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New Rumen Ciliate, *Entodinium kastamonicum*, New Species (Ophryoscolecidae: Entodiniomorphida) from Turkish Domestic Cattle, *Bos taurus taurus*

Gözde Gürelli
Biology Department, Faculty of Science and Art, Kastamonu University, 37100 Kuzeykent, Kastamonu, Turkey

Abstract.- In the course of examining the rumen contents obtained from 25 domestic cattle (*Bos taurus taurus*) in the vicinity of Kastamonu, Turkey, a new species belonging to the genus *Entodinium, viz., Entodinium kastamonicum* new species with seven morphotypes was identified. The seven new morphotypes of this new species, *E. kastamonicum* m. kastamonicum n. m., *E. kastamonicum* m. lobatum n. m., *E. kastamonicum* m. rudidorsospinatum n.m., *E. kastamonicum* m. monospinosum n. m., *E. kastamonicum* m. bifidum n. m., *E. kastamonicum* m. bispinosum n.m. and *E. kastamonicum* m. trispinosum n. m. were identified on the basis of differences in their caudal spines and lobes. The morphological characteristics of these protozoa are described and their relationship to similar entodiniid species are discussed.

Key words: *Entodinium kastamonicum* n. sp., rumen ciliate, *Bos taurus taurus*.

INTRODUCTION

Other than several reports from Turkey on rumen ciliate protozoa in domestic cattle (Göçmen and Öktem, 1996; Göçmen *et al.*, 2001a, b, 2003; Öktem and Göçmen, 1996), no additional research in this respect from various locations in Turkey has been published. Obviously, knowledge of rumen ciliate composition and the establishment and the comparison of the faunal similarities and differences between the hosts would yield specific information on the geographical distribution of both the hosts and the ciliates, the feeding habitats and physiology of the hosts and their specific ciliates. Specifically, some species and morphotypes have been detected only in certain areas and appear to be host specific (Dehority, 1978; Dogiel, 1927; Göçmen and Öktem, 1996; Imai, 1988; Ito and Imai, 1990).

Faunation of young ruminants or transfaunation between adults occurs only as a result of direct contact between animals. A mother can transfer protozoa to her young by grooming in which case the protozoa present in her mouth from rumination are passed in the saliva. Also, an adult can salivate on feed or pasture and another animal then ingests the protozoa with the feed (Dehority, 2003; Fuente *et al.*, 2006).

Rumen protozoa, which can play an important role by contributing nutrients to the host animal (Hungate, 1966; Ogimoto and Imai, 1981), also play a role in the digestion of carbohydrate and protein containing feedstuffs by secreting sacharolytic and proteolytic enzymes (Coleman and Laurie, 1974; Nagasawa *et al.*, 1992; Shinchi and Abe, 1987a, b; Shinchi *et al.*, 1986; Williams, 1979).

The aim of this study was to describe a new ciliate species and its morphotypes belonging to family Ophryoscolecidae from cattle in the vicinity of Kastamonu and in addition, to clarify their relationship with those species already known.

MATERIALS AND METHODS

Samples of rumen contents were obtained from 25 domestic cattle (*Bos taurus taurus*) at the slaughterhouse near Kastamonu, Turkey between March 2012 and December 2012.

The rumen wall was cut with a knife and a sample removed via a catheter as soon as possible of the animal was killed (Dehority, 1984). A well-mixed sample of the rumen contents was diluted with an equal volume of 18.5% formalin. A portion of each samples was also immediately fixed and stained in methylgreen formalin saline (MFS)

* Corresponding author: ggurelli@yahoo.com, ggurelli@kastamonu.edu.tr
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solution for total and differential counts (Göçmen and Gürelli, 2009; Ogimoto and Imai, 1981). The MFS served as a nuclear stain and Lugol's iodine was used to stain skeletal plates. This procedure was used to preserve the integrity of the cell and its internal structure.

Total cell counts were made at 400x magnification with a Neubauer hemocytometer counting chamber. The Neubauer hemocytometer counting chamber has slender grooves cut at regular intervals. The number per 1 ml of rumen contents can be calculated by the following formula:

$$N = 10 / 4 \times a \times d$$

where N is number of ciliates per 1 ml of rumen contents, a is number of ciliates in 4 divisions on the Neubauer hemocytometer and d is sample dilution.

Differential counts of species were estimated from smear slides with a total of 400 to 650 cells identified for each species (Göçmen and Gürelli, 2009).

All cell measurements were made and specimens were examined with a Leica DM 3000 microscope and imaging system.

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

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

RESULTS

Entodinium kastamonicum, new species

Description

Table I lists measurements and morphometric ratios of *Entodinium kastamonicum.* The body is ovoid and slightly tapered anteriorly and posteriorly. The posterior end rounded with lobes or spines. Ventral and dorsal body sides are convex. Oral lips are thick and do not protrude beyond the convex curve of the anterior end. The oral ciliary zone extends for approximately 1/3 of the cell length from the anterior end. The vestibulum is funnel-shaped, bending dorsally. The macronucleus is spherical or occasionally elipsoidal, lies on the dorsal side and is located near the middle of the cell. The micronucleus is elipsoidal in shape and is located ventrally at the anterior end of the macronucleus. The contractile vacuole lies above the macronucleus. The cytoproct is narrow at angle of ca. 45° to main body axis and terminated bending towards the dorsal side of the body.

Table I.- Dimensions of *Entodinium kastamonicum* new species from Turkish domestic cattles.

	Mean±SD (n= 25)	Range	SE
Length	44.2±5.1	32.3-60.0	1.0
Width	36.2±3.04	30.5-42.1	0.6
Length-weight	1.2±0.1	1.0-1.4	0.1
Macronucleus length	10.3±1.7	6.4-12.6	0.3
Macronucleus width	8.8±1.2	5.9-11.0	0.2
Micronucleus length	2.0±0.6	1-3.5	0.1
Micronucleus width	1.7±0.6	0.6-2.9	0.1

Habitat, type host and type locality

The rumen of domestic cattle (*Bos taurus taurus*) in Kastamonu, Turkey.

Etymology

Entodinium kastamonicum, new species is named after the location where the new species was found.

Type material

The speciemens are held in the endocommensal ciliates of herbivorous collection of the Department of Biology, Faculty of Science and Art, Kastamonu University, Kastamonu, Turkey. Holotype and paratypes are on the slide numbered as ERC-1.

Occurrence

E. kastamonicum, new species occurred only in Turkish domestic cattle no:14 (frequency of apearance is 4%) and constituted 55.4 % of total ciliate protozoa, 61.0 $x10^4$ protozoa per ml rumen contents.

Seven morphotypes may be distinguished based on the number and shape of caudal processes.

1. *Entodinium kastamonicum* morphotype kastamonicum n. m. (Figs. 1A, 2a)

Two round lobes at the posterior end of the body, not at the same level. Ventral lobe is more posterior than dorsal lobe.

2. *Entodinium kastamonicum* morphotype lobatum n.m. (Figs. 1B, 2b)

Slightly pointed ventral lobe is present.

3. *Entodinium kastamonicum* morphotype rudidorsospinatum n.m. (Figs. 1C, 2c)

Pointed spine located on the ventral posterior end.

4. *Entodinium kastamonicum* morphotype monospinosum n.m. (Figs. 1D, 2d)

A second spine is located on the ventral posterior end, on the right side.

5. *Entodinium kastamonicum* morphotype bifidum n.m. (Figs 1E, 2e)

Two spines are located on the ventral side at the posterior end.

6. *Entodinium kastamonicum* morphotype bispinosum n.m. (Figs. 1F, 2f)

Two spines are on the ventral posterior end and a sharp spine on the dorsal posterior end.

7. *Entodinium kastamonicum* morphotype trispinosum n.m. (Figs. 1G, 2g, h)

Three sharp spines of about equal length on the posterior end.

DISCUSSION

Entodinium kastamonicum, new species closely resembles *Entodinium constrictum* Dehority 1974, in shape of the body and macronucleus and the position of contractile vacuole. However, it does not have a constriction or identation in the body wall on the ventral side at the level of vestibulum. The length and width ratio is much lower (Table I). *E. kastamonicum* m. trispinosum closely resembles *E. triacum* m. triacum Buisson, 1923, but the shape of macronucleus and the location of micronucleus are different. *E. kastamonicum* is also closely related to *E. orbicularis* Bush and Kofoid, 1948 and *E. protuberans* Bush and Kofoid, 1948, because of macronucleus shape and location of contractile vacuole. However, *E. kastamonicum* n. sp. is narrower than *E. orbicularis* and the location of micronucleus is different. The length and the width of the *E. kastamonicum* n. sp. is larger than *E. protuberans* and also the location of micronucleus is different.

Many reports (Dogiel, 1927; Göçmen and Öktem, 1996; Imai *et al.*, 2004; Ito and Imai, 1990) suggested that the shape and number of caudal spines were poor characters for the determination of species and subspecies, 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 and subspecies.

The variation of caudal processes has been recognized in protozoa from the same host by Ito and Imai (1990) and Göçmen and Öktem (1996). For these reasons, it has been suggested that the term “morphotype” is more appropriate for the classisfications of rumen ciliates according to the caudal processes (lobes or spines). The reason for the formation of caudal spines in rumen ciliates has not been determined. Ito *et al.* (1995) suggested that the feeding habit of animals might increase the percentage of ciliates without caudal spines. Lubinsky (1957) advocated that when animals are fed a diet with low starch value, ciliates without caudal appendages are predominant, while ciliates with well-developed caudal spines become predominant when the food is rich in starch. Coleman (1980) considered that *Entodinium* species develop caudal spines to protect against engulfment. However, Imai *et al.* (2002) concluded that spine formation in rumen ciliates is not a response to predation, host diet or ciliate densities.

To date, no species has been found in the literature that is identical to the new species found in this study. On this basis, it has been described as a new species and named *Entodinium kastamonicum*, with seven morphotypes.

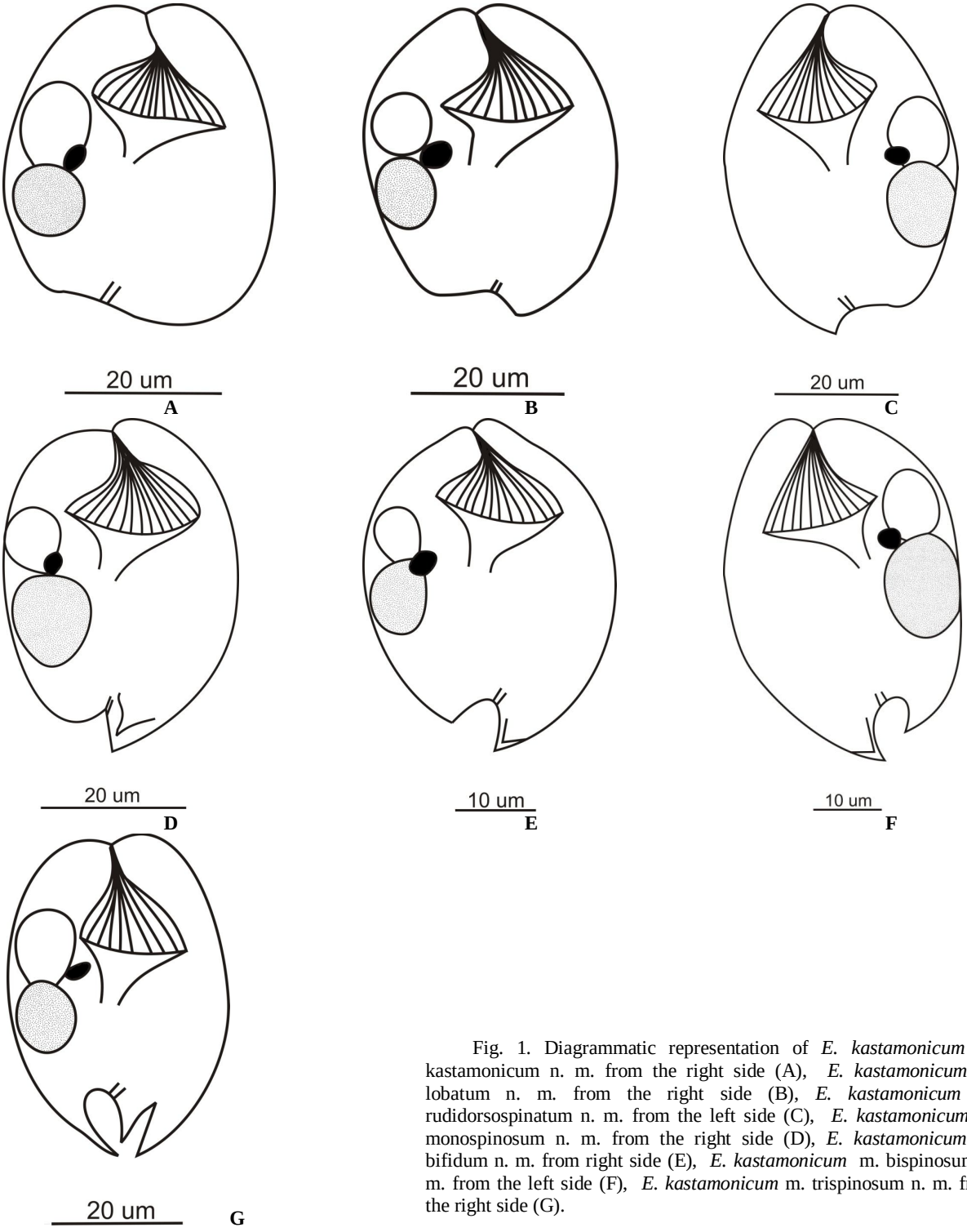

Fig. 1. Diagrammatic representation of *E. kastamonicum* m. kastamonicum n. m. from the right side (A), *E. kastamonicum* m. lobatum n. m. from the right side (B), *E. kastamonicum* m. rudidorsospinatum n. m. from the left side (C), *E. kastamonicum* m. monospinosum n. m. from the right side (D), *E. kastamonicum* m. bifidum n. m. from right side (E), *E. kastamonicum* m. bispinosum n. m. from the left side (F), *E. kastamonicum* m. trispinosum n. m. from the right side (G).

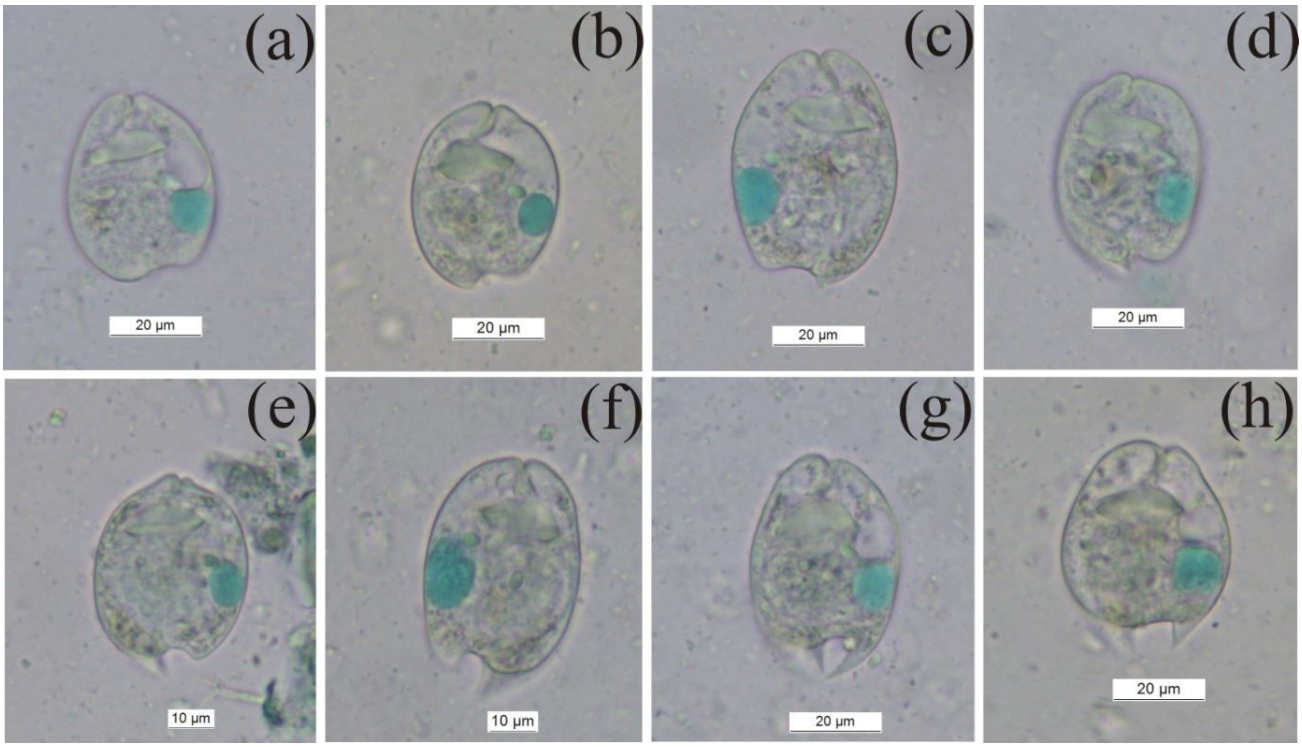

Fig. 2. Micrographs of *Entodinium kastamonicum* n. sp., fixed and stained with MFS, **(a)** *E. kastamonicum* m. kastamonicum n. m. from left side, **(b)** *E. kastamonicum* m. lobatum n. m. from left side, **(c)** *E. kastamonicum* m. rudidorsospinatum n. m. from right side, **(d)** *E. kastamonicum* m. monospinatum n. m. from left side, **(e)** *E. kastamonicum* m. bifidum n. m. from left side, **(f)** *E. kastamonicum* m. bispinosum n. m. from right side, **(g)-(h)** *E. kastamonicum* m. trispinosum n. m. from left side.

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