

## Rumen Entodiniid Ciliated Protozoan Fauna (Entodiniomorphida: Entodiniidae) of Domestic Sheep (*Ovis ammon aries* L.) from Northern Cyprus, with a Description of a New Species, *Entodinium cypriensis* sp. nov.

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**Abstract:** Rumen contents obtained from 10 adult domestic sheep (*Ovis ammon aries* L.) at a slaughterhouse in Lapethos-Kyrenia (Girne), Northern Cyprus were surveyed for entodiniid ciliate protozoa. As a result of our survey 27 species, including 33 forms, were identified. An unusual form of *Entodinium* was observed in 2 sheep and is described as a new species, *E. cypriensis* sp. nov. Its relationship to similar *Entodinium* species is discussed. This study also reports new host records for *E. imai*, *E. oektemae*, *E. ogimotoi*, *E. quadricuspis*, *E. rectangulatum* f. *dubardi*, and *E. rostratum*. Furthermore, we report for the second time the presence of *E. biconcavum*, *E. costatum*, *E. dalli* f. *rudidorsospinatum*, *E. protuberans*, *E. rectangulatum* f. *lobosospinosum*, *E. simulans* f. *lobosospinosum*, *E. triacum* f. *dextrum*, *E. williamsi* f. *williamsi*, and *E. w. f. turcicum* in sheep hosts. All species detected in this study are first reports in Cypriot sheep hosts. The entodiniid ciliate fauna of Cypriot domestic sheep appears to have greater diversity compared to those of sheep in different geographical locations (Turkey, China, Japan, Scotland, USA, Canada, and Alaska).

**Key Words:** *Ovis ammon aries*, sheep, ciliate protozoa, Entodiniidae, fauna, rumen, Cyprus

### Yeni Bir Tür, *Entodinium cypriensis* n. sp. Tanımıyla Birlikte Kuzey Kıbrıs Evcil Koyunlarının (*Ovis ammon aries* L.) İşkembe Entodiniid Silli Protozoan Faunası (Entodiniomorphida: Entodiniidae)

**Özet:** Kuzey Kıbrıs, Lapta Belediyesi Salhanesi (Girne)'nden elde edilen 10 ergin evcil koyunun (*Ovis ammon aries*, L.) işkembe içerikleri entodiniid siliyat protozoonlar açısından araştırılmıştır. Araştırma sonucunda 33 form ve bu formların dahil olduğu 27 tür tespit edilmiştir. İki koyunda *Entodinium*'a dahil alışımlı türler haricinde yeni bir tür gözlemlenmiş ve *E. cypriensis* sp. nov. olarak tanımlanmıştır. Benzer *Entodinium* türleriyle olan akrabalığı tartışılmıştır. Ayrıca *E. imai*, *E. oektemae*, *E. ogimotoi*, *E. quadricuspis*, *E. rectangulatum* f. *dubardi* ve *E. rostratum* türleri için yeni bir konak tür kaydı rapor edilmiştir. Bunların ötesinde *E. biconcavum*, *E. costatum*, *E. dalli* f. *rudidorsospinatum*, *E. protuberans*, *E. rectangulatum* f. *lobosospinosum*, *E. simulans* f. *lobosospinosum*, *E. triacum* f. *dextrum*, *E. williamsi* f. *williamsi* ve *E. w. f. turcicum*'un koyunlarda ikinci kez belirlenmişlerdir. Bu çalışmada belirlenen bütün türler, Kıbrıs koyunları için ilk kez rapor edilmişlerdir. Kıbrıs evcil koyunundaki entodiniid siliyat faunası, farklı coğrafi bölgelerde (Türkiye, Çin, Japonya, İskoçya, USA, Kanada ve Alaska) yaşayan koyunlardakilerle karşılaştırıldığında yüksek bir tür çeşitliliğine sahip olduğu görülür.

**Anahtar Sözcükler:** *Ovis ammon aries*, koyun, silli protozoan, Entodiniidae, fauna, işkembe, Kıbrıs

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## Introduction

The species of ciliate protozoa found in the rumen of all wild and domesticated ruminants also occur in the stomach of pseudo-ruminant camelids, and in the gastrointestinal contents of some non-ruminants, such as the hippopotamus and capybara (Thurston and Noirot-Timotheé, 1973; Ogimoto and Imai, 1981; Dehority, 1987; Williams and Coleman, 1992). These organisms can be important due to their digestive capabilities by contributing nutrients to the host animals (Williams, 1979; Shinchi et al., 1986; Nagasawa et al., 1992; Williams and Coleman, 1992). Rumen ciliate species composition varies both by host species and geographic region. Transfaunation of ciliates has been assumed to occur only by direct contact between hosts (Imai, 1981; Ogimoto and Imai, 1981; Imai, 1984; Ito et al., 1994). Although Dogiel (1927) reported that rumen ciliate composition is determined by phylogenetic factors and geographical distribution, Dehority (1978) showed that species composition appears to be primarily controlled by the type and amount of feed consumed. Ogimoto and Imai (1981) reported that the fauna was influenced by physiological conditions of the host, and that if the host was exposed to any kind of feed-related stress (starvation, rumen acidosis, etc.), ciliate protozoa composition in the rumen may be altered (Williams and Coleman, 1988, 1992). A large number of ciliate species have been observed in different host animals and under different conditions; however, Dehority and Orpin (1997) reported that the number of species in a specific animal is generally limited to 35 or fewer.

Several reports of the complete rumen ciliate fauna of different breeds of sheep have been published. Two of these studies were of animals from China and Japan (Hsiung, 1931; Imai et al., 1978), 2 from Europe [Scotland and Portugal] (Eadie, 1957; Marinho, 1983), 3 from North America [USA and Canada] (Bush and Kofoid, 1948; Dehority, 1974; Imai et al., 1989), and 2 from Turkey (Öktem et al., 1997; Göçmen et al., 1999). To date, it has been reported that worldwide approximately 120 species of Entodiniidae (based on the classification scheme proposed by Grain, 1994) have been observed in the rumen of animals (Williams and Coleman, 1992). During the last decade there have been a number of new additions to this list (Ito and Imai, 1990; Ito et al., 1994; Dehority, 1995; Göçmen and Öktem, 1996; Öktem and Göçmen, 1996; Öktem et al., 1997; Göçmen et al.,

2001a; Göçmen and Rastgeldi, 2004). Imai et al. (1989) and Göçmen et al. (2005) reported that the ciliate populations inhabiting the rumen of domestic cattle, sheep, and goats are generally similar; however, there are also characteristic variations in the occurrence of some species, according to geographical location.

Only a few reports on the rumen ciliate species composition of Cypriot domestic sheep (*Ovis ammon aries* L.) and Cypriot domestic goats (*Capra hircus* L.) have been conducted (Göçmen et al., 2001b; Göçmen, 2003; Göçmen and Sezgin, 2006; Mermer et al., 2006). Because of the large number of species present within Entodiniidae, only those ciliates belonging to the families Ophryoscolecidae and Isotrichiidae were identified to the species/form level in our previous studies (Göçmen et al., 2001b; Göçmen, 2003). Therefore, in the present study, those protozoa belonging to the genus *Entodinium* were identified in the rumen contents of the same Cypriot domestic sheep.

## Materials and Methods

Samples of rumen contents were obtained from 10 mature domestic sheep (*Ovis ammon aries*) at a slaughterhouse in Lapitos-Kyrenia (Girne). The animals grazed on the plateaus all day and were fed 0.5-1 kg of wheat straw and barley fracture twice a day, at 0600 and 2100. The sheep were generally slaughtered between 1500 and 1600, and samples were taken immediately thereafter. Sample collection, fixation, storage, and counting methods were previously described (Göçmen et al., 2001b). Specimens were examined using a Jena NF-binocular microscope and an Olympus BX51-Altra 20 Soft Imaging System. All cell measurements were made with a calibrated ocular micrometer.

Classification of species within the order Entodiniomorphida was based on the scheme proposed by Grain (1994), in which the sub-family Entodiniinae has been deleted from the family Ophryoscolecidae and elevated to family status with 2 genera—*Entodinium* and *Parentodinium*. The identification of species and forms was based on previously published descriptions and taxonomic lists (Dogiel, 1927; Kofoid and MacLennan, 1930; Das-Gupta, 1935; Bush and Kofoid, 1948; Lubinsky, 1958; Dehority, 1974; Imai, 1981; Ogimoto and Imai, 1981; Imai, 1984; Williams and Coleman, 1992; Grain, 1994; Dehority, 1995; Göçmen and Öktem,

1996; Öktem and Göçmen, 1996; Göçmen et al., 2001a; Göçmen and Rastgeldi, 2004). Drawings of the new ciliate species were based on photomicrographs and observations of cells stained with MFS (Ogimoto and Imai, 1981). All cell measurements were made with a calibrated ocular micrometer, according to Dogiel (1927), Kofoid and MacLennan (1930), and Dehority (1974). The terminology and orientation used in describing the structures of the new ciliate species followed the conventional system for ciliates proposed by Dogiel (1927) and Grain (1994).

The following morphological characters were measured: Body length (L), body width (W) (dorsoventral diameter), body length/width ratio (L/W), macronucleus length (MaL), macronucleus width (MaW), macronucleus length/width ratio (MaL/MaW), and body length/macronuclear length ratio (L/MaL). Cluster analysis (Jaccard, group average) using the Jaccard distance measure, based on the "presence/absence data" (Pielou, 1984) within the Biodiversity Professional Program (NHM and SAMS, 1997), was used to detect the similarities or/and distances between the entodiniid fauna from different geographical locations.

## Results

The total number and distribution of ciliate protozoa in the rumen contents of 10 domestic sheep from Cyprus are presented in Table 1. Although there was a range in total number,  $16.75-85.75 \times 10^4$  ciliates/ml of rumen fluid, family distribution was very similar, with the exception of sheep no. 3 and 4, which did not contain any isotrichiids (3 and 4) or ophryoscolecids (3); the majority of ciliates present in all 10 animals belonged to the family Entodiniidae (52.3%-100%).

As a result of our survey, 27 species of *Entodinium*, including 33 forms, were identified in Cypriot domestic sheep (Figure 1). Distribution by percentage of rumen entodiniid ciliate species was determined for all animals examined (Table 2). Considerable variation was noted in the fauna of these 10 domestic sheep. For example, there were many differences in the ciliate species detected and the frequency with which they were observed. Among the 27 species, only 1 (*E. parvum*) occurred in all 10 sheep, 2 (*E. caudatum* and *E. exiguum*) occurred in 9 sheep, 3 (*E. dubardi*, *E. minimum*, and *E. nanellum*) occurred in 8 sheep, 2 (*E. dilobum* and *E. rectangulatum*) occurred in 7 sheep, 3 (*E. longinucleatum*, *E. simplex*, and *E. simulans*) occurred in 6 sheep, 1 (*E. ovinum*) occurred in 5 sheep, and the other remaining species were observed in 1-3 sheep.

The number of rumen ciliate species per sheep ranged between 6 (in sheep no. 5) and 17 (in sheep no. 2) (mean:  $11.3 \pm 1.12$  per sheep). Two or 3 forms of the species *E. anteronucleatum*, *E. caudatum*, *E. simulans*, and *E. williamsi* were identified in the Cypriot domestic sheep. An unusual form of entodinia was observed in 2 sheep and was identified as a new species. The description of the new species is given below, based on the measurement of 25 specimens.

*Entodinium cypriensis* sp. nov. (Figures 1 and 2)

### Diagnosis

Body roughly ovoid to ellipsoid, generally widest at the 1/3 anterior level of the body; ventral side of the body convex, dorsal side generally has a weak and wide depression in the body wall at the midpoint along the length of the body; the posterior end is smoothly rounded; however, the ventral side has a small but pronounced lobous margin. In contracted specimens, the

Table 1. Concentrations and familial distribution of ciliate protozoa in the rumen contents of 10 sheep from northern Cyprus.

	Sheep no.									
	1	2	3	4	5	6	7	8	9	10
Total ciliates ( $\times 10^4$ )/ml	85.75	16.75	51.50	46.75	23.25	40.25	34.00	30.75	59.75	29.75
Family distribution (%)										
Isotrichidae	12.5	20.0	-	-	20.0	30.0	10.7	24.3	2.6	3.4
Ophryoscolecidae	9.2	23.0	-	0.4	40.0	17.0	8.8	23.4	36.8	42.7
Entodiniidae	78.2	57.0	100.0	99.6	40.0	53.0	80.5	52.3	60.6	53.9

Table 2. Distribution by percentage of species and forms of rumen ciliates belonging to the family Entodiniidae/genus *Entodinium* in the rumen contents of 10 sheep from northern Cyprus.

Sp. #	Species Form	Sheep no.									
		1	2	3	4	5	6	7	8	9	10
1	<i>E. anteronucleatum</i> Dogiel f. <i>leave</i> Dogiel	-	-	-	-	-	-	-	1.77	1.17	0.91
	<i>E. anteronucleatum</i> Dogiel f. <i>dilobum</i> Dogiel	-	-	-	-	-	-	-	0.89	-	-
2	<i>E. biconcavum</i> Kofoid & MacLennan	-	1.93	-	-	-	-	5.65	-	-	-
3	<i>E. bursa</i> Stein	-	-	-	-	-	-	1.41	-	-	-
4	<i>E. caudatum</i> Stein f. <i>caudatum</i> Stein	7.49	1.93	-	15.85	6.86	6.46	14.12	6.21	3.50	6.39
	<i>E. caudatum</i> Stein f. <i>lobosospinosum</i> Dogiel	-	0.97	-	6.79	3.43	5.17	1.41	0.89	-	1.83
5	<i>E. costatum</i> MacLennan	-	3.86	-	-	-	-	-	2.66	1.17	-
6	<i>E. cypriensis</i> n. sp.	0.83	-	-	-	-	-	2.82	-	-	-
7	<i>E. dalli</i> Dehority f. <i>rudidorsospinatum</i> Göçmen & Öktem	-	-	-	2.26	-	-	-	0.89	-	-
8	<i>E. dilobum</i> (Dogiel)	1.66	2.90	26.56	-	-	1.94	5.65	1.77	-	0.91
9	<i>E. dubardi</i> Buisson f. <i>dubardi</i> Buisson	9.15	6.76	20.31	20.37	4.57	-	9.89	-	8.16	5.48
10	<i>E. ekendrae</i> Das-Gupta	-	-	-	-	-	-	-	-	1.17	-
11	<i>E. exiguum</i> Dogiel	4.16	1.93	9.38	2.26	-	2.59	-	3.55	16.32	7.31
12	<i>E. imaii</i> Göçmen, Tosunoglu & Falakali	2.50	-	-	-	-	1.29	-	-	-	-
13	<i>E. longinucleatum</i> Dogiel f. <i>longinucleatum</i> Dogiel	11.65	4.83	14.06	-	-	-	5.65	-	5.83	8.22
14	<i>E. minimum</i> Schuberg	8.32	0.97	-	-	10.29	4.52	4.24	4.43	4.66	7.31
15	<i>E. nanellum</i> Dogiel	4.99	3.86	7.81	-	-	9.05	9.89	13.30	2.33	4.57
16	<i>E. oektemae</i> Göçmen, Tosunoglu & Falakali	-	-	-	-	-	-	-	0.89	-	-
17	<i>E. ogimotoi</i> Imai	-	-	-	-	-	-	-	0.89	-	-
18	<i>E. ovinum</i> Dogiel	2.50	1.93	3.13	-	3.43	-	2.82	-	-	-
19	<i>E. parvum</i> Buisson f. <i>parvum</i> Buisson	5.82	3.86	3.13	2.26	5.71	2.59	15.54	4.43	3.49	3.65
20	<i>E. protuberans</i> Bush & Kofoid	-	-	-	-	-	-	-	-	1.17	-
21	<i>E. quadricuspis</i>	-	0.97	-	4.53	-	-	-	0.89	-	-
22	<i>E. rectangulatum</i> Kofoid & MacLennan f. <i>rectangulatum</i> Kofoid & MacLennan <i>E. rectangulatum</i> Kofoid & MacLennan f. <i>lobosospinosum</i> Lubinsky <i>E. rectangulatum</i> Kofoid & MacLennan f. <i>dubardi</i> Lubinsky	1.66	-	-	18.11	4.57	5.82	-	-	4.66	2.74
		2.50	0.97	-	11.32	1.14	3.88	-	-	3.50	1.83
		4.99	3.86	-	6.79	-	1.29	-	-	2.33	-
23	<i>E. rostratum</i> Fiorentini	-	1.93	-	-	-	-	-	-	-	-
24	<i>E. simplex</i> Dogiel	6.66	9.66	15.63	-	-	-	1.41	1.77	-	2.74
25	<i>E. simulans</i> Lubinsky f. <i>lobosospinosum</i> Lubinsky <i>E. simulans</i> Lubinsky f. <i>caudatum</i> Lubinsky	1.66	0.97	-	4.53	-	3.88	-	0.89	-	-
		1.66	1.93	-	4.53	-	4.52	-	3.54	1.17	-
26	<i>E. triacum</i> Buisson f. <i>dextrum</i> Dogiel	-	0.97	-	-	-	-	-	-	-	-
27	<i>E. williamsi</i> Göçmen & Öktem f. <i>williamsi</i> Göçmen & Öktem <i>E. williamsi</i> Göçmen & Öktem f. <i>turcicum</i> Göçmen & Öktem	-	-	-	-	-	-	-	1.77	-	-
		-	-	-	-	-	-	-	0.89	-	-
Total no. of species		14	17	8	8	6	9	12	15	13	11
Total no. of formae		17	20	8	12	8	13	13	19	15	13

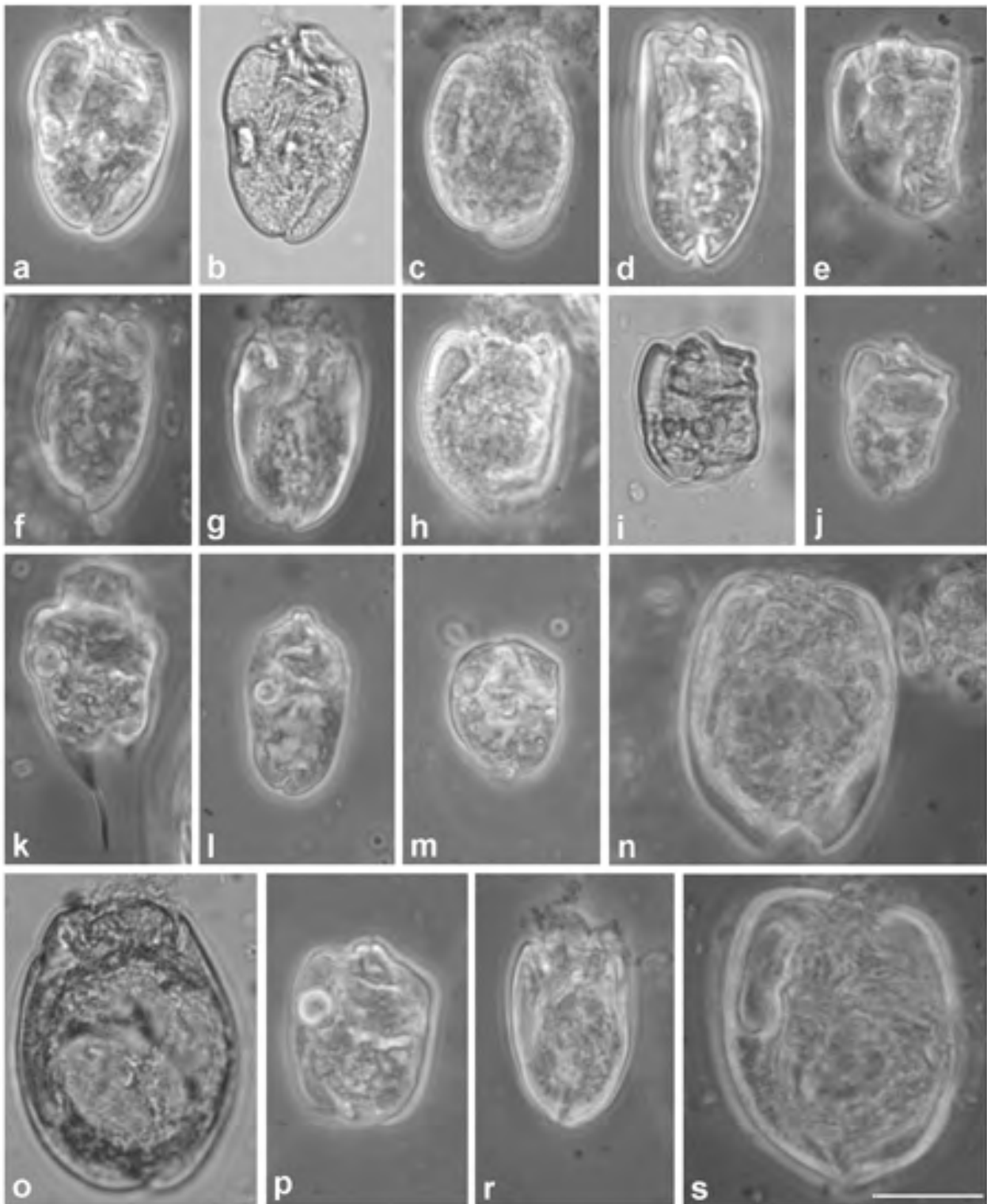


Figure 1. Photomicrographs of some entodiniid rumen ciliates observed in the rumen contents from Cypriot domestic sheep. a and b: *Entodinium cypriensis* sp. nov.; c: *E. biconcavum*; d: *E. costatum*; e: *E. ekendrae*; f: *E. rectangulatum* f. *dubardi*; g: *E. quadricuspis*; h: *E. imai*; i: *E. ogimotoi*; j: *E. anteronucleatum* f. *dilobum*; k: *E. oektemae*, l: *E. protuberans*; m: *E. dalli* f. *rudidorsospinatum*; n: *E. dilobum*; o: *E. ovinum*; p: *E. williamsi* f. *williamsi*; r: *E. simplex*; s: *E. dubardi*. All photos were taken from the right side. Bar is 20  $\mu$ m in all photos.

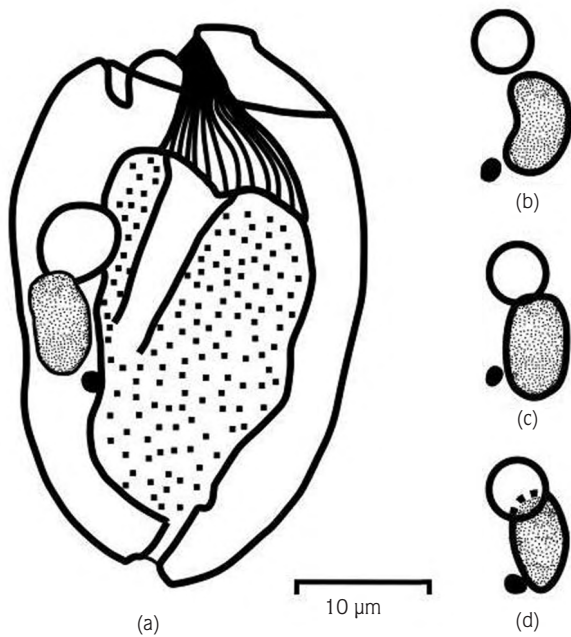


Figure 2. Drawings of *Entodinium cypriensis* sp. nov. made from the right side (a), variations seen in both the shape and location of the macro- and micronucleus, and the contractile vacuole from as seen from the left side (b-d).

adoral lips, especially the dorsal one, protrude beyond the concave curve of the anterior end. Macronucleus is ovoid to ellipsoid in side view and situated at the mid-level of the dorsal side of the body. The micronucleus is spherical in shape and generally located near the ventral posterior edge of the macronucleus. The contractile vacuole lies to the anterior side, and partially left and ventral of the macronucleus. The ectoplasm (central periplasm) does not penetrate beneath the adoral ciliary zone (ACZ).

### Description

In side view (Figure 1a, b, and Figure 2a, b), the body is roughly ovoid to ellipsoid; generally the widest diameter occurs at the 1/3 anterior level of the body. Body length is 39.20 (35.00-45.00) μm, body width is 25.90 (22.50-30.00) μm, and L/W ratio is 1.51 (1.27-1.70) (n = 25). The ventral side of the body is convex, but the dorsal side generally has a weak and wide depression in the body wall at the midpoint along the length of the body. The posterior part of the body is relatively narrower than the anterior part. The posterior end of the body has a small but pronounced ventral lobous margin that protrudes beyond the posterior end of the cytoproctal tube.

The macronucleus is ellipsoid and partially bean-shaped, is occasionally ovoid in side view, and lies on the dorsal side, generally at the mid-level of the body or occasionally somewhat posterior of this position. The L/MaL ratio is 3.13 (2.42-3.75).

When viewed from the dorsal side of the body, sometimes the macronucleus covers the micronucleus (Figure 2c, d). The micronucleus is spherical in shape (2.5 μm) and generally located near or occasionally on the ventral posterior edge of the macronucleus.

The contractile vacuole generally lies to the anterior side and partially left and ventral of the macronucleus (Figure 2a, c, d). Occasionally it is situated near the anterior side of the macronucleus (Figure 2b).

The vestibulum and nasse (esophagus) are a long funnel-shape and bend towards to the dorsal side and macronucleus, without having a pronounced curvature, terminating in a region near the posterior end of the macronucleus. The endoplasm (peripheral periplasm) penetrates the zone beneath the ACZ, which is at an angle approximately 20° to the main body axis. The ectoplasm (central periplasm) occupies about 2/3 of the cell. In contracted specimens the adoral lips, especially the dorsal one, protrude beyond the concave curve of the anterior end.

The cytoproctal tube is relatively long and narrow, at an angle of about 45° to the main body axis and opens near the end of the dorsal side margin.

Measurements of 25 cells of *Entodinium cypriensis* sp. nov. from Cypriot domestic sheep no. 7 are given in Table 3.

### Variations

The species appears to be fairly consistent in size and general morphology, although the macronucleus shape can be ovoid (Figure 2d) (12%). Additionally, in some specimens the contractile vacuole is located near the anterior side of the macronucleus (Figure 2b) (36%), and the micronucleus is located on the ventral posterior edge of the macronucleus (Figure 2d) (32%).

### Type Host, Locality, and Habitat

Domestic sheep, *Ovis ammon aries* L., in Lapethos, Kyrenia, Northern Cyprus, habitat rumen.

Table 3. Measurements and ratios of *Entodinium cypriensis* sp. nov. L: body length, W: body width (dorsoventral diameter); MaL: macronucleus length; MaW: macronucleus width; L/W: body length/width ratio; MaL/MaW: macronucleus length/width ratio; L/MaL: body length/macronuclear length ratio; SE: standard error of mean; SD: standard deviation.

Characters	Mean	Range	SE	SD
L	39.20	35.00-45.00	0.60	3.03
W	25.90	22.50-30.00	0.37	1.89
MaL	12.70	10.00-17.50	0.37	1.89
MaW	6.70	5.00-7.50	0.23	1.19
L/W	1.51	1.27-1.70	0.02	0.10
MaL/MaW	1.93	1.33-3.00	0.08	0.43
L/MaL	3.13	2.42-3.75	0.07	0.38

### Occurrence

This new species constituted 0.83% and 2.82% of the total ciliate protozoa in sheep no. 1 and no. 7, respectively, with an appearance frequency of 20%.

### Etymology

*Entodinium cypriensis* sp. nov. is named for the island upon which the new species was found.

### Type Material

The holotype and paratypes are kept on the slides numbered ZSBEU-RCS.2/PN 1-2 and in MFS-glycerin media (in a 1:1 ratio) dated 28 May 1996. The specimens are stored in the rumen ciliates of sheep (RCS) collection of the International ZDEU Museum [Zoology Section, Department of Biology, Faculty of Science, Ege University (ZDEU-ZSBEU), Bornova, Izmir, Turkey.

### Remarks

*Entodinium cypriensis* sp. nov. closely resembles *E. constrictum* Dehority, 1974, *E. protuberans* Bush and Kofoid, 1948, and *E. exiguum* Dogiel, 1925 in terms of body size, general shape of the macronucleus, and position of the contractile vacuole and cytoproctal tube. Nonetheless, it is differentiated from all species mentioned by its general body shape and is differentiated from both *E. constrictum* and *E. exiguum* by the position of its micronucleus, which is situated at the anterior or anterior part of the macronucleus. Additionally, the macronuclear shape shows wide variation (elliptic to elongated) in *E. exiguum* (Lubinsky, 1958). Additionally, one of the principal distinguishing characteristics of *E. exiguum* is that the esophagus is parallel to the body length axis; however, the macronuclear shape is very

consistent in the new species. *E. cypriensis* sp. nov. also differs from *E. protuberans* by having an oblique and long funnel-shaped “vestibulum and nasse” (esophagus), more elliptical macronucleus, and a contractile vacuole located approximately at the level of 2/3 of the body length, instead of at the anterior end of the body. In addition to the differences mentioned above, the new species also differs from *E. constrictum* in the following characteristics: (a) A weak and wide depression in the body wall on the dorsal side; (b) in contracted specimens, the adoral lips protrude beyond the concave curve of the anterior end; and (c) the esophagus is long, exceeding the level of the posterior half of the body and the posterior end of the macronucleus. *E. cypriensis* sp. nov. also resembles *E. truncatum* Bush and Kofoid, 1948 in the shape and position of its macronucleus; however, the posterior end of the body of *E. truncatum* is truncate, and the micronucleus is located at the middle of the macronucleus, which has a wide depression.

### Discussion and Conclusion

In our previous study on Cypriot domestic sheep (Göçmen et al., 2001b) we determined that the overall mean concentration of ciliates ( $37.84 \times 10^4 \text{ ml}^{-1}$ ) was somewhat lower than reports about Turkish domestic sheep ( $53.9 \times 10^4 \text{ ml}^{-1}$ , Öktem et al., 1997) and Turkish domestic cattle ( $59.52 \times 10^4 \text{ ml}^{-1}$ , Göçmen et al., 2003). Nonetheless, the mean ciliate concentration reported by Göçmen et al. (2005) in Turkish domestic goats ( $33.21 \times 10^4 \text{ ml}^{-1}$ ) was slightly lower. These variations may be a result of differences in geographical location, feeding habitats, and feedstuffs.

In the course of examining 10 Cypriot sheep, 27 species of *Entodinium*, including 33 forms, were identified (Table 2). An unusual form of *Entodinium* was observed in 2 animals and is described as a new species, *E. cypriensis* sp. nov. The new species appears to be unique to the rumen of Cypriot domestic sheep, but was only observed in 2 of the 10 sheep examined.

Among the 27 species identified, 12 species (*E. parvum*, *E. caudatum*, *E. exiguum*, *E. dubardi*, *E. minimum*, *E. nanellum*, *E. dilobum*, *E. rectangulatum*, *E. longinucleatum*, *E. simplex*, *E. simulans*, and *E. ovinum*) were observed in at least 50% of Cypriot domestic sheep. These species also have worldwide distribution in many ruminants (Dogiel, 1927; Ogimoto and Imai, 1981; Williams and Coleman, 1992).

The present study reported new host records for *E. imai*, *E. oektemae*, *E. ogimotoi*, *E. quadricuspis*, *E. rectangulatum* f. *dubardi*, and *E. rostratum* (Table 4). Both *E. imai* and *E. oektemae*, originally identified in Turkish domestic cattle (Göçmen et al., 2001a, 2003), were observed in the rumen of Cypriot domestic sheep. *Entodinium ogimotoi* was originally observed and described in water buffalo from Japan (Imai, 1981), and was observed in Cypriot domestic sheep, accounting for a new host record for sheep. *Entodinium quadricuspis* was commonly observed in deer (Dogiel, 1925, 1927; Lubinsky, 1958; Westerling, 1970; Dehority, 1986a, 1986b; Dehority et al., 1999), but rarely in cattle (Latteur, 1969; Göçmen et al., 2003) and giraffes (Kleynhans and Van Hoven, 1976); however, it was observed in sheep during the present study. Moreover, *E. rectangulatum* f. *dubardi* has been typically observed in cattle (domestic and zebu) worldwide (Imai and Ogimoto, 1984; Dehority, 1986b; Göçmen et al., 2003), in Brazilian water buffalo (Dehority, 1979), and in Turkish domestic goats (Göçmen et al., 2005). The present study reported a new host record for Cypriot sheep. *Entodinium rostratum*, which is considerably less common (Williams and Coleman, 1992), is widely distributed in many host species (cattle, water buffalo, African reedbeek, and tsessebe) (Imai et al., 1978; Göçmen et al., 2003) and was a new host record from sheep rumen contents.

Our investigation also reports for the second time the presence of *E. biconcavum*, *E. costatum*, *E. dalli* f. *rudidorsospinatum*, *E. protuberans*, *E. rectangulatum* f. *lobosospinosum*, *E. simulans* f. *lobosospinosum*, *E.*

*triacum* f. *dextrum*, *E. williamsi* f. *williamsi*, and *E. w. f. turcicum* in sheep hosts (Table 4). All species detected in the present study represent first reports for their occurrence in Cypriot sheep. The 27 *Entodinium* species (Table 4) from Cypriot sheep appears to be greater than the number of species observed in sheep from other geographical locations: Turkey [18 species] (Öktem et al., 1997), Portugal [18 species] (Marinho, 1983), Japan [12 species] (Imai et al., 1978), China [11 species] (Hsiung, 1931), Canada [8 species] (Imai et al., 1989), Scotland [8 species] (Eadie, 1957), Alaska [8 species] (Dehority, 1974), and the USA [5 species] (Bush and Kofoid, 1948).

Similarity and/or distance of the entodiniid ciliate fauna compositions from different geographic localities were determined and summarized with a dendrogram (Figure 3) and a similarity matrix (Table 5). According to Jaccard's cluster analysis results, the degree of similarity of the sheep entodiniid protozoa faunas from the different geographical localities varied widely (Table 5); however, these similarities were grouped into 3 clades, as demonstrated in the cluster analysis dendrogram (Figure 3). It is possible to say that the similarity was high in neighboring areas and that species number and/or diversity was quite similar. This positive correlation between the similarity of faunas and the number of species in neighboring areas indicates that rumen ciliate composition is determined by geographical distribution, as indicated by Dogiel (1927). When the results obtained in this Cypriot study are compared with those from other countries, the number of species observed in Cypriot sheep was much higher than that observed in all other countries. Apparently, the total number of species exhibits an equatorial-polar gradient in species richness. This finding also indicates that climatic factors play an important role in species diversity.

Among the 27 species recorded from the island of Cyprus, 16 (59.26%) and 17 (62.96%) are taxonomically identical to their conspecifics on the mainland of Portugal and Turkey, respectively. The remaining species are also known from other areas, with the exception of one, which was described as a new species, *E. cypriensis* sp. nov. This means that they have not undergone widespread specific differentiation since they first reached the island. This zoogeographic pattern of insular endemism is similar to that observed in other groups (e.g. reptiles and mammals) (Spitzenberger, 1979; Göçmen and Böhme, 2002; Göçmen et al., 2007a,



Table 4. Distribution of species and forms of entodiniid ciliates in the rumen contents of sheep at various locations throughout the world.

Geographical locations References*	China (1)	Scotland (2)	Alaska (3)	USA (4)	Japan (5)	Portugal (6)	Canada (7)	Turkey (8)	Cyprus (9)
<i>E. angustatum</i>	+	-	-	-	-	-	-	-	-
<i>E. anteronucleatum</i> f. <i>dilobum</i>	-	-	+?	-	-	+?	-	-	+
<i>E. anteronucleatum</i> f. <i>laeve</i>	-	-	+?	-	-	+?	-	+	+
<i>E. babicii</i>	-	+	-	-	-	-	-	-	-
<i>E. bicaudatum</i>	-	-	+	+	-	-	-	-	-
<i>E. biconcavum</i>	-	-	-	-	-	+	-	-	+
<i>E. bicornutum</i>	-	-	-	-	-	+	-	-	-
<i>E. bimastus</i>	+	-	-	-	-	-	-	-	-
<i>E. bursa</i>	+	-	-	-	+	+	+	+	+
<i>E. caudatum</i> f. <i>caudatum</i>	+	+	-	+	+	+	+	+	+
<i>E. caudatum</i> f. <i>lobosospinosum</i>	+	+	-	-	+	-	+	+	+
<i>E. constrictum</i>	-	-	+	-	-	-	-	+	-
<i>E. costatum</i>	-	-	-	-	-	+	-	-	+
<i>E. cypriensis</i> n. sp.	-	-	-	-	-	-	-	-	+
<i>E. dalli</i> f. <i>dalli</i>	-	-	+	-	-	-	-	-	-
<i>E. dalli</i> f. <i>rudidorsospinatum</i>	-	-	-	-	-	-	-	+	+
<i>E. dilobum</i>	+	-	-	-	+	+	-	+	+
<i>E. dubardi</i> f. <i>dubardi</i>	+	+	-	-	+	+	-	+	+
<i>E. ekendrae</i>	-	-	-	-	+	+	-	-	+
<i>E. ellipsoideum</i>	-	+	-	-	-	-	-	-	-
<i>E. exiguum</i>	-	+	+	-	+	+	+	+	+
<i>E. imaii</i>	-	-	-	-	-	-	-	-	+
<i>E. longinucleatum</i> f. <i>longinucleatum</i>	+	+	-	-	+	+	+	+	+
<i>E. minimum</i>	+	-	-	-	+	-	-	+	+
<i>E. nanellum</i>	-	-	-	-	+	+	+	+	+
<i>E. nanum</i>	-	+	+	+	-	-	-	-	-
<i>E. oektemae</i>	-	-	-	-	-	-	-	-	+
<i>E. ogimotoi</i>	-	-	-	-	-	-	-	-	+
<i>E. orbicularis</i>	-	-	+	+	-	-	-	-	-
<i>E. ovinum</i>	+	-	-	-	+	+	+	+	+
<i>E. ovoideum</i>	-	-	-	-	-	-	+	-	-
<i>E. parvum</i> f. <i>parvum</i>	-	-	-	-	+	+	+	+	+
<i>E. protuberans</i>	-	-	+	-	-	-	-	-	+
<i>E. quadricuspis</i>	-	-	-	-	-	-	-	-	+
<i>E. rectangulatum</i> f. <i>dubardi</i>	-	-	-	-	-	-	-	-	+
<i>E. rectangulatum</i> f. <i>lobosospinosum</i>	-	-	-	-	-	-	-	+	+
<i>E. rectangulatum</i> f. <i>rectangulatum</i>	-	-	-	-	-	+	-	+	+
<i>E. rostratum</i>	-	-	-	-	-	-	-	-	+
<i>E. semahatae</i>	-	-	-	-	-	-	-	+	-
<i>E. simplex</i>	+	+	-	+	+	+	+	+	+
<i>E. simulans</i> f. <i>caudatum</i>	-	-	-	-	-	+	-	+	+
<i>E. simulans</i> f. <i>lobosospinosum</i>	-	-	-	-	-	-	-	+	+
<i>E. triacum</i> f. <i>dextrum</i>	+	-	-	-	-	-	-	-	+
<i>E. williamsi</i> f. <i>turcicum</i>	-	-	-	-	-	-	-	+	+
<i>E. williamsi</i> f. <i>williamsi</i>	-	-	-	-	-	-	-	+	+
Total no. of species	11	8	8	5	12	18	8	18	27
Total no. of all forms	12	9	8	5	13	18	9	22	33

\*References: (1) Hsiung, 1931; (2) Eadie, 1957; (3) Dehority, 1974; (4) Bush and Kofoid, 1948; (5) Imai et al., 1978; (6) Marinho, 1983; (7) Imai et al., 1989; (8) Öktem et al., 1999; (9) Present study.. Data is not obvious in forms.

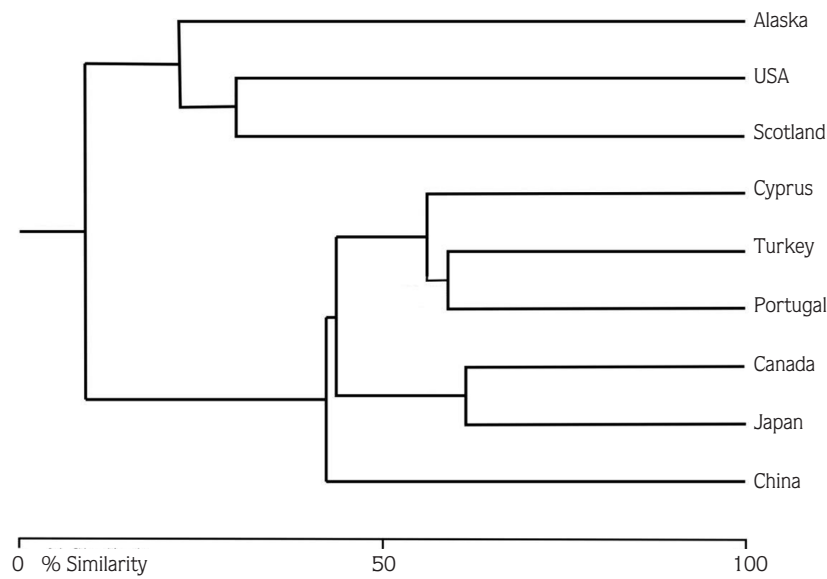


Figure 3. Cluster analysis dendrogram showing the similarities or/and distances of the rumen ciliate faunas from different geographical localities based on the data in the references given in Table 4.

Table 5. Jaccard's similarity matrix, indicating the similarity percentage values of the rumen ciliate faunas from different geographical localities based on the data in the references given in Table 4.

	China	Scotland	Alaska	USA	Japan	Portugal	Canada	Turkey	Cyprus
China	-	26.67	5.56	14.29	53.33	40.00	33.33	45.00	35.71
Scotland	-	-	14.29	30.00	33.33	25.00	30.77	23.81	16.67
Alaska	-	-	-	30.00	5.26	8.70	6.25	18.18	12.90
USA	-	-	-	-	13.33	10.00	16.67	9.52	6.67
Japan	-	-	-	-	-	61.11	61.54	57.89	44.44
Portugal	-	-	-	-	-	-	44.44	59.09	57.14
Canada	-	-	-	-	-	-	-	42.11	28.57
Turkey	-	-	-	-	-	-	-	-	55.17
Cyprus	-	-	-	-	-	-	-	-	-

b, 2008). Spitzenberger (1979), and Göçmen and Böhme (2002) have postulated that a land bridge to the opposite mainland explains the existence of several specific endemics. The geological history suggests that the last connection between Cyprus and the mainland dates back to the Miocene salinity crisis, which would lead us to expect many more endemics than we actually know. Therefore, additional studies of other Cypriot ruminants, especially endemic sheep such as Cyprus mouflon (*Ovis ammon ophion* Blyth 1841) that live only on Troodos Mountain (Southern Cyprus) need to be conducted.

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