

TABLE S1 Relative abundances of ciliate genera in fraction 1 of the rumen samples obtained from sheep S4 and cow C1 as observed by microscopic counts and 454-based pyrosequencing.

Ciliate genus	Relative abundance in sample from sheep S4		Relative abundance in sample from cow C1	
	Microscopy	Pyrosequencing	Microscopy	Pyrosequencing
Anoplodinium-Diplodinium	11.9%	18.6%	7.5%	7.1%
<i>Charonina</i>	- ^a	-	2.0%	0.8%
<i>Dasytricha</i>	11.4%	5.3%	-	-
<i>Entodinium</i>	38.9%	8.7%	88.0%	35.2%
<i>Epidinium</i>	24.6%	58.1%	-	0.5%
<i>Eudiplodinium</i>	10.1%	5.4%	-	-
<i>Isotricha</i>	3.1%	3.8%	0.5%	1.5%
<i>Metadinium</i>	-	-	-	24.9%
<i>Ostracodinium</i>	-	-	1.5%	25.0%
<i>Polyplastron</i>	-	-	0.5%	5.1%

^aNot detected.

FIG S1

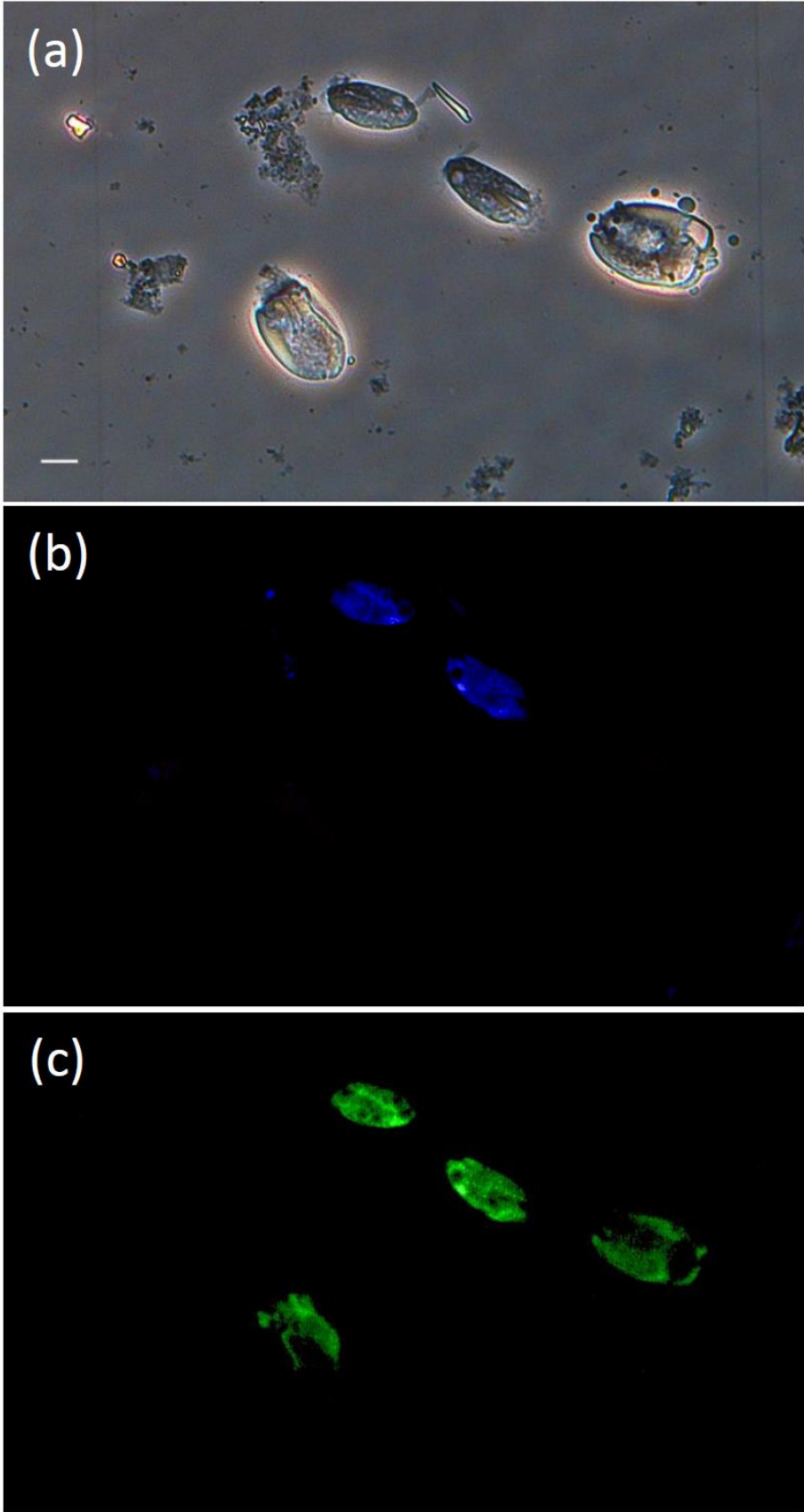


FIG S1 Bright field and single fluorescence *in situ* hybridization microscopic images from Fig. 2. Ciliates were simultaneously hybridized with the universal Eukarya probe EUKb1193 (Cy5) and the *Charonina ventriculi*-specific probe CHA1350 (Cy3). The images were taken from the same field of fraction 3 of a rumen sample collected from cow C1. (a) Bright field image. The scale bar represents 10 μm . Color replacement was used to show cells stained with CHA1350 in blue (b) and those stained with EUKb1193 in green (c) in the same field as shown in panel (a).

FIG S2

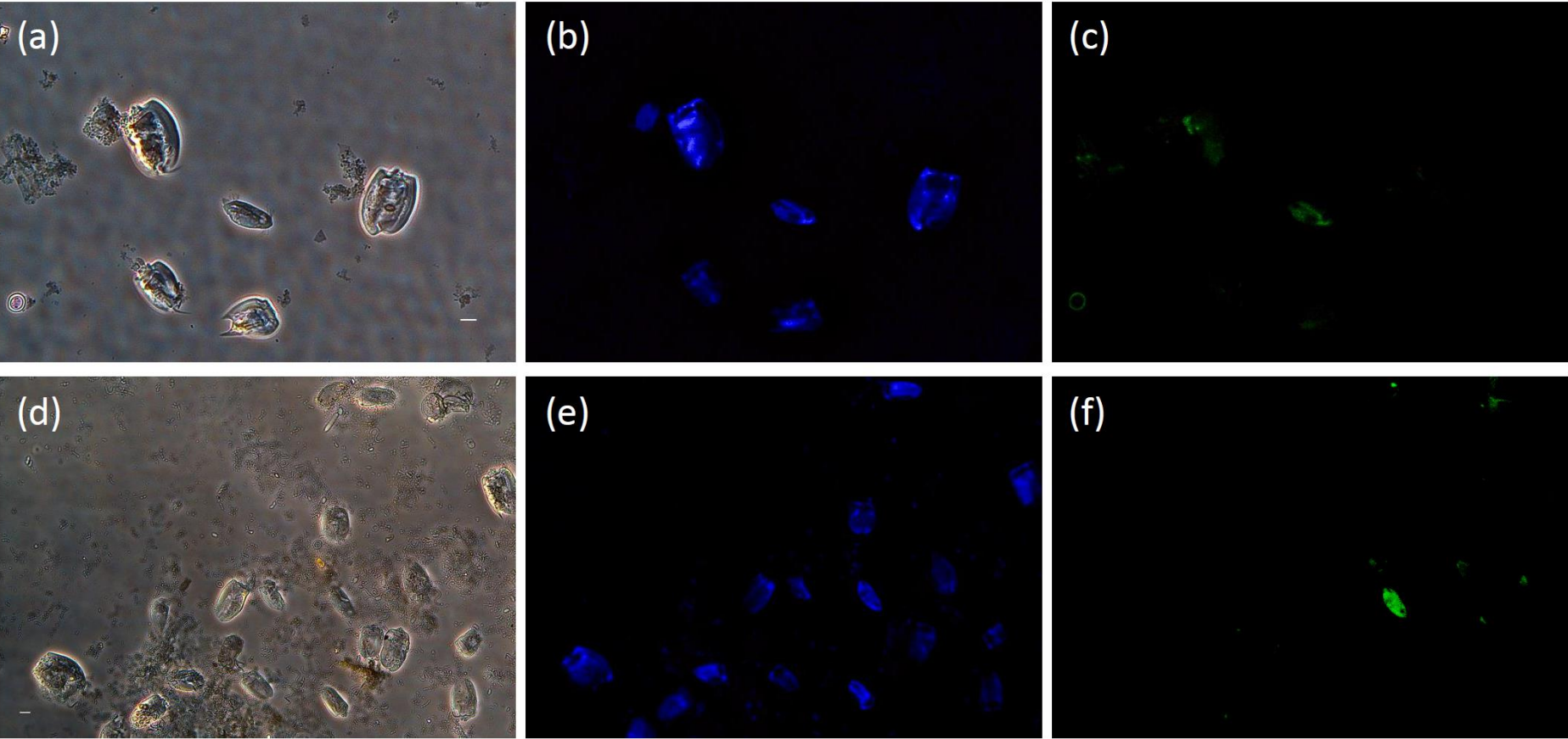


FIG S2 Bright field and fluorescence *in situ* hybridization microscopic images. Ciliates were simultaneously hybridized with the universal Eukarya probe EUKb1193 (Cy5) and the *Charonina ventriculi*-specific probe CHA1350 (Cy3). Images were obtained from two different fields (panels a-c and d-f) of fraction 3 of a rumen sample collected from cow C1. For each field, a bright field image (a, d) was collected together with one image using the Cy3 filter (b, e) and one image using the Cy5 filter (c, f). The scale bar represents 10 μm . Color replacement was used to show cells stained with EUKb1193 in blue, and those stained with CHA1350 in green.

FIG S3

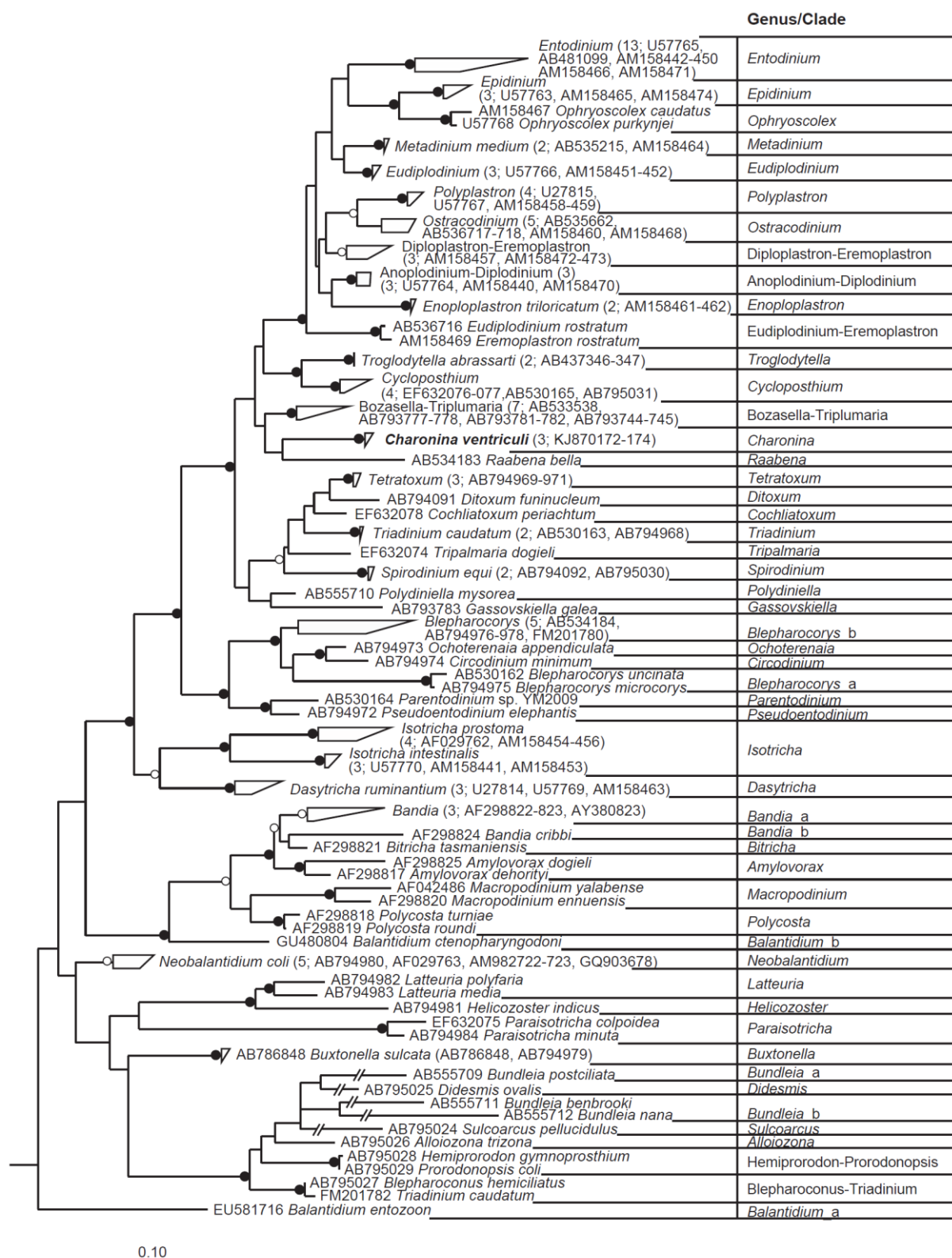


FIG S3 Randomized Accelerated Maximum Likelihood tree as shown in Fig. 4. This tree provides the genus/clade level designations used in File S1.

FILE S1 Accession numbers, taxonomic classifications and 18S rRNA gene sequences of all 168 almost full-length ($\geq 1,500$ bp) intestinal ciliate 18S rRNA gene sequences used in this study. All sequences contained *S. cerevisiae* positions 83-1,727, except for the sequences from a previous study, which were slightly shorter and contained positions 317-1,727 (1). Column A (“Cluster in tree”) represents the cluster that each sequence belongs when clusters in the tree are numbered from top to bottom, and allows sorting of sequences according to the phylogenetic tree. A taxonomy file and a sequence file (requires conversion into .fasta format) are provided in tabs “tax_file” and “seq_file”, respectively. This database is compatible with software such as QIIME (2) and may be used for BLAST-based taxonomic assignment of environmental sequences collected, for example, by using high-throughput next-generation sequencing technologies.

REFERENCES

1. **Tymensen L, Barkley C, TA McAllister.** 2012. Relative diversity and community structure analysis of rumen protozoa according to T-RFLP and microscopic methods. *J Microbiol Meth* **88**:1–6.
2. **Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Gonzales-Pena A, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widman J, Yatsunenko T, Zaneveld J, Knight R.** 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**:335–336.