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New Rumen Ciliates from Turkish Domestic Cattle (*Bos taurus* L.): 2. *Epidinium graini* n. sp. (Ophryoscolecidae, Entodiniomorphida)*

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Abstract: In the course of examining rumen contents obtained from 30 domestic cattle (*Bos taurus* L.) in the vicinity of Izmir, Turkey, somewhat similar but unusual forms of epidinia were observed. These ciliated protozoa were observed in six of the animals with a frequency of 20.00%, and this study is concerned with their classification into a species, *Epidinium graini* n. sp. All the protozoa identified as belonging to this species were divided into three formae, *E. graini* f. *graini* n. f., *E. graini* f. *caudatricoronatum* n. f. and *E. graini* f. *caudaquadricoronatum* n. f.. These species have 2-4 transversally periplasmic pellicle foldings resembling coronets or skirts in shape and 4-5 longitudinal groovelets extending posteriorly from the mid-level of the body on the left surface. These or similar characteristics had been described not in the genus *Epidinium* but in the genus *Ophryoscolex*. This fact may stem from some of the characteristics showing the phylogenetic relationship between these two genera.

Key Words: *Epidinium graini* n. sp., New Rumen Ciliates, *Bos taurus*.

Türk Evcil Sığırlarından (*Bos taurus* L.) Yeni İşkembe Siliyatları: 2. *Epidinium graini* n. sp. (Ophryoscolecidae, Entodiniomorphida)

Özet: İzmir (Türkiye) civarındaki 30 evcil sığırdan elde edilen işkembe içeriklerinin incelenmesi esnasında, epidiniumlara benzer fakat farklı olan bazı formlar gözlenmiştir. Bu silli protozoonlar incelenen hayvanların altısında %20.00'lik bir görülme sıklığı ile belirlenmiştir. Bu çalışma sözkonusu siliyatların tek tür, *Epidinium graini* n. sp. halinde sınıflandırılmasını bildirir. Bu türe dahil olacak şekilde tayin edilmiş protoozoonların tamamı 3 form *E. graini* f. *graini* n. f., *E. graini* f. *caudatricoronatum* n. f. ve *E. graini* f. *caudaquadricoronatum* n. f.. halinde bölünmüştür. Bu yeni tür şekil olarak etek veya taç yapısını andıran 2-4 adet enine pelikül katlantısına ve sol yüzeyi üzerinde vücudun orta düzeyinden itibaren posteriora doğru uzanan 4-5 adet uzunlamasına olukçuklara sahiptir. Bu veya benzeri özellikler *Epidinium* cinsinde tanımlanmamış olmasına rağmen *Ophryoscolex* cinsinde tanımlanmıştır. Bu durum iki cins arasındaki filogenetik akrabalığı gösteren bazı özellikler olabilir.

Anahtar Sözcükler: *Epidinium graini* n. sp., Yeni işkembe siliyatları, *Bos taurus*.

Introduction

In a previous report (2), we described three new formae and one new species belonging to the genus *Entodinium* (family Entodiniidae, order Entodiniomorphida) of rumen ciliates collected from domestic cattle (*Bos taurus* L.) in Izmir, Turkey. After that report, I investigated new rumen samples and restudied previous samples from the same animals and obtained an additional new species with three formae belonging to the genus *Epidinium* (family Ophryoscolecidae, order Entodiniomorphida).

The present paper deals with these new ciliates and includes their descriptions.

Material and Methods

Five new and 25 previously reported (2) adult rumen content samples were studied. Other information on the material and methods used in this study has been described previously and, thus, is not described further here.

Abbreviations used in the text, table and figures are as follows: ACZ-adoral ciliary zone, ANOVA-analysis of variance, Cy-cytoproct (=cytoppyge), Cyt-cytoproctal tube (=Rectum), CD-the coefficient of difference, CV-contractile vacuole, DCZ-dorsal ciliary zone, L/MaL-ratio of length to macronucleus length, L/W-ratio of length to width, M-macronucleus, m-micronucleus, MaL-

* This study is a part of the Ph. D. thesis which was completed in 1996 (1).

macronucleus length, MiL-micronucleus length, MaW-macronucleus width, N-nasse, n-specimen number, L-body length, LGs-longitudinal groovelets on the posterior left body surface, P-peristome, RG-right groove on the right body surface, SD-standard deviation of the mean, SE-standard error of the mean, Sp₁-dorsal skeletal plate (=Primitiva), Sp₂-median (right) skeletal plate (=Sternum), Sp₃-ventral skeletal plate (=Parasternum), T₁-primary transversal periplasmic pellicle folding, T₂-secondary transversal periplasmic pellicle folding, T₃-tertiary transversal periplasmic pellicle folding, T₄-quaternary transversal periplasmic pellicle folding W-width or dorsoventral diameter of body, VS-ventral caudal spine (=preanal spine).

Results

Epidinium graini n. sp.

Diagnosis: There are 2-4 transversally periplasmic pellicle foldings resembling coronets or skirts in the posterior half of the body (caudal region) except on the ventral side. The free ends of these foldings are generally indented (69.23%, in 36 of the examined 52 specimens) or occasionally smooth (30.77%). The longest ventral plate (*Parasternum*, Sp₃) of the skeletal complex arises near the left ventral edge of the oral area (Adoral Ciliary Zone, ACZ) and extends posteriorly to the level of the proximal tip of cytoproctal tube, passing the posterior end of the macronucleus. On the left side of the body, mostly (88.46%) there are 4-5 narrow longitudinal pellicular groovelets extending only as far as the primary transversal periplasmic pellicle folding from the mid-level of the body; rarely (11.54%) these longitudinal groovelets are absent.

Description: The body is elongated (length 1.83-2.73 times as great as width) and slightly tapered posteriorly (Figs. 1-5). The ventral surface is slightly concave, the dorsal and left surfaces are convex and the right surface is almost smooth (Fig. 1). The body length (L) is 92.41 (70.00-125.00) µm, and the width (W) 43.85 (35.00-50.00) µm, with an L/W ratio of 2.13 (n=52). ACZ is slanted to the ventral side at angles 35°-45° to main body axis.

The DSZ lies behind the anterior end of the body near the level of the anterior end of the macronucleus. It arises near the dorsal edge of the skeletal complex on the right surface of the body and extends to the left for about 1/3-1/2 of the circumference of the body. One caudal spine with a length of 18.97 (6.25-32.50) µm arises from the posteroventral side of the body. It shows wide variation

in length, shape and direction (Fig. 2). It generally has a heavy proximal base and a tapering distal part. In the posterior part of the body, except the area at which the ventral caudal spine (*Preanal Spine*) is located, there are 2-4 transversally periplasmic pellicle foldings (T₁-T₄) resembling coronets or skirts (Latin *Corona*) (Figs. 1-5). The free ends of these foldings directed towards the posterior end of the body are generally indented (69.23%) or occasionally smooth (30.77%) (Figs. 1-2). In addition to the fine longitudinal striations that extend over the pellicle (Fig. 3b), on the left side of the body there are mostly (88.46%) 4-5 narrow longitudinal pellicular groovelets extending only as far as the primary transversal periplasmic pellicle folding (T₁) from the mid-level of the body. Rarely (11.54%) these longitudinal groovelets are absent. On the right side of the body, a prominent longitudinal groove or folding arises near the ventral edge of the DSZ and extends posteriorly. There are three skeletal plates on the right and the ventral sides (Fig. 1). The longest ventral plate (= *Parasternum*, Sp₃) arises near the left ventral edge of the ASZ and extends posteriorly and to the right. It terminates near the level of the proximal tip of the cytoproctal tube (=rectum) (Figs. 1, 2, 4b&d). The large median plate (= *Sternum*, Right plate, Sp₂) arises along the right side of the ASZ and extends posteriorly parallel and adjacent to the ventral plate except in the anterior third of the body, where there is a short and wide lenticular undifferentiated central periplasmic window between the two plates (Figs. 1d & 4d). The narrow dorsal plate (= *Primitiva*, Sp₁) arises along the dorsal side of the oral area and extends posteriorly between the median plate and macronucleus. The dorsal and median plates lie close together except in the anterior half of the body, where there is a long and narrow lenticular window of undifferentiated central periplasm between these plates (Fig 1d). The peripheral periplasm (=endoplasm) and the central periplasm (=ectoplasm) are separated by a continuous and prominent fibrillar boundary. The peripheral periplasm (=endoplasm) of the anterior part of the body where the ASZ and the DSZ occur is thicker than on the other sides of the body.

The macronucleus is situated on the right dorsal side adjacent to the edge of the dorsal skeletal plate (Figs. 1 & 4b). Its anterior end is blunt, while its posterior part gradually tapers to the posterior end of the body. There is a notch in the mid-region of the macronucleus and the ellipsoidal micronucleus is located within. Two contractile vacuoles are on the dorsal side of the body in a longitudinal array (Figs 1-5).

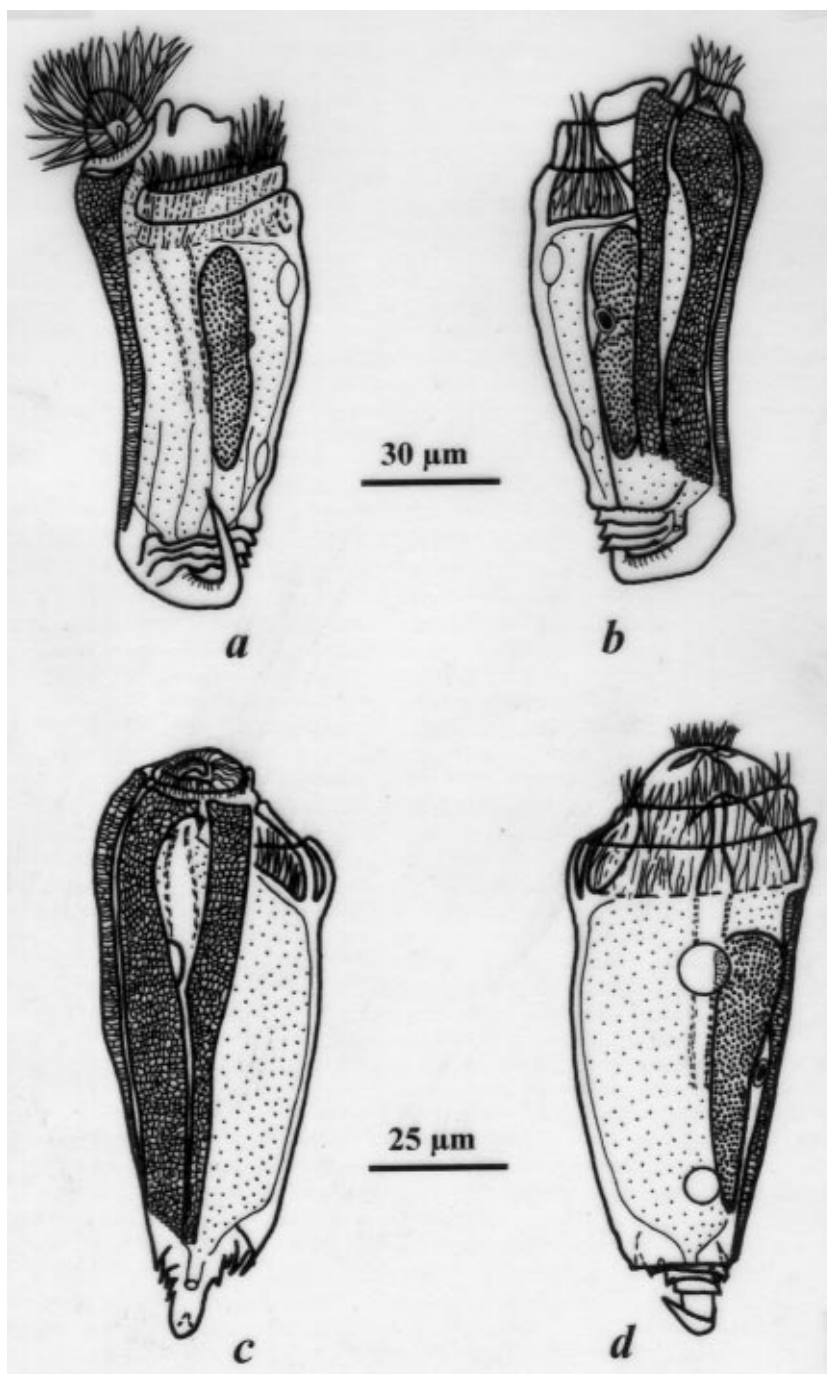


Figure 1. Drawings of *Epidinium graini* f. *caudaquadricoronatum* n. sp., n. f. from the left (a), right (b), ventral (c) and dorsal (d) sides illustrating the variation of the caudal spine in the posterior end of the body.

The vestibulum plus nasse (oesophagus) is cylindrical and bends to the dorsal, generally terminating in the region either posterior to the macronucleus or rarely near to the anterior of the posterior contractile vacuole (Figs. 1 & 5a).

The narrow cytoproctal tube or rectum (2.30-3.75 µm in length) and ellipsoidal cytoproct (=cytopyge) are

situated at the posteroventral end of the body, while the cytoproctal tube is at an angle of about 10°-15° to the main body axis.

Measurements were made on 52 specimens from cattle no. 27 selected at random. The results obtained are given in Table 1.

Table 1. Some morphometric data on the populations of *Epidinium graini* n. sp. and *Epidinium ecaudatum* from the same animal (cattle no. 27). All the measurements are in micrometers (μm). The protozoa are significantly similar in all characteristics ($P>0.05$).

Features	<i>Epidinium graini</i> n. sp. [Cattle no. 27] n=52				<i>Epidinium ecaudatum</i> [Cattle no. 27] n=171				
	Range	Mean	SD	SE	Range	Mean	SD	SE	CD
L	70.00-125.00	92.41	11.66	1.62	55.00-142.50	102.58	17.63	1.35	0.35
W	35.00-50.00	43.85	3.73	0.52	25.00-65.00	43.60	7.63	0.58	0.02
MaL	26.25-63.75	42.16	8.75	1.21	27.50-90.00	54.63	13.97	1.07	0.55
MaW	7.50-18.75	10.84	2.05	0.29	5.00-15.00	10.98	1.99	0.15	0.04
MiL	3.00-6.25	4.66	0.75	0.10	2.50-10.00	5.98	1.69	0.13	0.54
L/MaL	1.65-3.00	2.24	0.29	0.04	1.19-8.75	1.96	0.58	0.04	0.32
L/W	1.83-2.73	2.13	0.24	0.03	1.67-3.18	2.37	0.28	0.02	0.46

Variations: This species appears to be fairly constant in size and morphological characteristics, although the body shape can be regularly elongate (Figs. 1-3, 4c&d, 5) or stumpy in a few individuals (Fig. 4a&b) (L/W ratio=1.83-2.73), and the length and direction of the preanal (=ventral) caudal spine varies between specimens (Figs. 1 & 2). The proximal heavy base of the preanal spine is always directed towards the dorsal side, whereas the distal tapering part of the preanal spine generally bends either to the dorsal (36.54%) or to the left body surface (32.27%). Occasionally it is directed posteriorly at different angles (30.77%).

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

Occurrence: *Epidinium graini* n. sp. Constituted 6.20%, 2.80%, 2.00%, 1.56%, 6.45% and 1.40% of total ciliate protozoa in Turkish domestic cattle nos. 1, 2, 3, 4, 27 and 28, respectively, with a frequency of 20.00%. Total ciliate numbers per ml rumen contents in these cattle were 4.35×10^5 , 5.30×10^5 , 6.45×10^5 , 6.55×10^5 , and 1.75×10^5 , respectively.

Etymology: The name of this new species is attributed to the surname of the rumen protozoologist Professor Dr. Jean GRAIN (*Université Blaise Pascal, Aubière Cedex, France*) who made valuable international contributions to protozoology.

Type material. The holotype is deposited in the rumen of the cattle collection (RCC) of the Zoology Section, Department of Biology, Faculty of Science, Ege University (ZSBEU), Izmir, Turkey, on slide no. ZSBEU-

RCC.1/PN 1 dated March 21st, 1990. Paratypes are kept on the slides numbered ZSBEU-RCC.1/PN 12 and RCC.4/PN 324-329 dated March 21st, 1990, and April 3rd, 1995, respectively.

This species is divided into three formae on the basis of the number of the transversal central periplasmic (ectoplasmic) pellicle foldings.

(1) *Epidinium graini* f. *graini* n. sp., n. f.

(Figs. 2a-h&3)

Diagnosis: There are 2 transversal periplasmic pellicle foldings resembling coronets or skirts in the posterior half of the body except the posteroventral side, and a ventral (=preanal) caudal spine showing variations in length, shape and direction.

Occurrence: It is detected in 20.00% of the cattle (nos. 1, 2, 3, 4, 27 and 28) surveyed with the percentage composition of 0.30%-2.80%.

Etymology: This forma is named after Professor Dr. Jean GRAIN and considered a "nominata" form by us.

(2) *Epidinium graini* f. *caudatricoronatum* n. sp., n. f.

(Figs. 2i-1 & 4)

Diagnosis: There are 3 transversal periplasmic pellicle foldings resembling coronets or skirts in the posterior half of the body except the posteroventral side, and a ventral (=preanal) caudal spine showing variations in length, shape and direction.

Occurrence: It is detected in 16.67% of the cattle (nos. 1, 2, 3, 4 and 27) surveyed with the percentage composition of 0.52%-3.60%.

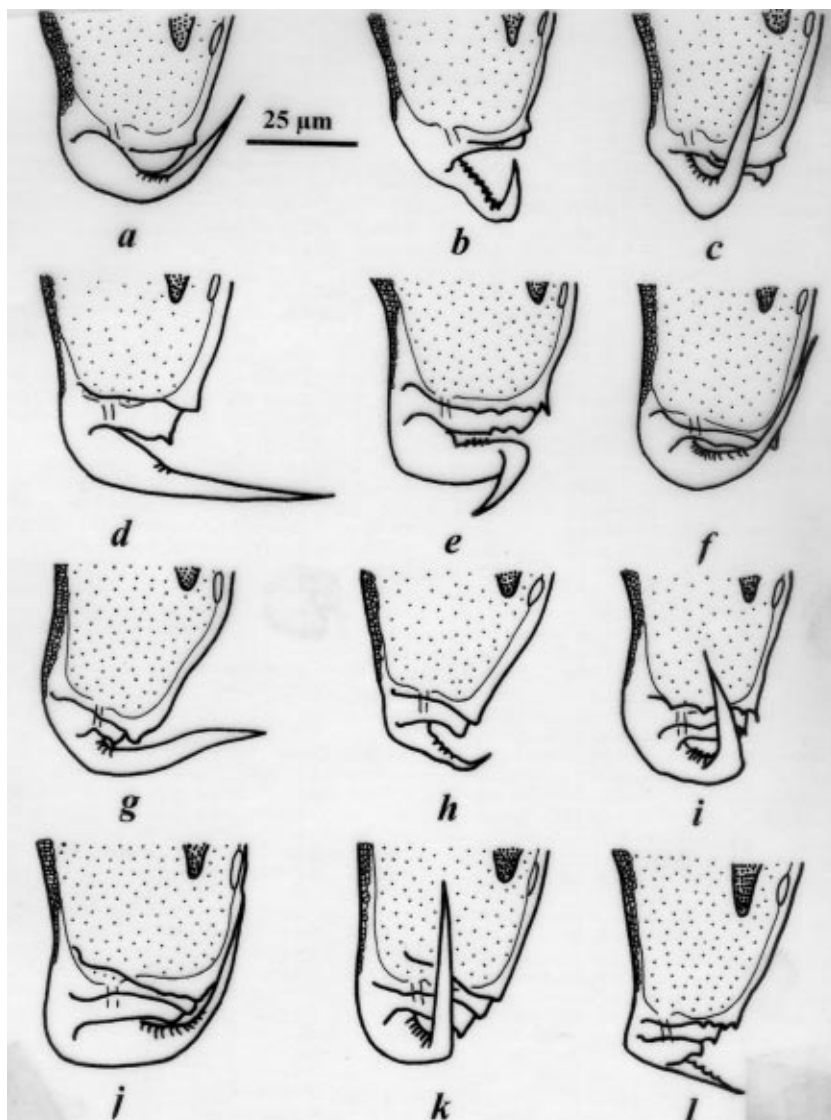


Figure 2. Drawings of *Epidinium graini* n. sp. from the left side showing the variation of the caudal spination and the free ends of the transversal pellicle foldings. a-h: *Epidinium graini* f. *graini* n. sp., n. f., i-l: *Epidinium graini* f. *caudatricoronatum* in the posterior end the body.

Etymology: This forma is named for the possession of three transversal periplasmic foldings resembling coronets or skirts and one caudal spine (in Lat. *coron*-coronet or skirt; *cauda*-, tail or posterior end; *tri*-, three).

(3) *Epidinium graini* f. *caudaquadricoronatum* n. sp., n. f.

(Figs. 1 & 5)

Diagnosis: There are 4 transversal periplasmic pellicle foldings resembling coronets or skirts in the posterior half of the body except the posteroventral side, and a ventral (=preanal) caudal spine showing variations in length, shape and direction.

Occurrence: It is detected in 6.67% of the cattle (nos. 1 and 27) surveyed with the percentage composition of 0.70%-1.00%.

Etymology: This forma is named for the possession of four transversal periplasmic foldings resembling coronets or skirts and one caudal spine (in Lat. *coron*-coronet or skirt; *cauda*-, tail or posterior end; *quadri*-, four).

Discussion

The main morphological characteristics such as the macro- and micronucleus shape, the location and number of the contractile vacuoles, the general body shape and

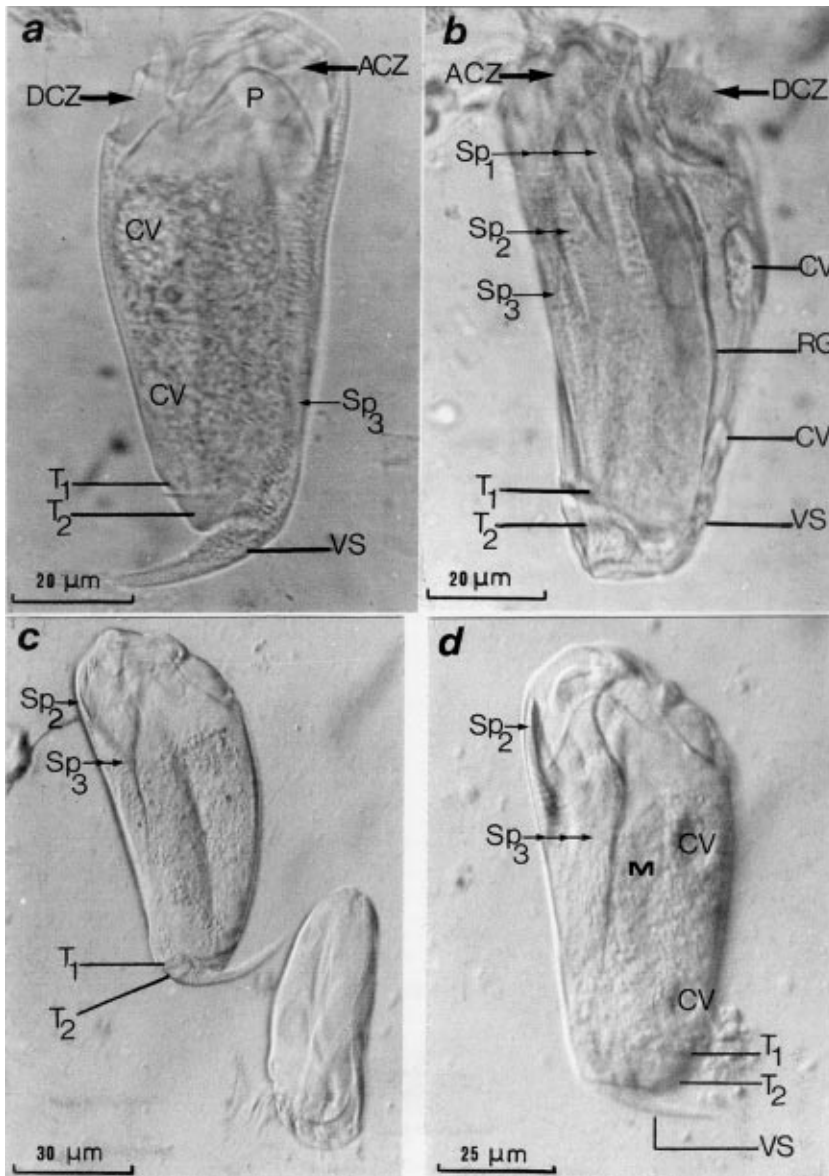


Figure 3. Photomicrographs of *Epidinium graini* f. *graini* n. sp., n. f. from Turkish domestic cattle. a from right side, b from left side showing the preanal spine, which lies anteriorly adjacent to the dorsal body surface, c&d from left side (the smaller protozoa in photo c is *Epidinium ecaudatum* f. *ecaudatum*).

organization except the posterior extremity, the number and orientation of the skeletal plates, and also the body size of *Epidinium graini* n. sp. are similar to those of *Epidinium ecaudatum*, which was the sole species of the genus and its previously described formae (1, 3-20), but there are differences in the surface structure of the posterior half of the body and the length of the ventral skeletal plate (*Parasternum*). These differences are significant enough to create a new species and formae belonging to the species in the taxonomical protozoology of the rumen ciliates (1, 2, 7, 12, 15, 20-24).

This new species is a unique ophryoscolecid species. The elongated body shape and general aspects, and also the presence of a ventral (=preanal) caudal spine resembles that in the forma *Epidinium ecaudatum* f. *caudatum* (9), which is the simplest member of the species in terms of caudal spination. This and other previously described formae, however, are lacking in the transversal pellicle foldings and the longitudinal groovelets on the posterior left surface of the body. These are the most characteristic features of the new species and its formae. There are no known species or

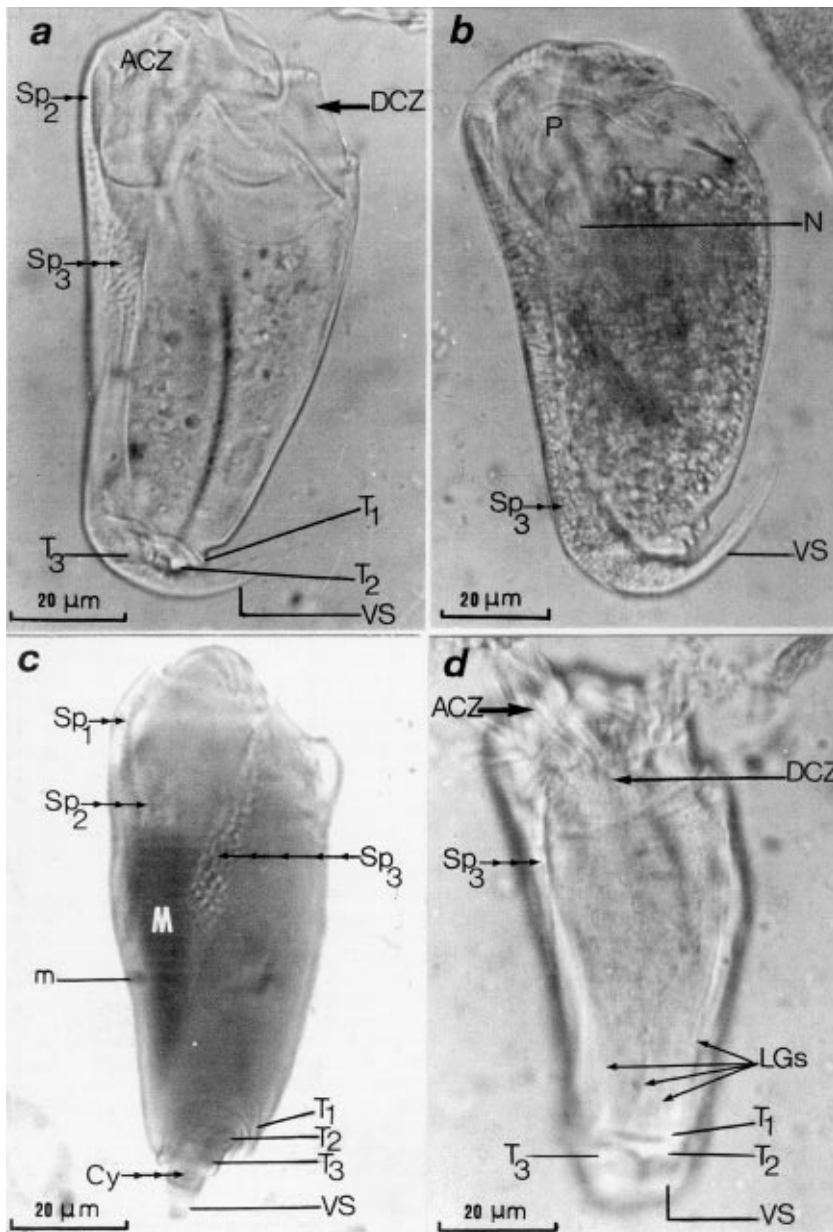


Figure 4. Photomicrographs of *Epidinium graini* f. *caudatricoronatum* n. sp., n. f. from Turkish domestic cattle. a, b&c from left side showing the variation of the body shape and the caudal spination. Cell in photo c focused on the left surface of the body to indicate the longitudinal groovelets. d from the ventral side to show the orientation of the skeletal plates, the macronucleus and the micronucleus.

formae having such features. Species or formae with transversal periplasmic pellicular foldings and longitudinal groovelets on the body surface have been found not in the genus *Epidinium*, but similar structures, such as the circlets of the caudal spines (*coronation*) and the longitudinal grooves extending posteriorly from the DSZ which divide the body surface into superficial plates (=antimeres) (*antimerisation*) in the genus *Ophryoscolex* (1, 12, 15, 20, 21). Thus, in position and shape, it seems

that these structures of *Epidinium graini* n. sp. give a transition to the coronation of the caudal spines and the antimerisation of the body surface. This fact may stem from some of the characteristics showing the phylogenetic relationship between the genera of *Epidinium* and *Ophryoscolex*.

On the other hand, the main skeletal complex of *Epidinium graini* n. sp. resembles that of *Epidinium ecaudatum* (9) and its formae, but the ventral plate of

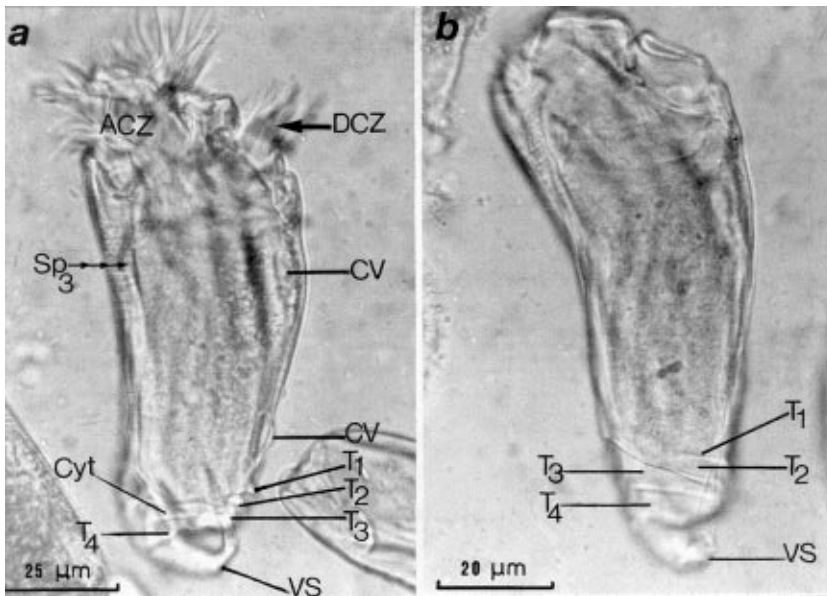


Figure 5. Photomicrographs (a&b) of *Epidinium graini* f. *caudquadricoronatum* n. sp., n. f. found in Turkish domestic cattle from the left side showing the variation in body shape.

this complex extends posteriorly to the level of the proximal tip of the cytoproctal tube, as does that of the genus *Ophryoscolex*, while the length of this plate is relatively short extending either to the level of the posterior end of the macronucleus or somewhat posteriorly to this location (1, 12, 15, 20, 21). This is another characteristic that may be significant enough to affect the species designation indicating the phylogenetic tendency of *Epidinium graini* n. sp. Though this species and its formae resemble the members of the genus *Ophryoscolex* in the characteristics mentioned above, they clearly differ from it in many characteristics: the number of contractile vacuoles, the size and position of the ciliary zones (especially in DSZ), the caudal spination, the surface armature and the orientation of the structures (see 1, 12, 15, 20, 21).

Data on various morphometrical characteristics that are important in species designation for the ciliate taxonomy (1, 2, 7, 12, 20, 21, 23, 24) of the populations *Epidinium graini* n. sp. (n=52) and *Epidinium ecaudatum* (n=171) from the same animal are summarized and compared in Table 1. There are no significant differences between the two species in all criteria, since the results of ANOVA are significantly similar ($P > 0.05$), and the CD values are lower than the

conventional level of difference of 1.28, ranging between 0.02-0.55. Therefore, it can be said that the present new species is clearly distinguished by qualitative features rather than quantitative ones.

Epidinium garini n. sp., which were detected with a high appearance (20.00%) and occurrence ratio (3.40% on average) only from Turkish domestic cattle, seem to be characteristic species in the ciliate fauna of the host, since the species has not been found in Turkish domestic sheep (*Ovis ammon aries*) (25, 26), which share the same distribution area.

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