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Endosymbiotic Intestinal Ciliates (Ciliophora) of Domestic Horses (*Equus caballus* Linnaeus, 1758) in Libya

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Abstract: In this study, the composition and distribution of intestinal ciliates, which are endosymbionts, were investigated in fecal samples from 30 domestic horses in Susah, Libya. One morphotype and 16 ciliate species belonging to 13 genera and 5 families were identified. Five of the horses did not harbor any ciliates. The genus *Cycloposthium* was present in 23 of the remaining horses, represented by the species *C. bipalmatum*, with a prevalence of 76.7%. In contrast, *Holophryoides macrotricha*, *Tetratoxum parvum*, and *Triadinium caudatum* were each observed in only one horse, with a prevalence of 3.3%. The number of species identified per horse ranged from 0 to 9, with an average of 4.3 ± 2.1. The average density of intestinal ciliates in the horses was 7.0 ± 6.2 × 10^4 cells mL^{-1}. This is the first report on intestinal ciliates in horses in Libya.

Keywords: Coprophagy, endosymbiont, microorganism, protist, Suctoria, Trichostomatia.

Libya'daki Evcil Atların (*Equus caballus* Linnaeus, 1758) Endosimbiyotik Bağırsak Siliyatları (Ciliophora)

Öz: Bu çalışmada, endosimbiyont olan bağırsak siliyatlarının kompozisyonu ve dağılımı Libya'nın Susah kentindeki 30 evcil attan alınan fekal örneklerde araştırılmıştır. Beş familya ve 13 cinse ait 16 siliyat türü ve 1 morfotip teşhis edilmiştir. Atların beşinde herhangi bir siliyat tespit edilmemiştir. *Cycloposthium* cinsi, kalan atların 23'ünde *C. bipalmatum* türü ile saptanmış olup yaygınlığı %76.7 olarak belirlenmiştir. Buna karşılık, *Holophryoides macrotricha*, *Tetratoxum parvum* ve *Triadinium caudatum* türlerinin her biri %3.3 yaygınlıkla yalnızca birer atta gözlenmiştir. At başına teşhis edilen tür sayısı 0 ile 9 arasında değişmekte olup ortalama 4.3 ± 2.1'dir. Atlardaki bağırsak siliyatlarının ortalama yoğunluğu 7.0 ± 6.2 × 10^4 hücre mL^{-1}'dir. Libya'daki atların bağırsak siliyatları ilk kez rapor edilmiştir.

Anahtar kelimeler: Koprofaji, endosimbiyont, mikroorganizma, protist, Suctoria, Trichostomatia.

1. Introduction

Horses are hindgut fermenters and the most microbial activities occur in the large intestine, an immensely enlarged fermentative chamber that constitutes approximately 60 percent of the gastrointestinal tract (GIT) and contains a highly abundant and diverse community of anaerobic microorganisms (Dicks et al., 2014; Fliegerova et al., 2016). The intestinal community comprises of archaea, bacteria, ciliated protists (also known as ciliates), fungi, and viruses (Julliand & Grimm, 2016, 2017). Fecal matter is widely used to study the microbial composition of the horse's large intestine and is generally considered a reliable indicator of the equine gut microbiome (Fliegerova et al., 2016). Numerous ciliate species are known to inhabit the horse's large intestine (Gassovsky, 1919; Hsiung, 1930; Strelkow, 1939; Adam, 1951; Ozeki, 1977) and the ciliate composition in feces is thought to reflect that of the large intestine (Ike et al., 1981, 1983a, b, c; Tung, 1992; Ito et al., 1996; Imai et al., 1999; Gürelli & Göçmen, 2010, 2011, 2012). A rigid cortex helps ciliates maintain their cell shape during defecation, enabling them to survive briefly in external environments (Kornilova, 2004; Kornilova et al., 2019). Even though these ciliates cannot produce resistant cysts, they are still able to reach and establish themselves in the large intestine of foals through coprophagy (Ike et al., 1985; Egan et al., 2010). The ability of these ciliates to digest cellulose and starch in the large intestine enables them to form an endosymbiotic relationship with their equine host (Ozeki et al., 1973; Dehority, 1986). They also act as medical regulators of the prokaryotic population in the intestine (Gassovsky, 1919).

Although the composition of the intestinal ciliate communities in horses has been studied in various geographic regions (Gassovsky, 1919; Hsiung, 1930; Strelkow, 1939; Adam, 1951; Ozeki et al., 1973; Ike et al., 1981; Ike et al., 1983a, b, c; Ike et al., 1985; Tung, 1992; Ito et al., 1996; Imai et al., 1999; Kornilova, 2006; Gürelli & Göçmen, 2011, 2012; Göçmen et al., 2012; Gürelli, 2012; Gürelli et al., 2015, 2019; Cedrola et al., 2019; Kornilova et al., 2019; Gürelli & Aydın, 2021; Kornilova et al., 2022; Kornilova & Chistyakova, 2024), no research has yet been conducted on the ciliate fauna of horses in Libya. Thus, the objectives of this study are to investigate the intestinal ciliate composition in domestic horses (*Equus caballus* Linnaeus, 1758) in Libya and to compare the results with those in the existing literature.

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2. Material and Method

Fecal samples were collected from 30 domestic horses (*Equus caballus* Linnaeus, 1758) inhabiting different farms in Susah, Libya between February 2016 and April 2016. The horses were fed barley and oats, supplemented with grass obtained through grazing and freshly cut forage. Fecal samples were immediately fixed and stained with twice the volume of methyl green formalin saline (MFS) solution compared to their original volume after defecation. The MFS solution served as a fixative and nuclear stain. This procedure was used to preserve cell integrity and internal structures (Ogimoto & Imai, 1981; Gürelli & Göçmen, 2012). The samples were filtered through a 2.56 mm mesh gauze in the laboratory. A 2% Lugol's iodine solution was applied to the samples to highlight the skeletal plates of the ciliates (Ogimoto & Imai, 1981; Gürelli & Göçmen, 2010; Gürelli et al., 2019).

The prevalence of ciliate species was calculated as previously described by Bush et al. (1997). Total cell counts were determined using a Neubauer hemocytometer counting chamber (Gürelli & Göçmen, 2011). Differential counts of each species were estimated from smear slides containing 37–369 cells (Gürelli & Aydın, 2021). Statistical analyses were performed using SPSS 23. Ciliate species and genera were identified and classified primarily according to the descriptions of Hsiung (1930), Kornilova (2004), Lynn (2008), Ozeki (1977), and Strelkow (1939).

The ciliates were observed at 400× magnification using a light microscope equipped with an imaging system (Zeiss Primo Star, Oberkochen, Germany). All data for Figures 1–3 were prepared in Excel worksheets. The online software Table Viewer (https://mk.bcgsc.ca/tableviewer/) was used to generate Figure 1 and SankeyMATIC (https://sankeymatic.com/build/) was used for Figure 2. Figure 3 was created using Excel graphics.

3. Results and Discussion

In this study, a total of 1 morphotype and 16 ciliate species belonging to 13 genera and 5 families were identified from fecal samples collected from 30 domestic horses in Susah, Libya. Five of the horses (horses 8, 12, 20, 21, 24) did not harbor any ciliates. The genus *Cycloposthium* was present in 23 of the remaining horses with the species *C. bipalmatum*, and its prevalence was 76.7%. However, *Holophryoides macrotricha*, *Tetratoxum parvum*, and *Triadinium caudatum* were each observed in only one horse, with a prevalence of 3.3% (Table 1, Fig. 1). Strelkow (1939) distinguished *Tetratoxum parvum* into two morphotypes based on the longitudinal rows on the body surface; however, in the present study, only one morphotype (*T. parvum* m. parvum characterized by longitudinal rows on both the dorsal and ventral surfaces) was identified. The relative abundance rate of *Cycloposthium bipalmatum* was high (i.e., 34.5%), whereas that of *Tetratoxum parvum* was low (i.e., <0.1%). Although no suctorian ciliates (class Phyllopharyngea, subclass Suctoria, order Exogenida, family Allantosomatidae) were found, all the species identified in this study belonged to the class Litostomatea and the subclass Trichostomatia, within the orders Vestibuliferida and Entodiniomorphida (Table 1). Similarly, no suctorian ciliates (*Allantosoma* spp., *Allantoxena* spp., *Arcosoma* spp., and *Strelkowella* sp.) were detected in horses from South Africa on the same continent (Kornilova et al., 2022) (Table 2, Fig. 2).

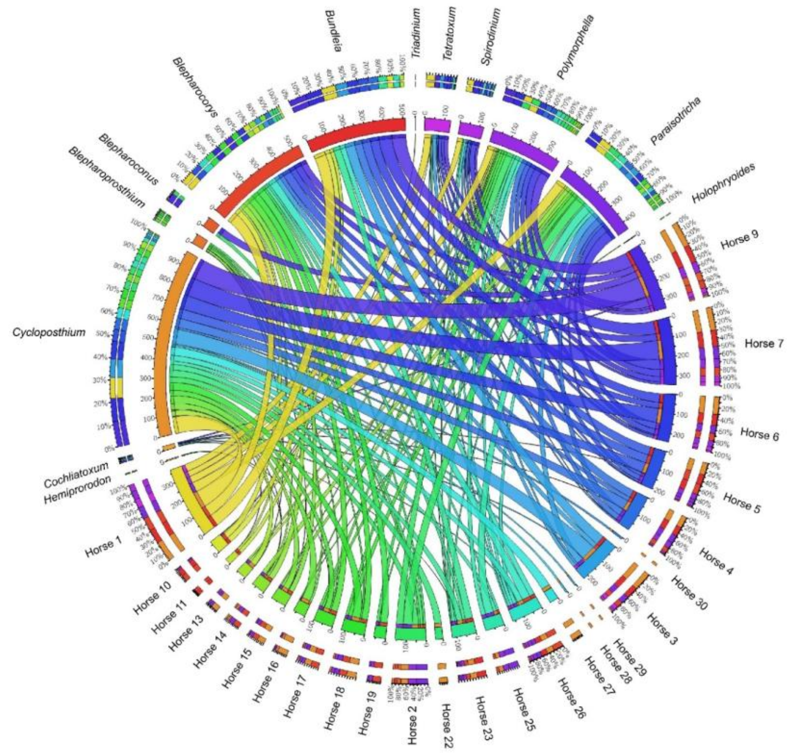

Figure 1. Chord diagram showing the number of ciliate protist species by genus in 30 horses from Libya. The lines within the circle represent the associations between hosts and genera. The numbers inside the circle indicate the number of species per genus in each host. The size of the bars outside the circle is proportional to the number of species per genus in each host. The gradients from golden yellow to dark blue and from orange to dark purple, both inside and outside the circle, represent the order of hosts and genera, respectively, in the diagram.

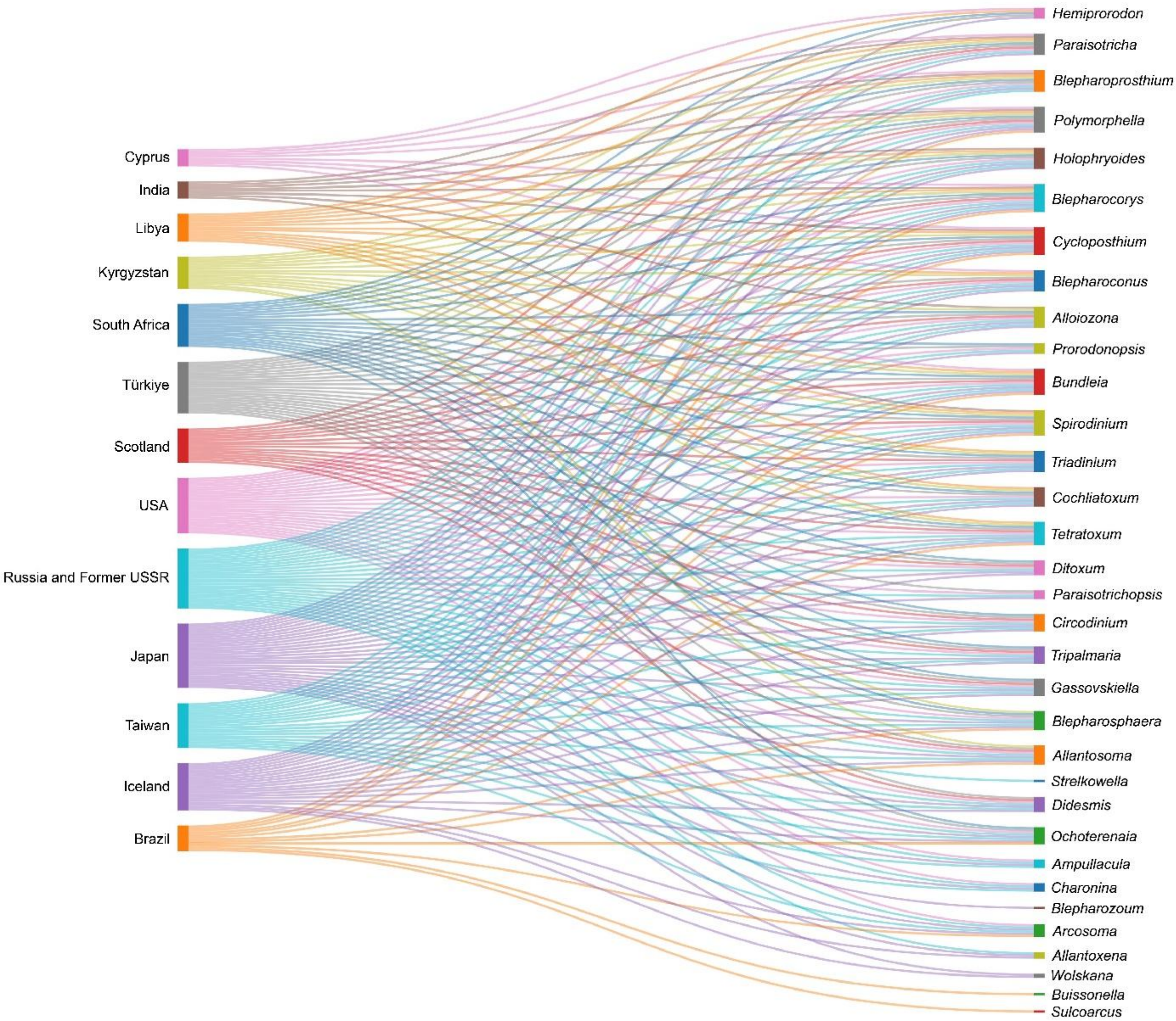

Figure 2. Sankey diagram showing the distribution of horse intestinal ciliate genera across different regions. Flows represent connections from countries to genera. The colors on the left indicate the countries. References: (USA) Hsiung, 1930; Gürelli et al., 2019; (Scotland) Adam, 1951; (Japan) Ike et al., 1981; Ike et al., 1983a, b, c; Ike et al., 1985; Imai et al., 1999; Ito et al., 1996; Ozeki et al., 1973; (Russia and Former USSR) Gassovsky, 1919; Kornilova, 2006; Strelkow, 1939; (Brazil) Cedrola et al., 2019; (Iceland) Kornilova et al., 2019; (India) Kornilova & Chistyakova, 2024; (Cyprus) Göçmen et al., 2012; (Türkiye) Gürelli & Göçmen, 2011, 2012; Gürelli, 2012; Gürelli & Aydın, 2021; (Kyrgyzstan) Gürelli et al., 2015; (Taiwan) Tung, 1992; (South Africa) Kornilova et al., 2022; (Libya) Present study.

Table 1. Prevalence and relative abundance of intestinal ciliates recorded in the feces of 30 domestic horses in Libya. Max, maximum; Mean, arithmetic mean; Min, minimum; SD, standard deviation.

Subclass/Order/Suborder/Family/Genus/Species/Morphotype	Prevalence (%)	Relative Abundance (%)	
		Mean ± SD	Min–Max Value
Subclass Trichostomatia Bütschli, 1889			
Order Vestibuliferida de Puytorac et al., 1974			
Family Paraisotrichidae da Cunha, 1917			
Paraisotricha Fiorentini, 1890	53.3	9.9 ± 11.7	0–44.9
colpoidea Fiorentini, 1890	53.3	9.9 ± 11.7	0–44.9
Order Entodiniomorphida Reichenow in Doflein and Reichenow, 1929			
Suborder Archistomatina de Puytorac et al., 1974			
Family Buetschliidae Poche, 1913			
Blepharoconus Gassovsky, 1919	6.7	0.7 ± 2.7	0–10.8
benbrooki (Hsiung, 1930)	6.7	0.7 ± 2.7	0–10.8
Blepharoprosthium Bundle, 1895	23.3	2.1 ± 4.5	0–17.5
pireum Bundle, 1895	23.3	2.1 ± 4.5	0–17.5

Table 1. (Continued)

Subclass/Order/Suborder/Family/Genus/ Species/Morphotype	Prevalence (%)	Relative Abundance (%)	
		Mean ± SD	Min–Max Value
Bundleia da Cunha & Muniz, 1928	36.7	9.8 ± 15.0	0–57.6
postciliata (Bundle, 1895)	36.7	9.8 ± 15.0	0–57.6
Hemiprorodon Strelkow, 1939	6.7	0.3 ± 1.1	0–5.6
gymnoposthium Strelkow, 1939	6.7	0.3 ± 1.1	0–5.6
Holophryoides Gassovsky, 1919	3.3	0.2 ± 0.9	0–4.8
macrotricha Strelkow, 1939	3.3	0.2 ± 0.9	0–4.8
Polymorphella Corliss, 1960	56.7	8.0 ± 8.3	0–26.7
ampulla (Dogiel, 1929)	56.7	8.0 ± 8.3	0–26.7
Suborder Blepharocorythina Wolska, 1971			
Family Blepharocorythidae Hsiung, 1929			
Blepharocorys Bundle, 1895	60.0	14.2 ± 16.4	0–61.7
curvigula Gassovsky, 1919	33.3	4.9 ± 9.1	0–33.3
microcorys Gassovsky, 1919	13.3	1.3 ± 4.1	0–19.1
uncinata (Fiorentini, 1890)	36.7	7.6 ± 15.4	0–61.7
Suborder Entodiniomorphina Reichenow in Doflein and Reichenow, 1929			
Family Cycloposthiidae Poche, 1913			
Cycloposthium Bundle, 1895	76.7	34.5 ± 34.2	0–100.0
bipalmatum (Fiorentini, 1890)	76.7	34.5 ± 34.2	0–100.0
Family Spirodiniidae Strelkow, 1939			
Cochliatoxum Gassovsky, 1919	20.0	0.4 ± 0.8	0–3.1
periachtum Gassovsky, 1919	20.0	0.4 ± 0.8	0–3.1
Spirodinium Fiorentini, 1890	30.0	1.6 ± 2.7	0–8.4
equi Fiorentini, 1890	30.0	1.6 ± 2.7	0–8.4
Tetratoxum Gassovsky, 1919	33.3	1.7 ± 2.9	0–10.3
parvum Hsiung, 1930	3.3	<0.1 ± <0.1	0–1.5
m. parvum	3.3	<0.1 ± <0.1	0–1.5
unifasciculatum (Fiorentini, 1890)	33.3	1.7 ± 2.9	0–10.3
Triadinium Fiorentini, 1890	3.3	0.1 ± 0.5	0–2.9
caudatum Fiorentini, 1890	3.3	0.1 ± 0.5	0–2.9
Total	5 families, 13 genera, 16 species, 1 morphotype		

Table 2. Distribution of horse intestinal ciliate species across different regions of the world (? is used for descriptions at the genus level only, not at the species level).

Subclass/Order/Suborder/Family/Genus/Species	Geographical Regions[a]												
	USA (1)	Scotland (2)	Japan (3)	Russia and Former USSR (4)	Brazil (5)	Iceland (6)	India (7)	Cyprus (8)	Türkiye (9)	Kyrgyzstan (10)	Taiwan (11)	South Africa (12)	Libya (13)
Subclass Trichostomatia													
Order Vestibuliferida													
Family Paraisotrichidae													
Paraisotricha beckeri	+	+	+	+	–	–	–	–	–	–	–	–	–
Paraisotricha colpoidea	+	+	+	+	–	–	+	+	+	+	–	–	+
Paraisotricha minuta	+	+	+	+	–	–	–	–	+	+	–	+	–
Order Entodiniomorphida													
Suborder Archistomatina													

Table 2. (Continued)

Subclass/Order/Suborder/Family/Genus/Species	Geographical Regions[a]												
	USA (1)	Scotland (2)	Japan (3)	Russia and Former USSR (4)	Brazil (5)	Iceland (6)	India (7)	Cyprus (8)	Türkiye (9)	Kyrgyzstan (10)	Taiwan (11)	South Africa (12)	Libya (13)
Family Buetschliidae													
Alloiozona trizona	+	+	+	+	–	+	+	–	+	+	+	+	–
Ampullacula ampulla	+	–	+	–	–	–	–	–	–	–	+	–	–
Blepharoconus benbrooki	+	+	+	+	–	+	–	+	+	+	+	?	+
Blepharoconus cervicalis	+	–	+	–	–	–	–	–	–	–	+	?	–
Blepharoconus hemiciliatus	+	–	+	+	–	–	–	–	+	–	–	?	–
Blepharoprosthium pireum	+	–	+	+	–	–	–	–	+	+	+	+	+
Blepharoprosthium polytrichum	+	–	+	+	–	–	+	+	+	+	–	–	–
Blepharosphaera ceratotherii	–	–	–	–	–	–	–	–	–	–	–	+	–
Blepharosphaera citriformis	–	–	+	+	–	–	–	–	–	–	–	–	–
Blepharosphaera ellipsoidalis	+	–	+	+	–	–	–	–	+	+	+	–	–
Blepharosphaera intestinalis	+	–	+	+	+	+	–	–	–	–	–	–	–
Blepharozoum zonatum	–	–	+	+	–	–	–	–	–	–	–	–	–
Buissonella tapiri	–	–	–	–	+	–	–	–	–	–	–	–	–
Bundleia asymmetrica	–	–	+	+	–	–	–	–	–	–	–	–	–
Bundleia dolichosoma	–	–	+	+	–	–	–	–	+	–	–	+	–
Bundleia elongata	+	–	+	+	+	–	–	+	+	+	–	–	–
Bundleia inflata	–	–	+	+	+	+	–	–	+	+	–	+	–
Bundleia nana	+	–	+	+	+	–	–	–	+	+	–	+	–
Bundleia piriformis	+	–	–	+	–	–	–	–	+	–	–	+	–
Bundleia postciliata	+	+	+	+	+	+	–	+	+	+	+	+	+
Bundleia triangularis	–	–	–	+	–	–	–	–	+	–	–	–	–
Bundleia vorax	–	–	+	+	–	+	–	–	–	–	–	+	–
Didesmis ovalis	+	+	+	+	–	–	–	–	+	–	+	–	–
Didesmis quadrata	+	+	+	+	–	+	–	–	–	–	+	–	–
Didesmis spiralis	+	–	+	+	–	–	–	–	–	–	+	–	–
Hemiprorodon gymnoposthium	–	–	+	+	–	–	–	+	+	–	–	+	+
Holophryoides macrotricha	+	–	+	+	–	–	–	–	+	–	+	+	+
Holophryoides ovalis	+	–	+	+	–	+	+	–	+	+	+	+	–
Paraisotrichopsis composita	+	–	+	+	–	–	–	–	+	–	–	–	–
Polymorphella ampulla	+	+	+	+	+	+	+	+	+	+	–	+	+
Prorodonopsis coli	+	–	+	+	–	–	–	–	+	–	–	+	–
Sulcoarcus pellucidulus	–	–	–	+	+	–	–	–	–	–	–	–	–
Wolskana tokarensis	–	–	+	–	–	+	–	–	–	–	–	–	–
Suborder Blepharocorythina													
Family Blepharocorythidae													
Blepharocorys angusta	+	+	+	+	+	+	–	–	+	–	+	+	–
Blepharocorys cardionucleata	+	–	+	+	+	–	–	–	–	–	+	–	–
Blepharocorys curvigula	+	+	+	+	+	+	–	+	+	+	+	+	+
Blepharocorys jubata	+	+	+	+	+	–	–	–	–	–	+	–	–
Blepharocorys microcorys	+	–	+	+	–	+	–	–	+	+	+	+	+
Blepharocorys uncinata	+	+	+	+	–	+	+	+	+	+	–	+	+
Blepharocorys valvata	+	–	+	+	+	+	–	–	–	–	+	+	–
Charonina equi	+	–	+	+	–	–	–	–	–	–	+	–	–

Table 2. (Continued)

Subclass/Order/Suborder/Family/Genus/Species	Geographical Regions[a]												
	USA (1)	Scotland (2)	Japan (3)	Russia and Former USSR (4)	Brazil (5)	Iceland (6)	India (7)	Cyprus (8)	Türkiye (9)	Kyrgyzstan (10)	Taiwan (11)	South Africa (12)	Libya (13)
Circodinium minimum	+	+	+	+	–	+	–	–	+	–	+	+	–
Ochoterenaia appendiculata	+	–	+	+	+	+	–	–	+	–	+	+	–
Suborder Entodiniomorphina													
Family Cycloposthiidae													
Cycloposthium affine	+	–	+	+	–	–	–	–	–	–	–	–	–
Cycloposthium bipalmatum	+	+	+	+	+	–	+	+	+	+	+	+	+
Cycloposthium dentiferum	+	+	+	+	+	–	–	–	–	–	+	+	–
Cycloposthium edentatum	+	+	+	+	+	+	–	–	+	+	+	+	–
Cycloposthium piscicauda	–	–	–	+	+	–	–	–	–	–	–	–	–
Cycloposthium plicatocaudatum	–	–	–	+	–	–	–	–	–	–	–	–	–
Cycloposthium ponomarevi	–	–	–	+	–	–	–	–	–	–	–	–	–
Cycloposthium scutigerum	+	–	+	+	–	–	–	–	–	–	+	–	–
Tripalmaria dogieli	+	+	+	+	–	+	–	–	+	–	+	+	–
Family Spirodiniidae													
Cochliatoxum periachtum	+	–	+	+	–	+	–	–	+	+	+	+	+
Ditoxum brevinucleatum	–	–	+	+	–	+	–	–	–	–	–	+	–
Ditoxum funinucleum	+	+	+	+	–	+	–	–	+	–	–	+	–
Gassovskiella galea	+	+	+	+	–	+	–	–	+	–	+	+	–
Spirodinium confusum	–	–	+	+	–	–	–	–	+	–	–	+	–
Spirodinium equi	+	+	+	+	+	+	+	–	+	+	+	+	+
Spirodinium magnum	–	–	+	–	–	–	–	–	–	–	–	–	–
Spirodinium nanum	–	–	+	–	–	–	–	–	–	–	–	+	–
Spirodinium uncinucleatum	–	–	–	–	–	–	+	–	–	–	–	–	–
Tetratoxum excavatum	+	+	+	+	?	+	–	–	+	–	+	–	–
Tetratuxum parvum	+	+	+	+	?	+	–	–	+	+	+	+	+
Tetratoxum unifasciculatum	+	–	+	+	?	+	–	–	+	+	+	+	+
Triadinium caudatum	+	+	+	+	–	+	–	–	+	+	+	+	+
Subclass Suctoria													
Order Exogenida													
Family Allantosomatidae													
Allantosoma cucumis	–	–	+	+	–	+	–	–	–	–	–	–	–
Allantosoma intestinale	+	+	+	+	+	+	–	–	+	+	+	–	–
Allantoxena biseriale	–	–	+	+	–	+	–	–	–	–	–	–	–
Allantoxena japonensis	–	–	+	+	–	–	–	–	–	–	–	–	–
Arcosoma brevicorniger	+	–	+	+	–	+	–	–	–	–	+	–	–
Arcosoma dicorniger	+	–	+	+	+	+	–	–	–	–	+	–	–
Arcosoma lineare	–	–	+	+	–	+	–	–	–	–	–	–	–
Strelkowella urunbasiensis	–	–	–	+	–	–	–	–	–	–	–	–	–

[a]References: (1) Hsiung, 1930; Gürelli et al., 2019; (2) Adam, 1951; (3) Ike et al., 1981; Ike et al., 1983a, b, c; Ike et al., 1985; Imai et al., 1999; Ito et al., 1996; Ozeki et al., 1973; (4) Gassovsky, 1919; Kornilova, 2006; Strelkow, 1939; (5) Cedrola et al., 2019; (6) Kornilova et al., 2019; (7) Kornilova & Chistyakova, 2024; (8) Göçmen et al., 2012; (9) Gürelli & Göçmen, 2011, 2012; Gürelli, 2012; Gürelli & Aydın, 2021; (10) Gürelli et al., 2015; (11) Tung, 1992; (12) Kornilova et al., 2022; (13) Present study.

The number of species identified per horse varied from 0 to 9, with an average of 4.3 ± 2.1 (SD). The average density of intestinal ciliates in the 30 horses was $7.0 \pm 6.2 \times 10^4$ cells mL^{-1}, with values ranging from 0 to 26.5×10^4 cells mL^{-1}

(Table 3, Fig. 3). When comparing the density reported in ciliate surveys conducted on horses from various countries, the average ciliate density in the hindgut of domestic horses in Libya ($7.0 \pm 6.2 \times 10^4$ cells mL^{-1}) was considerably lower than that reported for race horses in Japan (Ike et al., 1983b), riding horses in Taiwan (Tung, 1992), Kiso horses in Japan (Imai et al., 1999), Turk Rahvan horses in Türkiye (Gürelli & Göçmen, 2011), racing horses in Türkiye (Gürelli & Göçmen, 2012), domestic horses in Türkiye (Gürelli, 2012), domestic horses in Kyrgyzstan (Gürelli et al., 2015), and Thoroughbred horses in the USA (Gürelli et al., 2019). However, the average ciliate density observed in Libyan horses was higher than that reported for light horses in Japan (Ike et al., 1981), Tokara ponies in Japan (Ito et al., 1996), domestic horses in Cyprus (Göçmen et al., 2012), and domestic horses in Türkiye (Gürelli & Aydın, 2021) (Table 3).

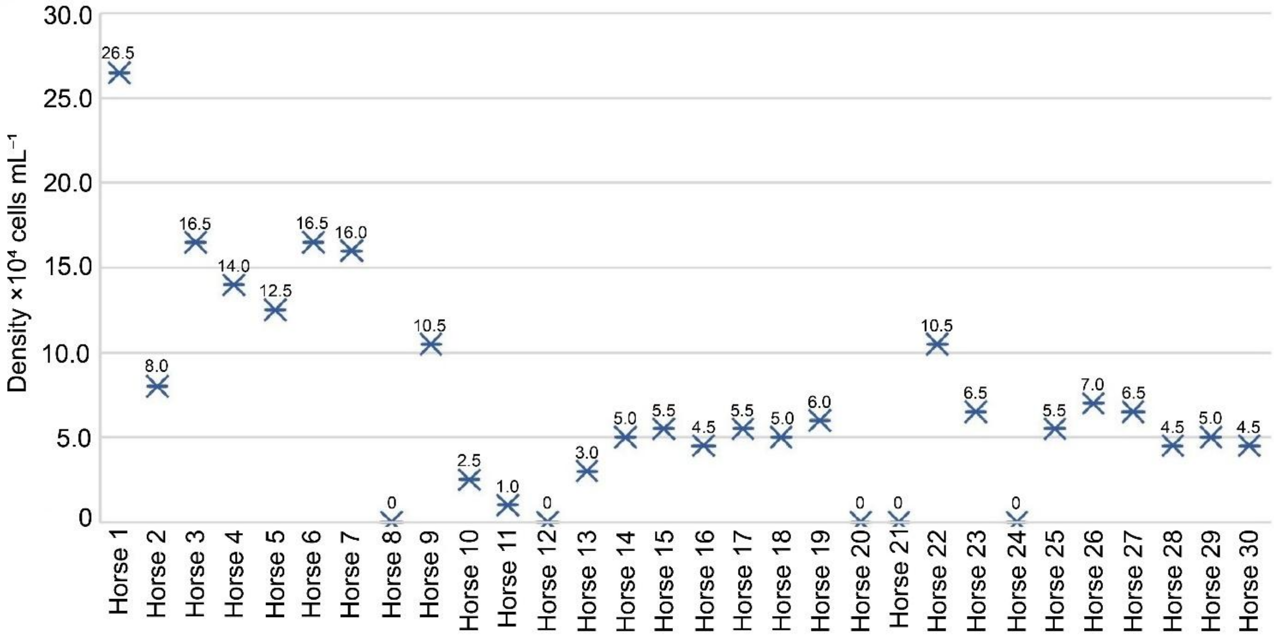

Figure 3. Ciliate densities ($\times 10^4$ cells mL^{-1}) in the intestinal contents of 30 horses in Libya.

Table 3. Average ciliate densities and the distribution of total ciliate genera and species found in the intestinal contents of horses from various regions worldwide ([a]Data not reported, [b]Mean ± SD).

Localities, Hosts	Average ciliate densities ($\times 10^4$ cells mL^{-1})	Min–Max value of densities ($\times 10^4$ cells mL^{-1})	Total no of genera	Total no of species	Number of animals	References
Japan, Light horses	3.4[a]	[a]	19	40	17	Ike et al., 1981
Japan, Race horses	9.0[a]	0.4–113.0	22	49	60	Ike et al., 1983b
Taiwan, Riding horses	38.1 ± 35.9[b]	0.3–127.0	19	38	40	Tung, 1992
Japan, Tokara ponies	1.4[a]	[a]	11	18	20	Ito et al., 1996
Japan, Kiso horses	140.0[a]	[a]	23	50	18	Imai et al., 1999
Türkiye, Rahvan horses	14.2 ± 13.9[b]	0–45.5	22	36	15	Gürelli & Göçmen, 2011
Cyprus, Domestic horses	5.5 ± 4.4[b]	1.5–12.5	9	11	5	Göçmen et al., 2012
Türkiye, Racing horses	26.4 ± 15.1[a]	0–54.5	21	37	15	Gürelli & Göçmen, 2012
Türkiye, Domestic horses	15.4 ± 10.8[a]	5.5–37.5	18	28	8	Gürelli, 2012
Kyrgyzstan, Domestic horses	14.1 ± 6.8[b]	4.5–28.5	14	23	15	Gürelli et al., 2015
USA, Thoroughbred horses	13.5 ± 13.7[b]	1.5–50.0	21	33	20	Gürelli et al., 2019
Türkiye, Domestic horses	5.6 ± 5.8[b]	1.0–29.5	20	34	11	Gürelli & Aydın, 2021
Libya, Domestic horses	7.0 ± 6.2[b]	0–26.5	13	16	30	Present study

This is the first report of intestinal ciliates in domestic horses in Libya. The ciliate composition observed in the examined animals is consistent with the findings reported from other regions of the world. The species composition was higher than that recorded in domestic horses in Cyprus but lower than that reported in horses from other regions of the world (Table 2). These differences in ciliate composition and density may be related to variations in host animals and their feeding habits. The selection of specific food types and the quantities consumed by different host species may play a crucial role in shaping the intestinal ciliate composition**.** Strelkow (1939) and Kornilova (2003) classified intestinal ciliates into four trophic groups based on their feeding habits. The first group comprises species that primarily utilize plant particles including *Bundleia piriformis*, *Bundleia triangularis*, *Blepharocorys microcorys*, *Cycloposthium* spp., *Triadinium caudatum*, *Gassovskiella galea*, *Tetratoxum parvum*, *Ditoxum* *funinucleum*, and *Cochliatoxum periachtum*. The second group consists of starch-feeding ciliates such as *Didesmis* spp., *Blepharoconus* spp., *Alloiozona trizona*, *Blepharoprosthium pireum*, *Holophryoides ovalis*, *Spirodinium* spp., *Paraisotricha* spp., and *Blepharocorys* spp. The third group includes species that feed mainly on bacteria including *Bundleia postciliata*, *Bundleia nana*, *Bundleia elongata*, *Bundleia inflata*, *Paraisotrichopsis composita*, *Didesmis quadrata*, *Polymorphella ampulla*, *Holophryoides macrotricha*, *Paraisotricha colpoidea*, *Paraisotricha minuta*, *Blepharocorys angusta*, *Blepharocorys curvigula*, *Blepharocorys cardionucleata*, and *Circodinium minimum*. The fourth group represents predatory ciliates. Suctorian ciliates (family Allantosomatidae) are considered obligatory predators, whereas *Blepharoprosthium polytrichum*, *Blepharoprosthium pireum*, and *Bundleia vorax* are facultative (optional) predators (Gürelli & Göçmen, 2012; Gürelli et al., 2015). In the present study, we observed *Cycloposthium bipalmatum* in 23 horses with high relative abundance, indicating that horses in Libya typically consume grass. If the feeding conditions of horses are known, the intestinal ciliate composition can be predicted.

Ozeki (1977) reported that the ciliate community in the large intestine can change when the host develops digestive disorders such as chronic intestinal catarrh. From this perspective, examination of fecal ciliate composition may serve as a useful indicator of digestive health and could have clinical relevance (Ike et al., 1983a). In the present study, 5 out of 30 horses lacked ciliates suggesting that these animals might have been experiencing illness, may have undergone medical treatments that resulted in intestinal defaunation, or possibly had not been exposed to adult animals early in life. In foals, coprophagy of their mother's feces serves as the main route for ciliate colonization, meaning that the composition of the ciliate community in foals is strongly influenced by that of their mother. Consequently, the ciliate fauna is largely transmitted from parents to offspring with little alteration. Transmission between unrelated hosts appears to be difficult as ciliates in adult horses are unlikely to survive passage through the stomach (Ike et al., 1985; Imai et al., 1999; Egan et al., 2010). It is possible that the digestive enzymes in the gastrointestinal tract of foals are less detrimental to ciliates than those in adult equids.

The five species identified in this study, *Bundleia postciliata*, *Polymorphella ampulla*, *Blepharocorys curvigula*, *Cycloposthium bipalmatum*, and *Spirodinium equi*, are widely distributed in horses worldwide. However, *B. postciliata* and *B. curvigula* have not been reported in horses in India, *P. ampulla* has not been recorded in horses in Taiwan, and *C. bipalmatum* has not been reported in horses in Iceland (Tung, 1992; Kornilova et al., 2019; Kornilova & Chistyakova, 2024) (Table 2).

Libya is located in the northern part of the African continent, while South Africa lies in the south. Despite being on the same continent, the intestinal ciliate composition of horses differs between these regions. Although *Paraisotricha minuta*, *Alloiozona trizona*, *Blepharosphaera ceratotherii*, *Bundleia dolichosoma*, *B. inflata*, *B. nana*, *B. piriformis*, *B. vorax*, *Holophryoides ovalis*, *Prorodonopsis coli*, *Blepharocorys angusta*, *B. valvata*, *Circodinium minimum*, *Ochoterenaia appendiculata*, *Cycloposthium dentiferum*, *C. edentatum*, *Tripalmaria dogieli*, *Ditoxum brevinucleatum*, *D. funinucleum*, *Gassovskiella galea*, *Spirodinium confusum*, and *S. nanum* have been reported from domestic horses in South Africa, these species were not recorded in domestic horses in Libya (Kornilova et al. 2022) (Table 2). This difference may result from variations in vegetation between South Africa and Libya, feeding habits, and interactions with other perissodactyl species in South Africa. This is supported by the fact that *B. ceratotherii* was originally discovered in the intestines of the African rhinoceros, while *Spirodinium nanum* was first recorded from zebras (Strelkow, 1931; Van Hoven et al., 1998).

Buissonella tapiri, *Blepharosphaera ceratotherii*, *Cycloposthium plicatocaudatum*, *C. ponomarevi*, *Spirodinium magnum*, *S. uncinucleatum*, and *Strelkowella urunbasiensis* have been reported from only one region in the world; therefore, they are considered very rare species (Strelkow, 1939; Ike et al., 1983a; Kornilova, 2006; Cedrola et al., 2019; Kornilova et al., 2022; Kornilova & Chistyakova, 2024) (Table 2).

In conclusion, the 16 species of intestinal ciliates recorded from domestic horses in Libya are also known from horses in other regions. Therefore, further studies on equine intestinal ciliates across the African continent and globally are needed to provide additional insights into potential hosts and to better understand the dispersal of these endosymbionts among different regions.

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