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Laboratory of Invertebrate Animals, Biological Research Institute of Leningrad State University, Leningrad V-164, USSR

L. N. SERAVIN and Z. P. GERASSIMOVA

A New Macrosystem of Ciliates

Synopsis. A new macrosystem of ciliates has been proposed. As the base for division of *Ciliophora* into macrotaxons two features have been used: particularities of the ultrastructure of the ectoplasmic fibrillar system and general character of structure of the ciliary system. The phylum *Ciliophora* has been divided into two classes: *Kinetodesmatophora* (having kinetodesms) and *Postciliodesmatophora* (having postciliodesms). The class *Kinetodesmatophora* includes two related subclasses: *Homoiotricha* with 4 orders previously assigned to the subclass *Holotricha* and having kinetodesms, and the second subclass *Peritricha*. The class *Postciliodesmatophora* includes 5 subclasses. As the initial one the subclass *Homotricha* is regarded; it embraces the orders previously attributed to *Holotricha* but having postciliodesms. Other subclasses assigned to this class are: *Dystricha* (— *Suctoria*), *Chonotricha*, *Spirotricha* and *Syntricha* (*Entodiniomorpha* and *Blepharocorythida*).

During last years a tendency for serious reconsideration of the ciliate macrosystem has appeared. A long time only the Stein's system created in 1855 and slightly modified later, has prevailed in protozoology. As it is known, Stein divided the ciliates into 4 main groups: *Holotricha*, *Heterotricha*, *Hypotricha*, and *Peritricha*. After Kahl (1930–1935), main taxons of ciliates (orders according to this classification) were the following: *Holotricha Spirotricha*, *Peritricha*, *Chonotricha*. The *Suctoria* were assigned the rank of a subclass. Similar systems were constructed by some other authors as Corliss (1961), Dogiel et al. (1962), Raabe (1964) etc.

Jankowski (1967), basing on some details of the buccal apparatus in ciliates, has deprecated from the traditional macrosystem giving a new one. He divided the subphylum *Ciliophora* into 3 classes: *Gymnostomea* (having no special preoral ciliature), *Ciliostomea* (having the preoral ciliature of various types), and *Tentaculifera* (deprived of primary mouth and cilia in mature stage, having tentacles). On the base of some particularities in the structure of the preoral ciliature Jankowski divided

the class *Ciliostomea* into 3 subclasses: *Fragmophora* (having kinetofragmon), *Tetrahymenophora* (having tetrahymenium), and *Polyhymenophora* (having polyhymenium).

Although Jankowski (1973) returned to the classical system, the basic ideas of his system have been accepted by other authors. De Puytorac et al. (1974) and Corliss (1974) have published new macrosystems based on Jankowski's ideas. The *Ciliophora* has been considered by them as a phylum comprising 3 classes: *Kinetofragmophora*, *Oligohymenophora* and *Polyhymenophora*. Under the influence of these works Jankowski (1975) proposed the new macrosystem, similar to that, published by him in 1967.

The previous as well as the present systems of ciliate classification seem to show that all protozoologists systematists consider this group of protozoans as a great monophyletic taxon (phylum or subphylum) of unicellular organisms. At the base of such systems a group of contemporary ciliates is laid (usually *Gymnostomata* at the rank of order or subclass) regarded as an initial one for more advanced *Ciliophora*.

In contemporary approaches to the problem of a new ciliate macrosystem formation the structure of the buccal apparatus is considered to be a fundamental feature. It includes: (a) position of the oral opening, (b) its connection with special ciliary system and (c) the character of structure of the preoral ciliature (kinetofragmon, oligo- or tetrahymenium, polyhymenium etc.). But, basing only on the characters as mentioned above, it is very difficult (practically impossible) to establish a correct macrosystem. Firstly, in many ciliates the oral opening and the buccal apparatus are strongly reduced or lacking (*Apostomata*, *Astomata*), or some other organelles, not homologous to the oral opening, serve for feeding, e.g., in *Suctoria*. No wonder that such groups are placed arbitrarily in the system. For example de Puytorac et al. (1974) and Corliss (1974, 1975) place suctorians within *Kinetofragmophora* (although *Suctoria* have no kinetophragmon), while Jankowski (1975) separates them into an idependent group at the same rank as *Kinetophragmophora*. But in fact there are no objective criteria for the first, as well as for the second opinion.

Up to now the knowledge of the structure of the oral apparatus in ciliates for systematic purposes is based mainly on light microscope investigations. It is very insufficient at present. Many authors have shown that undulating membranes and membranelles, connected with buccal apparatus, may have different ultrastructure in various ciliates, thus being not homologous (Lom and Corliss 1971, Grain 1972). This fact makes difficult proper arrangement of many ciliates within the system. For example the representatives of the order *Nassulida* have numerous

membranelles, so it would be logical to place them within the subclass *Polyhymenophora*. But as the structure of membranelles in nassulids differs from that of the typical *Spirotricha* Jankowski puts them into *Tetrahymenophora* (although they have no tetrahymenium). Their arrangement into *Kinetofragmophora* (de Puytorac et al. 1974, Corliss 1974) is also unjustified because nassulids have the structure analogous with developed polyhymenium.

In order to construct the proper system of ciliates it seems inevitable to find any morphological structure which, contrary to the buccal apparatus, persists in all forms (or in some developmental stages at least), and is fairly conservative in principal features. It would give the possibility to elucidate the phylogenetic affinities between greater taxons of ciliates.

In our investigations we have found that the ectoplasmic fibrillar system (EFS) of the ciliates may be used for these purposes (Seravin and Matvejeva 1971, Gerassimova 1975, Gerassimova and Seravin 1976, Seravin and Gerassimova 1976, Seravin and Gerassimova 1977 a, b).

Now let us gone to consider the principles of the EFS structure in ciliates as well as some particularities of its evolution within the phylum.

As it has been known due to numerous electron microscopic investigations (cf. Pitelka 1969, Grain 1969) somatic kinetosomes of ciliates have a characteristic standard (or classical according to some authors) pattern of fibrillar structures getting out from them at the same, proximal level. From each kinetosome there are 3 such derivatives going toward cytoplasm: kinetodesmal filament (KD filament), postciliary fibre (Pc fibre), and transverse fibre (T fibre).

The kinetodesmal filament, being in from of electron dense, transversely striped, thread-like structure gets off from the kinetosome at a zone of triplet 7 (according to Grain 1969). Sometimes however, the base of this filament is dilated, covering triplets 5–7. The postciliary fibre is composed of a band of parallel microtubules; it gets off always from triplet 9. The transverse fibre, beginning at a zone of triplets 3 and 4, is also composed of a band of microtubules. These three derivatives, getting off from numerous somatic kinetosomes and spreading in a defined way in the ectoplasm (cortex) of ciliates, form the ectoplasmic fibrillar net (EFS) beneath the pellicule.

In various species of ciliates particular derivatives of kinetosomes may be differently developed. When feebly developed the derivatives do not overpass the proper kinetosomal territory, while being strongly developed they may extend along some neighbouring kinetosomal territories.

In a series of cases some additional fibrillar elements appear in the

EFS. For example in *Rhynchodida,* beside the classical derivatives of kinetosomes, there are ribbons of subkinetal microtubules (Lom and Kozloff 1969). Sometimes, complete disappearance of some derivatives may be observed. The reduction of Pc fibrilles was observed in the representatives of the order Astomata (de Puytorac and Grain 1974, Gerassimova 1977).

We have tried already to distinguish the most common features of the EFS organization and to prepare their primary classification (Seravin and Matvejeva 1971, Gerassimova 1975, Gerassimova and Seravin 1976). It has been proposed to classify the types of EFS according to the development of the classical derivatives of kinetosomes. E.g., in the case of strongly developed KD filaments the EFS represents the kinetodesmal type (KD type of EFS), when Pc fibrilles are more developed the EFS belongs to the postciliary type (Pc type of EFS), etc.

As only three derivatives of kinetosomes (included into the classical pattern) are used in the analysis of the EFS structure, then, theoretically, 7 following types of the EFS might have been expected: (1) KD type, (2) Pc type, (3) T type, (4) KD-T type, (5) KD-Pc type, (6) Pc-T type, and (7) Pc-T-KD type.

Already the first investigators of subpellicular structures in ciliates have found the EFS which ought to be regarded as the KD type. In *Paramecium, Tetrahymena, Colpidium,* and others, long KD filaments were found, overcrossing several neighbouring kinetosomal territories along their ciliary rows. The Pc and T fibrilles in these forms were feebly developed (Pitelka 1961, 1963, 1965, Allen 1967, etc.). Long KD filaments, going off from 7th triplet of kinetosomes, run to the right from the ciliary row toward the anterior body end of a protozoan, joining one with the other and forming one organelle — the kinetodesma.

The Pc type of EFS is also well known in ciliates; it has been found for example in *Brooklinella, Dileptus, Tracheloraphis* etc. (Grain and Golińska 1969, Lom and Corliss 1971, Raikov et al. 1975). Strongly developed Pc fibrilles, going off from one ciliary row, join into an organelle, called by us postciliodesma (by analogy to kinetodesma) (Gerassimova and Seravin 1976).

The analysis of greater material (from literature and own data as well) has shown that there are no ciliate having simultaneously strongly developed KD filaments and Pc fibrilles — they would have not kinetodesms and postciliodesms at the same time. So we have come to a conclusion that the existence of such forms is not possible (Seravin and Matvejeva 1971, Gerassimova 1975). In this connection the "rule of closely lying triplets" has been formulated (Gerassimova 1975, Ge-

rassimova and Seravin 1976), showing that the more close the one from the other kinetosome, giving rise to any classical derivatives, is lying, the more pronounced is the inhibitory influence of one of them against the other. It is known that the bases of KD filaments and Pc fibrilles are close (triplets 7 and 9). So, according to the rule of closely lying triplets, not only Pc-KD type of EFS is impossible, but also Pc-T-KD type can not exist.

The electron microscopic studies have revealed the occurrence of Pc-T type of EFS in ciliates. It was found in a series of representatives of the order *Heterotricha* (Kennedy 1965, Yagiu and Shigenaka 1963, Peck et al. 1975). Together with typical postciliodesms these ciliates have T fibrilles going off from their ciliary rows toward neighbouring rows of cilia and crossing over several kinetosomal territories. Strong development of T fibrilles in *Heterotricha* has a secondary character and may be lacking. Just in *Stentor* (Grain 1968) these fibrilles are very short, ending within the ranges of their own kinetosomal territories. In such way the Pc-T type of the EFS seems to be derived from the Pc type.

Up to now the ciliates having T type and KD-T type of the EFS have not been found. There are some premises that T type of the EFS is "forbidden" for ciliates — it can not exist at all. For this speaks the fact that in flagellates such as *Opalina* and *Hypermastigina,* having greater number of undullipodia (flagella or cilia) some rudiments of the EFS appear and their structural elements usually join the kinetosomes of one row, being thus longitudinal (Noirot-Timothée 1959, Wessenberg 1966, Hollande and Valentin 1967, Hollande and Carruette-Valentin 1971). Transverse derivatives of kinetosomes (analogous to T fibrilles) have been found only in *Hypermastigina,* but together with obligatory occurrence of longitudinal ones.

Although we do not know exactly the cause, in all studied cases (in flagellates as well as in ciliates) the longitudinal derivatives are the most important for junction of somatic kinetosomes, the transverse ones have only an auxiliary role. So, the occurrence of strongly developed T fibrilles simultaneously with feebly developed KD filaments and Pc fibrilles seems to be little probable. Then, it seems that the species having the EFS of the transverse type will never be found. Simultaneously there is no evidence against the hypothesis on the origin of KD-T type of EFS from the KD type. It is quite probable that such ciliates will be found during further investigations.

The ciliates appeared when somatic kinetosomes of their ancestors had assumed three standard derivatives (KD filament, Pc and T fibrilles). As in all contemporary *Ciliophora* these derivatives get off from

the same points of the kinetosome triplets there are no doubts that they all originated from the only one initial group, *Protociliophora*. These ancestrine forms might have had somatic kinetosomes with three feebly developed derivatives of the classical pattern. Further evolution of ciliates might have run along two independent ways. In some species the development of the EFS has been achieved by growing length of kinetosomal filaments anad kinetodesms formation, in the other ones — due to stronger growth of postciliary fibrilles leading to postciliodesms formation. According to the rule of close triplets any crossing of these two phyletic lines was not possible in the process of evolution.

Foundations of the New Macrosystem of Ciliates

Due to the electron microscope the EFS has been investigated up to now in all basic orders of ciliates. All the facts uphold the opinion about great conservativeness of the EFS type of structure and, what will be shown further, its preservation in larger taxons of *Ciliophora*. This gives the possibility to use the EFS type of structure as the foundation of the systems and phylogeny of ciliates.

If the type of structure of the EFS is used for construction of the ciliate macrosystem two main classes may be distinguished without any difficulty the first class *Kinetodesmatophora* which includes the ciliates having a characteristic ectoplasmic fibrillar system with kinetodesms, and the second one — *Postciliodesmatophora* comprising the ciliates with developed postciliodesms in the EFS.

In the classical systems (e.g., Dogiel et al. 1962) the group *Holotricha* was regarded as the initial class or subclass. In the contemporary systems this group is rather regarded as artificial or compound one (de Puytorac et al. 1974, Corliss 1974, Jankowski 1975). Electron microscopic investigations have ascertained such point of view. Only a part of orders, previously assigned to *Holotricha,* may be placed within *Kinetodesmatophora.*

Strongly developed KD filaments were found in all examined representatives of the order *Hymenostomata* (Pitelka 1961, 1965, 1969, Allen 1967, Nilsson 1969, Didier 1970, Kattar 1973, Beams and Kessel 1973, Didier and Detcheva 1974, Gerassimova 1976 a, b etc.). In *Astomata* the EFS is practically composed of kinetodesms, as it was described in a series of papers (de Puytorac 1961 a, b, 1963 a, b, Gerassimova 1975, 1977).

The kinetodesmal type of the EFS occurs also in ciliates of the order *Apostomata* (Bradbury 1966, 1974, Bradbury and Pitelka 1965, de Puytorac and Grain 1975). Finally, strongly developed

kinetodesms occur in the representatives of the suborder *Arhynchodina* (Lom et al. 1968, Khan 1970, Antipa 1971), previously arranged into a compound order *Thigmotricha*, regarded at present as independent order *Thigmotricha* (Jankowski 1975).

All these data bear witness for phylogenetic nearness of the above considered groups of ciliates. The totality of orders, previously attributed to *Holotricha* but having kinetodesms, we propose to separate into a new subclassis *Homoiotricha* (from Greek *homoios* = similar).

The recognition of structure of the buccal apparatus in the representatives of the subclass *Peritricha* has shown its relation with the structure of this organellum in *Homoiotricha*. So, some authors regard *Peritricha* to be phylogenetically related with *Homoiotricha* (de Puytorac et al. 1974, Corliss 1974, 1975). Such point of view is supported also by the data on the EFS structure in *Peritricha*. In mature forms of *Peritricha* somatic ciliature is completely reduced and the corresponding EFS is lacking, but it occurs in disperse stages of these ciliates. The electron microscopic investigation carried by us have revealed that in swarmers of *Vorticella* the EFS of locomotory ciliature (cyclokineties) is organized according to the kinetodesmal type. In *Opisthonecta*, retaining a zone of cilia in mature stage, the ectoplasmic fibrillar system is strongly modified and the type of its structure remains obscure (Bradbury 1965). Occurrence of kinetodesms in the EFS of the representatives of subclass *Peritricha* gives final account for including this group into *Kinetodesmatophora*.

To the class *Postciliodesmatophora* the group of these orders should be assigned at first which have been previously ranged within the class *Holotricha*, but have postciliodesms instead of kinetodesms. The postciliodesms have been found in all examined representatives of the order *Gymnostomata* (Grain and Golińska 1969, de Puytorac and Kattar 1969, Bohatier 1970, Rieder 1971, Sołtyńska 1971, Seravin and Matvejeva 1971, Raikov 1971–1972, Holt et al. 1973, Kink 1973, Bohatier and Njine 1973, Kovaleva 1974, Raikov et al. 1975, de Santa Rosa and Didier 1975, etc.). Postciliary fibrilles, taking a part in their construction, form narrow or fairly broad bands composed of microtubules.

It has been found that in the cortex of *Gymnostomata* the postciliary fibrilles are arranged in two different modes, but they may give also some transitory variants. In the first mode the Pc fibrilles are arranged subpellicularly. The narrow subpellicular Pc fibrilles occur only in these gymnostomatous ciliates which have the somatic ciliature formed of single cilia (Grain and Golińska 1969, de Puytorac and Kattar 1969, Bohatier 1970, Grain 1970, Seravin and Ma-

tvejeva 1971, Kink 1973, Bohatier and Njine 1973, Matvejeva 1973, etc.). In such case the Pc fibrilles rise up towards the pellicula and, lying one near the other, form a flat postciliodesma, lining up the inner membranella of the pellicule. The second mode of Pc fibrilles arrangement is lateral. In general it is characteristic of these *Gymnostomata* which have the somatic cilia arranged in pairs (or only the kinetosomes are paired), and give rise to wide Pc fibrilles. Such fibrilles are known in *Trachelocercidae* (Raikov and Dragesco 1969, Pitelka et al. 1971, Kovaleva 1974, Raikov et al. 1975), in *Loxodes* (de Puytorac and Njine 1970), in *Kentrophoros* (Raikov 1971–1972). As a rule, the lateral wide Pc fibrilles do not rise up towards pellicule but extend at the right side of each ciliary row forming postciliodesms, composed of thick bundles of parallel postciliary bands, each of them being formed of greater number of microtubules.

In *Brooklinella* the Pc fibrilles are in form of not so wide and numerous bands, lying in bundles at the left side of neighbouring interciliary row (Lom and Corliss 1971). This is an intermediate variant between subpellicular and lateral patterns of these structures. Thus, there exist three main types of arrangement of postciliodesms beneath pellicule (subpellicular, lateral and intermediate), characteristic of all ciliates having developed Pc fibrilles.

The junction of cilia into pairs ought to be regarded as the initial, progressive stage in the change of ciliature in ciliates foregoing the development of more complicated structures, such as membranelles, cirri, etc. So, it may be supposed that not wide subpellicular postciliodesms, occurring in some *Gymnostomata* in the kineties with singular cilia, have been the base for development of intermediate and lateral postciliodesms in other gymnostomatous ciliates.

The order *Trichostomata* comprises the ciliates having the EFS organized according to postciliary type, similar to gymnostomatous ciliates. However, in some representatives of this order some peculiar structures may be found in the organization of this system. In *Paraisotricha*, *Balantidium*, and some other forms (de Puytorac and Grain 1965, Grain 1966, Paulin and Kraschenninnikov 1973) the postciliodesms occupy intermediate position, as in *Brooklinella* (Lom and Corliss 1971). However, the EFS of such *Trichostomata* as *Colpoda cucculus* (Didier and Chessa 1970), *Colpoda steini* and *Tillina magna* (Lynn 1976, Gerassimova 1976 a, b) has somewhat different structure. In all three species the somatic cilia are paired and not wide postciliodesms are situated subpellicularly.

Recently, a tendency has arisen to separate *Colpoda* from *Trichostomata* and to establish an independent order *Colpodida* for these ciliates (de Puytorac et al. 1974, Corliss 1974). The electron microscope data ascertain such point of view. Besides, some already known facts prove that duplication of somatic cilia not necessarily leads to changes in position of postciliodesms (as it has been observed in *Gymnostomata).*

The postciliary type of EFS organization has been found in ciliates of the suborder *Rhynchodina*, formerly arranged within *Thigmotricha*, but now forming a special order *Rhynchodida* (de Puytorac et al. 1974, Corliss 1974, 1975, Jankowski 1975). The postciliodesms were found in all species of *Rhynchodida* that have been examined in this respect (Lom and Kozloff 1969, Khan 1969). The EFS of these ciliates is closely related to that found in gymnostomatids of the type *Brooklinella* (Lom and Corliss 1971) and in trichostomatids of the type *Paraisotricha* and *Balantidium* (Grain 1966).

We propose to combine the ciliates having postciliodesms and formerly included into subclass *Holotricha* (orders *Gymnostomata*, *Trichostomata* and *Rhynchodida*) into a subclass *Homotricha* (from Greek *homos* = equal). Thus, the former subclass *Holotricha* has been divided into two independent subclasses — *Homoiotricha* and *Homotricha* related to two different classes: *Homoiotricha* to *Kinetodesmatophora* and *Homotricha* to *Postciliodesmatophora.* The names of these subclasses, bring out their succession from *Holotricha.*

In most up to now examined representatives of the order *Heterotricha* (subclass *Spirotricha*) the EFS is alike. Out of the classical pattern of kinetosomal derivatives two elements are more strongly developed — wide Pc fibrilles, being lateral in most cases, and T fibrilles which extend subpellicularly. Consequently, the EFS of the postciliary-transverse type is formed. KD filaments in *Heterotricha* are feebly developed; they do not overpass their kinetosomal territories. There are no doubts that *Heterotricha* can not be regarded as descendants of *Hymenostomata*, as it has been supposed by some authors (de Puytorac et al. 1974, Corliss 1974, Jankowski 1973, 1975 and others). In contrast, all premises support the view that *Heterotricha* originated from any *Gymnostomata*, having wide postciliodesms (Gerassimova 1975, Raikov et al. 1975, Gerassimova and Seravin 1976).

The data, although not numerous, on the EFS structure in other representatives of *Spirotricha* (Tuffrau et al. 1968, Grain 1972, Laval-Pento 1975 and others) ascertain the supposition that this subclass ought to be placed within *Postciliodesmatophora.*

The suctorians (subclass *Suctoria* or *Tentaculifera*) have no cilia in mature stage. Remnants of the EFS are preserved in form of a field of

kinetosomes situated in the region of contractile vacuole (Millecchia and Rudzińska 1972). But in swimming migratory forms the structure of EFS is fairly well developed and corresponds to the postciliary type (Mignot and de Puytorac 1968, Batisse 1972 and others). Besides, the elements of EFS, by the grade of development, character of connections among them, and distribution under the pellicule, correspond with those in *Gymnostomata*. Consequently, the suctorians ought to be phylogenetically related with any *Gymnostomata*.

The representatives of the subclass *Chonotricha* have the ciliary system only in a zone of a "funnel". It is a ciliature of preoral apparatus, however a small part of somatic ciliature is also preserved. The electron microscopic study of the EFS in this body part, carried on *Chilodochona quennerstedti* (Grain and Batisse 1974), has ascertained that the ectoplasmic fibrillar system of chonotrichous ciliates is related with the postciliary type. Thus, the former supposition that *Chonotricha* are the derivatives of *Gymnostomata* (Jankowski 1973) has been confirmed by the structure of their EFS.

A peculiar feature of ectoplasmic fibrillar system in the migratory forms of suctorians and chonotrichs is the occurrence of additional fibrilles — subkinetal bands of microtubules. Similar fibrilles are present in some *Gymnostomata* (e.g., in *Brooklinella*) and in *Rhynchodida*. The occurrence of this element in the EFS, not present in other ciliates, gives the ground for supposition that these groups of protozoans are phylogenetically related.

Lately, the systematists have approached *Suctoria* to *Rhynchodida* (de Puytorac et al. 1974, Corliss 1974). The occurrence of common structures in the EFS of both these groups of ciliates confirms the genuiness of such point of view.

Taking into account the fact that *Suctoria* have lost (in adult stage) their ciliature in the process of evolution, we propose to change the name of this subclass into *Dystricha* (from Greek prefix *dys-* = to lose sth.). This name expresses, similarly as in other subclasses, the state of the ciliary system in a group of ciliates.

Excellent papers on *Blepharocorythidae* by Wolska (1966, 1967, 1971) have shown that these ciliates, previously included within the order *Gymnostomata*, ought to be separated into an independent order *Blepharocorythida*, closely related to *Entodiniomorpha*. Thus, already at light microscope level the relationships between these two orders and the most primitive representatives of *Postciliodesmatophora*, i.e., *Gymnostomata*, have been proved.

A common evolutive feature of both *Blepharocorythidae* and *Entodiniomorpha* seems to be oligomerization of ciliature and close approach of

kinetosomes of all kineties in a zone of the anterior and the posterior body ends (in *Ophryoscolecidae* the cilia at the posterior body end are completely reduced). In these body parts some larger, not fragmented fields of kinetosomes are formed (Noirot-Timothée 1960, Nouzarède 1965, Gerassimova and Seravin 1978 a, b). These fields of closely arranged kinetosomes (with or without cilia) we propose to name synal fields (from Greek *syn-* = together). In *Blepharocorythida* and *Entodiniomorpha* practically the whole ciliature is in form of synal fields of kinetosomes. So, the group comprising *Blepharocorythida* and *Entodiniomorpha* may be named *Syntricha*. The more so, the cilia in these ciliates are frequently fused into larger complexes — syncilia.

The EFS in *Syntricha* is greatly modified. Unfortunately, there are only a few electron microscopic investigations on the structure of cortex in these ciliates (Noirot-Timothée 1960, Roth and Shigenaka 1964, Nouzarède 1965, Senaud and Grain 1972 and others). These studies concern, however, only the cortex of *Ophryoscolecidae*. In all examined representatives of this group the kinetosomes of syncilia are provided with KD filament and T fibre. Both these derivatives are feebly developed. However, there is always one more, well developed derivative of a kinetosome, namely the so called retrociliary fibre, getting off from the proximal end of kinetosome and running toward endoplasm (Noirot-Timothée 1960). It is composed of microtubules. The complex of retrociliary fibrilles of each syncilium, forming an organellum, we propose to name retrociliodesme by analogy to kinetodesme and postciliodesme (Gerassimova and Seravin 1978 a, b).

The recent electron microscopic investigations on the representatives of the family *Ophryoscolecidae* — *Entodinium* simplex and *Epidinium ecaudatum* (Gerassimova and Seravin 1978 a, b) have fournished the data on possible homology of retrociliodesms with organelles of the buccal apparatus — nemadesms, as well as the EFS in *Syntricha*, composed of cuticular microtubules, with postciliodesms. However, further investigations are necessary to upheld this supposition.

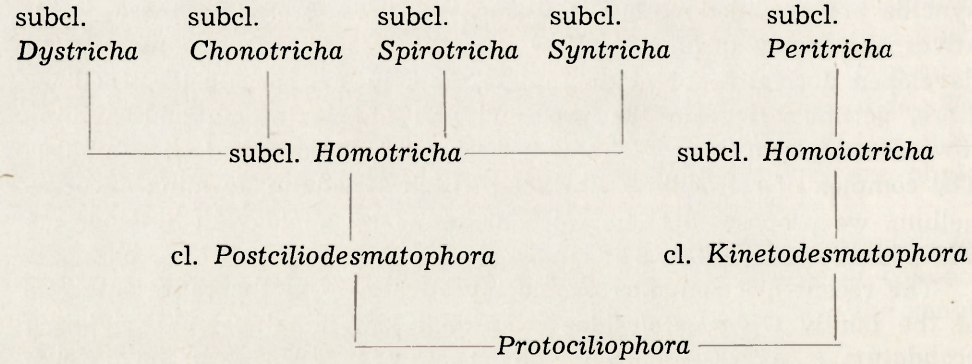
Particular structure of the ciliary system in *Syntricha* is connected with unique structure of the cortex and with other parts of their cytoplasm as well (Gerassimova and Seravin 1978 a, b). All these data prove that the opinion pronounced by some authors that *Blepharocorythidae* and *Entodiniomorpha* ought to be regarded as the members of one group at the rank of order (de Puytorac et al. 1974, Corliss 1974) is virtually right. We agree with Jankowski (1975) who places *Entodiniomorpha* (however, without *Blepharocorythida*) at the rank of subclass. In our opinion the *Syntricha* (*Blepharocorythida* and *Entodinio-*

morpha) ought to be arranged as the subclass within the class *Postciliodesmatophora*.

In such way we propose the following macrosystem of the phylum *Ciliophora*:

cl. *Kinetodesmatophora* Seravin et Gerassimova, 1977
(1) subcl. *Homoiotricha* Seravin et Gerassimova, 1977
(2) subcl. *Peritricha* Calkins, 1933
cl. *Postciliodesmatophora* Seravin et Gerassimova, 1977
(3) subcl. *Homotricha* Seravin et Gerassimova, 1977
(4) subcl. *Dystricha* Seravin et Gerassimova, 1977
(5) subcl. *Chonotricha* Calkins, 1933
(6) subcl. *Spirotricha* Bütschli, 1889
(7) subcl. *Syntricha* Seravin et Gerassimova, 1977

When we take into account that all contemporary ciliates originated from any closely related forms of *Protociliophora*, the phylogenetic relations of greater taxons of ciliates (at the level of classes or subclasses) would be following:



Thus, the evolution of contemporary ciliates has run through two main independent pathways. More advantageous appeared to be this one which had taken place in ciliates having postciliodesms.

Short Characteristic of Taxons

The distribution of ciliates into classes has been accomplished at the base of structure of the EFS and on some particularities of structure of the ciliature.

(A) Class *Kinetodesmatophora* — comprises ciliates which in adult or other developmental stage in the somatic ciliature have the most developed KD filaments out of the classical pattern; it leads to kinetodesms formation. This feature is expressed in the name of the class. The Pc

fibrilles never overpass the ranges of their kinetosomal territories; sometimes they are reduced. The kinetosomes of the preoral apparatus may have relatively well developed postciliary fibrilles.

(B) Class *Postciliodesmatophora* — comprises the ciliates which, in adult or any developmental stage, have the most developed Pc fibrilles in the somatic ciliature. This feature is emphasized in the name proposed for this class. The KD filaments never overpass the ranges of their kinetosomal territories; sometimes they may be reduced. In some groups, together with Pc fibrilles also T fibrilles may achieve stronger growth. It results in formation of the postciliary-transverse type of EFS which may be regarded as a descendant of the postciliary type. The kinetosomes of the preoral ciliature have fairly well developed T fibrilles.

In the process of evolution of ciliates the changes of structure of the somatic ciliature have taken place. In lower, more primitive ciliates of both classes the body is covered with more or less equally arranged rows of cilia. As the evolution proceeded two directions of changes in the ciliature might have been distinguished:

(1) Reduction or oligomerization of the ciliary apparatus.

(2) Approaching of ciliary rows and cilia.

The latter leads to the synal fields formation and, subsequently, to formation of other structures as syncilia, membranelles, cirri.

The processes of approaching of kinetosomes (and cilia) are the most common in formation of the buccal apparatus of ciliates. In a series of cases, especially in sedentary and parasitic forms, the reduction or oligomerization of the somatic ciliature reaches so far that the basic locomotory function of the ciliature is performed by the ciliary system connected with buccal apparatus.

Separation of ciliates into classes is done by us on the base of substantial modifications of the ciliature and the EFS, attained by ciliates in the process of evolution.

(A 1) Subclass *Homoiotricha*

In the adult stage the kineties of somatic ciliature preserve the EFS of the kinetodesmal type. The cilia, taking a part in the motion of ciliates (together with cilia of peristomal field), have the same or similar dimensions. This feature has contributed to the name of the taxon.

(A 2) Subclass *Peritricha*

The somatic ciliary apparatus of these ciliates is reduced totally or in greater part. In adult forms the KD filaments are lacking in the EFS. However, the kinetodesmal type of structure of the EFS occurs in dispersal stages (swarmers). The cilia of the peristomal field are arranged in rings contributing to the name of the taxon.

(B 1) Subclass *Homotricha*

In the adult stage the ciliates have the EFS of the postciliary type. The cilia (including these in peristomal field), taking a part in the motion of ciliates, have approximatively the same length. Hence the name of the taxon. Membranelles, if persist, in the oral aperture, are never spiral shaped.

(B 2) Subclass *Spirotricha*

In adult stage the ciliates preserve the postciliary type of the EFS, or, they assume often the postciliary-transverse type. Beside single or double somatic cilia in the movements of these ciliates take a part the peristomal membranelles forming a spiral. This has contributed to the name of the taxon. The somatic cilia may be grouped and some complexes are formed; the most complicated are cirri.

(B 3) Subclass *Syntricha*

The EFS of these ciliates is represented by transformed postciliodesms. The somatic ciliature is in a great part or totally reduced. The kinetosomes are grouped into greater synal fields and the cilia are joined together to form synal fields and syncilia. This feature is marked in the name of the taxon.

(B 4) Subclass *Chonotricha*

The somatic ciliature is strongly reduced, up to a small piece, a group of undullipodia. Their kinetosomes are connected with the EFS of the postciliary type. The cilia of the preoral apparatus are situated at a peculiar "funnel" which contributed to the name of the taxon.

(B 5) Subclass *Dystricha*

Former name — *Suctoria* or *Tentaculifera*. In adult stage the ciliates are completely devoid of cilia. Only a small synal field of kinetosomes with reduced EFS is preserved. In migratory juvenile stages the EFS of the postciliary type occurs.

Wide and intensive studies carried at present on the structure of the ciliature connected with the oral apparatus of ciliates have contributed to desintegration of old orders and to establishing numerous new ones. Thus, in the traditional system created by Dogiel et al. (1962) were 16 orders, in the system proposed by Jankowski (1975) this number was raised up to 40. We agree with the opinion that the structure of the ciliature of the buccal apparatus is one of the most important features for establishing the system at the level of orders. However, it is necessary, in order to avoid mistakes, to elucidate with the aid of electron microscope the basal principles determining the changes which occur in the EFS when the kinetosomes are approaching, e.g., in the formation of synal fields of various types, giving rise to various complicated ciliary structures as syncilia, membranelles, cirri etc.

A Review of the New Macrosystem

The macrosystem proposed by us basically differs from any other up to day existing systems by the use of a new feature for its formation — the type of the structure of the EFS. It gives the ground to suppose that the evolution of ciliates has been achieved by two independent pathways. However, about the *Protociliophora*, the ancestors of the contemporary *Ciliophora*, only retrospective extrapolation may give some information.

The approach accepted by us has shown that the principle of subsequent evolution of the ciliary system in connection with the buccal apparatus (which may be expressed in a following formula: kinetofragmon — tetrahymenium — polyhymenium), formulated by Furgason (1940) and serving up to now as a base of the ciliate system is not right. Due to the studies on the EFS of ciliates it has been found that *Spirotricha* (having polyhymenium) originated from *Gymnostomata* (having kinetofragmon) and not from *Hymenostomata* (having tetrahymenium).

The approaching of cilia, formation of synal fields of kinetosomes and membranelles or membrane-like organelles might have taken place independently or parallelly in various groups of ciliates. This is ascertained by electron microscope investigations (Nilsson 1969, Lom and Corliss 1971, Tucker 1971, Grain 1972, and others).

The macrosystem proposed by us is phylogenetic, i.e., based on relationships between greater taxons which was not the case of other systems based on peculiarities of the buccal apparatus structure. Thus, de Puytorac et al. (1974) and Corliss (1974) approach *Apostomata*, *Rhynchodida* and *Suctoria*, although the *Apostomata* are typical representatives of *Kinetophragmophora*, phylogenetically inevitably related with *Hymenostomata* and not with such representatives of *Postciliodesmatophora* as *Rhynchodida* and *Suctoria* are.

The systematists usually derive the *Astomata* and *Hymenostomata* from *Gymnostomata*, i.e., the forms having kinetodesms from those with postciliodesms. Taking into account the rule of approaching triplets it is impossible to accept such point of view.

Recognition of the EFS gives also the possibility to bring a series of important particularities in the macrosystem of ciliates and to establish correct phylogenetic relationships between greater taxons in contrast to previous attempts.

Finally, our macrosystem is fully consistent with other contemporary systems of ciliates what ascertains the entity of data, although the ways which have led to these results were substantially different.

РЕЗЮМЕ

Обосновывается новая макросистема инфузорий. При делении типа *Ciliophora* на макротаксоны использованы два признака: особенности ультратонкого строения эктоплазматической фибрилларной системы и общий характер строения ресничной системы. Тип *Ciliophora* разделен на два класса: *Kinetodesmatophora* (имеющих кинетодесмы) и *Postciliodesmatophora* (имеющих постцилиодесмы). Класс *Kinetodesmatophora* включает два родственных подкласса: п/кл *Homoiotricha*, к которому принадлежат 4 отряда инфузорий, ранее относившиеся к п/кл *Holotricha* и имеющие кинетодесмы, второй — п/кл *Peritricha*. Класс *Postciliodesmatophora* включает 5 подклассов. Исходным является п/кл *Homotricha* он содержит те отряды, которые ранее принадлежали к п/кл *Holotricha*, но имеющие постцилиодесмы. Остальные подклассы следующие: *Dystricha* (= *Suctoria*), *Chonotricha*, *Spirotricha*, *Syntricha* (*Entodiniomorphida*, *Blepharocorythida*).

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Institute of Zoology, Academy of Sciences of the Azerbaijan SSR, 5 Krylov Street, Baku 370122, and Institute of Cytology of the USSR Academy of Sciences, 32 Maklin Avenue, Leningrad 190121, USSR

F. G. AGAMALIEV

Morphology of some Free-living Ciliates of the Caspian Sea

Synopsis. The morphology of certain free-living ciliates of the Caspian sea has been studied with silver impregnation according to Chatton and Lwoff and with other cytological methods. A new species, *Plagiopyla binucleata* sp. nov., is described, as well as the morphology of 18 existing species which are characteristic of the Caspian sea microbenthos, periphyton and plancton: *Holophrya simplex, Enchelyodon trepida, Prorodon mimeticus, Chaenea tesselata, Coleps tesselatus, Chlamydodon triquetrus, Kentrophoros uninucleatum, Trithigmostoma cucullulus, Plagiopyla nasuta, Paramecium woodruffi, Uronema nigricans, Ophryoglena atra, Cyclidium citrullus, Anigsteinia salinara, Oxytricha marina, Oxytricha halophila, Stylonychia mytilus, Euplotes affinis.* Variability of some morphological characters of these species is stated.

The present paper contains descriptions of one new and 18 previously known ciliate species of the Caspian sea. Most of the latter have been described very briefly and/or using only *in vivo* observations. They are rather abundant either in the microbenthos, or in the plancton, or else in solid surface overgrowth (periphyton) of the Caspian sea and deserve more thorough morphological characterization. Most descriptions which follow are based on both *in vivo* observations and permanent preparations impregnated by the Chatton–Lwoff silver nitrate method (Chatton et Lwoff 1930), the latter method giving valuable information about the somatic and buccal infraciliature. The nuclear apparatus has been studied on Feulgen or Mayer's hemalum stained preparations.

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Descriptions of Species

Holophrya simplex Schewiakoff, 1893 (Fig. 1)

This species was first found by Schewiakoff on the Hawaii. It is abundant in both the mesopsammon and the periphyton of the Caspian sea. Both living and fixed animals were studied.

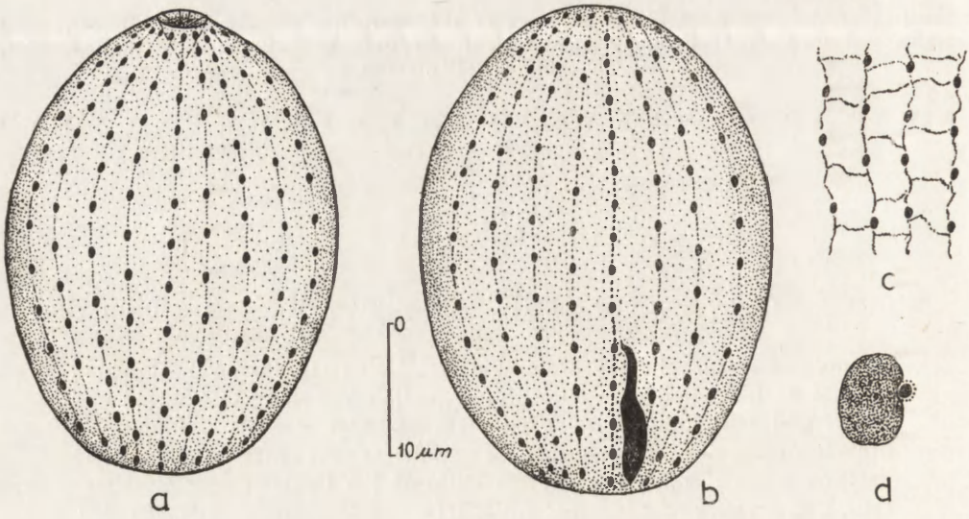


Fig. 1. *Holophrya simplex* Schewiakoff, 1893, a — ventral view, b — dorsal view, c — ventral argyrome (silver impregnated whole mounts); d — nuclei (Feulgen reaction)

Body form ellipsoid, sometimes almost spherical (Fig. 1, a); cytoplasm transparent. Cytostome apically located, funnel-shaped, and surrounded with indistinct fibrils.

Ciliature uniform, consisting of 18–20 meridional rows of cilia (Fig. 1 a, b). The meshes of the argyrome are more or less rectangular (Fig. 1 c). Contractile vacuole single, terminal.

One oval macronucleus, about 5 µm long, and one spherical micronucleus adjacent to it (Fig. 1 d).

Body length of living animals, about 50 µm; fixed ones measure 35–40 µm in length and 25–30 µm in width.

Common in fine and very fine sand of the Caspian sea, as well as in overgrowth of rocks, stones, hydrotechnical constructions, and experimental glass slides.

Enchelyodon trepida (Kahl, 1925) Borror, 1965
(Fig. 2, Pl. I 1–3)

This ciliate has been described by Kahl (1928) as *Trachelocerca trepida*. Borror (1965) transferred it into the genus *Enchelyodon* and gave its re-description. Numerous specimens of this ciliate have been found in overgrowth of natural objects (rocks, stones) on the west coast of Middle Caspian (near Sumgait). They are described using *in vivo* observations and silver impregnated material.

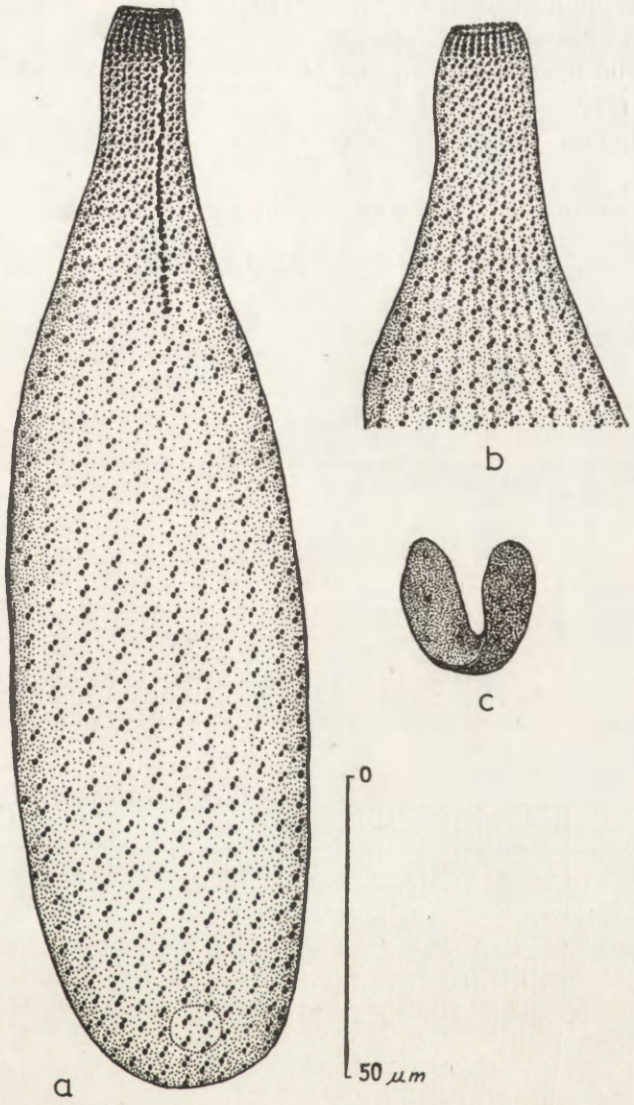


Fig. 2. *Enchelyodon trepida* (Kahl, 1933) Borror, 1965, a — ventral view, b — anterior end, dorsal view (silver impregnation); c — macronucleus (Feulgen reaction)

Body form bottle-like, symmetrical, with tapering neck-like anterior and broadly rounded posterior end (Fig. 2 a, Pl. I, 1). Contractile vacuole single, terminal (Fig. 2 a). Cytostome apical, surrounded by 4 or 5 girdles of prominent closely spaced kinetosomes bearing long cilia (Fig. 2 a, b, Pl. I 2). Anterior end usually filled with refringent granules. No pericytostomal fibrils have been found, neither *in vivo*, nor in silver impregnated preparations. A thigmotactic row starts at the mouth rim and proceeds

meridionnally along the entire "neck", for about 50 μm (Fig. 2 a; Pl. I 1, 2). It consists of closed-up kinetosomes bearing longer cilia.

Ciliature uniform, consisting of 24–26 meridional kineties with paired kinetosomes (Fig. 2 a, b, Pl. I 2, 3).

One C-shaped macronucleus (Fig. 2 c); no micronucleus has been found.

Body length 150–200 μm. Common in the Caspian sea periphyton.

Prorodon mimeticus Kahl, 1930 (Fig. 3)

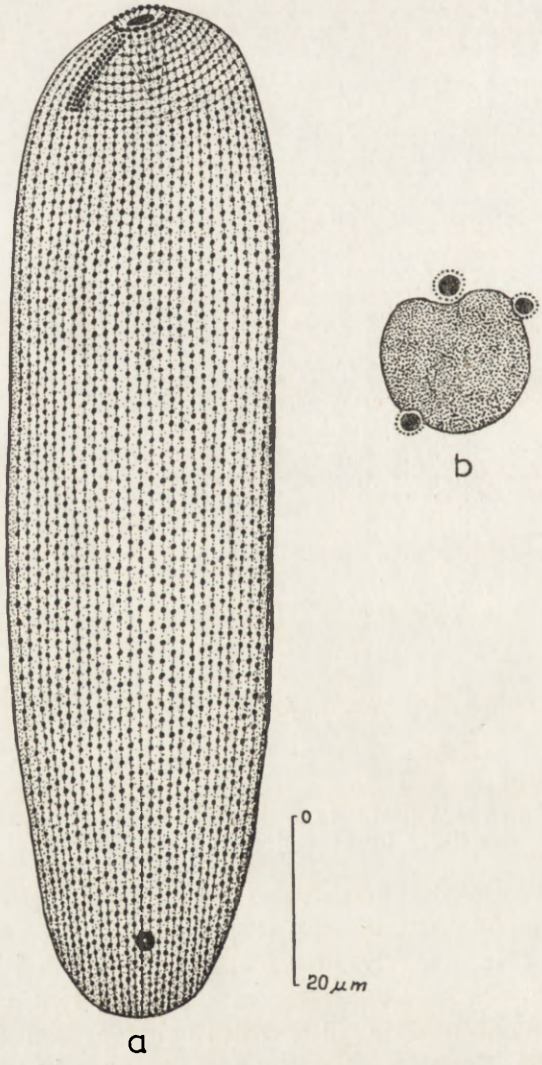


Fig. 3. *Prorodon mimeticus* Kahl, 1930, a — general aspect (silver impregnated whole mount), b — nuclei (Hemalum staining)

This brackish-water ciliate has been found in the Northern and other regions of the Caspian sea.

Body cylindrical (Fig. 3 a). Cytostome subapical, very slightly displaced to one side, funnel-shaped, surrounded by a girdle of strong cilia. Cytopharyngeal basket visible.

Thigmotactic apparatus consisting of three very closely adjacent rows of kinetosomes bearing longer cilia (Fig. 3 a). The anterior kinetosomes of the somatic kineties are clearly aligned into several concentric pericytostomal circles across the actual kineties (the so-called "adesmokineties").

Ciliature dense, uniform consisting of 65–80 meridional rows starting at the mouth rim. Two shorter kineties start behind the thigmotactic apparatus (Fig. 3 a).

Macronucleus single, spherical, about 15 μm in diameter; several micronuclei adjacent to it (Fig. 3 b).

Body length 120–150 μm, sometimes up to 200 μm.

Biotop: fine and medium sand and periphyton of the Caspian sea.

Chaenea tesselata (Kahl, 1933) Dragesco, 1965
(Fig. 4, Pl. I 4–5)

This species, first described by Kahl (1933) as *Trachelocerca tesselata*, has later been transferred by Dragesco (1965) to the genus *Chaenea* and provided with a brief description based on *in vivo* observations. We found this ciliate in big quantities at the west coast of the Middle Caspian (Sumgait region). It is described using both observations of living animals and studies of silver impregnated preparations (Chatton and Lwoff's technique).

Body fusiform (Fig. 4 a, b, Pl. I. 4). Living ciliates are transparent except the anterior region which is filled with inclusions. "Head" conical, with apical cytostome surrounded with short "trichites" which are seen only *in vivo*. Thigmotactic apparatus consisting of a single row of large closely implanted cilia (Fig. 4 b, c). Rear third of the body more narrow, contains a single terminal contractile vacuole.

The ciliature consists of 18–21 meridional kineties; "adesmokineties" (transverse alignments of kinetosomes) exist throughout the body. The kinetosomes seem to be paired (Fig. 4 b, Pl. I 5).

Single macronucleus (Fig. 4 d). No micronucleus has been found in our material.

Body length of living ciliates 300–350 μm, of fixed ones, 140–150 μm.

Biotop: overgrowth of stones.

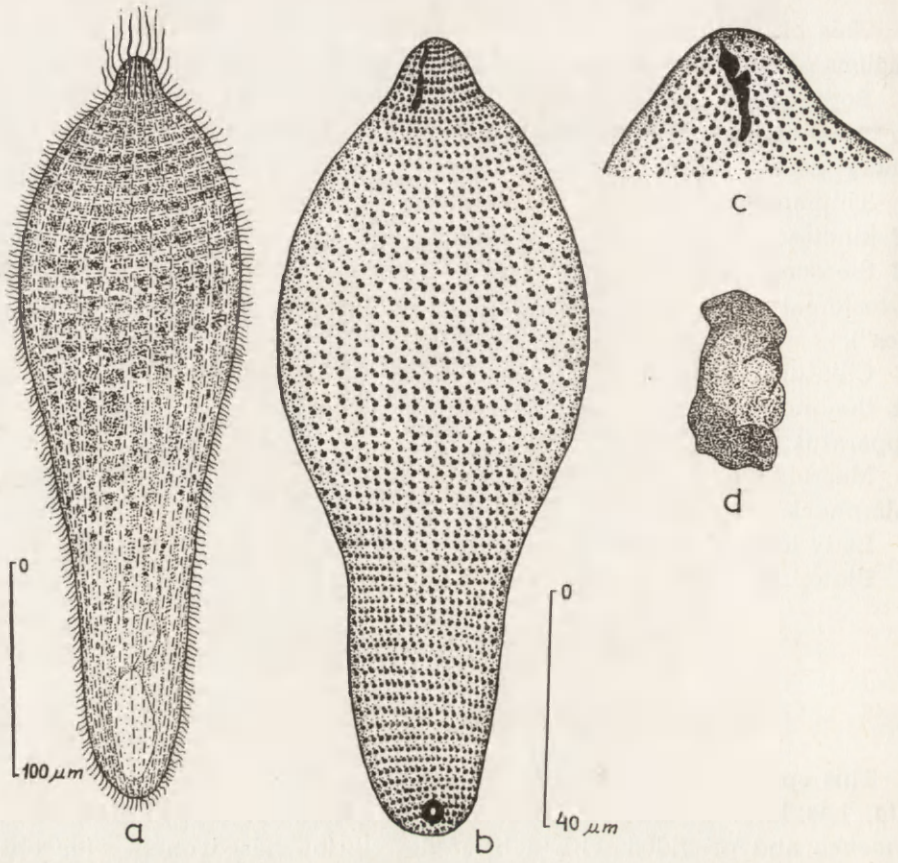


Fig. 4. *Chaenea tesselata* (Kahl, 1933) Dragesco, 1965, a — living animal, b — general aspect of a silver impregnated whole mount, c — anterior body end (silver impregnated), d — macronucleus (Feulgen reaction)

This species may deserve separation into a new genus, since, in other species of *Chaenea*, no thigmotactic apparatus is known, and the anterior ends of the somatic kineties are helical.

Coleps tesselatus Kahl, 1930 (Fig. 5, Pl. II 6)

This ciliate is widely distributed in the Caspian sea, being abundant in the microbenthos as well as in the plancton and the periphyton.

Body oval (Fig. 5 a, Pl. II 6), covered by skeletal plates. Living animals appear dark in incident light. Cytoplasm filled with inclusions. Cytostome large, apical.

Ciliature uniform, consists of 18–20 longitudinal kineties. Each kinetosome is accompanied by two smaller argentophilic granules (Fig. 5 a;

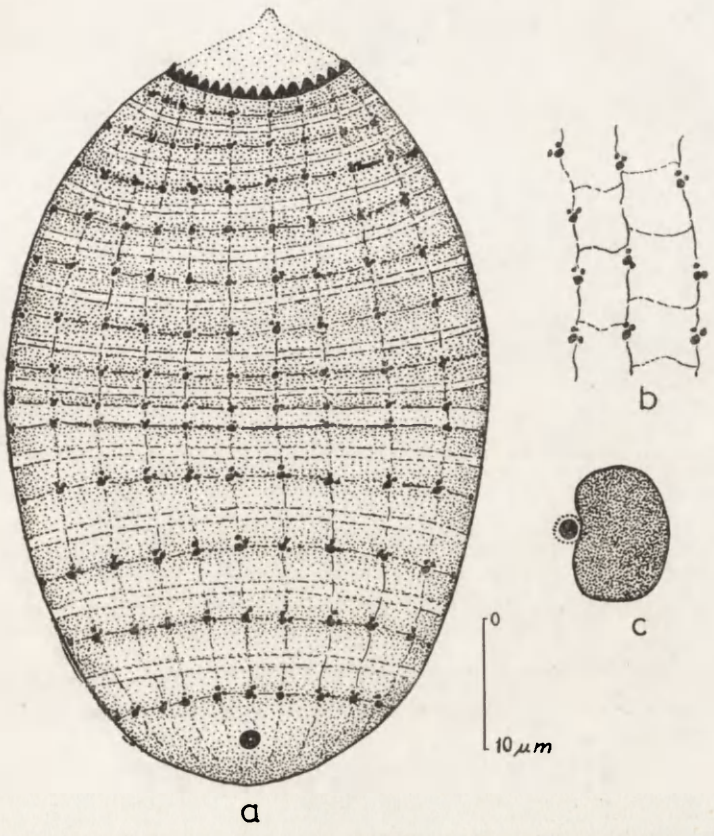


Fig. 5. *Coleps tesselatus* Kahl, 1930, a — general view of a silver impregnated specimen, b — argyrome, c — nuclei (Feulgen reaction)

Pl. II 6). The kinetosomes are aligned into 12 or 13 regular transverse rows throughout the body. The argyrome consists of rectangular meshes (Fig. 5 b).

Nuclear apparatus consisting of an oval macronucleus, about 10 µm long, and of an adjacent spherical micronucleus (Fig. 5 c).

Body length 50–60 µm, body width 38–40 µm. Contractile vacuole single, terminal; its pore is prominent in impregnated animals (Fig. 5 a).

Biotop: fine saprobic sand, surface overgrowth and plancton; especially abundant in spring and autumn. The Caspian form seems to be entirely identical to the previously described forms of this species.

Chlamydodon triquetrus O. F. Müller, 1786 (Fig. 6, Pl. II 7)

Mesosaprobic species often found also in polysaprobic sands of various seas. Dragesco (1963) first gave a detailed description of this species

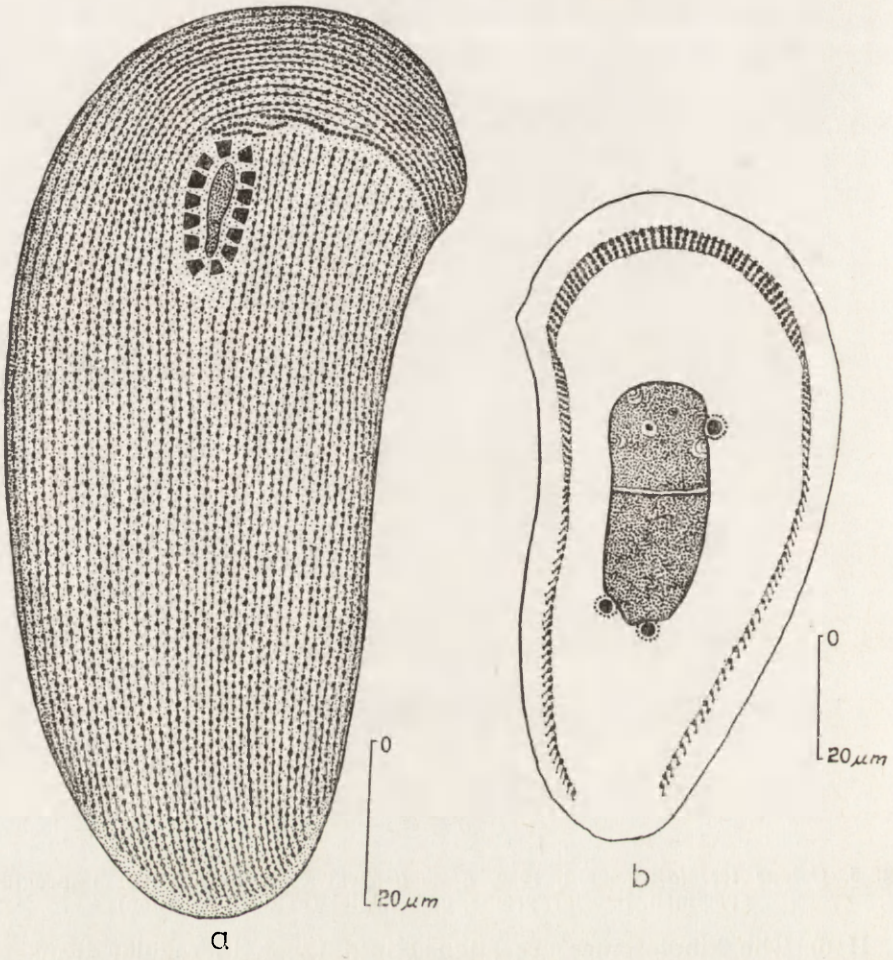


Fig. 6. *Chlamydodon triquetrus* O. F. Müller, 1786, a — ventral view, b — dorsal view (silver impregnation); nuclei from a Feulgen stained animal are shown in b

using silver impregnated material from Roscoff. Found in the Caspian sea microbenthos, plancton, and periphyton. The description below is based mainly on Chatton–Lwoff silver impregnated animals.

Body lanceolate, strongly dorso-ventrally flattened (Fig. 6 a, Pl. II 7). Anterior body edge curved, with a weak projection at the left side. Posterior body end vaguely pointed. Living ciliates yellowish-white in incident light. Cytoplasm granular, opaque.

Cytostome oval, opening in the anterior third of the ventral body side and surrounded with a pharyngeal basket of 13–15 nemadesmata.

The ciliature is largely ventral (Fig. 6 a). It consists of 48–50 kineties which slightly bend to the left near the posterior end. Five kineties start

from behind the cytostome and reach the rear body end; 16–18 kineties pass left of the cytostome, and 26–28 kineties right of it. Anterior to the mouth, the latter bend to the left and end against the left body edge. The preoral ciliature consists of three specialized kineties located along the suture between the left and the right ciliary fields (Fig. 6 a).

The dorsal body side carries only the so-called striated ribbon, running along the body edges but interrupted posteriorly (Fig. 6 b). It consists of many short rows of closely packed kinetosomes, 3 to 4 in each row.

Macronucleus single, oval, 25–30 μm long, heteromerous (consisting of an orthomere and a paramere). It is surrounded by 3 or 4 spherical micronuclei (Fig. 6 b).

Body length 120–140 μm, width 45–50 μm.

Biotopes: fine homogeneous sand, algal detritus, surface overgrowth, and plancton of the Caspian sea.

Kentrophoros uninucleatum Raikov, 1962 (Fig. 7)

This species, first found by Raikov (1962) in the White sea, has been discovered also at the Apsheron peninsula coast, Caspian sea.

Body form lanceolate, elongated, dorso-ventrally flattened (Fig. 7 a). Anterior end forming a thin and pointed rostrum. Rear end pointed and forms a transparent "tail" (Fig. 7 a).

Ciliature unilateral, consists of 15–17 longitudinal rows of cilia. Nonciliated left (physiologically dorsal) body side covered with a uniform layer of sulphur bacteria, attached perpendicularly to the body surface. Only the rostrum and the "tail" are free from bacteria.

Nuclear apparatus represented by a single nuclear complex ("complex nucleus") located in the middle region of the body. The nuclear complex consists of 2 central micronuclei and several peripheral macronuclei with indistinct boundaries, containing nucleoli and chromatin granules (Fig. 7 b). Body length 500–600 μm.

Biotop: fine, moderately saprobic sand (0.52% of organic matter).

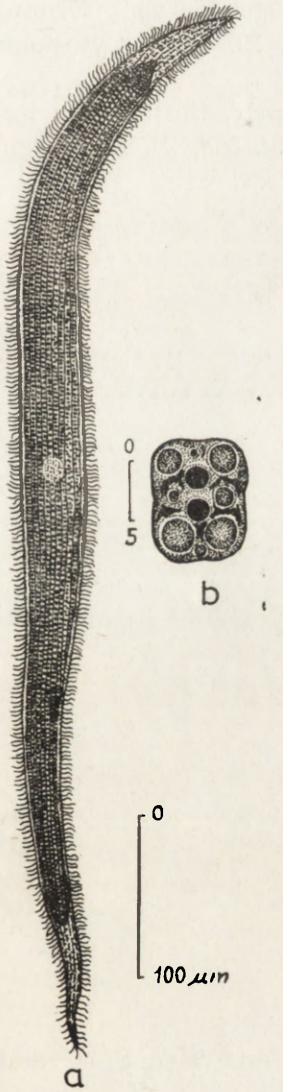


Fig. 7. *Kentrophoros uninucleatum* Raikov, 1962, a — general aspect of living ciliate, b — nuclear complex (hemalum staining)

Trithigmostoma cucullulus (O. F. Müller, 1786) Jankowski, 1967 (Fig. 8, Pl. II 8, 9)

This ciliate has long been considered a species of *Chilodonella*, until Jankowski (1967) separated it into a new genus. *T. cucullulus* is mainly a freshwater form; nevertheless, it has been found in big quantities in both the plancton and the periphyton of the North Caspian and of the west coast of the South Caspian (near the Kura delta).

Body lanceolate, strongly flattened dorso-ventrally (Fig. 8, a, Pl. II 8). Living ciliates brownish-white in incident light. Cytoplasm filled with inclusions and ingested diatoms. Anterior body end curved, forming a slight bulge at the left-anterior edge (Fig. 8 a, Pl. II 8). Cytostome circular, located near the anterior end.

The entire ventral body side is ciliated. Anteriorly, the kineties terminate against both sides of the preoral suture (Fig. 8 a, Pl. II 8). Ten

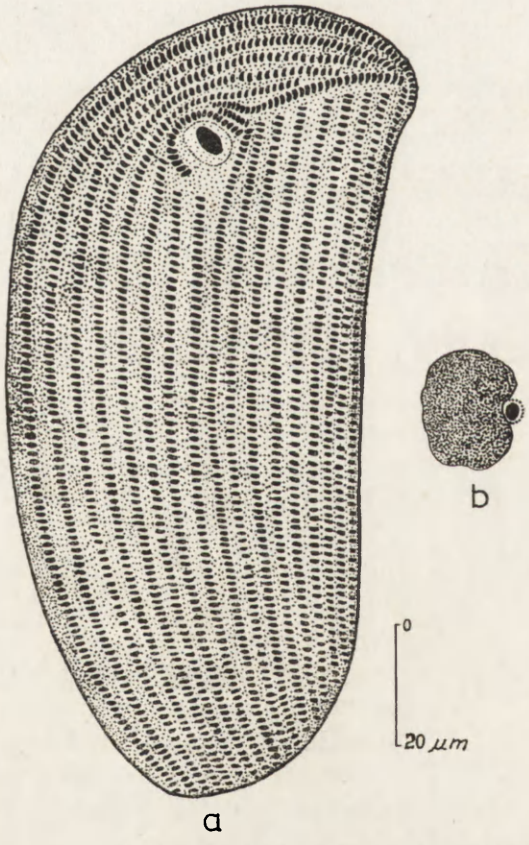


Fig. 8. *Trithigmostoma cucullulus* (O. F. Müller) Jankowski, 1967, a — ventral view of silver impregnated specimen, b — nuclei (Feulgen reaction)

kineties pass to the left of the cytostome, six kineties, to the right of it. Three kineties begin behind the cytostome (Fig. 8 a, Pl. II 9). In all, there are 19 to 20 ventral kineties. The kinetosomes are large, narrowly spaced (Fig. 8 a, Pl. II 9). The preoral specialized kineties are typical of *Trithigmostoma*; the third one of them skirts the cytostome anteriorly (Fig. 8 a, Pl. II 8).

Macronucleus single, ovoid, about 10 μm long; micronucleus single, spherical (Fig. 8 b). Body length 130–150 μm, body width about 60 μm.

Biotops: overgrowth of algae and the plancton of the Caspian sea.

Plagiopyla nasuta Stein, 1860 (Fig. 9)

Descriptions of this species have been given by Kahl (1931), Jankowski (1964), and Borror (1972 a). It proved to be abundant in the Caspian sea periphyton. Silver impregnated material has been used for the following description.

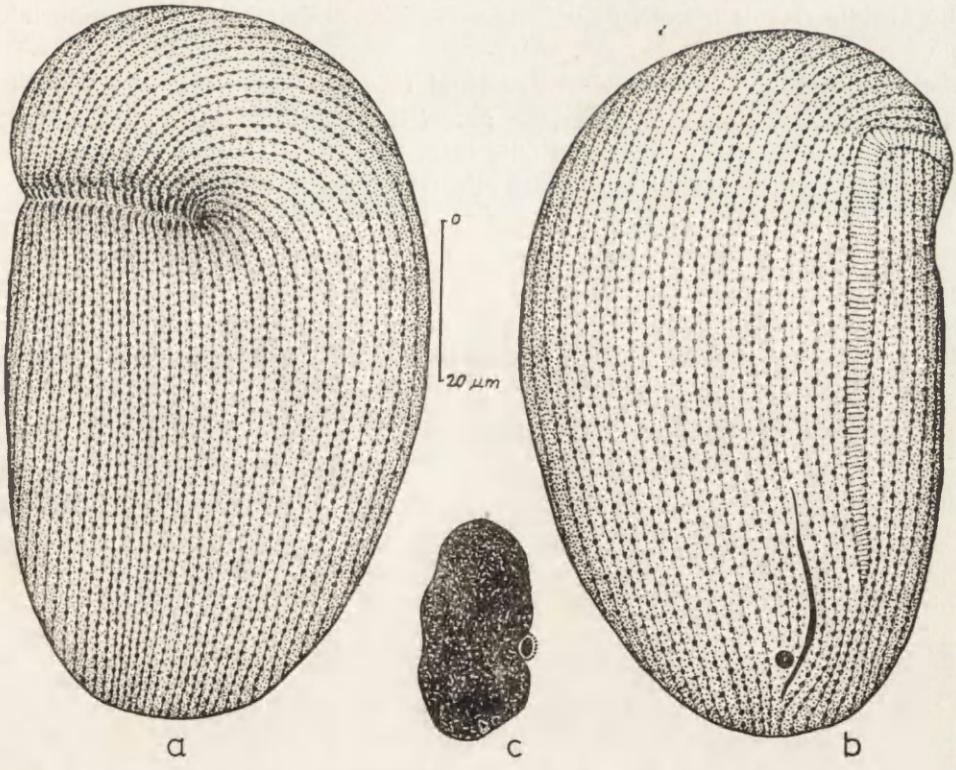


Fig. 9. *Plagiopyla nasuta* Stein, 1860, a — ventral view, b — dorsal view (silver impregnated whole mounts); c — nuclei (Feulgen reaction)

Body form oval, with rounded extremities (Fig. 9 a). Living ciliates opaque in incident light, cytoplasm filled with inclusions. Mouth slit-like, surrounded by closely set anterior cilia of the somatic kineties. Cytopharynx short, displaced to the left side of the buccal cavity. A wide, curved, transversely striated ribbon approaches the buccal field from the dorsal body side. Posteriorly, this ribbon ends at 20 μm distance from the rear body end (Fig. 9 b).

Ciliature dense, consisting of 60–70 kineties. Some of them reach the rear body end, others terminate against other kineties not far for it (Fig. 9 b).

The nuclear apparatus occupies the body centre and consists of a single oval macronucleus and of an adjacent micronucleus (Fig. 9 c).

Body length 90–110 μm, body width 50–60 μm.

Biotops: epiphytic on both natural objects and experimental slides.

Plagiopyla binucleata sp. nov. (Fig. 10, Pl. III 10, 11)

This ciliate has been observed in the periphyton of the west coast of the Middle Caspian. Both *in vivo* observations and impregnated material have been used.

Body form oval, somewhat flattened (Fig. 10 a, Pl. III 10). Mouth transverse, slit-like, located in the anterior body third, and surrounded by a zone of numerous enlarged cilia (Fig. 10 a, Pl. III 10). The latter are derivatives of the closely set anterior kinetosomes of the somatic kineties. Cytopharynx short, conical, visible on both living and fixed animals.

Cytoplasm opaque, filled with inclusions. Contractile vacuole single, posteriorly located.

Ciliature dense, consisting of 60–70 meridional kineties. Ventral meridians start behind the mouth slit and proceed parallel to each others until the rear end. Dorsal kineties, which also start from the mouth slit, proceed at first anteriad, then bend onto the dorsal side (Fig. 10 a, b. Pl. III 11). A wide, curved, transversely striated ribbon, characteristic of the genus *Plagiopyla*, joins the mouth slit from the dorsal side. Ventral argyrome formed by irregular meshes (Fig. 10 c).

Nuclear apparatus consisting of two rounded macronuclei and a single, intermediate, spherical micronucleus (Fig. 10 d).

Body length 80–100 μm, width 60–70 μm.

Biotop: periphyton on stones.

This species is characterized by presence of two macronuclei and a single micronucleus. No *Plagiopyla* species with such a nuclear apparatus have so far been described. This form shows also a peculiar shift of the contractile vacuole pore — cytopyge complex towards the rear body end,

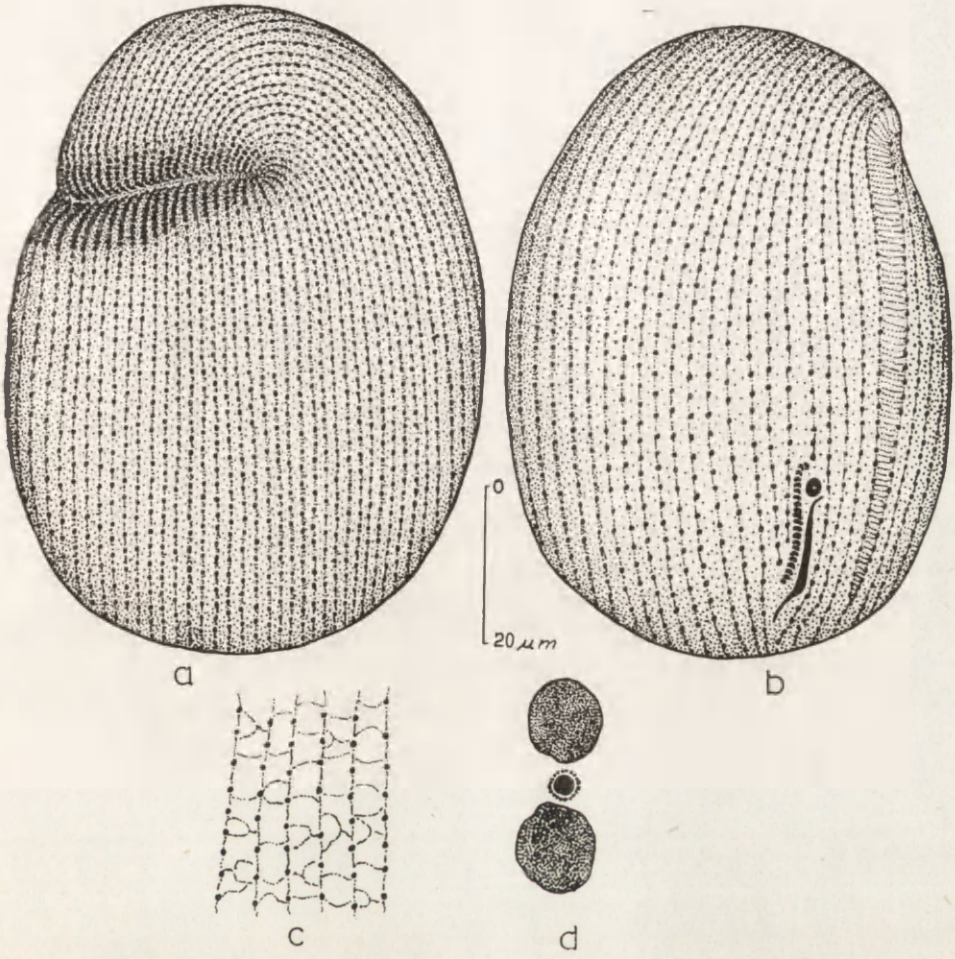


Fig. 10. *Plagiopyla binucleata* sp. nov., a — ventral view, b — dorsal view, c — argyrome (silver impregnation); d — nuclei (Feulgen reaction)

as well as a greater length of the dorsal cross-striated ribbon which here almost reaches the rear end. In other *Plagiopyla* species this ribbon usually terminates somewhat behind the body equator.

Paramecium woodruffi Wenrich, 1928 (Fig. 11, Pl. III 12, 13)

Polysaprobic brackish-water species, described by Wenrich (1928) from salt marshes of the USA. Later Jankowski (1969) observed it in slightly brackish localities of the Leningrad district; he also cultivated this ciliate and studied its morphology.

We observed *P. woodruffi* in mass quantities in polluted regions of the

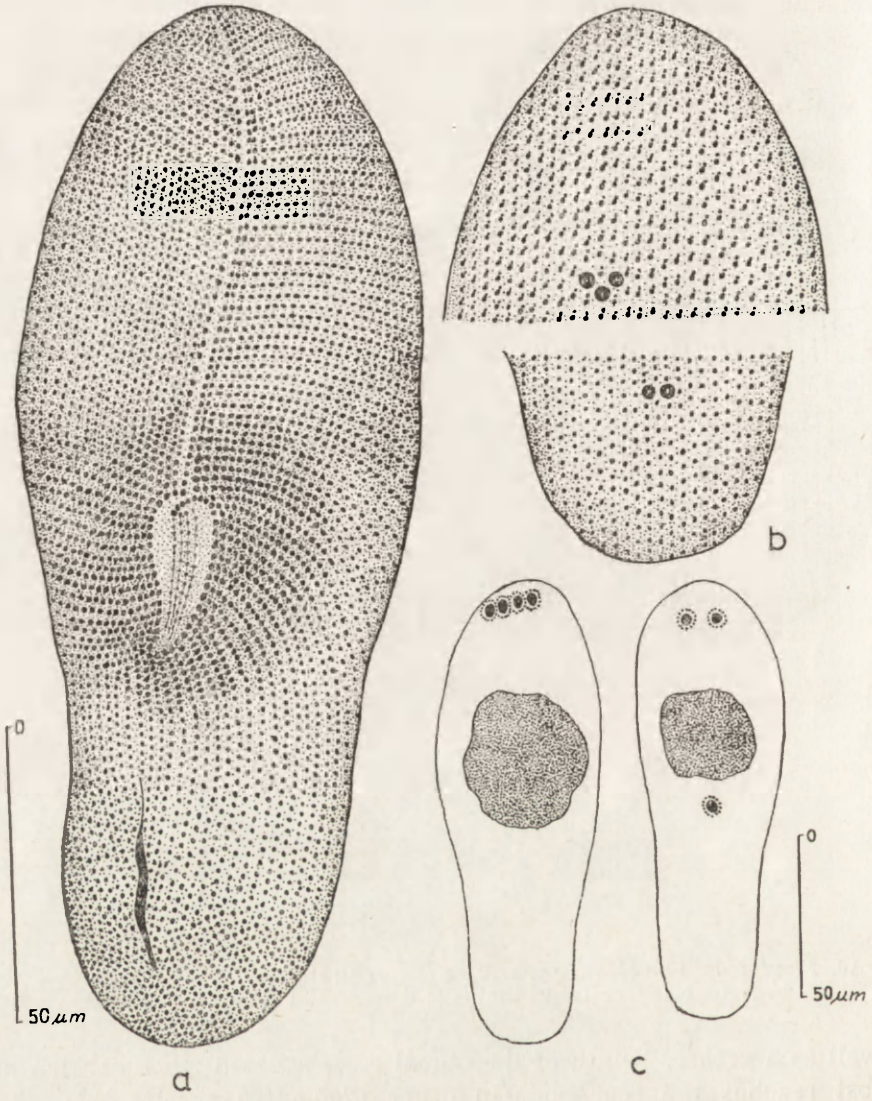


Fig. 11. *Paramecium woodruffi* Wenrich, 1928, a — ventral view, b — front and rear ends (silver impregnated whole mounts); c — nuclei (Feulgen reaction)

Caspian sea, especially in saprobic sands near Sumgait and on the Peschanyi island. It is abundant also in overgrowth of stones in the above regions.

Body shoe-shaped with rounded ends (Fig. 11 a). Living ciliates brownish-white in incident light. Endoplasm filled with inclusions. Trichocysts apparent in living specimens but lacking in fixed ones.

Ciliature dense, consisting of 80–85 kineties. Mouth slightly behind

the body equator. A well-developed system of secondary peristomal rows of kinetosomes (the adesmokineties), perpendicular to the real kineties, is apparent in the peristome region (Fig. 11 a, Pl. III 12). There are 18–20 adesmokineties at the right side of the peristome. Usually two contractile vacuoles, but sometimes 4, 5, and even 6, their pores are well visible in impregnated animals (Fig. 11 b, Pl. III 13). Kinetosomes of the dorsal somatic kineties appear to be paired (Fig. 11 b).

There is one spherical macronucleus, 25 μm in diameter, and 3 or 4 micronuclei located near the anterior body end. The micronuclei either form a compact group near the body end, or surround the macronucleus (Fig. 11 c). In the former case, the distance between the macronucleus and the micronuclei is about 20–25 μm.

Body length 160–180 μm, body width, about 70 μm. Biotop: fine polysaprobic sand and periphyton of the Caspian sea.

Uronema nigricans O. F. Müller, 1786 (Fig. 12, Pl. IV 14, 15)

Detailed descriptions of this ciliates have been given by Thompson and Evans (1968) and Puytorac et al. (1974). It has been found in mass quantities in the microbenthos, the plancton, and the periphyton of the Caspian sea and is described using silver impregnated material.

Body oval, with rounded ends (Fig. 12, Pl. IV 14, 15). Buccal apparatus,

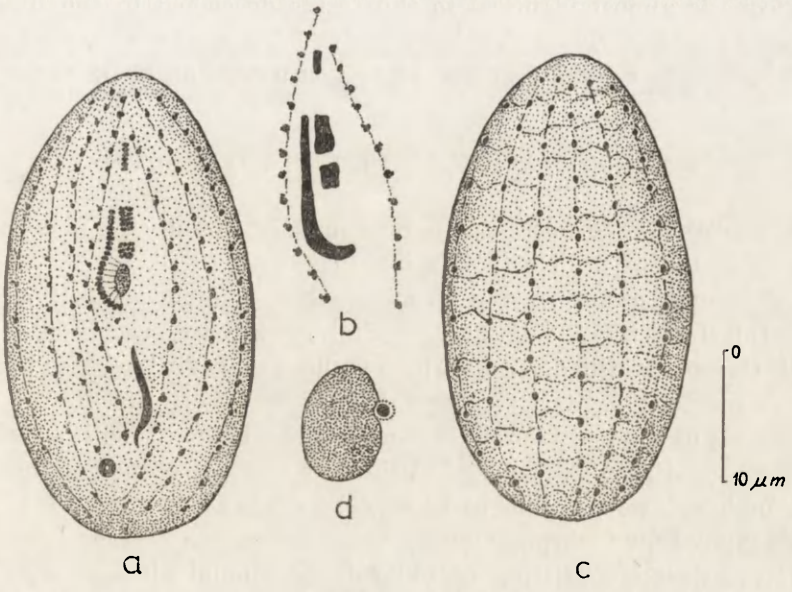


Fig. 12. *Uronema nigricans* O. F. Müller, 1786, a — ventral view, b — buccal infraciliature, c — dorsal view (silver impregnation); d — nuclei (Feulgen reaction)

reaching until the body equator, consists of an undulating membrane (UM) and three membranelles (M_1, M_2, M_3) (Fig. 12, a, b, Pl. IV 14). The UM is about 10 μm long, and the whole body cavity, about 18–20 μm long. The M_1 is rather anteriorly placed and consists of a single row of argentophilic granules. It is separated from the M_2 by a space about 2.5–3 μm. The M_2 begins at the same level as the UM and consists of two rows of granules. The M_3 is near the M_2 and is formed by irregularly placed granules (Fig. 12 a, b).

The somatic ciliature comprises 13 kineties; two of them, located to the right of the buccal cavity, do not reach the rear body end and terminate at the level of the rear end of the cytopyge. The first kinety to the left of the buccal cavity starts at the level of the anterior end of the M_1 and not at the apical pole. The rest of the kineties reach the edges of both polar nonciliated plates, the front one and the rear one. Each ventral kinety contains at average 17 kinetosomes. Contractile vacuole single, posterior, with a pore which opens at the end of the right second kinety (Fig. 12 a).

Dorsal body side carrying 5–6 kineties, with 12 to 14 kinetosomes in each. Argyrome with rectangular meshes (Fig. 12 c).

Nuclear apparatus central, consisting of a single oval macronucleus and a single micronucleus (Fig. 12 d).

Body length of fixed animals about 30–40 μm, width 20 μm.

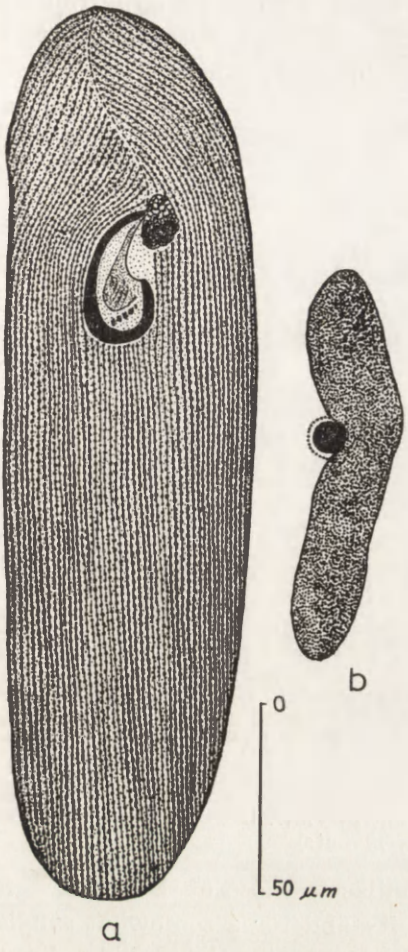
Biotop: fine sand, periphyton, and plancton. The Caspian form of *U. nigricans* is almost identical to the forms described in the literature. However, it is slightly larger than both Thompson and Evans' form (21–29 μm) and the form of Puytorac et al. (20–30 μm).

Ophryoglena atra Kahl, 1932 (Fig. 13, Pl. IV 16)

This fresh-water form proved to be abundant in both the microbenthos and the plancton of the North Caspian.

Body oblong, almost non-contractile, with rounded ends (Fig. 13 a, Pl. IV 16). Left body side always slightly convex, right side nearly straight. Cytoplasm filled with various inclusions. Living ciliates brownish white in incident light. Cytostome located between the first and the second thirds of the body length. A watchglass-like body, with an adjacent black pigmented spot, is localized at the left anterior corner of the buccal cavity; both are well visible in fixed cells (Fig. 13 a, Pl. IV 16). Buccal ciliature typical for *Ophryoglena*.

Ciliature dense, consisting of 200–230 meridional kineties with small, closely inserted kinetosomes. Fourteen to 16 kineties begin behind the



buccal cavity and proceed until the rear body end. The other kineties start anteriorly at both sides of the preoral suture (Fig. 13 a). Endoplasm strongly vacuolized. Contractile vacuoles numerous, with pores clearly visible in impregnated animals.

Macronucleus single, elongate (up to 100 µm long), with an adjacent large micronucleus (Fig. 13 b). Body length about 200–250 µm, body width 50–60 µm.

Main biotop: very fine and fine sand of the Caspian sea.

Cyclidium citrullus Cohn, 1865
(Fig. 14)

Detailed descriptions of both the somatic and the buccal ciliatures of this species have been given by Berger (1959) and Czapik (1963). Our form, found in the periphyton of the west coast of the Middle

Fig. 13. *Ophryoglena atra* Kahl, 1932, a — ventral view of a silver impregnated specimen, b — nuclei (hemalum staining)

Caspian, proved to be somewhat different from the forms already known; it is here described using both living and impregnated material.

Body form ovoid, with rounded ends (Fig. 14). Buccal cavity 20–25 µm long, occupies about 2/3 of the body length. Buccal organelles represented by an undulating membrane and three membranelles (Fig. 14 a). Cytopyge located immediately behind the buccal cavity, on a surface free from somatic kineties (Fig. 14 a).

Ciliature consisting of 14–15 meridional rows. All of them start at the level of the front end of the undulating membrane and proceed until the level of the rear end of the cytopyge. Two kineties, one on each side of the buccal cavity, have more closely implanted cilia than others. Contractile vacuole single, posterior; its pore opens behind the second right kinety (Fig. 14 a). Dorsal body side with 7–8 kineties (Fig. 14 b).

Nuclear apparatus anteriorly located, consisting of one large rounded macronucleus (8 μm in diameter) and a single micronucleus adjacent to it.

Body length of fixed animals about 30 μm, body width 20–25 μm.

Biotop: overgrowth of rocks, stones, underwater constructions, and experimental slides.

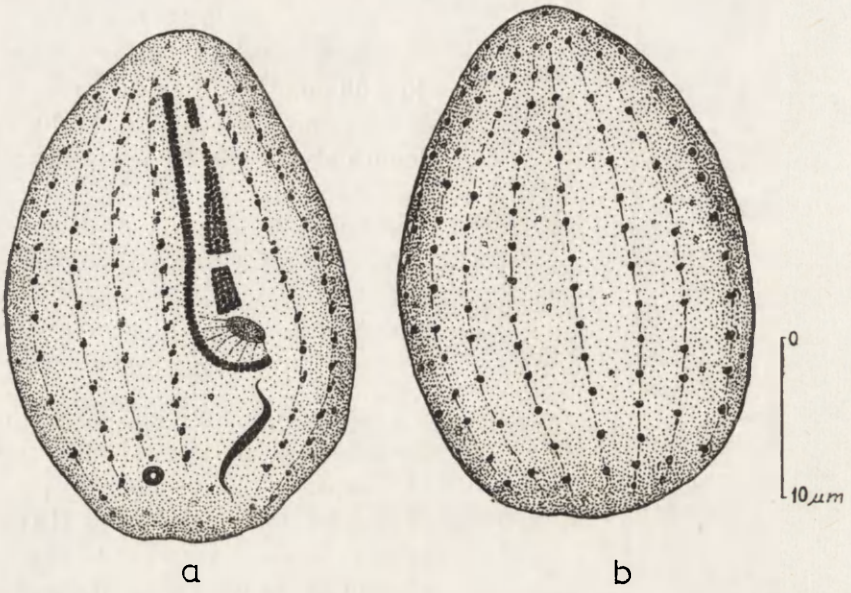


Fig. 14. *Cyclidium citrullus* Cohn, 1865, a — ventral view, b — dorsal view (silver impregnated whole mounts)

The Caspian form of *C. citrullus* is similar to those of Berger and Czapik, except for a larger buccal cavity and for a somewhat smaller number of kineties (14 to 15 in our form, vs. 16 in Czapik's form).

Anigsteinia salinara (Florentin) Isquith, 1968
(Fig. 15, Pl. IV 17)

This species, formerly known as *Blepharisma salinarum*, has been separated by Isquith (1968) into a new genus *Anigsteinia* (together with *B. clarissimum*). *A. salinara* is a polysaprobic form, widely distributed in polluted regions of the Caspian sea. The description is given using both living and fixed material.

Body lanceolate, laterally flattened (Fig. 15 a, Pl. IV 17). Rear end with a single large contractile vacuole. Posterior body region transparent, anterior region brownish, filled with inclusions. Adoral zone of membranelles (AZM) proceeding along the ventral body edge for a distance less

Fig. 15. *Anigsteinia salinara* (Florentin) Isquith, 1968, a — left side view of a silver impregnated specimen, b — nuclei (Feulgen reaction)

than or equal to the half of the body. Undulating membrane (UM) occupying the right side of the peristomal cavity. AZM consisting of 50–60 membranelles.

The somatic ciliature consists of 20 to 26 kineties. Kineties of both the right and the left body sides terminate against the post-oral suture (Fig. 15 a).

Many (50 to 130) small macronuclei, scattered in the anterior body region (Fig. 15 b). Body length (fixed cells) about 170–200 µm.

Biotop: very fine and fine sands as well as periphyton of the Caspian sea.

Oxytricha marina Kahl, 1932 (Fig. 16)

This species is widely distributed in the periphyton, the microbenthos, and the plancton of the Caspian sea.

Body elongate oval, dorso-ventrally flattened (Fig. 16 a). Cytoplasm opaque, filled with ingested diatoms and other inclusions. Peristome large (45 μm), occupies slightly less than a half of the body length. AZM consisting of 30 to 35 membranelles. Ventral body side carrying two rows of marginal cirri, 24–26 cirri in the right row and 18–20 in the left one. Eight frontal, 5 transversal, and 5 ventral cirri are present. Contractile vacuole single, with a pore located to the right of the peristome (Fig. 16 a). Dorsal body side with three kineties, each carrying 16 to 23 bristles (Fig. 16 b). A single short row of very closely inserted argentophilic granules is apparent in the left posterior region of the dorsal side (Fig. 16 b).

Two oval macronuclei, with two or three micronuclei (Fig. 16 b). Body length 90–120 μm, width about 50 μm. The Caspian form seems to be almost identical to the forms described by Kahl (1932) from the Baltic sea and by Borror (1972 b) from the Atlantic coast of the USA.

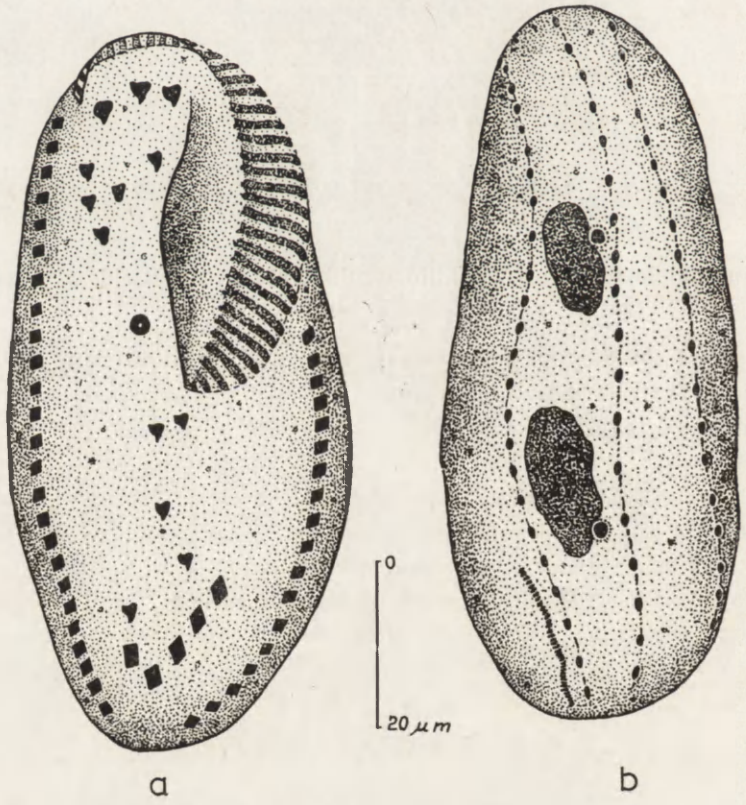


Fig. 16. *Oxytricha marina* Kahl, 1932, a — ventral view, b — dorsal view (silver impregnated whole mounts; nuclei included in b from a Feulgen staining preparation)

Oxytricha halophila Kahl, 1933 (Fig. 17)

A brief *in vivo* description of this species has been given by Kahl (1933). It is abundant in the Caspian sea microbenthos, plancton, and periphyton. Silver impregnated material has been used for the following description.

Body elongate, dorso-ventrally flattened (Fig. 17 a). Living ciliates yellowish-white in incident light; cytoplasm filled with various inclusions. Contractile vacuole single, with a pore opening to the left of the rear part of the AZM (Fig. 17 a). Anterior body end rounded, slightly bent

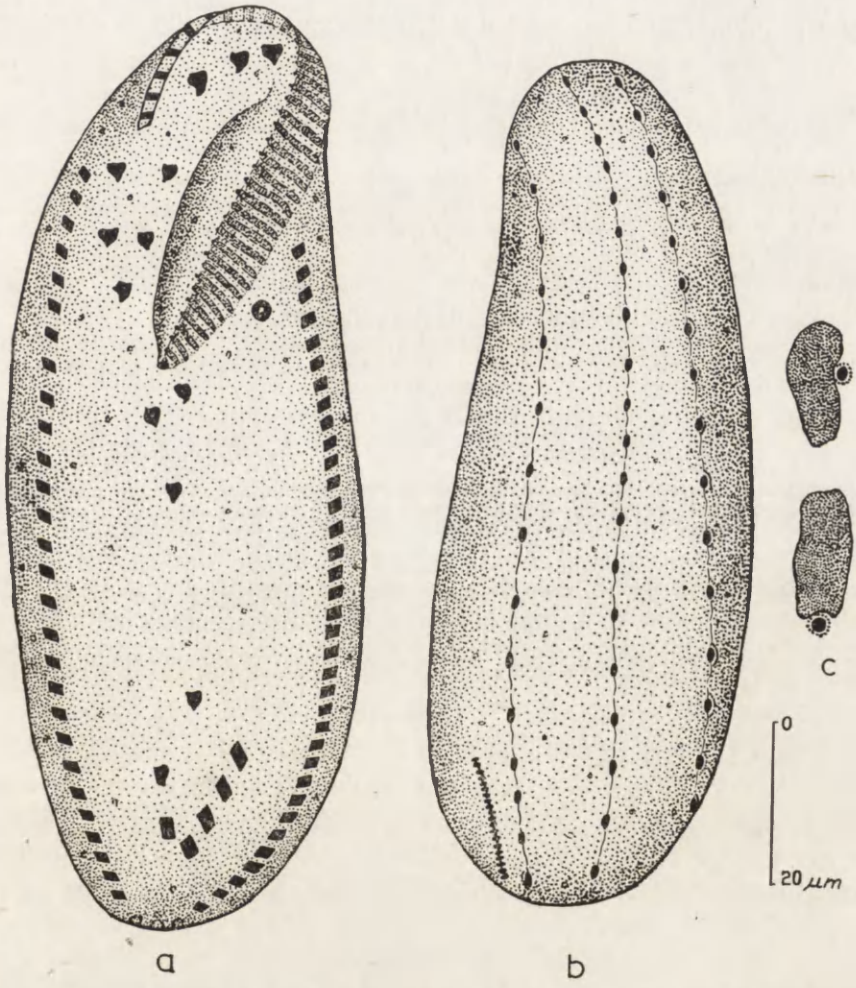


Fig. 17. *Oxytricha halophila* Kahl, 1933, a — ventral view, b — dorsal view (silver impregnated whole mounts); c — nuclei (hemalum staining)

to the left. Peristome relatively small, about 45 μm long, occupying about 1/3 of the body length. The AZM consists of 34 to 38 membranelles. The right anterior end of the AZM proceeds posteriad until or below the level of the third frontal cirrus (Fig. 17 a). There are two rows of marginals, with 28 to 35 cirri in each; 8 frontal, 5 ventral, and 5 transversal cirri are present (Fig. 17 a). Dorsal body side with three main rows of bristles, 17 to 20 units in each, and a short additional row of closely set bristles in the rear left region (Fig. 17 b). The number of the latter is 18–20.

Two oval macronuclei, and 2 or 3 micronuclei are present (Fig. 17 c).

Body length 130–140 μm (160–180 μm in living animals), body width 40–50 μm.

Main biotops: fine sand, algal detritus, and periphyton of the Caspian sea.

Stylonychia mytilus Ehrenberg, 1838 (Fig. 18)

This wide-known fresh-water species has been re-described by Kahl (1932), Tuffrau (1965), and Dragesco (1966). It was found in large quantities in both the periphyton and the plancton of the North Caspian.

Body elongate, with broader anterior end (Fig. 18 a). Peristome reaches almost to the half of the body length. The AZM consists of 36–38 membranelles. There are two rows of marginals, 23 to 33 cirri in each, as well as 8 frontal, 5 ventral, and 5 transversal cirri (Fig. 18 a). Dorsal side with three rows of bristles, 13 to 19 bristles in each (Fig. 18 b). Contractile vacuole single, with pore opening to the left of the rear end of the AZM (Fig. 18 a).

Two oval macronuclei and two micronuclei are present (Fig. 18 b). Body length of fixed animals 90–120 μm, body width about 40 μm.

Biotop: periphyton and plancton of low salinity regions of the Caspian sea.

Euplotes affinis Dujardin, 1842 (Fig. 19)

This fresh-water form has been found in the periphyton of both the North Caspian and the east coast of the South Caspian.

Body form oval (Fig. 19 a). Cytoplasm filled with inclusions. Peristome longer than a half of the body (about 25–30 μm in length). The AZM consists of 28–30 membranelles. The ventral side carries 9 fronto-ventral, 5 transversal, and 4 caudal cirri. Some specimens have 3 caudal cirri. Ventral argyrome with irregular meshes, of the type of *E. harpa*. Contractile vacuole single (Fig. 19 a).

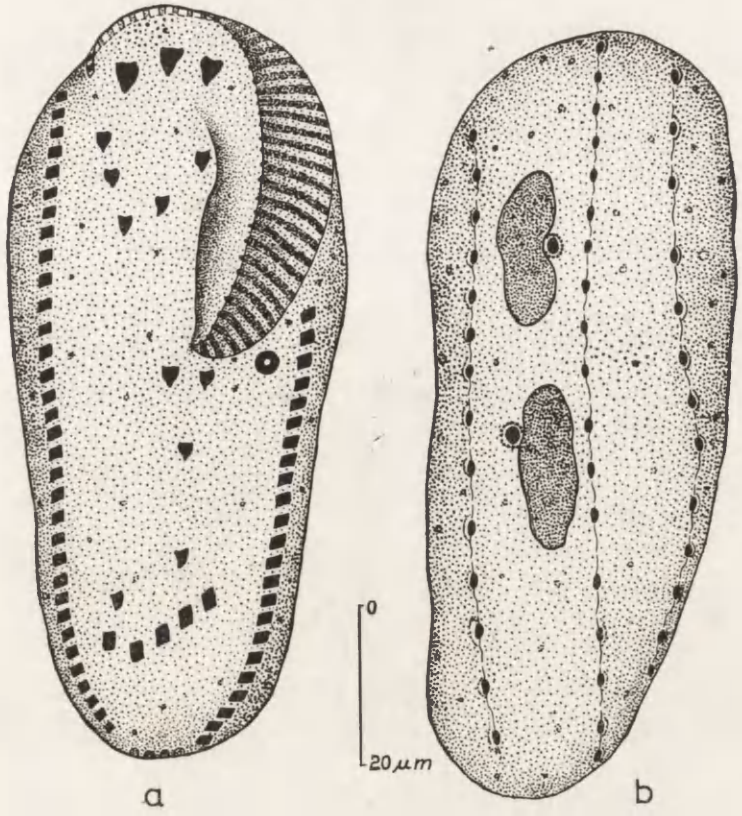


Fig. 18. *Stylonychia mytilus* Ehrenberg 1838, a — ventral view, b — dorsal view (silver impregnated whole mounts; nuclei included in b from a hemalum stained preparation)

There are 6 dorso-lateral rows of bristles, with 5 to 6 bristles in each (Fig. 19 a, b). Dorsal argyrome with two rows of large rectangular meshes in each interkinetal interval, thus belonging to the *E. harpa* type (Fig. 19 b).

Nuclear apparatus consisting of one C-shaped macronucleus and a single micronucleus (Fig. 19 c). Body length about 40 µm, width about 30 µm.

Biotop: overgrowth of both natural substrata and glass slides.

This species belongs to the group including *Euplotes bisulcatus*, *E. rotunda*, *E. moebiusi*, *E. elegans* var. *littoralis*, *E. gracilis*, and *E. zenkewitchi*. However, *E. affinis* differs from them by the structure of its dorsal argyrome, by the number of its dorso-lateral rows of bristles, and by the body size.

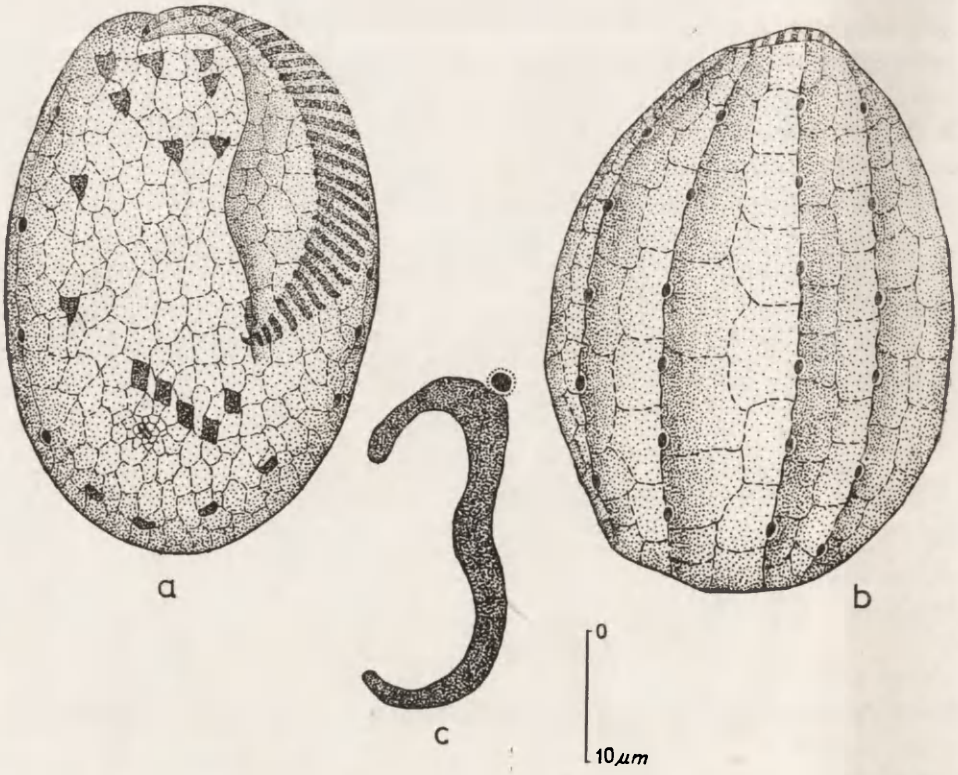


Fig. 19. *Euplotes affinis* Dujardin, 1842, a — ventral view, b — dorsal view (silver impregnated whole mounts); c — nuclei (Feulgen reaction)

РЕЗЮМЕ

Изучена морфология некоторых свободноживущих инфузорий Каспийского моря с помощью импрегнации по Шаттону и Львову, нуклеальной реакции Фельгена и окраски кислым гемалауном.

В статье дается описание одного нового (*Plagiopyla binucleata* sp. nov.) и 18 известных видов (*Holophrya simplex*, *Chaenea tesselata*, *Coleps tesselatus*, *Chlamydodon triquetrus*, *Kentrophoros uninucleatum*, *Trithigmostoma cucullulus*, *Plagiopyla nasuta*, *Paramecium woodruffi*, *Uronema nigricans*, *Ophryoglena atra*, *Cyclidium citrullus*, *Anigsteinia salinara*, *Oxytricha marina*, *Oxytricha halophila*, *Stylonychia mytilus*, *Euplotes affinis*), наиболее характерных для микробентоса, перифитона и планктона Каспийского моря. Отмечена вариабельность некоторых морфологических признаков у этих видов.

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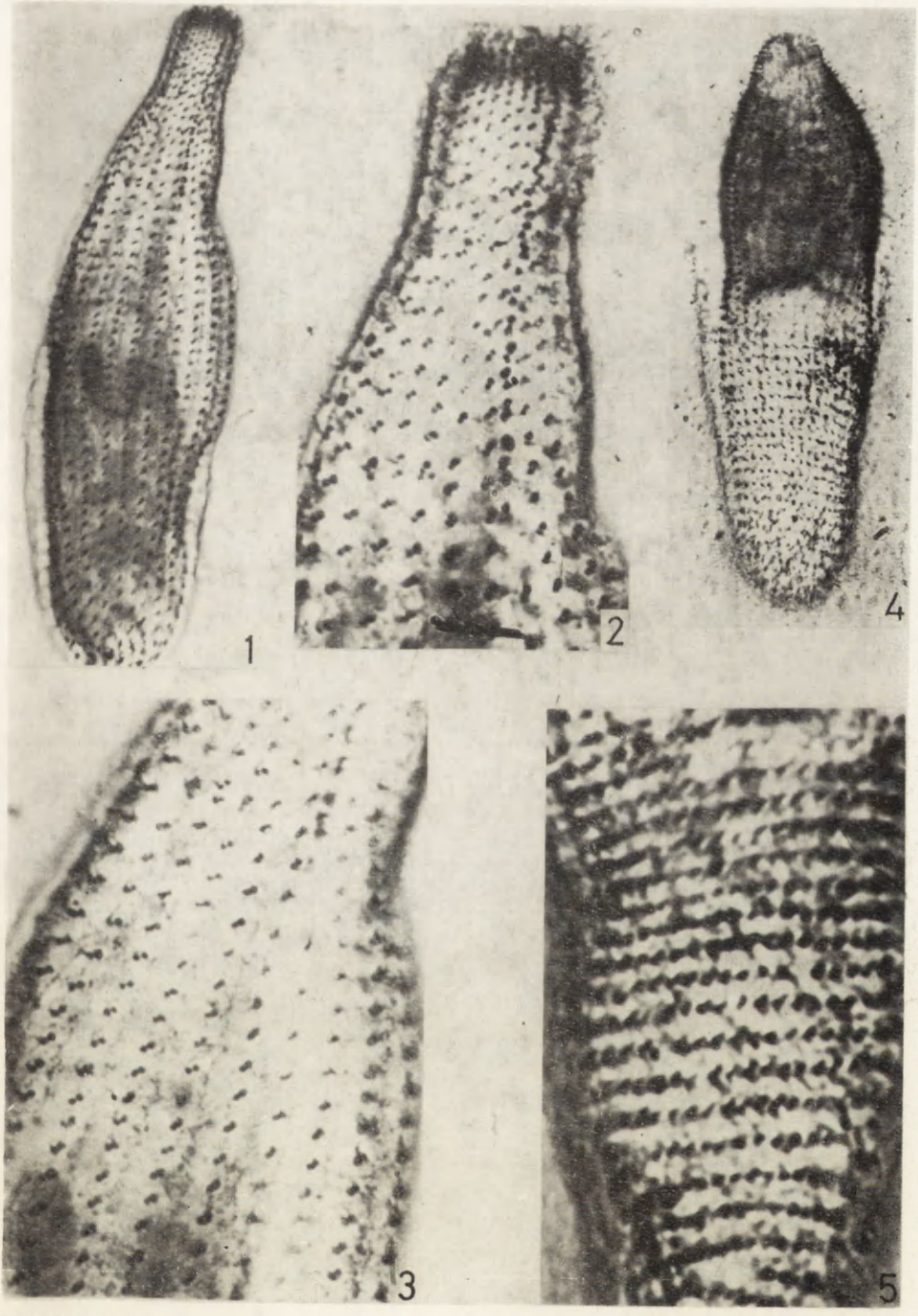
EXPLANATION OF PLATES I–IV

1–3: *Enchelyodon trepida* (Kahl) Borror, 1 — general view (silver impregnation, 800 ×), 2 — anterior body end (silver impregnation, 1350 ×), 3 — infraciliature (silver impregnation, 1350 ×)
4–5: *Chaenea tesselata* (Kahl) Dragesco, 4 — general view (silver impregnation, 800 ×), 5 — ciliature of the posterior body end (silver impregnation, 1350 ×)
6: *Coleps tesselatus* Kahl, general view (silver impregnation, 1350 ×)
7: *Chlamydodon triquetrus* O. F. Müller, ventral view (silver impregnation, 900 ×)
8–9: *Trithigmostoma cucullulus* (O. F. Müller) Jankowski, 8 — ventral view (silver impregnation, 800 ×), 9 — mouth region (silver impregnation, 1350 ×)
10–11: *Plagiopyla binucleata* sp. nov., 10 — ventral view (silver impregnation, 800 ×), 11 — dorsal view (silver impregnation, 800 ×)
12–13: *Paramecium woodruffi* Wenrich, 12 — circumoral adesmokineties (silver impregnation, 1350 ×), 13 — dorsal view showing contractile vacuole pores (silver impregnation, 800 ×)
14–15: *Uronema nigricans* O. F. Müller, 14 — ventral view (silver impregnation, 1800 ×), 15 — dorsal view (silver impregnation, 1800 ×)
16: *Ophryoglena atra* Kahl, ventral view (silver impregnation, 400 ×)
17: *Anigsteinia salinara* (Florentin) Isquith, left side view (silver impregnation, 800 ×)



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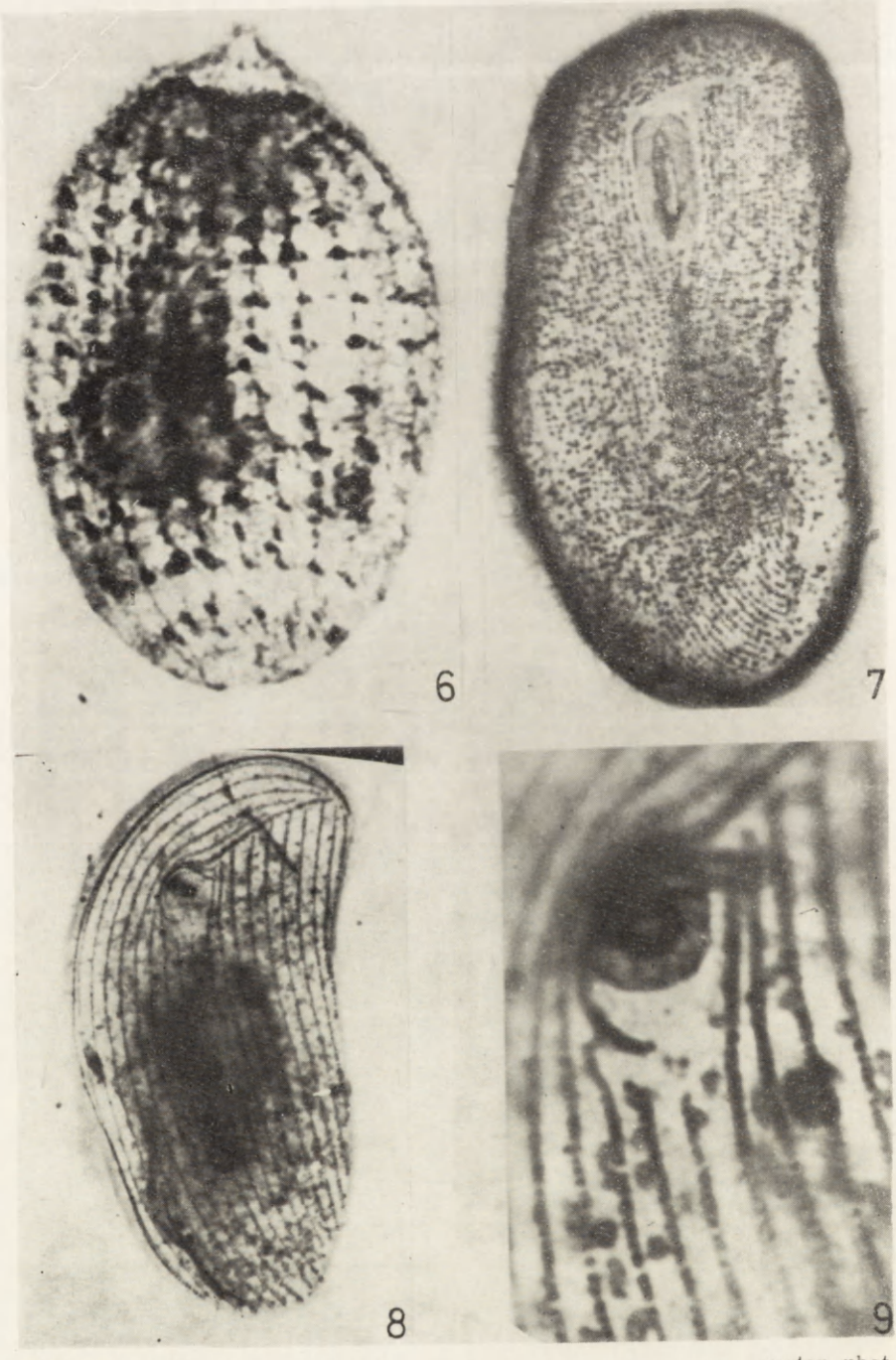

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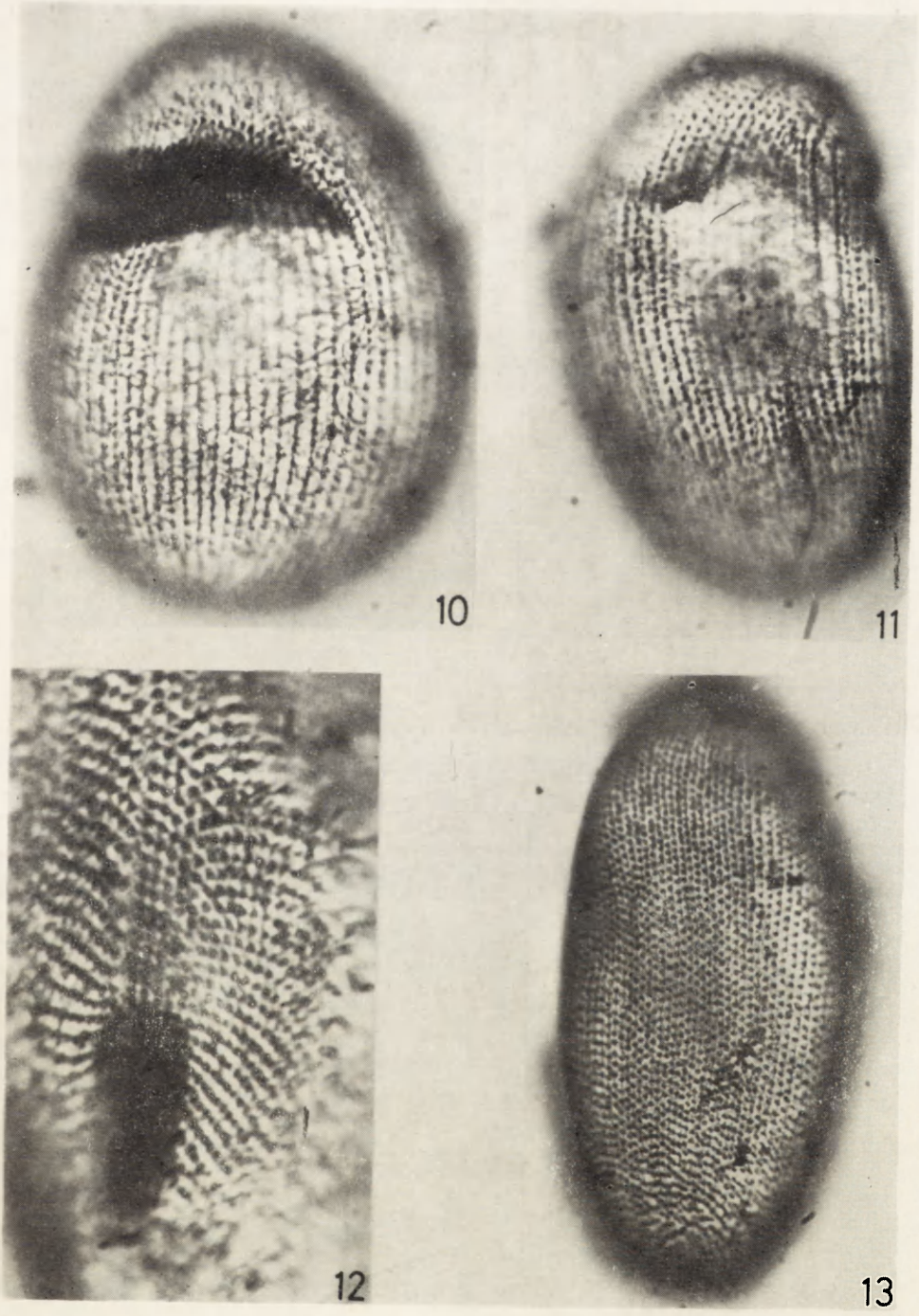

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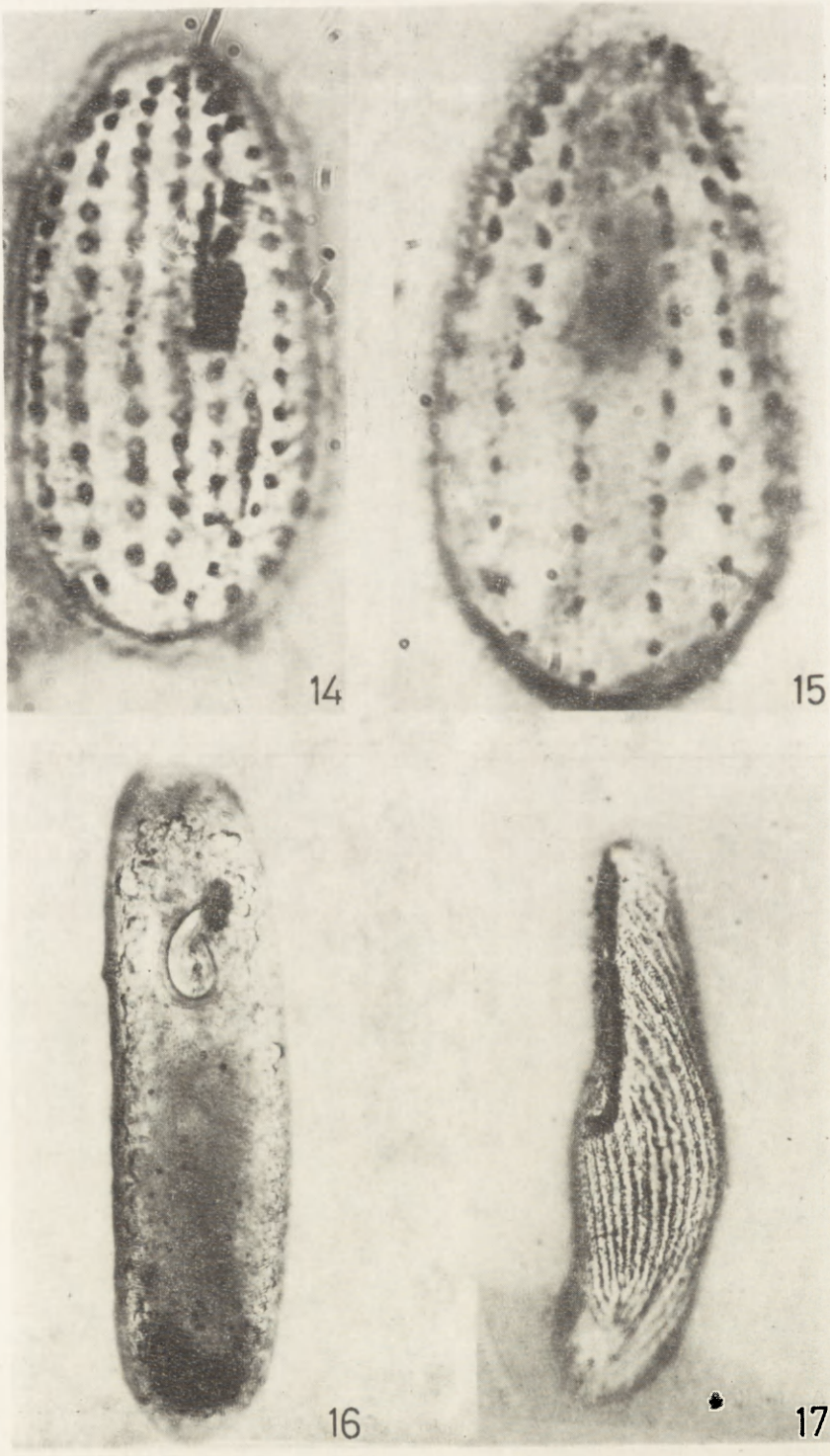

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Institute of the Environmental Biology, Department of Comparative Anatomy and Animal Ecology, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland

Maria WOLSKA

Triadinium caudatum Fiorent. Electron Microscope Examinations

Synopsis. In the cortex of *T. caudatum* strongly developed system of longitudinal microtubules and bars of the dense material underlying them is observed. Between the groups of microtubules the vesicles with grainy contents are regularly arranged. In deeper layer of the cytoplasm, beyond the ciliary zones, barren kinetosomes are scattered. A typically formed cytopharynx is surrounded by the "nasse" built of a layer of small nemadesmata and a structureless sheet. Large nemadesmata lie outside it. Both the cortex and the "nasses" are similar to those in *Cochliatoxum periachtum.* In the anterior part of the body, above the adoral zone there is a deep pouch with short swollen cilia. Presence of the pouch in *T. caudatum* is stated for the first time. It is supposed that the cilia enclosed in the pouch are sensory organs.

The need of investigations of the ultrastructure of ciliates inhabiting the intestine of *Equidae* have been accentuated by some authors (Senaud et Grain 1972, Wolska 1978 a). It is reasonable to perform such investigations on the species which have been studied with the aid of silver-impregnation technique. One of few species submitted to silver-impregnation is *Triadinium caudatum* (Wolska 1970). This species has been choosen as the object of the present study.

Basing on thorough morphological and cytological investigations, Strelkov (1939) separated genera *Ditoxum* Gass., *Tetratoxum* Gass., *Cochliatoxum* Gass. and *Triadinium* Fiorent. from the family *Cycloposthiidae* Poche (order *Entodiniomorphida*) and included them to the new established family *Ditoxidae.* Diagnostic features of this family were assumed to be the lack of the skeleton, and unretractable ciliary zones. Typical representatives of the family were recognized in the genera *Ditoxum*, *Tetratoxum* and *Cochliatoxum*, with their elongated bodies having the adoral zone situated at the anterior-end. The genus *Triadinium*, however, with its strongly shortened body and the adoral zone distinctly shifted to the posterior and situated at the ventral side was

considered by Strelkov as a descendant of the typical representative of *Ditoxidae* whose originally elongated body had been bent ventrally.

In the classification established by Strelkov several species were assigned to the genus *Triadinium* Fiorent., some of which (*T. caudatum* Fiorent., *T. minimum* Gass., *T. galea* Gass.) are endocommensals in horses.

Basing on results obtained with the silver impregnation technique, Wolska (1970) concluded, however, that the evolution of *T. minimum* must have followed quite a different course. The species has been separated from the genus *Triadinium* Fiorent. and included to a new genus *Circodinium* (Wolska 1971).

The new-named species, *Circodinium minimum* (Gassovsky), has been included to the family *Blepharocorythidae* Hsiung, the reason for such an decision being the structure of its oral apparatus.

As to the derivation of *T. caudatum*, the view of Strelkov seems to be justified, although Wolska (1970) ponders on another possibility.

Latteur et Dufey (1967) reformed the systematics of the family *Cycloposthiidae* Poche. The upshot was that the genera that belonged to the family *Ditoxidae* Strelkov were included into a new family *Spirodiniidae*, subfamily *Spirodiniinae*, together with the genus *Spirodinium* Fiorent. In the Strelkov's system *Spirodinium* Fiorent. belonged to the family *Ophryoscolecidae* which contains protozoa characteristic of ruminants. As can be seen, there is a number of different opinions on the taxonomy of Ciliata occurring in horse intestine and further studies in question are needed. Moreover, the comparison of ultrastructure between many species may be fruitful for phylogenetic consideration.

Triadinium caudatum Fiorent. (Pl. VII 29) has got, apart from the adoral zone of syncilia, the two additional somatic zones, and a tuft of cilia on the caudal process. The detail description of the species was given by Strelkov (1939) who used the classical, cytological technique. Wolska (1970) described the infraciliature in which a new group of kinetosomes, situated near the adoral zone, was recognized and considered as an equivalent of "free cilia" of other *Entodiniomorphida*, or as that of "special kinetosomes" of *Blepharocorythidae* (Wolska 1971).

The aim of the present study, was to examine just this group of kinetosomes (most likely bearing cilia), the structure of the cortex and the oral apparatus.

Material and Methods

Contents of horse colon were collected to a thermos bottle immediately after the horse was slaughtered at the knackery at Konstantynów near Łódź. The found

ciliates were prepared to electron microscope examinations according to the procedure recommended by Grain (1966).

Sections were out on III LKB ultramicrotome. Formwar-coated grids were examined with an electron microscope Tesla BS 513A.

Results

The body of *Triadinium caudatum*, Fiorent. is covered with a single membrane, more or less undulating, and coming off the underlying thin structureless sheet of epiplasma (Pl. I 1, 3). Deeper on regularly grouped longitudinal microtubules are situated. Each group may contain a dozen or so microtubules arranged in three or four layers, each of several tubules (Pl. I 1).

Around the ciliary zones the microtubules are less numerous. At the base of the lip surrounding the adoral ciliary zone the microtubules lie in two layers only, two or at most three in each (Pl. II 5). No microtubules occur in the internal side of the lips (Pl. II 7). Between the bundles of microtubules there are vesicles filled with grainy substance or empty (Pl. I 1, 2). The empty vesicles are open from the outside. Just beneath the bundles of microtubules, bars of electron dense material spread out (Pl. I 1, 2, 3). The bars are oblong, circular in cross-section, and very regularly arranged like the groups of microtubules (Pl. I 3, Pl. II 5, 6). Disturbances in the arrangement of bars are very rare (Pl. I 4). Transverse strands of structureless substance, which is less dense than that of the bars, connect them at the level of their distal surfaces (Pl. I 1, Pl. II 5, 8). Proximal surfaces of the bars are linked by the layer of microfibrils (Pl. I, 1, 2, Pl. II 5). Under this layer the barren kinetosomes are scattered throughout the body (Pl. I 2, Pl. II 6, 8). In some body regions greater agglomerations of microfibrils can be observed. They cross the base of lips surrounding cilia (Pl. II 5) and underlie the ciliary zones. Gathered in strands they interlace abundantly posterior part of the protozoan's body. Deeper away from the kinetosome layer flattened vesicles of ergastoplasm are arranged in rows parallel to the cell surface (Pl. II 5, 6).

The kinetosomes of *T. caudatum* are open. The base of each kinetosome is surrounded by a low ring of dense material which also partly underlies the kinetosome narrowing inside the diameter of kinetosome cylinder. From each ring the double longitudinal branches spring, linking the nearest kinetosomes of the same row, the lateral ones connect the parallel neighbouring rows (Pl. III 10, Pl. V 18), and still another ones are directed deep into the cytoplasm to interlock a thick strand (Pl. V 16, Pl. VI 23). All this forms a complicated spatial net.

Nemadesmata arise from the deep, thick strand, so not directly from

kinetosomes (Pl. III 9, Pl. V 19). Intermediary strands between the base of the kinetosome and the nemadesma may be very long as can be seen in Phot. 19. Each kinetosome contains one or more axial grains (Pl. III 9).

The examined kinetosomes are associated with transverse and kinetodesmal fibrils (Pl. III 10).

Primordia of the ciliary zones arise in infracytoplasmatic vacuoles (Pl. V 17).

The margin of the adoral zone is slightly and irregularly bent inwards a depression leading to the cytostome. Transverse fibrils originating from the marginal kinetosomes (Pl. IV 13, Pl. VI 22) produce "rideaux de tubules" which equip the barren part of the depression (vestibulum) and cytopharynx (Pl. III 11, 12, Pl. IV 13, 14, Pl. VI 22).

The cytopharynx is surrounded by small nemadesmata, whose connections with kinetosomes can, however, hardly be ascertained (Pl. VI 22). Along with small nemadesmata the structureless sheet adhering them from the outside, and the bundles of microfibrils lying a bit apart the structureless sheet, enter the cytoplasm (Pl. VI 22). In this way the cytopharynx is bordered with a wall consisting internally of nemadesmata and externally of the structureless sheet. This border may be compared with that surrounding the cytopharynx of *Gymnostomata*, and will be termed as "nasse" after Senaud and Grain (1972) when describing *Cochliatoxum*. The "nasse" is surrounded by large nemadesmata that originate (indirectly) from the kinetosomes situated farther than the cytostome (Pl. VI 22) and alternating bundles of microfibrils. It can be seen in some sections that outside this system the ergastoplasm borders the layer of large nemadesmata and microfibrils (Pl. VI 22).

The "rideaux de tubules" disappear very soon but the folded "nasse", large nemadesmata and microfibrils continue one's way (Pl. IV 15). The cytoplasm that fills the cytopharynx and the space outside it, enclosed with the large nemadesmata, have a character of phagoplasm (Pl. VI 21, Pl. IV 15). At the terminal part the "nasse" is not bordered with large nemadesmata, but only with the layer of microfibrils (Pl. VI 21).

On the right body side, above the adoral zone near the ventral margin, that is in the place where the silver impregnation blackens a group of kinetosomes (Wolska 1971), the electron microscope revealed a hollow (a deep pouch) which communicates with the outer world through a short duct (Pl. V 20). The duct is covered with the same sheets as the rest of the body, and the pouch is lined with cell membrane, epiplasma, and a single layer of microtubules (Pl. VII 25). The opening of this duct is directed backward similarly as the mouth. The bottom of the pouch is longitudinally folded. In furrows between the folds there lie rows of kine-

tosomes with short, deformed cilia. The kinetosomes are open and surrounded by dense material at their proximal ends; they contain axial grains but typical septa and axosomes are lacking (Pl. VII 26). The cilia are swollen or flattened, being trapeziform, triangular, or elliptic in crosssection (Pl. VII 24, 25). The number of peripheral fibres not always equals nine. The fibres are irregularly arranged. In strongly flattened cilia they lie in the same row and join together. Because of such an arrangement of fibres and variations in their number, the presence or absence of central fibres can not be ascertained. Only in a few cilia the peripheral fibres are regularly arranged, in such cases the cilia lack the central fibres (Pl. VII 25–28). Dense material surrounds the proximal parts of kinetosomes and branches out sidewardly and downwardly. Nemadesmata arise from the deep layer of dense material (Pl. VII 27). Perhaps they form greater agglomeration, which can be visible with the light microscope as a thick strand linking the adoral zone with the anterior wall of the body, and running between the anterior end of the nucleus and the pouch of "free cilia". Some nemadesmata of this strand can be seen in Pl. V 20.

Discussion

The cortex of *Triadinium caudatum* resembles much that of *Cochliotoxum periachtum* investigated by Senaud and Grain (1972). This resemblance consists first of all in the presence of longitudinal bars of the dense material lying between the microtubular and microfibrilar layers. Dense material is better developed in *Cochliatoxum*, where it is in form of plates going deep into the cytoplasm. In *Triadinium caudatum* the layer of bars of dense material is thinner, the difference being most probably due to different body sizes of the two species. Both *Cochliatoxum* and *Triadinium*, like all the *Ditoxidae*, lack a polysaccharide skeleton which reinforce the bodies of other *Entodiniomorphida*. The dense material seems to substitute it. As large protozoan as *Chochliatoxum periachtum* is, (being up to 380 μm in length and 170 μm in width according to Senaud and Grain 1972), needs to have its body surface consolidated with a well developed system of the dense material. *Triadinium caudatum*, with its much smaller size (its dimensions do not usually exceed 100 by 60 μm) and more compact body, does not need so much developed supporting structures.

Another similarity in the cortex between *T. caudatum* and *C. periachtum* consists in the presence of secretory vesicles. Their function in *C. periachtum* has been convincingly elucidated by Senaud and

Grain (1972). As concerns *T. caudatum*, one may understand their significance from that the cell surface happens to be covered with a thin sheet of fuzz.

In *T. caudatum* like in *C. periachtum* the electron micrographs revealed kinetosomes situated in different body regions. The presence of barren kinetosomes in naked body regions was exhibited in *Tripalmaria dogieli* as well (Wolska 1978 b). Whether this is a character of all the *Entodiniomorphida*, it cannot be stated yet. Electron microscope studies on *Cycloposthium* by Grain (1966) and on *Ophryoscolecidae* by Noirot-Timothee (1960) have brought no certain information in question, but the possibility of the occurrence of kinetosomes in non-ciliated regions is quite conceivable. *T. caudatum* is characterized by the occurrence of undulating cell membrane on the greater part of its body surface and by the presence of a thin, osmiophilic structureless sheet preceding a layer of microtubules. This is a difference between the cortex of *T. caudatum* and that of *C. periachtum* as in the latter the cell membrane adhere immediately to the layer of microtubules. That is just a similarity between the cortex of *T. caudatum* and that of *Cycloposthium* (Grain 1966) and *Ophryoscolecidae* (Noirot-Timothé 1960).

Kinetosomes of *T. caudatum* are partly open like in *C. periachtum*. The system of dense material enveloping kinetosomes is much the same as that in *C. periachtum*.

According to Strelkov (1939), in *Ditoxidae* the ectoplast containing the nucleus and contractile vacuoles is strongly reduced and situated anteriorly on the right body side. In other body regions the cytoplasm appears to be not differentiated into the ectoplast and the digestive cytoplasm, i.e., the endoplast. The "cuticula", as named by Strelkov, adhere nearly all around to endoplasmic sack. It is confirmed by electron-microscope observations. Most sections obtained from *T. caudatum* revealed no delimitation of ectoplasm by fibrous layer. The cytoplasm in which the barren kinetosomes occur, is separated from the digestive plasm only by vesicles of ergastoplasm. Sometimes, in frontal sections, a fine layer of microfibrils can be seen nearer the right body side, which confirms the of external nemadesmata being richer in *C. periachtum*.

The oral apparatus of *T. caudatum* is similar to that of other *Cycloposthiidae* Poche. The bare part of the vestibulum and the cytopharynx is equiped with the "rideaux de tubules". The folded "nasse" of *T. caudatum* is formed similarly as that of *C. periachtum* except for the layer of external nemadesmata being richer in *C. periachtum*.

New ciliary zones originate in a way characteristic of *Entodiniomorphida*, that is, in vacuoles.

The altered, swollen cilia of *T. caudatum*, that lie in folds of the pouch,

should be considered as sensory cilia. Such structure of single cilia, which are assumed to be receptors, were described in some free-living Ciliates as "soies sensorielles" (Grain and Golińska 1969), "cils claviformes" (Grain 1970), or "clavate cilia" (Holt et al. 1973).

Among *Entodiniomorphida* the ultrastructure of cilia of the similar type was already recognized in some *Ophryoscolecidae* by Bretschneider (1962) and by Roth and Shigenaka (1964). Bretschneider named that group of cilia "Paralabialorgan" supposing them to do a sensory function. Roth and Shigenaka described them in details but did not express their opinion on the role the cilia could play. The ultrastructure of swollen cilia of *T. caudatum* fits this description very well. Another mention about short, swollen cilia of *Ophryoscolecidae* can be found in Noirot-Timothée (1960), who observed them to occur in foldings of the cuticula outside the adoral zone; her research, however, did not comprise a study of the infraciliature in silver-impregnated preparations. Noirot-Timotheé believed those cilia to be a continuation of the adoral zone of syncilia.

Earlier, using the silver impregnation technique, Fernandez-Galiano (1958) indicated that the adoral zone in *Cycloposthium edentatum* is differentiated, and described an isolated group of kinetosomes ("cinecias independientes") situated near the main part of the adoral zone. He considered them to be a remnant of somatic cilia. Wolska (1965) proved that in the infraciliature of *Ophyoscolecidae* this very group, the so-called "free cilia", stood apart the synciliary zone. Small, groups of kinetosomes distinguished in *T. caudatum* and *Tripalmaria dogieli* were subsequently described by Wolska (1970, and 1978 a, respectively).

The observations presented herein as well as other, so far unpublished ones, allow us to believe that the presence of this group of cilia is characteristic of all the *Entodiniomorphida*. Considering also descriptions of their ultrastructure, it can be assumed that *Entodiniomorphida* have got receptors of some stimulus. Moreover, in *Ophryoscolecidae* and *Cycloposthiidae* these cilia lie at the base of the ciliophore surrounded with plasmatic lips, so they are protected from any mechanical injury even when the ciliophore becomes devaginated. In *T. caudatum*, however, they lie in a considerable distance of the adoral zone, beyond the lips surrounding it, but still they are sheltered by the special pouch they are in. Such a location of the "free cilia" proves that they cannot simply be the remnants of somatic cilia, but rather the organelles having essential meaning for the protrozoan.

In my earlier studies on ciliates of horse intestines with the use of optical microscope, I admited that *Entodiniomorphida* are closely related to *Blepharocorythidae*. Corliss (1974, 1975) in his new classification

of ciliata, having raised the *Blepharocorythidae* to the rank of the suborder *Blepharocorythina*, places these two groups of ciliates close together. A question arises whether the "special kinetosomes" (should read: "special cilia"), as described by Wolska (1966), are of the same nature in *Blepharocorythina* as the "free cilia" are in *Entodiniomorphida*. A trial of answer to this question will be a topic of the next researches.

RÉSUMÉ

Très abondant système de microtubules longitudinales et de travées denses sous-jacents caractérise le cortex de *T. caudatum*. Entre les groupes de microtubules se trouvent des vésicules régulièrement disposées avec le matériel granuleux. Dans la couche de protoplasme plus profonde, horse de zones ciliaires, des cinétosomes glabres sont dispercées. Le cytopharynx d'une structure tipique est entouré par le "nasse" formée d'une couche de petites némadesmes et d'une couche amorphe; exterieurement sont disposées des grandes némadesmes.

Par le charactère du cortex et de la "nasse" *T. caudatum* ressemble Cochliatoxum *periachtum*.

Dans la partie anterieur du corps, non loin de la zone adorale, s'ouvre une profonde poche avec les cils courts et gonflés. La présence de la poche chez *T. caudatum* est décrite pour la première fois. Les cils eachées dans la poche sont considerés comme les cils sensoriels.

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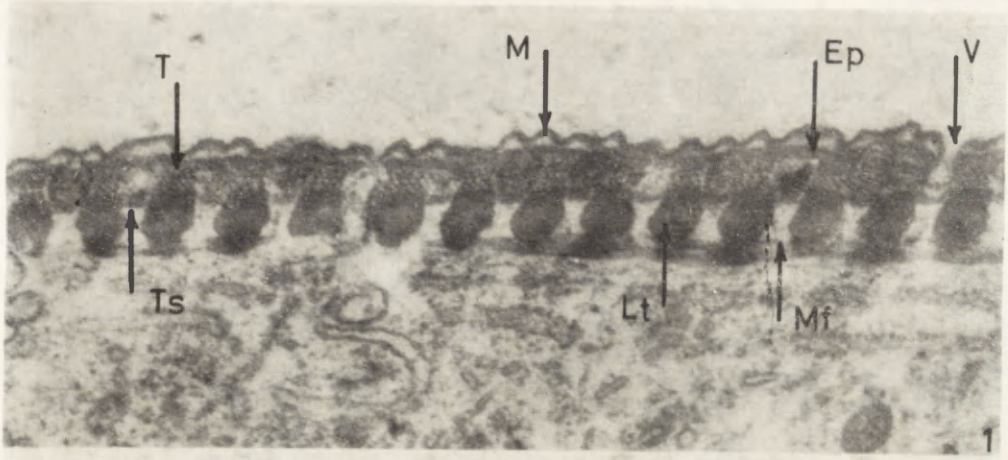
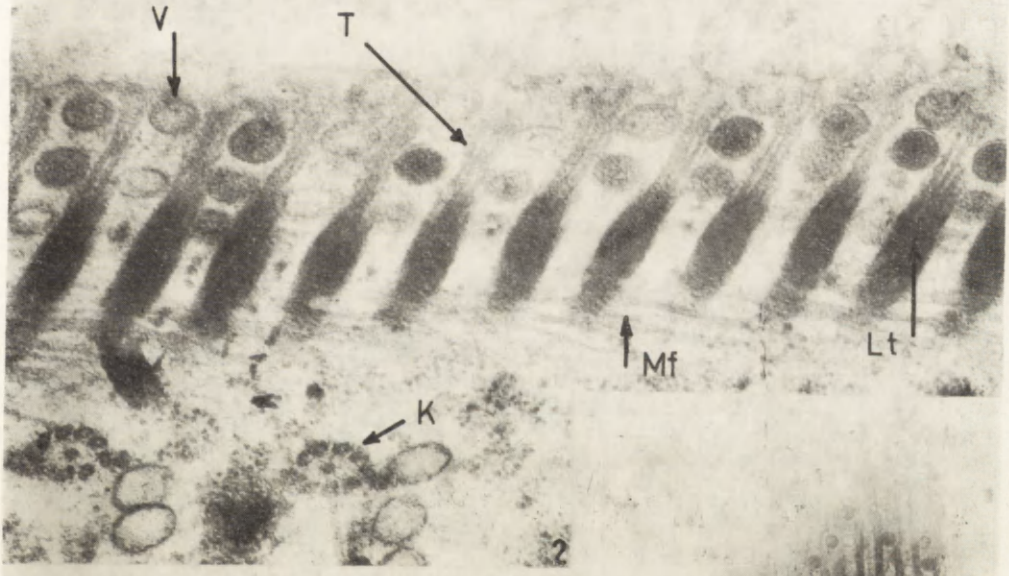
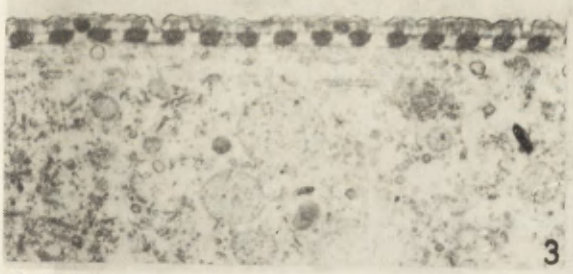
EXPLANATION OF PLATES I–VII

Triadinium caudatum Fiorentini

1: The section perpendicular to the surface of the non-ciliary part of the body. Cell membrane (M), epiplasm (Ep), microtubules (T), longitudinal bars of the dense material (Lt), transverse strands of the dense material (Ts), microfibrils (Mf), vesicles (V), × 40 200
2: The section parallel to the surface (slightly oblique). Microtubules (T), longitudinal bars of the dense material (Lt), microfibrils (Mf), vesicles (V), kinetosomes (K), × 44 200
3: The section perpendicular to the surface, × 11 200
4: The section parallel to the surface, × 11 200
5: The section through the region of the lip of the adoral zone (the front of the body). Microtubules (arrowed), transverse strands of the dense material (double arrowed), × 21 200
6: The section parallel to the surface. Kinetosomes (arrowed), × 11 200
7: The section through the lip of the adoral zone on the ventral side, × 21 200
8: The oblique section through the region of the lip of the adoral zone. Transverse strands of the dense material (arrowed), × 12 300
9: The longitudinal section through the cilia in the somatic zone. Nemadesmata (arrowed), × 11 200
10: The section perpendicular to the kinetosomes of the adoral zone. Transverse fibrils (Tr), kinetodesmal fibril (Kd), longitudinal strands of the dense material (arrowed), × 12 300
11: The slightly oblique section through the wall of the vestibulum, × 11 200
12: A fragment of phot. 11, × 44 200
13: The section through the vestibulum and the beginning of the cytopharynx, × 12 300
14: The cross-section through a fragment of the vestibulum, × 12 300
15: The oblique section through the "nasse", × 11 200
16: The slightly oblique section through kinetosomes of the somatic zone, × 12 300
17: The section through the anlage of the ciliary zone, × 11 200
18: The oblique section through kinetosomes of the adoral zone. Transverse strands of the dense material (arrowed), × 25 500
19: The longitudinal section through kinetosomes of the adoral zone (the anterior part), × 23 200
20: The section through the pouch and "free cilia", × 5800
21: The oblique section of the "nasse" in its terminal part, × 25 500
22: The section in frontal plane through the vestibulum and the cytopharynx bordered with the "nasse" and large nemadesmata, × 11 200
23: The section at the level of the deep braid of the dense material, × 18 600
24: A fragment of the section through the pouch. In the middle a triangular outline of cilium, × 23 300
25: The cross-section through the folds and cilia of the pouch. Microtubules in the wall of the pouch and in folds (arrowed), × 23 300
26: The section through the pouch. Kinetosomes and cilia cut longitudinaly, slightly obliquely, × 23 300
27: The oblique section through kinetosomes of the pouch. Nemadesmata (arrowed), × 12 300
28: A fragment of phot. 20 × 15 600
29: A general view of *T. caudatum*, left side. Photographed in optical microscope, × 1000







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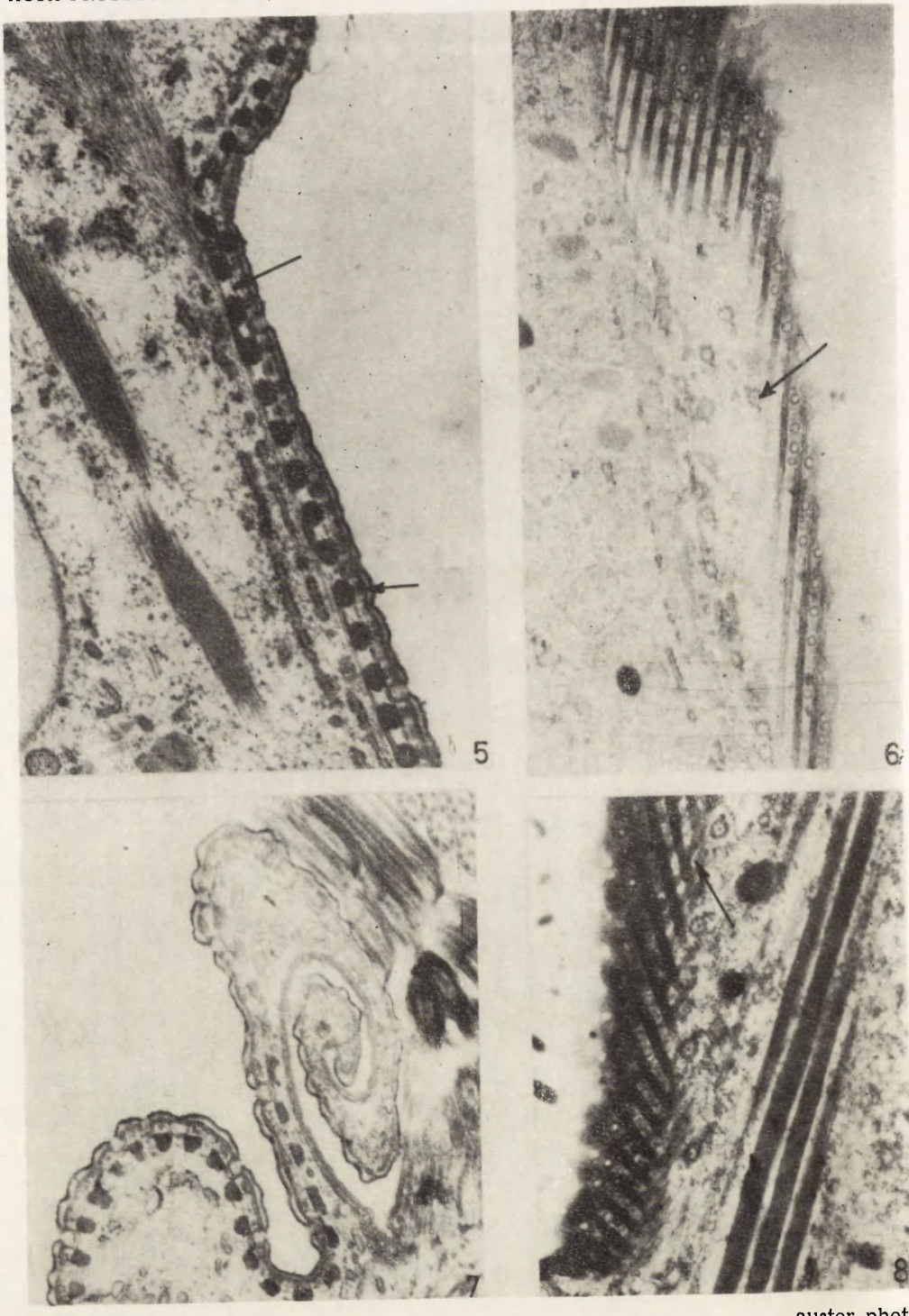

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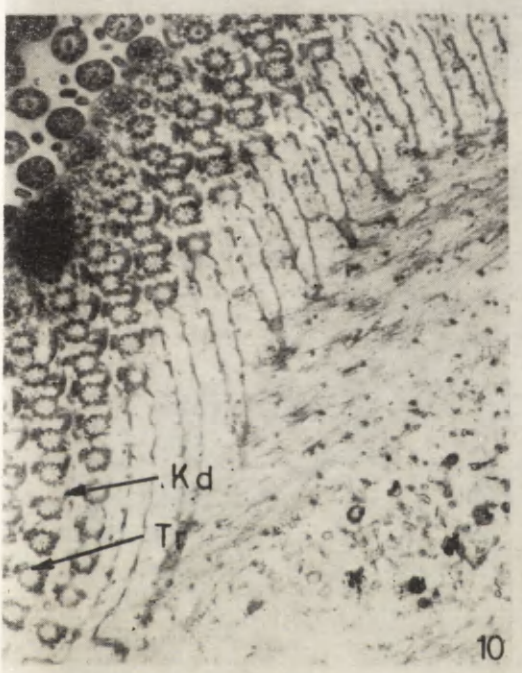

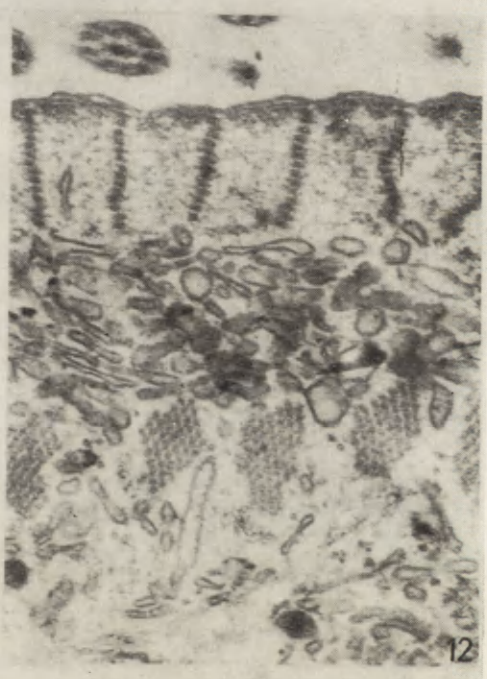

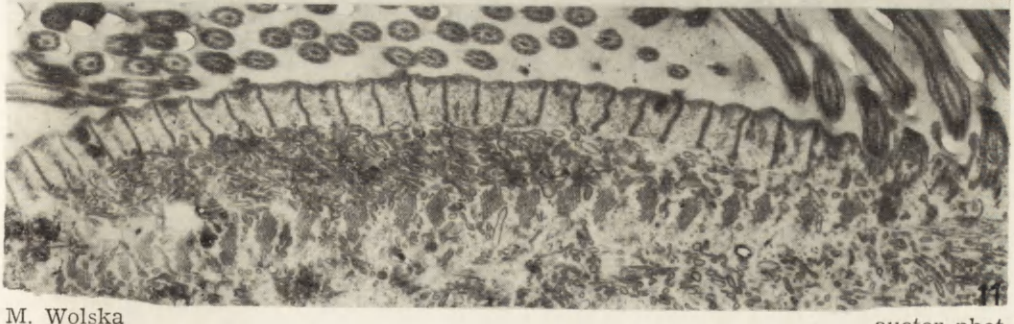

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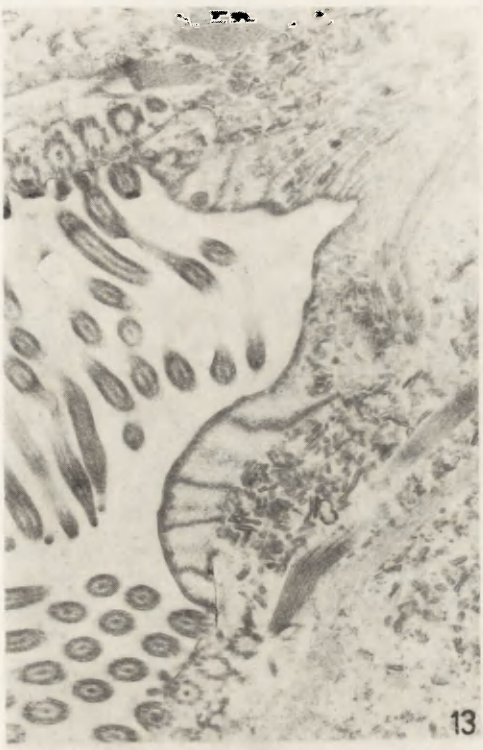

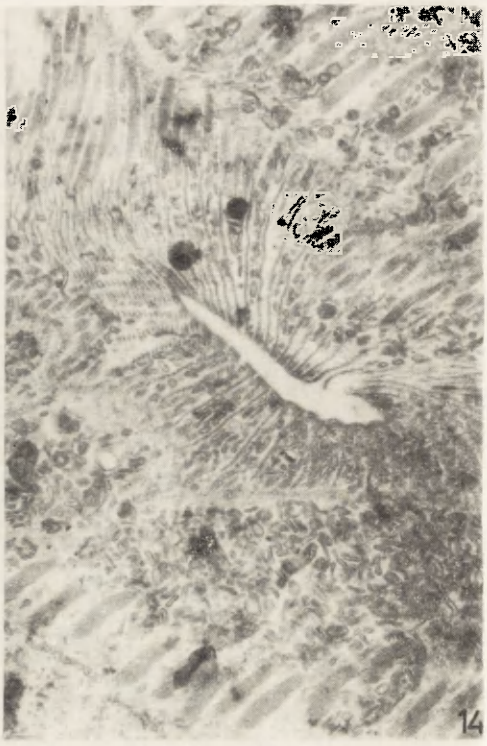

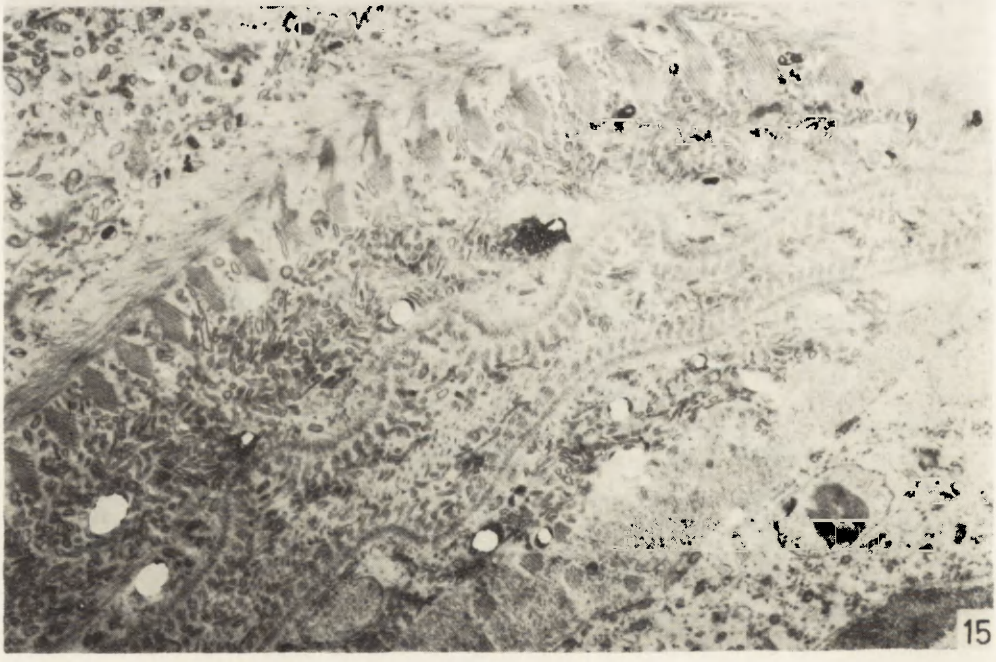

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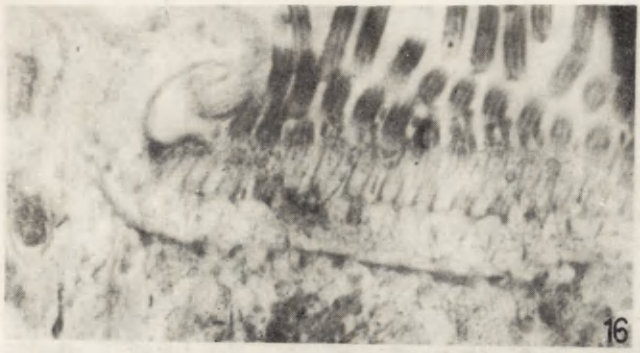

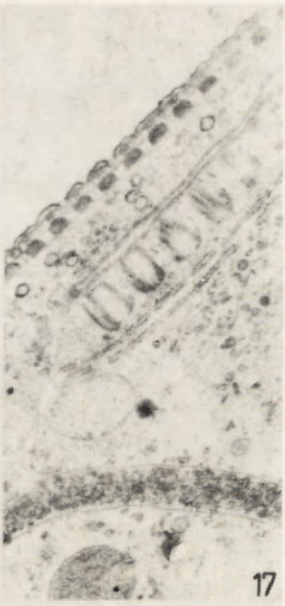

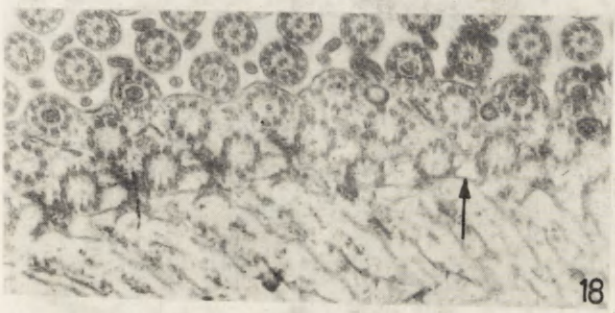

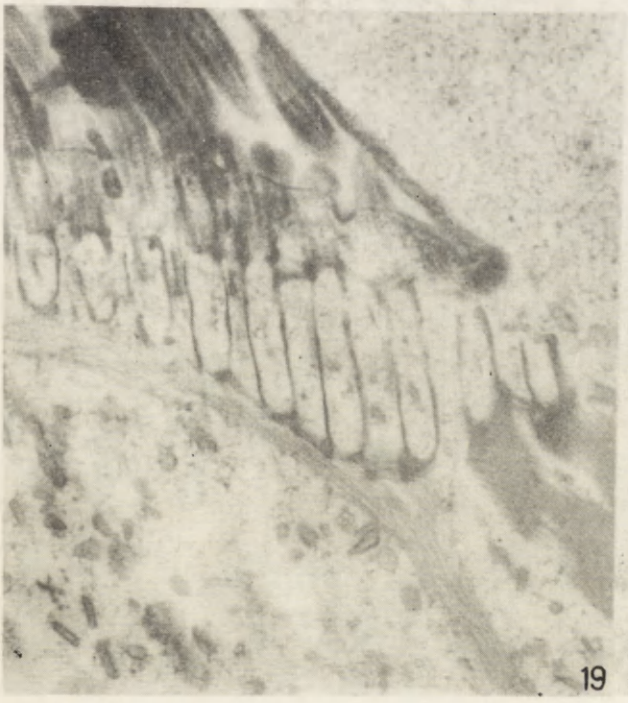

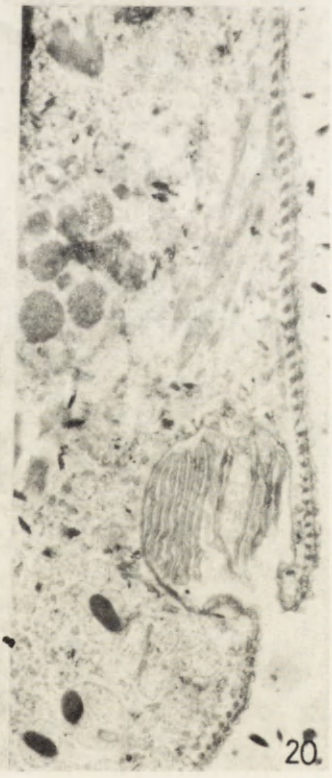

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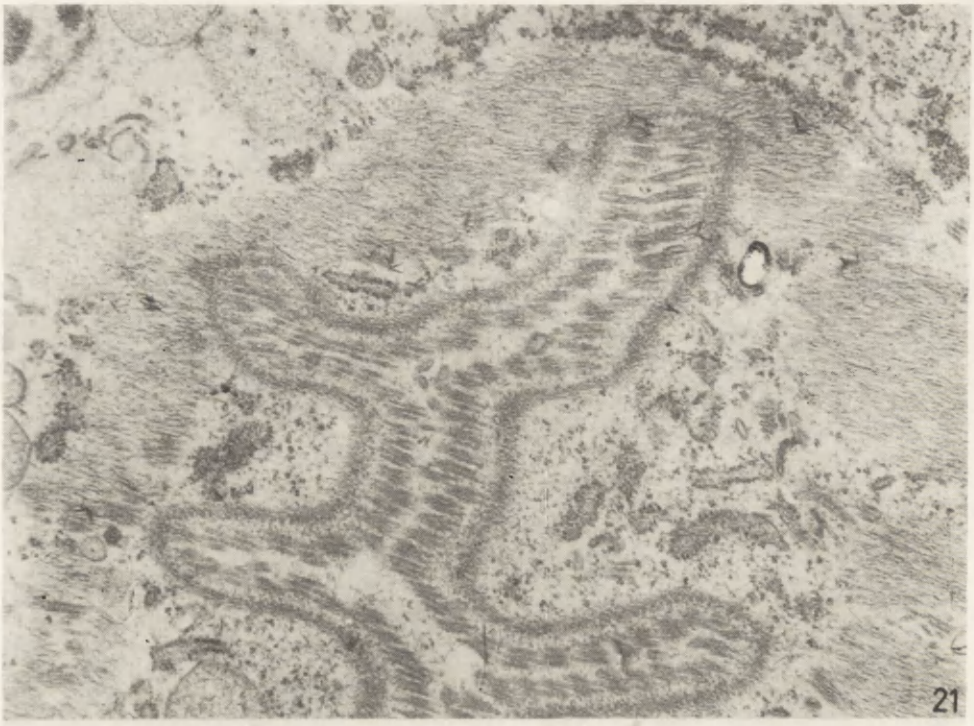

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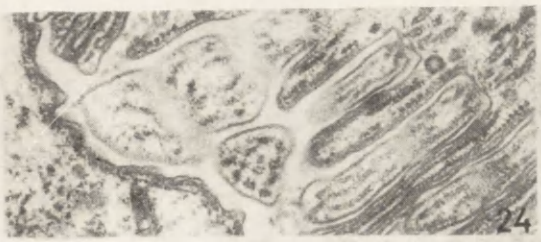

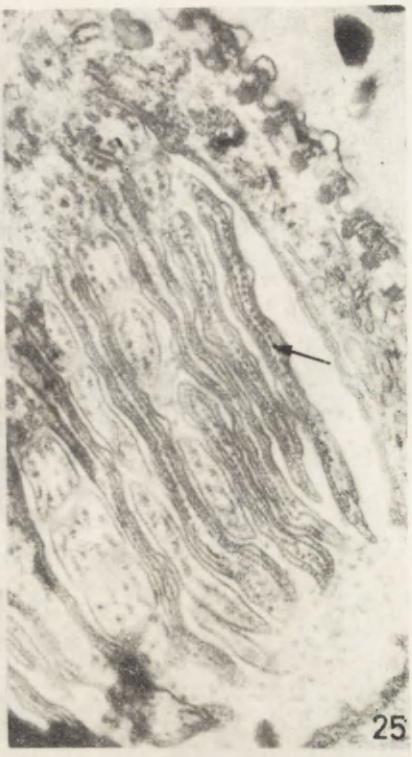

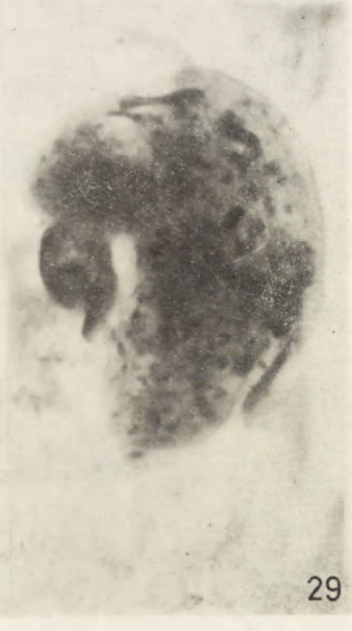

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Department of Zoology, Andhra University, Waltair, Andhra Pradesh, India

C. KALAVATI and C. C. NARASIMHAMURTI

A New Microsporidian, *Octosporea porcellioi* sp. n. from *Porcellio laevus* Latr. (*Oniscidae, Isopoda, Crustacea*) [1]

Synopsis. The morphology and life-history of a microsporidian parasite, *Octosporea porcellioi* sp. n. infecting the hypodermal cells lining the inside of the body wall, the cells found in the haemocoelic fluid, the cells lining the outside of the gut wall and the body muscles of the terrestrial isopod, *Porcellio laevus* Latr. (*Oniscidae, Isopoda, Crustacea*) is described. The parasite causes loss of tonus of the body muscles of the host, limping and finally death.

Microsporidian parasites are reported from different groups of arthropods but a perusal of the literature showed that three has so far been only one microsporidian parasite belonging to the genus *Mrazekia* from isopods (Leger and Hesse 1976). The present paper gives an account of the morphology and life-history of another microsporidian, *Octosporea porcellioi* sp. n. from the terrestrial isopod, *Porcellio laevus* Latr. (*Oniscidae, Isopoda, Crustacea*).

Material and Methods

The isopods were collected from underneath the dried and decaying leaves, slightly buried under the loose soil among the cashew-nut plants in the Andhra University Campus at Waltair (Andhra Pradesh, India) during July 1975–July 1976. As soon as they were brought to the laboratory they were isolated and kept singly in petri dishes at the bottom of each of which a moist blotting paper was placed. They remained active for about 7–10 days.

Smears of the haemocoelic fluid and the internal organs of the infected hosts were either air-dried, fixed in methyl alcohol and stained with Giemsa or fixed either with Schaudinn's or Carnoy's fluid and stained either with Heidenhain's iron haematoxylin or according to PAS technique. Material for sectioning was fixed in

[1] Abstract presented at the IX International Colloquium on Invertebrate Pathology held at Kingston, Ontario, Canada, during August–September 1976.

alcoholic Bouin's fluid, sectioned at 8 μm thickness and stained with iron haematoxylin. All the drawings were made with the aid of a camera lucida.

Observations

Octosporea porcellioi sp. n.

Host: *Porcellio laevus* Latr.
Site of infection: Hypodermal cells
Locality: Andhra University Campus at Waltair (Andhra, India)
Type slides: Zoology Department Andhra University, Waltair.

2–3% of the isopods examined were found infected with a microsporidian parasite belonging to the genus *Octosporea*. Healthy isopods were very active and when disturbed darted forwards or alternatively curled themselves up in the form of a small pebble so that they easily escaped attention in their natural surroundings. The infected forms on the other hand appear flaccid because they have lost the tonus of the body muscles.

The parasites are found in the hypodermal cells lining the inside of the body wall, in the cells found in the haemocoelic fluid (oenocytes and haemocytes), in the cells lining the outside of the gut wall and in the body muscles.

Infected hypodermal cells are narrow at the base and are expanded distally and appear bulb-like (Fig. 1). When the bulb-like portion is filled with pansporoblasts the cell is nipped off at the base and is released into the haemocoelic cavity. All the stages of development of the parasite are found in these cells.

The cells lining the outside of the gut wall are conspicuously hypertrophied and are nearly twice the size. The nucleus is pushed to a side and the nuclear membrane of the host cell nucleus sometimes disintegrates releasing the chromatin material into the cytoplasm (Fig. 3). In early infections a periparasitic vacuole is seen around the growing schizonts (Fig. 2).

Infected haemocytes do not show hypertrophy. Inspite of examining several infected cells the earlier stages of development of the parasite were not observed in these cells. All of them showed only the pansporoblasts. It is assumed that on account of the phagocytic habit of these cells they engulfed the pansporoblasts present in the haemocoelic cavity which are released by bursting of the infected cells from different sources. Even though the sporoblasts were phagocytosed they apparently remained viable as evident by the fact that such spores also released the polar filaments when treated appropriately. This shows that the cells found in the haemocoelic fluid which engulfed the pansporoblasts were only partly successful in that they were able to engulf the spores but not digest them.

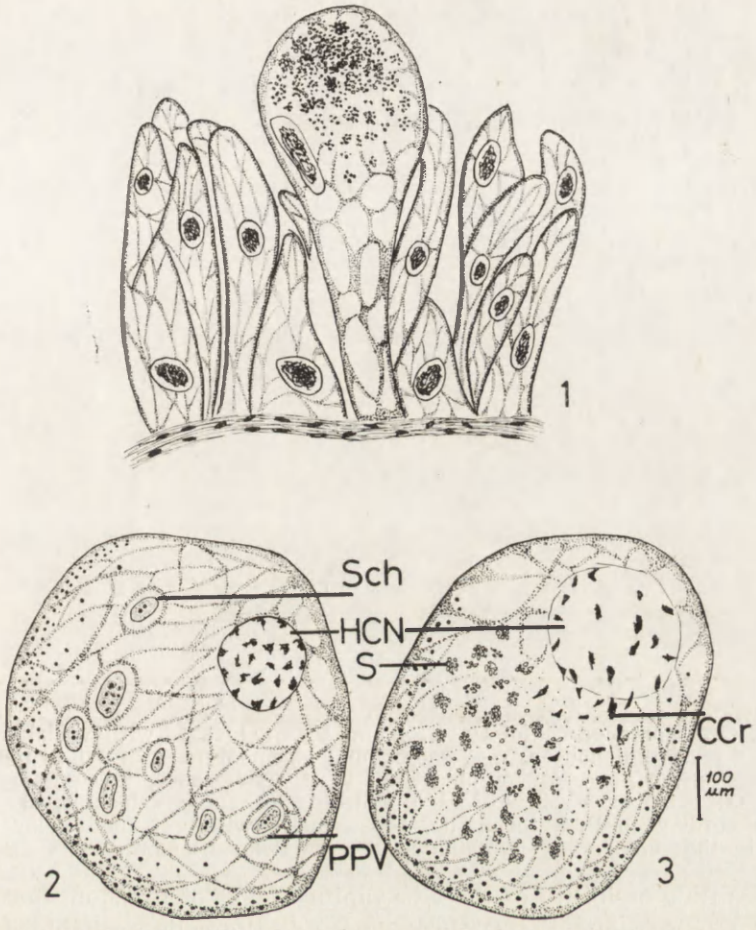

Fig. 1–3. *Octosporea porcellioi* sp. n. 1 — Section showing parasitised hypodermal cell, 2 — Oenocyte with growing schizonts: Note the periparasitic vacuole, 3 — Oenocyte with pansporablasts. Explanation: CCr — Chromatin released into the cytoplasm, HCN — Host cell nucleus, PPV — Periparasitic vacuole, S — Spores, Sch — Schizont

The earlier stages in the life-history of the parasite found in the hypodermal cells and oenocytes are elongated and have an amoeboid appearance. They range in size from 5.0–12.5 × 3.0–3.6 µm and have 4–8 deeply stained nuclei placed in the strongly basophilic cytoplasm (Fig. 4, 5). During the course of further development they undergo division and give rise to binucleate (Diplokaryon) bodies measuring 3.6 × 1.6 µm (Fig. 6). Diplokarya in which the two deeply stained nuclei are placed closely together and also show a small filamentous structure between them. The nuclei have an incipient but intact nuclear membrane which

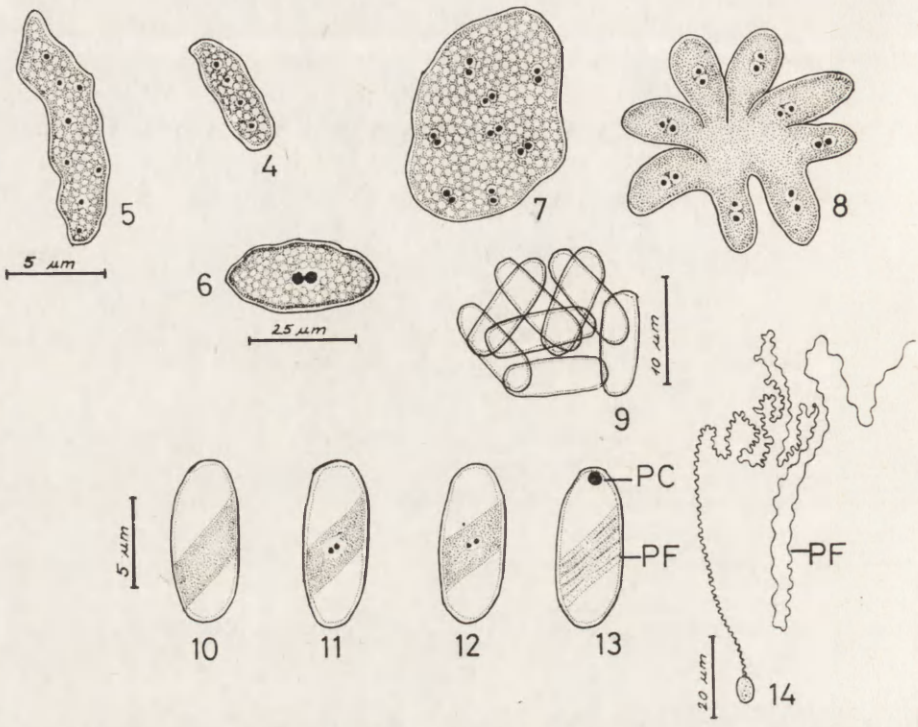

Fig. 4–14. *Octosporea porcellioi* sp. n., 4 and 5 — Schizonts with variable number of nuclei, 6 — A diplokaryon, 7 — Sporogonial plasmodium with paired nuclei, 8 — A rosette stage, 9 — A pansporoblast, 10 — A fresh spore, 11 — Spore stained with haematoxylin, 12 — Spore stained according to Feulgen's technic, 13 — Spores stained according to PAS technique, 14 — Spore with extruded polar filament. Fig. 5–8 in the same scale. Explanation: PF — Polar filament, PC — Polar cap.

eliminates the possibility of their becoming stages of division. Such stages are seen fairly frequently. Diplokarya inside the cells probably divide to form sporogonial mother cell. However, stages leading to the formation of the sporogonial plasmodium have not been observed. Numerous sporogonial plasmodia, each containing 8 paired nuclei and measuring 9.0–10.5 × 7.0–10.0 μm are seen in smears prepared from the haemocoelic fluid (Fig. 7). The cytoplasm is finely alveolated and the nuclei are deeply stained and are surrounded by a clear halo all round. The sporoblasts which are formed from the sporogonial plasmodium are arranged in the form of a rosette, a characteristic feature of the family *Caudosporidae*. The rosette stages vary in size from 11.6–15.2 × 9.5–14.0 μm. In subsequent stages of development of the rosette stages, the cytoplasm is thrown into 8 finger shaped lobes and each lobe contains a pair of nuclei (Fig. 8). The cytoplasm is granular and the nuclei are deeply stained. Each nucleus is surrounded by a clear halo, but a distinct nuclear membrane could not be seen.

The pansporoblast measures 12.0–14.0 × 10.0–12.0 μm. An outer limiting membrane is not seen clearly in the fresh preparations (Fig. 9). The usual number of sporoblasts formed from each pansporoblast is 8 but the number may vary between 6–12. The spores have a typical shape of *Octosporea*. They are cylindrical, sometimes slightly curved and without any ornamentation. The spores measure 9.0–10.0 × 3.6–4.0 μm in the fresh condition. They have a large anterior polaroplast and a small posterior vacuole (Fig. 10). In haematoxylin stained preparations the sporoplasm is seen extending in the form of a band extending between the polaroplast and the posterior vacuole. The nuclei are faintly stained (Fig. 11). Spores stained by the Giemsa and Feulgen's stain also revealed essentially the same structure (Fig. 12). Spores treated according to PAS technique showed a PAS positive polar cap lying anterior to the polaroplast. A faintly stained polar filament could be seen coiled inside the spore (Fig. 13). The polar filament is released by the addition of a drop of hydrogen peroxide to the wet or air dried smears. The polar filament is uniformly thin and measures 220–300 μm in length and the proximal $^3/_4$ part of the polar filament is highly sinuous.

Discussion

The microsporidian infecting the terrestrial isopod, *Porcellio laevus* Latr. is placed in the family *Cougarellidae* because the sporoblasts are in the form of a rosette and also because of the absence of pansporoblast membrane. It is placed in the genus *Octosporea* because the spores are cylindrical, sometimes slightly arched and each sporont gives rise to variable number of spores although the usual number is 8 (Sprague 1974). Several species of *Octosporea* have been described from arthropod hosts but so far there is no record of a microsporidian parasite belonging to this genus from isopods and from the present host and hence it is considered a new species for which the name *Octosporea porcellioi* sp. n. is proposed.

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Our thanks are due to Prof. K. Hanumantha Rao, Head of the Department of Zoology, Andhra University, Waltair for giving necessary facilities to carry out this work.

RÉSUMÉ

On décrit la morphologie et le cycle de developpement de l'*Octosporea porcellioi* sp. n., microsporidie parasitaire de l'isopode terréstre *Porcellio laevus* Latr. (*Oniscidae, Isopoda, Crustacea*). Le parasite infeste les cellules hypodermiques tapissant l'intérieur de la paroi du corps, l'exterieur de la paroi de l'intestin, et les muscles. Sa présence provoque une perte du tonus musculaire chez l'hote, son exténuation et finalement sa mort.

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Department of Zoology, Andhra University, Waltair, Andhra Pradesh, India

C. C. NARASIMHAMURTI and C. KALAVATI

A New Microsporidian, *Pleistophora blatellae* sp. n. from the Malpighian Tubules of *Blatella germanica* [1]

Synopsis. A new species of a microsporidian parasite, *Pleistophora blatellae* parasitic in the epithelial cells of the Malpighian tubules of *Blatella germanica* is described.

During the period 1972–1974 cockroaches belonging to the genus *Blatella* have been examined on several occasions for microsporidian parasites and on two occasions they were found harbouring a microsporidian parasite belonging to the genus *Pleistophora* which for reasons given below is considered new to science and the name *Pleistophora blatellae* sp. n. after the host is proposed.

Material and Methods

The host cockroaches, *Blatella germanica* were collected from the kitchens and store rooms in Waltair (Andhra Pradesh, India). There are no external indications of infection and hence all the specimens had to be decapitated, dissected and examined microscopically before infection could be detected. Smears were prepared from the infected organs and examined in the fresh condition both under bright field and under dark ground illumination. Smears were air dried, fixed in methyl alcohol and stained with Giemsa solution. Smears were also wet-fixed in Schaudinn's or Carnoy's fluid, hydrolysed in 1 NHCl at 60°C for 10 min and stained either with Heidenhain's iron haematoxylin or Feulgen's stain. Smears showing the spores were stained according to the PAS technique. Material for sectioning was fixed in alcoholic Boin's fluid, sectioned at 8 μm thickness and stained with Heidenhain's iron haematoxylin. Measurements of the spores were made in the fresh condition. The polar filaments were released by using conventional methods.

Observations

Pleistophora blatellae sp. n.

Host: *Blatella germanica*
Site of infection: Epithelial cells of Malpighian tubules

[1] Abstract presented at the IX International Colloquium on Invertebrate Pathology held at Kingston, Ontario, Canada during August–September 1976.

Locality: Waltair (Andhra, India)
Type slides: Zoology Department Andhra University, Waltair.

2–3% of the cockroaches, *Blatella germanica* examined revealed an infection with a microsporidian parasite belonging to the genus *Pleistophora*. The infection was usually restricted to the Malpighian tubules but on one occasion the infection was found to extend to the hepatic caeca and adipose tissue also. Infected tubules appeared opaque white and slightly bulged in contrast to the transluscent appearance of the healthy tubules. On microscopic examination it was found that the appearance is due to the tubules being filled with masses of pansporoblasts and spores of the parasite. Even the pressure of the cover slip resulted in bursting of the tubule and the release of the pansporoblasts and spores. Sections of the Malpighian tubules showed that the parasite undergoes its development in the epithelial cells. The host cell is hypertrophied and the host cell nucleus is pushed to a side or to the base of the cell (Fig. 1). With the growth and differentiation of the parasite, the cell cytoplasm is utilized and the cell is filled with pansporoblasts and spores of the parasite. Heavily infected cells burst and release the spores into the lumen of the Malpighian tubules.

The pansporoblast in the fresh condition is irregular in shape and measures 20–28 × 16–20 μm. It has an outer delicate limiting membrane, clearly seen in smears stained according to the PAS technique. The pan-

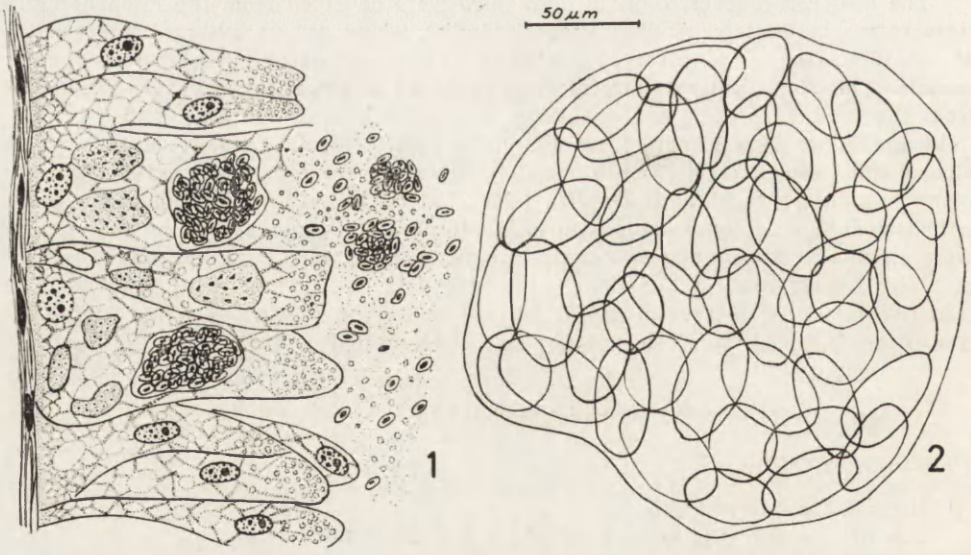

Fig. 1–2. *Pleistophora blatellae* sp. n. *1* — Section of the Malpighian tubule showing the pansporoblasts and other stages in the life-cycle. Note a burst cell releasing the spores, *2* — A pansporoblast

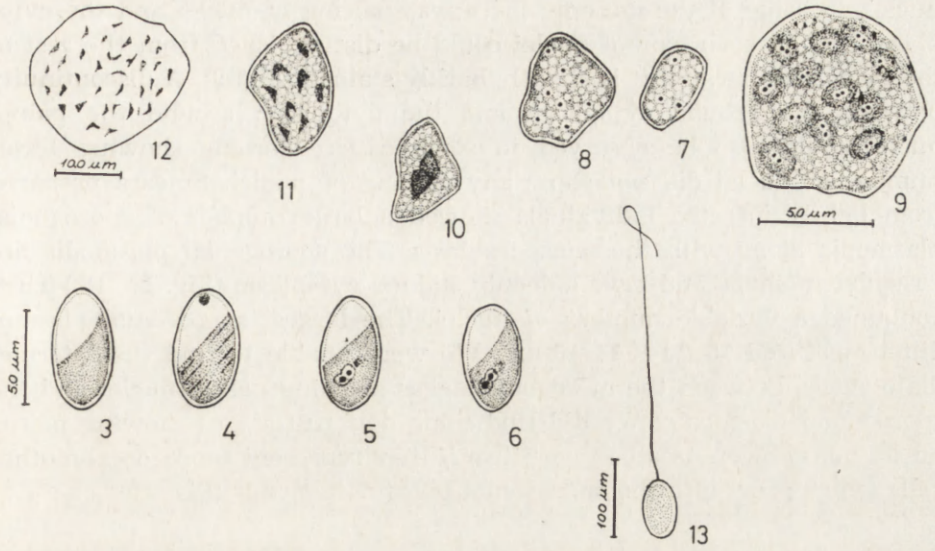

Fig. 3–13. *Pleistophora blatellae* sp. n. *3* — A fresh spore, *4* — A spore stained according to PAS technique, 5 — A spore stained with Giemsa, *6* — A spore stained according to Feulgen's technique, 7–8 — Stages of merogony showing variable number of nuclei, *9* — A meront showing pairing of nuclei, 11–12 — Stages of sporogony, *13* — A spore with extruded polar filament

sporoblast encloses about 32–48 spores (Fig. 2). The spores are oval with rounded corners and measure 6.5–8.5 × 2.3 µm. A large conspicuous polaroplast which appears in the form of a clear vacuole is present at the anterior end (Fig. 3). A posterior vacuole could not be seen either in the fresh or stained preparations. The entire space beneath the polaroplast is occupied by the sporoplasm. In Giemsa and Feulgen stained preparations the sporoplasm showed two deeply stained nuclei placed towards one side in the sporoplasm (Fig. 5, 6). Spores treated according to the PAS technique showed a PAS positive polar cap lying at the anterior end in front of the polaroplast (Fig. 4). The polar filament is released by the addition of a drop of hydrogen peroxide either to the fresh or air dried smears. The polar filament is uniformly thin and measures 45–50 µm in length (Fig. 13).

Different stages of merogony were observed in the epithelial cells of the Malpighian tubules, particularly in light infections. The earliest stage in the development of the parasite was irregular in shape with lightly stained finely alveolated cytoplasm and containing 4 dot-like nuclei 9 (Fig. 7, 8). Each of the nuclei is surrounded by an incipient nuclear membrane. Meronts of different sizes containing a variable number of nuclei have been observed. The largest meront measured was nearly rounded and measured 9–14 × 8–13 µm and contained a maximum number of 24

nuclei. In some of the meronts there was pairing of nuclei and the cytoplasm around each pair of nuclei could be distinguished from the rest of the cytoplasm because it is slightly darkly stained (Fig. 9). A discontinuity between the ground cytoplasm and the cytoplasm around the paired nuclei could also be observed in some cases. Meronts showing lesser number of nuclei did not show any pairing of nuclei. Smears prepared from lightly infected individuals showed a large number of sporogonial plasmodia along with the pansporoblasts. The sporogonial plasmodia are irregular in shape and have a deeply stained cytoplasm (Fig. 11, 12.) They contained a variable number of nuclei. The largest sporogonial plasmodium measured 15–20 × 14–18 μm. We were unable to find the intermediate stages between the merogonic stages showing paired nuclei and the sporogonial plasmodia. We did find some doubtful stages showing paired nuclei but we were unable to confirm if they represent sporogonial mother cells which grow into the sporogonial plasmodia or not (Fig. 10).

Discussion

Pernin (1906) reported a microsporidian belonging to the genus *Pleistophora* from the midgut of two species of cockroaches, *Periplaneta americana* and *P. orientalis* collected in England and Brazil. This was first reported as *Nosema periplanetae* by Lutz and Splendore (1903) (= *Pleistophora* Mercier). Georgevitch (1927) described the schizonts, spores and polar filaments in *P. periplanetae*. Subsequently a number of workers studied the sporozoan parasites of cockroaches and discussed the taxonomic position of *Pleistophora* and *Coelosporidium* Schaudinn, 1902 (*Haplosporidia*). Sprague (1940) discussed at length the taxonomic position of *Coelosporidium periplanetae* and stated that "... Georgevitch reported that he had observed both the polar capsule and polar filament and all other characters of a true cnidosporidian. These observations are entirely at variance with the results obtained by all other workers. One is strongly impelled to agree with Debaisieux (1927) who believed that Georgevitch was probably dealing with a mixed infection..." Sprague and Ramsay (1941, 1942) described *P. kudoi* from the fat bodies of *Blatella orientalis*. They stated that "... this parasite may be identical with the one of undetermined nature observed by Pernin (1905) although the spores he saw were about 1.2 × 0.5 μm in permanent preparations which were somewhat smaller than those described below in the living condition...". The authors did not make any mention of Georgevitch's (1927) paper. Weiser (1960) added another species *P. blattae* from the gut epithelium and caeca of *Blatta orientalis*

from the USA. Thomson (1960) and Weiser (1961) listed all the microsporidian parasites of insects including both *P. periplanetae* and *P. kudoi*. Weiser (1961) giving due consideration to Georgevitch (1927) stated that "... Auf Grund des beschriebenen Materials wei auch auch eigenen material gehort die Art zur Gattung *Pleistophora*. Es ist cosmopolitisch verbreitet wir ihr wirt." (On the basis of the described material, however, the unique material belongs to a species of the genus *Pleistophora*. It is cosmopolitan in distribution). Unfortunately we have not seen the original descriptions of Pernin (1905) and Georgevitch (1927) and hence followed Weiser (1961) and treated *P. periplanetae* as a microsporidian.

A comparison of a 11 species of microsporidians described from cockroaches shows that the one described in the present paper differs considerably from them. The spore in the present case is bigger than both that of *P. kudoi* and *P. blattae*. *P. kudoi* is from the gut epithelium and caeca of *Blatta orientalis* occurring in west Virginia, USA and the spores are reniform and they measure 3.2 × 1.75 µm. *P. blattae* is from the adipose tissue of *Blatta orientalis* occurring in the USA and the spores measure 5–6 × 2.5–3.0 µm. *P. periplanetae* is the only species described from the Malpighian tubules of two species of cockroaches, *P. periplaneta* and *P. orientalis*. The spores are dimorphic and occur in two sizes: the microspores measuring 5–6 × 2.5–3.0 µm and the macrospores measuring 6.5–7.0 × 3.5 µm and the polar filament is 150 µm in length. In the present form the spores are larger than even the macrospores and the fully released polar filament is only 45–50 µm long in contrast to 150 µm in *P. periplanetae*. The details of the pansporoblasts are not given for *P. periplanetae* and hence a comparison could not be made with that of the present form. In view of these differences between the present form and those that have already been described from cockroaches and also because the present form is from a new host and a new geographical locality it is considered a new species for which the name *Pleistophora blatellae* n. sp. after the host is proposed.

ACKNOWLEDGMENTS

Our thanks are due to Prof. K. Hanumantha Rao, Head of the Department of Zoology, Andhra University, Waltair for the facilities provided to carry out this work.

RÉSUMÉ

Une nouvelle espéce de microsporidien est décrite, *Pleistophora blatelle* sp. n. parasitaire des cellules epitheliales de les Malpighian tubules du *Blatella germanica*.

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Institute of Molecular Biology, Jagiellonian University, Grodzka 53, Krakow, Poland

Barbara KALISZ-NOWAK

Experimental Study on Locomotion of *Amoeba proteus*. II. Reactions to some External Stimuli in *Amoeba proteus* and its Fragments from which a Part of the Cytoplasm has been Removed

Synopsis. Primary responses to mechanical stimulation, pseudopodia-inducing chemotactic agents and pinocytose-inducing solutions were observed and analysed in intact specimens of *Amoeba proteus* and its fragments, from which endoplasm had been removed by high speed centrifugation. It was found that the fragments in which the ectoplasmic cylinder was absent reacted to mechanical stimulation and chemotactic agents in a rather similar manner as did the intact specimens. The primary responses to chemotactic agents were found to be localized in the plasmalemma and hyaline ectoplasm. The hyaline pseudopodia were formed in the intact specimens before any distinguishable changes in the ectoplasmic cylinder and endoplasmic streaming were detected. These last always followed and never proceeded the localized changes in cell periphery and took place only when a pseudopodium had just been formed and had begun to extend. Absence of the ectoplasmic cylinder in amoeba fragments made these fragments insensitive to pinocytose — inducing solutions. These results are discussed and it is suggested that pseudopodium initiation and extension is a multistep phenomenon.

In the recent work of Kalisz and Korohoda (1976) it has been shown that small, nucleated fragments of *Amoeba proteus* can locomote even when their granular endoplasm has been removed by high speed centrifugation. These fragments, though originating from *Amoeba proteus*, showed significant differences in the pattern of their locomotion when compared to the intact specimens. As in small amoebae their cytoplasm did not differentiate into a relatively stationary ectoplasmic cylinder and a streaming endoplasm but was transported in volume at a speed similar to the speed of progressive migration of the fragment. Moreover, the surface of these fragments was smooth in the posterior regions. Because of these differences it seemed desirable to compare responses to some external stimuli in these fragments with responses in the intact specimens. The results of these experiments are presented in this communication and

discussed from the point of view of functions of hyaline ectoplasm and of the ectoplasmic cylinder in motile activities of *Amoeba proteus*.

Material and Methods

Amoeba proteus (Princeton strain) was cultured as already described (Kalisz and Korohoda 1976). For the experiments the intact specimens washed in Chalkleys medium and the amoeba fragments obtained according to the method described by Kalisz and Korohoda (1976) were used. Responses to mechanical stimulation of amoebae and their fragments as well as to the chemotactic agents were observed and photographed as described by Korohoda (1977). For induction of pinocytotic activity in amoebae solution of 0.125 M NaCl and 0.125 M glutamate adjusted to pH 8.0 with NaOH were used (cf. Chapman-Andresen 1962, Kalisz 1973).

Results

Stimulation of *Amoeba proteus* and its Fragments with a Microneedle

When the intact specimens of *Amoeba proteus* are stimulated with a microneedle the cell response depends on the strength of the stimulus and on the position of the cell region to which the stimulus is applied (Jennings 1904, Hyman 1917, Goldacre 1952). In agreement with all previous descriptions of the responses to mechanical stimulation in the intact specimens of *Amoeba proteus* (Jennings 1904, Hyman 1917, Goldacre 1952) it was observed that when the stimulus was applied to the tip of an extending pseudopodium the first cell response was to cease for a while endoplasmic streaming and cell movement. Then, if the stimulus was not too strong, a new pseudopodium was formed and extended near the originally dominant one. The stimulated pseudopodium at the same time retracted and gradually withdrew (Pl. I 1 a–c). When a stronger stimulus was applied to the side of an extending pseudopodium, after primary inhibition of all cell motion, several new pseudopodia were formed at the opposite side to the stimulated one. This response is shown in Pl. I 2 a, b and it corresponds to that described by Jennings (1904). When the pseudopodium was very gently touched with a microneedle, only sporadically did not retract and even reassumed normal extension after a short cessation of motion (less than one second).

The immediate inhibition of all motile activities was also observed when the nucleated, monopodially locomoting hyaline fragments were stimulated. This was followed by an appearance of some folding of the

fragments surface. The inhibition of movement lasted up to 5 s and only then was a new pseudopodium formed and the old one retracted as shown in Pl. 3 a–d. Full reversion of the direction of the fragments' locomotion was never observed.

Primary Responses of *Amoeba proteus* and its Fragments to Locally Applied Anaesthetics

General anaesthetics such as benzene, chloroform or ether when locally applied were found to induce pseudopodia formation in intact specimens of *Amoeba proteus* as well as in their nucleated and enucleated fragments (K o r o h o d a 1972, 1977). Therefore it seemed interesting to observe the first responses of the amoebae to the local application of benzene.

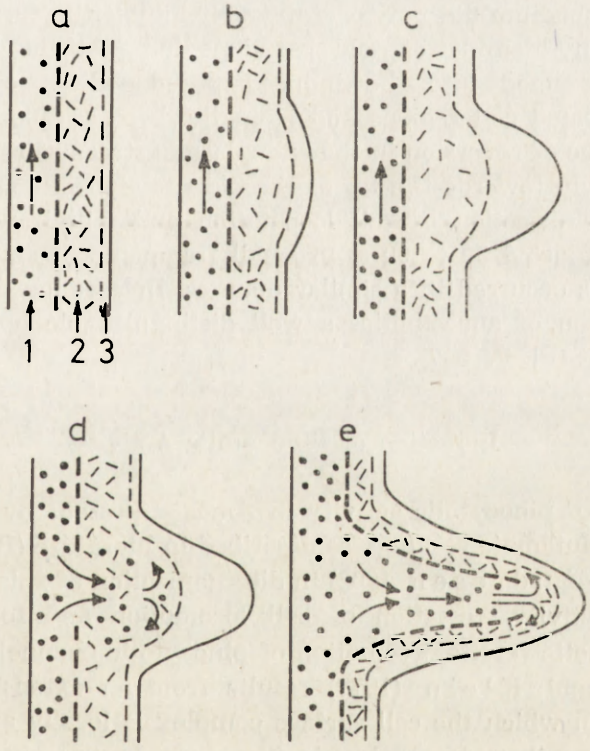

Fig. 1. Schematically presented, sequence of events during formation of pseupodia induced by locally applied anaesthetic. Arrows point to: *1* — endoplasmic streaming, *2* — ectoplasmic cylinder, *3* — hyaline ectoplasm; b and c correspond to primary stages in the response when endoplasmic streaming and the ectoplasmic cylinder are not disturbed and when the cell reaction is restricted to the hyaline ectoplasm; d and e correspond to the stages when, during further extension of the induced pseudopodium, a volume inflow of endoplasm starts and normal conversion of the endoplasm into ectoplasmic cylinder begins

When a very narrow capillary (approx. 2 um in diameter) filled with the benzene — saturated medium, was placed on one side of a monopodially locomoting intact *Amoeba proteus*, the first cell response involved only the plasmalemma and hyaline ectoplasm. This last locally increased in the thickness and became convex. Then small hyaline pseudopodia-like protuberances of this hyaline ectoplasm were formed and these extended towards the tip of the microcapillary (Pl. III 4 a–d). At that time the ectoplasmic cylinder had not yet changed and endoplasmic streaming continued in the original direction, perpendicular to the direction of extension of the hyaline pseudopodia. Only when these pseudopodia had fused together and a visible break in the structure of the cylinder took place did the granular endoplasm start to flow into the recently induced pseudopodium, which gradually started to dominate (Fig. 1 and Fig. 1 in Korohoda 1977). It should be pointed out that even the extension of a large pseudopodium did not for a moment inhibit the further extension of an originally dominating, old pseudopodium. At the beginning the endoplasm streamed into the induced pseudopodium only from the posteriorly located cell regions and from the base of this pseudopodium whereas a wave of reversion in the endoplasmic stream direction travelled relatively slowly towards the tip of the old pseudopodium. The complete change in the direction of cell locomotion and withdrawal of the old pseudopodium lasted 30 to 60 s. In small fragments of amoebae the primary reactions occurred in a similar manner. In pseudopodia induced by local application of anaesthetics a well distinguishable hyaline cap was always present (Pl. IV 5–7).

Induction of Pinocytotic Activity

Induction of pinocytotic activity in *Amoeba proteus* by 0.125 M NaCl and 0.125 M glutamate resulted, as described in literature (Brandt 1958, Chapman-Andresen 1962) in disorganization of polarization of cell locomotory activity, cessation of cell locomotion and formation of so called pinocytotic resette. Formation of pinocytotic channels, according to Stockem and Klein (1977) results from an extension of hyaline pseudopodia in which the cell surface complex is locally anchored to the cylinder by bundles of cytoplasmic filaments. It was observed that the pinocytose-inducing solutions which in intact *Amoeba proteus* induced rosette formation in 100% of specimens were quite ineffective for nucleated or enucleated hyaline fragments of *Amoeba proteus*, from which granular cytoplasm was removed by high speed centrifugation. These fragments in which differentiation of the loose cytoplasm into a cyto-

plasmic cylinder and streaming endoplasm was absent showed no response to these solutions. This cannot be explained as a result of cell enucleation since enucleated fragments obtained by amoeba dissection and containing normal, granular cytoplasm reacted normally (cf. aslo Chapman-Andresen 1962).

Discussion

Effects of factors which induce modifications in motile activities of *Amoeba proteus* were compared in intact specimens and fragments of *Amoeba proteus* from which granular cytoplasm had been removed by high speed centrifugation. These experiments were carried out to gain some insight into functions of the ectoplasmic cylinder and hyaline cortical ectoplasm in locomotion of *Amoeba proteus*. As has been shown (Kalisz and Korohoda 1976) the activity of the cell surface complex consisting of plasmalemma and cortical ectoplasm is responsible for locomotion of small fragments of *Amoeba proteus* (cf. also Grębecki 1976, 1977). The presence of an ectoplasmic cylinder as well as the occurrence of polarized conversions of streaming endoplasm into a stationary cylinder and vice versa seem to be associated with the locomotion of intact specimens and represent their accomodation to the problem of migration caused by their relatively great size and mass (cf. Kalisz and Korohoda 1976, Grębecki 1977).

The majority of workers studying mechanical stimulation of amoebae (Jennings 1904, Hyman 1917, Goldacre 1952, Mast 1926) have assumed that a cell response resulting in local "shortening" of the ectoplasmic cylinder accompanied by an outflow of cytoplasm from stimulated regions reflects contractile processes. However, it should be noticed that the mechanical stimulation itself lasts for a very short period of time and the observations concern the cell responses which occur after the stimulus ceases to act. Only the immediate inhibition of motile activity in *Amoeba proteus* and its fragments, which is not restricted to the cell region directly stimulated but extends over the whole amoeba, can be considered as the direct result of stimulation. Subsequent responses, that is the formation of new pseudopodia and retraction of the stimulated one (if the stimulus was strong enough) or the reassumption of extension by the weakly stimulated pseudopodium, represent amoebic reactions taking place during the recovery of the cells from the action of the stimulus. In particular the responses to very gentle mechanical stimulation seem to correspond to events occurring in *Xenopus laevis* eggs electrically stimulated for a short time, where short lasting contraction evoked by

the stimulus is followed by local relaxation whereas a contraction shifts posteriorly (cf. Korohoda and Rzehak 1972). If the above is true one can postulate that ectoplasmic contractions could be initiated in the vicinity of the tips of extending pseudopodia and gradually strengthen being transmitted posteriorly through the cell surface complex (cf. Marsland 1964, Korohoda and Stockem 1975). Observations that hyaline fragments of amoebae react to mechanical stimulation by cessation of movement and reassumption of locomotion in a new direction suggest that primary responses are associated with functions of the plasmalemma and subjacent hyaline ectoplasm. This does not, however, exclude involvement of the ectoplasmic cylinder in the responses of large, intact amoebae.

The responses of amoebae to locally applied anaesthetics concern cell motile activities which are observable when the stimulus continuously acts upon the cell. From the results shown in Pl. III and Fig. 1 is evident that the first changes in cell body contour associated with the pseudopodia initiation do not result from the endoplasmic streaming. Quite to the contrary the changes in endoplasmic streaming and the ectoplasmic cylinder always follow primary modifications of activity in the cell periphery. Local changes in the thickness of the hyaline ectoplasm correspond to those changes described in amoebae which have been immersed in an anaesthetic containing medium (Hüllsmann et al. 1976). Since anaesthetics are known to prevent cytoplasmic contraction by inhibition of calcium release from the smooth reticulum (Feinstein and Paimre 1969, Gail 1973, Braatz 1975) one can expect that induction of pseudopodia is associated with a decrease of contractile tension in cell periphery, which later is followed by local damage of the ectoplasmic cylinder. It is rather difficult to explain the formation of small hyaline pseudopodia on the edge of the cell as resulting from the gradient in the hydrostatic pressure within the cell. This last, which according to the hydraulic pressure theories of amoeboid locomotion is responsible for streamings of endoplasm (Jahn and Bovee 1964, Rinaldi et al. 1975), can be effective only at the stage at which a volume inflow of endoplasm into the pseudopodium which has just started to extend begins.

From observations of the primary events accompanying induction of pseudopodia by locally applied anaesthetics one can conclude that the basic phenomena which determine localization and extension of new pseudopodia take place in the plasmalemma and in the cortical hyaline cytoplasm. Therefore, the extension of pseudopodia in intact specimens of *Amoeba proteus* should be considered as a complex phenomenon in which several processes localized in particular cell structures, that is in the

plasmalemma, hyaline ectoplasm, ectoplasmic cylinder and streaming endoplasm, occur simultaneously and cooperate. Only under experimental conditions can these processes be separated one from another and, in future, be analysed in detail.

The inability of the amoebic fragments in which the ectoplasmic cylinder is absent to respond to pinocytose inducing solutions strongly supports a recent suggestion of Stockem and Klein (1977), who postulates the important role in the pinocytotic channel formation of the gelified ectoplasmic cylinder.

RÉSUMÉ

On a analysé les premières résponses à le stimulation mécanique et aux agents chimiques provoquant la formation des pseudopodes ou la pinocytose, chez les cellules intactes de l'*Amoeba proteus* et chez les fragments dont l'endoplasme a été évacué par la centrifugation. La réaction des fragments dépourvus du cylindre éctoplasmique aux stimulant mécaniques et chimiques est plutôt pareille à celle des amibes intactes. Le première réponse est localisée dans la plasmalemme et dans l'ectoplasme hyaline. Les pseudopodes hyalines sont formés avant que des changements dans le cylindre ectoplasmique et dans le courant endoplasmique soient visibles. Ces derniers sont toujours postérieurs à des changements localisés dans la périphére de la cellule et n'interviennent qu'après la formation et le début de l'extension du pseudopode. Les fragments sans cylindre ectoplasmique sont incapables de la pinocytose. La formation d'un pseudopode apparait comme un phénomène composé des plusieures étapes.

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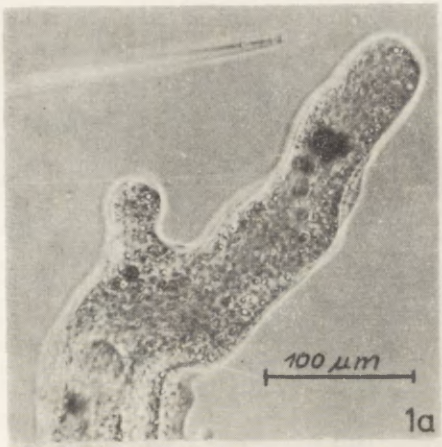
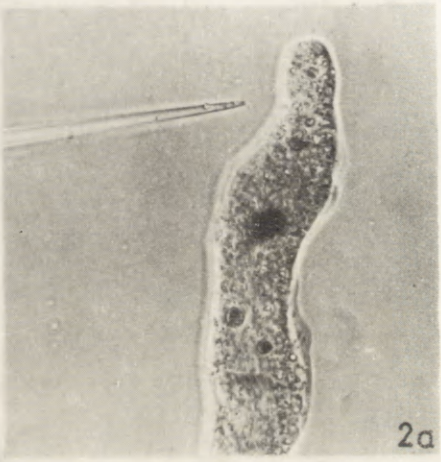
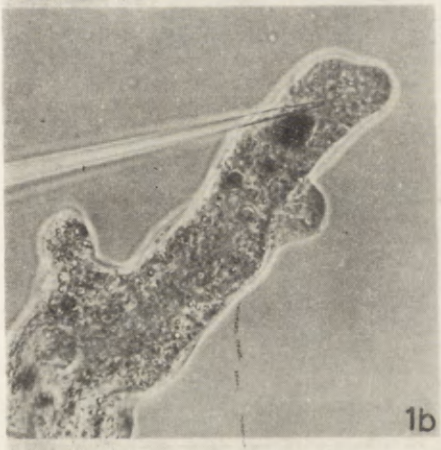
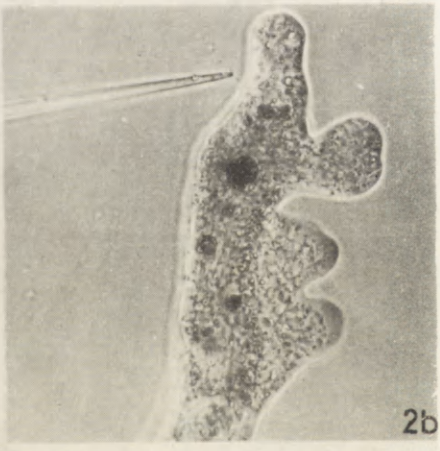
EXPLANATION OF PLATES I–IV

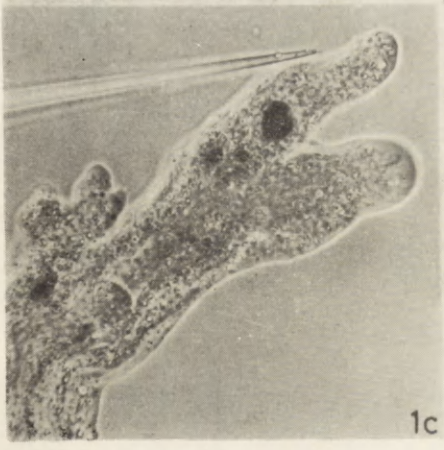
1 a–c: Reaction of *Amoeba proteus* after the moderate stimulation of an extending pseudopodium with a microneedle, 2 a, b — reactions of *Amoeba proteus* after strong stimulation of one side of dominating pseudopodium. Pictures taken at 30 s intervals
3 a–d: Response of a small, nucleated fragment of *Amoeba proteus* to stimulation with a microneedle. Pictures taken at 30 s intervals
4 a–d: Subsequent stages in primary response of *Amoeba proteus* to local application of benzene, Photographs taken at 2 s intervals
5–7: Responses of *Amoeba proteus* fragments obtained by high speed centrifugation (5 a, b) and by sectioning (6 a, b) to local application of benzene (7 a, b). In all induced pseudopodia a hyaline cap is clearly distinguishable











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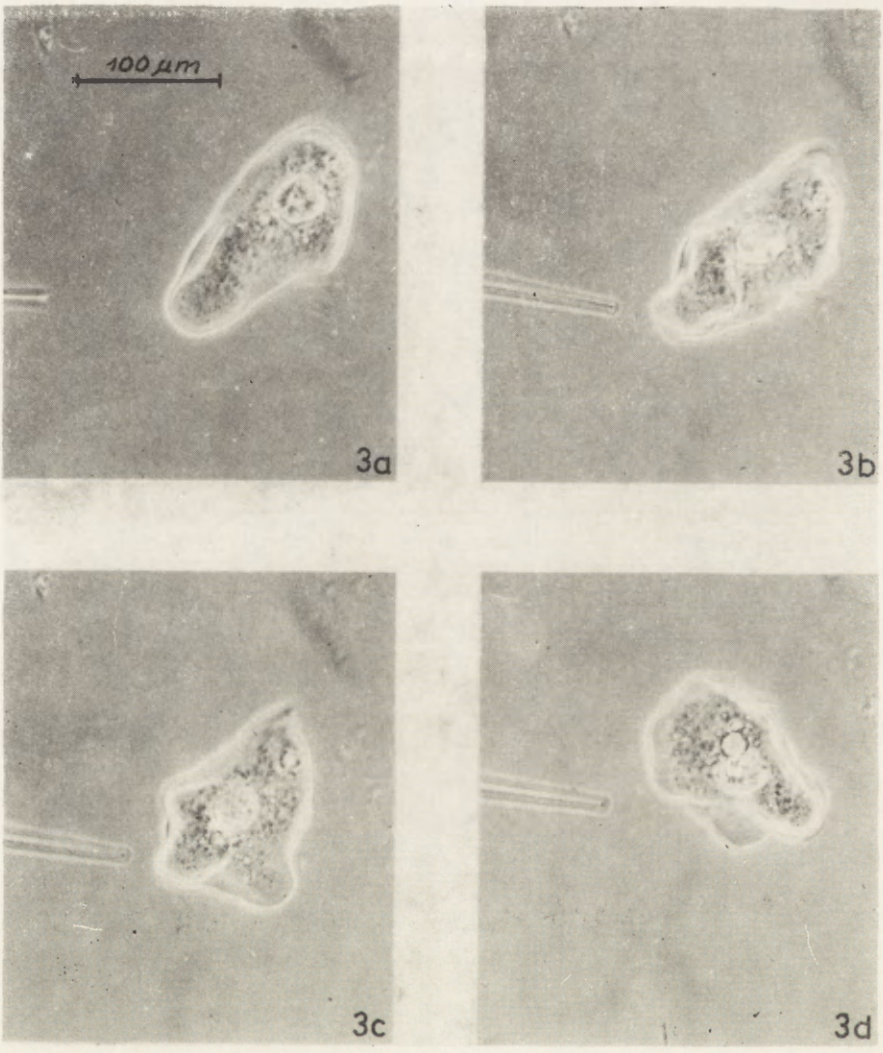

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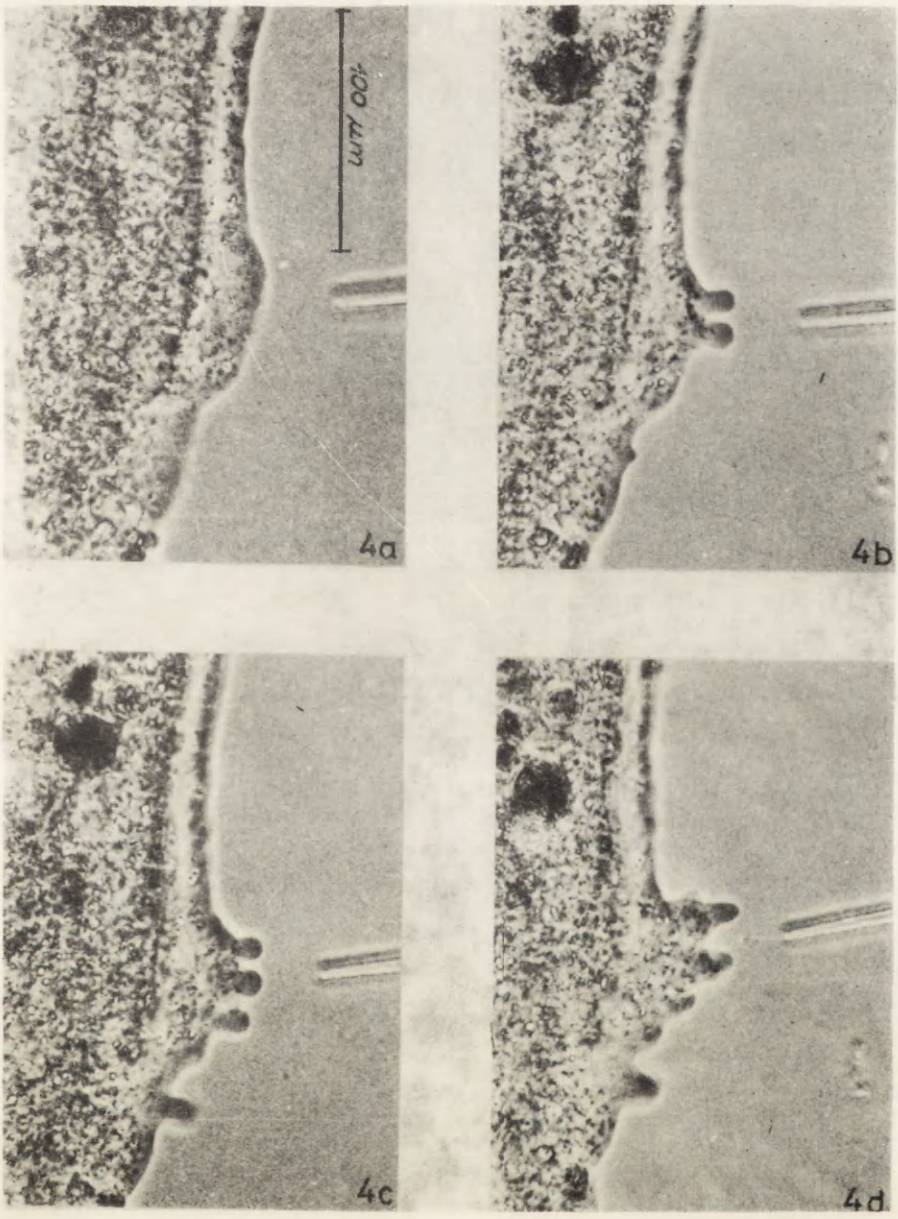

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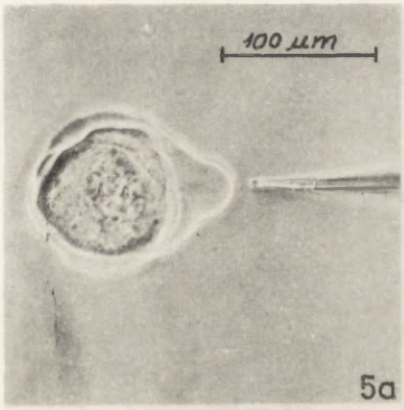

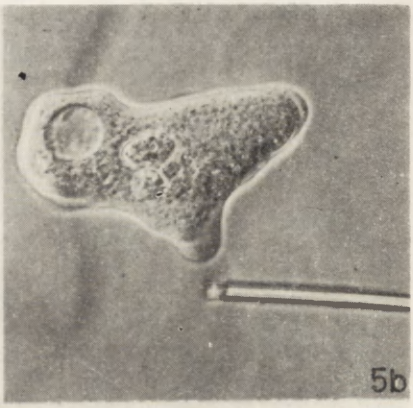

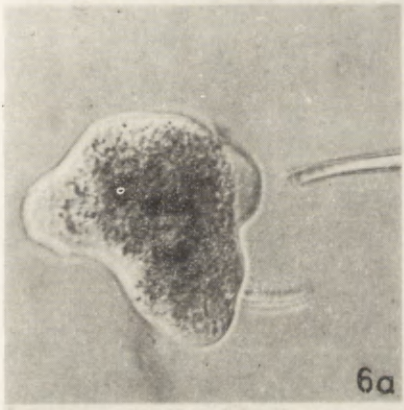

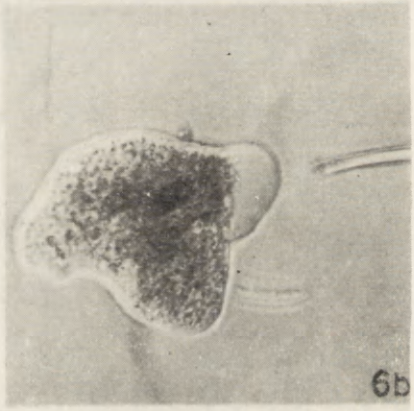

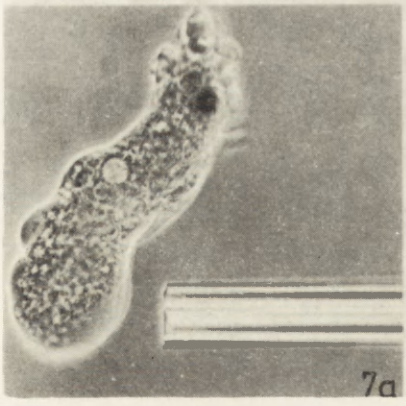

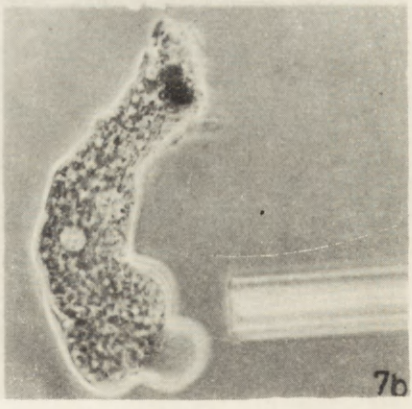

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Department of Biology, Nencki Institute of Experimental Biology, Pasteura 3, 02–093 Warszawa, Poland

Stanisław DRYL and Jacek KURDYBACHA

Galvanotactic Response in *Paramecium caudatum* at Various Levels of External Calcium Ions

Synopsis. In *Paramecium caudatum* the galvanotactic threshold was almost constant (voltage gradient 0.1–0.5 V cm^{-1}) within wide range of external calcium ions pCa 2–5. This finding suggests that some minimum depolarisation of the cell membrane on the cathodal side of animal and a minimum hyperpolarisation of the cell membrane on the anodal side — are responsible for induced ciliary response leading to cathodal orientation of ciliate in electric field. The galvanotactic threshold was 10–20 times higher in media containing low concentrations of calcium pCa 6–8. It is not clear whether the preserved response to d.c. stimulation should be attributed to an release of calcium ions from the hypothetical intracellular stores within cathodal region of cell or it depends on the persistence of augmented ciliary beat within anodal region of ciliate.

The significant role of external calcium Ca_{ex} for excitability and motor response of ciliates to potassium and other cations was stressed by a number of authors (Kamada and Kinosita 1940, Dryl 1961, Jahn 1962, Grębecki 1964, 1965, Kuźnicki 1966, Kinosita et al. 1964 a, b). It was found that potassium-induced ciliary reversal (CR) lasts longer at lower concentrations of Ca^{2+} but disappears in "absence" of calcium (Kamada 1940, Kamada and Kinosita 1940). Naitoh (1968) suggested that during induced CR calcium ions are released from their binding sites within cation exchange system in the cell membrane by other cations and are causing in this way CR due to direct action on the contractile elements within cilium in presence of ATP and calcium-activated ATP-ase. Eckert (1972) put forward hypothesis that ciliary reversal in *Paramecium* is caused by the transmembrane influx of external calcium ions due to an increase of calcium conductance of the cell membrane by the other cations or by external stimuli of other modality. This hypothesis found support in experimental results obtained by Naitoh

and Kaneko (1972), who demonstrated a direct role of calcium ions in inducing CR within paramecia models extracted by detergent Triton X-100, and afterwards exposed to various concentrations of calcium ions; CR appeared only at concentrations of calcium higher than 10^{-6} M. It should be pointed out in this respect that recently Hildebrand and Dryl (1976) noticed that in *Paramecium caudatum* potassium-induced CR can be evoked only at concentrations of external calcium higher than 0.3×10^{-6} M, and that previous incubation of animals in calcium reach medium does not change this threshold. Dryl and Kurdybacha (1977) found that *P. caudatum* and *P. aurelia* show linear increase of negative chemotactic thresholds in response to Quinine hydrochloricum solutions parallel to decrease of Ca_{ex} within range between pCa 2–pCa 5 while response to chemotactic stimuli is no more detectable in concentrations pCa 7–8. The aim of the present study was to check how important is the level of Ca_{ex} for response of *Paramecium* to d.c. stimulation.

Material and Methods

As an experimental material *Paramecium caudatum*, strain isolated in 1965 from surroundings of Warsaw, was used. Paramecia were cultivated in lettuce medium according to Sonneborn (1950). 18–24 h before starting the experiments, protozoans were washed and concentrated geotactically in 1 mM Tris (Flukka) — HCl + 1 mM $CaCl_2$ buffer solution of pH 7.1, until the dilution over 1000 times was obtained.

Experiments were carried out in experimental camera, similar to that used in previous experiments (Dryl and Kurdybacha, in press) with set of platinum-platinum black nonpolarized electrodes. The distance between electrodes was 6 cm. The cross section of the camera was 0.2 cm^2. Paramecia were introduced to the central part of the camera, separated from the other parts those with electrodes built in, by small sheets of paper, to eliminate the influence of the products of electrolysis on the animals.

To compare the strength of the stimulus in various mediums applied, the voltage gradient (v/cm) was measured (Korohoda and Kurowska 1970, Fabczak et al. 1973) during the series of experiments Paramecia were stimulated by d.c. of desired strength continuously during 15 s; various voltage gradients between 0.1–10 V cm^{-1} were applied. As a stimulus source a high capacity, specially built stimulator with set of stabilizers was used. The value of applied voltage was measured by Digital multimeter 1331. Conductivity of the medium was checked by the CDM–3 RADIOMETER Conductometer.

Experimental medium, contained Tris solution (Flukka) with various free calcium ions concentration, Ca/EGTA (Sigma) buffers, were prepared according to Portzehl et al. (1964), as it was described elsewhere (Dryl and Kurdybacha 1978, in press).

The movement of paramecia and direction of swimming was recorded during last 3 s of d.c. stimulation by long-exposure photomacrographic technique of Dryl

(1958), so that on film negatives the orientation of all paramecia present in the sample and numbers of animals swimming towards cathode or anode could be easy established (Pl. I 1).

The doubtful cases when animals were oriented transversally or perpendicularly to the lines of electric field, were omitted. Threshold value for each voltage gradient in each Ca^{2+} concentration was established arbitrary, when 70–80% of animals, were swimming towards cathode. The estimations of threshold value was repeated 10 times, and so each point of the cumulative diagram represents mean value from 10 calculations.

Results

Paramecia showed no disturbance of movement in concentrations of free calcium ions pCa 2–4. Spontaneous short lasting CR reactions (with frequency below 3–4 CR cycles per min) were observed at the level of calcium pCa 5. However, typical periodic ciliary reversal (PCR) appeared in animals exposed to external medium containing calcium ions in concentration pCa 6. It should be pointed out that paramecia showed forward movement (without any CR responses) at levels of free calcium ions lower than pCa 7; at these concentrations the animals could survive 3–5 min

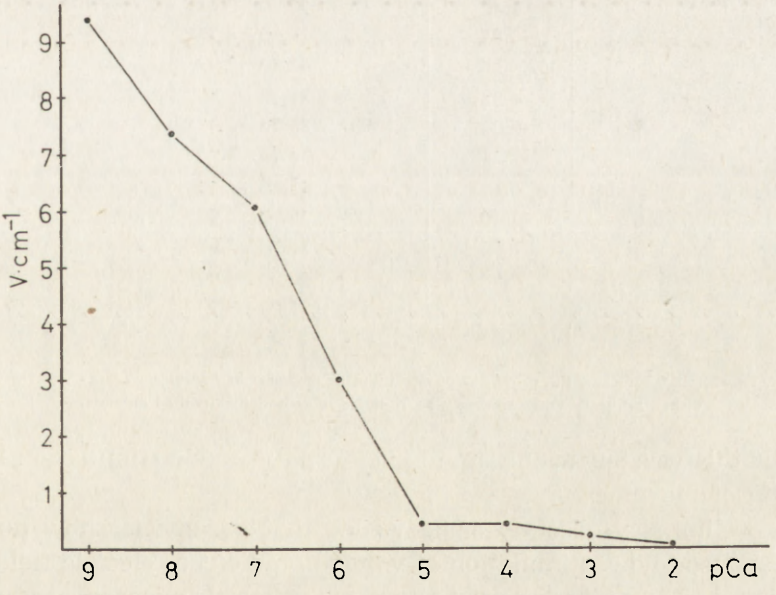

Fig. 1. Threshold of galvanotactic response in *Paramecium caudatum* at various concentration of free calcium ions (Ca–EGTA buffers) in external medium containing 1 mM Tris-HCl, pH 7.1. Threshold was established arbitrary in ca. 50 specimens as ratio 70–80% of cathodally oriented animals. Galvanotactic response was photo-recorded (Dryl 1958) 12–15 s after the beginning of d.c. stimulation. *Ordinate*: Voltage gradient of applied electric field ($V \cdot cm^{-1}$). *Abscissa*: Molar concentration of free calcium ions in external medium

Table 1

Threshold of galvanotactic response in *Paramecium caudatum* at various concentrations of free calcium ions (Ca-EGTA buffers)

pCa	V · cm^{-1}	Mean number SD in 50 specimens of *Paramecium caudatum* showing cathodal orientation. Data based on 10 series of experiments
2	0.02	26.20 2.74
	0.09	35.70 4.74*
	0.14	41.30 2.41
3	0.05	26.90 3.07
	0.24	35.40 5.48*
	0.38	43.30 3.09
4	0.29	33.50 4.50
	0.48	40.50 2.72*
	0.59	45.40 2.95
5	0.29	26.60 2.83
	0.48	36.20 4.26*
	0.59	43.30 3.16
6	2.40	27.80 2.44
	3.01	36.10 4.36*
	4.58	42.70 3.09
7	4.88	31.20 2.78
	6.09	36.70 4.35*
	7.34	38.70 3.95
8	6.17	33.80 4.34
	7.40	36.60 4.95*
	8.64	38.50 3.69
9	7.89	30.20 3.99
	9.44	36.00 3.77*
	10.52	38.50 3.69

* Indicates the threshold of galvanotactic response in *Paramecium* estabished arbitrary in 50 specimen as 70-80% of cathodally oriented animals.

only, but this was sufficient time for external electric stimulation and for recording of movement.

The preliminary observations revealed that paramecia show no detectable response to d.c. stimulation at voltage gradient of electric field below 0.2 V · cm^{-1}. The cathodal orientation of animals appeared gradually at higher voltage of applied electric current. The direct microscopic observations showed that the animals oriented parallel (or almost parallel) to lines of longitudinal electric field responded to d.c. stimulation at lower voltage than those oriented more or less perpendicularly. Physiological

state of animal could be also responsible for individual differences in sensitiveness to d.c. stimulation.

The arbitrary established threshold for galvanotactic response (i.e., 70–80% of animals swimming towards cathodal) proved to be very reliable and convenient method for measuring reaction at various external conditions.

The data included in Table 1 and Fig. 1 indicate that the threshold for galvanotaxis was 0.1 and 0.25 $V \cdot cm^{-1}$ for pCa 2 and pCa 3 and 0.48 $V \cdot cm^{-1}$ for pCa 4 and pCa 5 respectively. This is evident that within wide range of higher concentrations of calcium ions the threshold value for galvanotaxis was almost constant.

The dramatical increase of threshold for galvanotaxis at pCa 6 to 3.01 $V \cdot cm^{-1}$ was probably associated with PCR response which obviously inhibited oriented movement of animals towards cathode; PCR disappeared almost completely at applied electric fields of voltage equal or higher than threshold current.

Extremely high threshold for galvanotaxis at calcium ions level lower than pCa 7–6.1–9.4 $V \cdot cm^{-1}$ suggests that in this case we deal with strongly marked decrease of sensitivity of paramecia to d.c. stimulation. In medium with low concentration of calcium paramecia could resist stimulation with d.c. of high voltage gradient only for very short time, surviving usually not longer than 25–40 s. This made very difficult a careful analysis of polar effect of electric current on the ciliary activity at cathodal and anodal end of animal. Direct microscopical observations of slowly swimming animals towards cathode didn't reveal any evident signs of CR on cathodal side of the cell, although some augmentation of ciliary beat was noticed at the anodal end. However, it should be emphasized with this regard that it is not easy to observe cathodal CR at galvanotactic threshold d.c. stimulation even in animals kept in medium with higher content of calcium ions.

Discussion

Two interesting conclusions can be drawn from present study on galvanotactic response of *P. caudatum* at various Ca_{ex} levels.

(1) Unlike in the case of chemotactic stimuli (Dryl and Kurdybacha 1977), the galvanotactic threshold does not undergo much change within wide range of Ca_{ex} (pCa 2–5) and

(2) Galvanotactic response is still preserved even at lowest concentrations of Ca_{ex} (pCa 7–9), although its threshold is highly increased.

The first finding can be explained by assumption that a minimum of cell membrane depolarization on the cathodal side of the body and a minimum cell membrane hyperpolarisation on the anodal side — are necessary to evoke ciliary response which determines orientation of ciliates parallel to electric lines.

The preserved response to d.c. stimulation at very low levels of Ca_{ex} might be explained either by release of calcium ions from their hypothetical intracellular stores (Kuznicki 1973) or by persistance of augmented beat of cilia at anodal end of *Paramecium*. It is believed that this interesting problem will be elucidated by planned in the near future studies on galvanotactic response in behavioral mutants of *Paramecium aurelia*.

RÉSUMÉ

Le seuil de la galvanotaxie du *Paramecium caudatum* reste presque constant (entre 0.1–0.5 $V \cdot cm^{-1}$) dans des larges limites de concentration du calcium extérieur (pCa 2–5). Ce résultat suggère q'une dépolarisation minimale de la membrane céllularie du côté de la cathode et son hyperpolarisation minimale du côté de l'anode, présentent les facteurs qui provoquent la réponse ciliaire aboutissant à l'orientation catodique du cilié dans le champ électrique. Le seuil de la galvanotaxie dévient 10–20 fois plus élevé dans les milieux contenant moins du calcium (pCa 6–8). Il n'est pes clair si la persistance de la réaction à la stimulation électrique est due à la liberation des ions Ca^{2+} d'une hypothétique resèrve intracéllularie dans la région tournée vers la cathode ou bien si celle dépend de la persistance de l'intensification des battements ciliaires du côté anodique du cilié.

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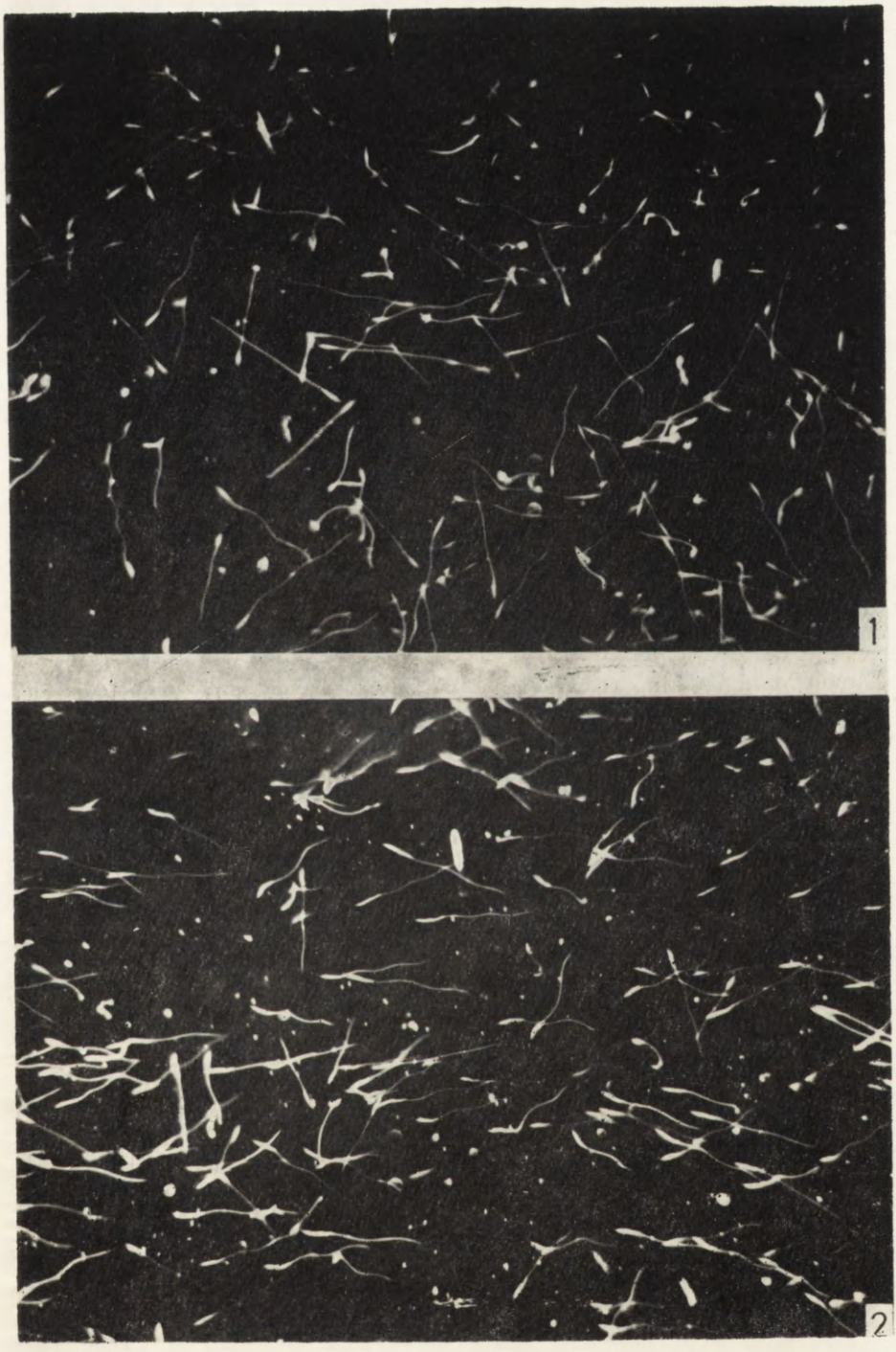

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EXPLANATION OF PLATE I

Macrophotographs of recorded movement of paramecia immersed in solution: 1 mM $CaCl_2$ + 1 mM Tris/HCl, pH 7.1 stimulated by d.c. during 15 s. Exposure time — 3 s. The bright dots at the ends of recorded paths indicate the direction of swimming. 1 — Paramecia stimulated by subthreshold d.c. with voltage gradient 0.05 V/cm. The ciliates are swimming various directions; no galvanotaxis. 2 — Paramecia stimulated by d.c. with voltage gradient 0.38 V/cm. The cathode on the left. Positive galvanotactic response

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