

## The Occurrence of the Rumen Ciliate *Entodinium constrictum* Dehority, 1974 (Entodiniidae, Entodiniomorphida) from Domestic Sheep (*Ovis ammon aries* L.) in Northern Cyprus

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**Abstract:** *Entodinium constrictum* was observed in rumen contents from a single sheep out of eleven living in Northern Cyprus. It constituted 0.08% of the total ciliate population and had a 9.09% frequency of appearance. The size and percentage occurrence of this species in the present study is compared to those previously reported for other animal hosts living in different geographical locations. This study reports for the first time the presence of *E. constrictum* in Cypriot domestic sheep.

**Key Words:** *Entodinium constrictum*, sheep, ciliate, rumen, Cyprus.

### Introduction

In a previous report (Göçmen & Gürelli 2009) we surveyed the rumen Entodiniid ciliate fauna of Domestic sheep (*Ovis ammon aries* L.) from Northern Cyprus and determined 27 species, including 33 forms. In addition, an unusual form of *Entodinium* was observed in 2 sheep and is described as a new species, *E. cypriensis*. After that report, we investigated new a domestic sheep rumen sample from Northern Cyprus and obtained an additional species *Entodinium constrictum* Dehority, 1974.

*Entodinium constrictum*, belonging to the family Entodiniidae, order Entodiniomorphida was first described from Alaska Dall Mountain Sheep (*Ovis dalli*) by Dehority (1974). *E. constrictum* has also been reported from Turkish domestic sheep (*Ovis*

*ammon aries*) (Öktem et al. 1997), from Turkish domestic cattle (*Bos taurus*) (Göçmen et al. 2003) and from Turkish domestic goat (*Capra hircus*) (Göçmen & Karaoğlu 2005).

The identification of rumen protozoan populations in different areas provides information about the geographical distribution of ciliates, their physiology, feeding habits of hosts and the specificity and phylogeny of individual ciliate species (Göçmen et al. 2002).

The aim of this study is to determine the morphological and taxonomic position of *E. constrictum* occurring in domestic sheep (*Ovis ammon aries* L.) from northern Cyprus, which is an island in the Mediterranean Sea. With this aim in mind, the results were compared with those of similar works previously conducted in Turkey and some

other regions to establish similarities and differences.

#### Materials and Methods

Samples of rumen contents were obtained from 11 mature domestic sheep (*Ovis ammon aries*) at the slaughterhouse in Lapitos-Kyrenia (Girne). The animals were allowed to graze on the plateaus all day and fed 0,5-1 kg of wheat straw and barley fracture twice a day at 06<sup>00</sup> and 21<sup>00</sup> h. The sheep were generally slaughtered between 15<sup>00</sup> and 16<sup>00</sup> h. and samples taken immediately thereafter. The rumen wall was cut with a knife and a sample of rumen contents was removed via a catheter (Göçmen 1991). A well-mixed sample of the rumen contents was diluted with an equal volume of 50% formalin (18,5% formaldehyde) as soon as possible after the animal was killed (Dehority 1984). A portion of each sample was also immediately fixed and stained in methylgreen formaline saline (MFS) solution (Ogimoto & Imai 1981) for total and differential counts. The MFS served as a nuclear stain. Differential counts of species were estimated from smear slides, with a total of 1200 cells identified.

Specimens were examined using a Jena "NF-binocular" microscope and Olympus BX51-Altra 20 Soft Imaging System. All cell measurements were made with a calibrated ocular micrometer. The identification of species was based on previously published descriptions (Dehority 1974, Öktem et al. 1997) and drawings of new ciliate species based on photomicrographs and observations of the cells stained with MFS (Ogimoto & Imai 1981).

Terminology for orientation used in describing the structure of the ciliate species conforms to the conventional system of the ciliate phylum proposed by Dogiel (1927) and Grain (1994).

#### Results

This study reports for the first time the presence of *Entodinium constrictum* in domestic sheep (*Ovis ammon aries* L.) in Northern Cyprus and only for the third time in a sheep. *E. constrictum* was found in only one of the eleven domestic sheep

examined and thus had a frequency of appearance of 9.09%. *E. constrictum* constituted 0.08% of the total ciliate protozoa in Northern Cyprus domestic sheep no. 11.

The cell dimensions of *E. constrictum* in Cypriot domestic sheep are given in Table 1. The body of *E. constrictum* is ovoid (Fig. 1); the dimensions measured in this study were 46.80 x 28.80 µm, length/width ration= 1.62; dorsal side is slightly convex. The posterior end smoothly rounded without lobes or spines. Ventral side has a marked indentation or constrictum, just level with the base of the adoral ciliary zone or anterior end of the nasse. The widest part of the ciliate is just posterior to this indentation or constrictum. Adoral lips are thick and do not protrude beyond the convex curve of the anterior end in contracted cells. Adoral ciliary zone is at dorsal angles to the main body axis and extend for approximately 1/3 of the length of the cell. The funnel-shaped vestibulum and nasse bend to the dorsal side, slightly towards the macronucleus. Nasse is short, terminating into the anterior half of the cell.

The macronucleus spherical, occasionally ellipsoidal, lies on the dorsal side and located nearly in the middle of the cell. Micronucleus is ovoid and in most of the specimens (48%) it lies near the posterior end of the macronucleus closer to the ventral side of its axis. In 40% of the specimens it is located near the anterior end of the macronucleus at the ventral side of its axis. In 12% of the specimens it lies below the macronucleus. Contractile vacuole lies above the macronucleus on the dorsal side, generally anterior to the base of the adoral ciliary zone. A boundary layer between ecto- and endoplasm was not observed. Cytoproctal tube is narrow, at angle of 45° to main body axis and terminated at the

cytoproct which bends towards the dorsal side of the body (Fig. 2a, b).

### Discussion and Conclusion

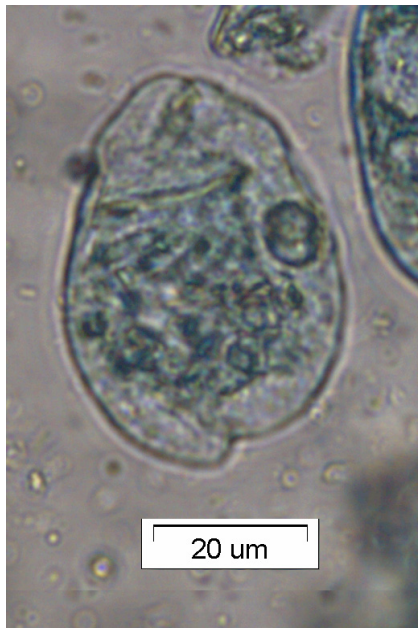
Previously, *E. constrictum* was observed in 2 Dall Mountain sheep (*Ovis dalli*) studied in Alaska (100%) (Dehority 1974), three of 7 domestic sheep (*Ovis ammon aries*) in Turkey (42.86%) (Öktem et al. 1997), four of 28 domestic cattle (*Bos taurus*) in Turkey (14.29%) (Göçmen et al. 2003) and five of 8 goats (*Capra hircus*) in Turkey (2.50%) (Göçmen & Karaoğlu 2005). Occurrence rates were 10.10-14.00%, 0.22-0.57%, 0.08%, 0.68-7.40% respectively. Both the frequency of appearance (9.09%) and occurrence rate (0.08%) of this species in the Cypriot domestic sheep were lower than that reported from other host species and geographical areas. This may be the result of the low number of hosts investigated or is related to the physiological conditions in the rumen (Göçmen et al. 2003). Perhaps this may be also result of competition with other protozoan species in sheep rumen.

Dimensions of *E. constrictum* from different hosts are given in Table 2. Specimens in the present study are larger than those from Dall Mountain sheep in Alaska and domestic sheep in Turkey. These differences can be related to the type of food ingested, the animal species, geographical variations or a combination of these factors (Dehority 1974, Rastgeldi & Göçmen 2003).

There are some differences on the location of the micronucleus. These variations are not significant enough to affect species designation. Only a few reports including the complete rumen ciliate fauna of the different kind of sheep have been published. Two of the studies were from animals in the Far East (China and Japan) (Hsiung 1931, Imai et al. 1978), two from Europe (Scotland and Portugal) (Eadie 1957, Marinho 1983), three from America (U.S.A., Alaska and Canada) (Bush & Kofoid 1948, Dehority 1974, Imai et al. 1989), two from Asiatic (Turkey) (Öktem et al. 1997, Göçmen et al. 1999) and one from Northern Cyprus (Göçmen & Güreli 2009). To date, *E. constrictum* has been reported only in

**Table 1.** Dimensions ( $\mu\text{m}$ ) of *E. constrictum* from domestic sheep rumen contents (n=25).

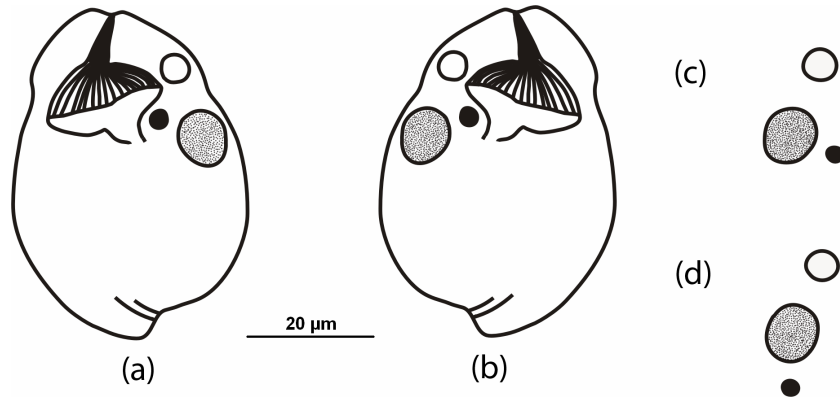
Characters	Mean	Range	SE	SD
Length (L)	46.80	37.50-52.50	0.77	3.85
Width (W)	28.80	22.50-32.50	0.56	2.80
Length of macronucleus (MaL)	8.50	5.00-12.50	0.32	1.61
Width of macronucleus (MaW)	8.30	5.00-10.00	0.27	1.39
L/W	1.62	1.38-2.00	0.03	0.15
MaL/MaW	1.02	1.00-1.33	0.01	0.08
L/MaL	5.69	4.50-7.50	1.17	0.87



**Figure 1.** Photomicrograph of the left aspect of *Entodinium constrictum* from Cypriot domestic sheep (*Ovis ammon aries* L.) (fixed and stained with MFS solution).

Alaska (Dehority 1974) and Turkey (Öktem et al. 1997) in the rumen of sheep. Recently, Göçmen & Gürelli (2009) reported on the fauna of Entodiniid ciliates in Cypriot domestic sheep. However it has not been reported in the rumen of ten Cypriot sheep. When the 11th. rumen sample was re-searched, *E. constrictum* was detected. So we suggest that the sample size of the re-searched host is important to detect ciliates which are in low numbers.

*E. constrictum* was first found in Alaska (Dehority 1974); later it was reported from Turkey (Öktem et al. 1997, Göçmen et al. 2003, Göçmen & Karaoğlu 2005) and now it is reported from Northern Cyprus. Turkey and Northern Cyprus are close geographically, however Alaska is in a different continent. The occurrence of *E. constrictum* in such widely divergent geographical locations is perplexing. Göçmen et al. (2003) and Göçmen & Karaoğlu (2005) have found it in the rumen of domestic cattle and goats.



**Figure 2.** Drawings of *E. constrictum* from the left side (a) and from the right side (b), illustrating the different locations of micronucleus (c-d).

**Table 2.** Comparison of *E. constrictum* dimensions from different hosts in various locations.

	Host/Location		
	Dall Mountain Sheep (Alaska) (Dehority, 1974)	Domestic sheep (Turkey) (Öktem et al. 1997)	Domestic sheep (Northern Cyprus) (Present study)
Number of samples	15	35	25
Length (µm)	37.6±3.8	39.83±4.95	46.80±3.85
Range in length	30.8-43.00	27.50-50.00	37.50-52.50
Width (µm)	24.2±2.5	23.72±2.97	28.80±0.56
Range in width	19.8-28.6	16.30-31.30	22.50-32.50
Length/width ratio	1.56±0.12	1.69±0.14	1.62±0.15
Range in L/W ratio	1.40-1.87	1.37-2.11	1.38-2.00
Length of macronucleus	9.4±2.4	11.35±2.62	8.50±1.61
Range in length of macronucleus	6.6-13.2	7.50-17.50	5.00-12.50

However no other researchers have found it in the rumen of cattle and goats (Das-Grupta 1935, Imai et al. 1978, Dehority 1986, Imai 1988, Imai et al. 1989, Ito et al. 1993, Ito et al. 1995, Selim et al. 1999, Fuente et al. 2006). In conclusion, further studies on the fauna of both wild and domestic ruminants in Cyprus, Turkey and Alaska might provide additional information about possible intermediate hosts and help explain the migration of this species to different continents.

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