

ORIGINAL ARTICLE

Ciliates from faeces of kiang *Equus kiang* kept in a zoo, with analysis of interspecific differences of 18S sequences among trichostomatids (Litostomatea)

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| Submitted August 20, 2023 | Accepted September 24, 2023 |

Summary

The species composition of ciliates - endobionts of the digestive tract of kiang *Equus kiang* was studied for the first time. In total, 21 ciliate species were found; all of them are characteristic representatives of the fauna of endobiotic ciliates of equids. *Paraisotrichopsis composita* and *Triadinium magnum* were previously found mainly in horses of the Asian region. The level of differences in 18S RNA sequences was established in representatives of various genera and families of ciliates, endobionts of the digestive tract of herbivorous mammals, primarily odd-toed ungulates and ruminants.

Key words: endobiotic ciliates, *Equus kiang*, equids, evolutionary distances analysis

Introduction

The modern species diversity of ciliates - endobionts of vertebrates was formed mainly as a result of protists adaptation to life in various parts of the digestive tract of herbivorous mammals (Kornilova, 2004a, 2006; Newbold et al., 2015). In these unique habitats, complex microbiomes are formed that are characterized by a rich species composition of both pro- and eukaryotes involved in the digestion of plant matter (Newbold et al., 2015). The species composition of communities of endobiotic ciliates differs among representatives of different orders of vertebrates (Vd'ačný, 2018). The vast majority of

endobiotic ciliates do not form cysts and remain viable in the external environment for a very short time, and the transfer of endobionts from one host to another occurs mainly due to grooming and coprophagy (Kornilova, 2004a).

In this regard, the most important factors determining the structure of communities of endobiotic ciliates include the taxonomic position of the host and the intensity of intra- and interspecific interactions of host individuals. In addition, endobiont communities appear to be somewhat influenced by the host's diet. In connection with the peculiarities of the lifestyle and distribution of ciliates – endobionts of different vertebrates, the study of the processes

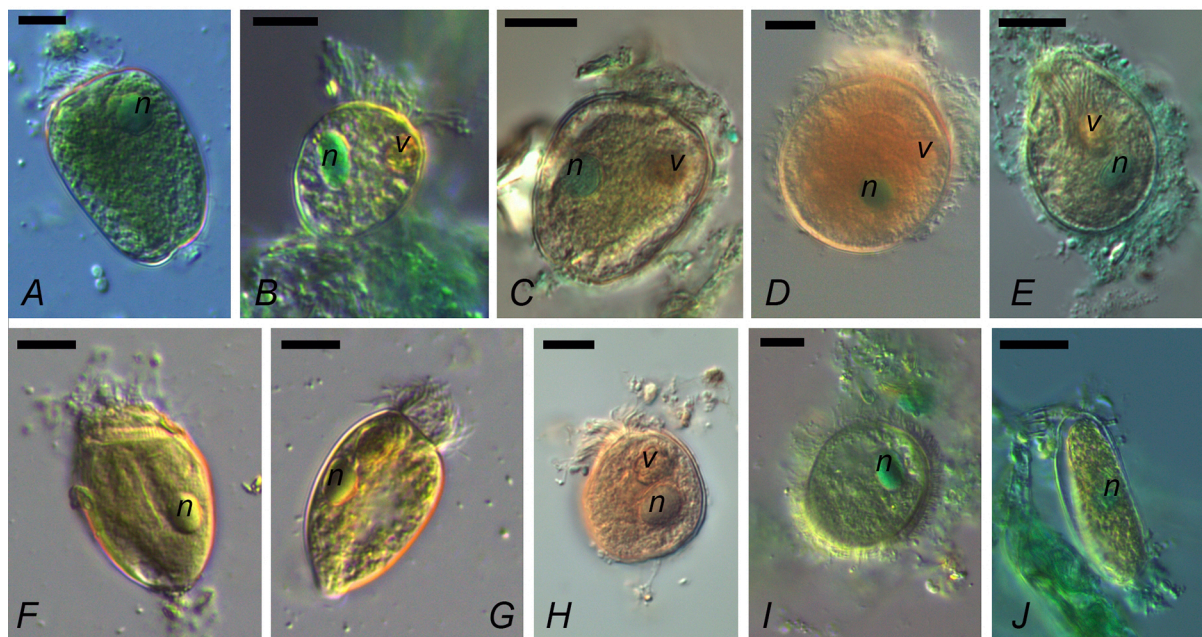


Fig. 1. Ciliates from the faeces of kiang *Equus kiang* (Buetschliidae, Allantosomatidae); DIC, staining with methyl green. A – *Bundleia postciliata*; B – *B. nana*; C – *B. triangularis*; D – *Hemiprorodon gymnoposthium*; E – *Polymorphella ampulla*; F – *B. inflata*; G – *B. dolichosoma*; H – *B. benbrookii*; I – *Paraisotrichopsis composita*; J – *Allantosoma intestinale*. Abbreviations: n – macronucleus, v – concretum vacuole. Scale bars: 10 µm.

of interpopulation isolation and speciation in this group of protists is of considerable interest. One of the possible approaches in research in this area could be to determine the structure of ciliate-endobiont communities of closely related host species. It should be noted, however, that the fauna of endobiotic ciliates of many herbivorous mammals has not yet been studied. For example, the genus *Equus* (the only currently existing genus in the family Equidae) includes several modern equine species: *Equus ferus*, *E. caballus*, *E. hemionus*, *E. asinus*, *E. quagga*, *E. zebra*, *E. grevi*, *E. kiang*, and *E. africanus*. The ciliate fauna of some of them was studied well enough (e.g., *E. caballus*), but others were studied incompletely (e.g., *E. grevi*), or even not investigated at all (e.g., *E. kiang*) (Kornilova, 2004a; Cedrola et al., 2019). Of undoubted interest in this regard is the study of the species diversity of ciliates – endobionts of rare animal species from hard-to-reach habitats kept in zoos.

This work for the first time presents the results of studying the species composition of ciliates isolated from the faeces of kiangs from the Moscow Zoo, in comparison with the ciliate fauna of other equids. We also assessed for the first time the level of differences in 18S RNA sequences in representatives of various genera and families of ciliates, endobionts

of the digestive tract of herbivorous mammals. Such an analysis, in our opinion, is an important and necessary component in studies of this group of protists with the use of methods of molecular phylogeny.

Material and methods

Samples of faeces were collected in June 2023 from two female eastern kiangs (*E. kiang holdereri* Matschie, 1911) kept in Moscow Zoological Park. One of them was born in Berlin Tierpark in 1999 and moved to Moscow Zoo in 2002. The other was born in Moscow Zoo in 2000. Since 2002, they have been living together in one corral. The samples of their faeces were put in 50 ml test tubes and fixed with 96% ethanol immediately after defaecation to prevent the destruction of intestinal ciliates. After a short time, the fixative in the test tubes was completely changed. Additional samples were preserved in formaldehyde at a final concentration 4%. The ciliates were stained with methyl green 1% solution in 1% acetic acid to visualize the nuclei. Ciliates were observed and photographed on glass object slides using a Leica DM 2500 microscope equipped with differential interference contrast (DIC) and digital camera Leica DFC495 (8.0MP).

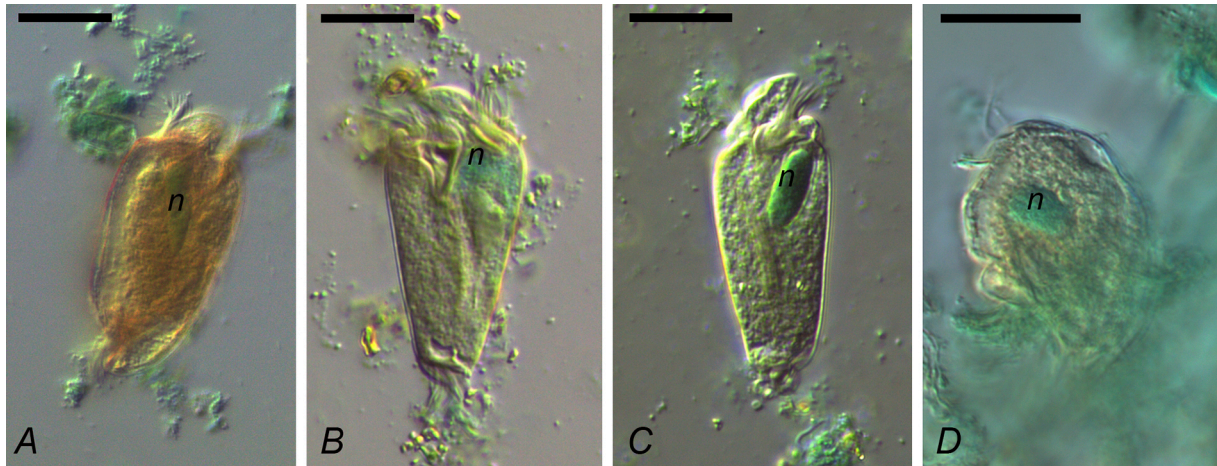


Fig. 2. Ciliates from the faeces of kiang *Equus kiang* (Blepharocorythidae); DIC, staining with methyl green. A – *Blepharocorys microcorys*; B – *B. curvigula*; C – *B. angusta*; D – *Circodinium minimum*. Abbreviation: n – macronucleus. Scale bars: 20 μ m.

The total number of ciliates in a fixed volume of liquid (100 μ l) was counted on the slides. Since the number of ciliates in the samples was very low, we quantified the results in the following manner: + about 10 cells per ml, ++ about 100 cells per ml, +++ many more than 100 cells per ml. Identification and taxonomy of ciliate species and genera were mainly based on the studies of Gassovsky (1919), Hsiung (1930), Strelkow (1931, 1939), and Lynn (2008).

For evolutionary distances analysis, we chose 18S sequences of Trichostomatia available from NCBI Genbank. The analysis included most of the groups identified in the traditional morphological system, whose composition was confirmed by the results of molecular phylogenetic analysis. We combined the selected sequences into three types of groups: sequences from the same family (e.g., Isotrichidae, Cycloposthiidae), sequences from the same genus (*Ostracodinium* sp., *Blepharocorys* sp., etc.), and those from the same species (*Isotricha intestinalis*, *Spirodinium equi*). Cycloposthiidae were analyzed three times: as a single group, and separately as a group including mainly *Cycloposthium* sp. and as a group including mostly *Triplumaria* sp. The detailed information of sequences included and NCBI accession numbers are given in Table S1. Nucleotide alignment was created for each group of sequences with the online tool Clustal Omega (Goujon et al., 2010). When compiling the summary Table 2, the values for specimens, whose systematic position was uncertain based on the results of the analysis, were not taken into account. Sequence information for geographic isolates of the same species is available

only for several ciliates; therefore, we considered it unnecessary to dwell on the discussion of this information.

The pairwise (for family groups) and mean (species and genus groups) evolutionary distances were estimated in MEGA X (Kumar et al., 2018). Two types of distances were calculated: (1) the number of base differences per sequence (divided by the number of total positions of final alignment), and (2) the number of base substitutions per site using the Maximum Composite Likelihood model. The parameters for both estimates were as follows: inclusion of transitions and transversions, gamma-distributed rates among sites (gamma shape parameter 1), and partial deletion of missing sites (cutoff 95%). Standard error estimates were obtained by a bootstrap procedure (100 replicates). The resulted distances and standard error estimates are provided in the Table S1.

Results and discussion

The species composition of endobiotic ciliates in different representatives of the genus *Equus* is generally the same; the vast majority of ciliate species were found in all studied species of this group of hosts (Cedrola et al., 2019). Some species of endobiotic ciliates, however, have a certain specificity in relation to their hosts. Thus, *Trifascicularia cycloposthium*, *Triadinium elongatum*, *Ditoxum hamulus*, and *Spirodinium ferrumequinum* were found only in the zebra *E. quagga* (Strelkow, 1931; Kornilova et al.,

Table 1. List of species of intestinal ciliates of different equids.

	Host species	1	2	3	4	5	6	7	8
	Family/genus/subgenus/species								
	Buetschliidae Poche, 1913								
	<i>Alloiozona</i> Hsiung, 1930								
1	<i>A. trizona</i> Hsiung, 1930		+			+	+		
	<i>Blepharoconus</i> Gassovsky, 1919								
2	<i>B. cervicalis</i> Hsiung, 1930		+						
3	<i>B. hemiciliatus</i> Gassovsky, 1919		+	+					
4	<i>Blepharoconus</i> sp.						+		
	<i>Blepharosphaera</i> Bundle, 1895								
5	<i>B. intestinalis</i> Bundle, 1895		+	+			+		
6	<i>B. ellipsoidalis</i> Hsiung, 1930		+	+					
7	<i>B. citriformis</i> Strelkow, 1939		+						
8	<i>B. ceratotherii</i> Van Hoven et al., 1998		+				+	+	
	<i>Holophryoides</i> Gassovsky, 1919								
9	<i>H. ovalis</i> (Fiorentini, 1890)		+	+		+	+		
10	<i>H. macrotricha</i> Strelkow, 1939		+	+		+	+	+	
	<i>Polymorphella</i> Corliss, 1960								
11	<i>P. ampulla</i> (Dogiel, 1929)	about 10 cells per ml	+	+		+			
	<i>Blepharozoum</i> Gassovsky, 1919								
12	<i>B. zonatum</i> Gassovsky, 1919		+			+			
	<i>Hemiprorodon</i> Strelkow, 1939								
13	<i>H. gymnoposthium</i> Strelkow, 1939	about 10 cells per ml	+	+					
	<i>Blepharoprosthium</i> Bundle, 1895								
14	<i>B. pireum</i> Bundle, 1895		+	+		+	+		
15	<i>B. polytrichum</i> Strelkow, 1939		+						
	<i>Bundleia</i> Cunha & Muniz, 1928								
	subgen. <i>Bundleia</i> Strelkow, 1939								
16	<i>B. postciliata</i> (Bundle, 1895)	about 10 cells per ml	+	+	+	+	+	+	
17	<i>B. piriformis</i> Strelkow, 1939		+			+	+	+	
18	<i>B. nana</i> Strelkow, 1939	about 10 cells per ml	+	+		+		+	
19	<i>B. vorax</i> Strelkow, 1939		+			+			
20	<i>B. asymmetrica</i> Strelkow, 1939		+		+				
	subgen. <i>Fibrillobundleia</i> Strelkow, 1939								
21	<i>B. benbrooki</i> (Hsiung, 1930)	about 10 cells per ml	+	+		+	+		
22	<i>B. inflata</i> Strelkow, 1939	many more than 100 cells per ml	+	+		+	+	+	
23	<i>B. dolichosoma</i> Strelkow, 1939	about 10 cells per ml	+					+	
	subgen. <i>Chlamydebundleia</i>								
24	<i>B. elongata</i> Strelkow, 1939		+	+		+			
25	<i>B. triangularis</i> Strelkow, 1939	many more than 100 cells per ml	+	+		+			
	<i>Prorodonopsis</i> Gassovsky, 1919								
26	<i>P. coli</i> Gassovsky, 1919		+	+					
	<i>Sulcoarcus</i> Hsiung, 1935								
27	<i>S. pellucidulus</i> Hsiung, 1935		+	+		+			

Table 1. Continuation.

	Host species	1	2	3	4	5	6	7	8
	Family/genus/subgenus/species								
	<i>Paraisotrichopsis</i> Gassovsky, 1919								
28	<i>P. composita</i> Gassovsky, 1919	about 10 cells per ml	+			+			
	<i>Fiorentinus</i> Jankowski, 1986								
29	<i>F. ovalis</i> (Fiorentini, 1890)		+	+		+			
	<i>Didesmis</i> Fiorentini, 1890								
30	<i>quadrata</i> Fiorentini, 1890		+	+		+			
31	<i>spiralis</i> Hsiung, 1929		+						
	<i>Wolskana</i> Ito, Ogimoto, Nakahara, 1996								
32	<i>W. tokarensis</i> Ito, Ogimoto, Nakahara, 1996		+						
	Paraisotrichidae da Cunha, 1917								
	<i>Paraisotricha</i> Fiorentini, 1890								
33	<i>P. minuta</i> Hsiung, 1930		+	+		+		+	
34	<i>P. colpoidea</i> Fiorentini, 1890		+	+		+			
	<i>Rhizotricha</i> Wolska, 1964								
35	<i>R. beckeri</i> (Hsiung, 1930)		+			+			
	Blepharocorythidae Hsiung, 1929								
	<i>Blepharocorys</i> Bundle, 1895								
36	<i>B. uncinata</i> (Fiorentini, 1890)		+						
37	<i>B. curvigula</i> Gassovsky, 1919	about 100 cells per ml	+	+	+	+		+	+
38	<i>B. angusta</i> Gassovsky, 1919	many more than 100 cells per ml	+	+	+	+	+	+	+
39	<i>B. microcorys</i> Gassovsky, 1919	about 100 cells per ml	+	+	+	+		+	+
40	<i>B. valvata</i> (Fiorentini, 1890)		+	+		+			
41	<i>B. cardionucleata</i> Hsiung, 1930		+			+		+	
42	<i>B. jubata</i> Bundle, 1895		+		+	+		+	
	<i>Ochoterenaiia</i> Chavarria, 1933								
43	<i>O. appendiculata</i> Chavarria, 1933		+		+	+		+	
	<i>Circodinium</i> Wolska, 1971								
44	<i>C. minimum</i> (Gassovsky, 1919)	about 100 cells per ml	+	+	+	+		+	+
	<i>Charonnautes</i> Strelkow, 1939								
45	<i>C. equi</i> (Hsiung, 1930)		+	+		+		+	
	Cycloposthiidae Poche, 1913								
	<i>Cycloposthium</i> Bundle, 1895								
46	<i>C. bipalmatum</i> (Fiorentini, 1890)	about 10 cells per ml	+	+	+	+		+	
47	<i>C. edentatum</i> Strelkow, 1928	many more than 100 cells per ml	+	+	+	+		+	
48	<i>C. dentiferum</i> Gassovsky, 1919		+		+	+			
49	<i>C. affine</i> Strelkow, 1929		+	+		+			
50	<i>C. corrugatum</i> Hsiung, 1930		+						
51	<i>C. hemioni</i> Kornilova, 2001					+			
52	<i>C. ponomarevi</i> Kornilova, 2001					+			
53	<i>C. piscicauda</i> Strelkow, 1928		+						

Table 1. Continuation.

	Host species	1	2	3	4	5	6	7	8
	Family/genus/subgenus/species								
83	<i>S. urunbasiensis</i> Kornilova, 2004		+						
	Total number of species	21	75	41	17	58	13	36	9

Notes: 1 – *E. kiang* (this paper), 2 – *E. caballus* (Cedrola et al., 2019), 3 – *E. asinus* (Strelkow, 1939, Kornilova, 2003, Gürelli, Göçmen, 2010, Gürelli, 2012), 4 – *E. ferus* (Kornilova, 2003), 5 – *E. hemionus* (Kornilova, 2003), 6 – *E. zebra* (Kornilova et al., 2020), 7 – *E. quagga* (Kornilova et al., 2021), 8 – *E. grevi* (Kornilova, 2003).

The number of ciliates is indicated only for *E. kiang*, for other equids only the presence or absence of a certain species of ciliates is indicated.

* Due to the neuter gender of the name *Allantosoma* we use species name “*intestinale*” according to the Article 30.1.2 of the International Code of Zoological Nomenclature (Aescht, 2001).

2021; Gürelli, 2023). *Spirodinium nanum* was also found mainly in zebras, but sporadic findings of this species in the domestic horse are also known (Ike et al., 1985; Kornilova et al., 2021, 2022; Gürelli, 2023). *Cycloposthium hemioni* and *C. ponomarevi* were first isolated and described from the intestines of the wild ass *E. hemionus* and have so far been found only in this host (Kornilova, 2003). The largest number of ciliate species was registered in the domestic horse *E. caballus* (Table 1). This is probably due to the worldwide distribution of this host species and frequent interactions of horses from different populations. At the same time, some species of ciliates were found only within certain isolated populations of horses. For example, *Wolskana tokarensis* was found only in Tokara ponies (native Japanese horses living on the Tokara islands) and horses from Iceland, and *Strelkowella urunbasiensis* – in endemic Yakut horses exclusively (Ito et al., 1996; Kornilova, 2004b; Kornilova et al., 2019).

In the faeces of kiangs, we found 21 species of ciliates, representatives of the families Buetschliidae, Spirodiniidae, Cycloposthiidae, Blepharocorythidae, and Allantosomatidae (Table 1; Figs 1–3). The number of ciliates in the samples was generally small and amounted to 1400 ciliates per ml. The most numerous species were *Bundleia inflata*, *B. triangularis*, *Blepharocorys angusta*, and *Cycloposthium edentatum*.

All ciliates found in the faeces of kiangs from the Moscow Zoo are typical representatives of the fauna of equine endobiotic ciliates. It is interesting to note the finding of *Paraisotrichopsis composita*, previously discovered mainly in horses of the Asian region (Cedrola et al., 2019). The same applies to the *Triadinium magnum*. It should be emphasized that when keeping the vertebrate hosts in zoos, a depletion of their ciliate composition is sometimes

observed (Modrý et al., 2009). Some species of endobiotic ciliates, such as *Triadinium elongatum* and *Spirodinium ferrumequinum*, were found in their specific hosts (the zebra *E. quagga*) only in natural habitats (Strelkow, 1931; Kornilova et al., 2021). Thus, to obtain a complete picture of the ciliate composition, it is certainly important to investigate endobiont samples from free-grazing kiangs in their natural habitat (Tibet and adjacent regions).

Studying the genetic variability of the isolated populations of endobiotic ciliates from different host species is impossible without investigating the level of interspecific differences between representatives of various genera and families of trichostomatid ciliates. Of course, to obtain a more or less complete and adequate idea of the nature of the differences between taxa of various ranks and the significance of the morphological differential characters observed, it is necessary to use a multigene phylogeny. However, the analysis using the 18S RNA gene sequence (the only one possible based on the available data) also seems relevant; its results are presented in Table 2, Fig. 4, and Table S1.

Endobiotic ciliates of mammals can be divided into two large groups, depending on the nature of their localization in the digestive tract of the host: hindgut (predominantly inhabitants of the intestines of odd-toed ungulates, elephants, damans, primates, and rodents) and foregut (inhabitants of the foregut fermentation chambers of ruminants, hippos, camels, and marsupials) (Kornilova, 2004a; Newbold et al., 2015). Among the hindgut ciliates included in the analysis, the species of the genus *Bundleia* (Buetschliidae) stand apart – the level of differences between them is much higher than the differences between the representatives of one genus, and even family, of other trichostomatids. The differences between other representatives of the

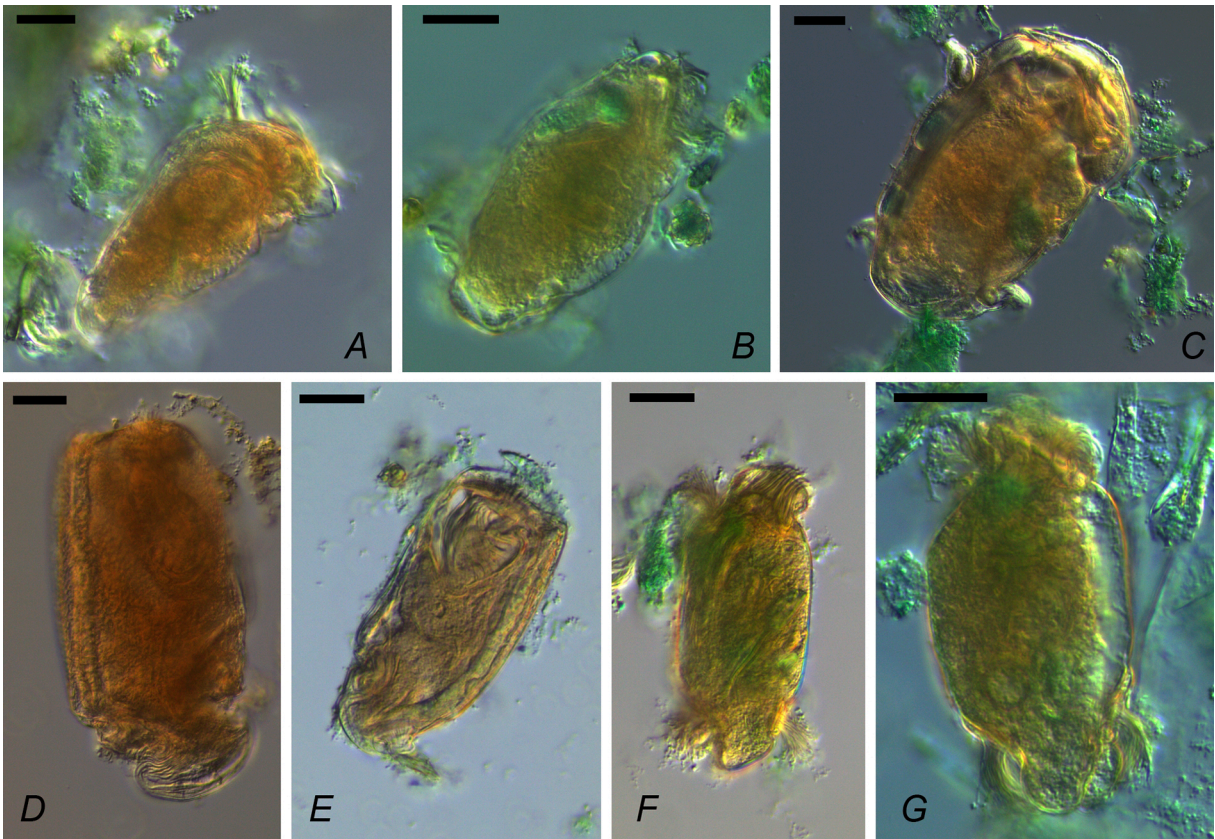


Fig. 3. Ciliates from the faeces of kiang *Equus kiang* (Spirodiniidae, Cycloposthiidae); DIC, staining with methyl green. A – *Triadinium magnum*; B – *Ditoxum funinucleum*; C – *Tripalmaria dogieli*; D – *Cycloposthium edentatum*; E – *C. bipalmatum*; F – *Tetratoxum parvum*; G – *T. unifasciculatum*. Scale bars: 20 μm .

family Buetschliidae are also generally higher than between representatives of other families of hindgut ciliates. A situation similar to *Bundleia* sp. is observed for species of the genus *Blepharocorys*, although the differences between them are not so great. The range of differences between representatives of the families Blepharocorythidae (except the genus *Blepharocorys*), Spirodiniidae and Cycloposthiidae is approximately the same. It is interesting to note that the level of differences between *Troglo-dytella abressarti* (Troglo-dytellidae) and different cycloposthiids appears to be approximately the same as between representatives of the genera *Cycloposthium* and *Triplumaria* (Cycloposthiidae). Moreover, according to the results of molecular phylogenetic analysis, species of the genus *Triplumaria* form a clade, which is separate from other cycloposthiids on the phylogenetic tree (Fregulia et al., 2021; Kornilova et al., 2023). Evolution divergence between species within the genera *Cycloposthium* and *Triplumaria* turns out to be approximately two times less than between representatives of different genera

of cycloposthiids. The species *C. compressum* stands out; evolutionary differences for it are approximately twice as large as that of other representatives of the group. Notably, the level of differences between *Cycloposthium* from equids and rodents turns out to be approximately the same.

Among foregut ciliates, 18S RNA sequence data are available primarily for endobionts of ruminants. For the family Isotrichidae, the level of differences between representatives of different genera (and species of the same genus *Isotricha*) is higher than the upper limit of the range of evolutionary divergence between representatives of different genera within the families Spirodiniidae and Cycloposthiidae. Within the family Ophryoscolecidae, three groups are clearly distinguished: a group of species from the genera *Polyplastron-Ostracodinium*, species of the genus *Epidinium*, and species of the genus *Entodinium*. The differences between the species of the first group correspond to the differences between representatives of the families Spirodiniidae and Cycloposthiidae. Evolution divergence between representatives

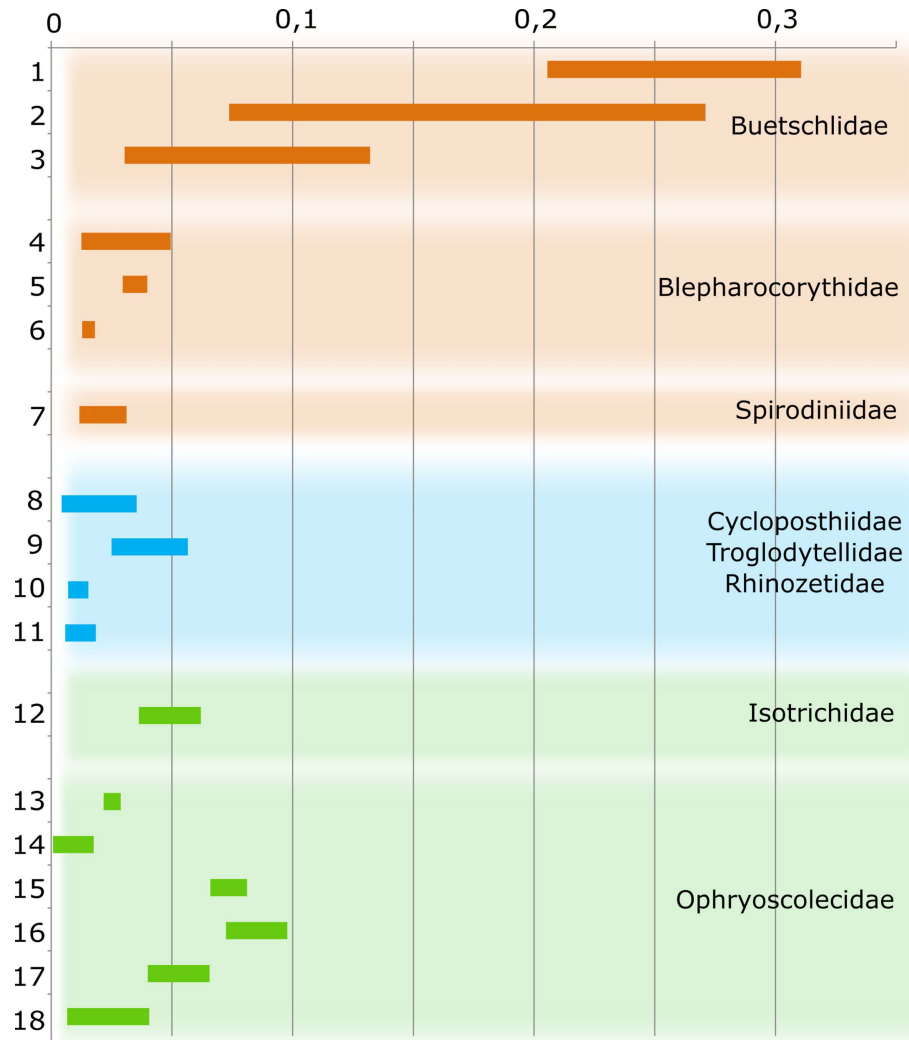


Fig. 4. Pairwise estimates (min/max) of the number of substitutions per site for selected groups. 1 – Buetschliidae, *Bundleia* sp. intragenus; 2 – Buetschliidae, *Bundleia* sp. and species of other genera; 3 – Buetschliidae, other genera; 4 – Blepharocorythidae, *Blepharocorys* sp. intragenus; 5 – Blepharocorythidae, *Blepharocorys* sp. and species of other genera; 6 – other genera; 7 – Spirodiniidae; 8 – Cycloposthiidae/Troglodytelliidae/Rhinozetidae, all species without *C. compressum*; 9 – Cycloposthiidae/Troglodytelliidae/Rhinozetidae, *C. compressum* with other species; 10 – *Cycloposthium* sp. (without *C. compressum*); 11 – *Triplumaria* sp.; 12 – Isotrichidae; 13 – Ophryoscolecidae, Polyplastron/Ostracodinium, 14 – Ophryoscolecidae, Ostracodinium sp., 15 – Ophryoscolecidae, *Polyplastron/Ostracodinium – Epidinium*; 16 – Ophryoscolecidae, *Polyplastron/Ostracodinium – Entodinium*; 17 – Ophryoscolecidae, *Epidinium – Entodinium*; 18 – Ophryoscolecidae, *Entodinium* sp.

of the groups *Polyplastron-Ostracodinium – Epidinium*, *Polyplastron-Ostracodinium – Entodinium* and *Epidinium – Entodinium* is at least twice as large. At the same time, the range of differences between the species of the genus *Ostracodinium* turns out to be somewhat wider than between the species of the genera *Cycloposthium* and *Triplumaria* of hindgut ciliates. The differences between some species of the genus *Entodinium* significantly exceed the differen-

ces between representatives of different genera of the families Spirodiniidae, Cycloposthiidae, and even Blepharocorythidae.

Thus, to conclude, the ranges of interspecific and intergeneric differences vary among representatives of different families of hindgut and foregut ciliates. Meanwhile, for three out of the four analyzed families of hindgut ciliates, these ranges (numeric values for these ranges) is approximately the same.

Table 2. The range (max/min) of interspecific and intergeneric differences between representatives of different families of hindgut and foregut ciliates (for explanations see Material and methods). Full table presented in Supplement (Table S1).

Ciliates	max	min
Buetschliidae <i>Bundliea</i> sp. intragenus <i>Bundliea</i> sp. and species of other genera Other genera	0.3106/14.38 <i>B. nana</i> – <i>B. benbrooki</i> 0.2710/13.27 <i>B. benbrooki</i> – <i>Sulcoarcus pellucidulus</i> 0.1321/8.63 <i>S. pellucidulus</i> – <i>D. ovalis</i> (<i>Florentinus ovalis</i>)	0.2055/11.24 <i>B. postciliata</i> – <i>B. benbrooki</i> 0.0737/5.69 <i>B. postciliata</i> – <i>Alloiozona trizona</i> 0.0304/2.75 <i>A. trizona</i> – <i>Blepharocorys hemiciiliatus</i>
Blepharocorythidae <i>Blepharocorys</i> sp. intragenus <i>Blepharocorys</i> sp. and species of other genera Other genera	0.0495/4.58 <i>B. microcorys</i> – <i>B. curvigula</i> 0.0398/3.74 <i>B. microcorys</i> – <i>Circodinium minimum</i> 0.0131/1.29 <i>O. appendiculata</i> – <i>C. minimum</i>	0.0125/1.23 <i>B. curvigula</i> – <i>B. jubata</i> 0.0296/2.84 <i>B. jubata</i> – <i>Ochoterenia appendiculata</i> 0.0131/1.29 <i>O. appendiculata</i> – <i>C. minimum</i>
Spirodiniidae	0.033/4.27 <i>Ditoxum funinucleum</i> – <i>Gassovskiella galea</i>	0.0128/1.75 <i>Cochliatoxum periachtum</i> – <i>Triadinium caudatum</i>
Cycloposthiidae+Troglodytelliidae+Rhinozetidae All species without <i>C. compressum</i> <i>C. compressum</i> with other species <i>Cycloposthium</i> sp. (without <i>C. compressum</i>) <i>Triplumaria</i> sp.	0.0354/3.37 <i>C. caudatum</i> , <i>C. cristatum</i> , <i>C. elongatum</i> – <i>Triplumaria harpagonis</i> 0.0566/5.16 <i>C. compressum</i> – <i>T. fulgora</i> , <i>T. harpagonis</i> 0.0154/1.5 <i>C. bursa</i> – <i>C. cristatum</i> 0.0185/1.8 <i>T. fulgora</i> – <i>T. harpagonis</i>	0.0043/0.43 <i>Cycloposthium incurvum</i> – <i>Monoposthium cynodontum</i> 0.0257/2.44 <i>C. compressum</i> – <i>C. elongatum</i> , <i>C. caudatum</i> 0.0007/0.07 <i>C. lenticularis</i> – <i>C. minutum</i> 0.0058/0.58 <i>T. fulgora</i> , <i>T. selenica</i> – <i>T. dvojnosi</i> , <i>T. sukuna</i> – <i>T. solea</i>
Isotrichidae	0.0620/5.7 <i>Isotricha prostoma</i> – <i>Dasytricha ruminantium</i>	0.0363/3.43 <i>I. prostoma</i> – <i>I. intestinalis</i>
Ophryoscolecidae <i>Polyplastron/Ostracodinium</i> <i>Ostracodinium</i> sp. <i>Polyplastron/Ostracodinium</i> – <i>Epidinium</i> <i>Polyplastron/Ostracodinium</i> – <i>Entodinium</i> <i>Epidinium</i> – <i>Entodinium</i> <i>Entodinium</i> sp.	0.0288/2.76 <i>P. multivesiculatum</i> – <i>O. gracile</i> 0.0176/1.71 <i>O. mammosum</i> – <i>O. gracile</i> 0.0811/7.17 <i>P. multivesiculatum</i> – <i>E. ecaudatum</i> 0.0978/8.42 <i>P. multivesiculatum</i> – <i>E. caudatum</i> 0.0656/5.92 <i>E. ecaudatum</i> – <i>E. caudatum</i> 0.0406/ <i>E. caudatum</i> – <i>E. simplex</i>	0.0217/2.1 <i>P. multivesiculatum</i> – <i>O. clipeolum</i> 0.0007/0.07 <i>O. gracile</i> – <i>O. clipeolum</i> 0.0659/5.92 <i>O. clipeolum</i> – <i>E. ecaudatum</i> , <i>E. caudatum</i> 0.0724/6.44 <i>O. clipeolum</i> – <i>E. furca monolobum</i> 0.04/3.75 <i>E. ecaudatum</i> – <i>E. furca monolobum</i> 0.0066/0.66 <i>E. dubardi</i> – <i>E. furca dilobum</i>

Within individual families, the ranges of differences between members of different genera and species of the same genus have been reported to overlap substantially; the differences between some species of the same genus are sometimes significantly greater than between species belonging to different genera of the same family.

Acknowledgements

The work was supported by the Russian Science Foundation (grant No 23-24-00240, <https://rscf.ru/project/23-24-00240/>). The research was carried out using the equipment of the Core Facilities Centre “Taxon” at the Zoological Institute, Russian Academy of Sciences (St. Petersburg, Russia).

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Supplementary material

Table S1. Estimates of evolutionary divergence for Trichostomatia representatives.