicular nuclei and (3) the absence of epimerite in trophozoites and primites.

This gregarine is ascribed to *Nematopsis* since its trophozoites and sporadins are like those of *Nematopsis* and its gametocysts formed gymnospores. The gregarine has resemblance to *N. mizoulei* (Théodoridès, 1964) Sprague and Couch, 1971 from *Solenocera membranacea*. But it

differs from *N. mizoulei* in (1) the size and shape of trophozoites and sporadins, (2) in forming smaller gametocysts and gymnospores and (3) in having spherical to oval nuclei with one or two endosomes. Besides, the host of the two species are different and are from two geographically separated regions. The gregarine is, therefore, a new species and is reported here as *Nematopsis indicus* sp.n.

Cephalolobus indicus sp.n.

The present gregarine from *Penaeus indicus* has trophozoites and associative sporadins which are septate and attached to the filter of host's stomach; early development is extracellular; anterior end of protomerite modified into a subcylindrical hyaline proto-epimerite. These characters justify its inclusion in the genus *Cephalolobus* Kruse, 1959 of the family *Cephaloidophoridae* Kamm, 1922.

Three species of *Cephalolobus* are known from prawns. The present gregarine from *Penaeus indicus* closely resembles *Cephalolobus penaeus* Kruse, 1959 from *Penaeus aztecus* and *P. duorarum* and *C. petiti* Théodoridès, 1964 from *Solenocera membranacea*. It is different from *C. penaeus* in shape and size of sporadins and in having (1) sub-cylindical, hyaline proto-epimerite, (2) nuclei enclosing 10—15 endosomes and (3) syzygy with 2—5 satellites attached to the primite. The gregarine differs from *C. petiti* in having (1) spherical nucleus with 10—15 endosomes, (2) syzygy with 2—5 satellites attached to one primite and (3) spherical gametocyst. The gregarine is a new species and is named as *Cephalolobus indicus* sp.n.

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Effect of *Microsporidium sitophili* on Molting of *Sitophilus oryzae*

S. GHOSH

Protozoology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, W. B., India

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Synopsis. In the study of effects of microsporidian infection on the molting of *Sitophilus oryzae* (L.), *Microsporidium sitophili* was experimentally applied on the 2nd instar larvae at the dose of 15^{10} spores gram of food material and 2×10^5 spores larva respectively. It was observed that infected larvae molted less frequently but pupated earlier.

Sitophilus oryzae (L.) (*Coleoptera, Curculionidae*) is a common and highly destructive stored grain pest in India. Recently a pathogenic microsporan, *Microsporidium sitophili* has been recorded from this pest (Ghose 1989). The parasite damages the malpighian tubules and fat bodies of the host and ultimately affects the physiology of the insect.

The present study deals with the effects of *M. sitophili* on the molting of *Sitophilus oryzae*.

Material and Techniques

Maintenance of insects

Uninfected larvae of *Sitophilus oryzae* were collected from a disease free stock reared on a medium containing sterilised food. Larvae were reared in a 100 ml glass container until 2nd instar.

Collection of spores of *Microsporidium sitophili*

Spores of *M. sitophili* were collected from laboratory reared *Sitophilus oryzae* (L.) which had died of microsporidiosis. The dead insects were homogenized in 0.005 M solution of ascorbic acid in 0.135 M NaCl solution. The homogenate was washed in a progressively diluted solution of ascorbic acid until the material was

transferred to distilled water (Vávra and Maddox 1976). The resulting homogenate was filtered through two layers of cheesecloth and purified by differential centrifugation. Spores were stored in distilled water at 4°C. Number of spores /ml of spore suspension were counted using a hemocytometer as described by Cantwell (1970).

Experimental infection

Thousand 2nd instar larvae were retrieved from the stock culture, starved for 24 h to induce hunger and to eliminate weak individuals. The food material was uniformly mixed with spores (15^{10} spores/gram food material) of *M. sitophili*. Half of the 2nd instar larvae were allowed to feed ad libitum on the food material for additional 24 h. After the inoculation period, each larva was removed from the infective material, brushed free of excess food particles and placed individually on spore free food material in glass vials, which were kept in an incubator at 28°C ± 2°C and 58% ± 3% r. h. The other 500 larvae were put directly on spore free food and reared simultaneously.

A 2nd method adopted for infecting the larvae with the spores of *M. sitophili* was to mix the measured amount of food with 1 ml of spore suspension (1 ml of spore suspension contains 3×10^6 spores) and to place the mixture in a petridish containing 10 larvae. After allowing to feed for 3 h the larvae were transferred to fresh vials containing sterilised food. The approximate dose/larva was calculated by the consumption of food materials by the larva. In this way 100 larvae were fed with *M. sitophili* spores. These were maintained in the same manner as former. It was calculated that each larva consumed 2×10^5 spores with food material. Control larvae were treated in the same manner except that they were given glucose water.

Each vial was checked every 2 days and the presence of pupae were recorded. At death or pupation, each larva was examined microscopically for the presence of microsporan spores. The data were plotted in Tables 1 and 2.

Results

For uninfected larvae, a total of 1507 and 326 molts were recorded between 2nd and 28th days. Altogether, 92.0—93.0% larvae pupated between 16th and 26th days and remainder died between 4th and 22nd days (Tables 1 and 2).

For infected larvae, 978 and 200 molts were recorded between 2nd and 28th days and 38.6—40.0% larvae pupated between 14th and 26th days. Mortality was 60.0—61.4% but occurred later in life, with all deaths taking place between 16th and 28th days (Tables 1 and 2). All these larvae were heavily infected with microsporan spores.

Thus in this experiment it is clearly demonstrated that in both methods of feeding infected larvae molted less frequently than healthy ones, but developed more quickly to pupate earlier (Tables 1 and 2). The parasite mainly infected the fat bodies of the larvae. The infected fat cells lost their connection with adjoining cells and in some cases whole fat body became fragmented. The parasites multiplied rapidly

Table 1

The number of molts, deaths and pupae recorded for batches of 500 larvae of *Sitophilus oryzae* (L.) infected with *M. sitophili* at the dose of 15^{10} spores/gram of food materials

Days	Molts				Deaths				Pupae			
	Infected		Uninfected		Infected		Uninfected		Infected		Uninfected	
	n	% of molting larvae	n	% of molting larvae	n	% of death	n	% of death	n	% of larvae pupated	n	% of larvae pupated
2	216	43.2	234	46.8	0	0	0	0	0	0	0	0
4	177	35.4	290	58.0	0	0	1	0.2	0	0	0	0
6	101	20.2	205	41.0	0	0	2	0.4	0	0	0	0
8	86	17.2	177	35.4	0	0	6	1.2	0	0	0	0
10	64	12.8	142	28.4	0	0	4	0.8	0	0	0	0
12	58	11.6	118	23.6	0	0	5	1.0	0	0	0	0
14	55	11.0	102	20.4	0	0	9	1.8	16	3.2	0	0
16	42	8.4	119	23.8	0	0	3	0.6	39	7.8	49	9.8
18	40	8.0	120	24.0	40	8.0	2	0.4	44	8.8	179	35.8
20	38	7.6	0	0	105	21.0	2	0.4	35	7.0	117	23.4
22	35	7.0	0	0	78	15.6	1	0.2	30	6.0	100	20.0
24	28	5.6	0	0	44	8.8	0	0	29	5.8	20	4.0
26	20	4.0	0	0	32	6.4	0	0	0	0	0	0
28	18	3.6	0	0	8	1.6	0	0	0	0	0	0
Total	978		1507		307	61.4	35	7.0	193	38.6	465	93.0

Table 2

The number of molts, deaths and pupae recorded for batches of 100 larvae of *Sitophilus oryzae* infected with *M. sitophili* at the dose of 2×10^5 spores/larva

Days	Molts				Deaths				Pupae			
	Infected		Uninfected		Infected		Uninfected		Infected		Uninfected	
	n	% of molting larvae	n	% of molting larvae	n	% of death	n	% of death	n	% of larvae pupated	n	% of larvae pupated
2	44	44.0	49	49.0	0	0	0	0	0	0	0	0
4	35	35.0	58	58.0	0	0	2	2.0	0	0	0	0
6	21	21.0	43	43.0	0	0	0	0	0	0	0	0
8	18	18.0	35	35.0	0	0	1	1.0	0	0	0	0
10	13	13.0	28	28.0	0	0	0	0	0	0	0	0
12	11	11.0	24	24.0	0	0	1	1.0	0	0	0	0
14	12	12.0	21	21.0	0	0	1	1.0	3	3.0	0	0
16	9	9.0	24	24.0	2	2.0	1	1.0	8	8.0	0	0
18	8	8.0	24	24.0	5	5.0	2	2.0	8	8.0	6	6.0
20	8	8.0	20	20.0	13	13.0	0	0	7	7.0	36	36.0
22	7	7.0	0	0	16	16.0	0	0	6	6.0	25	25.0
24	6	6.0	0	0	13	13.0	0	0	4	4.0	21	21.0
26	4	4.0	0	0	5	5.0	0	0	4	4.0	4	4.0
28	4	4.0	0	0	6	6.0	0	0	0	0	0	0
Total	200		326		60	60.0	8	8.0	40	40.0	92	92.0

in the fat bodies and consumed it. The depletion of these reserves inhibited molting and led to early pupation.

The infected larvae showed symptoms of infection on 14th day post application. Most of the larvae became brownish in color and some dark spots along the ventral side of their abdomen were noted. The larvae were very much lethargic and consumed less food. There was a marked loss of mobility in the infected larvae.

The difference in the number of molts in infected and uninfected larvae was insignificant on 2nd day but significant by the 4th day and became more pronounced as the experiment progressed. These data support the finding of Milner (1972) and George and Townes (1976) by showing that microsporan infection do not increase molting.

Discussion

Effects of microsporan on the molting of insects have been noted by many workers. Canning (1962) reported that molting was delayed in grasshopper infected with *Nosema locustae* and Ashford (1967) reported that protozoan infections can reduce the rate of larval growth in insects. However, Fisher and Sanborn (1964) claimed that larvae of *T. castaneum* infected by *N. whitei* grew faster and had more molts than uninfected larvae. In their study, larvae infected when four days old grew to twice the weight of uninfected larvae in 20 days. Fisher and Sanborn further claimed that these changes were caused by a juvenile hormone- like substance produced by *N. whitei*. However, Milner (1972) could not confirm these findings, because none of his infected larvae of *T. castaneum* grew faster than the control.

In the present study, it is clear that *Microsporidium sitophili* adversely affects the molting of *Sitophilus oryzae*. Histological studies reveal that besides fat bodies the parasite also infects gut epithelia, malpighian tubules and muscles. Experimental application of this microsporidium for the control of a serious stored grain pest, *Oryzaephilus mercator* shows that at the dose of 4×10^6 about 70.0% larvae and 60.0% adults are very much susceptible to infection and die within 168 h post application. Experiments are now being carried in the laboratory to develop this pathogenic microsporan on other serious pests.

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Studies on Histopathology and Development of a Microsporan, *Microsporidium lesiodermi*, sp. n., from a Coleopteran Insect, *Lesioderma sericorne* (L.) Infesting Common Spice, *Coriandrum sativum* (L.)

S. GHOSH

Protozoology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, W.B., India

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Synopsis. Observations have been made on the development of *Microsporidium lesiodermi* sp. n., described from *Lesioderma sericorne* (*Coleoptera, Curculionidae*). The parasite causes general infection in the host and this ultimately leads to mortality. Schizonts are multinucleated and the nuclei are diplokaryotic. Each sporogonial plasmodium contains 10—12 single nuclei. Most spores are macrospores, 3.6—5.3 (4.4) μm × 1.3—3.6 (3.0 μm, but a small number of sporophorous vesicles contain microspores, 1.6—3.3 (2.2) μm × 0.8—1.6 (1.4) μm. Both the spore types possess a single nucleus in the middle and a vacuole at the posterior region. The polar filament is thin and 25—30 μm long.

Lesioderma sericorne is a well known serious stored grain pest in India. A knowledge of pathogens of this pest is therefore important for the development of biological control methods.

Previously a nonpathogenic gregarine was recorded from this host (Haldar and Gupta, unpublished), but no one has yet recorded any pathogenic microsporan from it.

In this communication a report has been made on the development of a pathogenic polysporoblastic microsporan in the fat bodies, gut epithelium and hemocytes of *L. sericorne*.

Material and Methods

Host insects were collected from a warehouse at Chandannagore (West Bengal, India) in 1987. The insects were reared in containers in the laboratory. The

freshly collected insects moved actively, consumed sufficient amount of food and reproduced normally, but interestingly enough many adults and larvae were found dead after 21 days. Microscopic examination of the smears of dead adults and larvae revealed the presence of microsporan spores. The microsporans were also studied in paraffin sections of the fat body and gut epithelium of the larvae. The shape and size of the spores were studied under a phase contrast microscope.

Small pieces of infested organs were smeared following the method of Hazard et al. (1981) and stained in Giemsa's solution (Weiser 1976). For haematoxylin staining the smears were fixed in Bouin-Duboscq-Brasil solution for 1 h, washed in distilled water, kept in mordant of 1% aqueous solution of iron alum overnight, stained in Heidenhain's haematoxylin for 5 h and destained in iron alum solution to the desired intensity. Some of the wet smears were fixed in Carnoy's fluid and stained in Schiff's reagent. The smears were mounted in De PeX.

For histological studies whole larvae were fixed in Carnoy's fluid, washed in 100% ethanol, transferred three times through butyl alcohol and embedded in paraffin. 5—6 μm thick sections were cut and stained in Heidenhain's haematoxylin. Some larvae were fixed in Bouin-Duboscq-Brasil solution overnight, washed in water, dehydrated in graded alcohol and embedded in paraffin. 6—8 μm thick sections were cut and stained by the modified polychromatic staining method of Vetterling and Thompson (1972). To improve the staining of spores the original nuclear staining was substituted with an abbreviated haematoxylin staining (2.5% iron alum for 1 h, haematoxylin for 1 h) and the original procedure was followed from point 4 onwards (Vetterling and Thompson 1972).

Abbreviations

SP — Spore
HE — Hemocyte
SPV — Sporophorous vesicle
SC — Schizont
SPL — Sporogonial plasmodium
SCPL — Schizont in plasmodia appearance
SPg — Sporogony
FSV — Fresh sporophorous vesicle
MSV — Stained sporophorous vesicle (micro)
MaSV — Stained sporophorous vesicle (macro)
FSP — Fresh spore
MSP — Microspore
MaSP — Macrospore
Nu — Nucleus
PV — Posterior vacuole

Observations

Gross pathology

No external symptoms of infection in the case of infected adults have been recorded so far. However, heavily infected larvae become

slightly whitish in color on the dorsal side, which under normal conditions is a brownish color.

Infected larvae and adults move slowly, consume less food and tend to aggregate. In most cases, the heavily infected larvae are unable to pupate and die.

Histopathology

Midgut epithelium

The midgut epithelia of infected larvae show a somewhat different appearance from healthy ones. The cell wall of the infected cells becomes transparent and the volume of the cells greatly increases due to infiltration of large numbers of spores. In most cases the nuclei of host epithelia become fragmented (Pl. I 1).

In the gut lumen of the heavily infected larvae a great number of spores is noticed (Pl. I 2). This may be due to the rupture of infected cells and due to the abnormal regeneration process in the infected midgut epithelium (Lipa et al. 1983).

Hemocyte

The hemocyte of the infected larvae are filled with parasites. The infected hemocytes become enlarged (Pl. I 3).

Fat body

The fat body of the larvae is the main site of infection. In heavily infected cases the fat body is completely dissolved by the parasites (Pl. I 4, 5).

Development

Presporal stages

Schizogony: Schizonts vary greatly in size. Small schizonts are 5—10 μm in diameter (Pl. I 5). While the largest one is 20—25 μm. All schizonts are multinucleated and of a plasmodium appearance (Pl. I 6). The diplokaryotic nuclei are very small and take a deeper stain than the cytoplasm with Giemsa.

Sporogony: Histological studies of heavily infected hosts show a large number of sporogonial plasmodia along with the sporophorous vesicles. Each sporogonial plasmodium is irregular in shape and con-

tains 10—12 single nuclei (Pl. I 5, 7), the largest sporogonial plasmodium measuring 10.2 μm × 7.8 μm.

Spore: The sporogony produces two types of uninucleate spores: micro- and macrospores. Each type of spore is normally produced in a sporophorous vesicle of its own. A spherical sporophorous vesicle contains 10 to 32 spores (Pl. II 8—10). It has been estimated that about 60% of the vesicle carry macrospores.

Table 1

Frequency distribution of the size of one sample of 80 macrospores of *Microsporidium lesiodermi*

Sample	Dimensionable groups in microns					
	length			width		
	3.6–4.6	4.9–5.1	5.1–5.3	1.3–1.6	2.3–2.9	3.1–3.6
Fixed and stained spores	48	24	8	16	12	52

Table 2

Frequency distribution of the size of one sample of 80 microspores of *Microsporidium lesiodermi*

Sample	Dimensionable groups in microns					
	length			width		
	1.6–1.9	2.0–2.4	2.6–3.3	0.8–1.1	1.3–1.4	1.5–1.6
Fixed and stained spores	24	24	32	16	28	36

Both micro- and macrospores are spindle — shaped with pointed anterior and broad posterior extremities (Pl. II 11, 12). Unfixed macrospores measure: length — 3.7 to 5.3 μm, $\bar{X}$ — 4.9 μm, SD — 0.46; breadth — 1.4 to 3.9 μm, $\bar{X}$ — 3.2 μm, SD — 0.62. Fixed and stained spore length — 3.6 to 5.3 μm, $\bar{X}$ — 4.4 μm, SD — 0.45; breadth — 1.3 to 3.6 μm, $\bar{X}$ — 3.0 μm, SD — 0.47. Unfixed microspores measure: length — 1.8 to 3.4 μm, $\bar{X}$ — 2.9 μm, SD — 0.4; breadth — 0.9 to 1.7 μm, $\bar{X}$ — 2.0 μm, SD — 0.25. Fixed and stained spore length — 1.6 to 3.3 μm, $\bar{X}$ — 2.2 μm, SD — 0.54; breadth — 0.8 to 1.6 μm, $\bar{X}$ — 1.4 μm, SD — 0.23.

Under the light microscope, the spore appears to be composed of a distinct wall however in the electron microscope two distinct walls, endo- and exospores are observed of which the latter is uniformly electron deuse in most genera (Larsson 1988). A rounded nucleus is present at the centre of the spore (Pl. II 13). After staining with Heidenhain's haematoxylin a large vacuole is revealed at the posterior region of the spore (Pl. II 14). The polar filament is uniformly thin measuring 25—30 μm. The frequency distribution of the size of the spores in several dimensionable groups are given in Tables 1 and 2.

Intensity of infection

30.5% of the reared hosts are infected by the parasites.

Discussion

Polysporoblastic microsporans with uninucleate spores and sporogony in sporophorous vesicle have for a long time been placed in the genus *Pleistophora* Gurley, 1893. Electron microscopic studies have shown that it is a heterogenous assemblage of different species. Canning and Nicholas (1980) reexamined the type species *P. typicalis* and characterized the genus *Pleistophora*. Weiser (1977) erected a new genus *Vavraia* and transferred the microsporan *P. culicis* to this genus. Canning and Hazard (1982) further investigated the polysporoblastic microsporan and concluded that the genus *Vavraia* is a valid one and redefined the genera *Pleistophora* and *Vavraia*. Larsson (1986a) investigated a microsporan *V. holocentropi* and added some new characters to the genera *Pleistophora* and *Vavraia*.

Following the definitions of *Pleistophora* and *Vavraia* given by Canning and Hazard (1982) and Larsson (1986a) the present microsporan from *Lesioderma sericorne* is seen to possess characters which are very close to both these two genera. However it was not possible to observe some of the distinguishing features of these two genera given by Canning and Hazard (1982) and Larsson (1986a) under light microscope, and it is proposed that the microsporan from *Lesioderma sericorne* should be given the collective name *Microsporidium lesiodermi* in this communication. According to Larsson (1986b), a microsporan for which electron microscopy cannot be carried out should be reported as *Microsporidium* without giving the Latin binomen to avoid nomen nudum.

A number of genera like *Pleistophora* Gurley, *Heterosporis* Schubert, *Pygmatheca* Hazard and Oldacre, *Amblyospora* Hazard and Oldacre, *Vavraia* Weiser and *Pleistosporidium* Cordeanu-Balcescu and Cordeanu

are known to possess macrospores and microspores during sporogony. In *Microsporidium lesiodermi* a similar phenomenon is observed. The two types of spores differ in measurements, but are otherwise morphologically identical. As such, these have not been considered as spores of two different species.

Type slide: In slide no. LM, LLM/1—5 Protozoology Laboratory, Department of Zoology, University of Kalyani.

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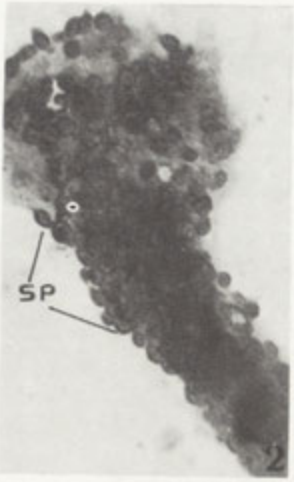
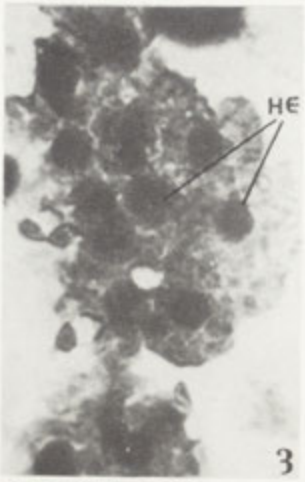
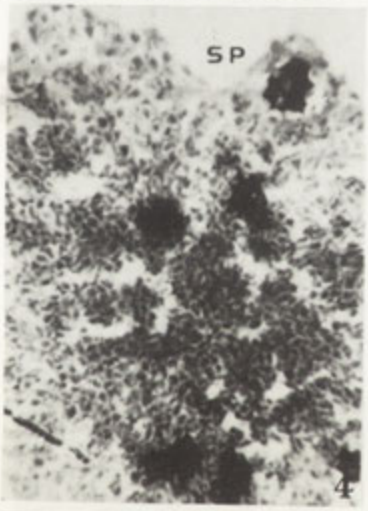
EXPLANATION OF PLATES I—II

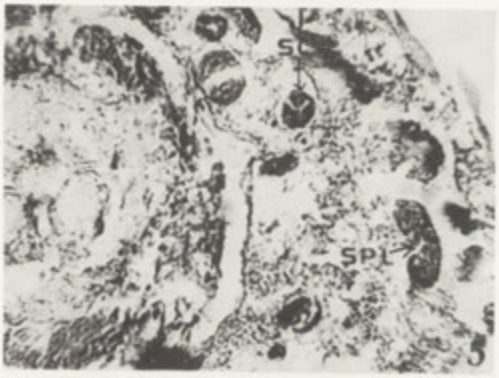
Microsporidium lesiodermi sp. n.

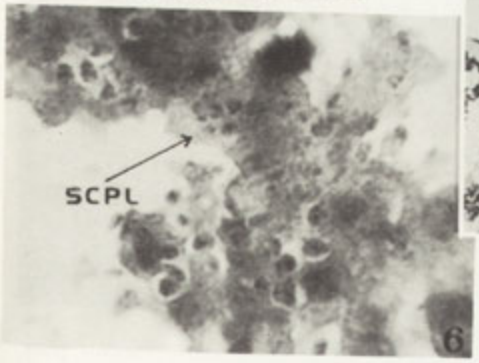
1: Infected host gut epithelium (× 1200)
2: Heavily infected gut showing cells with spores (× 1200)
3: Infected hemocytes (× 1500)
4: Heavily infected fat bodies (1× 1400)
5: Intracellular development of the parasite showing schizont, sporogonial plasmodia and the infection in the fat bodies (× 2500)
6: Schizonts in plasmodial appearance (× 2500)
7: Sporogonial plasmodium (× 2500)
8: Fresh sporophorous vesicle (× 1200)
9: Sporophorous vesicle containing microspores (× 2000)
10: Sporophorous vesicle containing macrospores (× 2000)
11: Fresh micro- and macrospores (× 1000)
12: Spores (both micro- and macro) stained with Giemsa's solution (× 1500)
13: Groups of spores showing centrally located nucleus (× 1000)
14: Spores stained with Heidenhain's haematoxylin showing posterior vacuole (× 1500)

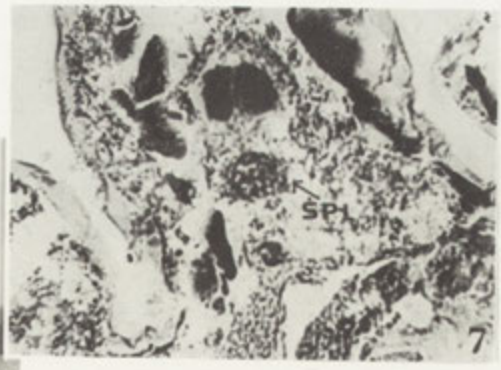












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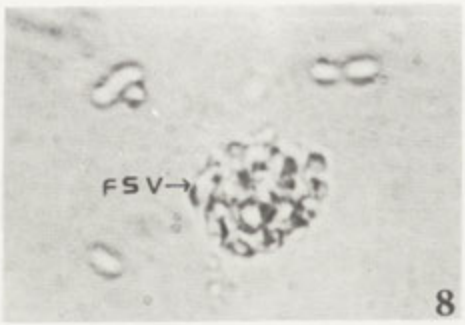

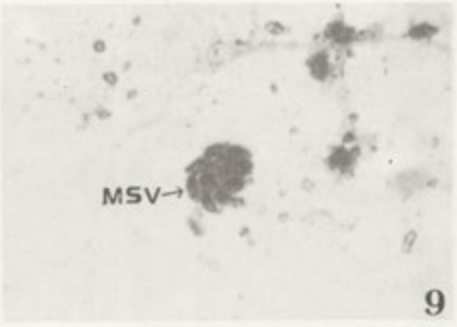

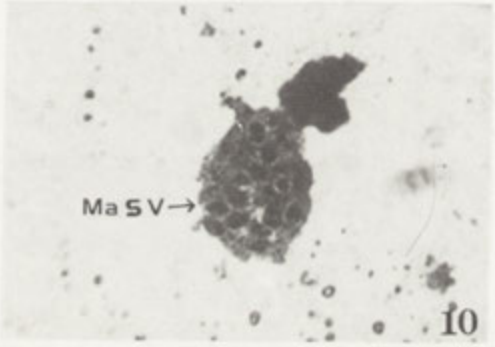

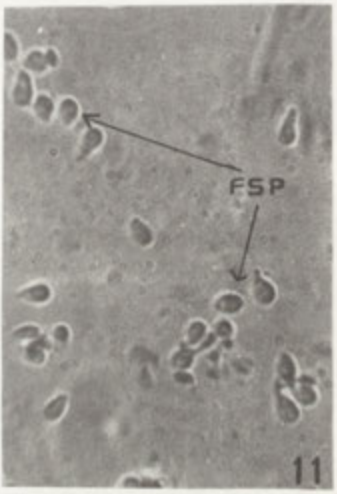

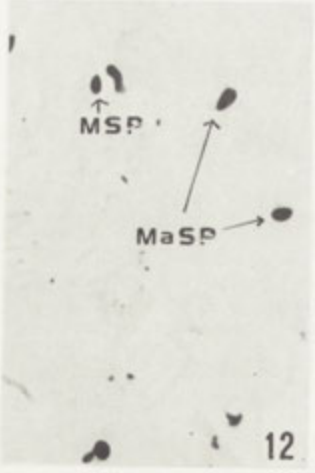

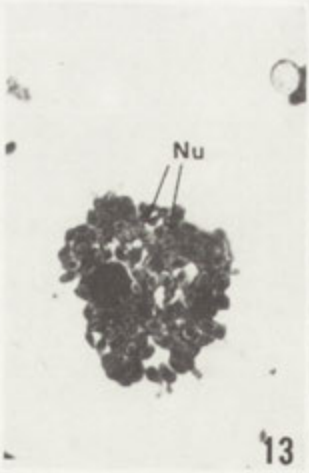

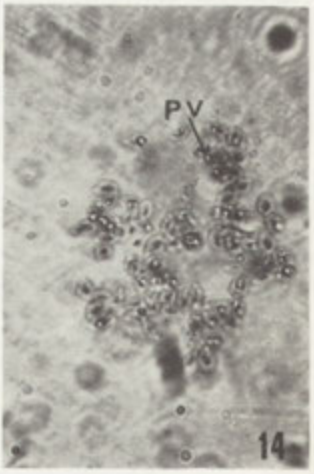

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