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Ciliates from the intestine of zoo-kept black rhinoceros, with immunofluorescence microscopic and molecular phylogenetic investigation of *Rhinozeta rhinozeta* (Litostomatea)

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Abstract

The study of endobiotic ciliates from the intestines of various zoo mammals is important for revealing the influence of various factors on endobiont communities. In this paper we describe the species diversity of endobiotic ciliates from the faeces of the eastern black rhinoceros *Diceros bicornis michaeli* (male and two females, mother and daughter) kept in the zoo. Seven species of the ciliates were found, among them *Rhinozeta rhinozeta*, *R. triciliata* and *Prototapirella clypeata* were observed in the rhinos only. The other two, *Monoposthium* sp. and *Triplumaria* sp., according to their morphology should have been identified as new ciliate species. Successful transmission of the endobionts from parents to the young rhino in the zoo was demonstrated. *R. rhinozeta* was investigated with the using of the methods of the light and immunofluorescence microscopy and molecular phylogeny. The composition and phylogeny of the family Cycloposthiidae were discussed.

Keywords: Endobiotic ciliates; Morphology; Molecular phylogeny; *Diceros bicornis*; Cycloposthiidae

Introduction

Endobiotic ciliates are an important component of the microbiome of the gastro-intestinal tract of herbivorous mammals. The composition of the microbiome influences the efficiency of digestion, including the process of breakdown of plant fibres (Williams 1986; Newbold et al. 2015). The species composition of endobiotic communities is determined both by the taxonomic position of the host and by factors associated with its lifestyle and the environment such as food preferences, gregariousness and contact with other herbivores (Williams and Coleman 1992; Vd'ačný 2018).

The diet of animals kept in zoos is often significantly altered as compared to the wildlife conditions, and the likelihood of horizontal transfer of ciliates between different host species is increased. Therefore, a comparative analysis of the community structure of endobionts in natural and artificial habitats can shed light on the impact of this factor on endobiotic fauna. In addition, zoo-kept animals offer an

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opportunity to trace the vertical transmission of endobiotic ciliates from parents to offsprings in family groups of hosts, including representatives of rare species.

Members of the family Rhinocerotidae possess a unique endobiotic ciliate community. Many of their endobionts have not been found in any other vertebrate host species (Van Hoven et al. 1988, 1998a, b; Ito et al. 2006; Fregulia et al. 2021). Therefore, the effect of captivity on the community structure of endobiotic ciliates of the rhinos, including the persistence of specific species in it, is a particularly interesting study topic.

We studied species diversity of endobiotic ciliates from the faeces of the eastern black rhino *Diceros bicornis michaeli* (male and two females, mother and daughter) kept in the Tallinn Zoo. Our results were compared with the literature data on endobiotic rhinoceros fauna in natural habitats. In addition, we investigated *Rhinozeta rhinozeta*, currently thought to be rhino-specific ciliate, with the use of immunofluorescence microscopy and molecular phylogeny techniques.

Material and methods

Samples of faeces were collected from three eastern black rhinos *Diceros bicornis michaeli* in the Tallinn Zoo in November 2021. The male “Kigoma” was born in 2010 in Berlin Zoo, the female “Kibibi” was born in 2010 in Krefeld Zoo in Germany and they were relocated to the Tallinn Zoo two years later. Their daughter “Kibeta” was born in 2017 in Tallinn Zoo. Fresh faeces from each rhino were collected separately, placed in plastic containers and fixed in 96° alcohol (1:50). The samples were transported to the laboratory, where the fixator was almost completely changed. The samples were stored in the dark at room temperature. Light-optical examinations and microphotography were performed using a Leica DM2500 microscope (Leica-Microsystems, Germany), equipped with a differential interference contrast and a Leica DFC495 (8.0MP) digital camera. During the study of cell morphology, the macronucleus was detected with the use of 0.1% solution of methyl green in 1% solution of acetic acid.

The ciliates were identified with the help of several published sources (Buisson 1923; Hoare 1937; Strelkow 1939; Thurston and Noirot-Timothe 1973; Van Hoven et al. 1987, 1988, 1998b; Timoshenko and Imai 1995; Ito and Imai 2000; Kornilova 2010; Ito et al. 2006, 2008, 2010, 2011; Fregulia et al. 2020; Ito 2022).

The organization of the tubulin cytoskeleton was studied with the use of immunofluorescence staining with antibodies against α-tubulin. The staining technique was described in detail in our previous study (Kornilova et al. 2021). 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) and a Leica TCS SP5 laser confocal scanning microscope. The images were processed using ImageJ software (Schneider et al. 2012).

For DNA isolation, ciliate cells from the sample, collected from the male, were picked one by one using a Nikon SMZ 1270 stereomicroscope (Nikon Corporation, Japan), washed in two changes of distilled water and placed in tubes with a lysing solution, 30–40 cells per tube, two tubes were prepared. Genomic DNA was extracted using PureLink Genomic DNA Kit (Invitrogen) for DNA extraction according to the manufacturer’s instructions.

SSU rRNA gene fragment was amplified with the use of universal eukaryotic primers EukA (5′-AACCTGGTT GATCCTGCCAGT-3′) and EukB (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) (Medlin et al., 1988). The PCR program was as follows: 1 cycle at 95 °C for 2 min; 40 cycles at 94 °C for 30 s, 50 °C for 60 s, 72 °C for 2 min; 1 cycle at 72 °C for 10 min. The PCR products were purified using Cleanup S-Cap kit (Evrogen) and Sanger-sequenced using EukA and EukB primers. The sequences obtained in this way were used for the phylogenetic analysis. The GenBank accession numbers for the new SSU rRNA gene sequences determined in this work are OQ821302-OQ821303.The dataset for phylogenetic analysis included 154 nucleotide sequences of 18S rRNA retrieved from the previous studies (Ito et al. 2014; Vd'ačný 2018; Fregulia et al. 2021) and sequences from this study. The list of the sequences used in this study is given in Supplementary Table 1. *Didinium nasutum* (Genbank accession number U57771) was selected as an outgroup according to Ito et al. (2014).

Multiple sequence alignment was performed using Clustal W algorithm with minor manual adjustments in the software package MEGA X (Kumar et al. 2018). Maximum likelihood (ML) trees were constructed on IQ-TREE v.1.6 web server (Trifinopoulos et al. 2016) with 1000 ultrafast bootstraps for branch support analysis (Hoang et al. 2017). The best nucleotide substitution model for tree inference was selected automatically by Akaike information criterion (AIC) score.

Bayesian analyses were conducted under the GTR + I + G evolutionary model on the CIPRES portal ver. 3.1 (https://www.phylo.org/) with MrBayes software (Ronquist and Huelsenbeck 2003) on XSEDE ver. 3.2.6 (Miller et al. 2010). Markov Chain Monte Carlo parameters were as follows: the two independent runs were done with four independent chains during 2,000,000 generations. Each 100th tree was sampled and 25% of the first trees were discarded as a burn-in.

Results and discussion

Seven ciliate species from the families Cycloposthiidae, Rhinozetidae and Paraisotrichidae were found in the faecal samples of the rhinos: *Triplumaria hamertoni* Hoare, 1937, *Triplumaria* sp., *Prototapirella clypeata* Buisson, 1923,

Table 1. Species composition of the ciliates – endobionts of black and white rhinos.

Host species	black rhinos *Diceros bicornis*		white rhinos *Ceratotherium simum*
Ciliate species	1	2	3
Buetschliidae Poche, 1913			
Blepharosphaera intestinalis Bundle, 1895		+	+
B. ceratotherii van Hoven et al., 1998		+	+
B. ellipsoidalis Hsiung, 1930		+	+
Fiorentinus ovalis (Fiorentini, 1890)		+	+
Didesmis quadrata Fiorentini, 1890		+	+
D. synciliata van Hoven et al., 1998			+
Holophryoides ovalis (Fiorentini, 1890)		+	+
Alloiozona trizona Hsiung, 1930			+
Polymorphella ampulla (Dogiel, 1929)		+	+
Blepharoconus cervicalis Hsiung, 1930		+	
B. dicerotos van Hoven et al., 1998		+	
Paraisotrichidae da Cuncha, 1917			
Helicozoster africanus van Hoven et al., 1998		+	+
H. apicalis van Hoven et al., 1998			+
Paraisotricha colpoidea Fiorentini, 1890	+	+	+
P. minuta Hsiung, 1930		+	+
Gilchristidae Ito et al., 2006			
Gilchristia artemis Ito et al., 2006			+
Digilchristia draconis Ito et al., 2006			+
Blepharocorythidae Hsiung, 1929			
Circodinium minimum (Gassovsky, 1919)			+
Charonina odotophora Gilchrist et al., 1994			+
C. tenuis Gilchrist et al., 1994			+
C. dicerotis Gilchrist et al., 1994		+	
C. tortuosa Gilchrist et al., 1994		+	
C. tetragona Gilchrist et al., 1994		+	
Cycloposthiidae Poche, 1913			
Lavierella klipdrifi van Hoven et al., 1998		+	+
L. africana Buisson, 1923		+	
Triplumaria hamertoni Hoare, 1937	+	+	+
T. selenica Latteur et al., 1970			+
T. corrugata van Hoven et al., 1998			+
T. alluvia Ito et al., 2008			+
T. grypoclunis Ito et al., 2008			+
Triplumaria sp.	+		
Monoposthium vulgaris van Hoven et al., 1987			+
M. latus van Hoven et al., 1987			+
M. bracchium van Hoven et al., 1987			+
Monoposthium sp.	+		
Cycloposthium bipalmatum (Fiorentini, 1890)			+
C. hydrochoeri Cunha, 1915			+
Arachnodinium noveni van Hoven et al., 1987			+
Phalodinium digitalis van Hoven et al., 1987			+
Prototapirella intestinalis Buisson, 1923		+	+
P. clypeata Buisson, 1923	+	+	
Tricaudalia brumpti Buisson, 1923		+	+
Bozasella rhinocerotis Buisson, 1923		+	+
Rhinozetidae van Hoven et al., 1988			
Rhinozeta addoensis van Hoven et al., 1988		+	+
R. unilaminatus van Hoven et al., 1988		+	+
R. caecalis van Hoven et al., 1988		+	
R. rhinozeta van Hoven et al., 1988	+	+	+
R. triciliata van Hoven et al., 1988	+	+	+
R. cristata van Hoven et al., 1988			+

R. multiplatus van Hoven et al., 1988			+
R. pedale van Hoven et al., 1998			+
Spirodiniidae Strelkov, 1939			
Gassovskiella galea (Gassovsky, 1919)			+
Ophryoscolecidae Stein, 1859			
Endoralium loxodontae Eloff, van Hoven, 1980		+	+
Telamodiniidae Latteur, Dufey, 1967			
Telamodinium onyx Latteur, Dufey, 1967		+	
Allantosomatidae Jankowski, 1967			
Allantosoma intestinale Gassovsky, 1919			+
A. multisuctores van Hoven et al., 1998		+	+
Allantoxena biseriale (Strelkow, 1939)			+

1 – present paper, 2, 3 – in according to van Hoven et al., 1988, 1998b, Ito et al., 2006, Fregulia et al., 2020.

Monoposthium sp., *Rhinozeta rhinozeta* van Hoven et al., 1988, *Rhinozeta triciliata* van Hoven et al., 1988, *Paraisotricha colpoidea* Fiorentini, 1890 (Figs. 1 - 4). To our surprise, no ciliates from the family Buetschliidae were found in the samples, though according to the literature, these endobiotic ciliates are numerous and diverse in the intestine of the black rhino (Van Hoven et al. 1998b). *Rhinozeta* spp. and *Monoposthium* sp. were the most abundant (about 10–30 cells per 100 µl of the sample), while each of the other species was represented by only a few individuals (less than 10 cells per 100 µl of the sample).

The species composition of ciliates in the faecal samples of all three rhinos was identical. It is interesting that, despite the fact that the rhinos had been kept in the zoo for a long time, most of their endobiotic ciliates were specific for rhinocerotids (Van Hoven et al. 1998b; Fregulia et al. 2020). Moreover, *Rhinozeta* spp. and *Monoposthim* sp. dominated in all samples. Thus, dietary change of zoo-kept rhinos does not appear to have a significant effect on this component of their endobiotic communities. In addition, endobiotic ciliates, including those specific to black rhinos, were successfully transmitted from parents to offspring. In zoo-kept

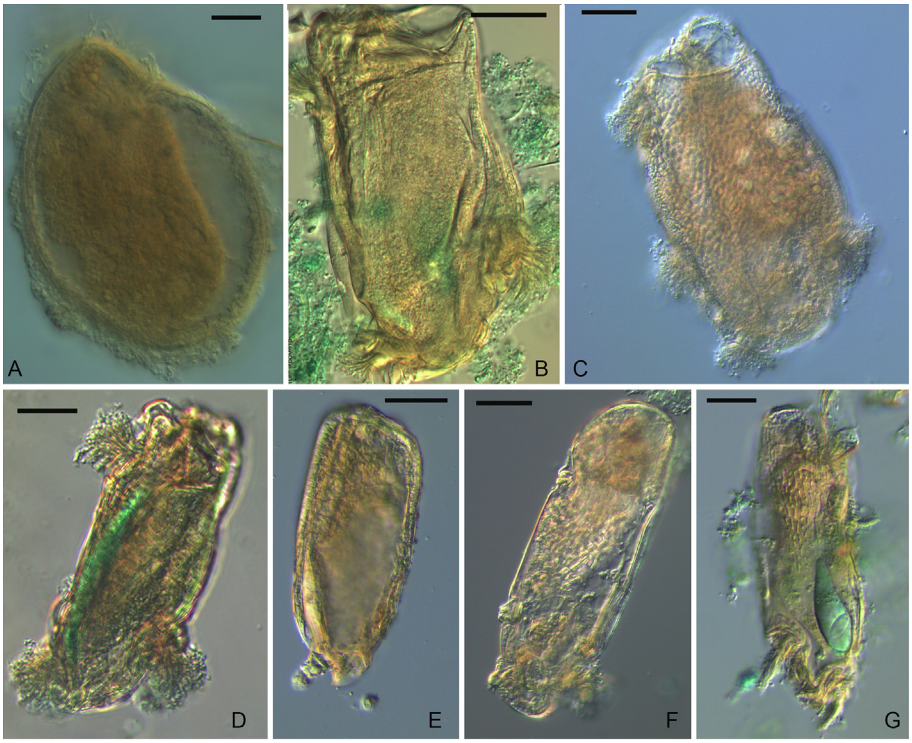


Fig. 1. Ciliates from the faeces of zoo-kept eastern black rhinos. A – *Paraisotricha colpoidea,* B - *Triplumaria hamertoni*, C - *Rhinozeta rhinozeta*, D - *Rhinozeta triciliata*, E - *Monoposthium* sp., F - *Prototapirella clypeata*, G - *Triplumaria* sp. DIC. Scale bars: A – 10 µm, B – G – 20 µm.

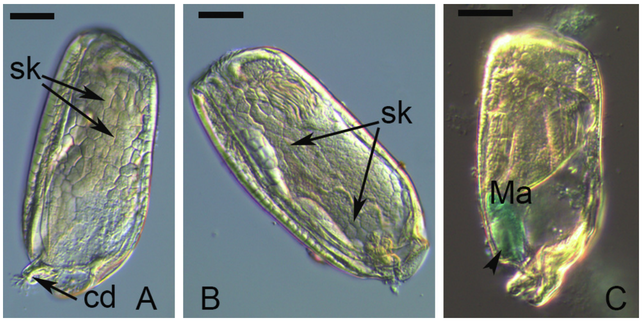


Fig. 2. Morphology of the *Monoposthium* sp. DIC. Ma – macronucleus, sk – skeletal plates, cd – caudalia, arrowhead – micronucleus. Scale bars 20 µm.

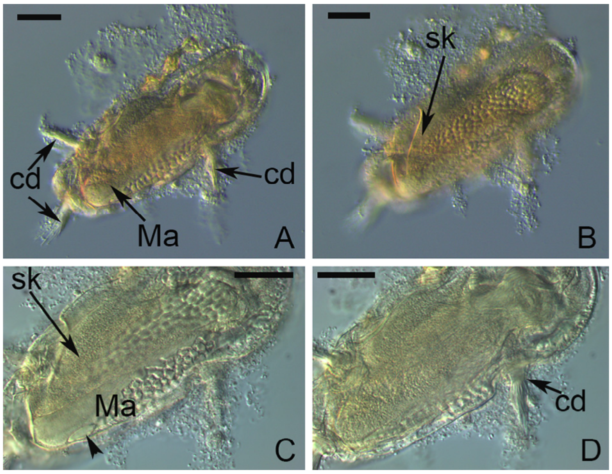


Fig. 3. Morphology of the *Triplumaria* sp. DIC. Ma – macronucleus, sk – skeletal plates, cd – caudalia, arrowhead – micronucleus. Scale bars 20 µm.

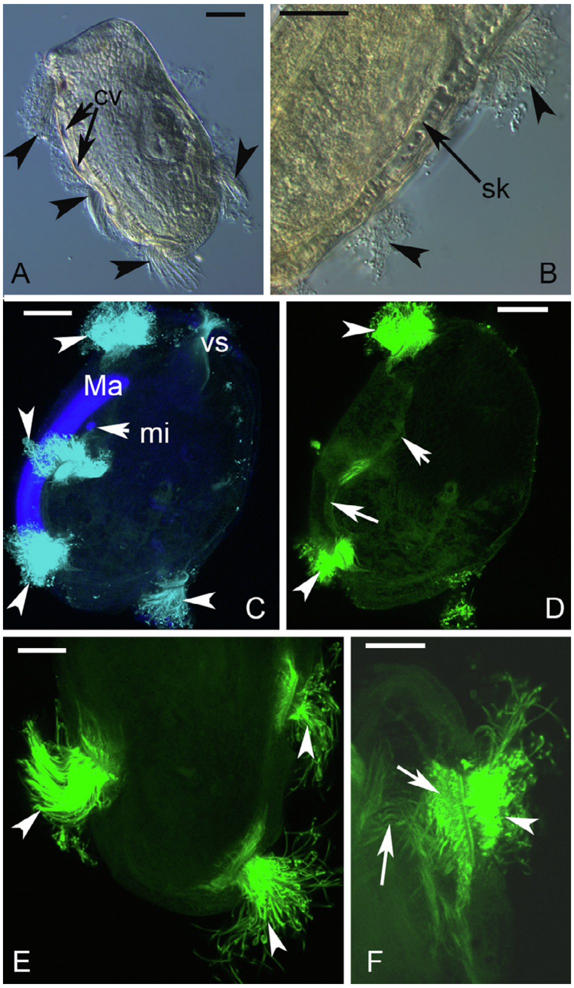


Fig. 4. Morphology of the *Rhinozeta rhinozeta*. A, B – DIC, C – F – CLSM. Ma – macronucleus, cv – contractile vacuoles, vs – vestibulum, sk – skeletal plate, mi – micronucleus, arrows – bundles of microtubules, arrowheads – caudalia. Scale bars: A – D – 20 µm, E, F – 10 µm.

rhinos the structure of the intestinal ciliate communities is maintained in the successive generations. It should be noted that the species diversity of endobiotic ciliates of black rhinos is, in general, much lower as compared to that of the white rhinos *Ceratotherium simum* (Table 1). A possible reason may be the differences in food preferences of these two rhino species: white rhinos mainly feed on grasses, whereas the black rhinos prefer leaves of trees and shrubs. The social behavior of rhinos may also influence the distribution of their endobionts. White rhinos often form herds of several individuals, while black rhinos tend to be solitary.

Monoposthium sp. found in the samples could not be assigned to any of the known species of this genus (Thurston and Noirot-Timothe 1973; Van Hoven et al. 1987; Ito and Imai 2000). Morphologically they were similar to *M. vulgaris* from the intestines of the white rhino (Van Hoven et al. 1987). However, according to the description (Van Hoven et al. 1987), *M. vulgaris* lacks skeletal plates, whereas in *Monoposthium* sp. skeletal plates, formed by polygons of different shape, were clearly visible on the cell surface (Fig. 2). A distinctive feature of *M. vulgaris* is a serrated edge of the trough on one side. We did not detect this feature in *Monoposthium* sp. In addition, the two species differ in the shape and position of the macronucleus. Morphometric characters of the *Monoposthium sp.* found in our samples have been given in Table 2.

We also found *Triplumaria* sp. differing from all the known representatives of this genus in the shape and position of the macro- and the micronucleus (Fig. 3). A characteristic feature of *Triplumaria* sp. is the position of the anterior caudalia closer to the middle of the cell, almost equidistantly from the anterior and the posterior end. Morphometric characters of the *Triplumaria* sp. found in our samples have been given in Table 2.

In his description of ciliates of the genus *Rhinozeta*, Van Hoven points out the presence of two skeletal plates covering the body on the sides (Van Hoven et al. 1988). In *R. tri-*

Table 2. Measurements (μm) and morphometric ratios (mean ± S.D. (minimum – maximum); n = 30 for cells and n = 15 for nuclei) of *Monoposthium* sp. and *Triplumaria* sp.

	Monoposthium sp.	*Triplumaria* sp.
Body length	119.80 ± 2.32 (95.00–147.00)	118.80 ± 2.11 (100.00–139.00)
Body width	48.50 ± 1.49 (32.50–65.00)	46.23 ± 1.56 (35.00–65.00)
Body length/body width	2.52 ± 0.07 (2.07–3.15)	2.56 ± 0.07 (1.85–3.21)
Macronuclear length	35.60 ± 1.40 (26.00–43.50)	31.00 ± 0.30 (30.00–35.00)
Macronuclear width	12.20 ± 0.20 (11.00–15.00)	9.40 ± 0.20 (8.50–13.00)

ciliata and *R. rhinozeta* we found one more narrow skeletal plate located near the dorsal side (Fig. 1D, 4B). The plate has a meshwork structure and lies between the macronucleus and the dorsal cell surface. The somatic ciliary arches radiating short disordered bundles of microtubules (nemadesmata) could be clearly identified by immunofluorescence microscopy (Fig. 4 C - F). Occasionally, separate microtubular bundles linking the ciliary arcs can also be seen (Fig. 4 D).

The distinguishing feature of the family Rhinozetidae is the organization of the somatic ciliature in the form of ciliary arches (Van Hoven et al. 1988). However, in other characters, such as the retractable oral cone, well-developed skeletal plates covering the cell from the sides and an elongated sausage-like macronucleus, *Rhinozeta* spp. are strikingly similar to representatives of the family Cycloposthiidae. The latter family presently contains species whose somatic ciliature can be organized as bundles or as arches (Lynn 2008; Fregulia et al. 2020). For instance, the caudalia of *Cycloposthium cristatum* Cunha, Muniz, 1927, described from the capybara *Hydrochoerus hydrochaeris*, are represented by long ciliary arches (Ito and Imai 2000). Thus, based on morphological characters, it seems more reasonable to include *Rhinozeta* spp. into the family Cycloposthiidae rather than into a separate family Rhinozetidae. However, allocating this genus to a certain family is complicated by the high morphological diversity of the *Rhinozeta* species.

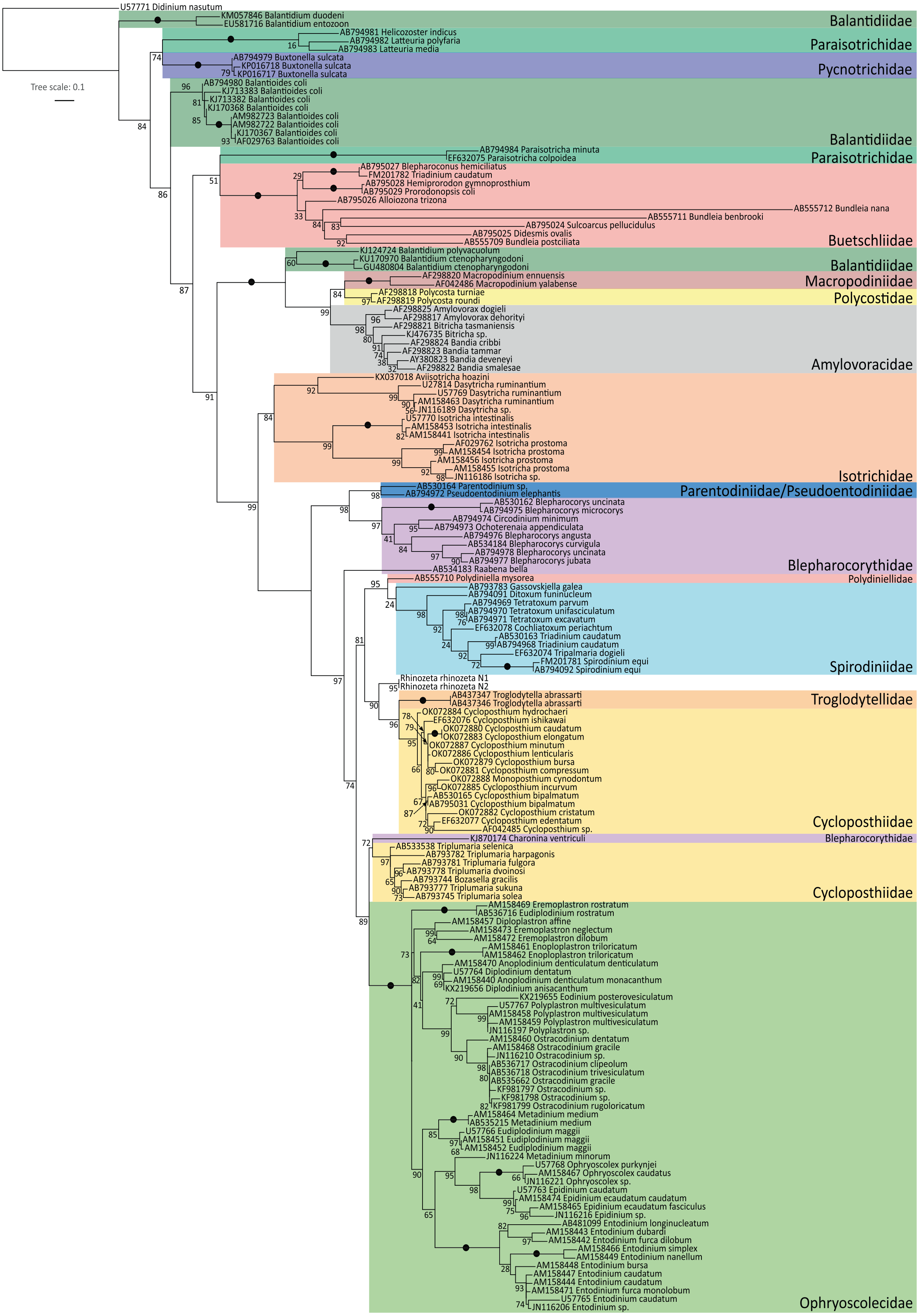
The current representatives of the genus *Rhinozeta* differ from each other based on such taxonomically important characters as number, shape and location of skeletal plates and ciliary bands (Van Hoven et al., 1988, 1998). Usually, these characters are decisive for allocation of a new genus. *R. rhinozeta*, *R. caecalis* and *R. pedale* form a separate group, having two wide skeletal plates, which enclose the cell from both sides, and four somatic ciliary bands. *R. addoensis*, *R. multiplatus*, *R. unilaminatus* and *R. cristata* have five somatic ciliary arches. At the same time, *R. addoensis* possesses two wide skeletal plates, *R. unilaminatus* has a single skeletal plate, and the cell of *R. multiplatus* contains a few narrow skeletal plates, located parallel to each other. In *R. cristata*, one skeletal plate is located in the left part of the cell along the macronucleus; this is a character that makes *R. cristata* similar to the species *Gilchristia artemis* and *Digilchristia draconis* (Ito et al., 2006). It should be emphasized that based on their characters all of the species mentioned above differ from currently known genera of endobiotic ciliates. An exception is *R. triciliata*, which is similar to the representatives of the genus *Triplumaria*, having the same amount of skeletal plates and ciliary bands; however, in *Triplumaria* species the somatic ciliature is composed of bundles, while *R. triciliata* has ciliary arches. It can be assumed that the Rhinozetidae family will be separated into a few independent genera in the future. For such a serious taxonomic revision, however, a complex research of this group of ciliates with the usage of both morphological and molecular phylogenetic methods is required.

PCR with EukA/EukB universal primers successfully amplified 18S fragment of ~ 1700 bp length in *Rhinozeta rhinozeta* samples. The Sanger sequencing in both directions resulted in two identical nucleotide sequences from N1 and N2 samples with 1370 and 1410 bp length after trimming.

Phylogenetic trees were based on 1670 base pairs alignment with 156 nucleotide sequences. The ML tree is shown in the Fig. 5 and the Bayesian tree is shown in Fig. 6. The bootstrap supports are given in percents. Taxonomy of the families follows Vd'ačný (2018). The newly described *Rhinozeta rhinozeta* is located on the one clade with Troglodytellidae and Cycloposthiidae. Bayesian and ML phylogenies confirm this state with 100% and 90% support, correspondingly.

It should be noted that the family Cycloposthiidae appears to be polyphyletic in its present composition, which agrees with the previous data (Vd'ačný 2018; Fregulia et al. 2021). *Triplumaria* spp. group with ciliates from the family Ophryoscolecidae, while *Tripalmaria dogieli* groups with

Fig. 5. Maximum likelihood phylogenetic tree inferred from 18S rRNA gene sequences by IQ-TREE, based on the General Time Reversible (GTR) model with gamma distribution and estimate of invariable sites. The numbers at the nodes are the ultrafast bootstrap values (the rate out of 1000 replicates). Branches marked by filled dots have 100% supports. The scale bar marks genetic distance.


U57771 Didinium nasutum
KM057846 Balantidium duodeni
EU581716 Balantidium entozoon
Balantidiidae
AB794981 Helicozoster indicus
AB794982 Latteuria polyfaria
AB794983 Latteuria media
Paraisotrichidae
AB794979 Buxtonella sulcata
KP016718 Buxtonella sulcata
KP016717 Buxtonella sulcata
Pycnotrichidae
Tree scale: 0.1
AB794980 Balantioides coli
KJ713383 Balantioides coli
KJ713382 Balantioides coli
KJ170368 Balantioides coli
AM982723 Balantioides coli
AM982722 Balantioides coli
KJ170367 Balantioides coli
AF029763 Balantioides coli
Balantidiidae
AB794984 Paraisotricha minuta
EF632075 Paraisotricha colpoidea
Paraisotrichidae
AB795027 Blepharoconus hemiciliatus
FM201782 Triadinium caudatum
AB795028 Hemiprorodon gymnoprosthium
AB795029 Prorodonopsis coli
AB795026 Alloiozona trizona
AB555712 Bundleia nana
AB555711 Bundleia benbrooki
AB795024 Sulcoarcus pellucidulus
AB795025 Didesmis ovalis
AB555709 Bundleia postciliata
Buetschliidae
KJ124724 Balantidium polyvacuolum
KU170970 Balantidium ctenopharyngodoni
GU480804 Balantidium ctenopharyngodoni
Balantidiidae
AF298820 Macropodinium ennuensis
AF042486 Macropodinium yalabense
Macropodiniidae
AF298818 Polycosta turniae
AF298819 Polycosta roundi
Polycostidae
AF298825 Amylovorax dogieli
AF298817 Amylovorax dehorityi
AF298821 Bitricha tasmaniensis
KJ476735 Bitricha sp.
AF298824 Bandia cribbi
AF298823 Bandia tammar
AY380823 Bandia deveneyi
AF298822 Bandia smalesae
Amylovoracidae
KX037018 Aviisotricha hoazini
U27814 Dasytricha ruminantium
U57769 Dasytricha ruminantium
AM158463 Dasytricha ruminantium
JN116189 Dasytricha sp.
U57770 Isotricha intestinalis
AM158453 Isotricha intestinalis
AM158441 Isotricha intestinalis
AF029762 Isotricha prostoma
AM158454 Isotricha prostoma
AM158456 Isotricha prostoma
AM158455 Isotricha prostoma
JN116186 Isotricha sp.
Isotrichidae
AB530164 Parentodinium sp.
AB794972 Pseudoentodinium elephantis
Parentodiniidae/Pseudoentodiniidae
AB530162 Blepharocorys uncinata
AB794975 Blepharocorys microcorys
AB794974 Circodinium minimum
AB794973 Ochoterenaia appendiculata
AB794976 Blepharocorys angusta
AB534184 Blepharocorys curvigula
AB794978 Blepharocorys uncinata
AB794977 Blepharocorys jubata
AB534183 Raabena bella
Blepharocorythidae
AB555710 Polydiniella mysorea
Polydiniellidae
AB793783 Gassovskiella galea
AB794091 Ditoxum funinucleum
AB794969 Tetratoxum parvum
AB794970 Tetratoxum unifasciculatum
AB794971 Tetratoxum excavatum
EF632078 Cochliatoxum periachtum
AB530163 Triadinium caudatum
AB794968 Triadinium caudatum
EF632074 Tripalmaria dogieli
FM201781 Spirodinium equi
AB794092 Spirodinium equi
Spirodiniidae
Rhinozeta rhinozeta N1
Rhinozeta rhinozeta N2
AB437347 Troglodytella abrassarti
AB437346 Troglodytella abrassarti
Troglodytellidae
OK072884 Cycloposthium hydrochaeri
EF632076 Cycloposthium ishikawai
OK072880 Cycloposthium caudatum
OK072883 Cycloposthium elongatum
OK072887 Cycloposthium minutum
OK072886 Cycloposthium lenticularis
OK072879 Cycloposthium bursa
OK072881 Cycloposthium compressum
OK072888 Monoposthium cynodontum
OK072885 Cycloposthium incurvum
AB530165 Cycloposthium bipalmatum
AB795031 Cycloposthium bipalmatum
OK072882 Cycloposthium cristatum
EF632077 Cycloposthium edentatum
AF042485 Cycloposthium sp.
Cycloposthiidae
KJ870174 Charonina ventriculi
Blepharocorythidae
AB533538 Triplumaria selenica
AB793782 Triplumaria harpagonis
AB793781 Triplumaria fulgora
AB793778 Triplumaria dvoinosi
AB793744 Bozasella gracilis
AB793777 Triplumaria sukuna
AB793745 Triplumaria solea
Cycloposthiidae
AM158469 Eremoplastron rostratum
AB536716 Eudiplodinium rostratum
AM158457 Diploplastron affine
AM158473 Eremoplastron neglectum
AM158472 Eremoplastron dilobum
AM158461 Enoploplastron triloricatum
AM158462 Enoploplastron triloricatum
AM158470 Anoplodinium denticulatum denticulatum
U57764 Diplodinium dentatum
AM158440 Anoplodinium denticulatum monacanthum
KX219656 Diplodinium anisacanthum
KX219655 Eodinium posterovesiculatum
U57767 Polyplastron multivesiculatum
AM158458 Polyplastron multivesiculatum
AM158459 Polyplastron multivesiculatum
JN116197 Polyplastron sp.
AM158460 Ostracodinium dentatum
AM158468 Ostracodinium gracile
JN116210 Ostracodinium sp.
AB536717 Ostracodinium clipeolum
AB536718 Ostracodinium trivesiculatum
AB535662 Ostracodinium gracile
KF981797 Ostracodinium sp.
KF981798 Ostracodinium sp.
KF981799 Ostracodinium rugoloricatum
AM158464 Metadinium medium
AB535215 Metadinium medium
U57766 Eudiplodinium maggii
AM158451 Eudiplodinium maggii
AM158452 Eudiplodinium maggii
JN116224 Metadinium minorum
U57768 Ophryoscolex purkynjei
AM158467 Ophryoscolex caudatus
JN116221 Ophryoscolex sp.
U57763 Epidinium caudatum
AM158474 Epidinium ecaudatum caudatum
AM158465 Epidinium ecaudatum fasciculus
JN116216 Epidinium sp.
AB481099 Entodinium longinucleatum
AM158443 Entodinium dubardi
AM158442 Entodinium furca dilobum
AM158466 Entodinium simplex
AM158449 Entodinium nanellum
AM158448 Entodinium bursa
AM158447 Entodinium caudatum
AM158444 Entodinium caudatum
AM158471 Entodinium furca monolobum
U57765 Entodinium caudatum
JN116206 Entodinium sp.
Ophryoscolecidae

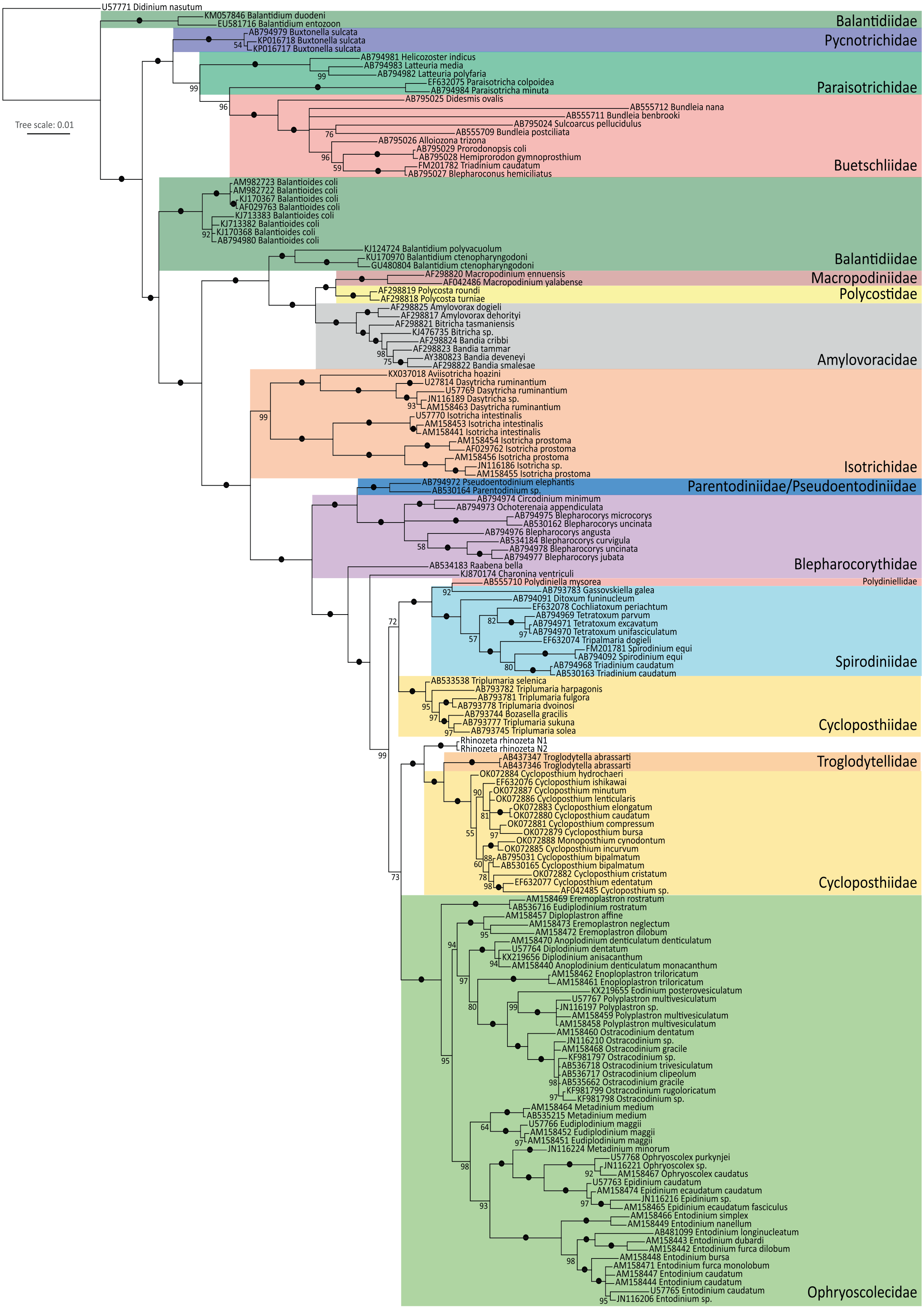


Fig. 6. Bayesian phylogenetic tree inferred from 18 s rRNA gene sequences. The posterior probabilities are given near branches. Branches marked by filled dots have 100% supports. The scale bar marks genetic distance.

spirodiniids. At present, it is difficult to identify any synapomorphy for these species groups due to the lack of morphological and molecular genetic data. Fregulia et al. (2021) have suggested that such features might be found in a detailed study of the ultrastructure of cycloposthiids, especially the cortex structure. Comparative analysis of the general plan of the cytoskeleton structure of different ciliates might presumable also be useful for revealing synapomorphies. For instance, many members of the clade *Cycloposthium* - *Monoposthium* - *Rhinozeta* tend to form somatic ciliary arches, in contrast to *Triplumaria* spp., which have ciliary bundles. In any case, given the lack of the data, a taxonomic revision of the families Cycloposthiidae and Rhinozetidae is premature.

CRediT authorship contribution statement

Olga A. Kornilova: Conceptualization, Investigation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Maria E. Belokon:** Investigation, Formal analysis, Visualization. **Maria A. Skazina:** Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Olga S. Alekseeva:** Investigation, Formal analysis, Visualization. **Ludmila V. Chistyakova:** Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Data availability

Data will be made available on request.

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.ejop.2023.126006.

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