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Fecal ciliate composition of Cypriot domestic horses (*Equus caballus* Linnaeus, 1758)

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Abstract: The species composition and distribution of large intestinal ciliates was investigated in feces from 5 Cypriot domestic horses living in Northern Cyprus. We identified 9 ciliate genera and 11 species. This is the first report on intestinal ciliates in Cypriot domestic horses, and no endemic species were observed. The genera *Cycloposthium*, *Spirodinium*, and *Paraisotricha* occurred in all animals. The mean number of ciliates was (5.5 ± 4.4) × 10^4 cells mL^{-1} of feces and the mean number of ciliate species per host was 7.2 ± 1.5. The characteristics of the domestic horse ciliates were almost identical to those reported in other equids from various regions around the world, suggesting that there is little or no pronounced geographic variation in the intestinal ciliate fauna of equids.

Key words: Intestinal ciliates, Cyprus, domestic horse, *Equus caballus*, feces

Kıbrıs evcil atının bağırsak siliyat faunası (*Equus caballus* Linnaeus, 1758)

Özet: Kuzey Kıbrıs'ta dağılış gösteren 5 Kıbrıs evcil atının arka bağırsak siliyat kompozisyonu ve dağılımı araştırılmıştır. Dokuz siliyat cinsi ve 11 tür tespit edilmiştir. Kıbrıs evcil atlarının bağırsak siliyatları üzerine yapılan ilk çalışmadır ve endemik bir türe rastlanmamıştır. *Cycloposthium*, *Spirodinium* ve *Paraisotricha* cinsleri tüm hayvanlarda tespit edilmiştir. Ortalama siliyat sayısı (5,5 ± 4,4) × 10^4 hücre mL^{-1} ve konak başına ortalama siliyat türü sayısı 7,2 ± 1,5'tir. Tespit edilen evcil at siliyatlarının özellikleri, dünyanın çeşitli bölgelerindeki diğer atgillerinkiyle neredeyse benzerdir ve bu durum Kıbrıs'ın evcil at bağırsak siliyat faunasında pek varyasyon olmadığını gösterir.

Anahtar sözcükler: Bağırsak siliyatları, Kıbrıs, evcil at, *Equus caballus*, dışkı

Introduction

Intestinal ciliates from the large intestine of the domestic horse were first reported by Gruby and Delafond in 1843. Since then, the intestinal ciliate fauna of equids has been surveyed from many areas around the world (Gassovsky, 1919; Strelkow, 1928, 1929, 1939; Hsiung, 1930; Grain, 1966; Ozeki et al., 1973). The ciliates invade the host due to oral ingestion (coprophagy) and then colonize the large intestine (Ike et al., 1985). Numerous studies have also shown that many of the ciliate species are excreted alive in the feces of equids (Ike et al., 1981, 1983a, 1983b; Tung, 1992; Ito et al., 1996; Imai et al., 1999).

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Although the composition of the intestinal ciliate community of various equids is known in general, no investigations have been conducted on the ciliate fauna in the Cypriot domestic horse, *Equus caballus* Linnaeus, 1758. The aim of this study was to identify and quantify the fecal ciliate fauna from those animals living on the island and compare the data with previous studies on equids from various other locations.

Materials and methods

Fecal samples were collected from 5 domestic horses (*Equus caballus* Linnaeus, 1758) located in Lefkoşa in Northern Cyprus. The samples were collected from December 2007 to June 2008. The fecal samples were collected immediately after defecation and fixed and stained in about 2 times as much methyl green formalin saline solution (MFS) as their original volume (Ogimoto and Imai, 1981; Ito et al., 1996). This procedure was used to preserve the integrity of the cell and its internal structures. The MFS served as a nuclear stain, and Lugol's iodine was used to stain skeletal plates. Fecal samples were passed through 562.5-μm mesh gauze and kept in the dark until examination.

Total cell counts were made at 400× magnification with a Neubauer hemocytometer counting chamber. Differential counts of species were estimated from smear slides with a total of 70 to 80 cells identified for each species (Göçmen and Gürelli, 2009; Gürelli and Göçmen, 2010). Details of the ciliate morphology were investigated at 1000× magnification using an oil immersion objective microscope. For detailed observation of species, the silver impregnation method was applied in addition to the ordinary light microscopic examination (Ito et al., 1996). We also examined species using pyridinated silver carbonate impregnation (Fernández-Galiano, 1976; Ito and Imai, 2006).

Ciliate genera and species were identified and classified based mainly on the descriptions of Hsiung (1930), Strelkow (1939), Ozeki (1977), Kornilova (2003, 2004b), and Lynn (2008).

Results

Ciliate composition of the fecal samples

The frequency of appearance (i.e. the number of hosts in which the species was detected/number of hosts examined) and the relative composition of genera and species are shown in Table 1. We identified

Table 1. Frequency of appearance*, percentage composition of genera, and species composition of intestinal ciliates in the feces of 5 Cypriot domestic horses.

Genus/Species	Frequency appearance (%)	Percentage composition (%)	
		Mean ± SD	Range
Bundleia	80	20.6 ± 24.2	0-50.0
postciliata (Bundle, 1895)	80	16.3 ± 18.3	0-37.5
elongata Strelkow, 1939	40	4.3 ± 6.6	0-15.0
Polymorphella	20	1.4 ± 3.0	0-6.8
ampulla (Dogiel, 1929)	20	1.1 ± 2.8	0-6.8
Blepharoprosthium	60	3.8 ± 4.3	0-10.0
polytrichum Strelkow, 1939	60	3.8 ± 4.3	0-10.0
Hemiprorodon	80	7.9 ± 6.9	0-16.7
gymnoposthium Strelkow, 1939	80	7.9 ± 6.9	0-16.7
Blepharoconus	20	3.3 ± 7.5	0-16.7
benbrooki Hsiung, 1930	20	3.3 ± 7.5	0-16.7
Paraisotricha	100	2.2 ± 1.6	1.2-5.0
colpoidea Fiorentini, 1890	100	2.2 ± 1.6	1.2-5.0
Blepharocorys	80	10.1 ± 9.5	0-22.9
curvigula Gassovsky, 1919	60	5.0 ± 7.0	0-17.0
uncinata (Fiorentini, 1890)	60	5.1 ± 7.8	0-18.8
Spirodinium	100	10.8 ± 7.0	5.4-20.0
equi Fiorentini, 1890	100	10.8 ± 7.0	5.4-20.0
Cycloposthium	100	10.2 ± 9.5	0-22.9
bipalmatum (Fiorentini, 1890)	100	48.4 ± 34.2	10.0-85.9
Total	9 genera 11 species		

*Number of hosts in which a species appeared divided by the total number of animals surveyed.

11 species belonging to 9 genera (Figures 1a-1n, Figures 2a-d, and Figures 3 and 4). The ciliate fauna consisted of 5 genera and 6 species of Buetschliidae; 1 genus and 1 species of Paraisotrichidae; 1 genus and 2 species of Blepharocorythidae; 1 genus and 1 species of Spirodiniidae; and 1 genus and 1 species of Cycloposthiidae. For individual horses, the total number of species per animal ranged from 5 to 9, with an average of 7.2 ± 1.5 (SD).

The average abundance of ciliates in the intestinal contents from the 5 Cypriot domestic horses was (5.5

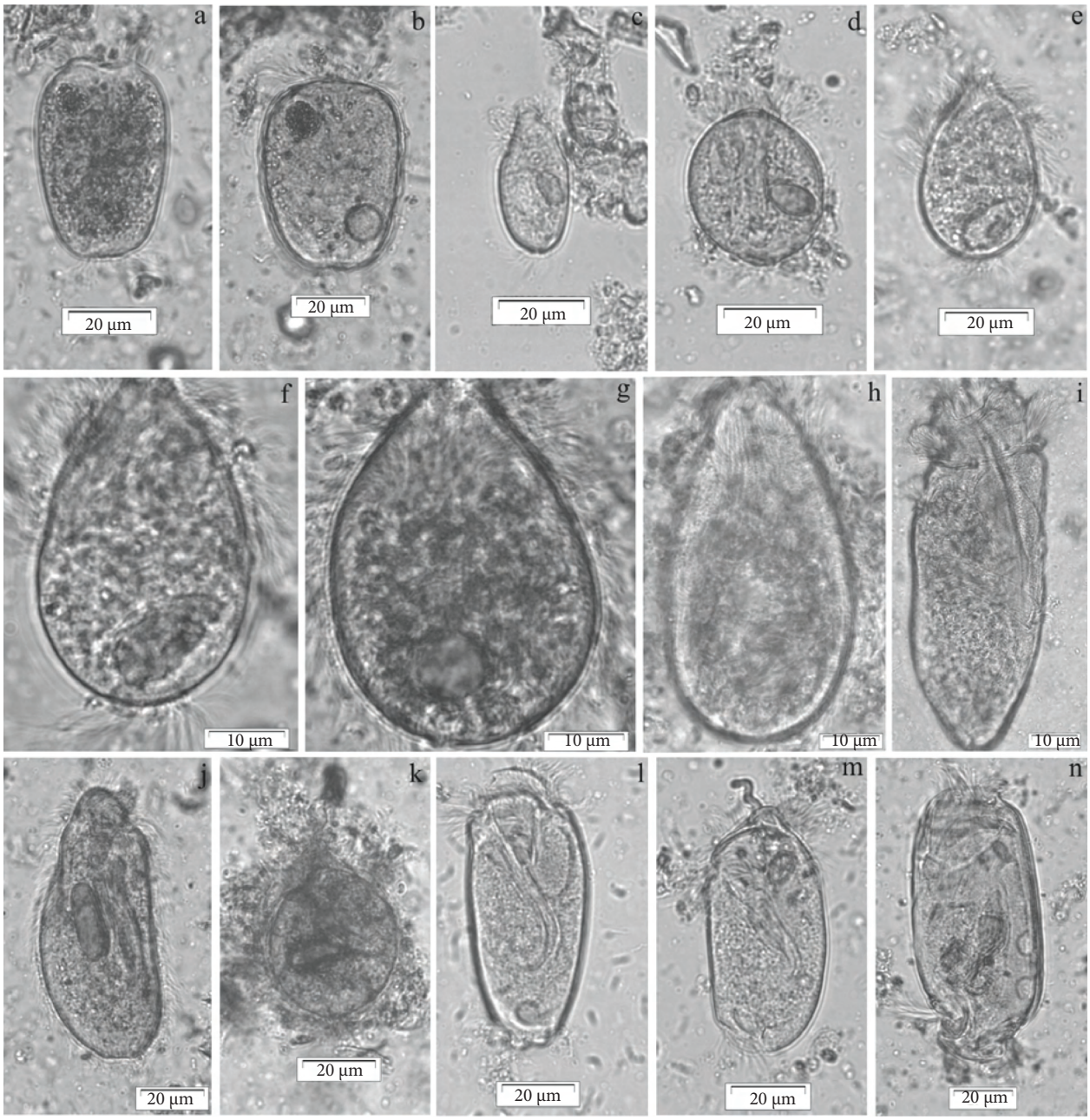

Figure 1. Photomicrographs from methyl green-formalin-saline fixed cells of the studied species: **a)** *Bundleia postciliata*, **b)** *Bundleia elongata*, **c)** *Polymorphella ampulla*, **d)** *Blepharoconus benbrooki*, **e and f)** *Blepharoprosthium polytrichum*, **g and h)** *Hemiprorodon gymnoposthium*, **i**) *Spirodinium equi*, **j and k)** *Paraisotricha colpoidea*, **l**) *Blepharocorys curvigula*, **m)** *Blepharocorys uncinata*, **n)** *Cycloposthium bipalmatum*.

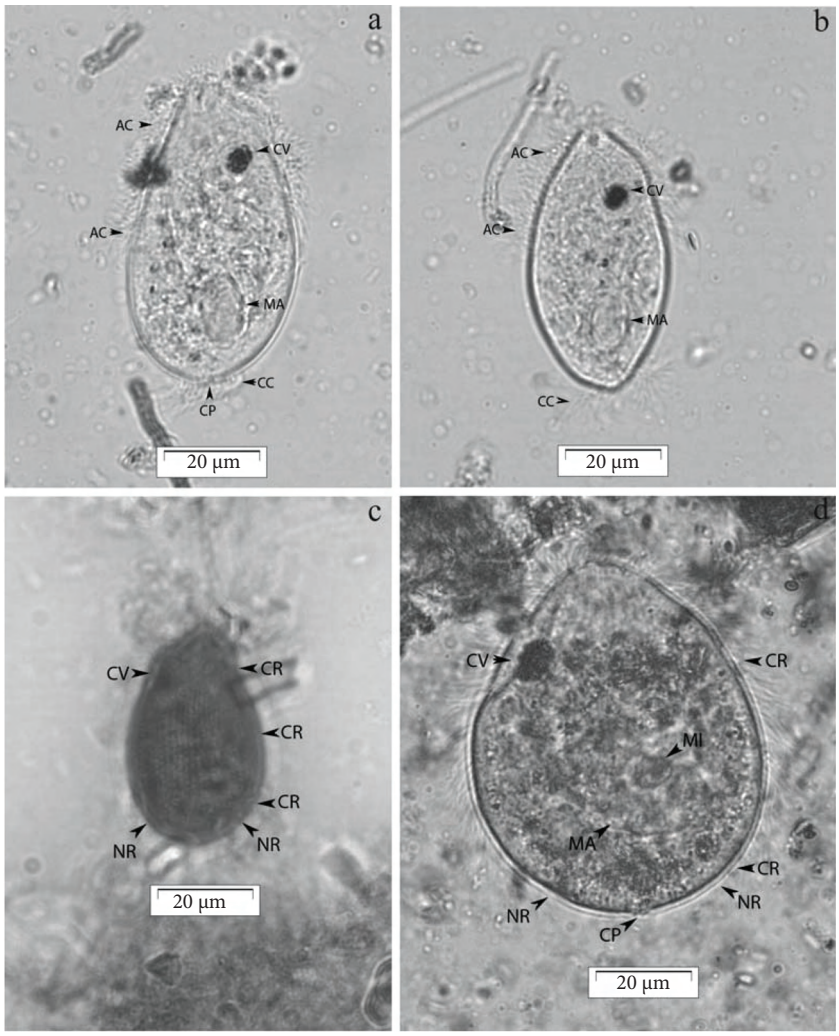

Figure 2. Photomicrographs of: **a and b)** *B. polytrichum* impregnated by pyridinated silver carbonate, **c)** *H. gymnoposthium* impregnated by silver nitrate, **d)** *H. gymnoposthium* impregnated by pyridinated silver carbonate. CV, concretion vacuole; AC, anterior ciliary zone; CC, caudal ciliary zone; CP, cytoproct; MA, macronucleus; MI, micronucleus; CR, ciliary rows of kinetosomes; NR, naked region.

± 4.4) × 10^4 cells mL^{-1}. Values ranged from 1.5 × 10^4 to 12.5 × 10^4 cells mL^{-1} (Table 2).

Description of *Blepharoprosthium polytrichum* Strelkow, 1939 (Fam. Buetschliidae) (Figures 1e, 1f, 2a, 2b, and 3)

The average body length was 45.1 ± 5.7 µm (35.0-55.0 µm, n = 20) and average body width was 23.9 ± 3.7 µm (17.5-30.0 µm, n = 20). Body is pear-shaped and rounded at the rear end. Anterior ciliary zone covers half of the body with longitudinal ciliary rows. Anterior polybrachykineties (infraciliary bands composed of numerous parallel kineties) extend the longitudinal body axis and completely encircle the cytostome. Small caudal (posterior) ciliary zone is at the posterior end of the body. The caudal kineties are around the cytoproct. Cytostome is at the anterior end of the body and is followed by a small cytopharynx. Concretion vacuole is situated in the anterior half of the body and in the middle of the anterior ciliary zone. Macronucleus is elongated and micronucleus is situated in the depression of the macronucleus. Its

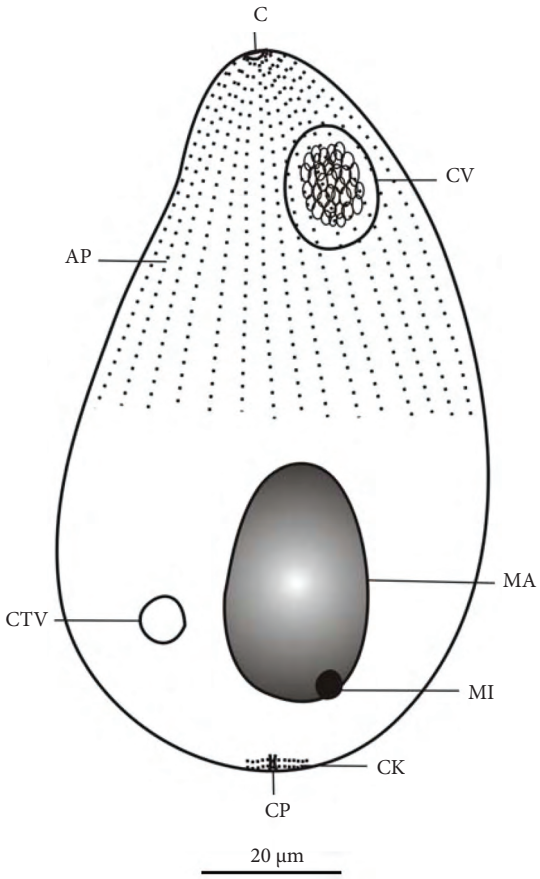

Figure 3. Drawing of *B. polytrichum*. MA, macronucleus; MI, micronucleus; CTV, contractile vacuole; AP, anterior polybrachykineties; CK, caudal kineties; C, cytostome.

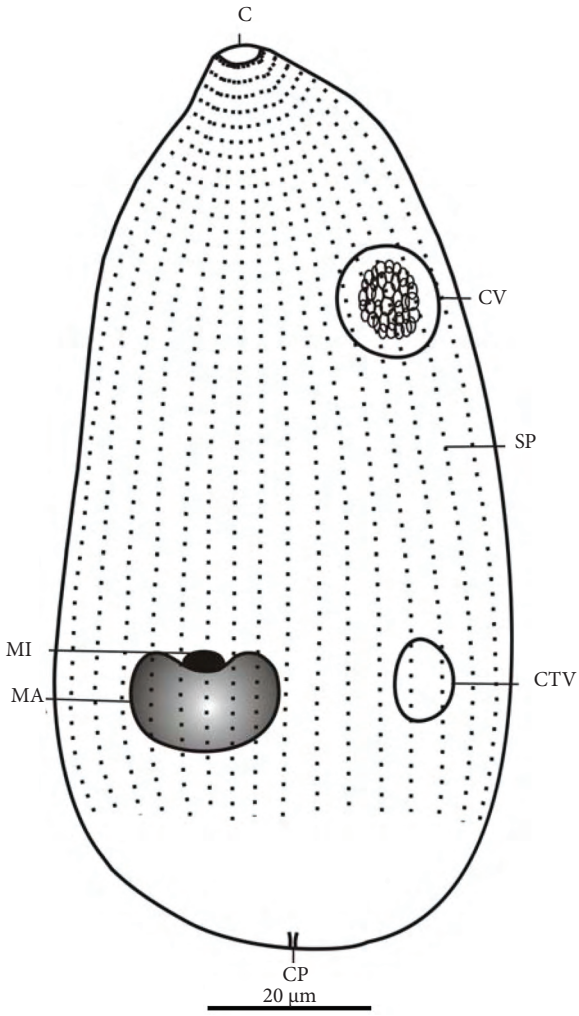

Figure 4. Drawing of *H. gymnoposthium*. SP, somatic polybrachykineties.

Table 2. Abundance of intestinal ciliates in the feces of 5 Cypriot domestic horses.

	Horse no.				
	1	2	3	4	5
Total ciliates ($\times 10^4$ cells mL^{-1})	2	6	1.5	12.5	5.5
Mean ± SD = (5.5 ± 4.4) × 10^4 cells mL^{-1}					

position is not constant in the cytoplasm. Its length is 10.8 ± 2.8 µm (5.0-15.0 µm, n = 20) and width is 10.9 ± 1.2 µm (10.0-12.5 µm, n = 20). Cytoproct is at the posterior end of the body. One or 2 contractile vacuoles are near the end of the body.

Description of *Blepharoprosthium polytrichum* Strelkow, 1939 (Fam. Buetschliidae) (Figures 1g, 1h, 2c, 2d, and 4)

The average body length was 57.4 ± 6.6 µm (47.5-75.0 µm, n = 20) and average body width was 38.5 ±

4.5 μm (30.0-47.5 μm, n = 20). Body is asymmetrical. It tapers toward the anterior end. Posterior end is broad and rounded. Cytostome is located at the anterior end of the body and is slightly bent to one side. Long somatic cilia cover the body in longitudinal rows except for approximately 1/6-1/7 of the body at the posterior end. Somatic polybrachykineties completely encircle the cytostome and overlie the longitudinal body axis, except the posterior end. Concretion vacuole is near anterior end of the body. Macronucleus is elliptic and its ends are rounded. Its position is not constant in cytoplasm. Its length is 14.3 ± 5.1 μm (7.5-25.0 μm, n = 20) and width is 12.1 ± 3.3 μm (7.5-17.5 μm, n = 20). Small micronucleus is situated in the depression of the macronucleus. One contractile vacuole is in the posterior half of the body. Cytoproct is at posterior end of the body.

Discussion and conclusions

In the present study, 11 ciliate species representing 9 genera were identified, but no new or endemic species were observed. All of the species detected in the present study represent first reports for their occurrence in Cypriot domestic horses.

The number of identified species was very low in comparison to previous reports from other equids (Table 3). One of the reasons for this may be due to the fact that only a small number of animals were sampled from the same geographical location. Feeding habitats and food-type differences may also be a factor.

When compared with ciliate surveys of other equids living in various countries, the average ciliate density in the intestine of Cypriot domestic horse ($(5.5 \pm 4.4) \times 10^4$ cells mL^{-1}) was considerably less than that of race horses (Ike et al., 1983a), riding horses (Tung, 1992), and Kiso horses (Imai et al., 1999). On the other hand, concentrations are higher than those in other equids such as the light horse (Ike et al., 1981), Tokara pony (Ito et al., 1996), and Cypriot wild donkey (Gürelli and Göçmen, 2010) (Table 3).

Table 3. Total ciliate abundance and distribution of the total number of genera and species of ciliates from intestine contents of equids from various locations around the world.

Locality[a]	Total ciliates ($\times 10^4$ cells mL^{-1})	Range ($\times 10^4$ cells mL^{-1})	Total no. of genera	Total no. of species	References[b]
China	[d]	[d]	19	30	1
Japan	3.4[d]	[d]	19	40	2
Japan	9.0[d]	0.4-113.0	22	49	3
Taiwan	38.1 ± 35.9[c]	0.3-127.0	19	38	4
Japan	1.4[d]	[d]	11	18	5
Japan	140.0[d]	[d]	23	50	6
Middle Asia	[d]	[d]	25	57	7
Cyprus	3.0 ± 2.5[c]	0.5- 8.5	16	22	8
Cyprus	5.5 ± 4.4[c]	1.5-12.5	9	11	Present study

[a]Number of animals and breed. China: 20 horses, donkeys, and mules; Japan: 17 light horses; Japan: 60 race horses; Taiwan: 40 riding horses; Japan: 20 Tokara ponies; Japan: 18 Kiso horses; Middle Asia: 184 kulans; Cyprus: 13 wild donkeys; Cyprus: 5 domestic horses.
[b]References. 1: Hsiung, 1935a, 1935b; Hsiung, 1936. 2: Ike et al., 1981. 3: Ike et al., 1983a. 4: Tung, 1992. 5: Ito et al., 1996. 6: Imai et al., 1999. 7: Kornilova, 2003. 8: Gürelli and Göçmen, 2010.
[c]Mean ± SD.
[d]Data not reported.

In general, the morphological characteristics of the ciliates observed in the present study are similar to the original descriptions, although there were minor differences in both length and width.

Of the 11 species found in 5 Cypriot horses, *Bundleia postciliata*, *B. elongata*, *Blepharoconus benbrooki*, *Polymorphella ampulla*, *Paraisotricha colpoidea*, *Blepharocorys curvigula*, *B. uncinata*, *Spirodinium equi*, and *Cycloposthium bipalmatum* have all been detected in various equids around the world (horses, donkeys, mules, and zebras) (Gassovsky, 1919; Hsiung, 1930, 1935; Strelkow, 1931, 1939; Kornilova, 2003). *Hemiprorodon gymnoposthium* was first described from the horse in Russia (Strelkow, 1939), and the present observation is only the second record of the species. *Blepharoprosthium polytrichum* was also first described from the horse in Russia (Strelkow, 1939) and has been subsequently observed by Kornilova (2004a) in Yakut horses, also in Russia. This study represents the third record of this species in the horse.

The occurrence of *H. gymnoposthium* and *B. polytrichum* in such widely divergent geographical locations is perplexing. These species were not found in the intestinal ciliate fauna of the Cypriot wild donkey (Gürelli and Göçmen, 2010). Further studies on the fauna of equids in Cyprus and Russia might provide additional information about possible intermediate hosts and help explain the migration of these species to different locations.

In conclusion, the 11 species of intestinal ciliates recorded from the Cypriot domestic horse are also known from other equids in different locations. The ciliates detected in our study do not appear to have undergone pronounced specific differentiation since they originally reached the island.

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