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Ciliates from *Bos indicus* the Genus *Metadinium* (Awerinzew & Mutafowa, 1914)

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Abstract: During the course of investigation of rumen fluid contents of 814 adult Indian cattle (*Bosindicus*) protozoan ciliates were studied from subfamily Diplodiniinae (Order: Entodiniomorphida). The present paper deals with the study of ciliates from the genus *Metadinium*. In all five species (*M. affine*,*M. minorum*Dehority, 1975, *M. medium*, *M. magnum*Dogiel, 1925 and *M. ypsilon*Dogiel, 1925) were described, of which *M. minorum* and *M. magnum* reported as new host record in cattle and reported first time in India. *M. affine* and *M. ypsilon* recorded first time in India. Morphology of all the species with variations is described and body dimensions are recorded from the specimen taken at random from different slides and compared with earlier reports.

Key words: Rumen, Protozoa, Ciliates, *Metadinium*

INTRODUCTION

The rumen micro flora comprises mainly bacteria, fungi and protozoa. The majority are ciliate protozoa with very few number of flagellates have an important role in contributing nutrients to the host animal (Imai[5]) also contribute in the digestion of carbohydrate and protein containing feedstuffs by secreting sacharolytic and proteolytic enzymes [1].

Gruby & Dalafond[2] in 1843 were first observed the protozoa in ruminants thereafter number of protozoa species have been reported by various workers from the different parts of the world. Dogiel[2], Becker &Talbott[3], Hsiung T.S.[4], Ogimoto & Imai[5] and Dehority [6,7]. A few studies of rumen ciliates from Indian

domesticated ruminants Kofoid & Maclennan[8-10] in *Bos indicus*, M. Das Gupta[11] in Indian Goat, A.K. Banerjee[12] in Indian buffalo, Misra *et al.*[13] Mukharjee & Sinha[14], Kulkarni[15] in Indian cattle have been reported. The present study reports the ciliate protozoa from the genus *Metadinium* in Indian cattle.

MATERIALS & METHOD

Samples of rumen fluid were collected from adult Indian cattle (*B.indicus*) 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 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 specimens 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 Hematoxylin stain. Identification of genera and species of rumen ciliates were based on description published by Dehority[7]. All the measures of the ciliates were based on a study of specimens taken at random from different slides with an calibrated ocular micrometer, line drawings were made with a camera lucida of magnification 10x X 40x.

RESULTS & DISCUSSION

Metadinium affine, Dogiel & Fedorowa 1925

Description of the species: The body of this species is rounded, slightly ovoid in shape. The adoral ciliary zone is of medium size it encloses mouth inclined ventrally at an angle of 30-40º. The left ciliary zone is slightly shorter that the adoral ciliary zone. The operculum distinct and shortly extends anterior to the oral zone. Both the body surfaces are convex. The dorsal surface is more convex than ventral surface. The ventral surface extends straight and posteriorly it forms a distinct blunt caudal lobe.**(Fig.1)**

Fig.1: Photomicrograph and line drawing of *M. affine*

The macronucleus is an elongate, rod shaped body lies under the dorsal surface of the body. The anterior end of the macronucleus is broader smooth rounded than the posterior narrow slightly pointed end. The micronucleus is an ellipsoidal body located at the middle of the dorsal side of macronucleus.

The two skeletal plates lie under the right surface. The dorsal plate extends from the edge of the dorsal half of oral area extends posteriorly across the midline of the body. It is composed of anteriorly 5-6 longitudinal rows of prisms. The ventral plate extends from the ventral half of the oral zone posteriorly to

the midline of the body. Both the plates posteriorly fuse together. The body dimensions and other measurements of *Metadinium affine* are given in **Table 1.**

Table- 1: The body dimensions and other measurements of *M. affine*, *M. minorum*, *M.medium*, and *M. ypsilon.* All measurements are in microns.

Sr. No.	Parameters	*M. affine* (n=50)	*M. minorum*(n=45)	*M. medium* (n=50)	*M. magnum* (n=9)	*M. ypsilon* (n=50)
1	Body					
	Length	60.8-108.8 (86.08)	70.4-118.4 (96.14)	172.8-323.2 (235)	192-272 (224.72)	92.8-147.2 (116.22)
	Width	48-86.4 (67.97)	64-96 (76.45)	121.8-233.6 (188.86)	156.8-224 (185.25)	60.8-96 (79.60)
	L/W Ratio	1.13-1.60 (1.24)	1.10-1.46 (1.26)	1.05-1.56 (1.25)	1.07-1.29 (1.22)	1.27-1.88 (1.47)
2	Macronucleus					
	Length	32-60.8 (44.32)	35.2-70.4 (53.76)	102.4-195.2 (148.99)	121.6-179.2 (179.2)	48-89.6 (66.97)
	%Length to the Body	38.46-62.50 (51.40)	38.71-62.07 (55.86)	55.41-75.81 (63.54)	58.21-78.94 (65.70)	50-70 (57.52)
	Diam. Ant. End.	6.4-14.4 (9.73)	8-17.6 (11.31)	16-41.6 (26.40)	17.6-32 (25.07)	6.4-16 (9.09)
	Diam. Post. End	3.2-11.2 (5.92)	6-12.8 (11.32)	9.6-35.2 (23.07)	12.8-30.4 (20.09)	4.8-14.4 (7.68)
3	Micronucleus	3.2-6.4 (4.48)	4.8-9.6 (6.76)	6.4-14.4 (9.51)	6.4-14.4 (8.18)	4.8-9.6 (6.56)
4	Adoralciliary zone (Mouth)	11.2-19.2 (15.39)	11.2-16 (13.58)	28.8-51.2 (37.35)	27.2-44.8 (35.38)	14.4-20.8 (16.64)
5	Left ciliary zone	8-14.4 (12.19)	8-12.8 (11.32)	16-35.2 (29.21)	22.4-32 (28.09)	9.6-17.6 (13.89)
6	Lobe/ Spine					
	Ventral lobe	1.6-6.4 (3.71)	--	--	--	--
7	Rectum	6.4-12.8 (10.15)	8-14.4 (11.60)	19.2-48 (26.02)	19.2-30.4 (23.82)	11.2-17.6 (14.93)
8	Skeletal Plate					
	Dorsal					
	Length	41.6-73.6 (56.56)	51.2-70.4 (64.64)	96-208 (134.65)	112-121.6 (116.98)	54.4-102.4- (72.63)
	Width	6.4-11.2 (9.31)	6.4-11.2 (8.46)	6.4-25.6 (16.13)	12.8-17.6 (15.82)	8-14.4 (11.39)
	Ventral					
	Length	41.6-73.6 (56.90)	51.2-70.4 63.18	80-192 (129.47)	112-121.6 (117.33)	54.4-102.4 (72.83)
	Width	4.8-12.8 (9.86)	6.4-11.2 (8.18)	9.6-19.2 (15.11)	12.8-22.4 (16.88)	8-14.4 (11.62)

Comments: Dogiel[16] reported firstly this species as *Diplodinium affine* and redescribed as *Eudiplodinium affine* in 1927 from cattle, sheep & goats. Kofoid & Maclennan[9] placed this species in a new genus

Diploplastron and named as *Diploplastron affine*. Dehority[6] described this species as *Metadinium affinie* from Brazilian cattle and redrawn in 1993. A comparison of the dimensions of *Metadinium affinie* described here and those given by earlier workers are shown in **Table 2**.

The table clearly shows that the length of the species recorded here is smaller than the length reported by Dogiel[2], Ogimoto & Imai[5], Dehority[6] however the width described here is slightly larger than the width recorded by Dogiel[2] and Dehority[6]. The L/W ratio is less as compared to the L/W ratio given by Dogiel[2] and Dehority[6].In the present studies, *Metadinium affine* is recorded for the first time from the rumen of cattle in India

Table 2: Comparative body dimensions of *Metadinium affine*

Parameters	Authors			
	Dogiel[2]	Ogimoto & Imai[5]	Dehority[6]	Present Study
Length	88-120 (105)	90-128	90-128 (105)	60.8-108.8 (86.08)
Width	47-65 (59)	65-87	47-87 (59)	48-86.4 (61.97)
L/W ratio	1.7	--	1.78	1.13-1.60 (1.24)

***Metadinium minorum*, Dehority 1975**

Description of the species: The body of *Metadinium minorum* is rounded, in shape. The adoral ciliary zone is smaller encloses mouth. The left ciliary zone is slightly small than the adoral ciliary zone. Both the ciliary zones are separated by a small but distinct operculum extended short distance to the oral area. Both the surfaces of the body are greatly convex especially in the middle region of the body. Posteriorly it becomes smooth rounded without formation of ventral lobe. The macronucleus is heavy, elongated structure. The macronucleus gets elevated from the middle of the dorsal side and forms a lobe like appearance. The anterior end of the macronucleus is smooth rounded. The posterior end narrows blunt. The micronucleus is an ellipsoidal body lies just anterior to lobed region of macronucleus. Two skeletal plates arise from the base of the ciliary zone fuses in the middle of the body and terminate near the posterior end of the macronucleus. The dorsal skeletal plate lies close to the macronucleus and the ventral skeletal plate is of nearly of equal size.(**Fig.2)**

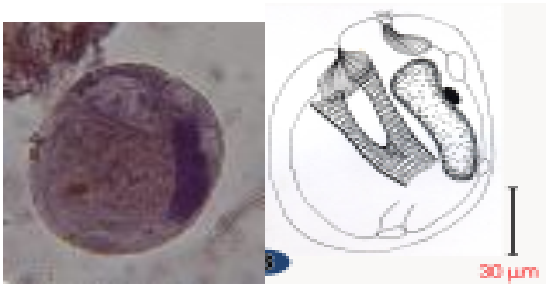

Fig.2: Photomicrograph and linedrawing of *M. minorum*

The body dimensions and other measurements of *Metadinium minorum* are given in **Table 1**.

Comments: Dehority[17] was first reported *Metadinium minorum* from the rumen of sheep and described as *Diplodinium* (*Ostracodinium*) *minorum* and in 1993 redrawn as *Metadinium minorum*. A comparison of the dimensions of the species described here and those given by Dehority[17] are shown in **Table 3.**

The table indicates that the length of the species recorded here is small as compared to the length given by Dehority[17] but the width of the species is exactly similar to the width given by Dehority[17]. The L/W ratio recorded here is less indicates that the species is more rounded as compared to the species described by Dehority[17]. The length of macronucleus measured here is also less as compared to the value given by Dehority[17] but the shape of the macronucleus is exactly similar to the shape described by Dehority[17].Dehority [17] described this species with the variation of presence of caudal lobe we found the specimens without any caudal lobe the posterior end is smoothly rounded. In the present studies, *Metadinium minorum* is recorded for the first time in cattle in India.

Table 3: Comparative body dimensions of *Metadinium minorum*

Parameters	Authors	
	Dehority[17]	Present study
Length	94.6-131.3 (113.1)	70.4-118.4 (96.14)
Width	64.9-89.1 (77.2)	64-96 (76.45)
L/W ratio	1.30-1.59 (1.46)	1.10-1.46 (1.26)
Ma.Nu. L	60.5-88.0 (74.4)	35.2-70.4 (53.76)

***Metadinium medium*, Awerinzew & Mutafowa 1914**

Description of the species: The body of this species is large heavy rounded in shape. The adoralciliary zone is larger encloses the mouth. The left ciliary zone is also large but smaller than adoralciliary zone with distinct lips. The operculum is medium and distinct extends anterior top of the oral zone. Both the body surfaces are flat to distinctly convex laterally they are slightly convex with maximum in diameter and posteriorly terminates into smooth rounded surfaces without any lobe.

The macronucleus is an elongate, heavy body, which is nearly of the body length. It lies along the dorsal edge of dorsal skeletal plate. There are three large lobes on dorsal side, one lies at anterior end, one in the middle and one found at posterior end of macronucleus. The anterior end of macronucleus is broader than the posterior end. The micronucleus is an ovoid body lying in a slight depression, formed just anterior to the middle lobe of macronucleus. The two skeletal plates start from the border of the oral area beneath the right surface towards the middle of the body. The anterior end of the dorsal plate extends from the base of the operculum across the dorsal half of the oral area extends up to the posterior end of the macronucleus. The ventral plate arises from the ventral base of the oral area extends up to the middle of the body and do not fuses with dorsal skeletal plate.**(Fig. 3)**

Fig.3: Photomicrograph and line drawing of *M. medium*

The body dimensions and other measurements of *Metadinium medium* are given in the **Table 1**

Comments: Dogiel[2] reported this species as *Eudiplodinium medium* f. *medium*. Kofoid& Maclennan[10] established new genus *Metadinium* and described this species as *Metadinium medium* from *B. indicus* in India, Ceylon and Colombo. A comparison of the body dimensions of the species described here and those given by earlier workers are given in **Table 4.**

The table reveals that the species described here is larger in body length & width, than the dimensions given by Dogiel[2], Becker& Talbott[3], Kofoid & Maclennan[10], Clarke[18], Ogimoto & Imai[5] and Dehority[6]. The L/W ratio is less as compared to the L/W ratio given by Kofoid & Maclennan[10], Clarke[18] Dehority[6]. The macronucleus of the present species is more elongated as compared to the macronucleus described by Kofoid & Maclennan[10] & Clarke[18] however; the diameter of the mouth is smaller than the dimension given by Kofoid & Maclennan[10].

Table 4: Comparative body dimensions of *Metadinium medium*

Parameters	Authors							
	Awerinzew& Mutafowa (1914)	Dogiel[2]	Becker & Talbott[3]	Kofoid & Maclennan[10]	Clark[18]	Ogimoto & Imai[5]	Dehority[6]	Present study
Length	187-272	150-225 (186)	187-270	180-224 (208)	162-230 (194.7)	170-210	150-272 (208)	172.8-323.2 (235)
Width	136-170	92-170 (140)	136-175	111-143 (134)	86-132 (109.3)	85-140	92-175 (134)	121.8-233.6 (188.86)
L/W ratio	--	--	--	1.25-1.78 (1.36)	1.63-2.07 (1.79)	--	1.25-1.78 (1.36)	1.05-1.56 (1.25)
Ma.Nu.L	--	--	--	107-155 (128)	89-143 (117)	--	--	102.4-195.2 (148.99)
Mouth	--	--	--	35-40 (44)	--	--	--	28.8-51.2 (37.35)

***Metadinium magnum*, Dogiel 1925**

Description of the species: The body of this species is large, heavy and oval. The adoralciliary zone is large comprising mouth. The left ciliary zone is shorter than adoralciliary zone. There is a distinct operculum separates both the ciliary zones. It is broad heavy and extends anterior top of the body. The body surfaces are convex. The ventral surface is slightly more convex than the dorsal surface posteriorly it is smoothly rounded without any lobe or spine. The greatest diameter found in the anterior third of the body.

The macronucleus is an elongate, heavy body with three dorsal lobes. The anterior end of the macronucleus is broad, while posterior end is narrower than anterior end smoothly rounded. The micronucleus is an ellipsoidal body, which lies in the slight depression just anterior to the middle lobe of macronucleus.

The two skeletal plates lie beneath the right middle surface of the body. They arise from the posterior border of the oral area and near the middle of the macronucleus; both the plates posteriorly fused together. The dorsal plate extends from the base of the operculum posteriorly across the half of the body. It is composed of 4-6 longitudinal rows of prisms. The ventral plate starts from the edge of the ventral half of the oral area posteriorly to the middle of the body comprising 4-6 longitudinal rows of prisms**(Fig.4).**

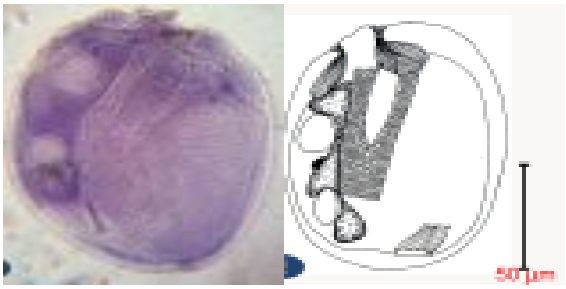

Fig.4: Photomicrograph and line drawing of *M. magnum*

The body dimensions and other measurements of *Metadinium magnum* are given in the **Table 1.**

Comments: Dogiel[2] described this species as *Diplodinium ypsilon* f. *magnum* and redescribed as *Euplidinium ypsilon* f. *magnum* from the reindeer from U.S.S.R. Kofoid& Maclennan[10] renamed this species in a new genus *Metadinium ypsilon* f. *magnum*. A comparison of the dimensions of the species described here and those given by Dogiel[2] are shown in Table 5.

The table indicates that the species described here is larger than the species described by Dogiel[2]. However, the L/W ratio is less indicates the species is more rounded in shape than the species described by Dogiel[2].

In the present studies, *M.ypsilon* f. *magnum* is recorded for the first time in cattle in India. Its frequency of appearance is less. Totally nine specimens of this species were found during the present study.

Table 5: Comparative body dimensions of *Metadinium magnum*

Parameters	Authors	
	Dogiel[2]	Present Study
Length	156-201 (183)	192-272 (224.7)
Width	92-135	156.8-224 (185.25)
L/W ratio	1.64	1.07-1.29 (1.22)

***Metadiniumypsilon*, Dogiel 1925**

Description of the species: The body of this species is elongate, ovoid slightly ellipsoidal. The adoralciliary zone relative large encloses the mouth. The left ciliary zone is shorter than the adoralciliary zone. The operculum is of medium size, which extends shortly anterior to the oral area separates both the ciliary zones. The body surfaces anteriorly are convex but laterally compressed. The ventral surface slightly convex posteriorly it narrows becoming smooth rounded without any formation of lobe.**(Fig. 5)**

The macronucleus is an elongate body lying adjacent to the dorsal edge of the skeletal plate. There is a small anterior lobe and a median lobe on the macronucleus no posterior lobe. The anterior end of the macronucleus is broad smooth while the posterior end is narrow than the anterior end with blunt end. The micronucleus is a small ovoid body lies in depression just anterior to the middle lobe of macronucleus.

The two skeletal plates arise from the border of the oral area beneath the right dorsal surface towards the middle of the body but posteriorly both the skeletal plates are fused extends together up to the posterior end of the macronucleus.

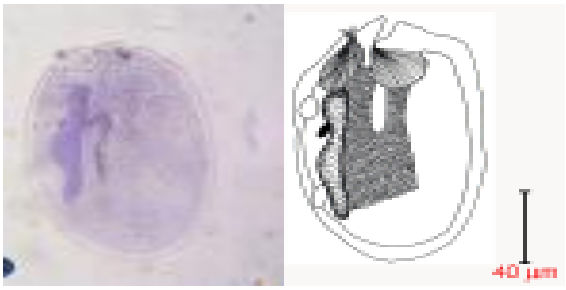

Fig.5: Photomicrograph and linedrawing of *M.ypsilon*

The body dimensions and other measurements of *Metadiniumypsilon* are given in **Table 1.**

Comments: Dogiel[16] described firstly this species as *Diplodiniumypsilon* f. *ypsilon* and redescribed in 1927 as *Eudiplodiniumypsilon* f. *ypsilon*from cattle in U.S.S.R. Kofoid& Maclennan[10] renamed this species in a new genus as *Metadiniumypsilon*. Ogimoto&Imai[5] described this species in cattle and water buffalo. A comparison of the body dimensions of the species described here and those given by the earlier workers are shown in **Table 6.**

It is clear from the table that the length of the species recorded is smaller than the length given by Dogiel[2]. The size range is smaller as compared to the size range given by Ogimoto& Imai[5]. However, the width recorded here is more than the width reported by Dogiel[2]. The L/W ratio is also less than the L/W ratio described by Dogiel[2].

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

Table 6: Comparative body dimensions of *Metadiniumypsilon*

Parameters	**Authors**		
	Dogiel[2]	Ogimoto & Imai[5]	Present Study
Length	110-152 (123)	110-160	92.8-147.2 (116.22)
Width	60-72 (70)	80-100	60.8-96 (79.6)
L/W ratio	1.8	--	1.27-1.88 (1.47)

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