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Ciliates from the faeces of the free-ranging dromedary from Oman: Morphology and molecular phylogeny

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ABSTRACT

Ciliates *Infundibulorium cameli* from the faeces of the free-ranging dromedary from Oman were studied using a set of methods of the light and immunofluorescence microscopy and molecular phylogeny. With the use of molecular genetic methods, it was confirmed that the cysts found in the samples simultaneously with trophozoites actually belong to the species *I. cameli.* Tubulin cytoskeleton organization of trophozoites and cysts of this species were described for the first time. A striking morphological similarity between species *I. cameli* and *Buxtonella sulcata* was demonstrated, including the organization of ciliature. Different isolates of *I. cameli* and *B. sulcata* formed a common clade on the phylogenetic tree. The level of evolutionary divergence between the 18 S rRNA sequences of *I. cameli, B. sulcata* and species closest to them according to the results of molecular phylogenetic analysis was estimated. It was demonstrated that the divergence between *I. cameli* and *B. sulcata* is extremely low compared to members of other genera included in the analysis. Taxonomic position of *I. cameli* and *B. sulcata* was discussed in according to the data of comparative morphology and molecular phylogeny

1. Introduction

Ciliates of the digestive tract of ruminants and camels are localized mainly in the foregut fermentation chambers, forming complex communities there, characterized by rich species diversity (Newbold et al., 2015; Vdachny, 2018). In the intestines and faeces of these herbivorous mammals, mainly *Balantioides coli* and *Buxtonella sulcata*, as well as *Infundibulorium cameli* are found. It should be noted that species *B. sulcata* and *I. cameli* are very similar in their structure; their characteristic feature, in contrast to *B. coli*, is the presence of a groove located along the longitudinal axis of the cell (Bozhenko, 1925; Jameson, 1926). In literature it has often been suggested that these two species should be united within the genus *Infundibulorium*. This was first pointed out by Dogiel (1934), who wrote: «Although *Buxtonella* has been found in the cattle and *Infundibulorium* in the camel, the data for both ciliates almost completely coincide. The general body shape, the characteristic cap-shaped protrusion of the oral end, the position of the mouth and an adjacent deep indentation, which Bozhenko designated as the anus, are identical in both species. Both species also have an oblique longitudinal groove on the body. The dimensions, as well as the direction of movement (with the aboral end facing forward) also coincide. Since no noticeable differences were noted between the two species, the name *Buxtonella* should be deleted, replacing it with the name *Infundibulorium* Bozhenko». Lubinsky had a similar point of view, however, he pointed out a need for a more detailed research of morphology for those two species of ciliates, so as to make a definitive conclusion on their systematic position (Lubinsky, 1957).

In his review, Grain directly points out the necessity of the merge of *I. cameli* and *B. sulcata* into a single genus (Grain, 1994). Jankowsky does the same in his ciliate taxonomy overview (Jankowsky, 2007). In spite of this, a formal taxonomic revision has never been made, and the species name *Buxtonella sulcata* is still widely used in scientific literature to this day. This could be explained by very little information about the structure of *I. cameli*; in fact, our knowledge of this species is limited by its first description. At the same time, information about *B. sulcata* in the literature is quite numerous. In addition to light microscopical data, the results of studying those ciliates using TEM and SEM were published, and the species position on the molecular phylogenetic tree was

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determined using the 18S rRNA gene sequence (Pomajbikova et al., 2013; Grim et al., 2015).

Recently, we proposed again to combine the species *B. sulcata* and *I. cameli* into one genus in brief report, based on the results of complex investigation of the ciliates from the faeces of free-ranging dromedaries in Oman (Chistyakova et al. 2023). Esteban-Sanchez et al., (2023) did the same, based on a study of ciliates from the faeces of dromedaries and bactrian camels from different zoos, mainly by the methods of molecular phylogeny. Importantly, these authors suggest to use the junior synonym of *Buxtonella* as a generic name due to the veterinary significance of *B. sulcata* (Esteban-Sanchez et al., 2023).

In this paper we present the results of investigations of ciliates from the faeces of free ranged dromedaries from Oman, obtained with the use of light and immunofluorescence microscopy, and methods of molecular phylogeny. We have carried out a detailed analysis of the available data on the structure of the species *I. cameli* and *B. sulcata*, including the peculiarities of the somatic cilia distribution. The position of these ciliates on the molecular phylogenetic tree is determined based on the analysis of the obtained sequence of the 18S rRNA gene, and an assessment is made of the level of differences in this sequence between the species *I. cameli* (including isolates from different habitats) and *B. sulcata* and some related representatives of the Vestibuliferida group. The data obtained are discussed in the context of the need to unite the species *I. cameli* and *B. sulcata* within one genus.

2. Materials and methods

Samples of faeces of two dromedaries *Camelus dromedarius* were collected in January 2022 in Oman (sampling locations 17.051510, 54.610565 and 17.077714, 54.437061). Fresh faeces were placed in 50 ml Falcon plastic tubes and fixated by 96° ethanol in the ratio of 1:50. After the samples were delivered to the lab, ethanol was almost entirely changed. The samples were then contained in the dark at room temperature. Light microscopy examinations and microphotography were performed using Leica DM2500 microscope (Leica-Microsystems, Germany), equipped with a differential interference contrast and a Leica DFC495 (8.0MP) digital camera. During cell morphology examination the nuclei were detected with the use of a 0.1 % solution of methyl green in a 1 % solution of acetic acid. The quantity of ciliates was established using the method of a "calibrated drop" (Kornilova, 2004a). For the study of tubulin cytoskeleton organization immunofluorescence staining with antibodies against α-tubulin was employed. The staining technique was described in detail in our previous study (Chistyakova et al., 2020). Slides were viewed under a Leica DM2500 microscope with a fluorescence module with the use of filter cubes B/G/R, N2.1 and I3 (Leica-Microsystems, Wetzlar, Germany) and a Leica TCS SP5 laser confocal scanning microscope. The images were processed using ImageJ software.

For DNA extraction, the cells were picked one by one with the use of a Nikon SMZ 1270 stereomicroscope (Nikon Corporation, Japan), then washed twice in distilled water and placed into tubes with a lysis buffer (45–50 cells a tube). Cysts detected in the samples were collected separately in the same manner. Genome DNA was extracted using PureLink Genomic DNA Kit (Invitrogen) for DNA extraction according to the manufacturer's instructions.

The ~ 1600 bp SSU rRNA gene fragment was amplified using the following primers: forward primer 82F (5′-GAAACTGCGAATGGCTC-3′; Elwood et al., 1985) and reverse primer EkyB (5′-TGATCCTTCTGCAGGTTCACCTAC-3′; Medlin et al., 1988) as in the protocol previously described by Ito et al. (Ito et al., 2014). The PCR products were purified using Cleanup S-Cap kit (Evrogen). Sanger sequencing was performed using 3 primers: amplification primer 82F, Jap2F (5′-TTTGCCAAGGATGTTTTC-3′ Ito et al., 2014) and 18S 5R Jap1R (5′-CTTGGCAAATGCTTTCGC-3′; Giribet et al., 1996). Obtained sequences were used for further phylogenetic analysis. GenBank accession numbers for the new SSU rRNA gene sequences determined in this work are OQ696201- OQ696205.

For phylogenetic analysis, we combined the obtained sequences with sequences previously deposited in the GenBank database (the accession numbers of the sequences used are listed in the Supplement 1). Alignment of 77 sequences of the SSU rRNA gene with a length of 1601 nucleotide sites was performed in MEGA X using MUSCLE under the default parameters (Kumar et al., 2018) (Supplement 2). The maximum likelihood tree reconstruction was performed in IQ-TREE v.1.6 (Nguyen et al., 2015) with the best evolutionary model (GTR + F + I + G4) selected using Bayesian information criterion by the built-in ModelFinder (Kalyaanamoorthy et al., 2017). Branch support was estimated using the ultrafast bootstrap method (10 000 replicates) (Hoang et al., 2018). Bayesian inference was accomplished in MrBayes v.3.2.7 under GTR + I + G model, with analysis run for 2,000,000 generations, trees sampled every 1000 generations and other parameters left in default states (Ronquist et al., 2003).

Estimates of evolutionary divergence (p-distance) between Vestibuliferida species and within species *I. cameli* (9 sequences), *Buxtonella sulcata* (8 sequences), *Buxtonella*-like sp. (9 sequences), *Balantioides coli* (8 sequences) and *Balantidium entozoon* (2 sequences) (Supplement 1) was performed in MEGA X (Kumar et al., 2018). 42 nucleotide sequences were involved in the analysis of the evolutionary divergence between species and 36 nucleotide sequences – within species (Supplement 3). There were a total of 892 positions in the final dataset. All positions with less than 95 % site coverage were eliminated, i.e., fewer than 5 % alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). The rate variation among sites was modeled with a gamma distribution (shape parameter = 5).

3. Results

In the samples of faeces of two dromedaries we found ciliates which were identified as *Infundibulorium cameli* Bozhenko, 1925 according to a variety of features. Cells are oval in shape or slightly pointed at the oral (posterior) end (Fig. 1). Their morphometric characteristics are given in Table 1. The cells are evenly covered by cilia, excluding the groove, which extends from aboral to oral end in a shape of a spiral (Fig. 2). The ridges which limiting the groove on the oral end of the cell form a loop where the peristome is located. Cytoproct is situated beyond the groove, close to the peristome (Fig. 2). The distance between each row of cilia is almost the same. On side of the cell opposite from the groove the rows of cilia continue parallel to each other along the longitudinal axis. Relative to the groove, the rows of cilia on one side are parallel to it, while on the other (where the cytoproct is) they remain at an angle (Figs. 2, 3). Cytopharynx is armed with bundles of microtubules that reach the center of the cell (Fig. 2). Microtubules which lie beneath the groove can be visualized by immunofluorescence staining (Fig. 2). Nemadesmata are well-noticeable by the cell surface, especially at the anterior (aboral) side. Cytoplasm is dense and granulated. Occasionally, 1–2 contractile vacuoles can be seen (Fig. 1). Macronucleus is oval in shape, and micronucleus adjoins it. In some cases, the micronucleus is located at an impression of the macronucleus (Fig. 1B).

In the samples, aside from trophozoites, we found cysts, possibly of *I. cameli*. Mature cysts are spherical and surrounded by a dense envelope (Fig. 4 A, B). Like in the trophozoites, the cytoplasm is dense and granulated, often detached from the envelope, with macro- and micronuclei visible. The results of the cysts morphometry are presented in Table 2. Presumably young cysts found in the samples are completely filled with the cytoplasm and have a less dense envelope (Fig. 4 C). A rounded "button" 4—5 μm in diameter can often be seen on their surface (Fig. 4 C). It probably corresponds to the fluorescent zone revealed in some cysts by immunofluorescence staining (Fig. 4 D). No other tubulin structures were found in the cysts.

We obtained three fragments of SSU rRNA for trophozoites of *I. cameli* and two fragments of this gene for its cysts; the fragment length ranged from 1537 to 1559 bp. The sequences were identical except for

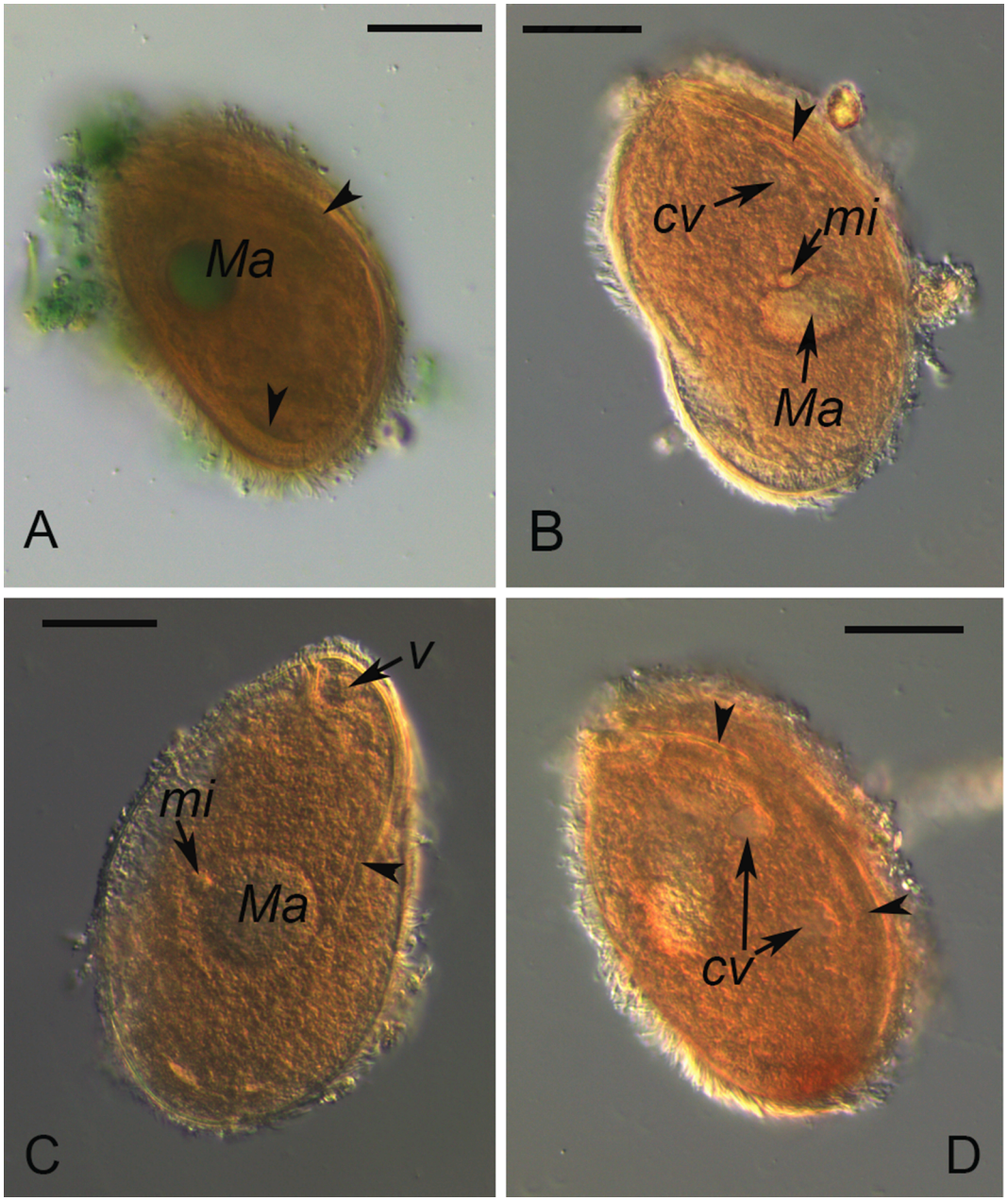

Fig. 1. Light microscopy of the *Infundibulorium cameli*. *Ma* – macronucleus, *mi* – micronucleus, *cv* – contractile vacuole, *v* – vestibulum, arrowhead – groove on the cell surface. DIC. Scale bars 20 μm.

Table 1
Morphometry of the ciliates: trophozoites and cysts; all dimensions are presented in μm.

	Trophozoites				Cysts			
	Min-Max	M	SD	SV	Min-Max	M	SD	SV
Cell length	60–166	117,2	4,9	4,2	59–110	77,5	2,3	2,9
Cell width	50–108	71,2	2,7	3,8	54–100	76,1	2,3	3,0
Macronucleus length	16,5–29	22,2	0,7	3,1	17–26	22,6	0,7	2,9
Macronucleus width	13,5–29	17,1	0,6	3,4	17–26	19,1	0,6	3,0
Micronucleus diameter	4,9–5,1	4,9	0	0	4,9–5,1	4,9	0	0

five loci that showed double peaks on the chromatograms. Two of these loci were detected in all the five voucher specimens, while two others were detected only in two specimens. BLAST analysis in GenBank showed that the sequences of *B. sulcata* were the closest to the sequences obtained in our study. The identity of the sequences of *I. cameli* and those of *B. sulcata* made up 99,03–99,29 %, while that of the five sequences of *B. sulcata* available in GenBank was 99,74–99,93 %. The difference between *I. cameli* and *B. sulcata* in the SSU rRNA gene fragment examined in our study was in six positions, three of which were possibly associated with the intra-species polymorphism of the latter species.

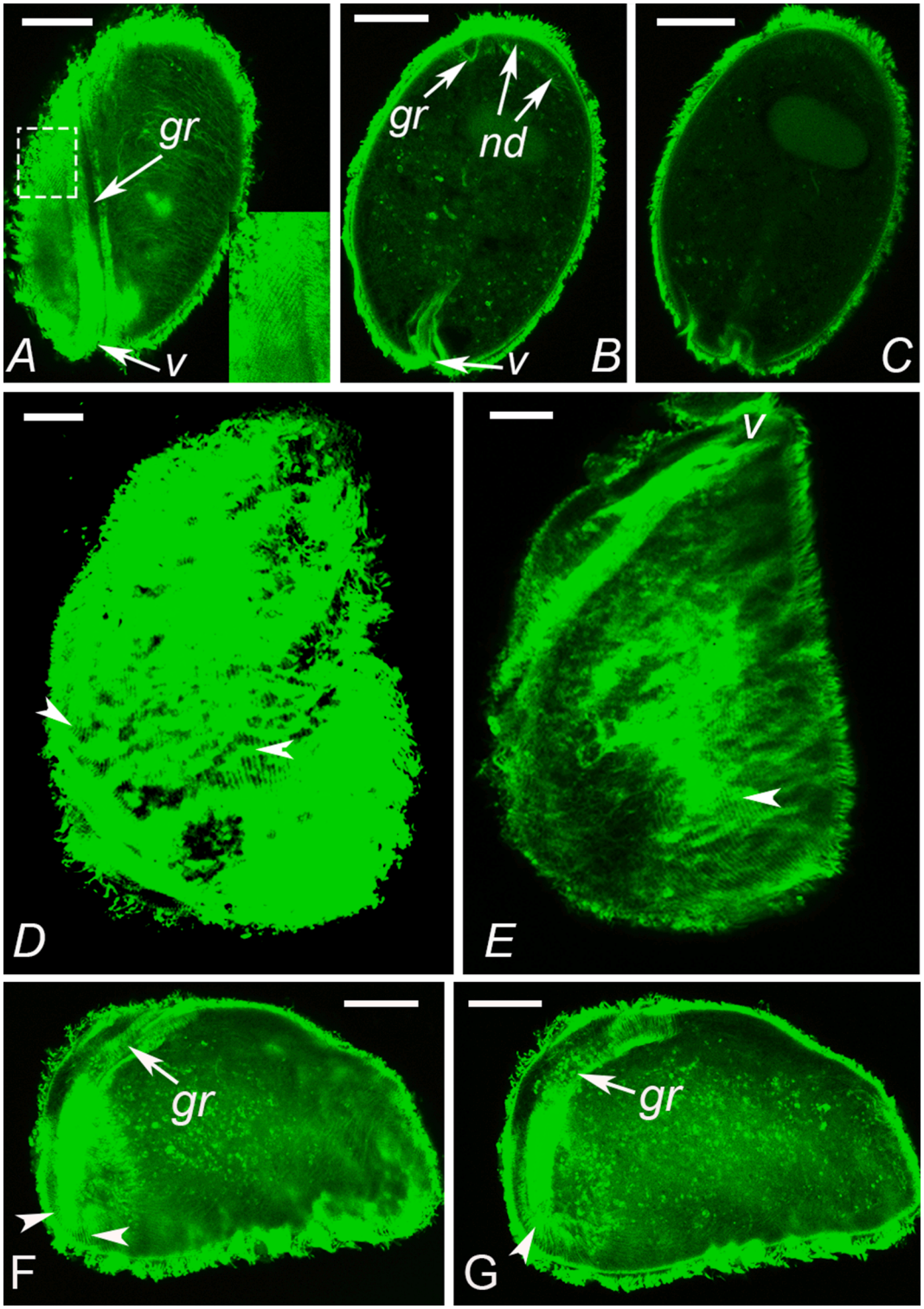

Fig. 2. Immunofluorescence microscopy of the *Infundibulorium cameli*. *v* – vestibulum, *cp* – cytoproct, *gr* – groove on the cell surface, *nd* – nemadesmata, arrowheads – rows of the cilia. A – C – the same cell. D – G – the same cell. D – the front side, E – the back side of the cell. Confocal microscope. Scale bars 20 μm.

The voucher specimens of *I. cameli* formed a common clade on the phylogenetic tree (the analysis was based on the sequences of the trophozoites and the cysts) (Fig. 5). This clade was sister to *B. sulcata*. Maximum likelihood and Bayesian analyses demonstrated good support of the branches of the phylogenetic trees.

We estimated the level of evolutionary divergence between the 18 S rRNA sequences of *I. cameli*, *Buxtonella sulcata*, *Buxtonella*-like species, as well as species closest to them according to the results of molecular phylogenetic analysis (Table 2). The level of intraspecific differences could only be assessed for *I. cameli*, *B. sulcata*, *Buxtonella*-like sp. and *Balantioides coli*, due to the lack of data for other species included in the analysis (Table 3). On average, the level of intraspecific differences for *I. cameli*, *B. sulcata*, and *Buxtonella*-like sp. varied slightly (0,0010–0,0019), while it was 3 times higher for *B. coli* (Table 3). The level of evolutionary divergence between different species of the same genus included in the analysis was 0,0079 for *Paraisotricha colpoidea* and

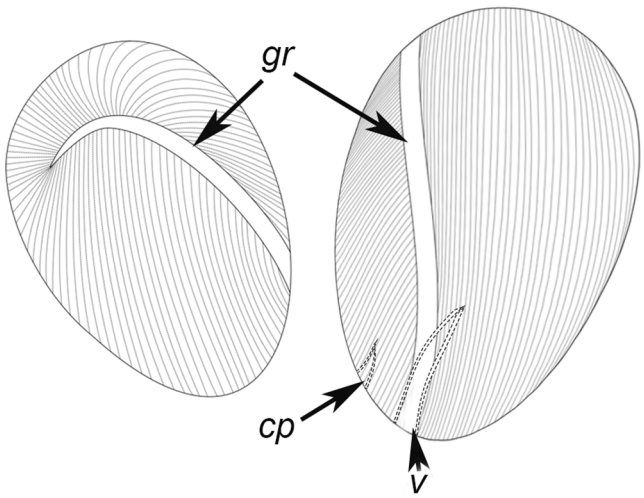

Fig. 3. Scheme of disposition of *Infundibulorium cameli* ciliature. The number of cilia rows per cell is about 160–170. *v* – vestibulum, *cp* – cytoproct, *gr* – groove on the cell surface.

P. minuta, 0,0185 for *Balantidium entozoon* and *B. duodeni,* and 0,0236 for *Latteuria polyfaria* and *L. media* (Table 2). The level of evolutionary divergence between *I. cameli/Buxtonella*-like sp. and *B. sulcata/Buxtonella*-like sp is relatively high, it is 0,0342 and 0,039 respectively (Table 2). At the same time, the level of evolutionary divergence between *I. cameli/B. sulcata* was significantly lower and amounted 0,0064. However, these values of evolutionary divergence between groups *I. cameli/B. sulcata* were higher than the level of evolutionary divergence within group *B. coli* (Table 2, 3). The level of evolutionary divergence between members of other genera included in the analysis ranged from 0.0168 between *L. media* and *Helicozoster indicus* to 0.0777 between *B. entozoon* and *P. colpoidea* (Table 2).

4. Discussion

Ciliates of the species *Infundibulorium cameli*, according to Bozhenko (Bozhenko 1925), are ovoid in shape (Fig. 5 A), with average sizes of 80–90 μm (max 128) in length and 50–60 μm (max 100) in width. The entire body of the ciliate is evenly covered with cilia. At the posterior end is a funnel-shaped peristome ending in the pharynx; next to the peristome is the anus. Beginning at the peristome, the groove continues along the cell. The cytoplasm is granulated, with several vacuoles. The macronucleus is oval, close to it is the micronucleus. Thus, according to the complex of morphological features, as well as taking into account the

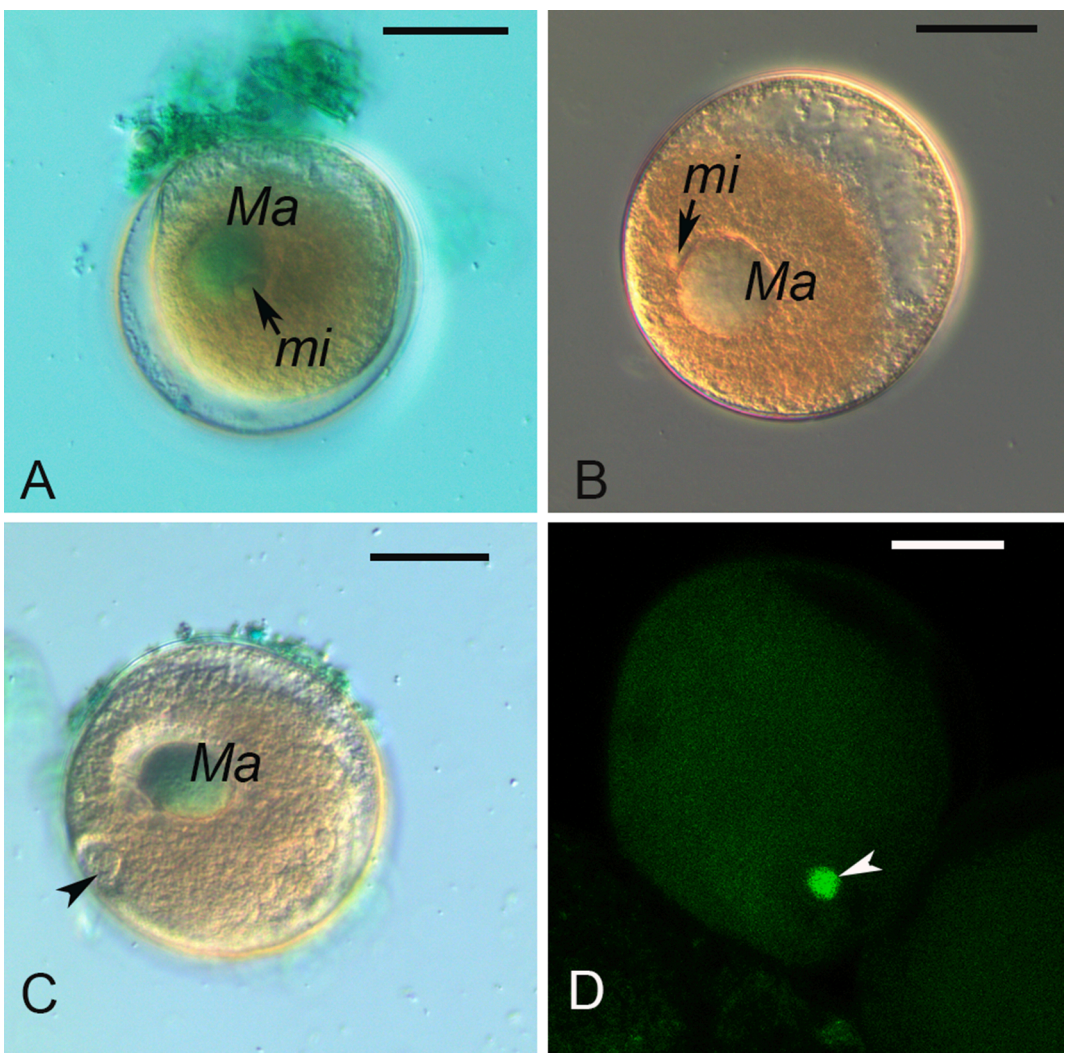

Fig. 4. Morphology of the cysts of *Infundibulorium cameli*. A – C – DIC, D - immunofluorescence microscopy. *Ma* – macronucleus, *mi* – micronucleus, arrowhead – "button" on the surface of the cyst. Scale bars 20 μm.

Table 2
Estimates of evolutionary divergence between 18 s rRNA sequences of Vestibuliferida species based on p-distances. Standard error estimates are shown above the diagonal.

	H. indicus	*L. polyfaria*	*L. media*	*P. colpoidea*	*P. minuta*	*I. cameli*	*B. sulcata*	*Buxtonella*-like sp.	*B. coli*	*B. entozoon*	*B. duodeni*
Helicozoster indicus		0,0049	0,0042	0,0082	0,0081	0,0071	0,0074	0,0071	0,0067	0,0072	0,0072
Latteuria polyfaria	0,0225		0,0051	0,0085	0,0088	0,0077	0,0079	0,0075	0,0075	0,0079	0,0078
Latteuria media	0,0168	0,0236		0,0080	0,0082	0,0073	0,0075	0,0074	0,0069	0,0075	0,0073
Paraisotricha colpoidea	0,0607	0,0665	0,0596		0,0030	0,0080	0,0080	0,0087	0,0082	0,0089	0,0086
Paraisotricha minuta	0,0619	0,0710	0,0630	0,0079		0,0081	0,0082	0,0089	0,0083	0,0087	0,0083
Infundibulorium cameli	0,0437	0,0528	0,0486	0,0578	0,0623		0,0024	0,0061	0,0053	0,0066	0,0066
Buxtonella sulcata	0,0477	0,0534	0,0500	0,0574	0,0620	0,0064		0,0065	0,0058	0,0068	0,0067
Buxtonella-like sp.	0,0461	0,0496	0,0506	0,0703	0,0725	0,0342	0,0390		0,0055	0,0064	0,0068
Balantioides coli	0,0429	0,0520	0,0466	0,0666	0,0666	0,0296	0,0341	0,0296		0,0058	0,0061
Balantidium entozoon	0,0539	0,0619	0,0600	0,0777	0,0766	0,0440	0,0470	0,0411	0,0345		0,0041
Balantidium duodeni	0,0494	0,0562	0,0527	0,0698	0,0676	0,0400	0,0409	0,0417	0,0344	0,0185	

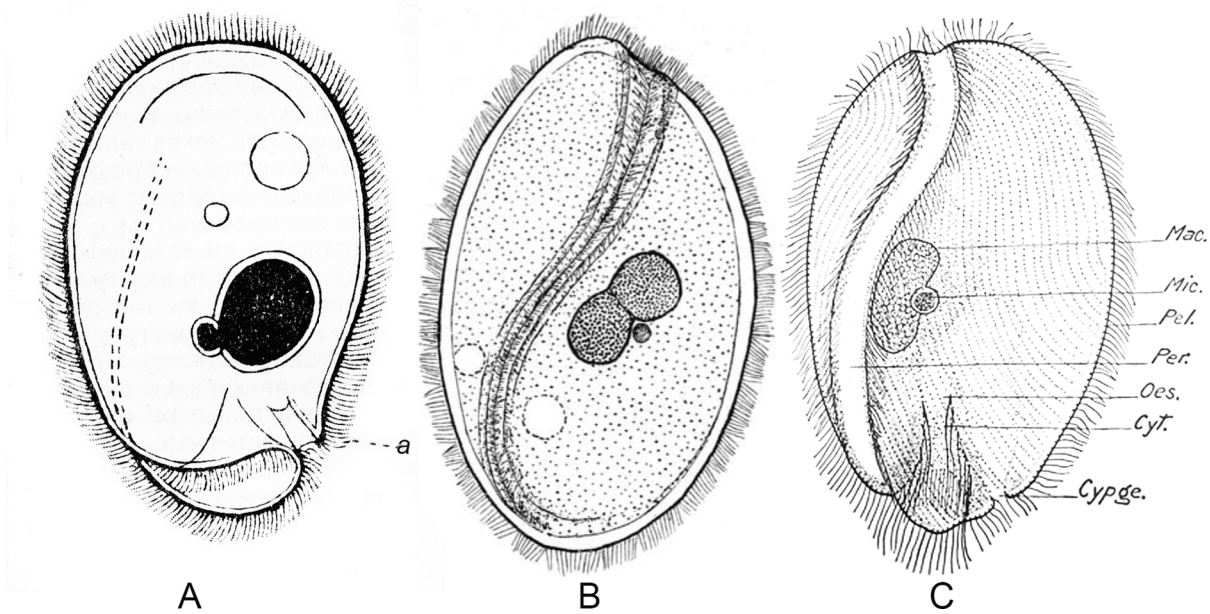

Fig. 5. Original images of *Infundibulorium cameli* (A - according to Bozhenko, 1925) and *Buxtonella sulcata* (according to B - Jameson, 1926 and C - Rees, 1930).

Table 3
Estimates of evolutionary divergence within 18 s rRNA sequences of Vestibuliferida species based on p-distances.

Species	p-distance	Standart error	Number of sequences
Infundibulorium cameli	0,0013	0,0007	9
Buxtonella sulcata	0,0019	0,0008	8
Buxtonella-like sp.	0,0010	0,0007	9
Balantioides coli	0,0043	0,0014	8
Balantidium entozoon	0,0169	0,0043	2

taxonomic affiliation of the host, the ciliates we found fully correspond to this description.

A comparative analysis of available data shows that the ciliates *I. cameli* are morphologically extremely similar to *Buxtonella sulcata*. The description of *B. sulcata* given by Jameson, in terms of the main morphological features, practically duplicates the description of Bozhenko, and includes information about the shape of the cell and its size, the ciliary coverage and the location of the groove, which, according to the author, is the main distinguishing feature of this species (Fig. 6 B). Jameson also describes the location of the cytostome, the structure of the nuclear apparatus, and points out the granular structure of the cytoplasm and the presence of two contractile vacuoles. Jameson does not indicate the location of the cytoproct; this was done later by Rees (1930), who also described the pattern of the ciliary rows in *B. sulcata* (Fig. 5 C).

In his work, Rees pointed out that on the side of the cell opposite to that where the ciliated groove is located, the rows of cilia are oriented parallel to each other along the longitudinal axis of the cell. On one side of the groove, the ciliary rows are parallel to it, and on the other, they are located at an angle (the cytoproct is also located on this side) (Fig. 5 C). Considering mutual orientation of the groove, cytostome, and cytoproct, the same arrangement of ciliary rows is typical for *I. cameli* (Figs. 3, 5). Differences in the shape of the groove in the drawings done by Jameson, Rees, and those shown in the present study can be explained by different orientation of the cells along the longitudinal axis. It should be noted that the shape and location of the groove in the images presented in this work correspond to those in the photographs of *B. sulcata* published by Pomajbikova et al. (2013). Based on the results of immunofluorescence staining, we can conclude that the transverse striation of the groove, which was noted by many researchers (Grim et al., 2015), is due to the microtubule bundles underlying the groove.

Cysts of *B. sulcata* were described by Becker and Hsiung (1929) and Rees (1930). They are also similar in structure and size to the cysts of *I. cameli*. Interestingly, in younger cysts Becker noted a thickening of the cytoplasm in the immediate vicinity of the vestibulum. This thickening might correspond to a rounded structure that we found in some cysts and is fluorescent when stained using labeled antibodies to α-tubulin (the nature of this structure remains unclear).

The morphometric data of *I. cameli*, obtained during this study,

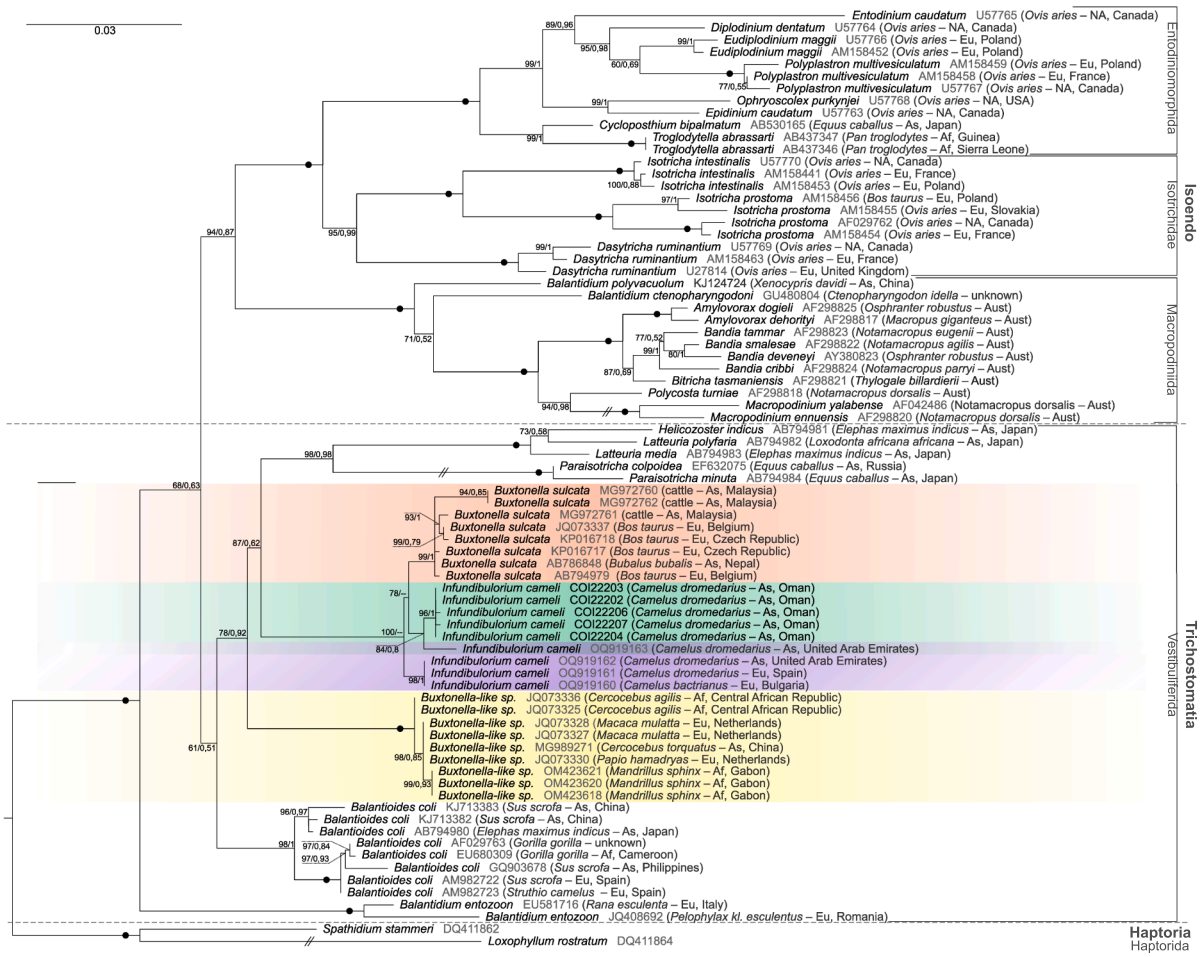

Fig. 6. Phylogenetic tree obtained by the maximum likelihood method from the alignment of the SSU rDNA sequences. The tree has been rooted using the sequences of free-living ciliates *Spathidium stammeri* and *Loxophyllum rostratum* as outgroup. The numbers at the nodes represent, respectively, the ultrafast bootstrap support as computed from 10,000 replicates for Maximum Likelihood and the posterior probability values of the Bayesian analysis; dashes indicate a different tree topology. The species obtained in the present study are highlighted in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

generally corresponds to the range of variability of both *I. cameli* (according to Bozhenko (1925) and Esteban-Sanchez et al. (2023)) and *B. sulcata* (according to different authors (Jameson, 1926; Becker and Hsiung, 1929; Rees, 1930; Lubinsky, 1957; Grim et al., 2015)) (Table 4). The exception is the work of Rees (1930), who writes that *B. sulcata* is almost twice as large as other researchers suggested. Unfortunately, there are no statistical data in literature on such an important morphometric value as the length-to-width ratio of ciliates (with the exception of the publication of Lubinsky (1957), who indicates a value of 1.39). However, analysis of the available data mostly suggests that *I. cameli* has a more elongated cell shape compared to *B. sulcata*.

Table 4
Morphometric data of *I. cameli* (1, 2) and *B. sulcata* (3 – 8), in according to different authors, all dimensions are presented in μm.

		Trophozoites		Cysts	
		Length (min–max)	Width (min–max)	Length (min–max)	Width (min–max)
1	Our data	60–166	50–108	58–70	58–70
2	Bozhenko, 1925	80–128	50–100		
3	Jameson, 1926	54–124	40–72	80–100	60–80
4	Becker and Hsiung, 1929	43–123	39–78	55–90	53–90
5	Lubinsky, 1957	63–135	71.8—0.86		
6	Rees, 1930	95–240	80–180		
7	Grim et al., 2015	65–132	50–84		
8	Esteban-Sanchez et al., 2023	131–167	60–84	58–122	58–122

Phylogenetic analysis has shown that specimens identified as *I. cameli* are positioned on the phylogenetic tree within subclass Trichostomatia, order Vestibuliferida, as the sister species of *B. sulcata*. The level of evolutionary divergence between these species is not very high; however, it is higher than the level of evolutionary divergence within individual species and *B. coli* in particular. That gives us reason to consider *I. cameli* an independent species. It should be noted that the differences in 18S rRNA sequences between *B. sulcata* and *I. cameli* are more pronounced than between different geographical isolates within the *I. cameli, B. sulcata*, *B. coli* species and *Buxtonella*-like ciliates isolated from primate faeces (Pomajbikova et al., 2013; Grim et al., 2015). At the same time, it should be emphasized that the level of differences in the 18S rRNA gene sequences between representatives of different genera of ciliates - vestibuliferids, analyzed in this study, is at least three times higher than the differences between *B. sulcata* and *I. cameli*. Thus,

molecular phylogenetic data unequivocally indicate that these two species of ciliates should be placed within the same genus. It should also be noted here that the values of evolutionary divergence between *B. sulcata* and *Buxtonella*-like ciliates correspond to differences at the level of individual genera, which suggests that *Buxtonella*-like ciliates found in primates are not *B. sulcata*, but are related to at least another species, or possibly even a genus.

The composition of endobiotic ciliate species in the digestive tract is specific for different orders of mammals. At the same time, the degree of specificity between individual endobiont species and different host species appears to fluctuate. For example, among the vestibuliferids, species of the genus *Paraisotricha* have been found in various equine hosts, as well as in rhinos (Kornilova, 2004b). On the other hand, species of the genus *Latteuria* have so far been found only in the African elephant (Timoshenko and Imai, 1997). It is important to note that our ideas about the nature of the distribution of trichostomatids among various host species are largely limited by the lack of information about the fauna of endobiotic ciliates in wild host species in natural habitats. In ruminants and camels, endobiotic ciliates inhabit mainly the foregut, where significantly diverse communities of these protists are formed (Vďačný, 2018). In the hindgut and faeces of ruminants, predominantly *B. coli* and *B. sulcata* species are found (Pomajbikova et al., 2013; Ahmed et al., 2020). *B. coli* is a ciliate that is distributed all over the world and has extremely low host specificity. It is most commonly found in pigs and primates, including humans (Pomajbikova et al., 2013). There are also known cases of infection of equids, ruminants, camels, carnivores, rodents and birds by these ciliates (Ponce-Gordo et al., 2011; Chistyakova et al., 2014; Ahmed et al., 2020). *B. sulcata* is also distributed worldwide but have only been found in cattle (representatives of the genera *Bos* and *Bubalus*). They have never been found in other ruminants, even when the latter were co-grazed with infected cows (Jameson, 1926; Lubinsky, 1957; Ganai et al., 2015). These observations suggest that the relationship of *B. sulcata* and its hosts is specific. The species *I. cameli* was discovered and described by Bozhenko in the territory of modern Kazakhstan, the described ciliates were found in several camels in different locations (Bozhenko, 1925). In addition, those ciliates have been found in the dromedary in India (Jankowsky, 2007), in dromedary and Bactrian camels in different zoo (Esteban-Sanchez et al., 2023), and, according to our study, in Oman.

Based on our findings and the literature data, we can therefore conclude that the species *I. cameli* and *B. sulcata* should undoubtedly be combined in the same genus. This conclusion is supported by the extreme similarity in all morphological features, with the possible exception of such a morphometric parameter as the ratio of cell length to its width. In addition, the level of differences in 18S rRNA sequences for representatives of different genera and even species within the same genus of vestibuliferids phylogenetically close to *I. cameli* and *B. sulcata*, is significantly higher than for the ciliate species under consideration. Earlier, we proposed to unite those two species within the genus *Infundibulorium*, in according to the principle of priority (art. 23 of the International Code of Zoological Nomenclature) (Chistyakova et al., 2023). However, *B. sulcata* is believed to cause diarrhea in cattle as the causative agent of buxtonellosis, and the species name is widely used in veterinary medicine. So, we join the opinion of Esteban-Sanchez et al. (2023) on the need to use a junior synonym of *Buxtonella* (instead of *Infundibulorium*) as a generic name; this genus should include the species *B. sulcata* and *B. cameli*, respectively.

5. Code availability

Monoclonal anti-α-Tubulin antibodies produced in mouse Sigma-Aldrich Cat# T5168, RRID: AB_477579, secondary antibodies Anti-Mouse IgG (whole molecule) –TRITC Sigma-Aldrich Cat# T5393, RRID: AB_261699.

CRediT authorship contribution statement

Olga A. Kornilova: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Anna I. Ganyukova:** Methodology, Formal analysis, Investigation, Data curation, Visualization. **Maria E. Belokon:** Formal analysis, Investigation, Visualization. **Vladimir V. Platonov:** Formal analysis, Investigation, Visualization. **Ludmila V. Chistyakova:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration.

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.protis.2023.125993.

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