https://www.doi.org/10.33910/2686-9519-2024-16-2-303-309
https://zoobank.org/References/4BF4C3D5-C5ED-4F7D-A319-40BD816B2AC2

UDC 593.17

Endobiotic ciliates of Marwari horses from the Thar Desert, India

O. A. Kornilova[1]✉, L. V. Chistyakova[2]

[1] Herzen State Pedagogical University of Russia, 48 Moika River Emb., 191186, Saint Petersburg, Russia
[2] Zoological Institute of Russian Academy of Sciences, 1 Universitetskaya Emb., 199034, Saint Petersburg, Russia

Authors

Olga A. Kornilova
E-mail: oakornilova@herzen.spb.ru
SPIN: 2056-5776
Scopus Author ID: 15053466800
ResearcherID: AAD-5485-2019
ORCID: 0000-0002-4537-6189

Ludmila V. Chistyakova
E-mail: pelomixa@mail.ru
SPIN: 2345-6416
Scopus Author ID: 8760549600
ResearcherID: AAE-2379-2020
ORCID: 0000-0001-7192-1198

Abstract. This paper examines the species composition of endobiotic ciliates in the faecal samples from domestic Marwari horses (*Equus caballus*) from the Thar Desert, Rajasthan, India. We discovered nine species of ciliates from the Cycloposthiidae, Buetschliidae, Blepharocorythidae, Spirodiniidae, and Paraisotrichidae families. We also emphasise that Marwari horses differ considerably from domestic horses in other geographical regions by the species composition of endobiotic ciliates. We assume that such difference may be due to their diet, which is largely based on peanut roughage.

Keywords: symbiotic ciliates, Trichostomatia, *Equus caballus*, Marwari horse, *Cycloposthium*, *Blepharocorys*, *Spirodinium*, *Paraisotricha*

Эндобионтные инфузории домашних лошадей породы маварри (пустыня Тар, Республика Индия)

О. А. Корнилова[1]✉, Л. В. Чистякова[2]

[1] Российский государственный педагогический университет им. А. И. Герцена, наб. реки Мойки, д. 48, 191186, г. Санкт-Петербург, Россия
[2] Зоологический институт РАН, Университетская наб., д. 1, 199034, г. Санкт-Петербург, Россия

Сведения об авторах

Корнилова Ольга Анатольевна
E-mail: oakornilova@herzen.spb.ru
SPIN-код: 2056-5776
Scopus Author ID: 15053466800
ResearcherID: AAD-5485-2019
ORCID: 0000-0002-4537-6189

Чистякова Людмила Валерьевна
E-mail: pelomixa@mail.ru
SPIN-код: 2345-6416
Scopus Author ID: 8760549600
ResearcherID: AAE-2379-2020
ORCID: 0000-0001-7192-1198

Аннотация. Исследован видовой состав эндобионтных инфузорий в пробах фекалий домашней лошади *Equus caballus* породы маварри из пустыни Тар (Республика Индия, Раджастхан). Обнаружено девять видов инфузорий — представителей семейств Cycloposthiidae, Buetschliidae, Blepharocorythidae, Spirodiniidae и Paraisotrichidae. Показано, что по видовому составу инфузорий-эндобионтов исследованные нами марварские лошади значительно отличаются от домашних лошадей из других географических регионов. Предположительно подобные различия обусловлены спецификой питания лошадей, основу рациона которых составляют грубые корма из отходов производства арахиса.

Ключевые слова: симбиотические инфузории, Trichostomatia, *Equus caballus*, лошади породы маварри, *Cycloposthium*, *Blepharocorys*, *Spirodinium*, *Paraisotricha*

Introduction

Among all the *Equus* species, the fauna of endobiotic ciliates is best studied in the domestic horse (*Equus caballus*). Endobiotic ciliate communities have been analysed in horses across various geographical regions, with a total of at least 78 ciliate species identified in the intestines of this host (Cedrola et al. 2019; Kornilova et al. 2019). However, data available on the species diversity of endobiotic ciliates in domestic horses from long-isolated geographical populations have been scarce and patchy so far. Meanwhile, such studies can be of great interest given the specific transmission pathway of endobiotic ciliates: most of them do not produce cysts and are mainly passed from one host to another via grooming or coprophagy (Kornilova 2004). We can thus assume a stark difference in the structure of endobiotic communities across various geographical domestic horse populations and even the presence of ciliate species that are specific to a certain host population (Kornilova 2006). Such differences may be linked to both the founder effect and the influence of various environmental factors such as diets.

We provide a comparative study of the species composition of endobiotic ciliates in the faecal samples of domestic Marwari horses (*E. caballus*) from the Thar Desert, India.

Material and methods

In January 2015, we collected faecal samples from six Marwari horses at the Mahansar Fort (Rajasthan, India) in the north-east of the Thar Desert (N28°12′0″, E75°2′53″). Local horses mostly feed on *Arachis hypogaea* L., 1753 peanut roughage — rhizoma peanut hay — and shelled peanuts. Marwari horses have been bred in the Thar Desert since the 12th century. Intensive selective breeding efforts produced sturdy cavalry horses with unique performance and external characteristics (Rousseau 2014). According to the analysis of the Marwari horse genome, this breed is closely related to Arabian and Mongolian horses in contrast with other native Indian horses (Jun et al. 2014).

In the 19th and early 20th centuries, the population of Marwari horses slumped, but was restored in the late 20th century. At present, Marwari horses are prohibited from being exported from India, but can be temporarily taken to international shows in very limited numbers (Rousseau 2014). The studied horses have never contacted any imported horses or left India.

The samples were fixed in 96% ethyl alcohol (1:1) and kept in the dark at room temperature. The ciliates were stained by methyl green 1% solution in 1% acetic acid in order to visualise the nuclei. The ciliates were observed and photographed on glass object slides using a Leica DM 2500 microscope equipped with differential interference contrast (DIC) and a digital camera Leica DFC495 (8.0MP).

To identify the features of the ciliature organization, the method of immunofluorescence microscopy was used. The samples for immunofluorescent staining and microscopy were prepared according to a previously published protocol (Kornilova et al. 2019).

The total number of ciliates in a fixed volume of liquid (100 μl) was counted on the slides. Identification and taxonomy of ciliate species and genera was mainly based on the studies of Gassovsky, Hsiung, Strelkow, and Lynn (Gassovsky 1919; Hsiung 1930; Strelkow 1931; 1939; Lynn 2008). Statistical data processing was carried out using Past 3.0.

Results and discussion

The faecal samples from Marwari horses revealed a total of nine ciliate species (Table 1, Fig. 1). All of them are typical representatives of the endobiotic ciliate fauna of this host. The ciliate species composition is generally similar in all the six horses although horses No. 1 and 2 had much fewer species than all the others. Meanwhile, the total number of endobiotic ciliate species in the studied horses from the Thar Desert was extremely low compared to the available data on the diversity of endobiotic ciliate species found in domestic horses from

 https://www.doi.org/10.33910/2686-9519-2024-16-2-303-309

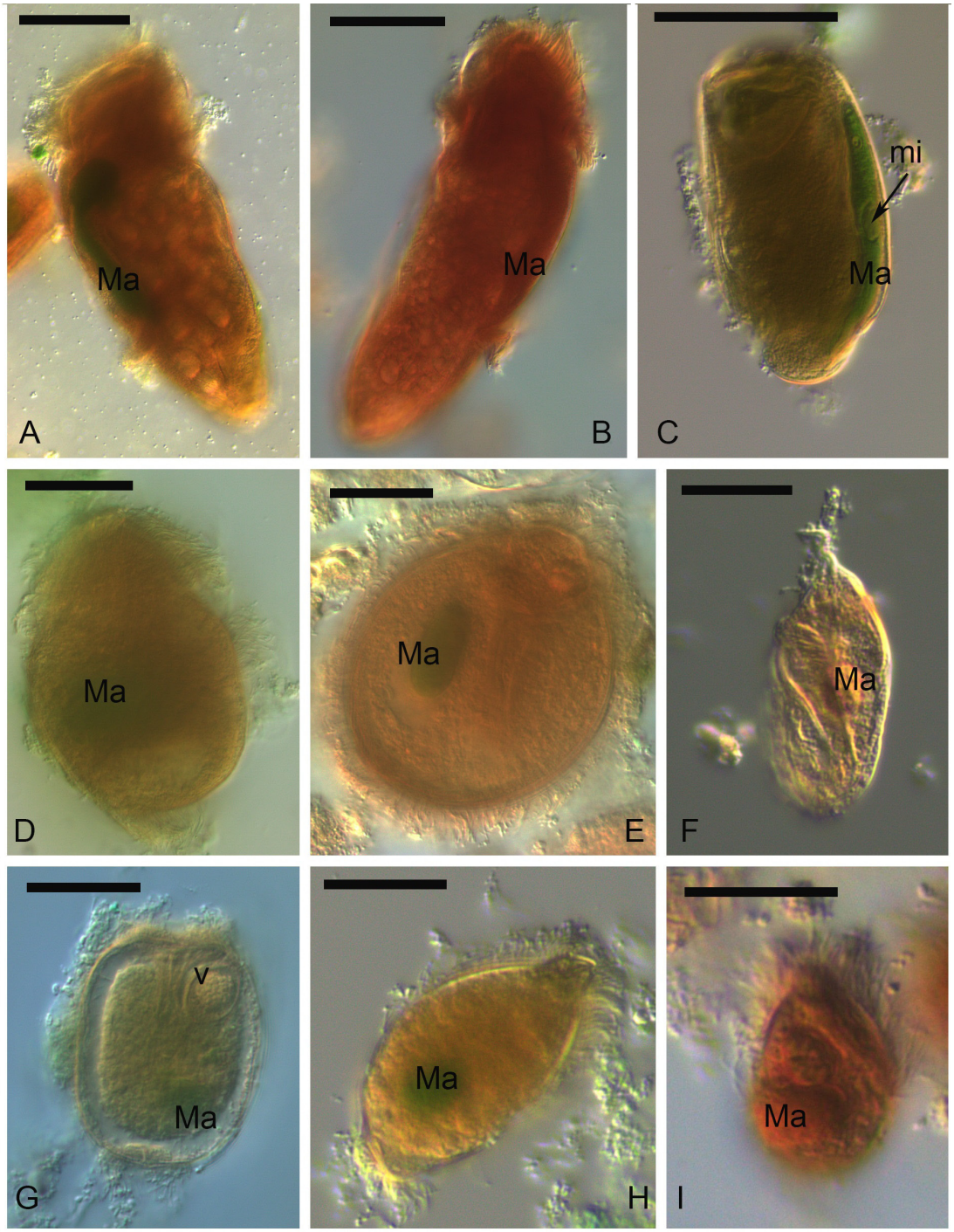

Fig. 1. Ciliates from the faeces of domestic Marwari horses from India: *A — Spirodinium uncinucleatum*; *B — Spirodinium equi; C — Cycloposthium bipalmatum*; *D — Alloiozona trizona*; *E — Paraisotricha colpoidea*; *F — Blepharocorys uncinata*; *G — Fiorentinus ovalis*, *H — Blepharoprosthium polytrichum*; *I — Polymorphella ampulla. Ma* — macronucleus; *mi* — micronucleus; *v* — vestibulum. Scale bars: *A–C* — 50 μm; *D–I* — 20 μm

Рис. 1. Инфузории из фекалий домашних лошадей породы марвари из Индии: *A — Spirodinium uncinucleatum*; *B — Spirodinium equi*; *C — Cycloposthium bipalmatum*; *D — Alloiozona trizona*; *E — Paraisotricha colpoidea*; *F — Blepharocorys uncinata*; *G — Fiorentinus ovalis*; *H — Blepharoprosthium polytrichum*; *I — Polymorphella ampulla. Ma* — макронуклеус; *mi* — микронуклеус; *v* — вестибулум. Масштабные линейки: *A–C* — 50 мкм; *D–I* — 20 мкм

Table 1

Composition and number of species of endobiotic ciliates in the faecal samples of domestic Marwari horses from India. The numbers indicate the number of cells in 100 μl, + — single ciliate individuals discovered

Таблица 1

Видовой состав и численность эндобионтных инфузорий в пробах фекалий домашних лошадей породы марвари из Индии

	Horse No. Family/Genera/Species	1	2	3	4	5	6
	Cycloposthiidae Poche, 1913						
	Cycloposthium Bundle, 1895						
1	*C. bipalmatum* (Fiorentini) 1890	20	50	50	40	50	50
	Spirodiniidae Strelkow, 1939						
	Spirodinium Fiorentini, 1890						
2	*S. equi* Fiorentini, 1890	–	–	10	10	20	10
3	*S. uncinucleatum* Hsiung, 1935	–	–	5	5	8	5
	Blepharocorythidae Hsiung, 1929						
	Blepharocorys Bundle, 1895						
4	*B. uncinata* (Fiorentini, 1890)	–	–	–	+	–	–
	Paraisotrichidae da Cunha, 1917						
	Paraisotricha Fiorentini, 1890						
5	*P. colpoidea* Fiorentini, 1890	–	–	20	20	+	20
	Buetschliidae Poche, 1913						
	Fiorentinus Jankowski, 1986						
6	*F. ovalis* (Fiorentini, 1890)		20	10	10	–	10
	Blepharoprosthium Bundle, 1895						
7	*B. polytrichum* Strelkow,1939	–	–	10	10	+	20
	Alloiozona Hsiung, 1930						
8	*A. trizona* Hsiung, 1930	–	–	10	10	+	10
	Polymorphella Corliss, 1960						
9	*P. ampulla* (Dogiel, 1929)	–	–	10	+	–	+
	Total species	1	2	8	9	6	8

Note: numbers — the number of cells in 100 μl; + — single ciliate individuals discovered

Примечание: числа — количество клеток на 100 мкл; + — обнаружены единичные особи инфузорий

other habitats (Cedrola et al. 2019; Kornilova et al. 2019). The samples showed a total of four species from the Buetschliidae family and no species of the *Bundleia* genus whatsoever, which is extremely rare for endobiotic ciliate communities in equine intestines. Meanwhile, the discovered buetschliids — *Fiorentinus ovalis*, *Polymorphella ampulla*, *Alloiozona trizona,* and *Blepharoprosthium polytrichum* — are rather frequent in Marwari horses.

The samples contained virtually no representatives of the equine endobiotic ciliate fauna typically found in horses, e. g., Blepharocorythidae family species. Horse No. 4 was the only one to show single individuals of the rather rare *Blepharocorys uncinate* species. No suctorians (Allantosomatidae family) were detected, either. The samples were clearly dominated by *Cycloposthium bipalmatum,* whereas no other cycloposthiids were found. Overall, the ciliate species composition discovered in the faecal samples collected from Marwari horses of the Thar Desert is largely different

 https://www.doi.org/10.33910/2686-9519-2024-16-2-303-309

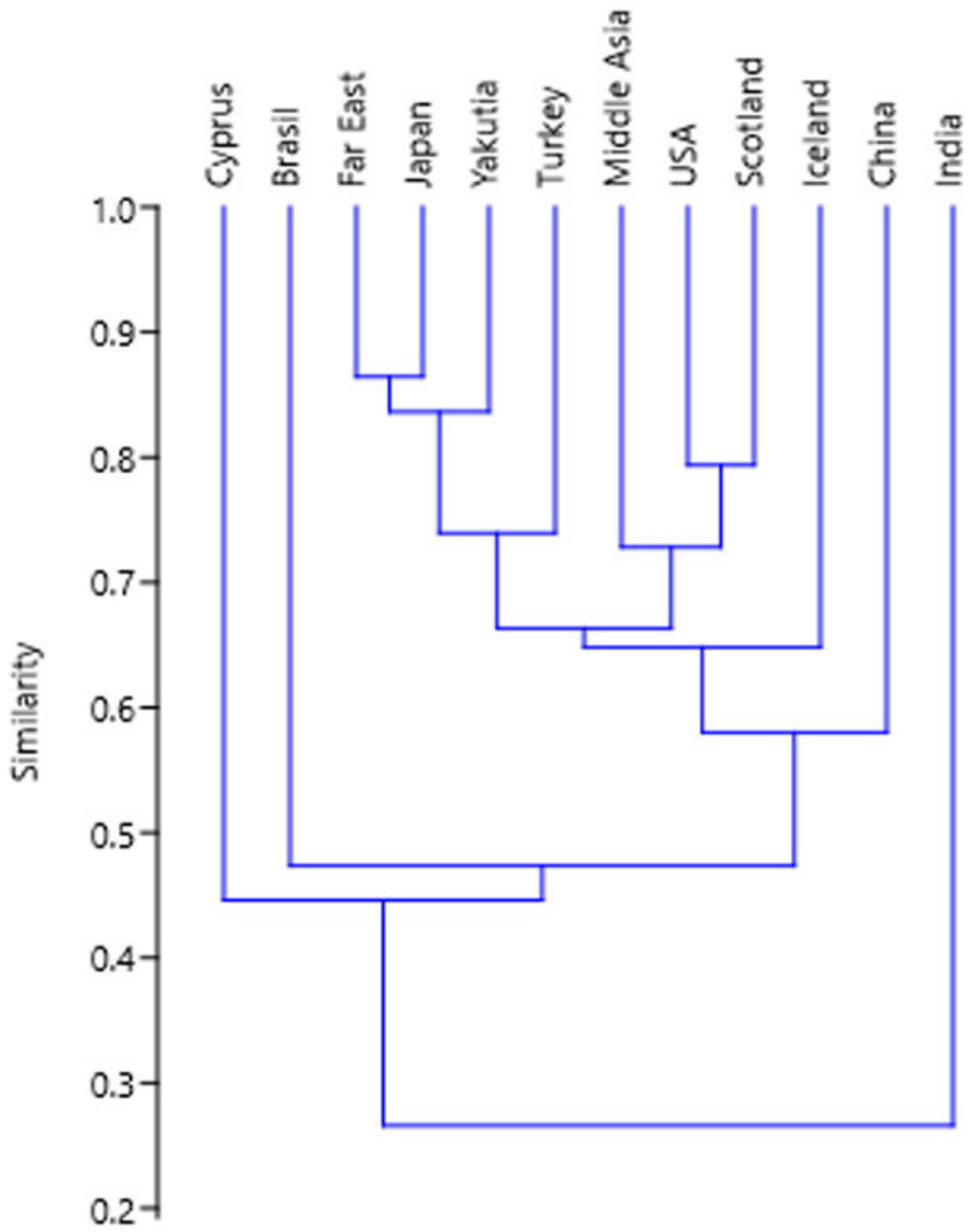

Fig. 2. Bray-Curtis dendrogram of cluster analysis of the species composition of endobiotic ciliate communities in *Equus caballus* from different geographical regions

Рис. 2. Дендрограмма, полученная по результатам кластерного анализа видового состава сообществ инфузорий-эндобионтов *Equus caballus* из разных географических регионов с использованием коэффициента Брея-Кёртиса

from the one in domestic horses from other geographical regions (Table 2). The lowest number of common species was identified in horses from Brazil, Iceland, and Cyprus.

According to the results of cluster analysis, the ciliate communities of horses from India form a separate branch on the dendrogram, demonstrating the presence of significant differences in species composition compared to the communities of endobiont ciliates of horses from other regions (Fig. 2).

The overwhelming majority of endobiotic ciliates in the mammalian intestinal tract are uncapable of producing cysts and are generally passed from one host to another via grooming and coprophagy (Kornilova 2004).

Table 2

Comparative analysis of the species diversity of endobiotic ciliates in domestic horses from different regions (localities)

Таблица 2

Сравнительный анализ видового разнообразия инфузорий-эндобионтов домашней лошади из разных регионов

	1	2	3	4	5	6	7	8	9	10	11	12
Number of species	9	25	30	56	37	56	65	39	34	50	21	24
Number of common species		7	7	8	8	8	8	7	5	8	2	3
Sørensen coefficient		0.44	0.38	0.25	0.36	0.25	0.22	0.33	0.26	0.27	0.17	0.22

Note: 1 — present study; 2 — Scotland (Adam 1951); 3 — China (Hsiung 1936); 4 — Far East; 5 — Middle Asia (Strelkow 1939; Gürelli et al. 2015); 6 — Yakutia, Russia (Kornilova 2006); 7 — Japan (Ike et al. 1983; 1985; Ito et al. 1996; Cedrola et al. 2019); 8 — Türkiye (Gürelli, Göçmen 2011; 2012; Gürelli 2012); 9 — Iceland (Kornilova et al. 2019); 10 — USA (Hsiung 1930; Cedrola et al. 2019; Gürelli et al. 2019); 11 — Cyprus (Cedrola et al. 2019); 12 — Brasil (Cedrola et al. 2019)

Примечание: 1 — настоящее исследование; 2 — Шотландия (Adam 1951); 3 — Китай (Hsiung 1936); 4 — Дальний Восток; 5 — Средняя Азия (Strelkow 1939; Gürelli et al. 2015); 6 — Якутия (Kornilova 2006); 7 — Япония (Ike et al. 1983; 1985; Ito et al. 1996; Cedrola et al. 2019); 8 — Турция (Gürelli, Göçmen 2011; 2012; Gürelli 2012); 9 — Исландия (Kornilova et al. 2019); 10 — США (Hsiung 1930; Cedrola et al. 2019; Gürelli et al. 2019); 11 — Кипр (Cedrola et al. 2019); 12 — Бразилия (Cedrola et al. 2019)

A certain species composition of endobiotic communities largely develops in foals during the first weeks after birth (Ike et al. 1985). Ciliates are transmitted to them from the horses that they are in close contact with during that period — their mothers, in the first place. Besides, the composition and structure of endobiotic ciliate communities depend on the host's diet and intensity of contacts with other species (Strelkow 1939; Kornilova 2004). We suppose that the species composition of ciliate communities in the studied Marwari horses is largely determined by their specific diet, which is dominated by peanut roughage. Therefore, it is worth mentioning that the cytoplasm of the vast majority of the ciliates we discovered was filled with nutrient granules.

The development of a certain endobiotic ciliate fauna in Marwari horses could have been driven by contacts with Mongolian and Arabian horses during selective breeding. It would therefore be instructive to examine endobiotic ciliates in Arabian and Mongolian horses in their native habitats and in Indian breeds that are beyond the scope of the reported study.

Acknowledgements

We would like to express our gratitude to Angad Deo and Anirudh Singh from Mandawa for their kind permission to conduct the research.

Funding

This work is part of the state-commissioned assignment, project No. 122031100260-0.

References

Adam, K. M. G. (1951) The quantity and distribution of the ciliate Protozoa in the large intestine of the horse. Parasitology, vol. 41, no. 3-4, pp. 301–311. https://doi.org/10.1017/S0031182000084158 (In English)

Cedrola, F., Bordim, S., D'Agosto, M., Dias, R. J. P. (2019) Intestinal ciliates (Alveolata, Ciliophora) in Brazilian domestic horses (*Equus caballus* L.) and a review on the ciliate communities associated with horses around the world. Zootaxa, vol. 4585, no. 3, pp. 478–488. https://doi.org/10.11646/zootaxa.4585.3.4 (In English)

Gassovsky, G. N. (1919) On the microfauna of the intestine of the horse. Travaux de la Société des Naturalistes de Pétrograd, vol. 49, pp. 20–37, 65–69. (In Russian)
Gürelli, G. (2012) Comparative study of hindgut ciliates in horses, mules and donkeys in Turkey. Global Veterinaria, vol. 9, no. 6, pp. 700–705. (In English)
Gürelli, G., Göçmen, B. (2011) Intestinal ciliate composition found in the feces of the Turk rahvan horse *Equus caballus* Linnaeus, 1758. European Journal of Protistology, vol. 47, no. 4, pp. 245–255. https://doi.org/10.1016/j.ejop.2011.04.005 (In English)
Gürelli, G., Göçmen, B. (2012) Intestinal ciliate composition found in the feces of racing horses from Izmir, Turkey. European Journal of Protistology, vol. 48, no. 3, pp. 215–226. https://doi.org/10.1016/j.ejop.2012.01.002 (In English)
Gürelli, G., Canbulat, S., Aldayarov, N. (2015) Fecal ciliate composition of domestic horses (*Equus caballus* Linnaeus, 1758) living in Kyrgyzstan. Zootaxa, vol. 4039, no. 1, pp. 145–156. https://doi.org/10.11646/zootaxa.4039.1.6 (In English)
Gürelli, G., Lyons, E. T., Kesbiç, F. I. (2019) Hindgut ciliate composition of thoroughbred mares in Kentucky, USA, and binary fission in Polymorphella ampulla. Zootaxa, vol. 4646, no. 2, pp. 369–384. https://doi.org/10.11646/ZOOTAXA.4646.2.11 (In English)
Hsiung, T. S. (1930) A monograph on the protozoan fauna of the large intestine of the horse. Iowa State College Journal of Science, vol. 4, pp. 359–423. (In English)
Hsiung, T. S. (1936) A survey of the ciliates of Chinese equines. Bulletin of the Fan Memorial Institute of Biology, vol. 6, pp. 289–304. (In English)
Ike, K., Imai, S. Ishii, T. (1985) Establishment of intestinal ciliates in new-born horses. Japanese Journal of Veterinary Science, vol. 47, no. 1, pp. 39–43. https://doi.org/10.1292/jvms1939.47.39 (In English)
Ike, K., Nuruki, R., Imai, S. et. al. (1983) Composition of intestinal ciliates and bacteria excreted in feces of the race-horse. Japanese Journal of Veterinary Science, vol. 45, no. 2, pp. 157–163. https://doi.org/10.1292/jvms1939.45.157 (In English)
Ito, A., Imai, S., Ogimoto, K., Nakahara, M. (1996) Intestinal ciliates found in the feces of Japanese native Tokara pony, with the description of a new genus and a new species. Journal of Veterinary Medical Science, vol. 58, no. 2, pp. 103–108. https://doi.org/10.1292/jvms.58.103 (In English)
Jun, J., Cho, Y. S., Hu, H. et al. (2014) Whole genome sequence and analysis of the Marwari horse breed and its genetic origin. BMC Genomics, vol. 15, suppl. 9, article S4. https://doi.org/10.1186/1471-2164-15-S9-S4 (In English)
Kornilova, O. A. (2004) Istoriya izucheniya endobiontnykh infuzorij mlekopitayushchikh [History of study of endobiotic ciliates of mammalia]. Saint Petersburg: TESSA Publ., 352 p. (In Russian)
Kornilova, O. A. (2006) Infuzorii iz kishechnika yakutskoj loshadi (*Equus caballus*) [Ciliates from the intestine of Yakut horse (*Equus caballus*)]. Parazitologiya, vol. 40, no. 5, pp. 472–478. (In Russian).
Kornilova, O. A., Chistyakova, L. V., Kamyshatskaya, O. G. (2019) Report on ciliates from the hindgut of horses in Iceland. Amurskij zoologicheskij zhurnal — Amurian Zoological Journal, vol. 11, no. 4, pp. 375–383. https://doi.org/10.33910/2686-9519-2019-11-4-375-383 (In English)
Lynn, D. H. (2008) The ciliated protozoa: Characterization, classification, and guide to the literature. 3rd ed. Dordrecht: Springer Publ., 627 p. https://doi.org/10.1007/978-1-4020-8239-9 (In English)
Rousseau, É. (2014) Tous les chevaux du monde: Près de 570 races et types décrits et illustrés. Paris: Delachaux et Niestlé Publ., 544 p. (In French)
Strelkow, A. A. (1931) Über die Fauna des Colons beim Zebra. Zoologischer Anzeiger, vol. 94, pp. 37–54. (In German)
Strelkow, A. A. (1939) Paraziticheskie infuzorii iz kishechnika neparnokopytnykh semejstva Equidae [Parasitical infusoria from the intestine of Ungulata belonging to the family Equidae]. Uchenye zapiski LGPU im. A. I. Gertsena — Scientific notes of the Herzen Leningrad State Pedagogical Institute, vol. 17, 261 p. (In Russian)

For citation: Kornilova, O. A., Chistyakova, L. V. (2024) Endobiotic ciliates of Marwari horses from the Thar Desert, India. *Amurian Zoological Journal*, vol. XVI, no. 2, pp. 303–309. https://www.doi.org/10.33910/2686-9519-2024-16-2-303-309

Received 28 February 2024; reviewed 30 March 2024; accepted 11 April 2024.

Для цитирования: Корнилова, О. А., Чистякова, Л. В. (2024) Эндобионтные инфузории домашних лошадей породы маравари (пустыня Тар, Республика Индия). *Амурский зоологический журнал*, т. XVI, № 2, с. 303–309. https://www.doi.org/10.33910/2686-9519-2024-16-2-303-309

Получена 28 февраля 2024; прошла рецензирование 30 марта 2024; принята 11 апреля 2024.