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Intestinal ciliates (Ciliophora) from the wild plains zebra (*Equus quagga*) in South Africa, with notes on the microtubule cytoskeleton organisation

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Abstract

Investigation of the ciliate communities from the digestive tract of different wild vertebrates is important in context of host-specificity of different ciliate species and the detection of any cases of non-specific infection. Here we present a description and analysis of the fauna of ciliates (Litostomatea, Trichostomatia) inhabiting the intestine of the wild plains zebra (*Equus quagga* Boddaert, 1785) in South Africa. Nineteen species belonging to 12 genera of five families were found. Five species were specific to *Equus quagga*; one was also found in *Equus zebra*; 29 are common to different equids; and one had been previously described from rhinoceros. For the first time, we used immunofluorescent staining to investigate microtubule cytoskeletons in trichostomatids. We found that this staining method is useful for the identification of trichostomatids.

Keywords: *Equus quagga*; Host-ciliate specificity; Microtubule cytoskeleton; Plains zebra; Trichostomatia

Introduction

Ciliates are found in various parts of the digestive tract of many herbivorous mammals such as ungulates, primates, elephants (Williams and Coleman 1991). It was previously generally accepted that the endobiotic ciliate fauna of mammals is characterized by significant specificity in relation to the host; however, there are many facts which contradict that suggestion. For example, *Opisthotrichum janus* (Dogiel, 1923) Dogiel, 1925 (Ophryoscolecidae), which had been regarded as specific to African antelopes, was recently found in Virunga mountain gorillas *Gorilla beringei beringei* (Ito et al. 2020). Six species of the family Ophryoscolecidae were detected in the intestine of capybaras kept in the zoo, and those rumen ciliates replaced capybara-specific hindgut endobionts (Dehority 1987). The horse-specific hindgut ciliate *Cycloposthium edentatum* Strelkow, 1928 was found in the foregut of 39% of examined wild black-striped wallaby *Macropus dorsalis* in Australia, and at the same time, no other ciliates non-specific to marsupials were detected in 263 animals belonging to another 20 macropodid species (Cameron et al. 2000).

Thus, the question of specific and non-specific ciliates in the digestive tract of vertebrate hosts, the type of interactions, and possible routes of infection remains open. In this context, it is important to investigate ciliate communities from the intestine of different mammalian hosts in nature, zoos, and biosphere reserves to determine the rate of

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specificity of different ciliate species and to detect events of non-specific infection. Ciliates from the digestive tract of the wild plains zebra *Equus quagga boehmi* from Kenya were collected in nature only once by Dogiel and Sokolow (1916) and investigated by Strelkow (1931). According to these data, some species of ciliates from the plains zebra were common to other equids, and some were described for the first time as unique to zebras only. Later, investigations of the fauna of intestinal ciliates from another subspecies of plains zebra, *Equus quagga chapmani,* and from *Equus grevyi* were made in different zoos and biosphere reserves in Europe and Asia (Kornilova 2003, 2006). The ciliates from the wild mountain zebra *Equus zebra* in Western Cape, South Africa, were also investigated (Kornilova et al. 2020).

In 2019, one of the authors visited South Africa and had the unique possibility to collect some fecal samples from wild zebras in nature. We decided to juxtapose the list of endobiotic ciliate species from the wild *Equus quagga,* which had been obtained by Strelkow (1931) in Kenya, with both material from the wild *E. quagga* from South Africa and the information regarding ciliates from the digestive tract of the wild mountain zebra *E. zebra* from South Africa. This gave us the opportunity to compare the faunas of closely-related species of hosts in their natural habitat. In this paper, we present a description and analysis of the fauna of endobiotic ciliates (Litostomatea, Trichostomatia) inhabiting the intestine of the wild plains zebra (*Equus quagga* Boddaert, 1785) in South Africa. We also note the use of immunofluorescent staining to investigate peculiarities of microtubule cytoskeleton organization in some small trichostomatids, predominantly species of the family Buetschliidae Poche, 1913. Our results suggest that this method can be very useful to distinguish the taxonomic position of trichostomatids.

Materials and Methods

In July 2019, some samples of feces were collected from three wild plains zebras (*E. quagga burchellii*) inhabiting Naval Hill Franklin Nature Reserve (S 29°5.923′ E 26° 14.129′) in Free State, South Africa. The behaviour of grazing zebras was observed in order to take fresh samples of feces from different individuals. Samples were immediately fixed with 96% ethanol within 5 min after defecation to prevent the destruction of ciliates. Ciliate species and genera were identified and classified mainly based on the descriptions of Gassovsky (1919), Hsiung (1930), Strelkow (1931, 1939), Van Hoven et al. (1998), and Lynn (2008).

Ciliates were stained by methyl green 1% solution in 1% acetic acid and by Lugol's iodine. We used an optical microscope MBI-11 (LOMO) and an optical inverted microscope Altami INVERT-3 (Altami) with ocular micrometer for the preliminary examination of the samples. Ciliates were observed and photographed on the glass object slides using a Leica DM 2500 equipped with differential interference contrast (DIC). Digital camera Leica DFC495 (8.0 megapixel) was used for taking microphotographs. The total number of ciliates was counted on slides in 1 ml of liquid. Because of the very low abundance, we used the following presentation of density results: +++, more than 10 cells per ml; ++, 2–10 cells per ml; +, single specimen. This type of presentation also facilitates the comparison of our data with other information about the ciliate fauna from zebras. The prevalence and the relative abundance of different ciliate species were not estimated because samples from only three zebras were taken into account.

For the purpose of immunofluorescent staining and microscopy, 50 μl of sample were put on the slides with polylizine cover and dried. Then, slides were put into ice-cold methanol for 30 min. After that, specimens were washed in PBS thrice for 5 min; treated with 1% Triton X-100 for 20 min; washed in PBS thrice; and blocked with 1% BSA for 10 min. Then, 20 μl of primary antibodies (monoclonal anti-α-tubulin antibodies produced in mouse (T5168, Sigma-Aldrich, USA) diluted with PBS 1:500) were added to the slides, which were then incubated at +4° C overnight. Next, the specimens were washed thrice in PBS; 20 μl of secondary antibodies anti-mouse IgG (whole molecule) –TRITC antibody produced in goat Sigma-Aldrich T5393 (diluted with PBS 1:100) were added; and the specimens were incubated in the dark at room temperature for 1.5 h. The preparations were then washed thrice in PBS and embedded into glycerol with addition of DAPI (1351303, Bio-Rad, USA) (2 μg/ml). Slides were viewed under a Leica DM2500 microscope with a fluorescent module with the use of filter cube B/G/R, N2.1, and I3 (Leica-Microsystems, Wetzlar, Germany).

Results and Discussion

We found 19 species of ciliates belonging to 12 genera of five families of the Trichostomatia Bütschli, 1889 (Litostomatea Small and Lynn, 1981) (Table 1, Fig. 1a–v, 2a–j). The largest number of species was found in the feces of zebra № 2. Only in that specimen the species *Bundleia dolichosoma, Holophryoides macrotricha, Triadinium elongatum,* and *Spirodinium equi* were detected. The species *Blepharosphaera ceratotherii, Bundleia nana, B. postciliata, Blepharocorys curvigula, Bl. angusta, Triadinium caudatum, Cochliatoxum periachtum,* and *Trifascicularia cycloposthium* were distinguished from all three zebras. Fig. 2.

The species composition was quite similar to that which had been described by Strelkow (1931). The species *Ditoxum hamulus*, *Spirodinium nanum,* and *Trifascicularia cycloposthium* were found among ciliates specific to the zebra only. It was interesting that *Trifascicularia cycloposthium* was common to wild plains zebras in nature, but it was detected only once in zoos in the feces of the young

Table 1. Occurrence of species of intestinal ciliates of zebras at various locations in nature and in captivity.[a]

№	Family/genus/species/morphotype	A	B	C	D	E	F	G	H[b]	I[b]
	Buetschliidae Poche, 1913									
	Alloiozona Hsiung, 1930									
1	*trizona* Hsiung, 1930				+					
	Blepharoconus Gassovsky, 1919									
2	sp.				+					
	Blepharoprosthium Bundle, 1895									
3	*pireum* Bundle, 1895				+					
	Blepharosphaera Bundle, 1895									
4	*ceratotherii* Van Hoven et al., 1998	+	+	+	+					
5	*intestinalis* Bundle, 1895				+	+				
	Bundleia Cunha & Muniz, 1928									
	subgen. *Bundleia* Strelkow, 1939									
6	*nana* Strelkow, 1939	++	++	++					+	
7	*piriformis* Strelkow, 1939				++				+	
8	*postciliata* (Bundle, 1895)	++	++	++	++	+			+	
	subgen. *Fibrillobundleia* Strelkow, 1939									
9	*benbrooki* Hsiung, 1930				+					
10	*dolichosoma* Strelkow, 1939		+							
11	*inflata* Strelkow, 1939	++	++	++	++				+	
	Holophryoides Gassovsky, 1919									
12	*macrotricha* Strelkow, 1939		+		+					
13	*ovalis* (Fiorentini, 1890)				+					
	Paraisotrichidae da Cunha, 1917									
	Paraisotricha Fiorentini, 1890									
14	*minuta* Hsiung, 1930		+	+						
	Blepharocorythidae Hsiung, 1929									
	Blepharocorys Bundle, 1895									
15	*angusta* Gassovsky, 1919					+	+	+	+++	++
	angusta m. triangulata				++					
	angusta m. ovata	+	+	+	++					
16	*cardionucleata* Hsiung, 1930								+	
17	*curvigula* Gassovsky, 1919	+++	+++	+++		+	+	+	++	+++
18	*jubata* Bundle, 1895					+			+	
19	*microcorys* Gassovsky, 1919						+		++	++
	Charonnautes Strelkow, 1939									
20	*equi* (Hsiung, 1930)								+	
	Circodinium Wolska, 1971									
21	*minimum* (Gassovsky, 1919)								+	++
	Ochoterenaia Chavarria, 1933									
22	*appendiculata* Chavarria, 1933								+	
	Cycloposthiidae Poche, 1913									
	Cycloposthium Bundle, 1895									

23	*bipalmatum* (Fiorentini, 1890)								++	
24	*edentatum* Strelkow, 1928							+	+	
	Trifascicularia Strelkow, 1931									
25	*cycloposthium* Strelkow, 1931[c]	+	+	++		+				
	Tripalmaria Gassovsky, 1919									
26	*dogieli zebrae* Strelkow, 1931[c]					+				
	Triplumaria Hoare, 1937									
27	sp. "A"				++					
28	sp. "B"				++					
	Spirodiniidae Strelkow, 1939									
	Cochliatoxum Gassovsky, 1919									
29	*periachtum* Gassovsky, 1919	+	++	+		+			+	+
	Ditoxum Gassovsky, 1919									
30	*brevinucleatum* Strelkow, 1931	+	++			+				
31	*hamulus* Strelkow, 1931[c]	+	++			+				
	Gassovskiella Grain, 1994									
32	*galea* (Gassovsky, 1919)		+	+		+			+	+
	Spirodinium Fiorentini, 1890									
33	*confusum* Hsiung, 1935						+		+	+
34	*equi* Fiorentini, 1890		+							+
35	*ferrumequinum* Strelkow, 1931[c]					+				
36	*nanum* Strelkow, 1931[c]	+	++		++	+				
	Tetratoxum Gassovsky, 1919									
37	*excavatum* Hsiung, 1930								+	+
38	*parvum* Hsiung, 1930								+	
39	*unifasciculatum* Fiorentini, 1890		++	++						
	Triadinium Fiorentini, 1890									
40	*caudatum* Fiorentini, 1890	+	++	++		+			+	
41	*elongatum* Strelkow, 1931[c]		+			+				
	Allantosomatidae Jankowski, 1967									
	Allantosoma Gassovsky, 1919									
42	*cucumis* Strelkow, 1939								+	
43	*intestinalis* Gassovsky, 1919					+			+	
	Arcosoma Jankowski, 1967									
44	*lineare* (Strelkow, 1939)								+	
	Total	12	19	12	13	16	4	3	23	9

[a] Abbreviations: A, *Equus quagga burchellii* № 1; B, *E. q. burchellii* № 2; C, *E. q. burchellii* № 3, Naval Hill Franklin Nature Reserve, South Africa; D, *E. zebra,* Gourikwa Natural Reserve, South Africa (Kornilova et al. 2020); E, *E. q. boehmi*, Kenya (Strelkow 1931, no density data); F, *E. q. chapmani*, Almaty Zoo, Kazakhstan (Kornilova 2003); G, *E. q. chapmani*, Leningrad Zoo, Russia (Kornilova 2003); H, *E. q. chapmani*, Askania-Nova biosphere reserve, Ukraine (Kornilova 2003); I, *E. grevyi*, Askania-Nova, biosphere reserve, Ukraine (Kornilova 2003); m, morphotype; +++, more than 10 cells/ml; ++, 2–10 cells/ml; +, 1 cell/ml or no abundance provided.

[b] The percentage occurrence of ciliates has been converted for comparison purposes: +++ (more than 20%); ++ (10–20%); + (less than 10%).

[c] Found in zebras only

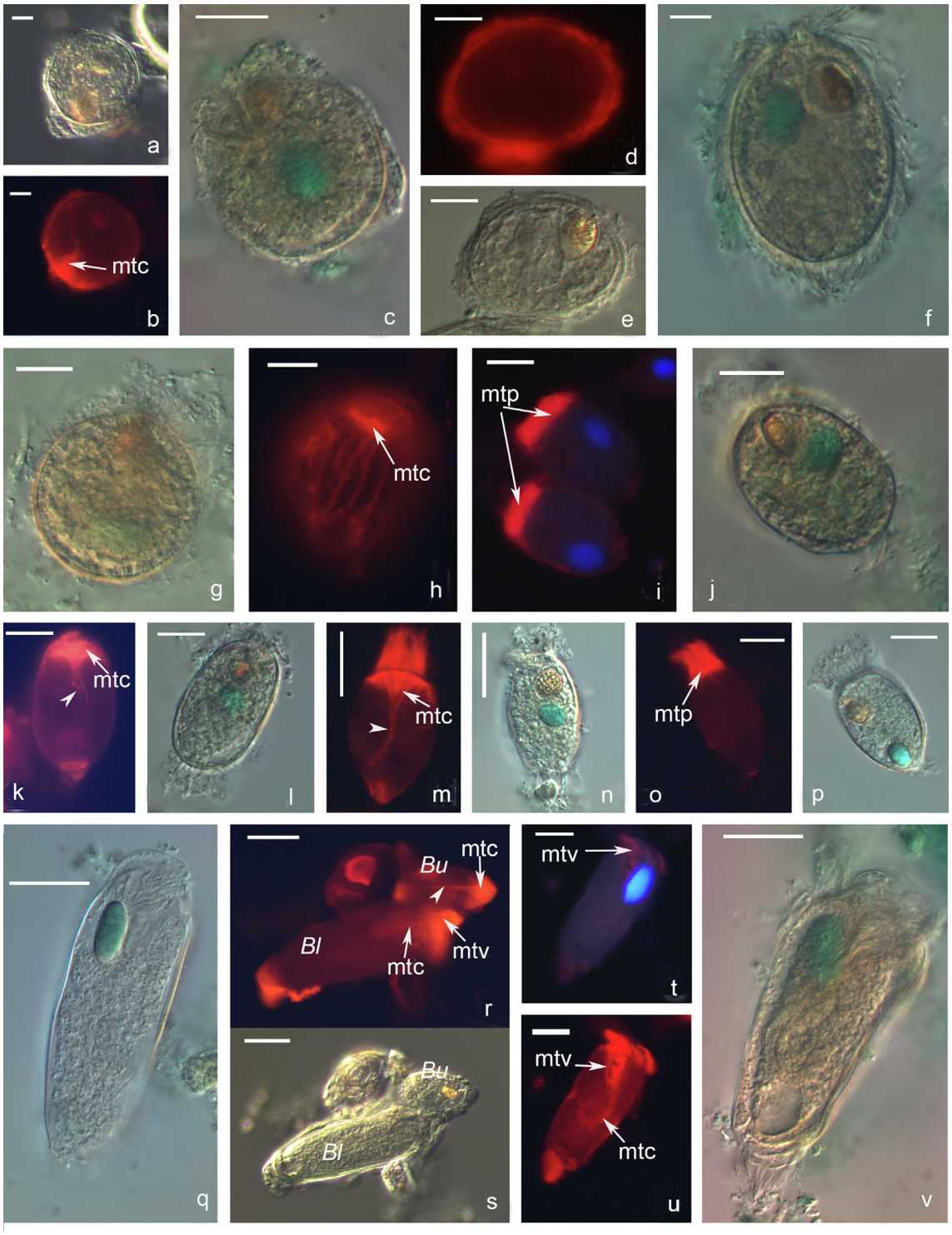

Fig. 1. a–v. Intestinal ciliates from *Equus quagga burchellii* (a and b, d and e, r and s, t and u, same individuals; a, c, e, f, g, j, l, n, p, q, s, v, differential interference contrast; b, d, h, k, m, o, r, u, immunofluorescent staining of microtubule cytoskeleton; i, t, immunofluorescent staining and DAPI staining). a–c: *Blepharosphaera ceratotherii*. d–f: *Holophryoides macrotricha.* g, h: *Paraisotricha minuta.* i, j: *Bundleia postciliata.* k, l: *B. inflata.* m, n: *B. dolichosoma.* o, p: *B. nana.* q: *Blepharocorys angusta* m. *ovata.* r, s: *Bl. angusta* m. *ovata*, *B. inflata.* t–v: *Bl. curvigula.* mtc – microtubules around cytopharynx, mtv – microtubules around vestibulum, mtp – microtubular plate, arrowhead – microtubule cord, *Bl* - *Blepharocorys, Bu* - *Bundleia*. Scale bars: a–p, 10 μm; q–v, 20 μm.

male *Equus quagga* in Kaliningrad Zoo (Kornilova 2006). The species *Spirodinium ferrumequinum* and *Tripalmaria dogieli zebrae,* which were specific to plains zebras according Strelkow (1931), were not found. Also, no representa-

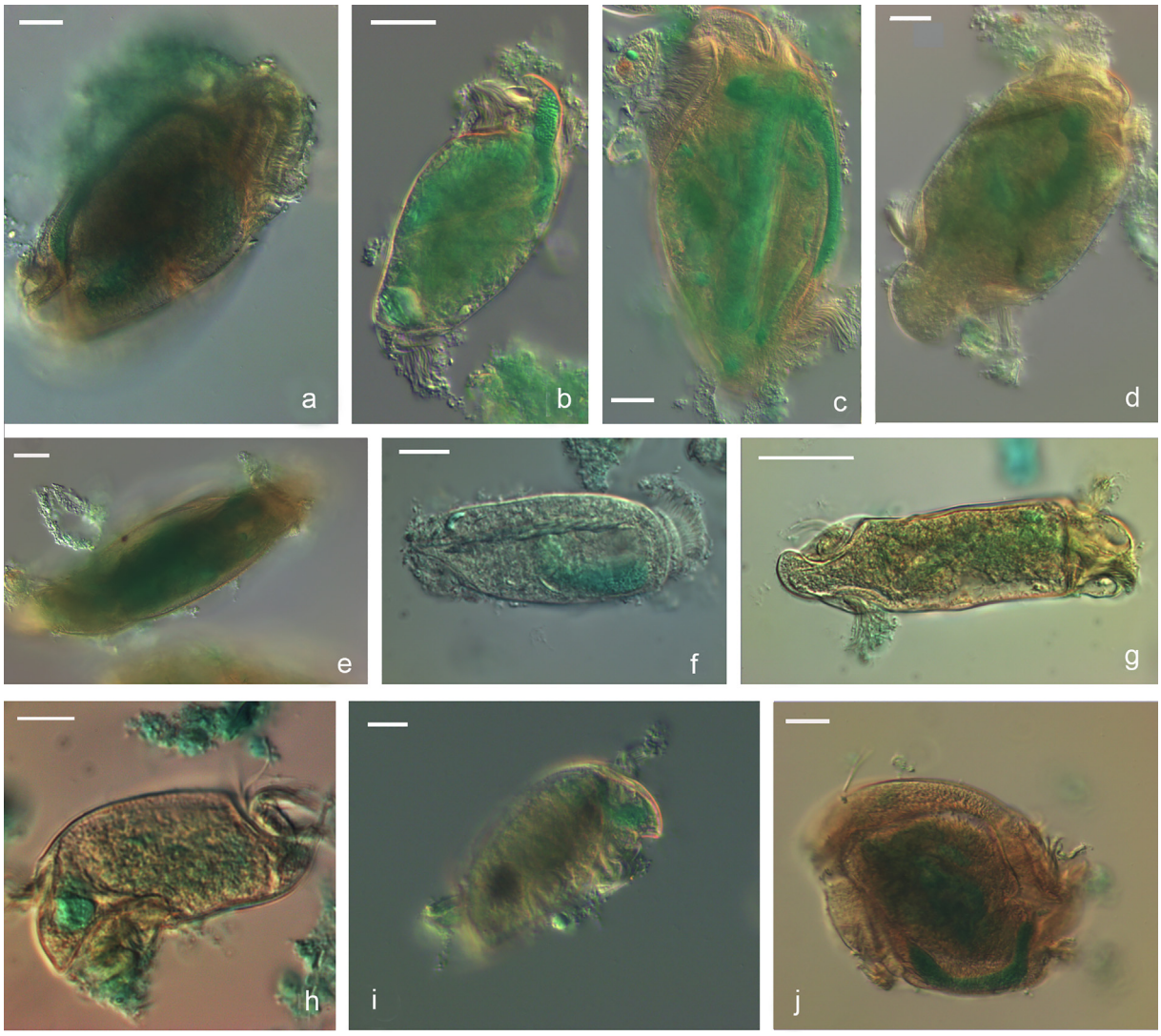

Fig. 2. a–j. Intestinal ciliates from *Equus quagga burchellii* (differential interference contrast). a: *Cochliatoxum periachtum*. b: *Ditoxum brevinucleatum*. c: *D. hamulus*. d: *Tetratoxum unifasciculatum*. e: *Spirodinium equi*. f: *S. nanum*. g: *Trifascicularia cycloposthium*. h: *Triadinium caudatum*. i: *T. elongatum*. j: *Gassovskiella galea*. Scale bars: a–e, j, 20 μm; f–i, 10 μm.

tives of allantosomatids were registered. At the same time, *Tetratoxum unifasciculatum, Bundleia dolichosoma,* and *Paraisotricha minuta* were detected, none of which had ever been found in zebras before. We identified the species of the genus *Blepharosphaera* from plains zebras as *B. ceratotherii* according to its dimensions, because the size of *B. ceratotherii* had been a main morphological feature used for separating it from *Blepharosphaera intestinalis* by Van Hoven et al. (1998). *Blepharosphaera ceratotherii* was originally found in the white rhinoceros *Ceratotherium simum* in South Africa, but it was later also recorded in mountain zebras in South Africa (Kornilova et al. 2020). It should be noted, however, that many trichostomatids are highly variable in size, so some questions arise about the real taxonomic position of *Blepharosphaera* species from different hosts. To date, this problem cannot be resolved because of the low number of studies.

According to our results, the total number of ciliate species from zebras' intestine increases to 44. In the list of ciliates from all analyzed zebras, 34 species are common to equids, one has been also found in rhinoceros, and nine are specific to zebras. Among the zebra-specific species, five are specific to *Equus quagga*, three are specific to *E. zebra,* and one has been found both in *E. quagga* and *E. zebra*. At present, most species of endobiotic ciliates specific to zebras were found in hosts in their natural habitats but not in zebras kept in zoos and biosphere reserves. We suppose that this could be due to the limited number of contacts between different zebras in captivity. It is interesting that the species composition of ciliates from wild plains zebras is quite different from that of wild cape mountain zebras from nearby regions of Africa. For example, no representatives of the genera *Ditoxum, Tetratoxum,* or *Triadinium* were found in fecal samples of cape mountain zebras, nor

were there any *Cochliatoxum periachtum* or *Gassovskiella galea*. *Trifascicularia cycloposthium,* which had been considered specific to zebras' ciliate fauna (Kornilova 2006), was also not found. On the contrary, two *Triplumaria* species, unusual for equids, were found in fecal samples of the wild cape mountain zebras. Some similarities in the species composition of the intestinal ciliates from these two zebra species were observed, mainly for the small representatives of the family Buetschliidae.

For the first time, we used immunofluorescent staining to investigate the microtubule cytoskeleton in trichostomatids. Overall, our data are consistent with the existing hypotheses about the organisation of the tubuline structures in different representatives of these ciliates (Gurelli and Gocmen, 2015; Strelkow 1939; Wolska 1964, 1965, 1971). The somatic ciliature (ciliary zones), as well as perivestibular microtubules were perfectly detected (Fig. 1b, d, h, i, k, m, o, r, t, u). Interestingly, in the studied species of the genus *Bundleia,* a plate of fluorescent material with a thickness of 3–5 μm was found at the anterior end of the cell, directly under the cilia (Fig. 1i, k, m, o). In representatives of the subgenus *B.* (*Fibrillobundleia),* microtubules surrounding the cytopharynx were detected inside that plate (Fig. 1k, m). Those microtubules formed a cone and apparently are arranged in two layers, between which there was a cavity. From the top of the cone deep into the cell, a thin fibrillar cord extended, which could sometimes reach the posterior end of the cell (Fig. 1k, m, r). These features of the organisation of the cytoskeleton of these species of trichostomatids have not been previously described.

We suggest that immunofluorescent staining of microtubular cytoskeleton can be very useful to describe a fauna of small endobiotic ciliates from different hosts. This method allows one to distinguish single cells of some species in the sample, to determine their taxonomic position, and makes counting of such ciliates easier. Immunofluorescent staining also allows the discovery of some cytoskeleton structures which cannot be detected by the silver impregnation method.

CRediT authorship contribution statement

Olga A. Kornilova: Conceptualization, Investigation, Supervision, Writing – review & editing. **Anton V. Radaev:** Visualization, Writing – original draft. **Ludmila V. Chistyakova:** Investigation, Methodology, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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