

Morphometric Studies of Some Diplodiniid (Diplodiniinae, Entodiniomorphida) Ciliates from the Rumen of Cattle (*Bos indicus*) in India

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Abstract

An investigation has been made to study the protozoan ciliates of subfamily Diplodiniinae (Order: Entodiniomorphida) from the rumen of cattle (*Bos indicus*). The present paper deals with the occurrence of some Diplodiniid ciliates from the rumen of cattle (*Bos indicus*) in India. The size and morphology of the species is compared to those previously reported in different geographical areas. In the present investigation *D. anacanthum* (Dogiel, 1927) and *D. triacanthum* (Dogiel, 1927) recorded first time in India from the rumen of cattle (*B. indicus*). *D. tetracanthum* (Dogiel, 1927) is reported from the rumen of cattle as a new host record in India.

Keywords: Rumen; Cattle; Ciliates; *Diplodinium*.

Introduction

The Rumen is anaerobic and the largest compartment of the stomach which occupies 80 per cent of the abdomen in the ruminant animals. The rumen does not secrete any enzyme but constantly receives the saliva. The pH of the rumen is in between 5-7.5 and temperature ranges from 38-41°C. In this way the rumen favors for microbial fermentation. Rumen micro fauna includes viruses, bacteria, fungi and protozoa. Of them, protozoa have large bodies with characteristic shape and about 10^5 , 10^6 per ml of rumen fluid. The impact of protozoa on the rumen digestion depends on their concentration and the generic composition of their population (J.P. Jouany and K. Ushida 1999).

Gruby and Delafond (1843) first reported the protozoa from ruminants since then a number of protozoan species have been reported from different parts of the world Dogiel (1927) Becker & Talbott (1927), Hsiung T.S. (1932) Clarke R.T.J. (1964), Ogimoto & Imai (1981) and Dehority (1993,2005) Gocman B. (1999a, 1999b, 2000) Gocman *et al.* (2005)

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Martenele I. *et al.* (2008), Gocman and Gurelli (2009), Dirk B. *et al.* (2010), Dirk B. and Dehority B.A. (2011) and Gurelli (2014), but very few studies have been made in India. Kofoid and MacLennan (1930, 1932, 1933), Dasgupta M. (1935), Banerjee A.K. (1955), Mathur C.S. (1963), Misra S.K.(1972), Mukherjee & Sinha (1989,1990) Sanghai P.K. and Kshirsagar H.S. (2015), Cedrola *et al.* (2016, 2017) studied rumen ciliates from different hosts. Kulkarni & Kshirsagar (2001) studied the genus *Entodinium* and reported 13 new species.

Although many studies have been reported on the rumen ciliates of different hosts from all over the world no investigations have been made on Diplodiniid ciliates of cattle in India. The objective of this study was to identify and compare the information with earlier reports from different parts of the world.

Materials and Methods

During the present study rumen fluid samples were collected from 814 adult Indian cattle slaughtered at abattoirs of Kannad, Dist. Aurangabad of Maharashtra State (India). After the removal of the stomach the rumen was slit open and 10-15 ml of rumen fluid was collected in a glass vial then the immediately the glass vial was closed airtight and brought to the laboratory. It was centrifuged and preserved by adding 1:1 glycerine alcohol solution. To determine the intensity of the ciliates live specimen were examined under the microscope by taking drop of fluid on a clean glass slide.

The permanent slides of the sample were made in duplicate stained by wet Tungstophosphoric Haematoxylin stain. Identification of genera and species of rumen ciliates were based on description published by earlier workers (Dehority 1993). All the measures of the ciliates were based on a study of 50 specimens (n=50) with an ocular micrometer, line drawings were made with a camera lucida at magnification 10x X 40x.

Result and Discussion

Diplodinium anacanthum, Dogiel 1927

Description of the species: (Photomicrograph 1)

The body of this species is rounded and medium in size. The adoral ciliary zone is inclined ventrally encloses the mouth. The left ciliary zone is comparatively shorter than adoral zone. The operculum is distinct, broad and extended a short distance anterior to the oral area. The surfaces of the body are convex. The dorsal surface is slightly convex than the ventral surface. The posterior half of the body is tapering, not truncated as in *D. dentatum*. The most striking feature of this species is the absence of caudal spine or lobe posteriorly it is smooth rounded. A distinct cuticular ridge is arising from the anus extending anteriorly in mid-dorsal line.

The endoplasmic sack enclosed by the boundary line along with the body surfaces. It occupies

almost all portion of the body. The boundary line is thin distinctly separates the ectoplasm.

The macronucleus is long, heavy, rod shaped body. It lies under the right dorsal surface of the body. Anterior third of the macronucleus slopes ventrally at an angle of 30-40°. The anterior end is smooth rounded while; the posterior end is blunt and narrow. The micronucleus is an ellipsoidal body lies in a small depression produced in the dorsal surface of anterior third bent region of the macronucleus. The two contractile vacuoles found in the ectoplasm left the macronucleus. The anterior contractile vacuole found just at the level of micronucleus, while the posterior contractile vacuole in the posterior third region of the body.

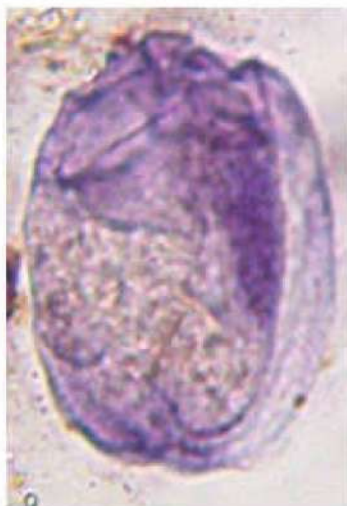
The body dimensions and other measurements of *Diplodinium anacanthum* are given in table 1.

Comments

Dogiel (1927) first described this species as *Anoploplodinium denticulatum* f. *anacanthum*. Kofoed & MacLennan (1932) named this species as *Diplodinium anacanthum*. The comparison of the body dimensions recorded during the present study and those given by Dogiel (1927) are shown in table 1.

The table indicates that the length described here is smaller than the length given by Dogiel (1927). The width recorded during the present study is larger than the width reported by Dogiel (1927). The L/W ratio of the present species is 1.31, which is less against the L/W ratio 1.6 recorded by Dogiel (1927).

In the present studies *D. anacanthum* is recorded for the first time from cattle in India.



Photomicrograph 1: *Diplodinium anacanthum*

Table 1: The body dimensions and other measurements of *D. anacanthum*, *D. triacanthum* and *D. tetracanthum*. All measurements are in microns.

Sr. No.	Parameters	<i>D. anacanthum</i> (n=50)	<i>D. triacanthum</i> (n=50)	<i>D. tetracanthum</i> (n=50)
1	Body			
	Length	57.6-86.4 (71.02)	51.2-83.2 (62.52)	54.4-105.6 (75.71)
	Width	48-67.2 (54.30)	41.6-67.2 (52.80)	44.8-76.8 (62.97)
	L/W Ratio	1.15-1.56 (1.31)	1.04-1.38 (1.23)	1.04-1.38 (1.22)
2	Macronucleus			
	Length	22.4-51.2 (36.03)	12.8-41.6 (28.18)	19.2-54.4 (35.84)
	% Length to the Body	31.82-63.64 (50.63)	25-59.09 (45.04)	30.0-61.11 (47.19)
	Diam. Ant. End.	6.4-16.4 (10.97)	6.4-12.8 (10.68)	6.4-16.0 (12.48)
	Diam. Post. End.	4.8-9.6 (6.66)	3.2-12.8 (6.75)	3.2-12.8 (9.47)
3	Micronucleus	1.6-6.4 (4.88)	1.6-6.4 (3.26)	1.4-6.4 (3.64)
4	Adoral ciliary zone (Mouth)	8-16 (11.30)	6.4-17.6 (11.04)	9.6-25.6 (16.12)
5	Left ciliary zone	6.4-9.6 (8.16)	3.2-12.8 (7.29)	6.4-12.8 (10.75)
6	Lobe/ Spine	--	3.2-12.8	3.2-12.8
	Ventral spine		(6.65)	(7.24)
	Middle spine (I lateral)		1.6-6.4 (3.36)	1.6-12.8 (4.57)
	II lateral		--	1.6-12.8 (4.41)
	Dorsal spine		3.2-9.6 (6.20)	3.2-6.4 (5.47)
7	Rectum	4.8-9.6 (7.40)	1.6-6.4 (4.03)	4.8-8.0 (6.36)

Table 2: Comparative body dimensions of *Diplodinium anacanthum*

Parameters	Authors	
	Dogiel (1927)	Present Study
Length	70-90 (80)	57.6-86.4 (71.02)
Width	40-60 (51)	48-67.2 (54.30)
L/W ratio	1.6	1.15-1.56 (1.31)

***Diplodinium triacanthum*, Dogiel 1927**

Description of the species: (Photomicrograph 2)

The body of this species is relatively short, heavy and rounded slightly tapering at posterior end. The adoral ciliary zone encloses mouth. The lips are weakly developed. The left ciliary zone is shorter

than the adoral ciliary zone. Both the ciliary zones are separated by the distinct, broad operculum. The operculum projects only a short distance anterior top of the body. The surfaces of the body are convex gives maximum body width in the middle of the body. This species is identified by the presence of three caudal spines one ventral, one dorsal and one on the right side. The ventral spine is long and slightly incurved. The dorsal spine is nearly of equal size to the ventral spine while the third spine is very short. A distinct cuticular ridge arises from the anus extending anterior in mid dorsal line.

The endoplasmic sack starts anteriorly at the level of the anterior end of the macronucleus. It becomes tapering at posterior half of the body. The ectoplasm is thin. It is differentiated by a thin boundary layer.

The macronucleus varies from short, heavy to long rod shaped body. It lies under the right dorsal surface. The anterior third of macronucleus is bent ventrally at an angle of 30-45°. The anterior end smooth rounded while posterior end is narrow with blunt end. An elliptical micronucleus lies in a notch of the dorsal surface of the anterior third bent region of the macronucleus. Two contractile vacuoles located at the left side of the macronucleus. The anterior contractile vacuole found situated at the level of micronucleus while the posterior vacuole placed at the level of the posterior end of the macronucleus.

The body dimensions and other measurements of *Diplodinium triacanthum* are given in table 1.



Photomicrograph 2: *Diplodinium triacanthum*

Comments

Dogiel (1927) firstly described it from the rumen of cattle from U.S.S.R. as *Anoplocladus denticulatus* f. *triacanthum*. Since then the species is described by many workers like Ogimoto K. & Imai S. (1981), Gureli G. (2016a) from water buffalo, and (2016b) from cattle in Turkey Cedrola *et al.* (2016) from sheep but the body dimensions are not given. The comparison of the dimensions of the species described here and those by earlier workers are shown in table 3.

The table reveals that the species described here is smaller than the species described by Dogiel (1927) and Clarke (1964) however; the maximum length of the species described here is close to the maximum length described by Dogiel (1927) and Clarke (1964). The width of the species recorded here is slightly smaller than the width described by Dogiel (1927) but it is larger than the width given by Clarke (1964). The L/W ratio described here is smaller than the L/W ratio given by Clarke (1964). The length of the macronucleus recorded here is shorter as compared to the length of the macronucleus given by Clarke (1964).

In the present studies, this species is described for the first time from the rumen of cattle in India.

Table 3: Comparative body dimensions of *Diplodinium triacanthum*.

Parameters	Authors		
	Dogiel (1927)	Clarke (1964)	Present Study
Length	70-85 (77)	65-81 (74)	51.2-83.2 (62.52)
Width	51-64 (55)	46-54 (49.5)	41.6-67.2 (52.80)
L/W ratio	--	1.33-1.65 (1.49)	1.04-1.38 (1.23)
Ma.nu.L.	--	35-40 (38.1)	12.8-41.6 (28.18)

Diplodinium tetracanthum, Dogiel 1927

Description of the species: (Photomicrograph 3)

The body of this species is medium, spheroidal in shape and pointed at the posterior extremities of the body. The adoral ciliary zone comprises the mouth which is larger as compared to the left ciliary zone. The inner lips are continuous with the operculum which is well developed broad and runs forward short distance to the oral zone. The dorsal surface is slightly convex than the ventral surface. The maximum width of the body found in the middle of the body. The identifying character of this species is the presence of four caudal spines

situated in posterior tapering part of the body. The ventral spine is the longest than all the spines, one dorsal and two lateral spines situated on the right side. A prominent cuticular ridge originating from the anus extending forward in mid dorsal line.

The endoplasmic sack originates near the anterior end of the macronucleus and occupies the posterior part of the body. The ectoplasm is slightly thick along the dorsal surface than the ventral surface of the body.

The macronucleus is heavy, long rod shaped body situated under the dorsal surface of the body. The anterior region slopes ventrally at an angle of 30°-45°. The anterior end of the macronucleus is slightly broader than the posterior narrow tapering end. The micronucleus is a small ellipsoidal body, which situated in the slight depression of macronucleus formed at anterior bent surface. The two ovoid contractile vacuoles located in ectoplasm at the dorsal side of macronucleus. Anterior contractile vacuole situated followed to the left ciliary zone and second one at the level of posterior end of macronucleus.

The body dimensions and other measurements of *Diplodinium tetracanthum* are given in table 1.



Photomicrograph 3: *Diplodinium tetracanthum*

Comments

Dogiel (1927) first reported *Diplodinium tetracanthum* from the rumen of cattle from U.S.S.R. as *Anoplocladus denticulatus* f. *tetracanthum*. Mukherjee & Sinha (1990) reported this species from the rumen of Goat in India but they have not

reported the body dimensions. A comparison of the body dimensions of the species described here and those given by earlier workers are shown in table 4.

The table reveals that the length of the species recorded during the present study is similar to the length given by Dogiel (1927) but it is slightly smaller than the length given by Clarke (1964). The width of the species described here is more than the width given by Dogiel (1927) and Clarke (1964) however the length of the macronucleus is less as compared to the length of the macronucleus given by Clarke (1964). The L/W ratio is also less than the L/W ratio given by Clarke (1964).

In the present studies, this species is described for the first time from the rumen of cattle as new host in India.

Table 4: Comparative body dimensions of *Diplodinium tetracanthum*

Parameters	Authors		
	Dogiel (1927)	Clarke (1964)	Present Study
Length	72-83 (76)	62-89 (78.8)	54.4-105.6 (75.71)
Width	52-61 (54)	40-59 (52.4)	44.8-76.8 (62.97)
L/W ratio	--	1.41-1.65 (1.51)	1.04-1.38 (1.22)
Ma.Nu. L.	--	27-51 (41.6)	19.2-54.4 (35.84)

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