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# Hindgut ciliate diversity of domestic horses in Kastamonu, Turkey and scanning electron micrographs

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**Abstract.** The hindgut ciliate diversity of domestic horses in Kastamonu, Turkey was investigated; 34 species and 3 morphotypes belonging to 20 genera were identified, but no novel species were detected. For individual domestic horses, the total number of ciliate species per animal ranged from 3.0 to 22.0, with an average of 13.9 ± 7.0 (SD). The average abundance of hindgut ciliates in the 11 domestic horses was $5.6 \pm 5.8 \times 10^4$ cells mL$^{-1}$, and the min.-max. values ranged from $1.0 \times 10^4$ cells mL$^{-1}$ to $29.5 \times 10^4$ cells mL$^{-1}$. Except for three individuals, all other domestic horses contained *Bundleia postciliata*, *Polymorphella ampulla*, *Blepharocorys curvigula*, and *Spirodinium equi*, each with a prevalence of 72.2%, whereas *Bundleia inflata*, *Tetratoxum excavatum* m. excavatum, and *Tetratoxum parvum* m. sulcatum were observed in only one horse, and their prevalence was 9.1%. Scanning electron microscopy images of the following species were analyzed: *Paraisotricha colpoidea*, *P. ampulla*, *B. postciliata*, *Bundleia piriformis*, *Blepharoprosthium pireum*, *Hemiprorodon gymnoposthium*, *Holophryoides macrotricha*, *Holophryoides ovalis*, *Blepharocorys angusta*, *B. curvigula*, *Blepharocorys microcorys*, *Blepharocorys uncinata*, *Cycloposthium bipalmatum*, *Cycloposthium edentatum* m. edentatum, *Tripalmaria dogieli*, *S. equi*, *T. excavatum* m. excavatum, *Tetratoxum unifasciculatum*, *Ditoxum funinucleum*, *Cochliatoxum periachtum*, *Triadinium caudatum*, *Circodinium minimum*, *Gassovskiella galea*, and *Allantosoma intestinale*.

**Key words:** ciliates, domestic horse, hindgut, SEM, Turkey

## Introduction

Horses are mammals of the family Equidae; they possess a well-developed hindgut composed of the cecum, large colon, small colon, and rectum (Moore et al. 2001, Costa & Weese 2012). The hindgut of horses is colonized by a diverse community of microorganisms, including viruses, bacteria, archaea, fungi, and ciliated protozoa (Julliand & Grim 2016, 2017, Kauter et al. 2019). Ciliated protozoa have larger sizes than the others and characteristic shapes (Imai 1998). A solid cortex can protect the cell shape of ciliated protozoa during defecation and help them survive for a short time in the environment (Kornilova 2004, Kornilova et al. 2019). These ciliates lack resistant cysts; they are transmitted to young foals by coprophagy (Ike et al. 1985, Egan et al. 2010). They are excreted alive in the feces; therefore, the ciliate composition in the feces reflects their population in the hindgut (Ike et al. 1981, 1983a, 1983b, Tung 1992, Ito et al. 1996, Imai et al. 1999, Gürelli & Göçmen 2010, 2011, 2012, Göçmen et al. 2012, Gürelli et al. 2015, 2019, Cedrola et al. 2019). These ciliates are endocommensals playing an important role in the digestion of cellulose and starch in the hindgut of horses (Ozeki et al. 1973, Dehority 1986).

Many light microscopic observations of the morphological structures of the hindgut ciliates of horses were carried out (Gassovsky 1919, Hsiung 1930, Strelkow 1939, Ozeki 1977, Kornilova 2004), but only a few studies used scanning electron microscopy (SEM) to observe the fine surface structures of these ciliates (Imai et al. 1979, Tung 1992, Gürelli et al. 2015, 2019). This study aimed to analyze the hindgut ciliate composition of domestic horses in Kastamonu, Turkey and examine them in detail using SEM.

### Materials and Methods

Fecal samples were collected from 11 domestic horses (*Equus caballus* Linnaeus, 1758) that inhabited different farms in Kastamonu, Turkey between September 2018 and February 2019.

The samples were collected immediately after defecation and fixed and stained in 2 times as much methyl green formalin saline (MFS) solution as their original volume. The MFS solution served as a nuclear stain. This procedure was used to preserve cell integrity and internal structure. The samples were filtered through a 2.56 mm mesh gauze at the laboratory. A 2% Lugol's iodine solution was added to the samples to reveal the skeletal plates of the ciliates (Ogimoto & Imai 1981, Gürelli & Göçmen 2012, Gürelli et al. 2019).

The average abundances of ciliates in each horse were determined using the hemocytometer counting chamber. The number of cells per mL of the fecal sample was calculated using the following formula: $N = (10/4) \times a \times d$, where a is the number of ciliates in four chambers of the hemocytometer, d is the sample dilution, and N is the number of ciliates per mL of the fecal sample (Gürelli & Göçmen 2011, Gürelli et al. 2019). The relative abundances of ciliate species in each horse were estimated from smear slides (Gürelli & Yürücüoğlu 2019). The prevalence calculation of ciliate species was performed as previously described by Bush et al. (1997).

Ciliate species and genera were identified and classified on the basis of the descriptions of Hsiung (1930), Kornilova (2003, 2004), and Strelkow (1939). Some fecal samples were examined in detail using a FEG 250 scanning electron microscope (FEI-Quanta, Hillsboro, OR, USA) following the method described by Imai et al. (1992).

## Results

A total of 34 species and three morphotypes belonging to 20 genera of ciliates were identified. The hindgut ciliate diversity comprised one genus and one species belonging to the family Paraisotrichidae; seven genera and fifteen species belonging to the family Buetschliidae; three genera and six species belonging to the family Blepharocorythidae; two genera, three species, and one morphotype belonging to the family Cycloposthiidae; six genera, eight species, and three morphotypes belonging to the family Spirodiniidae; and one genus and one species belonging to the family Allantosomatidae. Except for three individuals, all other domestic horses contained *Bundleia postciliata*, *Polymorphella* *ampulla*, *Blepharocorys curvigula*, and *Spirodinium equi*, each with a prevalence of 72.2%, whereas *Bundleia inflata*, *Tetratoxum excavatum* m. excavatum, and *Tetratoxum parvum* m. sulcatum were observed in only one horse, and their prevalence was 9.1%. In all domestic horses, the relative abundance rates of *B. postciliata*, *B. curvigula*, *Cycloposthium bipalmatum*, and *Cycloposthium edentatum* m. edentatum were high (i.e., >9.0%), whereas those of *Blepharoconus hemiciliatus*, *Cochliatoxum periachtum*, *T. excavatum* m. sulcatum, *T. parvum* m. sulcatum, and *Gassovskiella galea* were low (i.e., <0.3%). In general, the hindgut ciliate fauna of domestic horses in Kastamonu, Turkey was primarily composed of *Bundleia* species (mean relative abundance: 26.0%, min.–max. value: 0%–53.9%) (Table 1).

Table 1. Prevalence and relative abundance of hindgut ciliates in the feces of 11 domestic horses in Kastamonu, Turkey.

| Familia/genus/species/morphotype | Prevalence (%) | Relative abundance (%) | |
|---|---|---|---|
| | | Mean ± SD | Min.-Max. value |
| Paraisotrichidae | | | |
| *Paraisotricha* Paraisotricha, 1890 | 18.2 | 1.6 ± 5.2 | 0–17.3 |
| *P. colpoidea* Fiorentini, 1890 | 18.2 | 1.6 ± 5.2 | 0–17.3 |
| Buetschliidae | | | |
| *Bundleia* Cunha & Muniz, 1928 | 72.7 | 26.0 ± 20.0 | 0–53.9 |
| *B. dolichosoma* Strelkow, 1939 | 45.5 | 3.1 ± 4.0 | 0–10.3 |
| *B. elongata* Strelkow, 1939 | 45.5 | 5.1 ± 7.3 | 0–17.5 |
| *B. inflata* Strelkow, 1939 | 9.1 | 0.4 ± 1.2 | 0–4.0 |
| *B. piriformis* Strelkow, 1939 | 45.5 | 3.0 ± 4.4 | 0–12.7 |
| *B. postciliata* (Bundle, 1895) | 72.7 | 12.6 ± 10.9 | 0–24.8 |
| *B. triangularis* Strelkow, 1939 | 27.3 | 0.8 ± 1.7 | 0–5.1 |
| *Blepharoconus* Gassovsky, 1919 | 36.4 | 0.8 ± 1.5 | 0–4.8 |
| *B. benbrooki* (Hsiung, 1930) | 27.3 | 0.5 ± 1.1 | 0–3.3 |
| *B. hemiciliatus* Hsiung, 1930 | 27.3 | 0.3 ± 0.6 | 0–1.5 |
| *Blepharoprosthium* Bundle, 1895 | 72.7 | 2.7 ± 3.6 | 0–9.1 |
| *B. pireum* Bundle, 1895 | 27.3 | 0.4 ± 0.9 | 0–3.0 |
| *B. polytrichum* Strelkow, 1939 | 63.6 | 2.3 ± 3.2 | 0–9.1 |
| *Hemiprorodon* Strelkow, 1939 | 54.5 | 0.9 ± 1.2 | 0–3.0 |
| *H. gymnoposthium* Strelkow, 1939 | 54.5 | 0.9 ± 1.2 | 0–3.0 |
| *Holophryoides* Gassovsky, 1919 | 63.6 | 4.8 ± 5.6 | 0–18.2 |
| *H. ovalis* (Fiorentini, 1890) | 54.5 | 4.4 ± 5.7 | 0–18.2 |
| *H. macrotricha* Strelkow 1939 | 27.3 | 0.5 ± 0.8 | 0–2.1 |
| *Paraisotrichopsis* Gassovsky, 1919 | 18.2 | 1.2 ± 2.6 | 0–6.9 |
| *P. composita* Gassovsky, 1919 | 18.2 | 1.2 ± 2.6 | 0–6.9 |
| *Polymorphella* Corliss, 1960 | 72.7 | 5.1 ± 7.2 | 0–20.2 |
| *P. ampulla* (Dogiel, 1929) | 72.7 | 5.1 ± 7.2 | 0–20.2 |
| Blepharocorythidae | | | |
| *Blepharocorys* Bundle, 1895 | 81.8 | 18.8 ± 64.0 | 0–64.0 |
| *B. angusta* Gassovsky, 1919 | 45.5 | 4.4 ± 8.5 | 0–28.0 |
| *B. curvigula* Gassovsky, 1919 | 72.7 | 12.4 ± 19.9 | 0–64.0 |
| *B. microcorys* Gassovsky, 1919 | 36.4 | 1.2 ± 1.7 | 0–3.9 |
| *B. uncinata* (Fiorentini, 1890) | 18.2 | 0.6 ± 2.0 | 0–5.1 |
| *Ochoterenaia* Chavarria, 1933 | 45.5 | 0.6 ± 0.8 | 0–2.0 |
| *O. appendiculata* Chavarria, 1933 | 45.5 | 0.6 ± 0.8 | 0–2.0 |
| *Circodinium* Wolska, 1971 | 54.5 | 1.1 ± 1.7 | 0–5.4 |
| *C. minimum* (Gassovsky, 1919) | 54.5 | 1.1 ± 1.7 | 0–5.4 |
| Cycloposthiidae | | | |
| *Cycloposthium* Bundle, 1895 | 63.6 | 18.5 ± 35.4 | 0–99.3 |
| *C. bipalmatum* (Fiorentini, 1890) | 36.4 | 9.6 ± 20.0 | 0–52.8 |
| *C. edentatum* Strelkow, 1928 | 63.6 | 9.0 ± 16.7 | 0–52.4 |
| *C. edentatum* m. edentatum Strelkow, 1939 | 63.6 | 9.0 ± 16.7 | 0–52.4 |
| *Tripalmaria* Gassovsky, 1919 | 27.3 | 2.1 ± 4.7 | 0–15.3 |
| *T. dogieli* Gassovsky, 1919 | 27.3 | 2.1 ± 4.7 | 0–15.3 |
| Spirodiniidae | | | |
| *Cochliatoxum* Gassovsky, 1919 | 27.3 | 0.2 ± 0.3 | 0–0.3 |
| *C. periachtum* Gassovsky, 1919 | 27.3 | 0.2 ± 0.3 | 0–0.3 |
| *Tetratoxum* Gassovsky, 1919 | 63.6 | 3.0 ± 3.4 | 0–8.2 |
| *T. unifasciculatum* (Fiorentini, 1890) | 63.6 | 2.4 ± 3.3 | 0–8.2 |

Continued on the next page

Table 1. (continued).

| Familia/genus/species/morphotype | Prevalence (%) | Relative abundance (%) | |
|---|---|---|---|
| | | Mean ± SD | Min.-Max. value |
| *T. excavatum* Hsiung, 1930 | 9.1 | 0.1 ± 0.4 | 0–1.4 |
| *T.* excavatum m. excavatum Strelkow, 1939 | 9.1 | 0.1 ± 0.4 | 0–1.4 |
| *T. parvum* Hsiung, 1930 | 45.5 | 0.5 ± 0.6 | 0–1.4 |
| *T. parvum* m. parvum Strelkow, 1939 | 45.5 | 0.4 ± 0.5 | 0–1.4 |
| *T. parvum* m. sulcatum Strelkow, 1939 | 9.1 | <0.1 ± 0.1 | 0–0.4 |
| *Ditoxum* Gassovsky, 1919 | 36.4 | 0.6 ± 1.2 | 0–3.8 |
| *D. funinucleum* Gassovsky, 1919 | 36.4 | 0.4 ± 1.2 | 0–3.8 |
| *Spirodinium* Fiorentini, 1890 | 72.7 | 2.3 ± 3.0 | 0–9.2 |
| *S. equi* Fiorentini, 1890 | 72.7 | 2.3 ± 3.0 | 0–9.2 |
| *Triadinium* Fiorentini, 1890 | 54.5 | 1.7 ± 2.4 | 0–7.8 |
| *T. caudatum* Fiorentini, 1890 | 54.5 | 1.7 ± 2.4 | 0–7.8 |
| *Gassovskiella* Grain, 1994 | 18.2 | 0.2 ± 0.5 | 0–1.3 |
| *G. galea* (Gassovsky, 1919) | 18.2 | 0.2 ± 0.5 | 0–1.3 |
| Allantosomatidae | | | |
| *Allantosoma* Gassovsky, 1919 | 36.4 | 8.2 ± 17.4 | 0–53.1 |
| *A. intestinale* Gassovsky, 1919 | 36.4 | 8.2 ± 17.4 | 0–53.1 |
| Total | 20 genera<br>34 species<br>3 morphotypes | | |

Table 2. The average ciliate abundance and distribution of the total number of genera and species of ciliates from the hindgut contents of equids in Turkey (aMean ± SD).

| Locality and Host | Average ciliate abundance[a] (× $10^4$ cells $mL^{-1}$) | Min.-Max. value of abundance (× $10^4$ cells $mL^{-1}$) | Total number of genera | Total number of species | Number of animals studied | References |
|---|---|---|---|---|---|---|
| İzmir, Turkey (Turk rahvan horse) | 14.2 ± 13.9 | 0–45.5 | 22 | 36 | 15 | Gürelli & Göçmen 2011 |
| İzmir, Turkey (racing horse) | 26.4 ± 15.1 | 0–54.5 | 21 | 37 | 15 | Gürelli & Göçmen 2012 |
| İzmir, Turkey (domestic horse) | 15.4 ± 10.8 | 5.5–37.5 | 18 | 28 | 8 | Gürelli 2012 |
| İzmir, Turkey (mule) | 6.4 ± 4.6 | 0–12.5 | 16 | 27 | 8 | Gürelli 2012 |
| İzmir, Turkey (donkey) | 10.4 ± 13.2 | 0–39.0 | 16 | 20 | 8 | Gürelli 2012 |
| Kastamonu, Turkey (domestic horse) | 5.6 ± 5.8 | 1.0–29.5 | 20 | 34 | 11 | Present study |

For individual domestic horses, the total number of species per animal ranged from 3.0 to 22.0, with an average of 13.9 ± 7.0 (SD). The average abundance of hindgut ciliates in the 11 domestic horses was 5.6 ± 5.8 × $10^4$ cells $mL^{-1}$, and the min.– max. values ranged from 1.0 × $10^4$ cells $mL^{-1}$ to 29.5 × $10^4$ cells $mL^{-1}$.

Fine surface structures of *Paraisotricha colpoidea*, *P. ampulla*, *B. postciliata*, *Bundleia piriformis*, *Blepharoprosthium pireum*, *Hemiprorodon gymnoposthium*, *Holophryoides macrotricha*, *H. ovalis*, *Blepharocorys angusta*, *B. curvigula*, *B. microcorys*, *B. uncinata*, *C. bipalmatum*, *C. edentatum* m. *edentatum*, *Tripalmaria dogieli*, *S. equi*, *T. excavatum* m. excavatum, *T. unifasciculatum*, *Ditoxum funinucleum*, *C. periachtum*, *Triadinium caudatum*, *Circodinium minimum*, *G. galea*, and *Allantosoma intestinale* were analyzed using SEM. Compared with the light micrographs, the SEM images showed more clearly the ciliary rows and zones, pellicular grooves, ridges, striations, fold, and cavity, barren kinetosomes, frontal lobe, pore of the contractile vacuoles, surface pattern, the lip of ciliary arches and caudalia, cytoproct, tail flap, caudal lobes, anterior and caudal process, tentacles, and suckers (Figs. 1–6).

## Discussion

In the present study, 34 species and three morphotypes to 20 genera were identified, but no novel species were detected. The ciliate diversity was similar to reports from other equids in Turkey. The average ciliate abundance in the hindgut of domestic horses in Kastamonu (5.6 ± 5.8 × $10^4$ cells $mL^{-1}$) was considerably lower than that in other equids in Turkey (Gürelli & Göçmen 2011, 2012, Gürelli 2012). The total number of ciliate species identified from domestic horses in Kastamonu was lower than that from Turk rahvan horses and racing horses (Gürelli & Göçmen 2011, 2012), but higher than that from domestic horses, mules, and donkeys (Gürelli 2012) (Table 2). These variations in ciliate abundance and number of species may be due to differences between host animals, feeding habits of equids, number of hosts

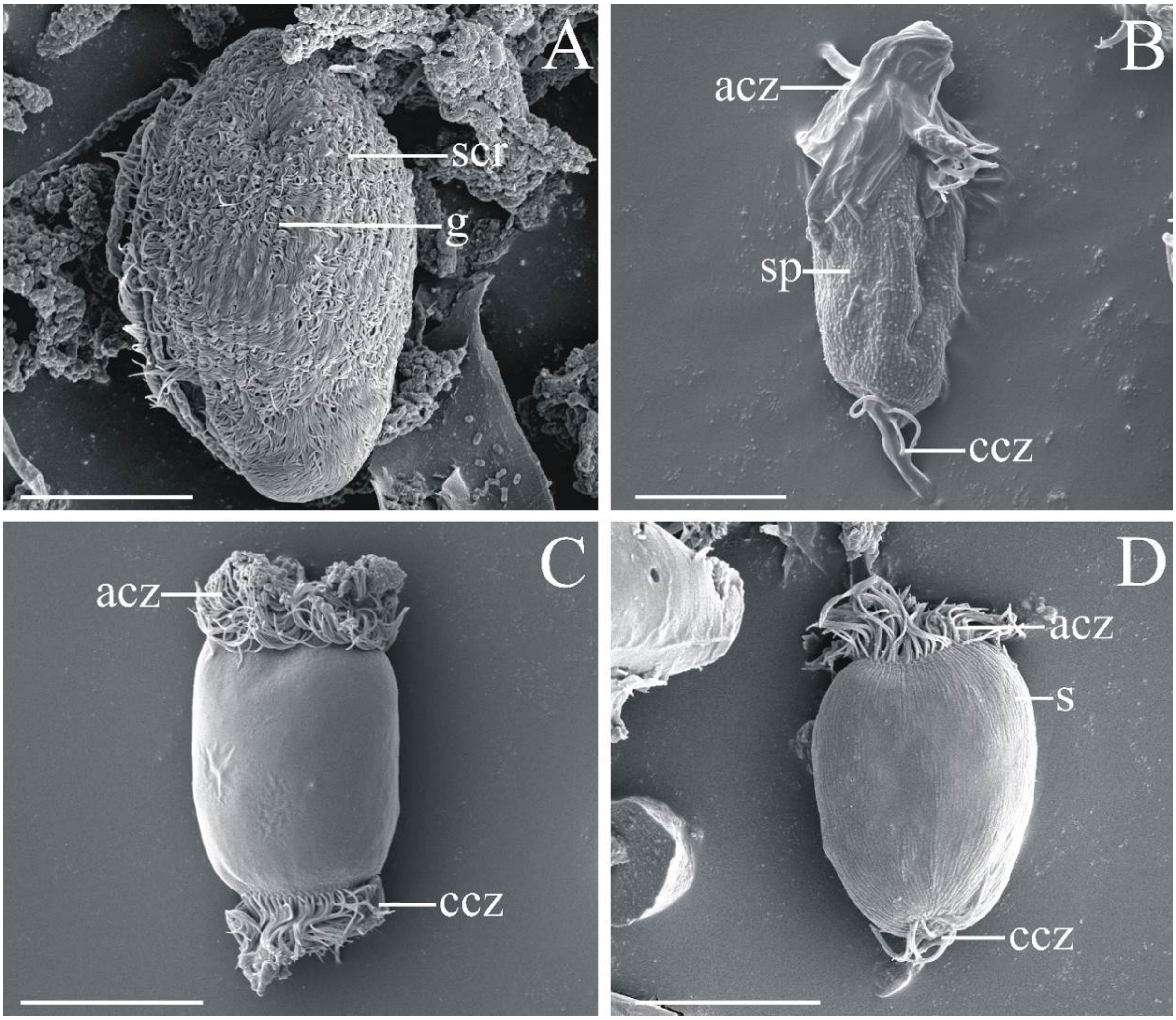

Figure 1. SEM images of A: *Paraisotricha colpoidea*, B: *Polymorphella ampulla*, C: *Bundleia postciliata*, D: *B. piriformis*. Abbreviations, acz: adoral ciliary zone, ccz: caudal ciliary zone, g: groove, s: striation, sp: surface pattern, scr: somatic ciliary row. Scale bars, A, C, and D: 20 μm; B:10 μm.

examined, or a combination of these factors (Gürelli et al. 2019).

In the present study, the SEM images of 24 hindgut ciliates of horses in Kastamonu, Turkey were analyzed. Of them, *B. piriformis*, *B. pireum*, *H. gymnoposthium*, *B. angusta*, *D. funinucleum*, *C. minimum*, and *G. galea* were first examined using SEM (Figs. 1–6).

*Paraisotricha colpoidea* was first reported in horses from Italy (Fiorentini 1890). This species belongs to the order Vestibuliferida and family Paraisotrichidae (Lynn 2008). The body of *P. colpoidea* is oval and covered with fine and dense cilia derived from slightly spiraling longitudinal somatic ciliary rows. These ciliary rows originate within pellicular grooves and are close together. Pellicular grooves are present between pellicular ridges (Fig. 1A).

*Polymorphella ampulla* was first observed in horses from Russia (Dogiel 1929). The body of *P. ampulla* is flask shaped and possesses two ciliary zones at the anterior and posterior extremities. The adoral ciliary zone occupies the anterior one-third of the body, whereas the caudal ciliary zone comprises a ciliary tuft at the posterior end of the body. The body has a surface pattern between the adoral and caudal ciliary zones (Fig. 1B).

*Bundleia postciliata* was first reported in horses from Germany (Bundle 1895). The body of *B. postciliata* is elongated and slightly flattened laterally. The anterior and posterior ends of the body are truncated with long, fine cilia. The adoral ciliary zone is more developed than the caudal ciliary zone (Fig. 1C).

*Bundleia piriformis* was first observed in horses in Russia (Strelkow 1939). The body of *B. piriformis* is elongated and pear shaped. The anterior end of the body is truncated, and the body sharply tapers toward the posterior end. The adoral ciliary zone is at the anterior end of the body, and the caudal ciliary zone comprises a ciliary tuft at the posterior end of the body. The body surface has longitudinal striations (Fig. 1D).

*Blepharoprosthium pireum* was first reported in horses in Germany (Bundle 1895). The body of *B. pireum* is elongated and pear shaped. The body tapers toward the anterior end, and the posterior end is rounded. The adoral ciliary zone occupies nearly the anterior half of the body or the anterior one-third of the body, whereas the caudal ciliary zone comprises a ciliary tuft at the posterior end of the body and

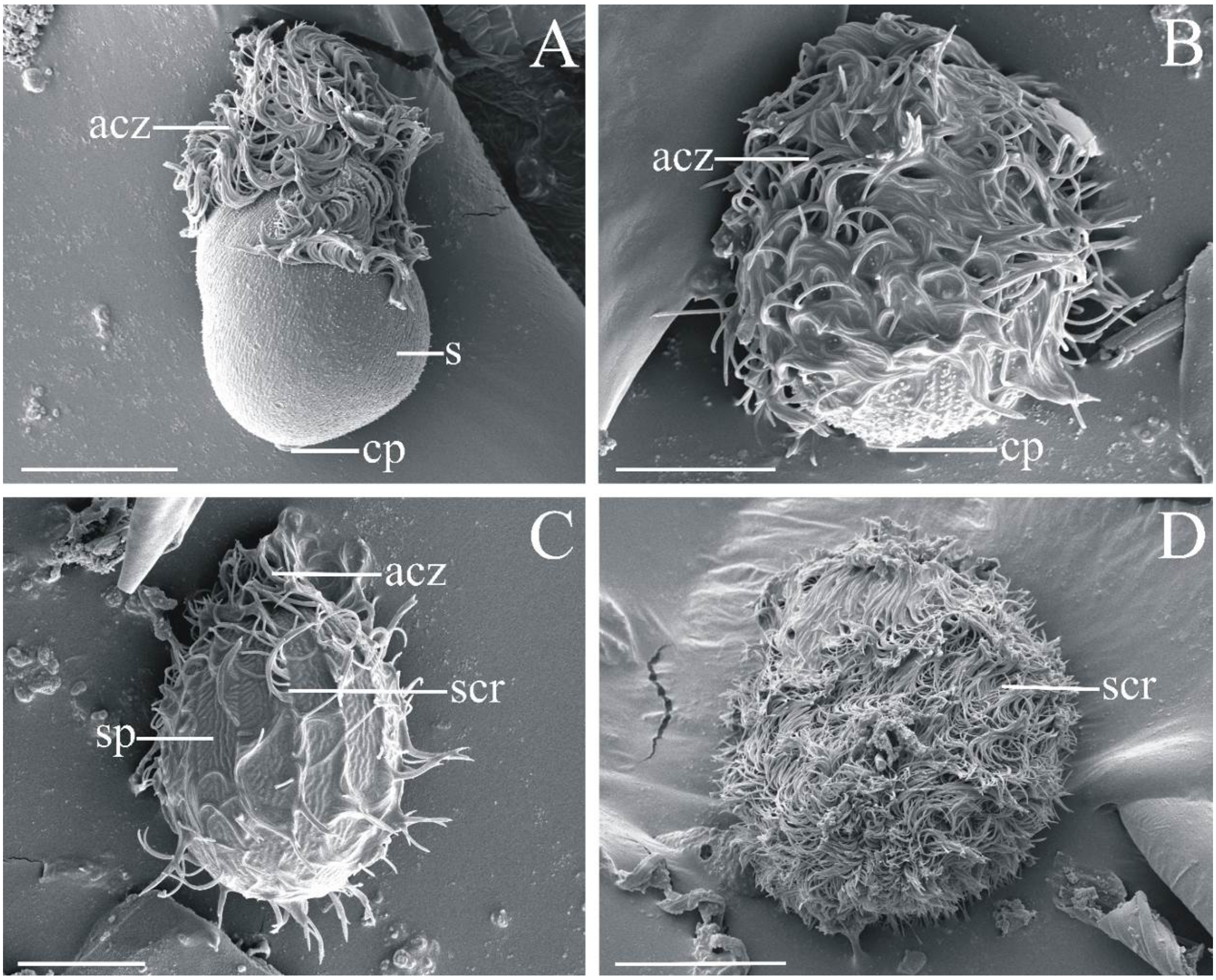

Figure 2. SEM images of A: *Blepharoprosthium pireum*, B: *Hemiprorodon gymnoposthium*, C: *Holophryoides macrotricha*, D: *H. ovalis*. Abbreviations, acz: adoral ciliary zone, cp: cytoproct, s: striation, sp: surface pattern, scr: somatic ciliary row. Scale bars, A: 20 µm; B and C: 10 µm; D: 30 µm.

surrounds the cytoproct. A ciliary tuft of the adoral ciliary zone is slightly apart from the adoral ciliary zone. The body surface has longitudinal striations (Fig. 2A).

*Hemiprorodon gymnoposthium* was first observed in horses in Russia (Strelkow 1939). The body of *H. gymonoposthium* is elongated and asymmetric, tapers to the anterior end, and the anterior end of the body directs to one side. The posterior end of the body is rounded. The adoral ciliary zone consists of long, fine cilia and covers the entire body with longitudinal ciliary rows, except for the posterior one-sixth or one-seventh part of the body. The posterior end of the body without cilia (naked section) has barren kinetosomes. These kinetosomes are with nonciliferous rows and are found between the ciliary rows in the ciliferous section. The cytoproct is at the posterior end of the body (Fig. 2B).

*Holophryoides macrotricha* was first reported in horses from the Soviet Union (Strelkow 1939). The body of *H. macrotricha* is rounded or oval and possesses somatic ciliary rows extending from the anterior end to the posterior end. Somatic ciliary rows are composed of long, fine cilia, but they are not dense and close together. The body has a wavy surface pattern between ciliary rows. The adoral ciliary zone surrounds the cytostome at the anterior end of the body (Fig. 2C).

*Holophryoides ovalis* was first observed in horses from Italy (Fiorentini 1890). The body of *H. ovalis* is rounded or oval and covered entirely with longitudinal ciliary rows extending from the anterior end to the posterior end. Somatic ciliary rows are composed of short, fine, dense cilia, which are very close together (Fig. 2D).

*Polymorphella ampulla*, *B. postciliata*, *B. piriformis*, *B. pireum*, *H. gymnoposthium*, *H. macrotricha*, and *H. ovalis* were classified in the order Entodiniomorphida and family Buetschliidae. Members of the family Buetschliidae and Paraisotrichidae have a concretion vacuole near the anterior end of the body. The concretion vacuole is the sensory organelle of the cell (Dogiel 1929, Anderson & Dumont 1966, Schrenk & Bardele 1987). The concretion vacuole is connected to the paralabial kineties, and these kineties are composed of specialized, large kinetosomes that lack cilia (Wolska 1964, Lynn 2008, Gürelli 2019, Gürelli et al. 2019). Although the paralabial kineties of the concretion vacuole of buetschliids were detected with SEM in the earlier studies (Gürelli 2019, Gürelli et al. 2019), they were not found in the present study.

*Blepharocorys angusta* was first observed in horses in Russia (Gassovsky 1919). The body of *B. angusta* is elongated, narrow, and flattened laterally. The width of the body decreases toward the posterior end. The frontal lobe on the anterior dorsal side is nearly triangular with its tip

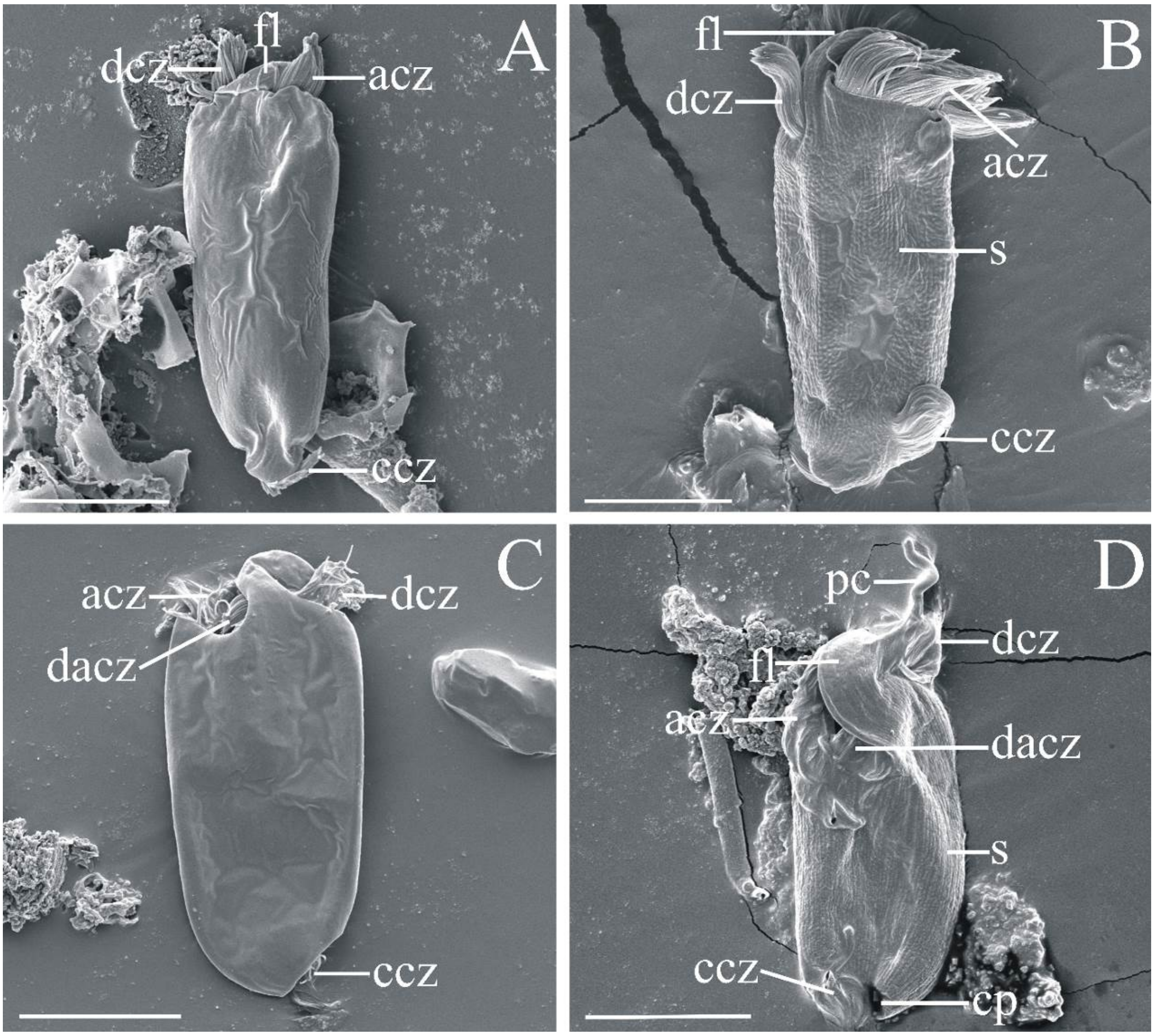

Figure 3. SEM images of A: *Blepharocorys angusta*, B: *B. curvigula*, C: *B. microcorys*, D: *B. uncinata*. Abbreviations, acz: adoral ciliary zone, ccz: caudal ciliary zone, cp: cytoproct, dacz: dorsoadoral ciliary zone, dcz: dorsal ciliary zone, fl: frontal lobe, pc: process, s: striation. Scale bars, A, B, C, and D: 20 µm.

projecting toward the ventral side. The adoral ciliary zone surrounds the right and ventral sides of the vestibular opening. The dorsoadoral ciliary zone is on the ventral side of the frontal lobe. The dorsal ciliary zone is on the dorsal side of the frontal lobe. The small caudal ciliary zone is on the left side of the posterior end (Fig. 3A).

*Blepharocorys curvigula* was first reported in horses in Russia (Gassovsky 1919). The body of *B. curvigula* is elongated and flattened laterally. The width of the body decreases toward the posterior end. It is the largest species of the genus *Blepharocorys*. The frontal lobe is on the anterior dorsal side, and its tip projects toward the ventral side. The adoral ciliary zone surrounds the right and ventral sides of the vestibular opening. The dorsoadoral ciliary zone is on the ventral side of the frontal lobe. The dorsal ciliary zone is on the dorsal side of the frontal lobe. The caudal ciliary zone is on the left side of the posterior end, but the ventral side cilia are bent to the right side. The body surface has longitudinal striations (Fig. 3B).

*Blepharocorys microcorys* was first observed in horses in Russia (Gassovsky 1919). The body of *B. microcorys* is elongated and flattened laterally. The dorsal side of the body is flat, whereas the ventral side is convex. The width of the body decreases toward the posterior end. The narrow frontal lobe is on the anterior dorsal side, and its sharpened tip projects toward the ventral side. The adoral ciliary zone surrounds the right and ventral sides of the vestibular opening. The dorsoadoral ciliary zone is on the ventral side of the frontal lobe. The dorsal ciliary zone is on the dorsal side of the frontal lobe. The caudal ciliary zone is short and at the right side of the postero-dorsal end (Fig. 3C).

*Blepharocorys uncinata* was first reported in horses from Russia (Gassovsky 1919). The body of *B. uncinata* is elongated and flattened laterally. The dorsal side of the body is slightly convex, and the ventral side is slightly concave. The width of the body decreases toward the posterior end. The frontal lobe is on the anterior dorsal side and extends toward the left dorsal side in a rounded shape. A corkscrew-like anterior process projects from the anterior end of the frontal lobe. This process makes two turns and is rounded at its anterior end. The adoral ciliary zone surrounds the right and ventral sides of the vestibular opening. The dorsoadoral

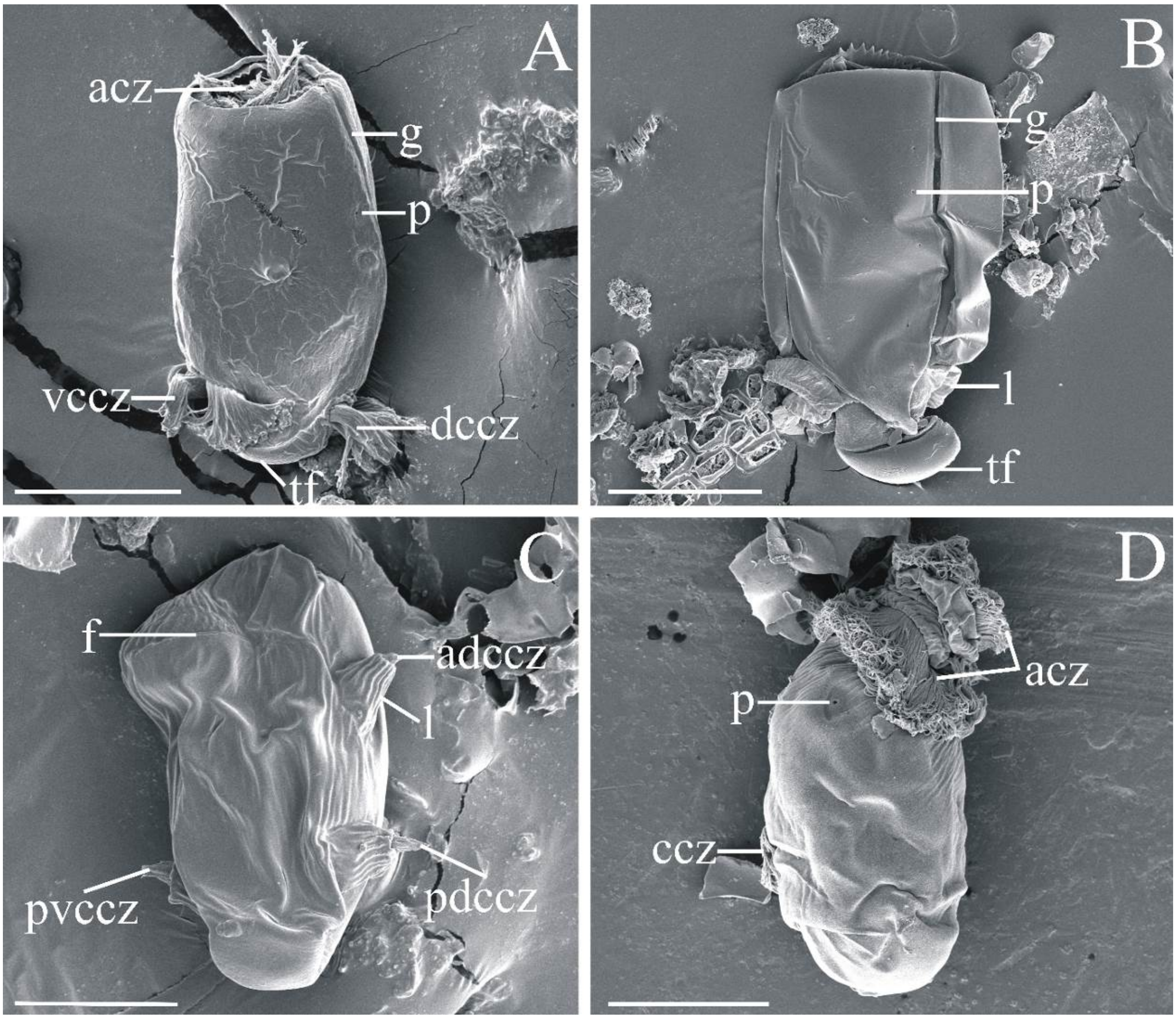

Figure 4. SEM images of A: *Cycloposthium bipalmatum*, B: *C. edentatum* m. edentatum, C: *Tripalmaria dogieli*, D: *Spirodinium equi*. Abbreviations, acz: adoral ciliary zone, adccz: anterior dorsal caudal ciliary zone, ccz: caudal ciliary zone, dccz: dorsal caudal ciliary zone, f: fold, g: groove, l: lip, p: pore of contractile vacuole, vccz: ventral caudal ciliary zone, pvccz: posterior ventral caudal ciliary zone, pdccz: posterior dorsal caudal ciliary zone, tf: tail flap. Scale bars, A and D: 40 µm; B: 50 µm; C: 30 µm.

ciliary zone is on the ventral side of the frontal lobe. The dorsal ciliary zone is on the dorsal side of the frontal lobe. The caudal ciliary zone is at the left side of the posterior end and the ventral side of the cytoproct. The body surface has longitudinal striations (Fig. 3D).

*Cycloposthium bipalmatum* was first observed in horses from Italy (Fiorentini 1890). The body of *C. bipalmatum* is rectangular and flattened laterally. The dorsal side of the body is convex, whereas the ventral side is flat. The anterior end of the body is truncated, and the posterior end tapers to form a tail. The dorsal and ventral caudalia are asymmetrically located at the dorsal and ventral sides of a constriction at the base of the tail flap. A pellicular groove extends from the anterior end to the posterior end at the left dorsal side of the body. Four contractile vacuoles are spaced along the ventral side of the groove on the left side. The retractible adoral ciliary zone surrounds the vestibular opening and is at the anterior end of the body. The dorsal and ventral caudal ciliary zones are ciliary tufts, non-retractable, and arise from the two caudalia. Both caudalia possess caudal lips (Fig. 4A).

*Cycloposthium edentatum* m. edentatum was first reported in horses and donkeys from the USSR (Strelkow 1939). The body of *C. edentatum* m. edentatum is rectangular and flattened laterally. The dorsal side of the body is convex, whereas the ventral side is flat. The anterior end of the body is truncated, and the posterior end tapers to form a tail. The dorsal and ventral caudalia are asymmetrically located at the dorsal and ventral sides of a constriction at the base of the tail flap. The tail flap is narrow after the dorsal and ventral caudalia, but it suddenly expands and appears similar to a horseshoe. The horseshoe-shaped tail flap is slantwise to the dorsal side, and its dorsal end is more posterior than its ventral end. A pellicular groove extends from the anterior end to the posterior end at the left dorsal side of the body. Five to seven contractile vacuoles are spaced along the ventral side of the groove on the left side. The retractible adoral ciliary zone surrounds the vestibular opening and is at the anterior end of the body. The dorsal and ventral caudal ciliary zones are ciliary tufts, retractable, and arise from the two caudalia. Both caudalia possess caudal lips (Fig. 4B).

*Tripalmaria dogieli* was first observed in horses from Russia (Gassovsky 1919). The body of *T. dogieli* is irregularly oval in shape, elongated, and laterally flattened. The greatest width of the body is the anterior one-third of the body, at the level of the anterior dorsal caudalium. The body width reduces toward the posterior end, and the body is rounded after the caudal ciliary zones. The anterior 1/5–1/7 part of the body is delimited from the remaining body by the transverse fold of the pellicle not reaching the dorsal edge of the body. The retractible adoral ciliary zone surrounds the vestibular opening and is at the anterior end of the body, slightly closer to the dorsal side. The posterior dorsal and ventral caudal ciliary zones are ciliary tufts, retractable, and arise from the two caudalia. The anterior dorsal ciliary zone is ciliary tuft, retractible, and arises from the caudalium lying in the anterior one-thirdth part of the body. The proximal part of the caudalia is covered by caudal lips (Fig. 4C).

*Spirodinium equi* was first reported in horses from Italy (Fiorentini 1890). The body of *S. equi* is elongated and cylindrical. The anterior end of the body is rounded and directs to the ventral side, whereas the body width slightly reduces toward the posterior end. The adoral ciliary zone is at the anterior end of the body and revolves two and a half times around the vestibulum. The caudal ciliary zone is at the dorsal side of the posterior half of the body, near the middle, and slantwise to the left side. Both ciliary zones arise from the arches surrounded by the lips. The contractile vacuole is at the right side of the body and behind the adoral ciliary zone (Fig. 4D).

*Tetratoxum excavatum* was first observed in horses from the USA (Hsiung 1930). Later, two morphotypes of this species, *T. excavatum* m. excavatum and *T. excavatum* m. sulcatum, were detected from horses in Russia (Strelkow 1939). The first morphotype *T. excavatum* m. excavatum has no longitudinal grooves on the body surface, whereas *T. excavatum* m. sulcatum has them. In the present study, only one morphotype, *T. excavatum* m. excavatum, was observed. The body of *T. excavatum* m. excavatum is elongated and irregularly elliptical in shape, with both ends rounded. It is thickest in the middle. The dorsal and ventral sides are convex. The special cavity formed by the invagination of the pellicle is at the anterior end and on the right side of the body between the adoral and dorsal ciliary zones. The dorsal and ventral sides of the body have strongly developed longitudinal grooves. Two caudal lobes are at the posterior end of the body, but their positions are not at the same level. The left caudal lobe is located more dorsally, whereas the right caudal lobe is more ventral. The dorsal ciliary zone is at the antero-dorsal end of the body. It is slantwise from the right side to the left side. The adoral ciliary zone is at the antero-ventral end of the body. It is derived from the right side, directs to the ventral side, passes to the left side, directs to the right side again, and ends near the starting point. The dorsal and ventral caudal ciliary zones are at the dorsal and ventral posterior ends, respectively. The ventral caudal ciliary zone is slantwise from the right side to the left side, whereas the dorsal caudal ciliary zone is slantwise from the left side to the right side. Every ciliary zone arises from the arch surrounded by the lips (Fig. 5A).

*Tetratoxum unifasciculatum* was first observed in horses in Italy (Fiorentini 1890). The body of *T. unifasciculatum* is elongated, slightly flattened laterally and irregularly elliptical, with both ends rounded. It is thickest in the middle. The dorsal and ventral sides of the body have longitudinal grooves. Two caudal lobes are at the posterior end of the body, but their positions are not at the same level. The left caudal lobe is more dorsal, whereas the right caudal lobe is more ventral. The dorsal ciliary zone is at the antero-dorsal end of the body. It is slantwise from the right side to the left side. The adoral ciliary zone is at the antero-ventral end of the body. It is derived from the right side, directs to the ventral side, passes to the left side, directs to the right side again, and ends near the starting point. The dorsal and ventral caudal ciliary zones are at the dorsal and ventral posterior ends, respectively. The ventral caudal ciliary zone is slantwise from the right side to the left side. The dorsal caudal ciliary zone is slantwise from the left side to the right side. Every ciliary zone arises from the arch surrounded by the lips (Fig. 5B).

*Ditoxum funinucleum* was first reported in horses in Russia (Gassovsky 1919). The body of *D. funinucleum* is elongated, elliptical in shape, with both ends rounded, flattened laterally, and the body width is slightly reduced toward the posterior end. One caudal lobe is at the posterior end of the body. The adoral ciliary zone is at the antero-ventral end of the body and surrounds the vestibulum. The dorsal ciliary zone is at the antero-dorsal end of the body and slantwise from the right side of the body to the left side. The caudal ciliary zone is at the postero-dorsal end of the body and slantwise from the left side of the body to the right side. Every ciliary zone arises from the arches surrounded by the lips (Fig. 5C).

*Cochliatoxum periachtum* was first observed in horses in Russia (Gassovsky 1919). The body of *C. periachtum* is oval, with the anterior end rounded and flattened laterally. The body width is slightly reduced toward the posterior end. It is the largest ciliate species in the hindgut of equids. One caudal lobe is at the posterior end of the body. The adoral ciliary zone is at the antero-ventral end of the body. It is derived from the right side, extends spirally to the ventral and left sides, and directs spirally to the right side again. The dorsal and ventral caudal ciliary zones are at the dorsal and ventral posterior ends, respectively. The ventral caudal ciliary zone is slantwise from the right side to the left side. The dorsal caudal ciliary zone is slantwise from the left side to the right side. Every ciliary zone arises from the arch surrounded by the lips (Fig. 5D).

*Triadinium caudatum* was first reported in horses in Italy (Fiorentini 1890). The body of *T. caudatum* is helmet shaped and slightly flattened laterally. It is thickest in the middle. The caudal process is at the posterior end of the body and possesses a ciliary tuft, and its posterior end appears similar to a tail. The crescent-shaped adoral ciliary zone is in the middle of the ventral side and surrounds the vestibulum. The dorsal ciliary zone is at the postero-dorsal side of the body and slantwise from the anterior to the posterior. The anterior ciliary zone is at the anterior end of the body. Every ciliary zone arises from the arch surrounded by the lips (Fig. 6A).

*Circodinium minimum* was first observed in horses in Russia (Gassovsky 1919). The body of *C. minimum* is helmet shaped. It is thickest in the middle. The caudal process is at the posterior end of the body and possesses a ciliary tuft,

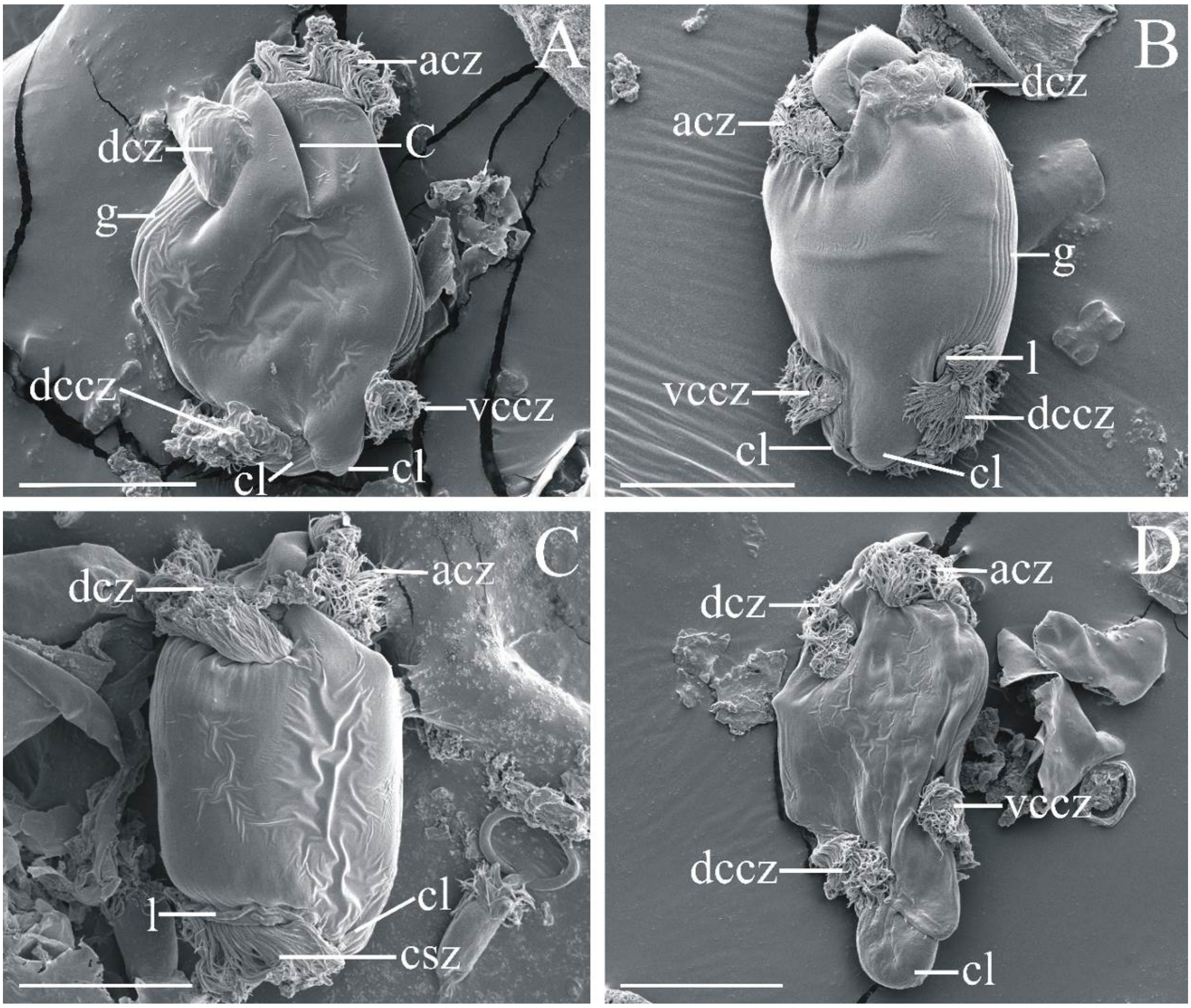

Figure 5. SEM images of A: *Tetratoxum excavatum* m. *excavatum*, B: *T. unifasciculatum*, C: *Ditoxum funinucleum*, D: *Cochliatoxum periachtum*. Abbreviations, acz: adoral ciliary zone, c: cavity, cl: caudal lobe, csz: caudal ciliary zone, dcz: dorsal ciliary zone, dccz: dorsal caudal ciliary zone, vccz: ventral caudal ciliary zone, g: groove, l: lip. Scale bars, A and C: 40 µm; B and D: 50 µm.

and its posterior end looks like a tail. The crescent-shaped adoral ciliary zone is in the middle of the ventral side and surrounds the vestibulum. The dorsal ciliary zone is at the postero-dorsal side of the body and slantwise from anterior to posterior. The anterior ciliary zone is at the anterior end of the body. The body surface of *C. minimum* has circular striations (Fig. 6B).

*Gassovskiella galea* was first reported in horses from Russia (Gassovsky 1919). The body of *G. galea* is helmet shaped and slightly flattened laterally. It is thickest in the middle. The adoral ciliary zone is at the postero-ventral end of the body. It is slantwise from the ventral side to the posterior side and surrounds the vestibulum. The dorsal ciliary zone is at the postero-dorsal end of the body and slantwise from the dorsal side to the posterior side. The anterior ciliary zone is at the anterior end of the body. Every ciliary zone arises from the arch surrounded by the lips (Fig. 6C).

*Allantosoma intestinale* was first observed in horses in Russia (Gassovsky 1919). The body of this suctorian species is elongated with convex and flattened sides. It possesses 7–16 tentacles at both ends of the body, and every tentacle has many suckers on the distal end. The body surface of *A. intestinale* resembles that of a honeycomb with numerous minute holes (Fig. 6D).

Although the lip surrounding the ciliary zones of the caudalia and arches is observed in members of the families Cycloposthiidae and Spirodiniidae, this feature was not detected in members of the families Buetschliidae and Blepharocorythidae. The hindgut ciliates *T. caudatum*, *C. minimum*, and *G. galea*, all having the characteristic helmet shape, however, are not in the same family. *T. caudatum* and *G. galea* belong to the family Spirodiniidae, whereas *C. minimum* is in the family Blepharocorythidae. SEM observations showed that *C. minimum* and *Blepharocorys* spp. have the same surface pattern, but *T. caudatum* and *G. galea* have no surface pattern.

Infraciliature patterns and molecular phylogenetic analysis of *T. caudatum*, *C. minimum*, and *G. galea* also supported the idea that the helmet-shaped body is a homoplastic character that may have arisen at least three times and thus may not reflect evolutionary divergences (Wolska 1969, 1971, 1981, Cedrola et al. 2020). In addition, *T. caudatum* and *G. galea* have the lip at the circumference of the ciliary arch, whereas *C. minimum* does not have it. Our SEM observations and other studies proved that *C. minimum* and

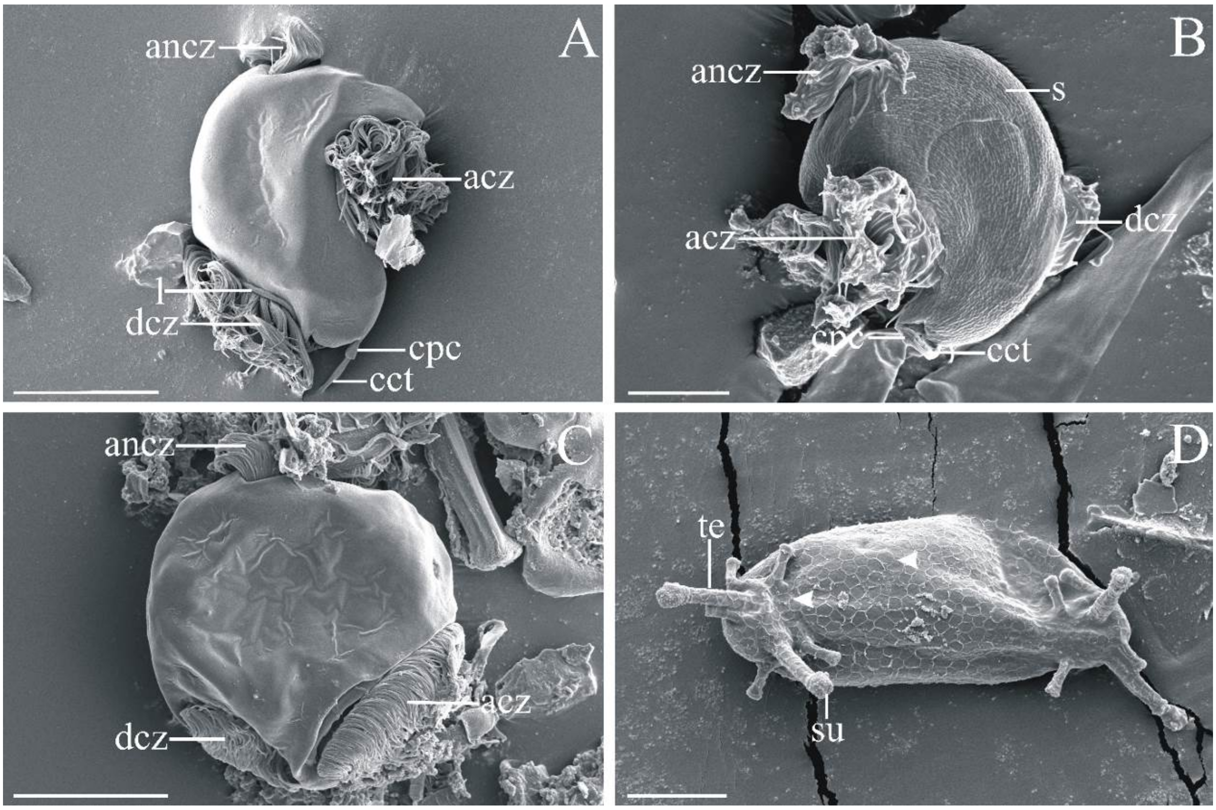

Figure 6. SEM images of A: *Triadinium caudatum*, B: *Circodinium minimum*, C: *Gassovskiella galea*, D: *Allantosoma intestinale*. Abbreviations, acz: adoral ciliary zone, ancz: anterior ciliary zone, cpc: caudal process, cct: caudal ciliary tuft, dcz: dorsal ciliary zone, l: lip, s: striation, te: tentacle, su: sucker, holes (arrowheads). Scale bars, A: 30 µm; B and D: 10 µm; C: 20 µm.

*Blepharocorys* spp. are phylogenetically close species and thus should be classified under the same family.

In conclusion, the fine surface structures of endocommensal ciliates of herbivores warrant further investigation to understand their evolution and the relationships among species.

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