(From the Department of Zoology, University of Calcutta.)

On the Morphology of *Balantidium depressum* (Ghosh) from a mollusc, *Pila globosa*, with a note on its nuclealreaction and cytoplasmic inclusions.

By

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(With 7 figures in the text and plate 1).

Introduction.

Ghosh (1921) described *Balantidium depressum* from the rectum of *Pila (Ampullaria) globosa* and placed it under the genus *Balantidiopsis*. Bhatia and Gulati (1927) however, placed it under the genus *Balantidium* as they found no justification of re-introducing the generic title of *Balantidiopsis*. Ghosh's account of this ciliate, as I found it while comparing my observations, is very inadequate and therefore, in order to keep the species alive, it has been considered necessary to give a detailed description of *B. depressum* in this paper. In addition, observations on its nucleal-reaction and cytoplasmic inclusions have also been given in way of further information that I have been able to gather about this organism. I have also indicated the presence of a boring apparatus in this ciliate, a feature, which was first described by Ray (1932) for *B. sushilii* and subsequently by Chakravarty (1933) for *B. elongatum*, *B. helenae* and *B. rotundum*.

Here I wish to express my indebtedness to Dr. H. N. Ray for his kind guidance and helpful suggestions.

Material and Methods.

The snail, *Pila globosa* was collected from the tanks in Calcutta and suburbs. Abundant supply of ciliates was obtained from the large intestine of these snails while a few were found in the rectum too. Observations on living organisms were made in normal saline under a cover-glass, the edges of which were sealed with vaseline. Smears were fixed in SCHAUDINN'S fluid for half an hour and stained in HEIDENHAIN'S haematoxylin. Smears were also fixed in Yocum ZENKER'S for half an hour and stained in MALLORY'S triple stain with a view to demonstrate the presence of myoneme fibres. In order to study the morphology in detail, the infected portions of the intestine were fixed in BOUIN-DUBOSCQ fluid for 24 hours and in CARNOY'S fluid for one hour. The latter fluid however, proved to be a better fixative of the two for the study of myoneme fibres. These tissues were then cut into sections 8μ thick and subsequently stained in HEIDENHAIN'S haematoxylin. Silver lining preparations were also made to show the arrangement of the ciliary rows. LUDFORD'S modification of FEULGEN'S re-action was carried out for the study of the distribution of chromatin in the nucleus of this ciliate. BORREL'S stain was also used for the above purpose. The method adopted by HALL (1929) for the vital staining of GOLGI bodies and mitochondria by neutral-red and Janus green respectively were applied with great success. MANN-KOPSCH and DAFANO methods were applied to obtain permanent preparations of GOLGI bodies and mitochondria respectively. In the former method the tissues were osmicated for 12 days and bleached in turpentine.

Tissues fixed in CARNOY'S fluid were cut 5μ thick and stained in BEST'S carmine and Iodine for the study of glycogen.

Observations on *Balantidium depressum* (GHOSH).

In shape and form this ciliate is more or less oval with either ends slightly pointed. GHOSH in his description has said that "there is a deep concavity on the ventral surface occupying the posterior third of the body" which however I could not see either during life or in stained specimens (both smear and sections). If the ciliate meets an obstruction during movement its shape is considerably changed due to the flexibility of its body, and this feature most probably, led GHOSH to make this error. GHOSH further states that "the left side is more convex than the right side, which is rather flattened in its anterior portions"; but as far as I can judge from

my own observations on both living and stained specimens, I find that both the sides are symmetrical and the body of the ciliate is perfectly circular in transverse section and not oval as described by Ghosh.

The macronucleus is bean-shaped in most of the forms while the micronucleus is oval or spindle-shaped lying usually in the notch of the former. The macronucleus, 16.5 μ in length and 8.2 μ in breadth, varies very much in its position in the body — sometimes it is lying at the extreme posterior end, while at others, it is in front of the anterior third of the body. The micronucleus is very small and measures 5.2 $\mu \times$ 2.06 μ. The long axis of the macronucleus may be transverse, oblique, or at right angles to the long axis of the body.

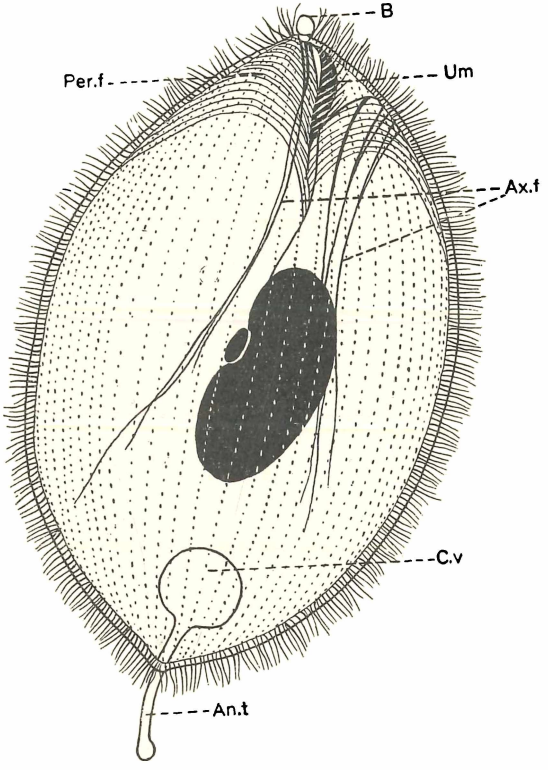

Fig. 1. Camera lucida drawing of *B. depressum* (Ghosh), made from a smear fixed in Schaudinn's fluid and stained in Heidenhain's haematoxylin. × 1666. Ax. f, axial system of fibres; An. t, anal tube; B, borer; C. v, contractile vacuole; Per. f, peripheral system of fibres; Um, undulating membrane.

There is a single contractile vacuole situated beyond the posterior third of the body. A long duct or anal tube, rectractile in nature, is sometimes seen to connect it with the exterior (see Text-Fig. 1).

The mouth is a cone-shaped depression placed slightly towards the right margin of the anterior end — the peristome beginning as a circular groove and gradually narrowing as it passes backwards

into the body. Its left margin is beset with a row of long cilia which in living specimen produce a current leading into the mouth. From below this row of cilia an undulating membrane hangs into the peristomial cavity. The table below shows the comparison between the original and amended description of *B. depressum*.

Table showing the difference between the original and amended description of *B. depressum* (GHOSH).

	Original description.	Amended description.
1.	Body simply or elongately oval, slightly narrowed and rounded anteriorly, wide and tapering to a point posteriorly.	Body more or less oval with either ends slightly pointed.
2.	Body oval in transverse section.	Body circular in transverse section.
3.	A deep concavity on the ventral surface occupying the posterior third of the body.	No such concavity could be found.
4.	Left side more convex than the right side, which is rather flattened in its anterior portion.	Both the sides are symmetrical.
5.	Macronucleus oval and central.	Macronucleus bean-shaped and its position is variable in the body.
6.	Micronucleus spherical and placed at the side of the macronucleus.	Micronucleus ovar or shpindle shaped lying usually in the notch of the macronucleus.
7.		Both axial and peripherial system of fibres are present.
8.		Boring apparatus is situated at the anterior extremity of axial system of fibres on the left side.

Both axial and peripheral system of fibres, as has already been described by RAY (1932) for *B. sushilii*, can to a certain extent, be demonstrated in this ciliate also.

Axial system of fibres.

These are embedded in the cytoplasm and consists of two or more fibres placed slightly towards the left of the peristome. They are either parallel or twisted against one another. The knob-like borer (Pl. 1 Fig. 3) is situated at the anterior termination of these fibres while towards the posterior end they are, in some specimens, found to be tethered to the pellicle (see Text-Fig. 2, 3 a). In addition to these there are four or more fibres originating from below the

pellicle of the right margin of the peristome which are found to run parallel to the axial system of fibres and similarly tethered to the pellicle of that side.

Peripheral system of fibres.

These fibres are arranged along the right and left anterior borders of the ciliates in the form of radial arches and, in all probabilities, serve the purpose of maintaining the rigidity of its peristomal end similar to what has already been pointed out by Ten Kate (1927) for *B. entozoon* and by Ray for *B. sushilii*. In the region of the peristome they appear to attach themselves to the outer peristomal wall on one hand and the pellicle on the other, while, beyond this area, they converge and run along the median line (see Text-Fig. 3, 3a, 4, 4a and Pl. 1 Fig. 5) for a short distance. The peripheral system of fibres are not traceable beyond certain limit and the fibres which form the extreme posterior boundary of the arches may be compared with what Ray has termed limiting membrane' in *B. sushilii* (see Ray, 1935, fig. 1, lm).

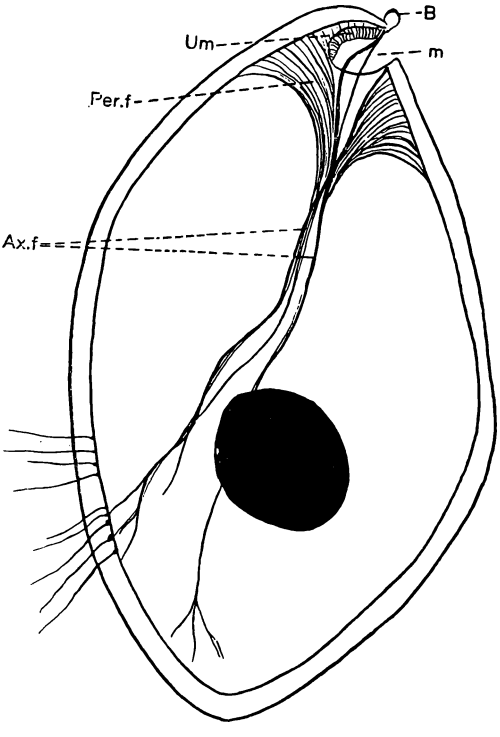

Fig. 2. Camera lucida drawing of longitudinal section of *B. depressum*. × 1666. m, mouth. Other letterings as in Fig. 1.

Nucleal-reaction.

In the Feulgen's reaction both the micro- and macronuclei give positive reaction. The micronucleus takes a diffuse homogeneous red stain showing no positive granules, while, on the other hand

the macronucleus is filled with red staining granules having several non-staining vacuoles among them. These red granules are densely packed together giving the resting nucleus a homogeneous appearance with the non-staining vacuoles in between them (see Text-Fig. 5). These red staining granules in the fixed material are therefore, indicative of the presence of phosphonucleic acid

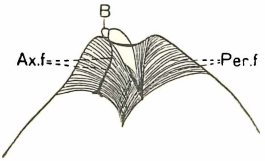

Fig. 3. Camera lucida drawing of longitudinal section of the anterior end of *B. depressum*. ×833. Lettering as in Fig. 1.

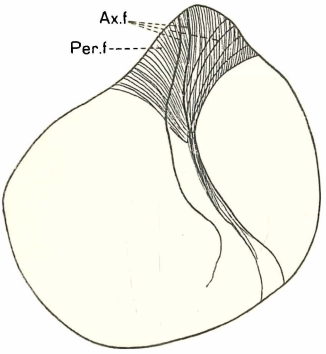

Fig. 3a. Camera lucida drawing of longitudinal section of *B. depressum*, × 833. Lettering as in Fig. 1.

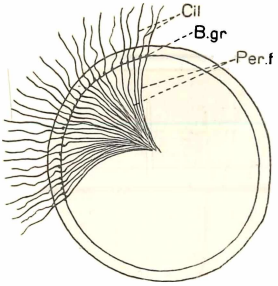

Fig. 4. Camera lucida drawing of transverse section of the anterior end of *B. depressum*. × 833. For lettering see Fig. 1. Cil, cilia; B. gr., basal granule.

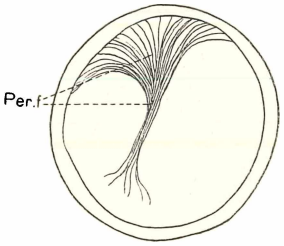

Fig. 4a. Camera lucida drawing of transverse section of the anterior end of *B. depressum*. × 833. For lettering see Fig. 1.

in the nucleus while the vacuoles that react negatively are devoid of this complex chemical.

BORREL'S stain gives the same picture as in FEULGEN reaction. The micronucleus takes a diffuse red stain and gives it a solid apparance, while the macronucleus exibits red staining granules with non-staining vacuoles in their interspaces. CALKINS (1930) in *Uroleptus Halseyi* has shown that these non-staining vacuoles take a green stain and may be called X-granules, but I have failed to observe such granules in this ciliate although CALKIN'S method was followed rigidly.

Cytoplasmic inclusions.

Golgi bodies and mitochondria.

In vital staining with neutral red a number of small globules were seen to take up the dye within a very short time. The smear was then exposed to osmic vapour by inverting the slide over a pot containing 2% osmic solution. The neutral red staining bodies were blackened after a time (see Text-Fig. 6). In staining with a mixture of neutral-red and Janus green a number of comparatively small granules, scattered throughout the cytoplasm, were seen to take up the green colour also. In a living specimen these granules showed Brownian movement while the neutral-red stained bodies were motionless. Mann-Kopsch method demonstrated osmicated globules

Fig. 5. Camera lucida drawing of the macro- and micronuclei of *B. depressum* as seen after Feulgen's reaction. × 1666.

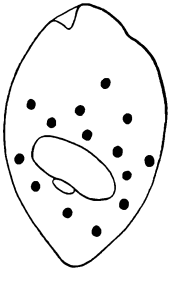

Fig. 6. Camera lucida drawing of *B. depressum*, showing neutral-red positive granules. × 1666.

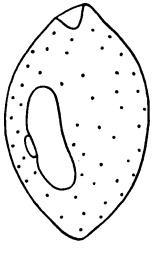

Fig. 7. Camera lucida drawing of *B. depressum*, showing mitochondria as stained by Janus Green. × 1666.

similar to those that were stained with neutral-red-the Golgi bodies (see Pl. 1 Fig. 6) In Dafano's method the silver impregnated granules corresponds in size and nature to those that were stained with Janus green and are therefore mitochondria (see Pl. 1 Fig. 7 and Text-Fig. 7).

Glycogen. — When stained intra-vitally with neutral-red and Janus green, either separately or in combination, some refractile large vacuoles did not take any stain, but when stained with Iodine or Best's carmine these vacuoles gave positive reactions. Besides these vacuoles the cytoplasm of this ciliate was seen to be packed up with iodine positive granules of smaller dimensions, which, however, became destained after a short time. These may therefore be supposed to contain paraglycogen while those that retained their stain even after a period of six months may be said to hold glycogen proper (Pl. 1 Fig. 2).

Summary.

1. Morphology of *Balantidium depressum* (GHOSH) from *Pila globosa* is described in detail. The following characters are noticeable.

a) Body oval with both the sides symmetrical and perfectly circular in transverse section.

b) The mouth is a cone-shaped depression slightly towards the right of the anterior end.

c) The macronucleus is bean-shaped and its position in the body is variable.

d) The micronucleus is oval and lies in the notch of the macronucleus.

e) The axial system of fibres consists of two or more fibres on the left side of the peristome with the borer at their termination and four or more fibres on the right side.

f) The peripheral system of fibres are in the form of radial arches along the right and left anterior borders of the ciliate. Beyond the peristome they form a sort of a "limiting membrane".

2. In FEULGEN-reaction the micronucleus takes a diffuse stain and appears solid while the macronucleus seems to be filled with red staining granules with non-staining vacuoles in their interspaces. BORREL'S stain also gives the same picture.

3. GOLGI bodies are several globules in the cytoplasm of the ciliate.

4. Mitochondria are numerous small granules scattered throughout the cytoplasm.

5. Presence of serveral iodine positive vacuoles and small granules has been demonstrated; these are glycogen and paraglycogen respectively.

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Explanation of plate.

Microphotographs of *Balantidium depressum* (Ghosh).

Plate 1.

Fig. 1. From a smear stained after Klein's silver lining method showing the arrangement of ciliary rows. × 870.

Fig. 2. From a smear fixed in Carnoy's fluid and stained in Iodine showing glycogen containing bodies. × 870.

Fig. 3. Longitudinal section. × 940. Note the borer and the axial system of fibres.

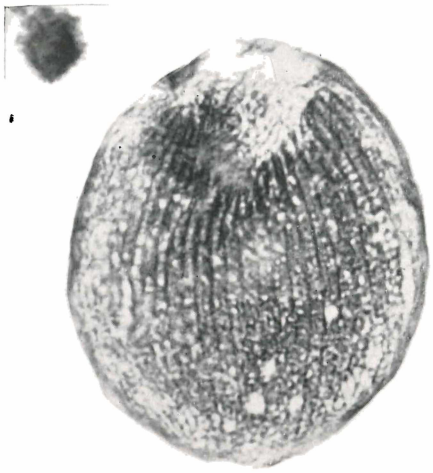
Fig. 4. From a smear. × 930. Note the 'V' shaped depression — the mouth, at the anterior end and the nucleus at the extreme posterior end.

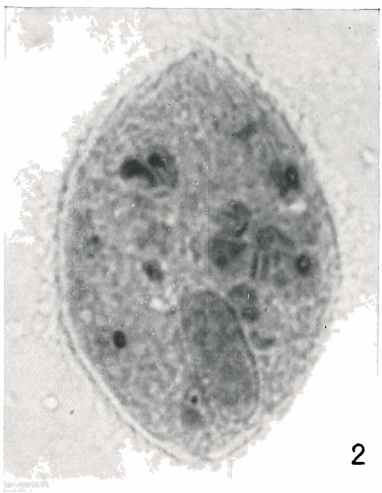
Fig. 5. Longitudinal section. × 940. Note the depression of the mouth and peripheral system of fibres.

Fig. 6. Transverse section. Stained after Mann-Kopsch method. × 940. Note the black globules which are Golgi bodies.

Fig. 7. Transverse section. Stained after Dafano's method. × 940. The small black stained dots are mitochondria.

Fig. 8. Transverse section. Top view of the mouth. × 940.





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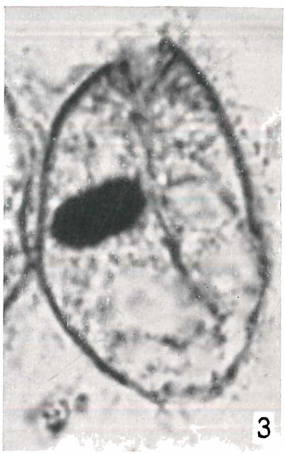

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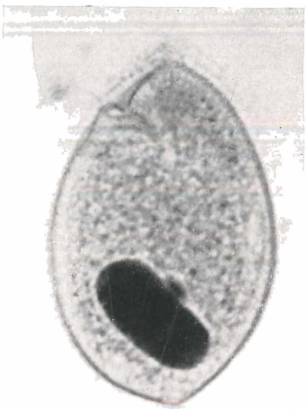

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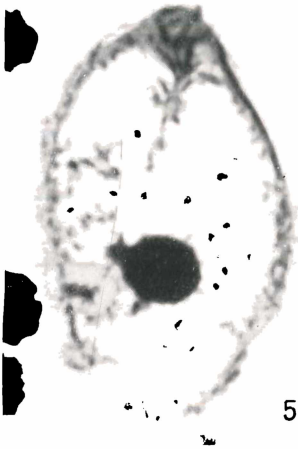

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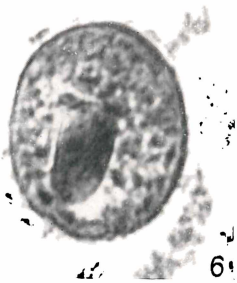

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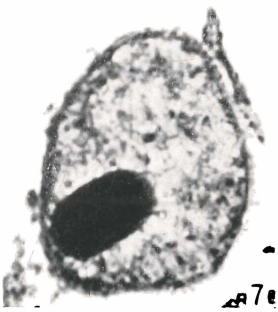

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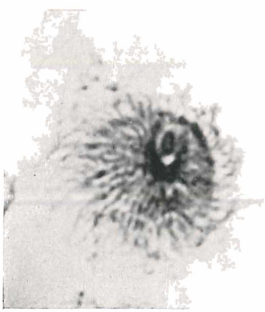

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Chakravarty.

VERLAG VON GUSTAV FISCHER IN JENA