

## ORIGINAL ARTICLE

# Endobiotic ciliates of the digestive tract of reindeer *Rangifer tarandus* from Yakutia

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## Summary

The fauna of ciliates - endobionts of the rumen of wild reindeer from Yakutia was investigated. In total, 23 species of ciliates belonging to 10 genera from the families Ophryoscolecidae and Isotrichidae were found. Among them, seven species of ciliates specific to reindeer have been identified. The comparative analysis of the species diversity of endobiotic ciliates in reindeers from different geographical regions was carried out. Based on molecular phylogenetic analysis using SSU and ITS-1 sequences, the question of the species status of *Epidinium gigas*, ciliates specific to reindeer, was discussed.

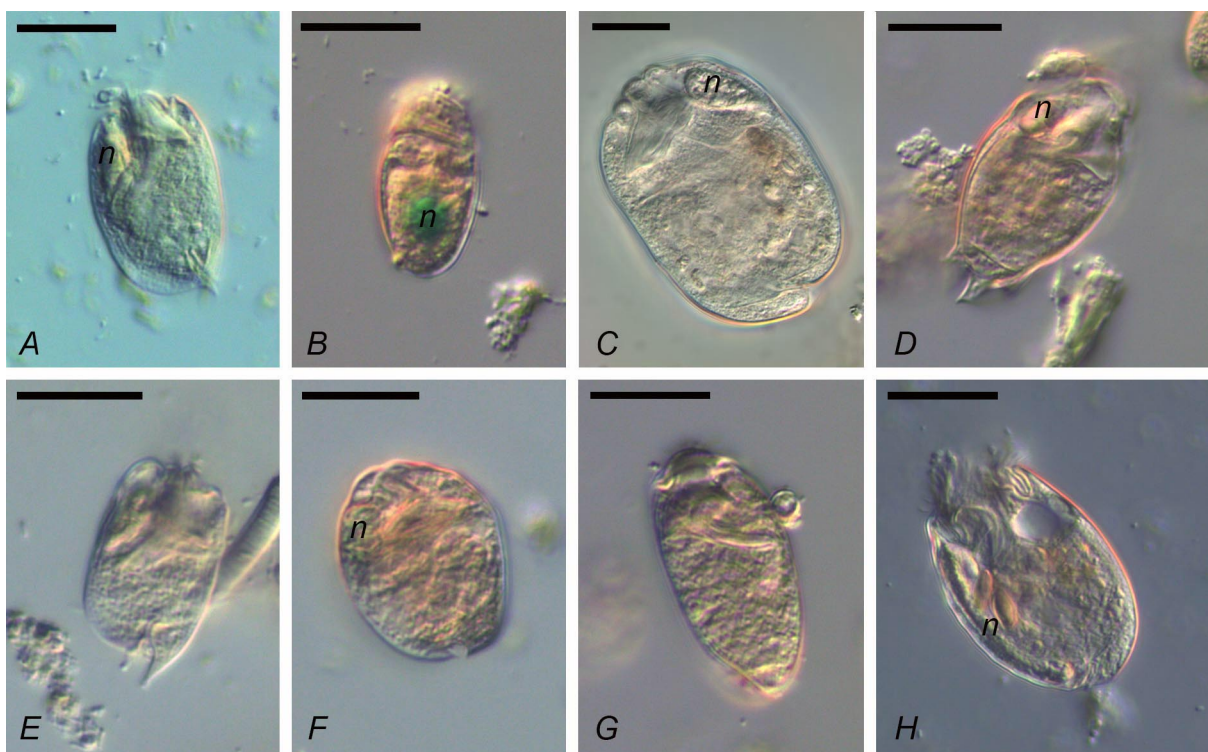
**Key words:** endobiotic ciliates, reindeer, species diversity, Yakutia, *Epidinium gigas*

## Introduction

Reindeer *Rangifer tarandus* is one of the few ruminants living in the Far North. In contrast to most other ruminants that mainly feed on higher plants, a considerable part of the reindeer diet consists of various lichens, especially in winter (Orpin and Mathiesen, 1990; Mathiesen et al., 2005). The ability of ruminants to effectively digest and assimilate plant foods, including cellulose, is due to the complex microbiomes in their forestomach (Sanjorjo et al., 2023). Assemblages of endobiotic ciliates are important components of these

microbiomes, and the specific diet of reindeer undoubtedly influences their structure.

Species composition of endobiotic ciliate assemblages has been studied in reindeers from Finland, Canada, European part of Russia, China, Alaska, Iceland, Spitsbergen, and Yakutia (Dogiel, 1925, 1929; Lubinsky, 1958 a, 1958b; Westerling, 1970; Imai et al., 2004; Kornilova et al., 2004; Mathiesen et al., 2005; de la Fuente et al., 2006; Machakhtyrov, 2009; Sleptsov et al., 2023). It has been shown that many ciliate species inhabiting the reindeer rumen are specific for this host (Dogiel, 1929; Lubinsky, 1958 a, 1958b; Imai et al., 2004). Nevertheless, the



**Fig. 1.** Endobiotic ciliates of the reindeer rumen; *Entodinium* spp. A – *Entodinium bicornutum*, B – *E. exiguum*, C – *E. anteronucleatum*, D – *E. quadricuspis*, E – *E. caudatum*, F – *E. furca*, G – *E. simplex*, H – *E. longinucleatum*. Scale bars: 20  $\mu$ m.

available data are insufficient for a comparative analysis of the structure of endobiotic ciliate assemblages in different subspecies of *R. tarandus* in wild and domestic reindeers. Meanwhile, this analysis would be useful for elucidating certain questions of general biological and practical importance. The former includes the study of possible dispersal routes and formation of the current species structure of *R. tarandus* in the light of the co-evolution of the host and its endobionts. In practical terms, understanding how endobiotic ciliate assemblages are formed may be helpful for elaborating the diet of reindeer in semi-free conditions. In addition, it remains unclear whether reindeer-specific ciliates are independent species that have evolved in association with their host or simply forms of the species inhabiting the forestomach of other ruminants. The study of endobiotic ciliates of reindeer using molecular genetic methods is of considerable importance in this regard.

Here we present the results of our study of the species composition of endobiotic ciliates of wild reindeer individuals from different regions of Yakutia. In addition, we ascertained the position of ciliate *Epitodinium gigas* on the molecular phylogene-

tic tree using the analysis of 18S RNA and ITS region sequences.

## Material and methods

Samples of rumen contents were collected from two individuals of wild reindeer from Eveno-Bytantai National District (sample 1, 2021) and Oymyakon (sample 2, 2022). Samples were fixed with 96% alcohol at a ratio of 1:20 (sample 1) and with 10% formalin at a ratio of 1:1 (sample 2) and stored in the dark at room temperature. To determine the species composition of the ciliates, a 100- $\mu$ l subsample was placed on a slide and viewed using a light microscope. Cells were stained with 1% methyl green solution in 9% acetic acid to reveal nuclei. The species of ciliates were determined according to Dogiel (1929) and Lubinsky (1958 a, b). Light-optical studies and microphotography were made using a Leica DM 2500 microscope with a Leica DFC 495 digital camera (Leica-Microsystems, Germany). Statistical processing of data was performed using Past 4.03 software (Hammer et al.,

**Fig. 2.** Endobiotic ciliates of the reindeer rumen; representatives of the families Ophryoscolecidae and Isotrichidae. A – *Diplodinium rangiferi*, B – *D. dogieli*, C – *Metadinium magnum*, D – *Epidinium ecaudatum*, E – *Dasytricha ruminantium*, F – *M. ypsilon*, G, H – *Eremoplastron spectabile*, I – *Ostracodinium obtusum*, J, K – *Enoploplastron confluens*, L, M – *E. triloricaum*, N, O – *Epidinium gigas*, P, Q – *Eremoplastron impalae*. Scale bars: A, C, N, O – 50 µm; B, D–M, P, Q – 20 µm.

2001). For DNA isolation, cells were collected one by one with a glass pipette using a Nikon SMZ 1270 stereomicroscope (Nikon Corporation, Japan). DNA extraction was performed using PicoPure™ DNA Extraction Kit (Thermo) according to the manufacturer's instructions. Amplification of the 18S DNA sequence was performed with primers 82F (5'-GAAACTGCGAATGGCTC-3'; Elwood et al., 1985) and EkyB (5'-TGATCCTTCTTCTGCAGGTTCCACCTAC-3'; Medlin et al., 1988) according to the protocol published by Ito et al. (2014). Sequence amplification of the ITS region was performed using primers SSU-end (5'-AAGGTWTCGGTCCGTAGGTGAACCTG-3') and LSU-start (5'-TAKTRAYA TGCTTAAG

TYCAGCG-3'), according to the protocol of Snoeyenbos-West et al. (2002). DNA purification was performed using the Cleanup S-Cap kit (Evrogen). Sanger sequencing was performed using three primers for 18S DNA: 82F, Jap2F (5'-TTTGCCAA GGAT GATGTTTTTC-3'; Ito et al., 2014), and Jap1R (5'-CTTGGGGCAAATGCTTTTCGC-3'; Giribet et al., 1996) and two primers for the ITS region. The 18S rRNA and ITS region sequences obtained in this way were stored at NCBI Genbank (PQ009215 and PQ009221, correspondingly) and used for phylogenetic analyses.

The phylogenetic analysis included sequences of other Ophryoscolecidae species available at NCBI GenBank. Among them were 47 18S rRNA



Table 1. Continuation.

| Endobiotic ciliates                  | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8 | 9  | 10 | 11 | 12 | 13 |
|--------------------------------------|----|----|----|----|----|----|----|---|----|----|----|----|----|
| <i>D. ruminantium</i> Schuberg, 1888 | +  | +  |    | +  | +  |    | +  |   | +  | +  | +  | +  | +  |
| Number of species                    | 18 | 20 | 20 | 19 | 15 | 18 | 19 | 6 | 10 | 15 | 18 | 20 | 17 |

**Notes:** 1 – China (Imai et al., 2004), 2 – Finland (Westerling, 1970), 3 – Canada (Lubinsky, 1957 a, b), 4 – Russia, European part, domestic reindeer (Dogiel 1925, 1929), 5 – Russia, European part, wild reindeer (Dogiel 1925, 1929), 6 – Alaska (Imai et al., 2003), 7 – Iceland (de la Fuente et al., 2006), 8\* – Svalbard (Mathiesen et al., 2005; this publication gives only dominant species, and they were not included in the analysis), 9 – Yakutia, Chukchi breed of reindeer (Sleptsov et al., 2023), 10 – Yakutia, Evenk breed of reindeer (Sleptsov et al., 2023), 11 – Yakutia, “Tabsylyn” reindeer farm (Kornilova et al., 2004; Machakhtyrov, 2009), 12 – Yakutia, Eveno-Bytantai National District, wild reindeer, 13 – Yakutia, Oymyakon, wild reindeer.

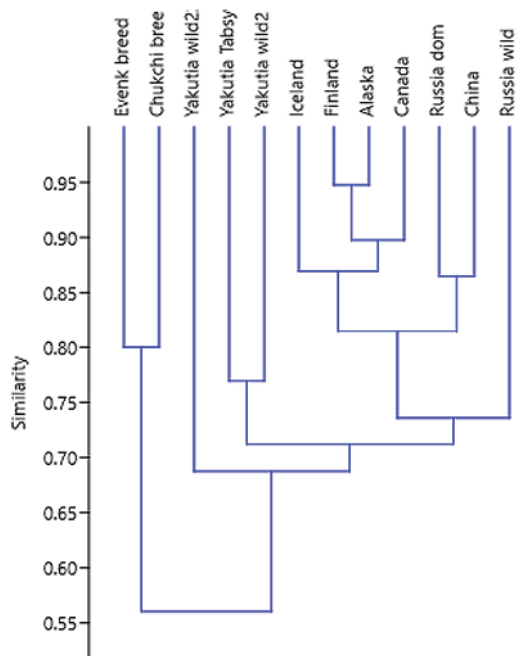
sequences (Supplementary Table S1) and 27 ITS region sequences (Supplementary Table S2). The nucleotide alignments were obtained using MUSCLE algorithm with standard parameters in AliView software (Larsson, 2014). The final length of alignment for 18s rRNA was 1663 bp and for ITS region 562 bp. A phylogenetic trees based on the maximum likelihood model were constructed in IQ-TREE v1.6 software (Nguyen et al., 2015), with the best evolutionary model TIM2+F+I+G4 selected by Bayesian information criterion by the inbuilt ModelFinder algorithm (Kalyaanamoorthy et al., 2017). Branch supports were calculated using the bootstrap method with 1000 repetitions (Hoang et al., 2018). Bayesian analysis was performed based on the evolutionary model GTR + I + G on CIPRES v.3.1 (<https://www.phylo.org/>) using MrBayes (Ronquist and Huelsenbeck, 2003) on XSEDE v.3.2.6 (Miller et al., 2010) with the following parameters of the MSMS algorithm: 2 independent runs with four independent chains at 2,000,000 generations, every 100th tree sampled and 25% of the first trees discarded (burn-in plot).

## Results and discussion

The rumen contents of wild reindeers from Eveno-Bytantai National District and Oymyakon were found to contain 20 and 17 species of endobiotic ciliates, respectively (Table 1, Figs 1, 2). The species composition of endobionts was quite similar to that of wild and domestic reindeers from other geographical regions (Table 1). We found almost all specific endobionts of reindeer in both samples, with the exception of *Metadinium magnum*, which was found only in the sample from Oymyakon. It is noteworthy that *Entodinium anteronucleatum*, *E. bicornutum*, *E. quadricuspis*, and *Diplodinium rangiferi* have been found in reindeers from every

population examined in this respect. Differences in the species composition of the endobiotic ciliates of the rumen in the wild and domestic reindeers from different regions of Yakutia can be explained, among other things, by differences in their diet. For instance, it has been reported that the species diversity of endobiotic ciliates in reindeer of the Chukchi breed living in the tundra is lower than in reindeer of the Evenk breed living in the mountain taiga zone, which is characterized by richer forage vegetation compared to the tundra (Sleptsov et al., 2023).

The results of the cluster analysis indicate that the greatest similarity in the species composition of endobiotic ciliates is observed in reindeers from Finland, Iceland, Alaska, and Canada (Fig. 3). It should be noted that in the late 19<sup>th</sup> and early 20<sup>th</sup> century, reindeer husbandry was promoted in Alaska and Canada (Swanson and Barker, 1992). For this, domestic reindeers from Siberia and Scandinavia were imported to Alaska (Swanson and Barker, 1992; Lincoln, 2014). By the summer of 1904, the total number of imported and breeding reindeer in Alaska exceeded 8,000 animals (Jackson, 1905). In mainland Canada, an attempt to import several dozen domestic reindeers from Norway was made in 1911. In 1935, over 2,000 domestic reindeers were brought into Canada from Alaska (Scotter, 1972). Importantly, reindeer husbandry involves free grazing, with domestic reindeers often joining herds of wild or feral individuals (Scotter, 1972). Thus, the exchange of endobiotic ciliates between domestic reindeers imported from Eurasia and American caribou is highly probable. It is interesting that mitochondrial DNA analysis indicates that a population of reindeer from China descended from domestic reindeer population inhabiting northern parts of central Russia (Wang et al., 2019). This may explain the high level of similarity in the species composition of ciliate endobionts of domestic reindeers from European Russia and China.



**Fig. 3.** LDendrogram based on the results of cluster analysis of the species composition of endobiotic ciliate communities of reindeer from different geographical regions using the Bray-Curtis coefficient.

The question of species vs. forms in the endobiotic ciliate fauna of reindeer deserves a special discussion. Entodiniomorphid ciliates are characterized by a high level of polymorphism, including cell size and the degree of development of various cell surface outgrowths (Kornilova, 2004). At the same time, these characters are often used to differentiate species of endobiotic ciliates. The boundary between species and forms in entodiniomorphids is therefore obscure. So far, even molecular phylogenetic analysis cannot provide unambiguous answers, mostly because of the scarcity of the data. The sequence of the 18S RNA gene has been determined only for a few endobiotic ciliates and, in most of these cases, only for one isolate of the species. Information on other markers is even more scarce. Therefore, the level of intra- and interspecific differences cannot be assessed even for the 18S RNA sequence.

In this study, we obtained the first sequences of 18S RNA and ITS region of *Epidinium gigas*, a specific endobiont of reindeer. This ciliate was described by Dogiel as a form of *E. ecaudatum*, namely *E. ecaudatum* f. *gigas* (Dogiel, 1925, 1929). This form was then elevated to the rank of the species

(Kofoid and MacLennan, 1932, 1933) and has been generally considered as such by most researchers (Lubinsky, 1958b; Imai et al., 2004; de la Fuente et al., 2006). However, our data suggest that *E. gigas* is actually a form of *E. ecaudatum*, although a definitive conclusion cannot be made yet.

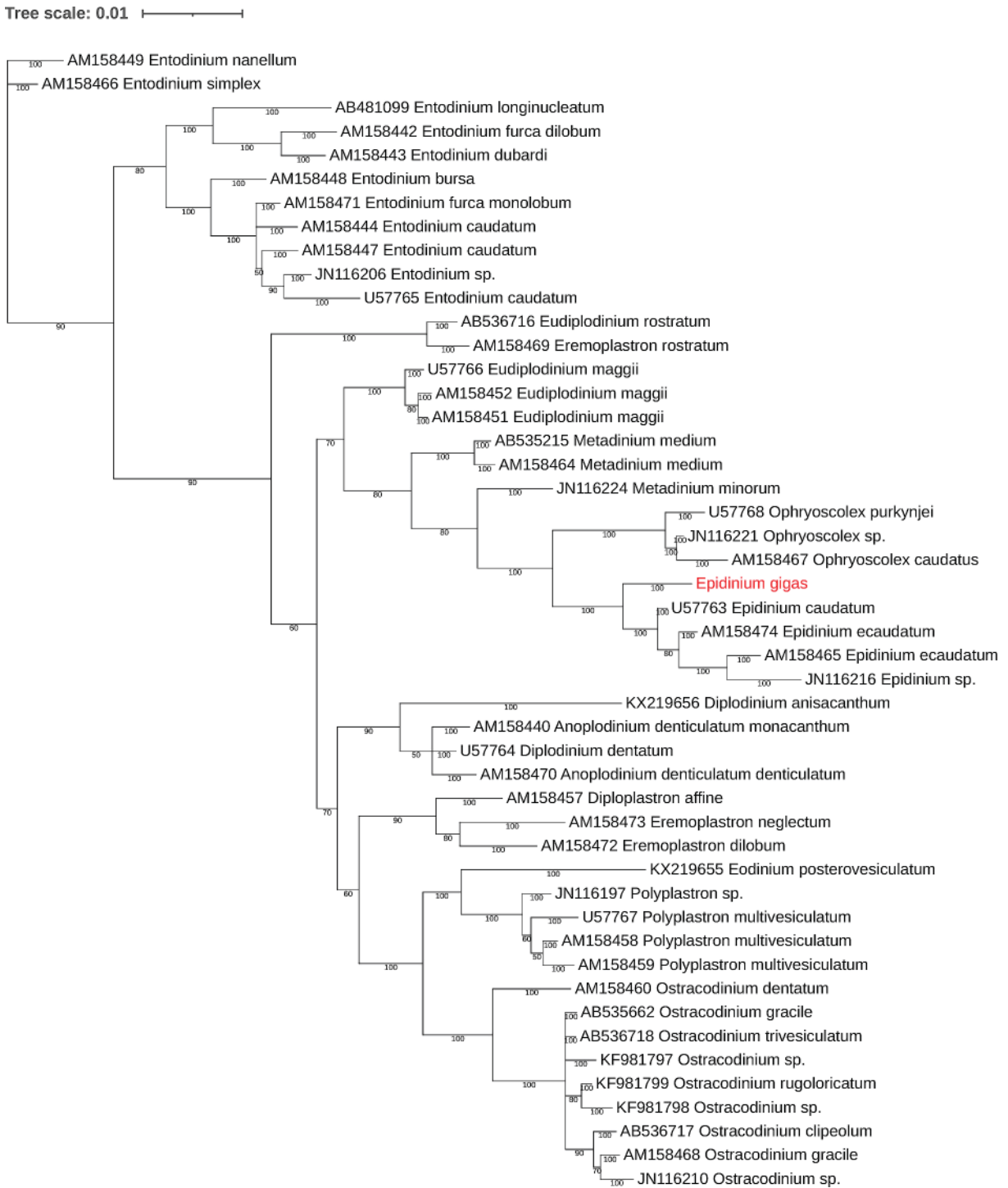
Based on the results of molecular phylogenetic analysis, *E. gigas* forms a single clade with *E. ecaudatum* and *E. ecaudatum caudatum* (Figs 4–6). These two forms differ in the presence of caudal spines, which is a variable character depending on environmental conditions. *E. ecaudatum* f. *gigas* reflects the tendency to gigantism, which is generally characteristic of endobiotic ciliates of reindeer (Dogiel, 1925).

### Acknowledgments

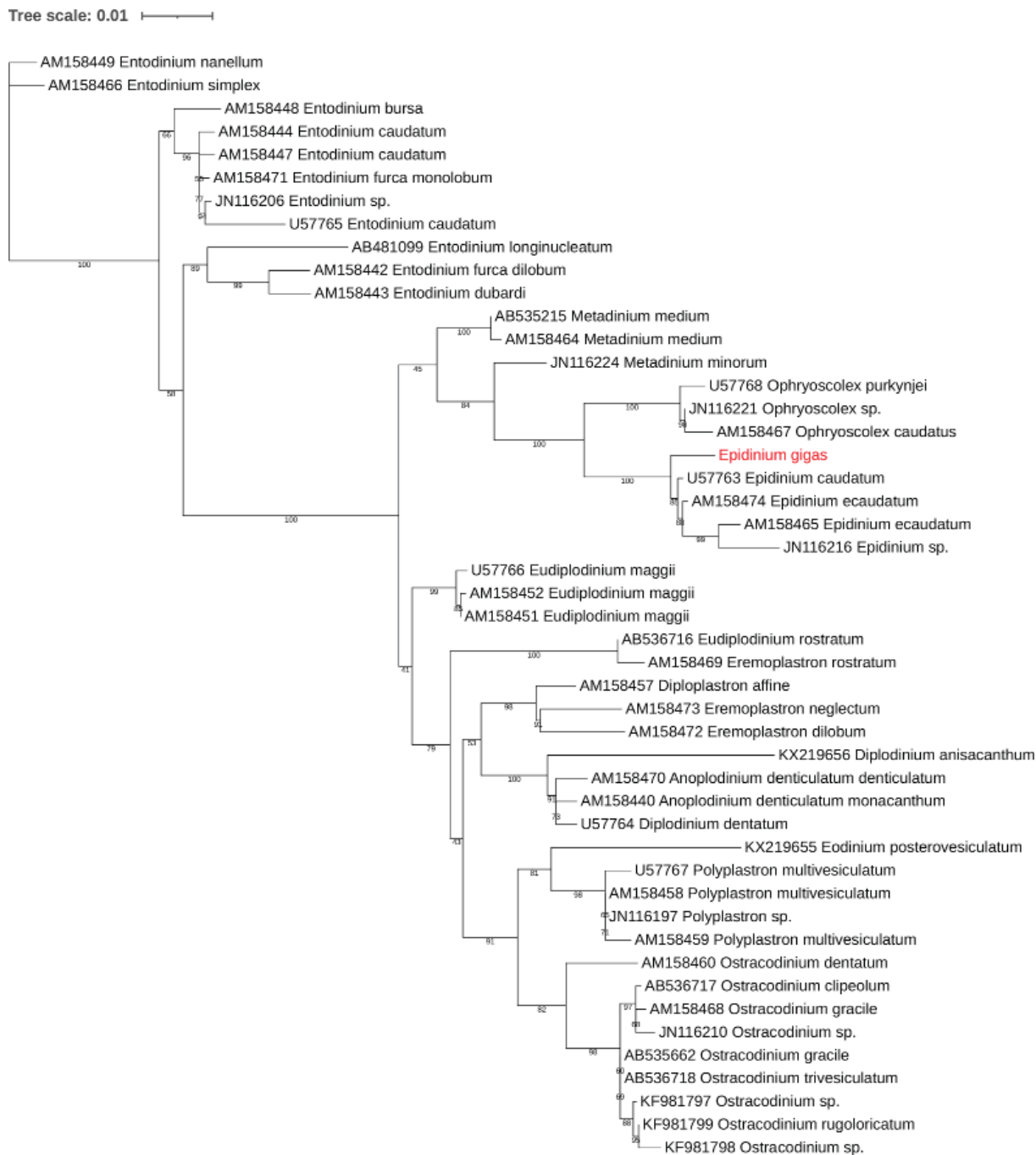
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**Fig. 4.** Phylogenetic tree of members of the family Ophryoscolecidae based on Bayesian analysis of SSU sequences. Values of posterior probabilities are given on the branches (in %). Scale bar reflects genetic distance (number of substitutions per nucleotide).



**Fig. 5.** Phylogenetic tree of members of the family Ophryoscolecidae based on maximum likelihood analysis of SSU sequences. Supports on branches were calculated by bootstrap method at 10,000 repetitions. Scale bar reflects genetic distance (number of substitutions per nucleotide).

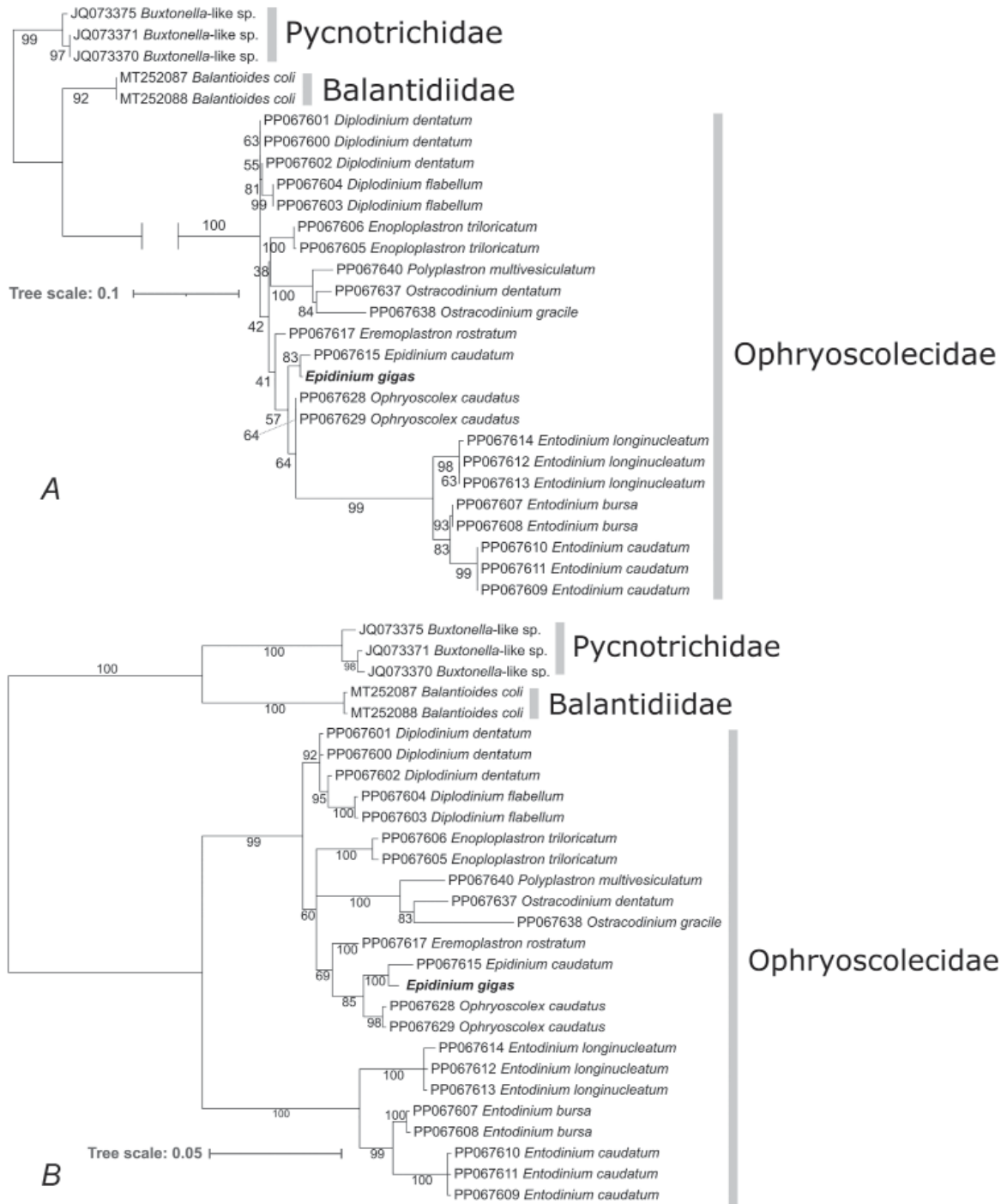
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**Fig. 6.** Phylogenetic trees of members of the family Ophryoscolecidae based on maximum likelihood analysis (a) and Bayesian analysis (b) of ITS region sequences. A – supports on branches were calculated by bootstrap method at 10,000 repetitions. Scale bar reflects genetic distance (number of substitutions per nucleotide). B – values of posterior probabilities are given on the branches (in %). Scale bar reflects genetic distance (number of substitutions per nucleotide).

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## **Supplementary materials**

**Table S1.** List of SSU sequences from GenBank used for phylogenetic analysis.

**Table S2.** List of ITS region sequences from GenBank used for phylogenetic analysis.