Turk J Zool
25 (2001) 269-274

New Rumen Ciliates from Turkish Domestic Cattle (*Bos taurus* L.): 3. *Entodinium oektemae* n. sp. and *Entodinium imaii* n. sp. (Entodiniidae, Entodiniomorphida)

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Received: 23.06.2000

Abstract: Certain entodiniid ciliates, somewhat different from those previously described (1), were observed the during examination of the rumen contents of 30 adult domestic cattle (*Bos taurus* L.) in the vicinity of Izmir. We propose that these new forms should be taxonomically named *Entodinium oektemae* n. sp. and *Entodinium imaii* n. sp. The frequencies *Entodinium oektemae* n. sp. and *Entodinium imaii* n. sp. were 6.67% 16.67% respectively. The main characteristics of these two species are described in detail and discussed in comparison with those of the previously reported ones.

Key Words: *Entodinium oektemae* n. sp., *Entodinium imaii* n. sp., New Rumen Ciliates, *Bos taurus*, Turkey

Türkiye Evcil Sığırlarından (*Bos taurus* L.) Yeni İşkembe Siliyatları: 3. *Entodinium oektemae* n. sp. ve *Entodinium imaii* n. sp. (Entodiniidae, Entodiniomorphida)

Özet: İzmir civarındaki toplam 30 ergin evcil sığır (*Bos taurus* L.)'dan elde edilen işkembe içeriklerinin incelenmesi sırasında, entodiniumlara benzer fakat önceden tanımlananlardan (1) farklı olan bazı siliyatlar gözlenmiştir. Bu çalışma söz konusu siliyatların 2 tür, *Entodinium oektemae* n. sp. ve *Entodinium imaii* n. sp. halinde sınıflandırılmasını rapor eder. *Entodinium oektemae* n. sp. incelenen sığır işkembe içeriklerinin %6.67'sinde, *Entodinium imaii* n. sp. ise %16.67'sinde gözlenmiştir. Bu türler ayrıntılı bir şekilde tanımlanmış ve önceden bilinen benzer türlerle karşılaştırmalar yapılarak tartışılmıştır.

Anahtar Sözcükler: *Entodinium oektemae* n. sp., *Entodinium imaii* n. sp., Yeni İşkembe Siliyatları, *Bos taurus*, Türkiye

Introduction

Three formae and one species belonging to the genus *Entodinium*, and a new species of *Epidinium* which contains three formae have been described in two preceding reports (1,2).

Upon reinvestigation of the rumen ciliate composition of certain domestic cattle, two new species have been recorded belonging to the genus *Entodinium* (Entodiniidae: Entodiniomorphida).

Therefore, the purpose of this study was to describe these new ciliates and, in addition, to clarify their relationship with those already known.

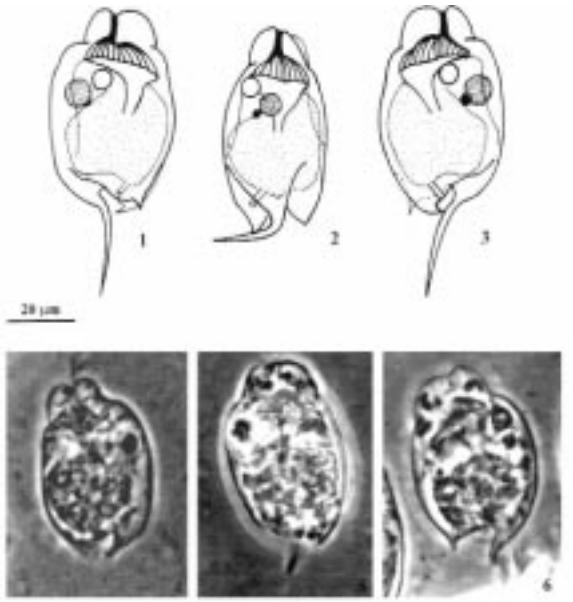
Materials and Methods

Samples of the prior study (2), which were obtained from the rumen of domesticated cattle, were reviewed (nos. 1-30). The details of the method are not given, because they were the same as those described in a previous paper (1).

Results and Discussion

(1) *Entodinium oektemae* n. sp. (Figs. 1-6 and Table 1)

Diagnosis: The body is ovoid-ellipsoidal and is widest at the midpoint. There are two central periplasmic foldings on either side, starting from the adoral cilliary zone (ACZ), which extends towards the posterior at the $^3/_4$ of the body circumference. A spine and two matching spinate lobes are present at the posterior end. The spine is seen on the dorsal side, whereas the lobes are located ventrally on both sides. The dorsal spine has a thick base which is mainly shaped by the distal part 5.00-28.75 µm in length, bending sometimes dorsally or towards the left side of the body.



Figures 1-6: *Entodinium oektemae* n. sp. 1-3, drawings from the right (1), dorsal (2) and left sides (3); 4-6, phase-contrast photomicrographs from the left (4, 6) and right (5) surfaces, showing some variations in the shapes of the body, and micro- and macronucleus and the shapes and localizations of the contractile vacuole, and micro- and macronucleus.

Table 1. Dimensions of *Entodinium oektemae* n. sp. based on the data of 25 (n) specimens. L=Length, W=Width, L/W=Length to Width ratio, MAL=Macronucleus Length, L/MAL=Length to Macronucleus Length, DSPL=Dorsal Spine Length, SE=Standard Error, SD=Standard Deviation.

	Mean	Range	SE	SD
L	63.22	52.50-75.00	1.15	5.87
W	40.53	33.75-46.25	0.60	3.07
L/W	1.56	1.40-1.67	0.02	0.08
MAL	11.83	8.75-17.50	0.47	2.40
L/MAL	5.55	4.21-7.66	0.16	0.83
DSPL	14.42	5.00-28.75	0.86	4.41

The adoral lips extend distinctively from the convex part of the body at the anterior end and form an outward protrusion as long as ca. 1/6 of the total body length. The macro- and micronuclei are spherical and the micronucleus is situated posteriorly or anteriorly on the left of the macronucleus, generally in its vicinity. The contractile vacuole is located before or at the level of macronucleus on its ventral side. The central periplasm penetrates beneath the ACZ and has a length approximately 2/4 of the body length.

Description: The body is ovoid to ellipsoid in side view. Both sides of the body are generally convex but the ventral side has a weak and wide shallow depression at the level of the ACZ base. The widest diameter occurs at the level of mid-body. Two central periplasmic foldings are present, starting from the base of the ACZ on the dorsal side of the cell and extending over the macronucleus, one on the left and the other on the right side down to $^{3}/_{4}$ of the posterior of the body. The body length (L) is 63.22 (52.50-75.00) mm and the width (W) is 40.53 (33.75-46.25) mm with a L/W ratio of 1.56 (1.40-1.67) (n=25).

The ACZ slants away from the macronucleus tilted ventrally and stretches to $^{1}/_{4}$-$^{1}/_{5}$ of the cell length from the anterior end. The adoral lips, the ventral one thicker than the dorsal, protrude beyond the convex curve at the anterior end of the body where they form a prominent projection $^{1}/_{6}$ the length of the body. There are a spine and two lobes in the posterior of the body. The spine is situated on the dorsal side. Because the edges of the lobes are acutely pointed, they are called spinate lobes. Although both are the same size, the left lobe has the shape of a crow's beak.

The caudal spine has a thick base and this part has a distal portion gradually tapering, 5.00-28.75 µm in length, which generally curves to the dorsal side (72.00%) but sometimes towards the left dorsal side (28.00%).

The macronucleus is generally spherical (96.00%) but occasionally ellipsoidal (4.00%) and lies on the dorsal side below or at the level of the base of the ACZ or at some distance. In the right side view, it partially covers the micronucleus. The micronucleus is spherical and is situated at the left posterior or left anterior side and at the proximity of the macronucleus.

The contractile vacuole lies between the macronucleus and ACZ, generally below the ACZ (80.00%), but sometimes at the same level as the macronucleus and at the dorsal side of the cell (anteropulsatum or lateropulsatum types). The vestibulum and nasse appear in a funnel shape that shows a curvature at angle of 15-20° towards the macronucleus. They extend for almost

$^1/_2$ of the length of the cell. The peripheral periplasm (endoplasm) does not penetrate into the caudal spine and spinate lobes. The central periplasm on the dorsal side is thicker than the ventral one, and also diffuses beneath the ACZ and within the thicker lips. The central periplasm occupies less than $^2/_4$ of the cell.

The cytoproctal tube is relatively long and narrow, and shows an approximate angle of 45° to the main body axis. The cytoproct opens at the middle part of the left spinate lobe or over its free edge.

The measurements and ratios of 25 cells of *Entodinium oektemae* n. sp. from Turkish cattle no. 23 are given in Table 1.

Variations: Although this new species has a regular appearance in terms of shape with projecting adoral lips, it shows variations in terms of some other features. The macronucleus is generally spherical (96.00%) but rarely ellipsoidal (4.00%) and its anterior end is either at the same level as the ACZ (32.00%) or at a certain distance (68.00%). The micronucleus has a constant shape but its location varies, either on the left posterior side (72.00%) of the macronucleus or in its viccinity.

The contractile vacuole is situated either on the left antero-lateral (anteropulsatum type) (80.00%) or on the left lateral (lateropulsatum type) (20.00%).

The dorsal spine mainly curves to the dorsal side (72.00%) or, occasionally to the left at an angle of 90° then to the dorsal side (28.00%).

Type Host and Locality: Domestic cattle, *Bos taurus*, in Izmir, Turkey.

Habitat: Rumen

Occurrence: *Entodinium oektemae* n. sp. is found to constitute 0.20% and 11.85% of total ciliate protozoa in Turkish domestic cattle nos. 1 and 23, respectively, with an appearance frequency of 6.67%. Total ciliate numbers per ml rumen contents in these cattle were 4.53×10^5 and 5.05×10^5, respectively.

Etymology: *Entodinium oektemae* n. sp. is named after to Prof. Dr. Nimet ÖKTEM (Ege University, Science Faculty, Biology Department, Zoology Section, 35100 Bornova, Izmir, Turkey) whose contributions to protozoology at national and international level are immeasurable.

Type Material: The holotype and paratypes are deposited in the Protozoology and Parasitology Laboratory in the Zoology Section, Biology Department, Faculty of Science, Ege University, Bornova, Izmir, Turkey, on the slides numbered ZSBEU-RCC. 3/PN, 98-100 and dated May 17, 1993.

Relationship with other Species: *Entodinium oektemae* n. sp. seems closely related to E. caudatum f. caudatum Lubinsky 1957 (3), E. ovoido-nucleatum Das Gupta 1935 (4), E. williamsi Göçmen & Öktem 1996 (1) and E. constrictum Dehority 1974 (5).

Entodinium oektemae n. sp., however, differ from those with certain characteristics, such as:

-having thick adoral lips extending anteriorly $^1/_6$ of the body.

-having two central periplasmic foldings that occupy $^3/_4$ of the cell and start from the base of the ACZ, stretching posteriorly, one to the left and the other to the right side.

-pertaining a central periplasm longer than $^2/_4$ of the body length.

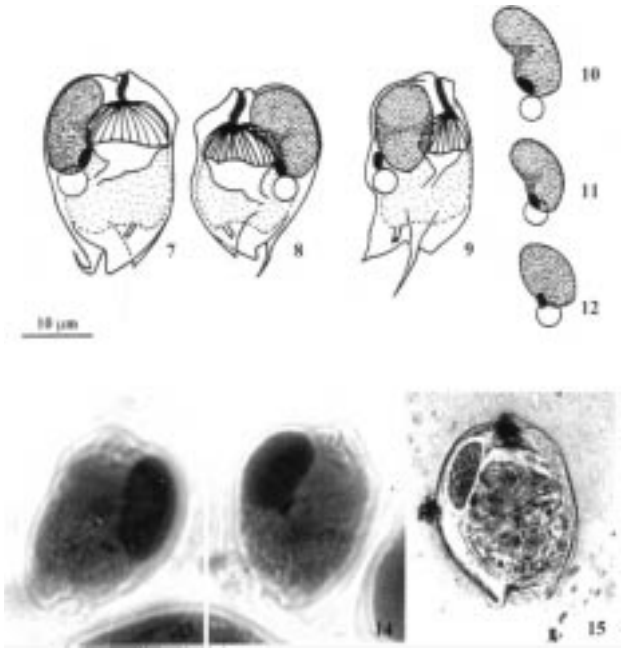
-possessing 2 spinate lobes at the posterior.

-having a contractile vacuole situation referable to the macronucleus (typed as anteropulsatum and lateropulsatum) (6, 7).

Entodinium oektemae n. sp. clearly differ from *E. caudatum* f. *caudatum,* because the latter has a bar-shaped macronucleus and only a lateropulsatum type of contractile vacuole (3), but can be discriminated from *E. ovoido-nucleatum,* which has an oval macronucleus and only a dorsopulsatum type contractile vacuole. It is also different from *E. williamsi,* which belongs to the lateropulsatum type by the presence of a contractile vacuole, and whose adoral lips do not project anteriorly, and the L/W ratio of 1.40 is smaller than the present form (1). This new species resembles *E. consrictum* (5) in the shapes of the macronucleus and the body. But the latter is smaller (37.6 x 24.2 mm) and its adoral lips do not form distinct projections. It is lacking right and left central periplasmic foldings, a spine and spinate lobes in the posterior half of the body. *E. constrictum* belongs to the cephalopulsatum type due to its contractile vacuole localization.

(2) *Entodinium imaii* n. sp. (Figs. 7-15 and Table 2)

Diagnosis: The body of *Entodinium imaii* n. sp. is ovoid and widest at $^1/_4$ of the body level. The dorsal side



Figures 7-15: *Entodinium imaii* n. sp. 7-9, drawings from the right (7), left (8) and dorsal (9) sides; 10-12, some basic variations in the macronuclear shape and related organelles; 13-15, photomicrographs from the left (13) and right side views (14, 15) showing some variations in the body, and micro- and macronuclear shapes [(13-14), Iron haematoxylen staining; 15, Silver impregnation technique].

Table 2. Dimensions of Entodinium imaii n. sp. based on the data of 25 (n) specimens. L=Length, W=Width, L/W=Length to Width ratio, MAL=Macronucleus Length, MAW=Macronucleus Width, MAL/MAW=Macronucleus Length to Macronucleus Width ratio, L/MAL=Length to Macronucleus Length, DSPL=Dorsal Spine Length, SE=Standard Error, SD=Standard Deviation.

	Mean	Range	SE	SD
L	27.30	18.75-33.75	0.72	3.57
W	20.19	18.75-25.00	0.47	3.33
L/W	1.36	1.07-1.65	0.03	0.16
MAL	13.62	10.00-20.00	0.56	2.80
MAW	7.65	5.00-11.25	0.31	1.56
MAL/MAW	1.80	1.33-2.60	0.06	0.28
L/MAL	2.05	1.38-2.67	0.07	0.35
DSPL	5.35	2.50-10.00	0.40	1.99

in convex and humpbacked anteriorly. The ventral side is almost straight but slightly depressed on the mid-surface.

In the posterior part of the body, there is one dorsal spine 5.35 (2.50-10.00) µm in length, extending outwardly but sometimes bending dorsally and towards the anterior part. Another triangular spine and a back-shaped lobe on the right side are also present. The right lobe is shorter than the secondary spine on the left.

The adoral lips do not protrude beyond the convex curve of the anterior end of the body. The ACZ is slightly slanted and extends approximately half the length of the body. The macronucleus, which is spherical, mostly concave on the ventral side and convex on the dorsal side, is located at almost the anterior tip of the body. It is bean-shaped but, rarely, is ellipsoidal. It is very large and occupies almost $^{1}/_{4}$-$^{1}/_{4}$ of the body volume but occasionally could be smaller. The micronucleus is ellipsoidal and is situated close to the left posterior of the macronucleus. The contractile vacuole lies left posterior (80.00%) or left (20.00%) of the macronucleus, which is lateropulsatum type.

In the very anterior, the central periplasm penetrates the left dorsal sides of the ACZ and the large-volumed macronucleus. This thick central periplasm is seen on $^{2}/_{4}$ of the anterior dorsal side of the body.

Description: In the side view, the body is roughly ovoid with a hump on the anterior dorsal part and its dorsal side is convex while the ventral is flat, but a slight depression is seen at the mid-level. The widest part of the body is $^{1}/_{4}$ from the anterior end. The body length (L) is 27.30 (18.75-33.75) mm, and the width (W) is 20.19 (18.75-25.00) mm with a L/W ratio of 1.36 (1.07-1.65) (n=25).

The ACZ lies almost at a right angle to the body axis. The adoral lips do not protrude beyond the convex curve of the anterior end of the body. The ACZ extends approximately _ of the body length in the anterior. There are two spines in the posterior end of the body, one elongated on the dorsal and the other on the left ventral, and a wide lobe exists on the right. The dorsal spine, 5.35 (2.50-10.00) µm in length, generally tapers outward from the body end, but occasionally it bends to the dorsal side (24.00%) and towards the anterior. The other spine, at the left ventral side of the body, is roughly triangular and has a base wide enough to reach the median.

The right lobe is formed by an expansion of the right posterior part of the body and is directed toward the back. This lobe is enlarged in the anterior half, which gradually narrows in the posterior half, and it resembles the beak of a crow. This right lobe is longer than the left ventral spine.

The dorsally located macronucleus lies to the anterior end of the body, which is ellipsoidal, generally having a concave ventral side and convex dorsal side (88.00%). Although it generally is bean-shaped, it occasionally is ellipsoidal (12.00%). It usually occupies $^1/_5$-$^1/_4$ of the body volume (72.00%) in some individuals. However, it is smaller (28.00%). The ellipsoidal micronucleus is situated on the left posterior side of the macronucleus (84.00%), but rarely is seen in its exact posterior (16.00%).

The contractile vacuole is on the left posterior (80.00%) or on the left (20.00%) of the macronucleus (lateropulsatum type). The vestibulum plus nasse is funnel-shaped and, on the right-side view, has an angle of 45° towards the macronucleus, and extends for approximately $^1/_2$ of the cell length. The peripheral periplasm does not penetrate into the caudal spines. The central periplasm on the dorsal side is very thick where the macronucleus resides.

The cytoproctal tube is relatively long and narrow, leaning at an angle of ca. 30-45° to the main body axis. The cytoproct has an opening near the midpoint of the left spine.

The measurements and calculated ratios of 25 cells of *E. imaii* from the domestic cattle no. 9 are given in Table 2.

Variations: The species shows fairly constant body shape and other morphological characters. However, there are some notable characteristics concerning the shape and size of the macronucleus, the site of the contractile vacuole with reference to the macronucleus and the direction of the dorsal spine.

Type Host and Locality: Domestic cattle (*Bos taurus*) in Izmir, Turkey.

Habitat: Rumen

Occurrence: *Entodinium imaii* n. sp. constituted 0.42, 0.20, 0.53 and 1.08% of total ciliate protozoa in 5 domestic cattle (nos. 4, 5, 8, 9 and 10, respectively) with a frequency of 16.67%. Total ciliate protozoa numbers per ml of rumen contents in these cattle were 6.55×10^5, 6.35×10^5 5.25×10^5, 5.05×10^5 and 7.80×10^5, respectively.

Etymology: This new species is named after Prof. Dr. Soichi IMAI (Nippon Vet., Zootechnical College, Tokyo, Japan), who has carried out much important research on rumen ciliate-protozoa.

Type Material: Holotype and paratypes are kept on the slides numbered ZSBEU-RCC, 1/PN. 34-48 and /PN. 63-102 and dated April-May 1990. These specimens are retained in the Protozoology & Parasitology Laboratory of the Zoology Section, Department of Biology, Science Faculty, Ege University, Bornova, Izmir, Turkey.

Relationship with other species: *Entodinium imaii* n. sp. resembles *E. caudatum* Stein 1958 (8) and *E. ekendrae* Das Gupta 1935 (4). This new species, however, differs from those previously known, because it has the widest diameter at $^1/_4$ level of the body and its macronucleus, which is situated in the uppermost part of the body, is relatively proportional to the body size, and characteristic with its bean-like shape. This species also varies from the others in terms of the relative positions of the contractile vacuole to the macronucleus. The rest-point of the contractile vacuole in *E. caudatum* and *E. imaii* is consistent and lateropulsatum type is seen in both species. The macronucleus in *E. caudatum* is elongated and the contractile vacuole is situated on its anterior tip its whereas in *E. imaii* n. sp. the bean-shaped macronucleus is ellipsoidal and the contractile vacuole is seen on posterior end.

The measurements of this new species are almost half those of *E. caudatum* (40.70 x 25.50 mm). Although *E. ekendrae* is rarely similar to *E. imaii* in terms of body size, macronucleus shape and dimensions, it differs absolutely with a cephalopulsatum type of contractile vacuole.

Because neither *E. oektemae* n. sp. nor *E. imaii* n. sp. have been reported to date, they seem to be endemic species of ciliated fauna in the rumens of Turkish domestic cattle.

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