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Entodinium dalli m. *monospinatum* n.m. and *Entodinium dalli* m. *triangulobatum* n.m., two new morphotypes of *Entodinium dalli* Dehority, 1974, from Turkish Cattle

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Abstract. In the course of examining the rumen contents obtained from 25 domestic cattle (*Bos taurus taurus*) in the vicinity of Kastamonu, Turkey, several new morphotypes of *Entodinium dalli* Dehority, 1974 were identified. The new morphotypes of this species, *E. dalli* m. *monospinatum* n.m. and *E. dalli* m. *triangulabatum* n.m. were identified on the basis of differences in their caudal spines and lobes. Including the previously described morphotype *Entodinium dalli* m. *rudidosospinatum* brings the total number of *E. dalli* morphotypes to four. The size and percentage occurence of this species in the present study is compared to those previously reported for other animal hosts living in different geographical locations.

Key words: *Entodinium dalli*, rumen, cattle, morphotype, Turkey.

Introduction

In addition to the bacteria and fungi, rumen protozoa contribute significantly to the digestion of feedstuffs in ruminants (Dehority 1986, Williams & Coleman 1992, Baraka 2012). The majority are ciliate protozoa, with much lesser numbers of flagellates present. The species of ciliates present varies with different feeds, i.e., diets high in concentrates seem to support populations of predominantly *Entodinium* species. Conversley, high forage diets generally are characterized by a population of mixed protozoal species (Dehority & Orpin 1988, Franzolin & Dehority 1996). Physiological conditions of the host such as rumen pH, turnover rate, level of feeding and frequency of feeding can also influence the ciliate population (Dehority & Orpin 1988, Towne et al. 1988).

Comparative studies of the rumen ciliate populations of various hosts in different regions should provide information on phylogenetic relationships between the rumen ciliates and the host ruminants. Specifically some species and morphotypes have only been detected in certain areas and a few appear to be host specific (Dogiel 1927, Dehority 1978, Imai 1988, Ito & Imai 1990, Ito et al. 1995, Göçmen & Öktem 1996).

The entodinia are the smallest, simplest and most common of the rumen ciliates and are found in almost all ruminants without exception. They are also the most difficult to classify into species because of the large number of closely related organisms and the difficulty in deciding between intraspecific and interspecific variation (Williams & Coleman 1992).

The aim of this study was to describe two new morphotypes of *Entodinium dalli* (Dehority 1974) identified in rumen contents from domestic cattle living in the vicinity of Kastamonu, Turkey.

Materials and Methods

Samples of rumen contents were obtained from 25 domestic cattle (*Bos taurus taurus*) at slaughterhouses around Kastamonu, Turkey between March 2012 and December 2012. As soon as possible after the animal was killed, the rumen wall was cut with a knife and a well-mixed sample of the rumen contents was removed via ladle. The sample was fixed by diluting with an equal volume of 18.5% formaldehyde aqueous solution (Dehority 1984). A second portion of each samples was also immediately fixed and stained in methylgreen formalin saline (MFS) solution for total and differential counts (Ogimoto & Imai 1981, Göçmen & Gürelli 2009). The MFS served as a nuclear stain. These procedures were used to preserve the integrity of the cell and its internal structure.

Differential counts of species were estimated from smear slides with a total of 300 to 500 cells identified for each species (Göçmen & Gürelli 2009).

All cell measurements were made and specimens were examined with a Leica DM 3000 microscope and imaging system. The identification of species was based on previously published descriptions (Dehority 1974, Göçmen & Öktem 1996).

Drawings of the new morphotypes were based on photomicrographs and observations of the cells stained with MFS (Ogimoto & Imai 1981).

Terminology for orientatiton used in describing the structure of the ciliate species conforms to the conventional system of ciliate phylum proposed by Dogiel (1927).

Results

Two species similar to *E. dalli* were observed in this study. They occurred in relatively low numbers, 17.0% and 12.6% of the total ciliate protozoa in cattle nos. 14 and 23, respectively. Caudal spines and lobes differred from the description of *E. dalli* Dehority 1974 and *E. dalli* f. *rudidorsospinatum,* Göçmen & Öktem 1996. In general, body shape and size; location, size and shape of the macronucleus; and location of the contractile vacuole and micronucleus were very similar, suggesting they were morphotypes of *E. dalli* (Dehority 1974). Descriptions of the four morphotypes of *E. dalli* are given below:

Morphotype 1: dalli type (Fig. 1)
In side view, the body is almost circular, 34 (28-38) µm long (L); 31 (23-41) µm wide (W); L/W = 1.1(0.9-1.2). Macronucleus is ellipsoidal to spherical in shape and lies on the dorsal side in the anterior half of the body. Micronucleus is ellipsoid or spherical and lies posterior to the macronucleus, generally in line with the ventral edge. Contratile vacuole lies ventral of the posterior end of the macronucleus, gener-

ally just anterior to the micronucleus. Oral lips are thick and do not protude beyond the convex curve of the anterior end. The oral ciliary zone is at a slight dorsal angle to the main body axis. The vestibulum is funnel-shaped, bending dorsally and terminating in the region just posterior to the micronucleus. Cytoproct is narrow, near the middle of the body, angled toward the dorsal side. A posterior dorsal spine, 22-25 µm in length, has a relatively narrow base and tapers to a point. A left ventral lobe follows the curvature of the ventral side body wall, terminating as a bluntly rounded triangle directed towards the dorsal side of the cell. A very short, acutely pointed lobe is present on the right ventral side of the posterior end of the cell. This is the typical form of *E. dalli* as described by Dehority (1974).

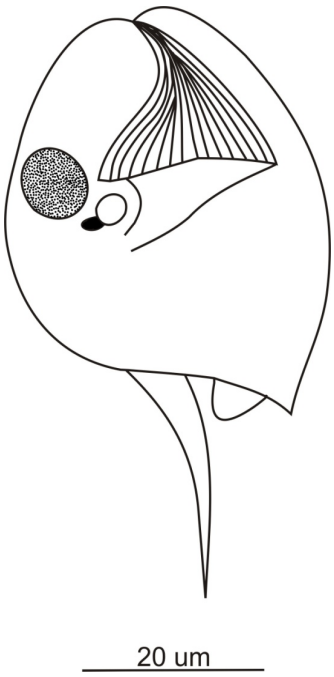

Figure 1. Drawing of *E. dalli* m. dalli from the right side (redrawn from Dehority 1974).

Morphotype 2: *rudidorsospinatum* type (Fig. 2, 5c)
With the characteristics of morphotype 1, except the dorsal spine is generally absent or rudimentary (2.5-3.0 µm in length), rarely with a very short spine with a length of 4.5-12.0 µm which curves to the dorsal and left side of the cell. The left and right ventral lobes are as described for *E. dalli* Dehority 1974. This morphotype is described by Göçmen & Öktem (1996).

Frequency: In 8% of the 25 cattle surveyed.

Morphotype 3: *monospinatum* type n.m. (Fig. 3, 5a)
With the characteristics of morphotype 1, except the posterior dorsal spine is short with a length between 2.5-7.9 µm. Ventral side of the posterior end is rounded without any lobes.

Frenquency: in 8% of the 25 cattle surveyed.

Morphotype 4: *triangulobatum* type n.m. (Fig.4, 5b)
With the characteristics of morphotype 1, except the posterior dorsal spine is short and curves to the left and

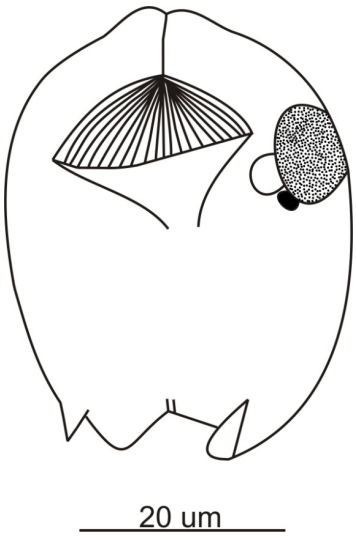

Figure 2. Drawing of *E. dalli* m. rudidorsospinatum from the left side.

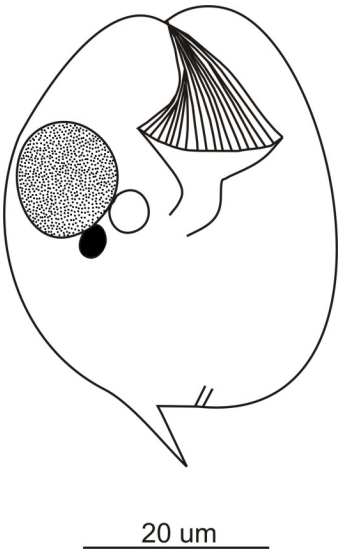

Figure 3. Drawing of *E. dalli* m. monospinatum n. m. from the right side.

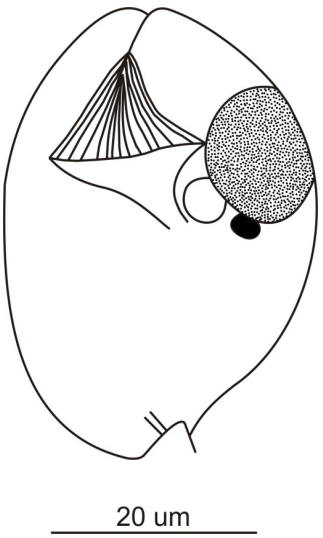

Figure 4. Drawing of *E. dalli* m. triangulobatum n. m. from the left side.

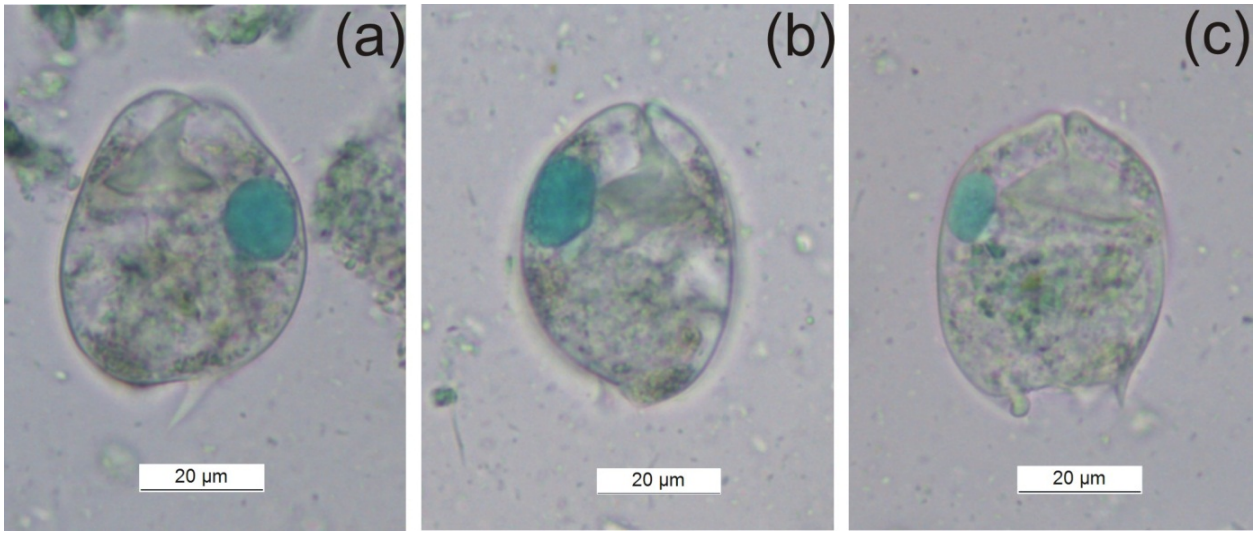

Figure 5. Photomicrographs of the morphotypes of *E. dalli*, showing caudal projections, **(a)** *E. dalli* m. monospinatum n.m. from left side, **(b)** *E. dalli* m. triangulobatum n.m. from right side, **(c)** *E. dalli* m. rudidorsospinatum from right side in MFS.

dorsal side of the cell. The ventral side of the posterior end of the cell has a blunt, slightly-rounded triangular lobe which protrudes outward from the main body axis at an angle of 45º.

Frequency: in 8% of the 25 cattle surveyed.

Table 1 shows measurements of the *Entodinium dalli* morphotypes observed in the present study. These are compared in Table 2 to the measurements previously published for *E. dalli* morphotype 1 (Dehority 1974) and *E. dalli* morphotype 2 (Göçmen & Öktem 1996).

Table 1. Dimensions of *Entodinium dalli* morphotypes from Turkish domestic cattles. L (length), W (width), L/W (length to width ratio), MAL (macronucleus length), MAG (macronucleus width), MIL (micronucleus length), MIW (micronucleus width).

	Mean (n= 25)	SD	Range
[L]	38.4	3.1	32.2-43.3
[W]	31.0	2.6	25.9-35.7
[L/W]	1.2	0.1	1.1-1.4
[MAL]	11.0	1.7	7.6-14.7
[MAW]	8.3	1.3	6.7-10.8
[MIL]	1.5	0.4	0.9-2.6
[MIW]	1.0	0.4	0.3-1.5

Discussion

Previously, *E. dalli* was observed in 2 Dall mountain sheep (*Ovis dalli*) studied in Alaska (100% frequency) (Dehority 1974), and in two of 25 domestic cattle (*Bos taurus*) from a slaughterhouse in Izmir, Turkey (8% frequency) (Göçmen & Öktem 1996). In the present study, the frequency of appearance of this species was also 8%, being present in two of 25 domestic cattle. The percentage of occurence in individual animals was 22.4 and 24.0% of total protozoa for the two Dall mountain sheep, 17.13 and 0.06% in the Turkish cattle living near Izmir and 17.0 and 12.6% in the present study. The values in Turkish cattle thus range from 0.06-17.13%. These differences may be related to the environmental conditions in the rumen or may be also result of nutritional competition with other protozoan species in cattle rumen (Göçmen & Gürelli 2009).

Specimens of *E. dalli* in the present study are slightly larger than those from Dall mountain sheep and domestic cattle living near Izmir (Table 2). The differences in size may simply reflect the amount and type of food ingested, the animal species, geographical variations or a combination of these factors (Dehority 1974, Göçmen & Gürelli 2009).

Many reports (Dogiel 1927, Imai et al. 1981, Ito & Imai 1990, Göçmen & Öktem 1996) suggested that the shape and number of caudal appendages were poor characters for the determination of species, because they are continuous, so they are considered to be unsuitable taxonomical features for classifying protozoa into species and subspecies. For these reasons, Ito & Imai (2003) and Imai et al. (2004) have used morphotypes to indicate differences in caudal spines and lobes for protozoa with all other characteristics in common.

Entodinium dalli (Dehority 1974) is similar to *E. bicaudatum, Entodinium ovido-nucleatum* and *E. ekendrae*, differing in caudal projections, location of the CV and body shape of *E. ovido-nucleatum* (Das Gupta 1935, Bush & Kofoid 1948). *Entodinium dalli* m. *rudidorsospinatum* (Göçmen & Öktem 1996) and the two new morphotypes described in this study are consistant in both location of the CV, body shape and size to

Table 2. Comparision of *E. dalli* morphotype dimensions from different hosts in Alaska and Turkey.

References	Country	Host	[L]	[W]	[L/W]
Dehority (1974)	Alaska (morphotype 1)	Dall sheep	34.3±3.5	30.7±3.9	1.1±0.1
Göçmen & Öktem (1996)	Turkey (morphotype 2)	Domestic cattle	34.5±4.1	30.5±3.1	1.1±0.1
Present study	Turkey (morphotypes 3 and 4)	Domestic cattle	38.4±3.1	31.0±2.6	1.2±0.1

the original description of *E. dalli.*

The factors that promote development of caudal appendages are not known. Different stimuli may be present in the rumen of ruminants. Lubinsky (1957) advocated that when the animals are fed a diet with low starch content, ciliates without caudal appendages are predominat, while ciliates with well-developed caudal appendages become predominant when the food is rich in starch. Coleman (1980) considered that the caudal appendages of *Entodinium* sp. are developed to protect against predation. It has been reported that the development of the caudal spines of *Diplodinium rangiferi* in the bovine rumen is not a response to predation, host diet or ciliate densities (Imai et al. 2002).

Entodinium dalli has now been reported to occur in Alaska, USA and two different locations in Turkey. Although four different morphotypes have been observed, the occurence of *E. dalli* in such widely divergent geographical locations is perplexing. Further studies on the fauna of both wild and domestic ruminants around the world might provide information on additional hosts of this species and help explain the migration of the species to different continents.

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