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A NEW SPECIES OF PROTOZOAN CILIATE ENTODINIUM CONICOSPINUM (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 observed to study the morphology of rumen ciliate protozoa belonging to genus *Entodinium*. The samples were collected from the Hingoli District Marathwada region (Maharashtra, India). The present paper deals with the morphology of and new species *Entodinium conicospinum* identified from the rumen of Indian cattle. The body measurements, Frequency distribution and variations of the characters are recorded. Critical comments are made on its specific identity. *Entodinium conicospinum* (sp.nov) is characterized by having small conical, elongated peg shaped body. The ventral surface is terminated posteriorly into a sharp, pointed caudal spine. The wedge shaped macronucleus is located high up near the base of the adoral lip.

Keyword: Cattle, Ciliates, Protozoa, Entodinium Rumen.

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 protozoal 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 (Jonney - 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. 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 haematoxylin 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, diameter, 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	:-	Vestibulifera
order	:-	Entodiniomorpha
Family	:-	Ophryoscolecidae
Subfamily	:-	Entodiniinae
Genus	:-	<i>Entodinium</i>

The following parameters were considered for observation.

- 1) Shape of the body
- 2) Length of the body
- 3) Width of the body
- 4) L/W ratio
- 5) Shape of nucleus
- 6) Length of nucleus
- 7) Position of micronucleus
- 8) Position of contractile vacuole
- 9) Location rectum and anus

RESULTS AND DISCUSSION

Entodinium conicosipinum (sp.nov)
(fig. 1a, 1b)

During the present study *Entodinium conicosipinum* is recorded as a new protozoan ciliate species belonging to genus *Entodinium* from the rumen of India cattle. Its morphology is described, the body dimensions and other measurements are recorded (Table-1). The variations in the body characters are recorded and critical comments are made on its specific identity. The observations are based on a study of 50 specimens taken at random from different rumen fluid samples.

MORPHOLOGY :

The body is small, elongated and cone shaped. The average body length is 30.73µm. The anterior end is conical with small mouth (4.20µm) located anteriorly in the mid-line of body, L/w ratio is 2.25.

Both the body surfaces are convex but dorsal surface is much more convex as compared to the ventral surface. The ventral side of the body is terminated posteriorly into a sharp spine (5.52µm) pointing posteriorly. The greatest diameter of the body is in the anterior half (13.65µm). In general body appears like peg or cone shaped; Conical anteriorly and narrow posteriorly.

The endoplasmic sack is surrounded by a closely applied boundary layer. The ectoplasm is thickened posteriorly near the spine. Rectum is a broad transverse slit located near the ventral spine.

Macronucleus is wedge shaped structure 17.03µm in length. It is 55.42 per cent to the body length and is closely applied with the dorsal body surface. It extends anteriorly very close from the base of adoral lip and posteriorly it reaches to the middle of the body. The anterior tip of macronucleus is broad and smoothly rounded, while the posterior end is narrow and pointed. The micronucleus is oval body located on the ventral edge of the anterior third of macronucleus. Contractile vacuole is located anteriorly to the left of macronucleus.

COMMENTS :-

This species is similar to *Entodinium pasurum* forma *monospinosum* (Imai, 1983) *Entodinium laterospinum*, (Kofoid and MacLennan 1930) *Entodinium brevispinum* (Kofoid and MacLennan 1930) *Entodinium rostratum* (Firoentini, 1889) in having single ventral spine.

However it differs from these species in having different characters.

This species differs from *E. parvum monospinosum* by following characters. The general shape of body of this species is elongated and cone shaped as against elongated and ellipsoidal shape of *E. parvum monospinosum*.

The macronucleus of this species is wedge shaped of medium length, extending anteriorly close from the base of adoral lip as against long, rod shaped macronucleus lying in the middle of the body of *E. parvum monospinosum*. The body of this species is elongated and narrow posteriorly as against the symmetrical and rounded posterior end of *E. parvum monospinosum*.

This species is also comparable in size, spine structure and shape of macronucleus with *E. brevispinum* and *E. laterospinum*, however it differs from *E. brevispinum* in its body shape; This species has the perfectly cone shaped body as against slender body with humpback appearance of *E. brevispinum* and wedge like body with large anterior end of *E. laterospinum*. The species also differs from these two species in the shape and length of ventral spine. This species has some what elongated sharp spine pointing in the posterior direction as against short ventral spine in *E. brevispinum* and long curved spine deflecting to right at an angle of 300 in *E. laterospinum*. The average L/W ratio of this species is 2.25 as against 1.71 in *E. laterospinum* and 1.70 in *E. brevispinum*.

This species also differs from *E. rostratum* in body shape and position macronucleus; curvature of spine and position of contractile vacuole.

The body of this species is cone shaped; macronucleus is wedge shaped located in the anterior half;

ventral spine is elongated, sharp pointing posteriorly; contractile vacuole located anteriorly to the left of macronucleus. As against, long and slim body with concave ventral surface; macronucleus is band like structure located in the middle half of the body, ventral spine is heavy blunt which points ventrally at its base but curves back so that tip points dorsally; and the contractile vacuole is directly anterior to the macronucleus in *E. rostratum*.

The comparison of the body dimensions of this species with the closely related species of *Entodinium* is indicated in (Table 2.0) In view of the distinct characters this species is considered new to the science.

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TABLE – 1
The Body Dimensions and other measurements of
Entodinium conicospinum (sp.nov) are given below.
(All the measurements in microns)

Sr. No.	Parameters	Minimum	Maximum	Average
1.	Body			
	Length	25.68	38.52	30.73
	Width	12.84	17.12	13.65
	Length width ratio	2.00	2.25	2.25
2.	Macronucleus			
	Length	12.84	23.54	17.03
	Percent length of body	50.00	61.11	55.42
	Dia. Ant. end.	2.14	3.00	2.98
	Dia. post. end.	2.14	2.14	2.14
3.	Mouth	4.28	4.28	4.28
4.	Spine	2.14	8.56	5.52

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