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A NEW SPECIES OF PROTOZOAN CILIATE ENTODINIUM BINATASUM (SP. NOV) FROM THE RUMEN OF INDIAN CATTLE, (BOS INDICUS)

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ABSTRACT

Rumen fluid samples from adult Indian cattle (Bos indicus) were collected and observed to study the morphology of rumen ciliate Protozoa belonging to genus Entodinium. The present paper deals with the morphology of a new species Entodinium binatasum from the rumen of Indian cattle. The body measurements, frequency distribution, and variations in the characters are recorded. Critical comments are made on its specific identity. Entodinium binatasum is characterized by having small oval to spherical body with two small, thick, triangular caudal lobes and having curved rod shaped long and thick macronucleus.

KEYWORDS: Cattle, Cilites, Protozoa, Rumen, Entodinium

INTRODUCTION

Protozoa are unicellular animals. The great majority of ciliates are free living, but a number are parasitic. The protozoa placed in the phylum ciliophora possess cilia, cirri or other compound ciliary structures which serve as organelles of locomotion. Two kinds of nuclei are present in all without exception. Nutrition is holozoic or saprozoic. Asexual reproduction is by binary fission or budding and sexual reproduction is by conjugation or autogamy in which micronuclei play an important role. The ciliates of rumen belongs to the families Buetchliidae, Isotrichidae, Paraisotrichidae, Blepharocarythidae, Ophryoscolecidae, and Cyclopostidae. The ciliates vary in size from a few micron to 2 mm or more in length. The anterior and posterior extremities are permanently differentiated, the rumen ciliates are obligate anaerobes.

Ruminants have a very complex ecosystem harboring a variety of microorganisms which are capable of bringing out diverse types of fermentation. Rumen, the largest of the four compartments of stomach in ruminants, serves as a closed fermentation vat in which ingested feed is attacked by the microflora.

The rumen microflora consist of mainly of bacteria, protozoa and fungi, which have a significant role to play in rumen fermentation. Of the total microbial biomass existing in rumen 40 to 80 percent is of protozoa origin (Jouney -1991, Punia et al, 1992), Protozoa
living in the rumen are essentially ciliates, flagellates are often less numerous, not well
known and are often confused with the flagellate stage of fungi (Journey - 1988) Fermentation
of starch and soluble sugars is regulated by rumen protozoa (Mackie et al 1978) and they are
held in controlling acidosis in the rumen. Rumen protozoa are generally proteolytic
(Balaraman, 1996).

The ciliates are established in the rumen within three weeks after the birth of a calf
(Kurar, 1996) provided that the pH is above 6.0. Entodinium population is abundant in the
rumen. It increases when the diet is rich in starch. Protozoa contributes about 40 to 60 percent
of total hydrolytic enzyme activity in rumen. In ruminants, protozoa were first observed by
Gruby and DalaFond in 1843 (Hungate, 1978); Since then a number of protozoal species have
been reported in rumen. Subsequently the taxonomic studies on the rumen protozoa was done
by various workers in different parts of the world; only a few studies have been carried in
domesticated Indian ruminants. Kofoid and MacLennan (1930,1932,1933) in Bos indicus,
Das Gupta (1935) in Indian Goat, Ajit Banerjee (1955) in Indian Buffalo; Kulkarni and
Kshirsagar (2004, 2005, 2006, 2008) in Bos indicus. There is much scope to do work on the
taxonomy of rumen ciliates. The taxonomical work on rumen ciliates of Cattle in India is
very scanty. The present research work deals with study of taxonomy of rumen protozoa from
Indian cattle.

MATERIAL AND METHODS

Rumen fluid samples were collected for the present study from Indian adult cattle
(Bos indicus) slaughtered at abattoirs in Hingoli district of Maharashtra state in India. On the
removal of stomach, rumen was slightly punctured and 10ml. rumen fluid was collected in a
vial. It was centrifuged and preserved adding 1:1 glycerine: alcohol solution. A drop of this
material was taken on a glass slide for observing ciliates in living condition under research
microscope. The permanent slides of the samples were made in duplicate, stained by
tungstophosphoric haemotoxylein stain. The staining procedure of Krier and Becker, 1987
was followed. The stained slides of ciliates were observed under research microscope for
their identification and morphology.

The general features used to classify the rumen protozoa into genus Entodinium are
as follows : (Dehority - 1993)

1. The Presence of single adoral zone.
2. Lack of skeletal plates.
3. Position of the macronucleus which lies between micronucleus and closest body side. Body measurements such as length, width, L/W ratio, length of the nucleus etc. were recorded with an ocular micrometer. Frequency distribution, body shape, location of contractile vacuole, rectum, mouth are also recorded.

**Taxonomical position of Entodinium Stein, 1858.**

| Subkingdom | - | Protozoa |
| Phylum | - | Ciliphora |
| Class | - | Kinetofragminophorea |
| Subclass | - | Vestibuliferia |
| Order | - | Entodinimorphida |
| Family | - | Ophryoscolecidae |
| Subfamily | - | Entodniinae |
| Genus | - | Entodinium |

**RESULTS AND DISCUSSION**

**Entodinium binetasum (Sp. Nov) (Fig. 1a, 1b)**

During the present study Entodinium binetasum is recorded as a new protozoan ciliate species belonging to genus Entodinium, from the rumen of Indian cattle (Bos indicus). It’s morphology is described and body measurements are recorded (Table-1). The variations in the body characters are observed and critical comments are made on it’s specific identity. The observations are based on a study of 50 specimens taken at random from different rumen fluid samples.

**MORPHOLOGY OF Entodinium binetasum**

Body of this species is small, oval to spherical in shape. Average body length 25.72\(\mu\)m. Mouth is broad (10.19\(\mu\)m) located in the midline with prominent adoral lips. The L/w ratio is 1.16. Both the body surfaces are equally convex. The greatest diameter of body is in the midline (22.08\(\mu\)m). The dorsal and ventral body surfaces are terminated posteriorly into small (2.25 \(\mu\)m) triangular lobes; which are quite separated from each other. Boundary layer is weakly developed with thin cytoplasm over the body; posteriorly near the lobes on both sides the ectoplasm is thickened. The rectum is lying posteriorly between the two lobes in the midline of the body.
Macronucleus is rod shaped, curved, long (14.48 µm) with thick body and is closely applied to the dorsal body surface. It is 71.85 per cent of the body length. Its anterior tip is near the base of adoral lip, which is broad as compared to the posterior narrow end. The micronucleus is oval shaped body placed in the anterior quarter of the left edge on macronucleus. Contractile vacuole is located to the anterior left of macronucleus.

**COMMENTS**

This species has two caudal lobes and thus can be compared with E.dilobum (Dogiel,1927) E.furca (Cunha,1914), E. biconvexum (Kulkarni,2005), E.flagi (Kulkarni, 2005). This species differs from E. dilobum in having oval to spherical body shape, in the absence of flanges and cuticular flods; in having short caudal lobes. Macronucleus is comparatively large, elongated curved rod shaped running nearly upto \(\frac{3}{4}\) th of body length, micronucleus placed in the anterior quarter of body. As against ellipsoidal body; right and left flanges with cuticular folds, two triangular well defined lobes; club shaped macronucleus about \(\frac{1}{2}\) of the body length and micronucleus placed in the middle position. The L/W ratio of this species is 1.16 as against 1.41 in E.dilobum. It also differs from E.furca in having small size and two short ill defined caudal lobes. As against large size and with two long prong shaped caudal spines. The dorsal and ventral body surfaces of this species are equally convex as against dorsal convex and ventral surface almost flat in E.furca.

When compared to E.biconvexum, the circular shape in this species is well marked as against oval body. The two caudal lobes are small sized as against smoothly rounded triangular unequal two caudal lobes having cuticular folds. Macronucleus is thick, elongated and rod shaped as against elongated, wedge shaped macronucleus of E.biconvexum.

It differs from E.flagi in having small size, circular body; as against long and elongated ellipsoidal body. In having both the body surfaces equally convex, as against less convexity of the ventral surface; in having two short caudal lobes as against two triangular pointed, large, unequal caudal lobes. These is no cuticular fold in this species as against a single broad cuticular fold running along the main body axis in E.flagi. The L/W ratio of this species is 1.16 as against 1.41 in. E.flagi.

The comparative body dimensions of the related species discussed here with those of E.binatasum (Sp. Nov.) are given in Table-2. frequency distribution of various size ranges in E.binatasum (Sp. Nov.) are shown in table-3. In view of these differences the species described herein is considered different from all the earlier species and is new to the science.
TABLE – 1
The Body Dimensions and other measurements of Entodinium binatasum
(Sp. Nov) are given below. (All the measurements in microns)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Body</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td>21.40</td>
<td>32.10</td>
<td>25.72</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>17.12</td>
<td>25.68</td>
<td>22.08</td>
</tr>
<tr>
<td></td>
<td>Length width ratio</td>
<td>1.15</td>
<td>1.25</td>
<td>1.16</td>
</tr>
<tr>
<td>2.</td>
<td>Macronucleus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length</td>
<td>12.84</td>
<td>21.40</td>
<td>18.48</td>
</tr>
<tr>
<td></td>
<td>Percent length of body</td>
<td>60.00</td>
<td>66.47</td>
<td>71.85</td>
</tr>
<tr>
<td></td>
<td>Dia. Ant. end.</td>
<td>3.00</td>
<td>4.28</td>
<td>3.89</td>
</tr>
<tr>
<td></td>
<td>Dia. post. end.</td>
<td>2.14</td>
<td>3.00</td>
<td>2.31</td>
</tr>
<tr>
<td>3.</td>
<td>Mouth</td>
<td>8.56</td>
<td>12.84</td>
<td>10.19</td>
</tr>
<tr>
<td>4.</td>
<td>Lobe</td>
<td>1.28</td>
<td>3.00</td>
<td>2.25</td>
</tr>
</tbody>
</table>

TABLE – 2
Comparative Body Measurements of closely reacted species of Entodinium with that of
Entodinium binatasum given by earlier workers and the present dimensions
(In microns)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Length</td>
<td></td>
<td>34-55 (44.19)</td>
<td>27</td>
<td>25.68-55.64 (36.51)</td>
<td>21.40-32.10 (25.72)</td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td></td>
<td>25-38 (30.94)</td>
<td>27</td>
<td>21.40-32.10 (25.55)</td>
<td>17.12-25.68 (22.08)</td>
<td></td>
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<tr>
<td>L/W ratio</td>
<td></td>
<td>1.1-1.7 1.42</td>
<td>--</td>
<td>1.20-1.73 (1.43)</td>
<td>1.15-1.25 (1.16)</td>
<td></td>
</tr>
<tr>
<td>Ventral Lobe</td>
<td></td>
<td>4-7 (4.81)</td>
<td>--</td>
<td>2.14-8.56 (4.83)</td>
<td>1.28-3.00 (2.25)</td>
<td></td>
</tr>
<tr>
<td>Dorsal lobe</td>
<td></td>
<td>6-9 (5.80)</td>
<td>--</td>
<td>1.28-4.28 (2.50)</td>
<td>1.28-3.00 (2.25)</td>
<td></td>
</tr>
</tbody>
</table>
TABLE – Frequency Distribution of Various size ranges in Entodinium binatasum (Sp.Nov.) (All the measurements are in microns)

<table>
<thead>
<tr>
<th>Width</th>
<th>21.40</th>
<th>23.54</th>
<th>25.68</th>
<th>27.82</th>
<th>29.96</th>
<th>32.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.12</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.26</td>
<td></td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.40</td>
<td>9</td>
<td></td>
<td>8</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>23.54</td>
<td></td>
<td></td>
<td>9</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.68</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

(Fig. 1a)

PHOTOGRAPH OF Entodinium binatasum (sp.nov)

(Fig. 1b: Line Drawing of Entodinium binatasum)
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