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REPORT ON CILIATES FROM THE HINDGUT OF HORSES IN ICELAND

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Abstract. Ciliates inhabiting the intestine of horses in Iceland were investigated. The population of horses on the island has been isolated for more than 1000 years, so their hindgut ciliates were also isolated all that time. The samples were taken from 6 horses on the territory of Iceland. More than 30 species belonging to 18 genera were found. One species is a very rare.

Keywords: intestinal ciliates, equine hindgut ciliates, trichostomatids, Trichostomatia, Allantosomatidae, Iceland, horses.

КРАТКОЕ СООБЩЕНИЕ ОБ ИНФУЗОРИЯХ ИЗ КИШЕЧНИКА ЛОШАДЕЙ В ИСЛАНДИИ

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Аннотация. Исследованы инфузории, обитающие в кишечнике лошадей в Исландии. Популяция лошадей была изолирована на острове на протяжении более 1000 лет, и их кишечные инфузории также были изолированы все это время. Пробы были взяты у 6 лошадей на территории Исландии. Найдено более 30 видов, относящихся к 18 родам. Один вид очень редкий.

Ключевые слова: инфузории кишечника, инфузории лошадиных, трихостоматиды, Trichostomatia, Allantosomatidae, Исландия, лошади.

INTRODUCTION

Intestinal horse ciliates were first opened at the end of 19th century and since that time more than 80 species were found in different equids, mostly in horses. Ciliates belong to 2 different groups – trichostomatids (Litostomatea, Trichostomatia) and suctorids (Suctorea, Exogenia). All horse ciliates are not able to form cysts, although they are transmitted by coprophagy. Probably a solid cortex can protect the cell during the process of defecation, helps to survive in the environment before being eaten by the new host. Outside the intestine ciliates can survive for very short periods - less than one hour, and usually they settle in a new host after they are swallowed by the member of the same herd. Keeping horses in herds increases the number and variabilty of species of their intestinal ciliates (Kornilova 2004).

The microfauna of intestinal ciliates from different native horses was investigated from many places on Earth but not from Iceland (we did not find such publications), so we decided to fill the gap. Because of some unique features in the history and the biology of the Icelandic horse population, their endobiotic ciliates are of particular interest.

Icelandic horses are one of the oldest breeds. Their ancestors were shipped from Norway to Iceland over 1000 years ago and since 982 AD they were never mixed with other breeds. According to DNA analysis there were some horses with some part of Mongolian breed among these ancestors (Petersen et al. 2013), probably they were brought to Norway from Russia by Swedish traders (Bjornstad et al. 2003). Many horse breeds in the world are in relation with Mongolian breed, for example Japanese native horse breeds (Tozaki et al. 2003, Li et al. 2008). Some features of Mongolian horses such as warm hairy winter coat, thick fat, seasonal changes of carbohydrate metabolism probably ensured the predisposition for surviving of the descendant breeds (Icelandic, Norwegian, Yakut etc.) on the territories with short summers and long cold winters (Librado et al. 2015).

The population of Icelandic horses has gone through several bottlenecks in its history, for example, the consequences of the Laki volcanic eruption in 1784-1785 have reduced their number to 8 000-9 000 (Hreidarsdottir et al. 2014). Last 70 years a lot of Icelandic horses were exported to Europe and North America. Currently in the world there are about 250 000 horses in at least 19 countries, the number of horses in Iceland is about 80 000 (Hreidarsdottir et al. 2014).

The diet for Icelandic horses consists of fibrous forage such as a diet for wild equids. In Iceland there are temperate grasses which are used for the horse grazing or for the harvesting and conservation. Meadow grass includes many different species and sowed grasses mainly include timothy grass. In spite of the short and cold summer the digestibility of grasses in Iceland is better than in some southern countries. The grass in Iceland is growing rapidly due to a longer day photoperiod, but the lignification process of the cell wall is slower in a cold climate (Ragnarsson 2009).

There are different ways for using horses in Iceland: as a working animals in riding tours and sheepherding, for leisure and sport competitions, for showing and racing, and also for horsemeat. Different purposes require a different food for horses, often it is pasture, but usually horses in Iceland are fed with hay and haylage (plastic wrapped forages). Grasses which are used for preparing forage in Iceland are mild and the parts of plants are thin, soft, and not very long. No other food is usually used when feeding Icelandic horses (Ragnarsson 2009).

Although the horses in Iceland were isolated (and in fact were quarantined) for more than 1000 years, we cannot say the same about all their parasites and infections. In last decades there have been several cases of epizootic diseases, which were mostly associated with contaminated clothes and shoes of riding tourists from other countries. In addition, helminth eggs can be carried by winds over long distances. But hindgut ciliates remain alive in the faeces only for a very short time (Kornilova 2004), therefore ciliates from

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Icelandic horses were isolated 1000 years like their hosts. So, it was very interesting to make a study of native microfauna of ciliates from Icelandic horses and compare it with cliates from horses from another places.

MATERIAL AND METHODS

In August 2018 one of the authors of this paper visited Iceland as a tourist and took part in a 6-days horse riding tour "Kjolur" from South to North of Iceland. In the riding tour horses worked hard, so every day their ration included hay or haylage, also the horses were grazing during the resting. The samples of faeces of 6 native Icelandic horses were collected. The pieces of warm faeces were taken immediately after defecation and fixed by alcohol. There was no possibility to take special fixing liquids from home because of some specific circumstances of the whole journey. So it was decided to buy some alcohol in Iceland. The "Jin Beefeater" was chosen — a noncoloured liquid contained 40% ethanol packed in small plastic bottles, which were very useful for transportation. The bottles were half-emptied and the free space was filled with the samples. We found that the result of using such unusual fixation was well enough for the further microscopic investigations. Unfortunately the material was not suitable for DNA sequencing.

We used an optical inverted microscope Altami Invert-3 with ocular micrometer for the preliminary exam of the samples. The selection of ciliates from the original samples was performed in Petri dishes under the stereomicroscope Leica M125 in the fixing liquid. Ciliates were observed and photographed on the glass object slides using a Leica DM 2500 equipped with differential interference contrast (DIC). DIC allow to obtain layer by layer images of the cell, like tomography, which is very useful for morphology investigation of trichostomatids, especially their cortical structures. We used digital camera Leica DFC495 (8.0MP) to prepare microphotographs. Measurements of fixed cells were taken from micrographs. Classification and identification of species were based on previously published species descriptions and taxonomic lists (Hsiung 1936; Strelkow 1939; Kornilova 2003, 2004; Lynn 2008).

RESULTS AND DISCUSSION

Our observations suggest that the modified fixation procedure is quite suitable for light microscopy investigations. Moreover the results of our fixation for this purpose were better than after fixation by 96% ethanol and similar to that after routine formalin fixation. We found that as a result of fixation with 96% ethanol many cells of trichostomatids, especially big ones, become unnaturally twisted, that strongly changes their morphometric data; therefore, this fixation method we try not to use for morphological studies. The method used by us made it possible to preserve the basic cellular structures used in determining the species affiliation, namely, the general morphology of the cell, including the location of ciliary complexes, and the structure of the nuclear apparatus, which are quite suitable for determining the species affiliation of ciliates.

We found 34 species belonging to 18 genera of the intestinal horse ciliates from Iceland (table 1).

Comparative analysis of ciliates fauna suggest that species composition was similar in different geographical regions (table 2)

In the intestine of Icelandic horses, the most frequent species of ciliates were *Polymorphella ampulla, Wolskana tokarensis, Bundleia benbrooki, Bundleia inflata, Blepharocorys microcorys, Gassovskiella galea, Tetratoxum excavatum, Allantosoma cucumis.* The ciliate composition of the Iceland horses examined in this study is similar to that of other horses around the world (table 2). Most of them are common for domestic horses. The species *W. tokarensis* was previously found only once in the Tokara ponies (native Japanese horses living on the Tokara islands) (fig. 1) but it was numerous in the Icelandic horses.

As we mentioned before, the Icelandic horses might have some Mongolian ancestors among others. And the detection of the species *W. tokarensis* in the Icelandic horses gives

Table 1
The species occurrence of the endobiotic ciliates in equids from some geographical regions Eurasia

Таблица 1
Встречаемость инфузорий–эндобионтов кишечник а лошадиных из некоторых географических регионов Евразии

no	Species	Scotland[1]	China[2]	Far East[3]	Middle Asia[4]	Yakutia[5]	Japan[6]	Turkey[7]	Barsa-kelmes[8]	**Iceland**
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*Holophryoides ovalis* (Fiorentini, 1890)							+	+	
2	*Holophryoides macrotricha* Strelkow, 1939			+++		+		+	+	
3	*Prorodonopsis coli* Gassovsky, 1919		+++				+			
4	*Paraisotrichopsis composita* Gassovsky, 1919		++	+		+			+	
5	*Hemiprorodon gymnoprosthium* Strelkow, 1939			++				+		
6	*Blepharozoum zonatum* Gassovsky, 1919		+	+		+			+	
7	*Alloiozona trizona,* Hsiung, 1930	+		+++	+	+		+	+	+
8	*Blepharosphaera intestinalis* Bundle, 1895					+				+
9	*Blepharosphaera ellipsoidalis* Hsiung, 1930		+	+++	+	+		+		
10	*Blepharosphaera citriformis* Strelkow, 1939			++						
11	*Blepharoprosthium pireum* Bundle, 1895		+	++	+	+	+	+	+	
12	*Blepharoprosthium polytrichum* Strelkow, 1939			++	+	+	+	+		
13	*Polymorphella ampulla* (Dogiel, 1929)	++	+	+++	+	+	+	+	+	+++
14	*Wolskana tokarensis* Ito, Ogimoto, Nakahara, 1996						+			+++
15	*Blepharoconus hemiciliatus* Gassovsky, 1919					+				
16	*Didesmis quadrata* Fiorentini, 1890	+++		+++	+	+			+	+
17	*Fiorentinus ovalis* (Fiorentini, 1890)	+++	+	+++	+	+		+		+
18	*Bundleia postciliata* (Bundle, 1895)	+++	+	+++	+	+	+	+	+	+

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Table 1. Continued
Таблица 1. Продолжение

1	2	3	4	5	6	7	8	9	10	11
19	*Bundleia piriformis* Strelkow, 1939			+		+		+	+	
20	*Bundleia vorax* Strelkow, 1939			+++		+				++
21	*Bundleia nana* Strelkow, 1939			+++	+	+			+	
22	*Bundleia asymmetrica* Strelkow, 1939			+						
23	*Bundleia elongata* Strelkow, 1939			+++	+	+	+	+	+	
24	*Bundleia triangularis* Strelkow, 1939			+++		+		+	+	
25	*Bundleia benbrooki* (Hsiung, 1930)	+	+	++	+	+	+	+		+++
26	*Bundleia inflata* Strelkow, 1939			+	+	+	+	+	+	+++
27	*Bundleia dolichosoma* Strelkow, 1939			+++				+		
28	*Sulcoarcus pellucidulus* Hsiung, 1935		+	+	+					
29	*Paraisotricha colpoidea* Fiorentini, 1890	++	+	+++	+	+		+	+	
30	*Paraisotricha minuta* Hsiung, 1930	+++	+	++	+	+		+	+	
31	*Rhisotricha beckeri* (Hsiung, 1930)	+		+	+	+				
32	*Blepharocorys uncinata* (Fiorentini, 1890)	+++	+	+++	+	+		+		+
33	*Blepharocorys jubata* Bundle, 1895	+		++	+	+				
34	*Blepharocorys curvigula* Gassovsky, 1919	+++	+	++	+	+	+	+	+	++
35	*Blepharocorys cardionucleata* Hsiung, 1930			+			+		+	
36	*Blepharocorys angusta* Gassovsky, 1919	+++	+	+++	+	+	+	+	+	+
37	*Blepharocorys valvata* (Fiorentini, 1890)			+++						++
38	*Blepharocorys microcorys* Gassovsky, 1919			+++	+	+		+	+	+++
39	*Ochoterenaia appendiculata* Chavarria, 1933			++		+		+		++
40	*Circodinium minimum* (Gassovsky, 1919)	+++	+	++		+		+	+	++
41	*Charonnautes equi* (Hsiung, 1930)		+	+++		+				
42	*Cycloposthium bipalmatum* (Fiorentini, 1890)	+++	+	+++	+	+	+	+	+	
43	*Cycloposthium edentatum* Strelkow, 1928	+++	+	+	+	+		+	+	+

Table 1. Continued
Таблица 1. Продолжение

1	2	3	4	5	6	7	8	9	10	11
44	*Cycloposthium corrugatum* Hsiung, 1930					+				
45	*Cycloposthium scutigerum* Strelkow, 1928			+	+					
46	*Cycloposthium affine* Strelkow,1929			+	+	+				
47	*Cycloposthium dentiferum* Gassovsky, 1919	+		+	+	+			+	
48	*Cycloposthium plicatocaudatum* Strelkow,1939				+					
49	*Cycloposthium ishikawai* Gassovsky, 1919		+			+				
50	*Cycloposthium hemioni* Kornilova, 2001								+	
51	*Cycloposthium ponomarevi* Kornilova, 2001					+			+	
52	*Tripalmaria dogieli* Gassovsky, 1919	+		+		+	+	+		++
53	*Ditoxum brevinucleatum* Strelkow,1931		+	+	+					+
54	*Ditoxum funinucleum* Gassovsky, 1919	++		+++	+	+		+	+	+
55	*Ditoxum gravinucleatum* Hsiung, 1935		+							
56	*Triadinium caudatum* Fiorentini, 1890	+++	+	+++	+	+	+	+	+	++
57	*Triadinium magnum* Hsiung, 1935				+				+	
58	*Gassovskiella galea* (Gassovsky, 1919)	+++	+	++	+	+		+	+	+++
59	*Cochliatoxum periachtum* Gassovsky, 1919		+	++	+	+		+	+	++
60	*Tetratoxum unifasciculatum* (Fiorentini,1890)		+	++	+	+	+	+		++
61	*Tetratoxum excavatum* Hsiung, 1930	+		++		+		+		+++
62	*Tetratoxum parvum* Hsiung, 1930	+	+	++	+	+		+	+	+
63	*Spirodinium equi* Fiorentini, 1890	+++	+	+	+	+	+			++
64	*Spirodinium uncinucleatum* Hsiung, 1935		+						+	
65	*Spirodinium confusum* Hsiung, 1935		+	+++		+		+		
66	*Spirodinium magnum* Ike, Imai, Ishii, 1983								+	

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Table 1. Completion
Таблица 1. Окончание

1	2	3	4	5	6	7	8	9	10	11
67	*Allantosoma intestinale* Gassovsky, 1919	+++		+++	+	+	+	+	+	+
68	*Allantosoma cucumis* Strelkow,1939					+				+++
69	*Allantoxena biseriale* (Strelkow,1939)			+		+			+	+
70	*Allantoxena japonensis* (Imai, 1979)					+				
71	*Arcosoma dicorniger* (Hsiung, 1928)			+		+			+	+
72	*Arcosoma brevicorniger* (Hsiung, 1928)					+			+	+
73	*Arcosoma lineare* (Strelkow,1939)			++		+				+
74	*Strelkowella urunbasiensis* Kornilova, 2004					+				

Data is in accordance with: 1 — Adam 1951; 2 — Hsiung 1936; 3 — Strelkow 1939; 4 — Strelkow 1939, Gurelli et al. 2015; 5 — Kornilova 2006; 6 — Ito et al. 1996; 7 — Gurelli, Gocmen 2011; 8 — Kornilova 2003. An estimate for the frequency of occurrence of species is given in the legend: “+++” often, “++” average, “+” single individuals

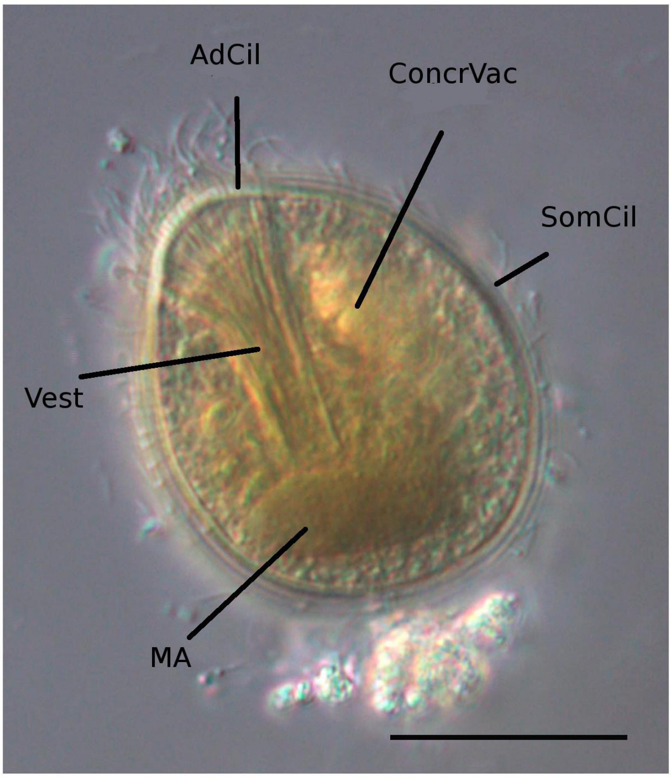

Fig. 1. *Wolskana tokarensis* from the hindgut of Icelandic horse (DIC). AdCil — adoral ciliature, ConcrCil — concrement vacuole, SomCil — somatic ciliature, Vest — vestibulum, MA — macronucleus. Scale bar 10 μm

Рис. 1. *Wolskana tokarensis* из кишечника исландской лошади (контраст Номарского). AdCil — адоральная цилиатура, ConcrCil — вакуоль с конкрециями, SomCil — соматическая цилиатура, Vest — вестибулюм, MA — макронуклеус. Шкала 10 μm

Table 2

The Sorensen-Dice similarity coefficient of the species composition of equine hindgut ciliates from Iceland and from some other geographical regions of Eurasia

Таблица 2

Индекс сходства Серенсена для видового состава сообществ инфузорий кишечника лошадиных из Исландии и из некоторых других регионов Евразии

Barsa-kelmes	Scotland	China	Far East	Middle Asia	Yakutia	Japan	Turkey
0.51	0.56	0.48	0.59	0.52	0.64	0.64	0.58

us the opportunity to assume that probably the horses from Iceland and the ponies from the Tokara islands had the common Mongolian ancestor with the same intestinal ciliates. Unfortunately, the fauna of the endobiotic ciliates of Mongolian horses has not been studied yet, so there are no data for a comparative analysis.

In addition we suggest that repeated sharp declines in the population of Icelandic horses in conditions of complete isolation on the island have led to the formation of specific features of the ciliates fauna, such as the conservation of *Wolskana* and the dominant position of this species in the community. This could also be caused by specific habitat conditions, including the diet of horses. So the population of Icelandic horses can be considered as a unique reservoir for the conservation of the rare population of ciliates such as *W. tokarensis* and others.

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