Europ. J. Protistol. 32, 513–522 (1996)
December 31, 1996

European Journal of
PROTISTOLOGY

New Rumen Ciliates from Turkish Domestic Cattle (*Bos taurus* L.) I. The Presence of *Entodinium dalli* Dehority, 1974 with a New Forma, *E. dalli* f. *rudidorsospinatum* n. f. and Comparisons with *Entodinium williamsi* n. sp.

Bayram Göçmen and Nimet Öktem
Zoology Section, Department of Biology, Faculty of Science, Ege University, Bornova-Izmir, Turkey

SUMMARY

In the course of examining rumen contents obtained from 25 domestic cattle (*Bos taurus*) in the vicinity of Izmir, Turkey, two somewhat similar but unusual forms of entodinia were observed. These ciliates were observed in three of the animals and this study reports on their classification into two species, *Entodinium dalli* Dehority, 1974 and a new species, *Entodinium williamsi* n. sp. All the protozoa identified as belonging to *E. dalli* were distinguished as a new forma, *E. dalli* f. *rudidorsospinatum* n. f. of the basis of their caudal spines. The species *Entodinium williamsi* n. sp. was also divided into two formae, *E. williamsi* f. *williamsi* n. f. and *E. williamsi* f. *turcicum* n. f. The morphological characteristics of these protozoa are described and their relationships to similar entodiniid species are discussed.

Introduction

The composition of the rumen ciliate protozoal populations of domesticated ruminants have been surveyed by many investigators [4, 6, 8–12, 15, 16, 20–30, 32–34, 37, 39, 44]. However, there is little published information on the ciliated protozoa occurring in domestic ruminants from Turkey, which is the bridge connecting the continents of Europe and Asia.

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 [31, 42]. It is evident from earlier studies on the ciliate populations of domesticated numinants that although the species composition was generally similar, some characteristic geographical variations in the occurrence of some species occurred. Specifically, some species and formae were detected only in certain areas and host species [24, 33].

As there was no data available on the rumen ciliate composition of Turkish domestic ruminants we examined the ruminal ciliates of domesticated cattle, *Bos taurus* [17, 43]. In this study, we describe a new species belonging to subfamily *Entodiniinae* (Ophryoscolecidae) and a new forma belonging to *Entodinium dalli* Dehority, 1974. Statistical analysis, as used by Dehority [14] to determine morphological variation lines within the species, *Entodinium dubardi* Buisson, 1923 in the blue duiker, was applied to determine interrelationships between these ciliates; these findings are discussed.

Material and Methods

Samples of rumen contents were obtained from 25 adult domestic cattle, *Bos taurus* which were slaughtered at the slaughterhouse of Izmir from March 1990 to April 1994.

0932-4739-96-0032-0513$3.50-0

The cattle had been fed approximately 4 kg of mixed food (composed of oat hay, clover and white beet molasses) twice a day at 08^{00} and 16^{00}. Samples of ruminal contents after the 08^{00} h feed were collected. The rumen wall was cut with a knife and the sample removed via a cathater. Samples were immediately fixed and stained in methylgreen-formalin-saline (MFS) solution [42]. A part of each sample was stained with Mayer's hematoxylin, Heidenhain's iron-hematoxylin, and Chatton-Lwoff's silver impregnation technique [42], and prepared as permanent slides. Some specimens were also stained with 2% borax-methylen blue [5] after fixation with 10% formaldehyde solution for photography. Total ciliate numbers were determined by means of a Neubauer hemocytometer. Differential counts of species were calculated using smear slides as the number per total 250 or 500 cells.

Drawings of new ciliates were based on the photomicrographs and observations of the cells stained with MFS. All of the cell measurements were made with a calibrated ocular micrometer according to the system used by Dogiel [15], Kofoid & MacLennan [34], and Dehority [11]. Specimens were examined by Jena "NF-binocular" microscope and "MF" photomicrography accessory.

The terminology for orientation used in describing the structure of new ciliate species conforms to the conventional system of the ciliate kingdom proposed by Dogiel [15] and Grain [18]. This system appears to be more logical for general usage since the same terms are used for all members within the ciliate kingdom. According to this convention, the buccal side is considered as the ventral one, while the opposite side or the body surface closest to the nuclear apparatus is termed the dorsal one. A new terminology used by Grain [18] for the morphological structure of the order Entodinimorphida was taken into consideration when describing new ciliates.

Identification of the genus *Entodinium* and the species *Entodinium dalli* was based mainly on the descriptions of Kofoid & MacLennan [34]. Bush & Kofoid [3] and Dehority [7], and also the original photomicrographs of *E. dalli* kindly provided by Prof. Dr. Burk A. Dehority (The Ohio State University, Ohio, U.S.A.).

Data of various morphological characteristics (i.e., the length of body [L], the width of body [W], the length to width ratio [L/W], the macronucleus length [MAL] and the length to macronucleus length ratio [L/MAL]) were analyzed by the least squares analysis of variance [19] and the coefficient differences values [40]. The graphics of least squares differences [LSD] range tests of the analysis of variance [ANOVA] were obtained using STATGRAPHICS procedures [45]. The frequency histograms were calculated using MINITAB procedures [41].

Results

This study reports for the second time the presence of *Entodinium dalli* Dehority, 1974 in herbivorous mammals; it is the first report of its occurrence in domesticated cattle, *Bos taurus*. The specimens of *E. dalli* were observed in low numbers (17.13% and 0.60% of total ciliate protozoa in cattle nos. 9 and 23, respectively), and appeared to differ from the species description with regard to the length of posterior spine on the dorsal side (Figs. 1, 2, 8 and 9). A new morphologically distinct form is, therefore, proposed. The original species description of *E. dalli* Dehority, 1974 [7] has been amended to include the differences observed in our specimens and comprises two formae, *E. dalli* f. *dalli* n. f. Dehority [7] and *E. dalli* f. *rudidorsospinatum* n. f. which was found in the rumen contents from *Bos taurus* in Turkey.

A second form resembling *E. dalli* was observed only in cattle nos. 23 and 24 (Figs. 3–7 and 10–13). However, some differences between the two ciliates were evident. The length, width and length to width ratio of the latter form was higher (Table 1) and the central periplasm (ectoplasm) on the dorsal side of the body was thicker than that on the ventral side. In addition, the caudal spination differed from that of *E. dalli*. Measurements made on *E. dalli* f. *rudidorsospinatum* n. f. from animal 9 and on the second form from animal 23 were analysed statistically. Figure 14 compares these values according to the frequency histograms of the ciliates in mixed models. The protozoal populations were significantly different in all characteristics ($p < 10^{-4}$), i.e., L, W, L/W ratio, MAL, L/MAL ratio (Table 1). Histograms of these criteria for total 52 cells (26 cells per each ciliate population) (Fig. 14) indicate conclusively that two different populations or species are present because the values for all criteria are bimodally distributed, with no indication of symmetrical or normal distribution. The differences in size, L/W ratio and MAL are significant. Therefore, the second form which was found in the rumen contents of *B. taurus* was considered to be a new species, *Entodinium williamsi* n. sp., with two formae. The descriptions of these new ciliates follow.

Entodinium dalli Dehority, 1974

Diagnosis. Body roughly spherical to ellipsoid; dorsal side of the body generally convex, ventral side generally has a weak and wide depression in the body wall at the level of the base of adoral ciliary zone (ACZ) or smoothly convex; posterior end terminates in a long or rudimentary spine on the dorsal side, a blunt lobe on the left ventral side and a short spine on the right ventral side. The left ventral lobe follows the curvature of the ventral side wall terminating as a bluntly rounded triangle directed towards the dorsal side of the cell and also protrudes outward from the main body axis. On the right ventral side of the posterior extremity a short, acutely pointed spine is present; macronucleus is spherical to ellipsoid lying generally at the level of the base of ACZ; micronucleus is ellipsoid or spherical and situated at some distance posteriorly from the base of the ventral edge of the macronucleus.

Type forma. Entodinium dalli Dehority, 1974 forma *dalli* n. f.

Entodinium dalli Dehority, 1974 forma *dalli* n. f.

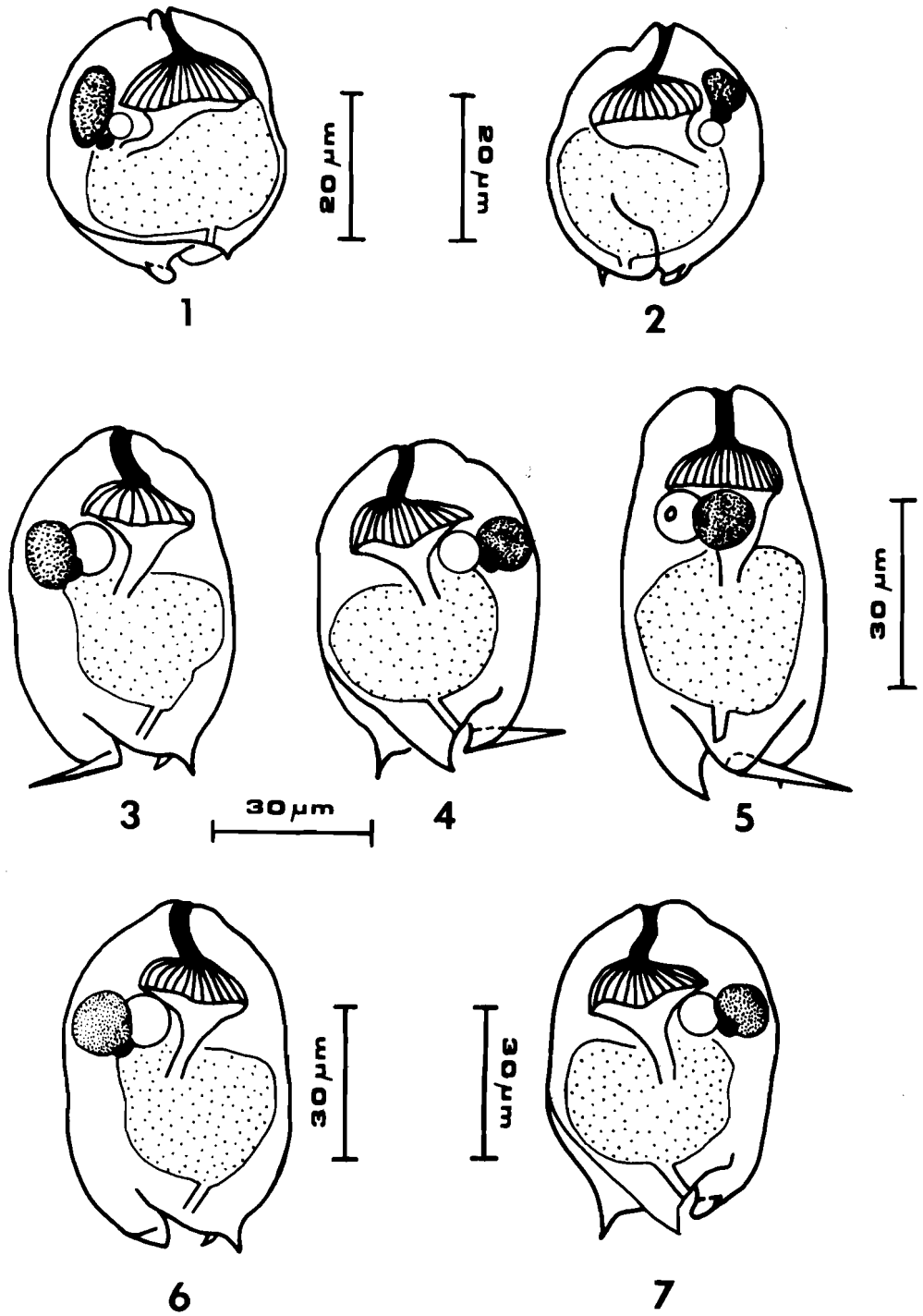
Diagnosis. On the dorsal side of the posterior end of the cells a long spine (22–25 μm in length) is present; A lobe and short spine are present on the ventral side. In

few individuals, the dorsal spine was absent or only rudimentary (unpublished data, personal communication Prof. Dr. B. A. Dehority).

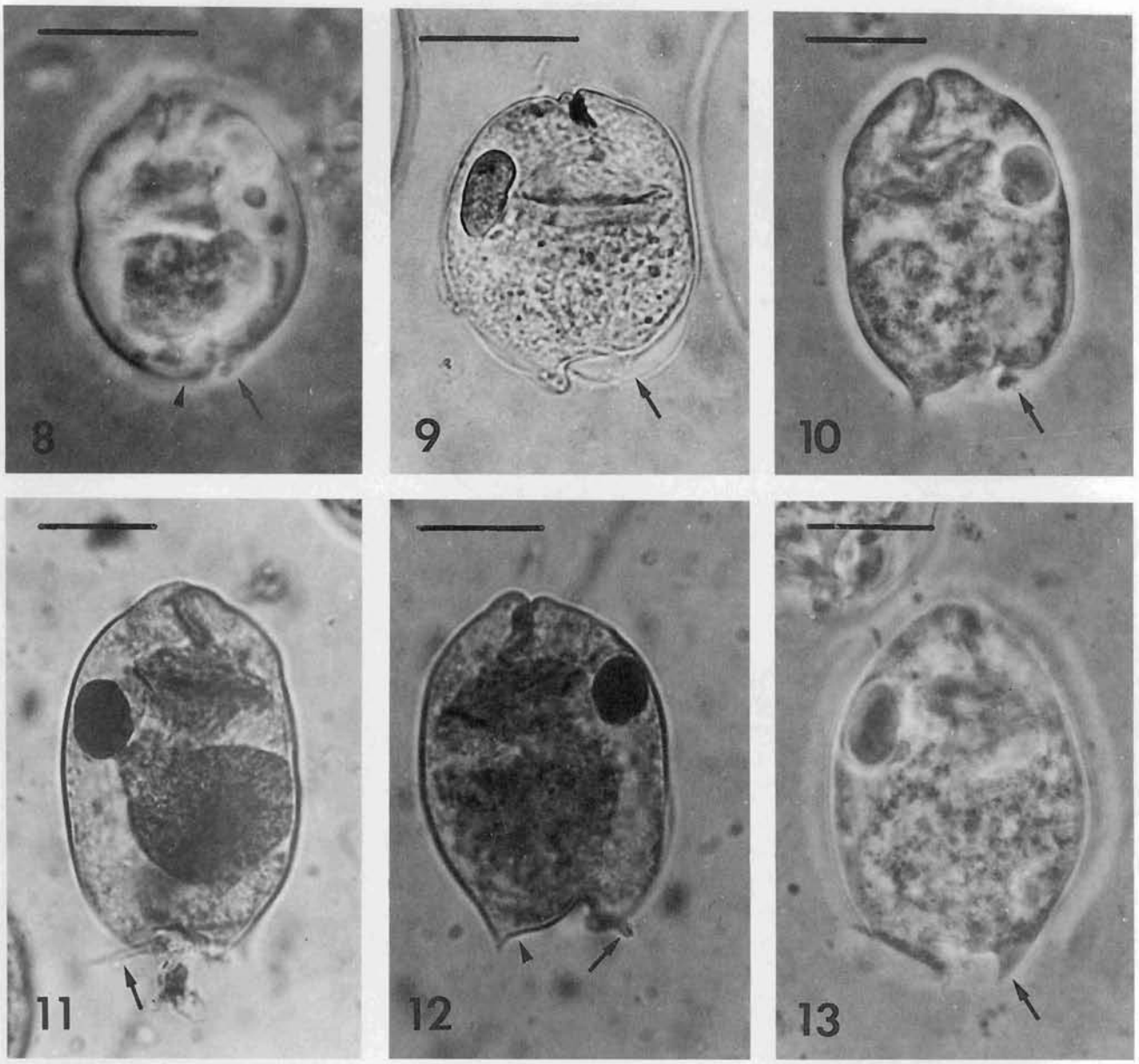
Description. E. dalli f. *dalli* n. f. is the "nominate" form and has been described by Dehority [7] and is not described further here.

Entodinium dalli Dehority, 1974 forma *rudidorsospinatum* n. f. (Figs. 1, 2, 8 and 9)

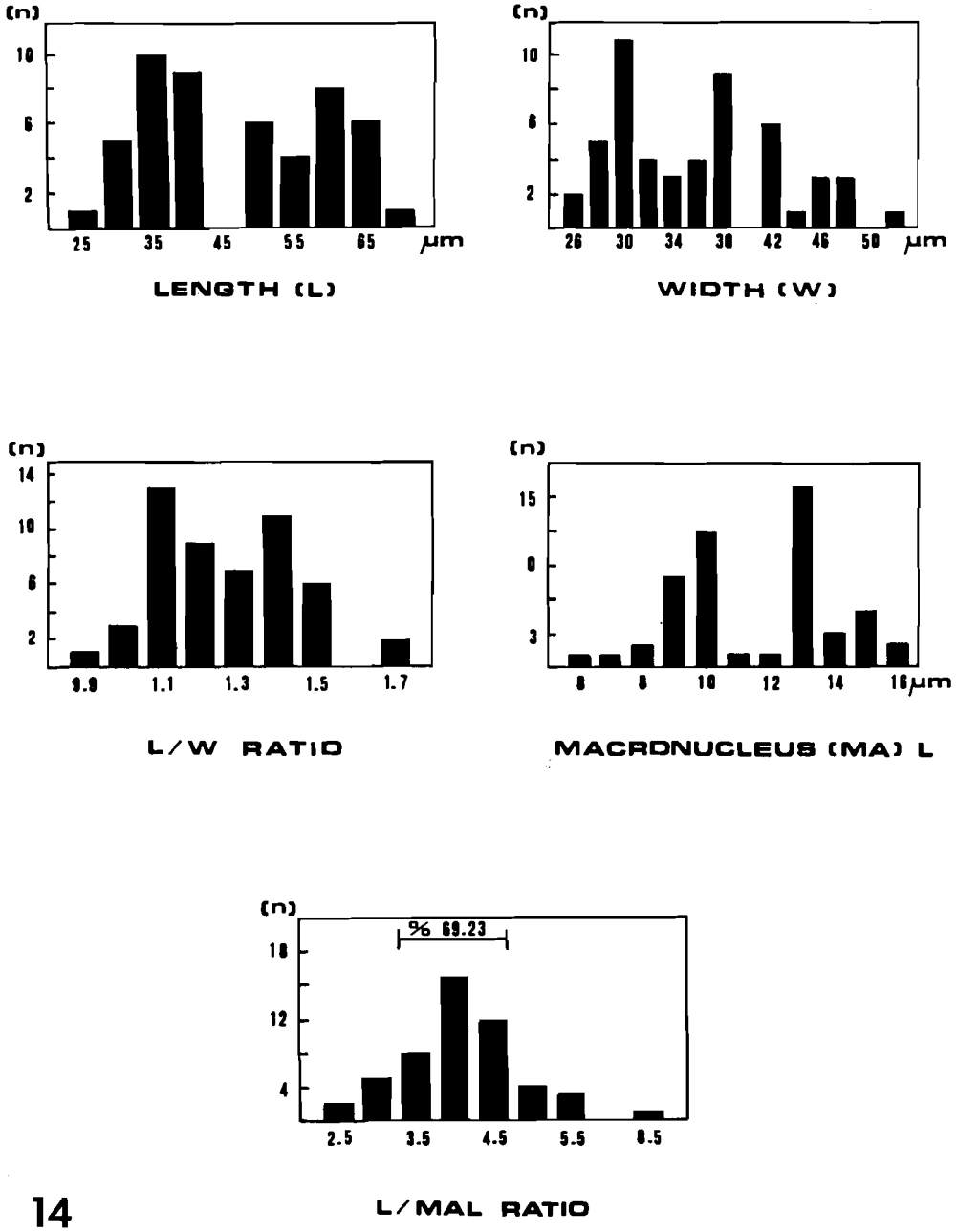
Diagnosis. The dorsal spine is generally absent or rudimentary (2.5–3.0 μm), rarely very short with a length between 4.5–12.0 μm. The rudimentary spine



Figs. 1–7. Drawings of *Entodinium dalli* f. *rudidorsospinatum* n. f. and *E. williamsi* n. sp. – Figs. 1, 2. *E. dalli* f. *rudidorsospinatum* n. f. from the right side (1) and from the left side (2), illustrating the variation of the location of the macronucleus as found in some specimens. – Fig. 3–7. *E. williamsi* n. sp. – Figs. 3–5. *E. williamsi* f. *williamsi* n. f. from the right (3), left (4) and dorsal (5) sides. Figs. 6–7. *E. williamsi* f. *turcicum* n. f. from the right (6) and left (7) sides.



Figs. 8–13. Photomicrographs of *Entodinium dalli* f. *rudidorsospinatum* n. f. and *E. williamsi* n. sp. from Turkish domestic cattle. – Figs. 8–9. *Entodinium dalli* f. *rudidorsospinatum* n. f. – Fig. 8. Cell focused on the left side to show the dorsal spine (arrow) and the left ventral lobe (arrowhead) (fixed and stained with MFS solution). – Fig. 9. Cell focused to show the short spine (arrow) on the right ventral side (fixed with Champy's solution and stained with silver impregnation method). – Figs. 10–13. *E. williamsi* n. sp. – Fig. 10. *E. williamsi* f. *turcicum* n. f. cell focused on the left side of the body to show the location of contractile vacuole, micro- and macronucleus (arrow indicates the rudimentary dorsal spine) (fixed and stained with MFS). – Figs. 11–12. *E. williamsi* f. *williamsi* n. f. cells fixed with 10% formaldehyde and stained with 2% borax – methylene blue, demonstrate that the central periplasm is lying beneath of the adoral ciliary zone (arrows show the tapering part of the dorsal spine and arrowhead indicates the spinated lobe on the right ventral side of the cell). – Fig. 11. From right side, – Fig. 12. From left side. – Fig. 13. A cell of *E. williamsi* f. *williamsi* n. f. from right side (fixed and stained with MFS) shows some variation in the general shape (arrow shows the left spine). Photos in Figs. 8, 10 and 13 were taken using phase contrast microscopy (Bars = 20 μm).



14

Fig. 14. Histograms of the mixed cell measurements of *Entodinium dalli* f. *rudidorsospinatum* n. f. and *E. williamsi* n. sp. (a total of 52 cells, 26 cells from each domestic cattle, nos. 9 and 23) indicating that two populations or species are present, since the frequency histograms do not show a gradual change, i. e., normal distribution curve in all characteristics (except L/MAL ratio). The horizontal bar at the top of L/MAL histogram represents the range in value that encompasses the 69.23% of all cells (n = number of cells).

curves to the dorsal and left side of the cell. A lobe and spine are present on the posterior ventral side as in the preceding form.

Description. The body is almost spherical to ovoid in left or right view (Figs. 1 and 2), but the ventral side generally has a weak and wide depression in the body wall at the level of the base of the adoral ciliary zone (ACZ). Body length 34.52 (26.00–41.25) µm, width 30.45 (25.00–37.50) µm, with L/W ratio 1.13 (0.87–1.13) (n = 26). A left ventral blunt lobe, a short right ventral spine and a rudimentary or very short dorsal spine are present at the posterior extremity of the body. The left ventral lobe follows the curvature of the ventral side wall, terminating as a bluntly rounded triangle directed towards the dorsal side of the cell; this lobe protrudes outward from the main body axis at an angle of ca. 45°. The right ventral spine is acutely pointed. The posterior dorsal spine is generally absent or rudimentary (69.23%) with a maximal length of 3.00 µm or occasionally very short (30.77%) with a length between 4.5–12.0 µm. It has a relatively narrow base. The rudimentary form curves to the left and dorsal side of the cells. The ACZ is nearly at a dorsal angle to the main body axis.

The macronucleus is ellipsoid to spherical in shape and located at the level of the ACZ. The micronucleus is ellipsoid or spherical and situated at some distance posteriorly from the base of the ventral edge of the macronucleus.

One contractile vacuole lies to the ventral of the posterior end of the macronucleus, generally just anterior to the micronucleus (Fig. 1) and rarely just posterior to it (Fig. 2).

The vestibulum and nasse (oesophagus) is funnel-shaped and bends to the dorsal, generally terminating in the region either posterior to the macronucleus or rarely just posterior of the contractile vacuole.

The peripheral periplasm (endoplasm) occupies most of the body, but does not enter into the spine or lobes. The central periplasm (ectoplasm) of the dorsal side of the body is thicker than that of the ventral side. The cytoproctal tube is short and terminated at the cytoproct which opens to near the base of the left ventral lobe.

Measurements of 26 specimens of *E. dalli* f. *rudidorsospinatum* n. f. from cattle no. 9 are given in Table 1.

Variations. Body size and shape are fairly constant. However, the dorsal caudal spine or this form can vary in length: It is rudimentary or very short. In a few cells, the contractile vacuole is situated just posterior of the micronucleus, but generally it is located to the anterior of the micronucleus. This variation was not, however, significant and does not affect species designation.

Type host and locality. Domestic cattle, *Bos taurus,* in Izmir, Turkey

Habitat. Rumen

Occurrence. This new form constituted 17.13% and 0.60% of total ciliate protozoa in cattle nos. 9 and 23, respectively, with an appearance frequency of 8.00%. Total ciliate protozoa numbers per millilitre rumen contents in both cattle were determine as 5.05×10^5.

Etymology. E. dalli f. *rudidorsospinatum* n. f. is named after the possession of a rudimentary dorsal spine (in Lat. *dorso-* or *dorsum,* dorsal; *rudimentum,* very imperfectly developed or represented only by a vestige and *spinum,* spine).

Type material. This is deposited in the rumen ciliates of cattle (RCC) collection of the Zoology Section, Department of Biology, Faculty of Science, Ege University (ZSBEU), Izmir, Turkey on the slides nos. ZSBEU – RCC. 1/PN 75–86 dated on May 9th, 1990.

Entodinium williamsi n. sp. (Figs. 3–7 and 10–11)

Diagnosis. Body ovoid or quadriangular to ellipsoid, generally widest at the midpoint along the length of the body; there are 2 spines and a "spinated lobe" at the posterior end of the body. One of the spines is situated on the dorsal side, the other one on the left side, and the spinated lobe on the right ventral side; the lobe and the left spine are approximately the same size. The lobe is acutely pointed as a spinated form and the left spine is roughly triangular or beak shape; the other spine has a heavy base and curves acutely towards the direction of the right surface and the dorsal side and then projects and tapers outward from the body axis with a length between 8.75–16.25 μm. The macronucleus is spherical to ellipsoid in side view. The micronucleus is usually ellipsoidal, rarely ovoid in shape and situated on the left ventral posterior edge of the macronucleus. The contractile vacuole lies to the ventral side and partially the left of the macronucleus. The central periplasm penetrates beneath the adoral ciliary zone (ACZ) and is very thick on the dorsal side of the body.

Description. In side view (Figs. 3, 4, 6, 7 and 10–12), the body is roughly ovoid or quadriangular to ellipsoidal in shape, generally the widest diameter occurs at the level of mid-body. Body length 56.73 (37.50–67.50) μm width 40.64 (28.75–51.25) μm, with L/W ratio 1.40 (1.14–1.69) (n = 26). Ventral and dorsal body sides are nearly smooth or occasionally convex (Fig. 13). The ACZ is slightly slanted away from macronucleus and extends approximately 1/3–1/4 of the cell length from the anterior end. The adoral (or paraciliar) lips are thick and do not protrude beyond the convex curve of the anterior end of the body. The posterior end of the body terminates in two spines and a lobe which has a sharply pointed end. Therefore, the lobe is named as a spinated lobe (Figs. 10 and 12). One of the two spines is situated on the dorsal side of the body and has a heavy base, it curves acutely to-

Table 1. Dimensions of *Entodinium dalli* f. *rudidorsospinatum* n. f. and *Entodinium williamsi* n. sp. L (length), W (width), L/W (length to width ratio), MAL (macronucleus length) and L/MAL (length to macronucleus length ratio) [n = 26 per each population; SE = standard error; SD = standard deviation; CD = coefficient differences]. The protozoa are significantly different in all characteristics ($p < 10^{-4}$)

	Entodinium dalli f. *rudidorsospinatum* n. f.				*Entodinium williamsi* n. sp.				
	Mean	Range	SE	SD	Mean	Range	SE	SD	CD
L	34.52	26.00–41.25	0.81	4.13	56.73	37.50–67.50	1.30	6.62	2.07
W	30.45	25.00–37.50	0.61	3.11	40.64	28.75–51.25	0.97	4.92	1.27
L/W	01.13	00.87–01.35	0.02	0.09	01.40	01.14–01.69	0.03	0.13	1.23
MAL	09.92	06.00–15.00	0.42	2.14	12.74	08.75–15.00	0.34	1.73	0.73
L/MAL	03.60	02.54–05.50	0.12	0.63	04.54	03.87–06.25	0.12	0.59	0.77

wards the right surface, bends to the dorsal side, and then immediately projects and tapers outward from the main body axis with a length between 8.75–16.25 μm; this tapering part of the spine is occasionally absent or rudimentary. The other spine at the left side is localized centrally and is roughly triangular, resembling the beak of a crow in shape. The spinated lobe on the right ventral side and the left spine are approximately the same size.

The macronucleus is spherical, occasionally ellipsoidal, and lies on the dorsal side generally below the base or rarely at the level of the ACZ. In the specimens viewed from the dorsal side of the body it covers the micronucleus. The micronucleus is ellipsoid or occasionally ovoid in shape and is situated ventral of the macronucleus.

The contractile vacuole lies below the ACZ on the left dorsal side of the cell and the ventral left surface of the macronucleus.

The vestibulum and nasse is funnel-shaped, they do not have a pronounced curvature towards the macronucleus as being more nearly parallel to the long body axis, generally at an angle of 15°–25°, and extend for approximately 1/2 of the length of the cell.

The peripheral periplasm does not penetrate into the caudal spines and spinated lobe. The central periplasm of the dorsal side is thicker and also the central periplasm diffuses the zone beneath the ACZ. The peripheral periplasm occupies approximately 1/2–2/3 of the cell.

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

Measurements of 26 cells of *Entodinium williamsi* n. sp. from Turkish domestic cattle no. 23 are given in Table 1.

Variations. This species appears to be fairly constant in size and morphological characters, although the general shape can be more regularly ellipsoidal (Fig. 13) or rectangular in few individuals and the direction of the dorsal spine varies between specimens. In some individuals, the dorsal spine curves sharply towards the left side, instead of the right side, and projects to the dorsal side at different angles to the body axis within the range between 45°–135°. In some cells, the tapering part of the dorsal spine was absent or rudimentary, but the heavy base was always present.

Type host and locality. Domestic cattle, *Bos taurus,* in Izmir, Turkey.

Habitat. Rumen.

Occurrence. Entodinium williamsi n. sp. constituted 8.87% and 1.20% of total ciliate protozoa in Turkish domestic cattle nos. 23 and 24, respectively, with the frequency appearance of 8.00%. Total ciliate protozoa numbers per ml rumen contents in these cattle were 5.05×10^5 and 3.75×10^5, respectively.

Etymology. Entodinium williamsi n. sp. is named after the rumen microbiologist Dr. Alan G. Williams (Hannah Research Institute, Ayr, Scotland, U.K.) in recognition of his work on various aspects of rumen protozoology.

Type material. Holotype and paratypes are kept on the slides numbered ZSBEU – RCC .3/PN 98–100 and in MFS-glycerine media (in a 1:1 ratio) dated on May 17th, 1993. The specimens are held in the rumen ciliates of cattle (RCC) collection of the Zoology Section, Department of Biology, Faculty of Science, Ege University (ZSBEU), Bornova, Izmir, Turkey.

Two formae may be distinguished on the basis of the shape of the dorsal spine.

Entodinium williamsi forma *williamsi* n. f. (Figs.11, 12 and 13)

Diagnosis. The dorsal spine has a heavy base. It curves acutely towards the right surface and bends to the dorsal side, and then immediately projects and tapers outward from the main body axis. The spine is 8.75–16.25 μm in length.

Frequency. In 8.00% of cattle (nos. 23 and 24) surveyed.

Etymology. This forma is named after the surname of Dr. Alan G. Williams and considered "*nominate*" form by us.

Entodinium williamsi forma *turcicum* n. f. (Figs. 6, 7 and 10)

Diagnosis. The tapering part of the dorsal spine is absent or rudimentary; the heavy base is always present.

Frequency. In 4.00% of cattle (no. 24) surveyed.

Etymology. This forma is named after the place, Turkey, where this new species was found.

Discussion

A new species of rumen ciliated protozoa, *Entodinium dalli,* was described by Dehority [7] in 1974. The organism was observed in the rumen contents of two Dall mountain sheep, *Ovis dalli* from the Alaskan mountain range, Alaska, USA. There have not been other reports on the occurrence of this species prior to the survey reported here. A new forma based on the caudal spination, was observed in the rumen contents from domestic cattle of Turkey. The main morphological characters such as macronucleus and micronucleus shape, the location of the micronucleus and the body shape are similar to those of *E. dalli,* but there are differences in the caudal armature structure and localization of the contractile vacuole. These differences are not significant enough to affect species designation.

Many reports [15, 24, 33, 46] suggested that the shape and number of caudal spines were poor characters for the determination of species, because wide and continuous variation has been observed in these characters in some rumen ciliates. Thus these features are

considered to be unsuitable taxonomical characteristics for an assignment to species. In addition, the variation of caudal projections has been recognized in protozoa from the same host by Ito & Imai [33]. A similar variation was also observed in the individuals of *Entodinium williamsi* n. sp. confirming the observations of Ito & Imai [33] and indicating that variations in these features are more appropriate for the classifications of formae, since it is a neutral category in systematic zoology [40].

Variations in the caudal projections of *E. dalli* therefore served as criteria for the creation of two new formae, *E. dalli* f. *dalli* n. f. which was described by Dehority [7] in Dall sheep and *E. dalli* f. *rudidorsospinatum* n. f., and of two new formae belonging to *E. williamsi* n. sp.; *e. williamsi* f. *williamsi* n. f. and *E. williamsi* f. *turcicum* n. f. which were found in the present study.

Dehority [7] describes the contractile vacuole position of *E. dalli* as being situated to the ventral of the posterior end of the macronucleus, and generally just anterior to the micronucleus. In this study, the contractile vacuole was rarely found to be posterior to the macronucleus. Latteur [35, 36] classified the entodiniid ciliates into several types according to the relative positions of the macronucleus and contractile vacuole. However, Poljansky & Strelkow [1938, cited in 14] and also Wilkinson & van Hoven [46] reported considerable variation in the vacuolar position in some entodiniid species. Therefore, this character does not always appear to be appropriate for distinguishing species in the family Entodiniidae. Because of the marked variability of the vacuolar position, it is better to detail the individual locations of the organelles, not their relative positions. Despite the above observations, there is a general agreement among researchers in this field on the constancy of the contractile vacuole position in entodiniid ciliates [3, 4, 6, 7, 14, 15, 20, 34–37, 42, 43].

E. dalli and its formae closely resemble to *Entodinium ekendrae* Das-Gupta, 1935 [6] and *Entodinium bicaudatum* Bush & Kofoid, 1948 [3] in body size and shape and the size, shape and location of the macronucleus; these latter two ciliates were observed and described from the rumen contents of two Indian goats, *Capra hircus* and a Sierra Nevada bighorn sheep, *Ovis canadensis*, respectively. However, *E. dalli* differs from these species because their contractile vacuoles are located on the anterior end of the macronucleus. It also differs in the variable possession of a posterior dorsal spine.

E. dalli also resembles *Entodinium williamsi* n. sp., since the shape and location of the macro- and micronucleus and the general morphology of the adoral region are similar. However, *E. williamsi* n. sp. is distinguished from *E. dalli* by the following points:

(1) The central periplasm occupies more of the body in *E. williamsi* than in *E. dalli*,

(2) The body of *E. williamsi* is more elliptical than that of *E. dalli*. The length to width ratio of the body of *E. dalli* f. *dalli* is given by Dehority [7] as 1.12 which is consistent with the figure of 1.13 found in this study for *E. dalli* f. *rudidorsospinatum* n. f. However, the L/W ratio determined for *E. williamsi* was 1.40,

(3) The location of the contractile vacuoles differs. It is situated on the ventral of macronucleus in *E. williamsi* n. sp.; whereas, in *E. dalli* it lies on the ventral side of the posterior end of the macronucleus, generally just anterior of the micronucleus and rarely just posterior of it.

(4) The length and orientation of the cytoproctal tube. It is relatively long and narrow at an angle of ca. 45° to the main body axis *in E. williamsi* n. sp.; whereas, in *E. dalli* it is short and slightly parallel to main body axis,

(5) The oblique bend in vestibulum + nasse and the location of its end. This organelle does not bend sharply towards the macronucleus and it is more nearly parallel to long body axis and also, its end is localized at the midpoint of the main body axis in *E. williamsi* n. sp.; whereas, in *E. dalli* it sharply bends to the macronucleus and terminates in the quite dorsal side of main body axis.

E. williamsi n. sp. is also closely related to *Entodinium fujitai* Imai, 1981 [25] and *Entodinium caudatum* Stein, 1859 [3, 34] in the relative positions of macronucleus and contractile vacuole, both species belonging to the same type of Latteur's classification [35, 36], the "*lateropulsatum*" type. It is, however, distinguished from *E. fujitai* by the shape of body, macronucleus and caudal spination. It is also easily distinguished from *E. caudatum* by the length and shape of the macronucleus. In addition, *E. williamsi* n. sp. resembles *Entodinium ovoido-nucleatum* Das-Gupta, 1935 [6] concerning shape of the body and possession of an anterior short macronucleus. However, it is very different from *E. williamsi* n. sp. by (1) the presence of its contractile vacuole on the anterior dorsal side of macronucleus and (2) the possession of a short and narrow cytoproctal tube. In other words, this species belongs to the "*dorsopulsatum*" type, while *E. williamsi* n. sp. is of the "*lateropulsatum*" type in the classification system proposed by Latteur [35, 36].

Several studies have indicated that the size of a ciliate can vary and is not a reliable taxonomic feature [1, 2, 4, 13, 33, 43]. Statistical analysis confirmed that two species were present and there was not a gradual change in size from the small form, *E. dalli* f. *rudidorsospinatum* n. f. to the large form, *E. williamsi* n. sp. The differences in L/W ratio and morphology support the contention that *E. williamsi* is not a larger form of *E. dalli* f. *rudidorsospinatum* n. f.

Acknowledgements

The authors wish to thank Prof. B. A. Dehority (Department of Animal Science, Ohio Agricultural Research and Development Center, Ohio State University, Ohio, USA) for his suggestions and assistance in the classification of the protozoa.

References

1 Awerinzew S. and Mutafówa R. (1914): Material zur Kenntnis der Infusorien aus dem Magen der Wiederkäuer. Arch. Protistenkd., *38*, 109–118.

2 Becker E. R. and Talbott M. (1927): The protozoan fauna of the rumen and reticulum of American cattle. Iowa state Univ. J. Sci., *1*, 345–373.

3 Bush M. and Kofoid C. A. (1948): Ciliates from the Sierra Nevada bighorn sheep, *Ovis canadensis sierra* Grinnel. Univ. Calif. (Berkeley) Publ. Zool., *53*, 237–262.

4 Clarke R. T. J. (19649): Ciliates of the rumen of domestic cattle (*Bos taurus* L.). N. Z. J. Agric. Res., *7*, 248–257.

5 Coleman G. S. and Hall F. J. (1971): A study in the light and electron microscope of the extruded peristome and related structures of the rumen ciliate *Entodinium caudatum*. Tissue & Cell, *3*, 371–380.

6 Das-Gupta M. (1935): Preliminary observations on the protozoan fauna of the rumen of Indian goat, *Capra hircus* Linn. Arch. Protistenkd., *85*, 153–172.

7 Dehority B. A. (1974): Rumen ciliate fauna of Alaskan moose (*Alces americana*), musk-ox (*Ovibos moshatus*) and Dall mountain sheep (*Ovis dalli*). J. Protozool., *21*, 26–32.

8 Dehority B. A. and Potter E. L. (1974): *Diplodinium flabellum:* occurrence and numbers in the rumen of sheep with a description of two new subspecies. J. Protozool., *21*, 686–693.

9 Dehority B. A. (1975): Rumen ciliate protozoa of Alaskan reindeer and caribou (*Rangifer tarandus* L.). In: Proceedings of the First International Reindeer/Caribou Symp., pp. 241–250. Biological Sciences Publication # 18, University of Alaska, Fairbanks.

10 Dehority B. A. (1978): Specificity of rumen ciliate protozoa in cattle and sheep. J. Protozool., *25*, 509–513.

11 Dehority B. A. (1979): Ciliate protozoa in the rumen of Brazilian water buffalo, *Bubalus bubalis*, Linnaeus. J. Protozool., *26*, 536–544.

12 Dehority B. A., Damron, W. S., and McLaren J. B. (1983): Occurrence of the rumen ciliate *Oligoisotricha bubali* in domestic cattle (*Bos taurus*). App. Environ. Microbiol., *45*, 1394–1397.

13 Dehority B. A. (1985): Rumen ciliates of musk-oxen (*Ovibos moschatus* Z.) from the Canadian Arctic. J. Protozool., *32*, 246–250.

14 Dehority B. A. (1994): Rumen ciliate protozoa of the blue duiker (*Cephalophus monticola*), with observations on morphological variation lines within the species *Entodinium dubardi*. J. Euk. Microbiol., *41*, 103–111.

15 Dogiel V. A. (1927): Monographie der Familie Ophryoscolecidae. Arch. Protistenkd., *59*, 1–288.

16 Eadie J. M. (1956): The mid-winter rumen microfauna of the seaweed-eating sheep of North Ronaldshay. Proc. R. Soc. Edinburgh Sect. B, *66*, 276–287.

17 Göçmen B. (1993): Sığır işkembesinde endosimbiyont yaşayan *Isotricha* spp. Stein 1859 (Isotrichidae, Trichostomatida) üzerine ışık mikroskobu düzeyinde morfolojik ve sitolojik gözlemler (in Turkish, with English summary) Doğa – Tr. J. of Zool., *17*, 289–301.

18 Grain J. (1994): Infusoires Ciliés, Ordre des Entodinimorphida. In: Grassé P. (ed.): Traité de Zoologie, 2(2) pp. 327–364. Masson ed., Paris.

19 Harvey W. R. (1982): Mixed model capabilities of LSML 76. Anim. Sci., *54*, 1279–1285.

20 Hsiung T.-S. (1931): The protozoan fauna of the rumen of Chinese sheep. Bull. Fan Mem. Inst. Biol., *2*, 29–43.

21 Hungate R. E. (1966): The rumen and its microbes. Academic Press, New York.

22 Imai S., Katsuno M., and Ogimoto K. (1978): Distributions of rumen ciliate protozoa in cattle, sheep and experimental transfaunation of them. Jpn. J. Zootech. Sci., *49*, 494–505.

23 Imai S., Katsuno M., and Ogimoto K. (1979): Type of the pattern of the rumen ciliate composition of the domestic ruminants and predator – prey interaction of ciliates. Jpn. J. Zootech. Sci., *50*, 79–87.

24 Imai S., Cheng C.-H., Wang J.-S., Ogimoto K., and Fujita J. (1981): Rumen ciliate protozoal fauna of the water buffalo (*Bubalus bubalis*) in Taiwan. Bull. Nip. Vet. Zootech. Coll., *29*, 77–81.

25 Imai S. (1981): Four new rumen ciliates, *Entodinium ogimotoi* sp. n., *E. bubalum* sp. n., *E. fujitai* sp. n. and *Oligoisotricha bubali* (Dogiel, 1928) n. comp. Jpn. J. Vet. Sci., *43*, 201–209.

26 Imai S., Shimizu M., Kinoshita M., Toguchi M., Ishii T., and Fujita J. (1982): Rumen ciliate protozoal fauna and composition of cattle in Japan. Bull. Nip. Vet. Zootech. Coll., *31*, 70–74.

27 Imai S. and Ogimoto K. (1983): *Parabundleia ruminantium* gen. n., sp. n., *Diplodinium mahidoli* sp. n. with two formae, and *Entodinium parvum* forma *monospinosum* forma n. from the zebu cattle (*Bos indicus* L., 1758) in Thailand. Jpn. J. Vet. Sci., *45*, 585–591.

28 Imai S. and Ogimoto K. (1984): Rumen ciliate protozoal fauna and bacterial flora of zebu cattle (*Bos indicus*) and the water buffalo (*Bubalus bubalis*) in Thailand. Jpn. J. Zootech. Sci., *55*, 576–583.

29 Imai S. (1985): Rumen ciliate protozoal fauna of bali cattle (*Bos javanicus domesticus*) and water buffalo (*Bubalus bubalis*) in Indonesia, with the description of a new species, *Entodinium javanicum* sp. nov. Zool. Sci., *2*, 591–600.

30 Imai S. (1986): Rumen ciliate protozoal fauna of zebu cattle (*Bos taurus indicus*) in Sri Lanka, with the description of a new species, *Diplodinium sinhalicum* sp. nov. Zool. Sci., *3*, 699–706.

31 Imai S. (1988): Ciliate protozoa in the rumen of Kenyan zebu cattle, *Bos taurus indicus*, with the description of four new species. J. Protozool., *35*, 130–136.

32 Imai S., Han S. S., Cheng K.-J. and Kudo H. (1989): Composition of the rumen ciliate population in experimental herds of cattle and sheep in Lethbridge, Alberta, Western Canada. Can. J. Microbiol., *35*, 686–690.

33 Ito A. and Imai S. (1990): Ciliated protozoa in the rumen of Holstein-Friesian cattle (*Bos taurus taurus*) in Hokkaido, Japan, with the description of two new species. Zool. Sci., *7*, 449–458.

34 Kofoid C. A. and MacLennan R. F. (1930): Ciliates from *Bos indicus* Linn. I. The genus *Entodinium* Stein. Univ. Calif. (Berkeley) Publ. Zool., *33*, 471–544.

35 Latteur B. (1968): Revision systematique de la famille des Ophryoscolecidae Stein, 1858. Sous-famille Entodiniinae Lubinsky, 1957. Genre *Entodinium* Stein, 1858. Ann. Soc. Roy. Zool. de Belgique, *98*, 1–41.

36 Latteur B. (1969): Revision systematique de la famille des Ophryoscolecidae Stein, 1858. Sous-famille Entodiniinae Lubinsky, 1957. Genre *Entodinium* Stein, 1858. Ann. Soc. Roy. Zool. de Belgique, *99*, 3–25.

37 Lubinsky G. (1957): Studies on the evolution of the Ophryoscolecidae (Ciliata: Oligoisotricha). I. A new species of *Entodinium* with "*caudatum*", "*loboso-spinosum*" and "*dubardi*" forms, and some evolutionary trends in the genus *Entodinium*. Can. J. Zool., *35*, 111–133.

38 Lubinsky G. (1958): Ophryoscolecidae (Ciliata: Entodiniomorphida) of reindeer (*Rangifer tarandus* L.) from the Canadian Arctic. I. Entodiniinae. Can. J. Zool., *36*, 819–835.
39 Marinho A. A. M. (1983): Ciliate protozoa in the rumen of grazing sheep. Rev. Port. Cienc. Vet., *78*, 157–165.
40 Mayr E. (1969): Principles of Systematic Zoology. Mac Graw – Hill Inc., New York.
41 MINITAB Reference Manual. (1991): PC version, release 8. Qickest Inc., Rosemont, Pennsylvania.
42 Ogimoto K. and Imai S. (1991): Atlas of rumen microbiology. Japan Scientific Societies Press, Tokyo.
43 Öktem N. and Göçmen B. (1996): Türkiye evcil sığır (*Bos taurus taurus*) işkembesinden yeni bir siliyat türü, *Entodinium basoglui* sp. nov. (Entodinimorphida: Ophryoscolecidae) ve yeni bir grup, *Entodinium dilobum* grubu hakkında (in Turkish, with English summary). Doğa – Tr. J. of Zoology, (in press)
44 Shimizu M., Kinoshita M., Fujita J., and Imaii S. (1983): Rumen ciliate protozoal fauna and composition of the zebu cattle, *Bos indicus* and water buffalo, *Bubalus bubalis*, in Phlippines. Bull. Nip. Vet. Zootech. Col., *32*, 83–88.
45 STATGRAPHICS Statistic Graphic System. (1992): PC version. 6. Manugustics, Inc. and Statistical Graphics Coorporation (STSC), Rockville, Maryland.
46 Wilkinson R. C. and van Hoven W. (1976): Rumen ciliate fauna of the springbok (*Antidorcas marsupialis*) in Southern Africa. Zool. Afr., *11*, 1–22.

Key words: *Entodinium dalli* – Turkish domestic cattle – *Entodinium dalli* f. *rudidorsospinatum* n. f. – *Entodinium williamsi* n. sp. – *Bos taurus* – Rumen ciliates of Turkey

Department of Biology, Zoology Section, Faculty of Science, Ege (Aegean) University, 35 100 Bornova – Izmir, Turkey