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## *Triadinium minimum* GASSOVSKY. ITS PHYLOGENETIC SIGNIFICANCE

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The genus *Triadinium* was created in the year 1890 by Fiorentini for the species *T. caudatum* from the horse intestine. This species was included to the family Cycloposthiidae by Hsiung in 1930; the diagnosis of the genus was also emended by Hsiung in 1935.

In the recent classification of ciliates the families Cycloposthiidae and Ophryoscolecidae belong to the order Entodiniomorpha (Corliss 1961, Raabe 1964), class Spirotricha according to Raabe 1964, subclass Spirotricha according to other authors. Following Strelkov's proposal 1939 supported by Noiro-Timothee 1960, genus *Triadinium* Fiorentini together with genera *Ditoxum* Gass., *Cochliatoxum* Gass., *Tetratoxum* Gass., excluded from the family Cycloposthiidae, should be transferred to family Ditoxidae in the order Entodiniomorpha.

In 1918 Gassovsky described two new species of the genus *Triadinium* from the horse intestine: *T. minimum* and *T. galea*. Strelkov 1931 described *T. elegantum* from zebra intestine. Hsiung 1935 described *T. magnum* found in mule feces and in 1936 found by the same author in horse intestine.

The morphology of species from genus *Triadinium* was described in details by their authors. The descriptions, however show one deficiency—they are deprived of the full particulars of infraciliation, because ciliates were not impregnated with silver.

Applying silver impregnation to horse intestine ciliates I had the occasion to impregnate species from the genus *Triadinium* found in my material. The impregnation results of *T. minimum* Gass. were specially interesting. They revealed a completely unexpected character of the buccal apparatus in the ciliate included in the order Entodiniomorpha.

In the first description by Gassovsky 1918 the mention can be found that in *T. minimum* the peristome leads to a long, bent, preoral space. In the descriptions by Hsiung 1930, 1935 it is mentioned that vestibulum and the long, bent esophagus are ciliated. From these short and general statements few conclusions can be drawn.

Leaving aside many details of the structure described by the authors and more deeply analyzed by Strelkov in his monograph 1939, I am going to present a description of the infraciliation of *T. minimum* Gass., as well as of the type-species *T. caudatum* Fior.

### MATERIAL AND METHODS

Material was provided from the content of horse colon from the slaughterhouses in Warsaw or in Łódź and from the horse feces of the Łódź district. The material was fixed in 10% formaline in the 1:1 ratio. When the bigger particles of the non-digested food were removed by filtration, the material was several times rinsed in water, and then concentrated by centrifugation. A drop containing the rinsed and concentrated ciliates was mixed on slide with warm gelatine (after Chatton) and was quickly spread into a thin layer on the slide. The preparation was immediately put into the refrigerator for about an hour. Subsequently the preparation was impregnated with ammoniacal silver solution of Bielszowski.

### RESULTS

#### *Triadinium minimum* Gass (Fig. 1)

The protozoon resembles a helmet rounded at the top and flattened laterally. The posterior part ends with a caudal projection narrowing at the end and provided with a bundle of cilia. A small group of anterior terminal cilia is

slightly moved to the left. Another similar group at the dorsal side, near the base of the caudal projection is moved to the right. A large buccal overture in the posterior part of the body on ventral side is surrounded by a half open ring of adoral cilia. The above mentioned zones lie in slight depression of the cytoplasm; their cilia usually cling to form bundles called by the authors membranella or cirri. The buccal overture leads to a large cavity, which gradually gets narrower and is directed to the front and then turns to the dorsal side. It looks like a horn. On the left side of the buccal overture there is a small triangular process. Oval macronucleus lies in the center of the body. The round micronucleus excavation of the macronucleus on the anterior dorsal side. One contractile vacuole lies in the caudal projection. Near the contractile vacuole on the dorsal side the cytophyge is situated.

Dimensions: length 31-40  $\mu$ , width 22-36  $\mu$ .

In the ciliate impregnated with silver the following features of the infraciliation can be state! (Fig. 2). Groups of cilia-anterior, dorsal, caudal and adoral look as it might be expected. Each of them looks like a ribbon composed of short, parallel, evenly spaced kineties. In the separate kineties the kinetosoms are densely disposed. In the zones-anterior, dorsal and adoral the kineties run obliquely to the long axis of the zone. The kinetosoms of the zones do not form any groups corresponding to membranella or cirri. So in each zone there are rows of cilia which do not cling to form permanent structures of higher level, but compose temporary bundles-syncilia, similar to those found in Entodiniomorpha (Noirot-Timothee, 1960). The basis of the cilia of a caudal bundle consists of a few very short kineties. The adoral zone is in the form of a long ribbon. It surrounds the right side of the buccal overture and passes to the left where it does not reach the triangular process. On the right side of the body, to the front of the adoral zone near the base of the caudal projection, very close to the zone but separated from it there are a few very short, dense kineties.

The ciliature of the buccal horn is composed of several parallel, long kineties situated very close to each other. They begin near the edge of the buccal overture on the ventral wall and run along the horn wall up to its end. Not all kineties begin on the same level. When we move further from the buccal overture we can discover that gradually one kinety after another appears but they all reach the end of the cone. At first they run obliquely (towards the dorsal and anterior side) across the right wall and subsequently they turn towards the left wall and run in arch along the curvature of the narrowed part of the buccal cavity.

Numerous fibres, probably connected with long kineties, run across the buccal wall in its narrowed part. In the part close to the buccal overture the fibres form a very complex system, which I am unable to describe. A particularly dense bundle of fibres enters the triangular process. In some other places the fibres form similar bundles (Fig. 3).

*Triadinium caudatum* Fior.  
(Fig. 4)

The protozoon is similar to that described above. The position of the buccal overture and the zones of somatic cilia are similar too. Similarly to *T. minimum* cytophyge lies on the dorsal side of the caudal projection. Macronucleus slightly elongated, irregularly shaped is situated in the anterior part closer to the ventral side. Its widened end is directed towards the ventral side, the narrowed end towards the dorsal side. At the narrowed end of the macronucleus, posterior to it around micronucleus is placed. Anterior to the narrowed end of the macronucleus there is a contractile vacuole. A funnel-shaped cytopharynx provided with fibres begins at the buccal overture and is directed towards the anterior part and slightly towards the dorsal side of the body.

Silver impregnation did not reveal here, similarly to *T. minimum* any existence of membranelles or cirri in ciliary zones (Fig. 5). These zones consist of parallel equally spaced kineties, oblique to the long axis of zones. The base of caudal bundle is composed of a few very short kineties. The adoral zone forms almost a closed ring around the buccal overture. Its ends meet on the left side of the body. In front of the adoral zone, near the ventral edge, on the right side of the body, a few short kineties get impregnated with silver very intensely. These short kineties composed of big kinetosomes are usually parallel to each other, but sometimes they have irregular course.

Dimension: length 45-67  $\mu$ , width 36-54  $\mu$ .

DISCUSSION

Strelkov 1939 in the chapter on comparative anatomy of Entodiniomorpha from a horse, discusses the relationship between families and genera from this order.

Speaking about genus *Triadinium* Strelkov says that it is a representative of Ditoxidae made shorter as a result of a strong bending of the body and the adoral zone being transferred back along the ventral side (p. 186). Cirri of the adoral zone are then often directed backwards, says Strelkov. The occipital zone occupies terminal position, nucleus together with contractile vacuole moves towards the anterior end, but the lay-out of the protoplast layers does not change and is characteristic of Ditoxidae (p. 239).

These considerations apply particularly well to species *T. magnum* and *T. caudatum* where the nucleus and contractile vacuole have anterior position. It should be added that besides the transformations mentioned by Strelkov other transformations should also take place. Preoral lobe of typical representative of Ditoxidae should disappear. In the case of *T. caudatum* the caudal projection with bundle of cilia should be formed. This bundle of cilia could be a new structure obtained in the course of evolution if we assume that *T. caudatum* is derived from a representative of Ditoxidae such as *Ditoxum* sp. where there is only one group of posterior cilia. This bundle could be only a reduced posterior ventral zone of a representative such as *Tetratoxum* sp., if this genus (with two posterior zones of cilia) were to be considered as the initial form.

Nevertheless as regards the *T. minimum*, these considerations have not been confirmed by facts which were found in reality. The character of the oral ciliature of *T. minimum* suggests that it is necessary to connect this species with another group of ciliates, more primitive than Entodiniomorpha.

In the structure and ciliature of the buccal apparatus of *T. minimum* a striking likeness to the buccal apparatus of Blepharocorythidae (strictly speaking to the genus *Blepharocorys*) has been found (Figs. 3 and 6). So it can be assumed that *T. minimum* is derived from a representative of the genus *Blepharocorys* by means of a bending of the anterior part of the body to the ventral side. The frontal lobe of *Blepharocorys* sp. would have to be reduced. The ciliary zone of the frontal lobe would change its position becoming the anterior zone with terminal position. The ciliary zone of the ventral lip of the *Blepharocorys* sp. would be the adoral zone. As a result of the fact that the buccal overture was moved backward, the buccal cavity would be remarkably curved.

In the buccal cavity of *T. minimum* longitudinal kineties typical of the genus *Blepharocorys* can be found in the same form but in a somewhat different position. In *Blepharocorys* sp. longitudinal kineties begin at the dorsal wall of the cavity and run either along its dorsal wall or encircle it spirally (Grain, 1966; Wolska, 1966). But the anterior part of the dorsal buccal wall of *Blepharocorys* sp. became the ventral one in *T. minimum* and it is at the ventral side that the longitudinal oral kineties begin now. The semicircular fibers, typical of *Blepharocorys* sp. preserve the same character. The only structure that is missing is the anterior buccal zone composed of short oblique kineties, always present in *Blepharocorys* species (Wolska, 1966).

The small group of dense kineties near the adoral zone at the base of the caudal projection is certainly the same structure which was described for the representatives of Blepharocorythidae (Wolska, 1966, 1967a, 1967b, 1968) near the ciliary zone of the ventral lip; the structure will be further called special kinetosomes. Special kinetosomes, which were found by me in all the species of the family Blepharocorythidae known to me, always lie at the ventral margin of the ciliate, backwards from the zone of the ventral lip. When the anterior part of the body is bent towards the posterior end, they would take approximately the position in which some short dense kineties at *T. minimum* are found.

The posterior ciliary zone of *Blepharocorys* sp. would slightly change its position, or it would remain almost without change in *B. valvata* this zone has a dorsal position in relation to the cytophyge, similarly to the *T. minimum* (Fig. 7). But in *T. minimum* there is a caudal projection with a bundle of cilia, which does not exist in the genus *Blepharocorys*. Supposing that *T. minimum* came from the representative of the genus *Blepharocorys*, it would be necessary to admit that besides the changes and reduction of the structures existing in *Blepharocorys* sp., mentioned above, a new structure has come into existence.

It is very attractive to assume that *T. minimum* arose from a representative of another genus from the family Blepharocorythidae, namely from the genus *Ochoterenaiia* Chavarria. This genus contains only one species *O. appendiculata* Chavarria (Fig. 8). In this case all the posterior part of the body would remain almost without any changes. In *O. appendiculata* in the posterior part of the body there is a caudal projection with a bundle of cilia; the posterior zone of the cilia has a dorsal position; cytophyge lies between this zone and the caudal projection. So the topography of the structures mentioned above is the same as in *T. minimum*.

*O. appendiculata* was not impregnated with silver (it did not appear in my material) so it cannot be exactly known what its buccal ciliature is. But there are no reason to suppose that there will be any essential differences in comparison with the genus *Blepharocorys*. It results from the description of Chavarria 1933 that there is a ciliation in

the excavation called vestibulum by the author. The same is stated by Strelkov 1939. Genus *Ochoterenaia* is so similar to *Blepharocorys* that Strelkov 1939 considered these two genera identical and included *Ochoterenaia appendiculata* in the genus *Blepharocorys* Fiorentini giving it the name *Blepharocorys appendiculata* (Chavarría). In my opinion the existence of such a characteristic projection and an additional group of cilia is sufficiently important to retain the separate genus *Ochoterenaia* Chavarría.

But taking into account the close similarity of these two genera we can expect their oral equipment to be similar too. All representatives of the family Blepharocorythidae from a horse and elephant studied by me (Wolska, 1966 1967b, 1968) have buccal ciliature either divided into two groups or constituting one set of kineties with a different course in the anterior and posterior region but the longitudinal kineties always dominate. All representatives have semicircular fibers. It is difficult to think that *O. appendiculata* has neither longitudinal kineties nor semicircular fibers. We cannot know for certain whether the small anterior group of buccal cilia disappeared already in *Ochoterenaia* sp. or whether it was reduced in the course of changes leading from *Ochoterenaia* sp. to *T. minimum*. The shape of macronucleus and the position of micronucleus in relation to the macronucleus are similar both in the *T. minimum* and the *O. appendiculata*. The location of the nuclear apparatus in *T. minimum* in the center of the body, in the inner curvature of the buccal horn, and the change of the direction of the nuclear axis would be a natural consequence of the bending of the body. So the transformation of *Ochoterenaia* sp. into *T. minimum* would require a minimum of changes.

Drawing general conclusions from the considerations presented above I put forward the statement that *T. minimum* proceeds from the representative of the family Blepharocorythidae.

The next problem to discuss is the relation of *T. minimum* (which should be excluded from the genus *Triadinium* Fiorentini), to *T. caudatum*. Looking at these two species one cannot resist the impression of their close relationship, so great is their external similarity. But it is known that external similarity may be deceptive. There are important internal differences between these species. *T. caudatum* has typical features of order Entodiniomorpha which consist in the presence of endoplasmic sac (it was studied by Strelkov 1939), characteristic cytopharynx and the manner of feeding on quite large particles of vegetable food. If *T. caudatum*, according to Strelkov 1939, has arisen from a typical representative of Ditoxidae, the similarity of *T. caudatum* and *T. minimum* would be a phenomenon of convergence.

Strelkov's view is very convincing indeed; the transition from a typical representative of Ditoxidae to *T. caudatum* could be realized without essential changes in the inner structure with changes in the outer structure only.

But another origin of *T. caudatum* cannot be excluded. The descent from the species of *T. minimum* type of structure, with changes in the inner structures (first of all with the reduction of the oral equipment of Blepharocorys-type and the development of cytopharynx of Entodiniomorpha-type), and without essential changes of the outer form is also possible.

We cannot exclude the possibility that the specialization of cytoplasmic layers, typical of Entodiniomorpha was produced in the course of evolution just here "on the way" from *T. minimum* to *T. caudatum*.

It seems to me that this second view is not without foundation. The similarity of the shape and topography of somatic ciliary zones is too prominent to be due to convergence only. The similarity of caudal projection is especially striking, this structure is rarely met in ciliates.

Besides there is one detail in the infraciliature of *T. caudatum* which deserves our attention. I am inclined to attach phylogenetic significance to this detail. In the anterior part of the body on its right side, near the ventral margin there is a small group of big kinetosomes (Figs. 9 and 10). The look of this group resembles special kinetosomes of Blepharocorythidae. Similarly to Blepharocorythidae these kinetosomes are arranged in some short parallel kineties but sometimes they follow an irregular course as if they were covering each other. I have not been able to detect cilia on these kinetosomes, I have not detected cilia on special kinetosomes of Blepharocorythidae either. There might be some very short cilia there which I have been unable to detect. Some time ago I described a similar group of kinetosomes without cilia in two species of Buetschliidae (Wolska, 1964). Later Grain 1966 studying these species in electron microscope stated the presence of very short cilia hidden in the furrows of ectoplasm in this part of the cell surface. It seems that similar furrows exist in Blepharocorythidae and in *T. caudatum*, on the territory where special kinetosomes occur. That is why the detection of cilia in light microscope is impossible. The furrows may account for the picture of the kinetosomes covering one another. The existence of special kinetosomes in *T. caudatum* as well as in *T. minimum* shows that there is a relationship between these two species and the family Blepharocorythidae, so it points to the possibility of such an evolutionary line: Blepharocorythidae, *T. minimum*, *T. caudatum*.

In the medial link of this chain, in *T. minimum* special kinetosomes are inconspicuous while in *T. caudatum* they are very well developed. In the *T. minimum* they take the position which they ought to take when the anterior part of *Blepharocorys sp.* is bent down. They lie close to the adoral zone at its dorsal part. In *T. caudatum* they lie in front of the adoral zone, secondarily removed to a large distance and placed at the ventral margin. It is difficult to say why they are removed, because nothing is known about their function here. If they were an organ of some sense, their position at the anterior pole of the ciliate could be justified.

Finally I assume that *T. caudatum* is related with *T. minimum*, although a long evolution separates it from *T. minimum*, the latter by way of less complicated changes has arisen from the family Blepharocorythidae; some structures existing in Blepharocorythidae continue their existence through *T. minimum* to *T. caudatum*.

No attempt has yet been made at determining the problem of the systematic position of *T. minimum*, I only want to point to the fact that *T. minimum* is a specie which shows a link between the family Blepharocorythidae and the family Ditoxidae from the order Entodiniomorpha.

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Fig. 1. *Triadinium minimum* Gass., left side view. magnification 1000x.

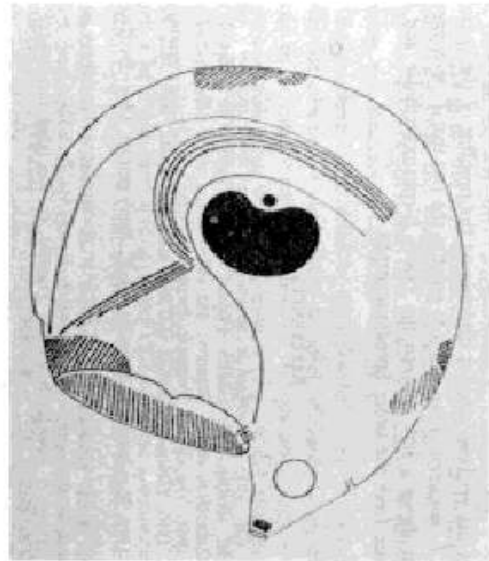


Fig. 2. *T. minimum* Gass. Scheme of ciliature fibers in the mouth part are omitted.

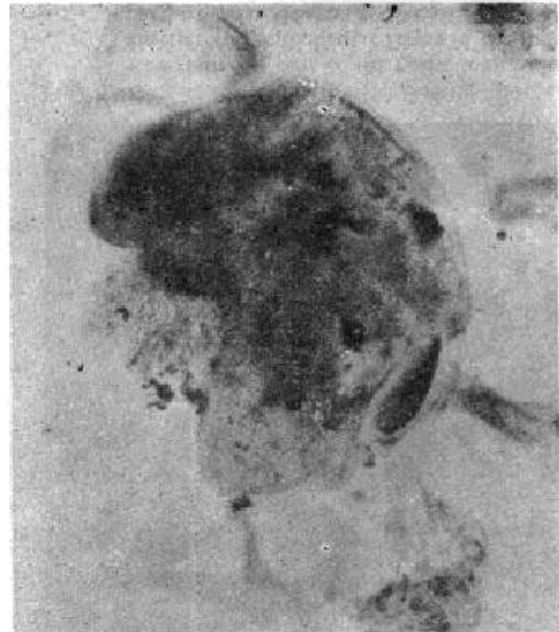
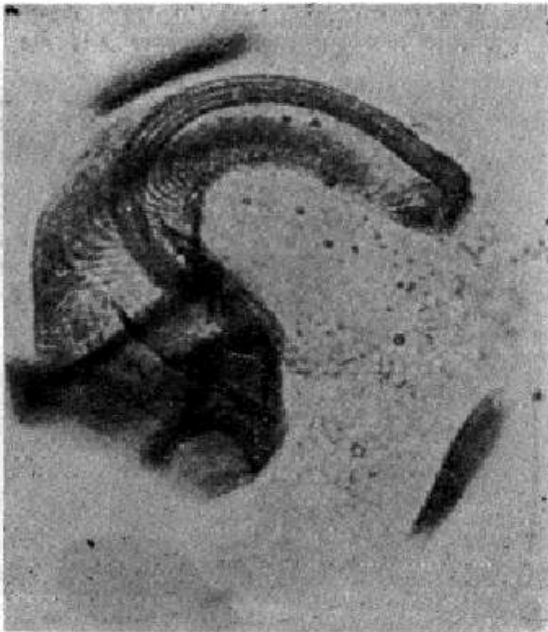


Fig. 3. *T. minimum* Gass., left side view, semi-circular fibers and longitudinal kineties in the mouth part are seen. Magn. 2000x.

Fig. 4. *T. caudatum* Fior., left side view. Magn. 1000x.

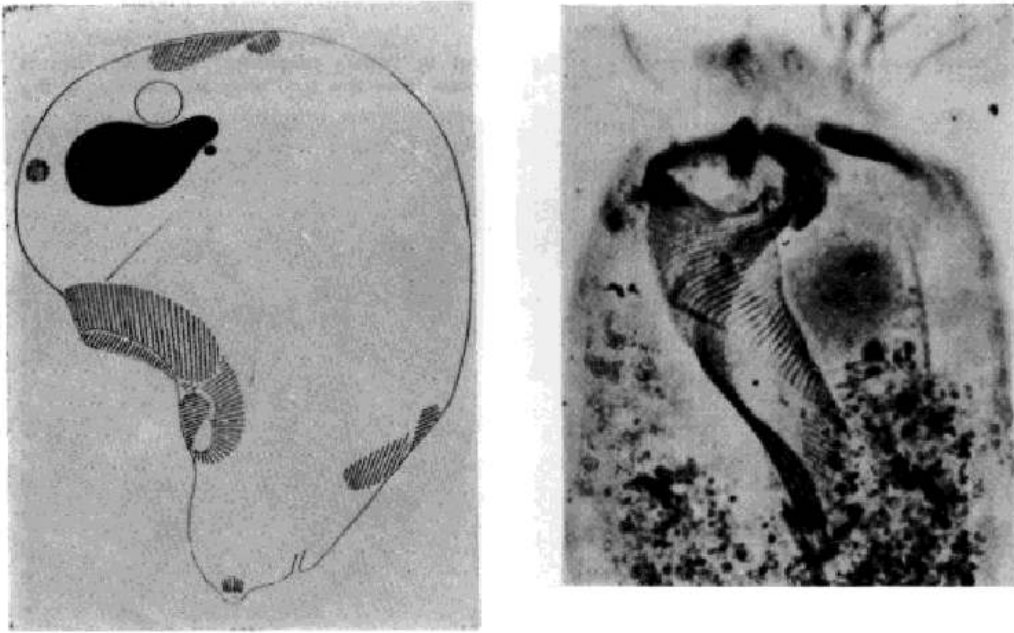


Fig. 5. *T. caudatum* Scheme of ciliation.

Fig. 6. *B. valvata* (Fiorentini) Bundle, left side view, anterior part of the body, longitudinal kineties and semicircular fibers of the mouth part are seen. Magn. 2000x.

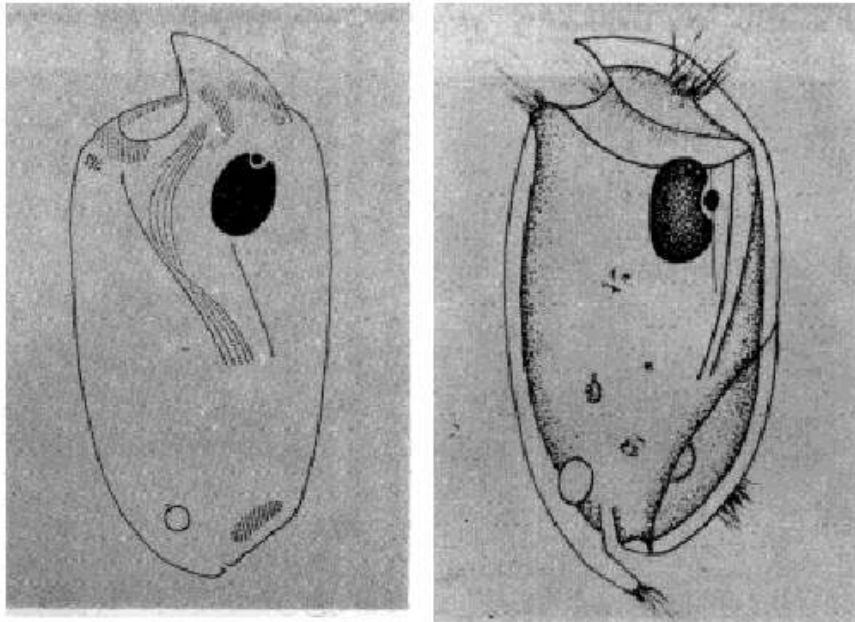


Fig. 7. *B. valvata* (Fiorentini) Bundle. Scheme of ciliature, fibers in a mouth part are omitted.

Fig. 8. *Ochoterenaiia appendiculata* Chavarría, drawn after Chavarría 1933, left side view.

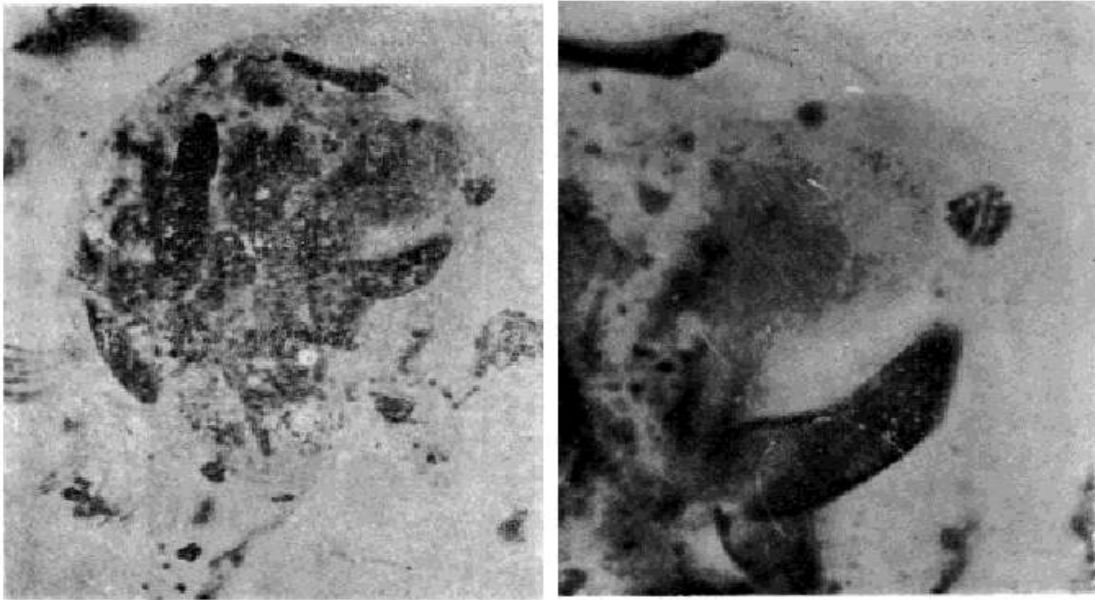


Fig. 9. *T. caudatum* Fior., right side view, specie kinestosomes are seen. Magn. 1000x.

Fig. 10. *T. caudatum* Fior. Anterior part of the specimen above. Magn. 2000x.