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Morphology and phylogeny of a new soil ciliate, *Colpodidium zelihayildizae* n. sp. (Ciliophora, Nassophorea, Colpodidiidae), from Van, Turkey

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Abstract: A new ciliate species *Colpodidium zelihayildizae* n. sp., which was discovered in a dry irrigation channel in Van (Turkey), was investigated morphologically using standard live and silver impregnation methods, and its SSU rRNA gene phylogeny was provided. This new species was characterized as follows: body size about 40–60 × 15–20 μm in vivo, elongated ellipsoidal with flat or slightly concave ventral surface; the buccal cavity slightly below equatorial plane; macronucleus spherical or slightly elliptical, usually located in the last third of the cell; cortex distinctly furrowed in the anterior half; 19–22 somatic kineties, 4 postoral kineties; paroral membrane has 20 dikinetids; 3 nassulid organelles (NOs), with NO1 very close to the left edge of the paroral membrane, which consists of 1 dikinetid pair. Phylogenetic analyses conducted on the SSU rDNA sequences determined that *Colpodidium zelihayildizae* was clustered with *Colpodidium caudatum* and formed a clade with *Apocolpodidium etoschense* and Colpodididae sp.

Key words: Soil ciliates, *Colpodidium zelihayidizae* n. sp., description, phylogeny, 18S rRNA

1. Introduction

Soil ciliates adapt to various terrestrial habitats due to their ability to form cysts. They can survive for a long time in arid environments and develop active populations in moist environments. These organisms are an important component of the nutrient network in soil habitats as they contribute to the mineralization of organic matter, feed on organisms that are smaller than them, and serve as prey for organisms that are larger than them (Foissner et al., 2002; Foissner et al., 2005; Kim et al., 2016; Mamedova and Alekperov, 2016). Although there are almost 1000 species of ciliates that have been discovered and defined from terrestrial habitats (Foissner, 1993; Foissner et al., 2002), it has been estimated that this number would be much higher if the unexplored regions of the world were taken into consideration (Finlay and Fenchel, 1999; Foissner et al., 2002; Chao et al., 2006; Foissner et al., 2008). In recent years, molecular taxonomic studies have shown that many of the ciliate species that are morphologically indistinguishable, and the number of ciliates is far beyond what has been predicted (Vďačný and Foissner, 2017; Warren et al., 2017).

The genus *Colpodidium* was established by Wilbert (1982), who described *Colpodidium caudatum*. The genus *Colpodidium* was assigned to the family Colpodidae (in class Colpodea) due to its somatic dikinetids and 2 ciliary fields, located in an inconspicuous vestibulum. However, Foissner (1990) reinvestigated the type species and reported that the description given by Wilbert contained serious mistakes and misinterpretations. Based on various morphological investigations, Foissner (1990) revealed that *Colpodidium caudatum* had monokinetal somatic ciliature, 3 adoral membranelles, and a tight irregular silverline system and therefore indicated that this species was closer to the family Furgasonidae (order Nassulida) than to the family Colpodidae (order Colpodida).

Later, Foissner (1995) isolated a population of *C. caudatum* from Africa (Mombasa, Kenya), described it in detail using various taxonomical techniques, and transferred the genus *Colpodidium* to the newly established family Colpodidiidae (class Nassophorea). After this, Foissner et al. (2002) separated the genus *Colpodidium* into 2 subgenera (*Colpodidium* and *Pseudocolpodidium*) and described 4 new species [*Colpodidium* (*Colpodidium*) *horribile*, *Colpodidium* (*Colpodidium*) *trichocystiferum*, *Colpodidium* (*Colpodidium*) *microstoma*, and *Colpodidium* (*Pseudocolpodidium*) *bradburyarum*].

The genus *Colpodidium* is characterized by a horn-shaped buccal cavity that is directed anteriorly or rightwards, slightly or distinctly curved paroral membrane surrounds the right and anterior side of the buccal cavity; NO3 is oriented obliquely or perpendicularly to the main

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body axis, some of the postoral kineties (at least postoral kinety 2, PO2), which commences in the buccal cavity) possesses dikinetids anteriorly (Foissner, 1995; Foissner et al., 2002).

In this study, which was conducted on soil samples that were obtained from a dry irrigation channel on the Van Yüzüncü Yıl University campus, a population of the genus *Colpodidium* was observed. After detailed investigations, using both live and silver impregnation techniques and phylogenetic analyses, it was confirmed that this population belonged to a new *Colpodidium* species.

2. Materials and methods

2.1. Sampling and cultivation

Soil samples were collected from a dry irrigation channel located on the Van Yüzüncü Yıl University campus (about 1675 m above sea level) in September 2016, in Van, Turkey. About 3 kg of soil sample was dried in-shade in the Laboratory of Protozoology. Ciliates were cultivated by employing the non-flooded Petri dish technique, as reported by Foissner (1987) and Vďačný and Foissner (2012), and semolina grains were added to the cultures to stimulate the bacterial growth that would feed the ciliates. The cultures were maintained for up to 5 weeks after which the aged cultures were discarded and new ones were made.

2.2. Morphological investigation

The ciliates were observed live under a Leica S8 stereo microscope (Wetzlar, Germany) at 20–80× magnification and a ZEISS Axio Imager 2 compound microscope (Oberkochen, Germany) at 400–100× magnifications using ordinary, phase contrast, and interference-contrast optics. During the live observations, special attention was paid to the general morphology of the cells, location and shape of the nuclear apparatus, the position of the oral cavity and contractile vacuole, and presence or absence of extrusomes. The Chatton–Lwoff silver nitrate and silver carbonate impregnation techniques were used to reveal the ciliature and nuclear apparatus (Foissner and Xu, 2007; Vďačný and Foissner, 2012; Foissner, 2014). Measurements of the cells were conducted using an ocular micrometer. Drawings of the cells were performed using Adobe Photoshop v.CS5 software (San Jose, CA, USA) on a computer, on the basis of photomicrographs and free-hand sketches. The observed cells were identified based on the descriptions of Wilbert (1982), Foissner (1995), and Foissner et al. (2002). The terminology and classifications were mainly performed based on those of Foissner (1995) and Foissner et al. (2002).

2.3. DNA isolation, PCR amplification, and gene sequencing

The nuclear DNA of *Colpodidium zelihayildizae* n. sp. was isolated from 1 or 2 cells. The cells of the new species were selected from the subsamples of non-flooded Petri dish cultures, washed repeatedly with sterile distilled water to avoid possible contamination, placed into 200 mL tubes with about 1–2 mL of distilled water, and stored in a deep freezer at –65 °C for several weeks. Nuclear DNA was extracted from the cells using the REDExtract-N-Amp Tissue PCR kit (Sigma-Aldrich, St. Louis, MO, USA), following the manufacturer's instructions and the modifications of Gong et al. (2007) and Yıldız (2018). Amplification of the SSU rDNA gene of *Colpodidium zelihayildizae* n. sp. was performed using EukA (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and EukB (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′) primers (Medlin et al., 1988). The cycling conditions were as follows: an initial denaturation at 94 °C for 2 min, 40 cycles at 95 °C for 45 s, 60 °C for 1 min, 72 °C for 3 min, and a final extension at 72 °C for 8 min (Yıldız, 2018). The PCR products were sequenced with PCR primers and the internal forward primer SR7R (5′-AGT TAA AAA GCT CGT AGT GT-3′). The final SSU rDNA gene sequence was obtained by assembling a total of 9 sequence fragments that were derived from 3 PCR products in a CodonCode Aligner (Centerville, MA, USA).

2.4. Phylogenetic analyses

The SSU rRNA gene sequence obtained from *C. zelihayildizae* n. sp. was aligned with the sequences of 60 other ciliates that were retrieved from GenBank (NCBI: National Center for Biotechnology Information) (see Figure 6 for the accession numbers) using in Mega 6.0 software (Tamura et al., 2013). As the outgroup, 2 karyorelict (*Loxodes striatus* and *Trachelorаphis* sp.) and 2 heterotrich (*Blepharisma sinuosum* and *Spirostomum ambiguum*) ciliates were used. The resulting alignment was masked using G-blocks v.091b, allowing gap positions within the final blocks (Talavera and Castresana, 2007). The maximum likelihood tree was computed using the TIM2+G+I+F4 model, with IqTree v.1.6.3 and 1000 nonparametric bootstrap replicates (Nguyen et al., 2015; Hoang et al., 2018). jModelTest v.2.1.7 selected TIM2+I+G as the best model according to the Akaike information criterion (Posada, 2008). Bayesian inference (BI) was conducted on the determined model with MrBayes v.3.2.2 (Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012), which was run with 2 sets of 4 chains for 3 million generations and a sampling of 100 generations. The first quarter of the sampled trees was eliminated as burn-in. The rest of the Bayesian trees were used to build a 50% majority rule consensus tree and calculate its posterior probabilities and branch length.

3. Results

Phylum: Ciliophora Doflein 1901
Class: Nassophorea Small and Lynn, 1981
Order: Colpodiidida Foissner et al., 2002

Family: Colpodiidae Foissner, 1995
Genus: *Colpodidium* Wilbert, 1982
Colpodidium zelihayildizae n. sp. (Figures 1a–1i, 2a–2h, and 3a–3f; Table)

Diagnosis: Size about 50 × 20 µm, in vivo; macronucleus in the last third of the cell. Cytoplasm colorless and densely granulated. No extrusomes. On average, 21 somatic ciliary rows and paroral membrane has 20 dikinetids.

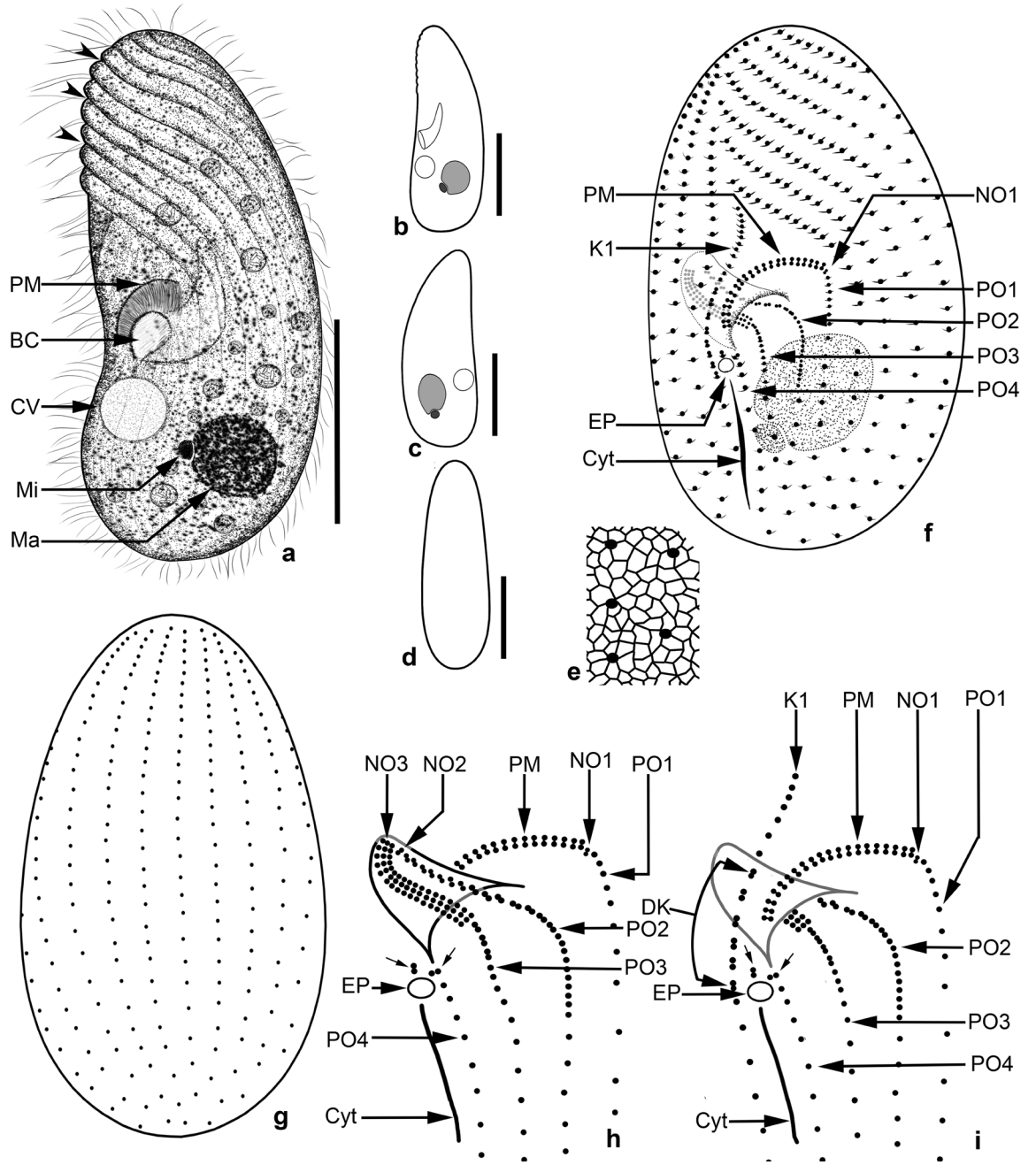

Figure 1. Morphology, infraciliature, and cortical structure of *C. zelihayildizae* n. sp. live (a– d), and silver nitrate- and silver carbonate-stained (f–j). a: Ventral view of a representative individual. b–d: Left lateral, right lateral, and dorsal view, respectively, to show general body outline, buccal cavity, contractile vacuole, and nuclear localization. e: Part of the pellicle to show silverline system. f, g: Infraciliature in ventral (f) and dorsal view (g). h, i: Oral ciliature in different focal levels. BC: buccal cavity, CV: contractile vacuole, Cyt: cytopyge, DK: dikinetid part of K1, EP: excretory pore, K1: the kinety on the right side of oral apparatus, Ma: macronucleus, Mi: micronucleus, NO1–NO3: nassulid organelles 1–3, PM: paroral membrane, PO1–PO4: postoral kinety 1–4. Small arrows (h, i) show dikinetids above the excretory pore. Scale bars 20 mm.

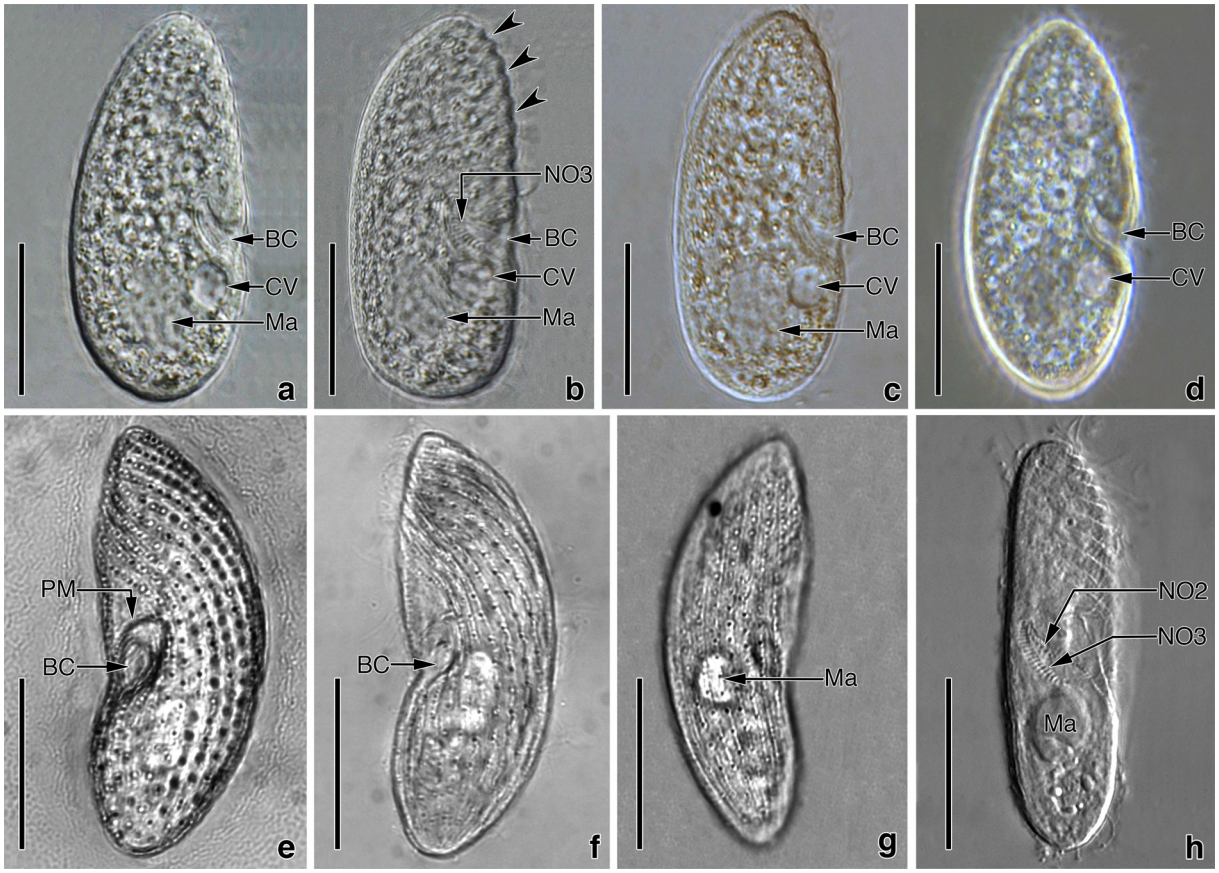

Figure 2. Micropotographs of *C. zelihayildizae* n. sp. live (a–d) and silver nitrate-stained (e–f). BC: buccal cavity, CV: contractile vacuole, Ma: macronucleus, NO2: nassulid organelle 2, NO3: nassulid organelle 3, PM: paroral membranelle. Arrowheads show the furrowed edge of the antero-ventral surface. Scale bars 20 mm.

Buccal opening slightly below the equatorial plane. NO3 composed of 16–17 ciliary rows, each with 3 basal bodies.

Type locality: A dry irrigation channel located in the grassed area of the Faculty of Agriculture of Van Yüzüncü Yıl University (38°34′22′′ N, 43°17′36′′ E).

Type slides: The holotype (2020/H01) and 2 paratypes (2020/P01 and 2020/P02) slide with silver nitrate impregnated specimens were deposited in Van Yüzüncü Yıl University, Ciliate Research Collection, Van, Turkey.

Dedication: The name of the species, *zelihayildizae*, was chosen in dedication to the author's mother, Zeliha Yıldız, who recently passed away.

Description: Size 40–60 × 15–20 mm, usually 50 × 20 µm in vivo. Shape fairly constant, lateral view elongated-ellipsoidal with more or less flat ventral and distinctly convex dorsal side (Figures 1a–1d and 2a–2h); length:width ratio 2.3–3.4:1, usually 3:1 in silver nitrate impregnated specimens (Table). Laterally slightly flattened, ventral and dorsal view elongated ellipsoidal (Figure 1d). Macronucleus underneath the mid-body, usually in the last quarter, ellipsoidal to spherical (Figures 1a–1c, 1f, 2a–2c, 2h, and 3a–3c), about 9 × 6 µm in size (Table), with the roundish nucleolus. Micronucleus globular to ellipsoidal (Figures 1a–1c), difficult to recognize because of the small and in shallow indentation of the macronucleus, about 1.75–2.0 µm across. Contractile vacuole subequatorial, underneath the buccal cavity (Figure 1a), surrounded by small vesicles during diastole; excretory pore in line with the paroral membrane underneath the right edge of buccal entrance (Figures 1f, 1h, and 1i). Cytopyge commences from underneath the excretory pore and extends up to almost the posterior pole (Figures 1f, 1h, and 1i). No extrusomes recognizable in either the live or impregnated specimens. Cortex distinctly furrowed by ciliary rows, especially in the anterior half (Figures 1a, 2b, and 2h). Cytoplasm colorless, contains many bright fat globules and food vacuoles with bacterial remnants (Figures 1a, and 2a–2d). Swims by rotation around the main body axis.

Somatic ciliary rows consist of 19–22 monokinetal ciliary rows and are equidistantly arranged (Figures 1f, 1g, and 3a–3c; Table), except for more widely spaced postoral kineties (Figures 1f, 1h, 1i, 3a, 3b, 3d, and 3e). Left antero-lateral kineties on the ventral surface (about 8–9 rows) sinistrally twisted, gradually shortened from the

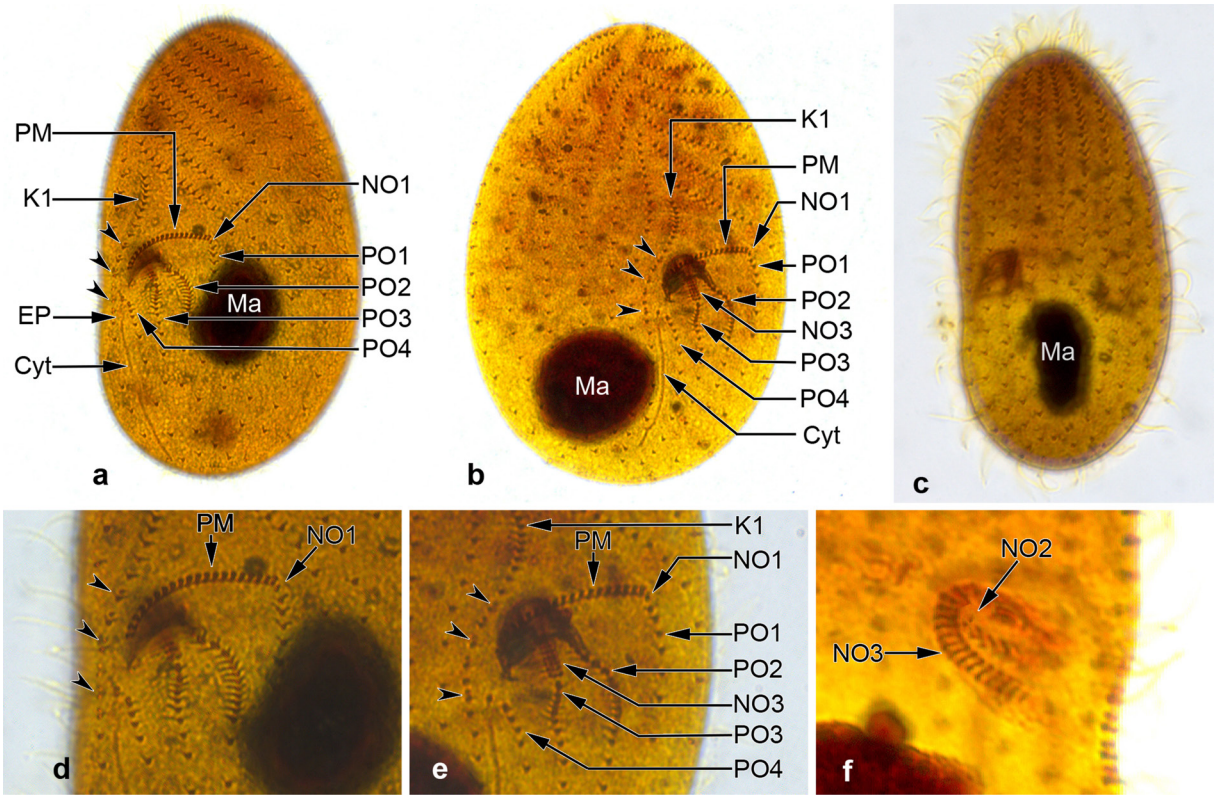

Figure 3. Microphotographs of *C. zelihayildizae* n. sp. with silver carbonate staining (a–f): a, b: Ventral ciliature of representative individuals. c: Dorsal ciliature of representative individual. d, e: Oral ciliature to show paroral membranelle, NO1 and NO3, and postoral kineties 1–4. f: Oral ciliature to show NO2 and NO3. Arrowheads in Figure d show the dikinetidal section of K1. Cyt: cytopyge, EP: excretory pore, K1: the kinety on the right side of oral apparatus, Ma: macronucleus, NO1–NO3: nassulid organelles 1–3, PM: paroral membrane, PO1–PO4: postoral kinety 1–4, Arrowheads in figure d show dikinetidal section of K1.

anterior body end to oral apparatus, and form a prominent preoral suture (Figures 1f, 2e, 2f, 2h, 3a, and 3b). Right ventro-lateral kineties almost parallel to the main body axis, except for K1, which is sigmoidally curved (Figures 1f, 1i, and 3a–3c). K1 commences pre-equatorially with closely spaced monokinetids, extends along the right edge of the oral apparatus with dikinetids restricted to the area between the right side of the paroral membrane and excretory pore of the contractile vacuole, and terminates close to the posterior pole with ordinarily spaced (5–7 kinetids) monokinetids (Figures 1f, 1i, 3a, 3b, 3d, and 3e). Three or 4 of the right ventro-lateral kineties shortened gradually from the anterior end to the oral apparatus (Figures 1f, 2e, 2f, 2h, 3a, and 3b). A blank area without kineties above the paroral membrane (Figures 1f, 2a, and 2b). Four postoral kineties (PO1–PO4): PO1 at the left edge of the buccal cavity, almost parallel to the main body axis, commences beneath NO1 and the proximal end of the paroral membrane, monokinetal throughout; PO2 commences in the proximal region of the buccal cavity, dikinetidal in the anterior half, monokinetidal in the posterior; PO3 commences at the distal end of NO3, dikinetidal anteriorly, monokinetidal posteriorly; and PO4 commences on the left side of the excretory pore of the contractile vacuole, monokinetidal throughout; 2 dikinetid pairs above the excretory pore (Figures 1f, 1h, 1i, 3a, 3b, 3d, and 3e).

Somatic cilia about 8–10 mm long in live specimens (Figure 1a), cilia very closely spaced in the anterior region of PO2 and PO3, and K1 (Figures 1f, 2a, 2b, 2d, and 2e).

The distance from the apex to the anterior end of paroral membrane and oral cavity is 43% and 50% of the body length, respectively (Table). Considering that the buccal opening is 5–6 µm in size, the oral apparatus is slightly under the equatorial plane. Buccal cavity horn-shaped (Figures 1a, and 2a–2d) and about 8–9 µm deep, contains NO2 and NO3, and the anterior portion of PO2 (Figures 1f, 1h, 1i, 3b, 3d, and 3e). NO1 between the left edge of paroral membrane and anterior end of PO1, small because it consists of only 2 kinetosomes, difficult to distinguish from the kinetosome pairs of the paroral membrane (Figures 1f, 1h, 1i, 3a, 3b, 3d, and 3e). NO2 is at the proximal end of PO2 and difficult to recognize (Figures 1h and 3f). NO3 extends on the dorsal wall of the

Table. Morphometric data for *C. zelihayildizae.*

Characteristics	x̄	M	SD	SE	CV	Min	Max	N
Body, length	51.8	52.0	4.9	0.9	9.4	40.0	60.0	27
Body, width	17.5	17.0	1.8	0.3	10.1	15.0	21.0	27
Body length:width ratio	3.0	3.0	0.3	0.1	9.3	2.3	3.4	27
Macronucleus, length	8.8	9.0	1.3	0.2	14.6	6.0	11.0	27
Macronucleus, width	6.4	6.0	1.2	0.2	17.9	5.0	9.0	27
Macronucleus length: width ratio	1.4	1.3	0.3	0.1	20.8	1.0	2.0	27
Anterior body end to macronucleus, distance (A)	31.4	31.0	4.1	0.8	13.0	24.0	40.0	27
Body length:A, ratio	1.7	1.6	0.1	0.0	8.5	1.4	2.0	27
Micronucleus, length	2.1	2.0	0.5	0.1	22.9	1.5	3.0	14
Micronucleus, width	1.8	1.5	0.4	0.1	24.4	1.5	3.0	14
Micronucleus length:width, ratio	1.2	1.3	0.2	0.0	15.4	1.0	1.5	14
Anterior body end to left end of paroral membrane, distance (B)	22.6	23.0	2.1	0.4	9.1	19.0	26.0	21
Body length:B, ratio	2.3	2.3	0.1	0.0	5.3	2.1	2.6	21
Cytostome, length	8.5	8.5	0.5	0.2	6.4	8.0	9.0	6
Anterior body end to cytostome, distance (C)	25.7	26.0	2.2	0.5	8.6	21.0	30.0	20
Body length: C, ratio	2.0	2.0	0.1	0.0	4.3	1.9	2.3	20
Anterior body end to excretory pore, distance (D)	33.3	33.0	3.1	0.7	9.3	28.0	40.0	21
Body length:D, ratio	1.6	1.6	0.1	0.0	5.5	1.4	1.7	21
Somatic kinety, number	20.6	20.0	1.0	0.2	5.1	19.0	22.0	22

CV: coefficient of variation; M: median; Max: maximum value; Min: minimum value; N: number of specimens evaluated; SD: standard deviation of arithmetic mean; SE: standard error of arithmetic mean; x̄: arithmetic mean.

buccal cavity to the cytostome, orientated obliquely to the main body axis, proximal end bent in a hook-like shape (Figures 1h, 2h, 3b, and 3e), composed of about 16–17 ciliary rows with 3 basal bodies each. Paroral membrane extends in the flat bow along the right and upper margins of the buccal opening conspicuously, composed of 20 dikinetids that are orientated perpendicularly to the kinety axis, equidistantly spaced (Figures 1a, 1f, 1h, 1i, 2e, 3a, 3b, 3d, and 3e). Silverline system tight and irregularly meshed (Figure 1e).

3.1. Phylogenetic analyses

The small subunit rDNA sequence of *C. zelihayildizae* n. sp. was recorded in GenBank under accession number MW411350. The length and GC ratio of the current sequence were 1657 bp and 44.72%, respectively. With the current new species included, the genus *Colpodidium* now comprises 6 species. Of these, the SSU rDNA gene sequences of the other species, except for *C. caudatum*, are currently unavailable. When the SSU-rDNA gene sequences of *C. caudatum* and *C. zelihayildizae* n. sp. were aligned, it was observed that this organism had a difference of 69 base positions (Figure 4) (3 insertions, 14 deletions, 29 transitions, and 23 transversions).

The topologies of both the maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees were similar, thus only the ML tree was presented with the bootstrap and posterior probability values on the branches in Figure 5. The phylogenetic tree topologies obtained using the different algorithms strongly supported the finding of monophyletic groups at the class level (except for Nassophorea) of taxa in phylum Ciliophora. Nassophorea was distributed into 3 clades and most of its members were clustered with different lineages. The clade Nassulida, where *Colpdidium zelihayildizae* n. sp. was included, was separated into 3 subclades, namely Colpodidiidae, Nasuliidae, and Furgasonidae. *Apocolpodidium etoschense* was branched as a sister group with the *C. caudatum* and *C. zelihayildizae* n. sp. clade (ML/BI, 86/0.70) and Colpodidae sp. formed a sister taxon to the branch that comprised the above 3 species (ML/BI, 100/1.00). *C. zelihayildizae* n. sp. showed the nearest relationship to *C. caudatum* (ML/BI 99/1.00), with a genetic distance of 3.6% based on the Kimura-2 parameter model.

4. Discussion

Foissner et al. (2002) separated the genus *Colpodidium* into 2 subgenera, comprising *Colpodidium* and

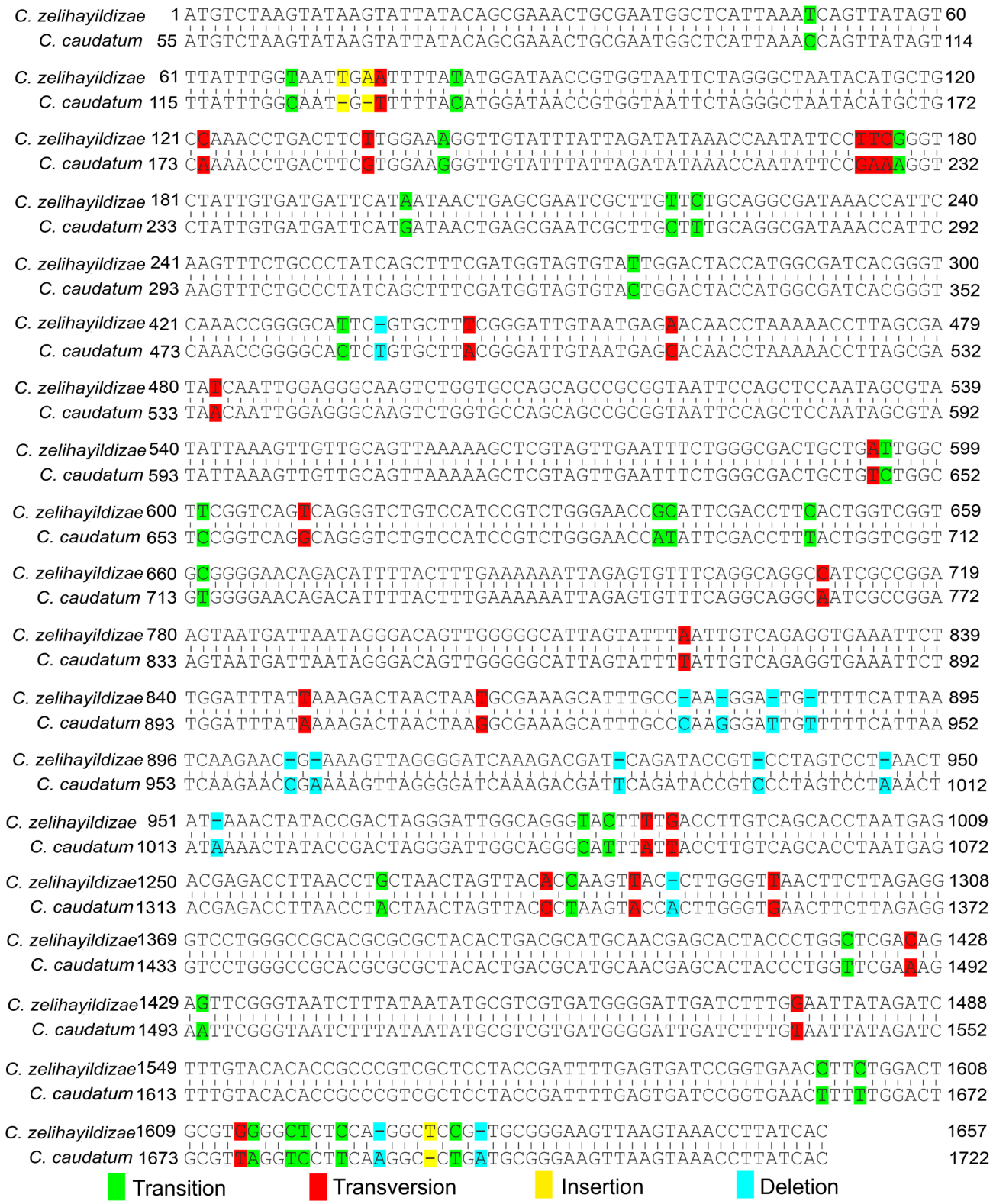

Figure 4. Alignment of the SSU-rDNA sequences of *C. caudatum* (EU264560) and *C. zelihayildizae* n. sp. (MW411350) to show the substitution positions.

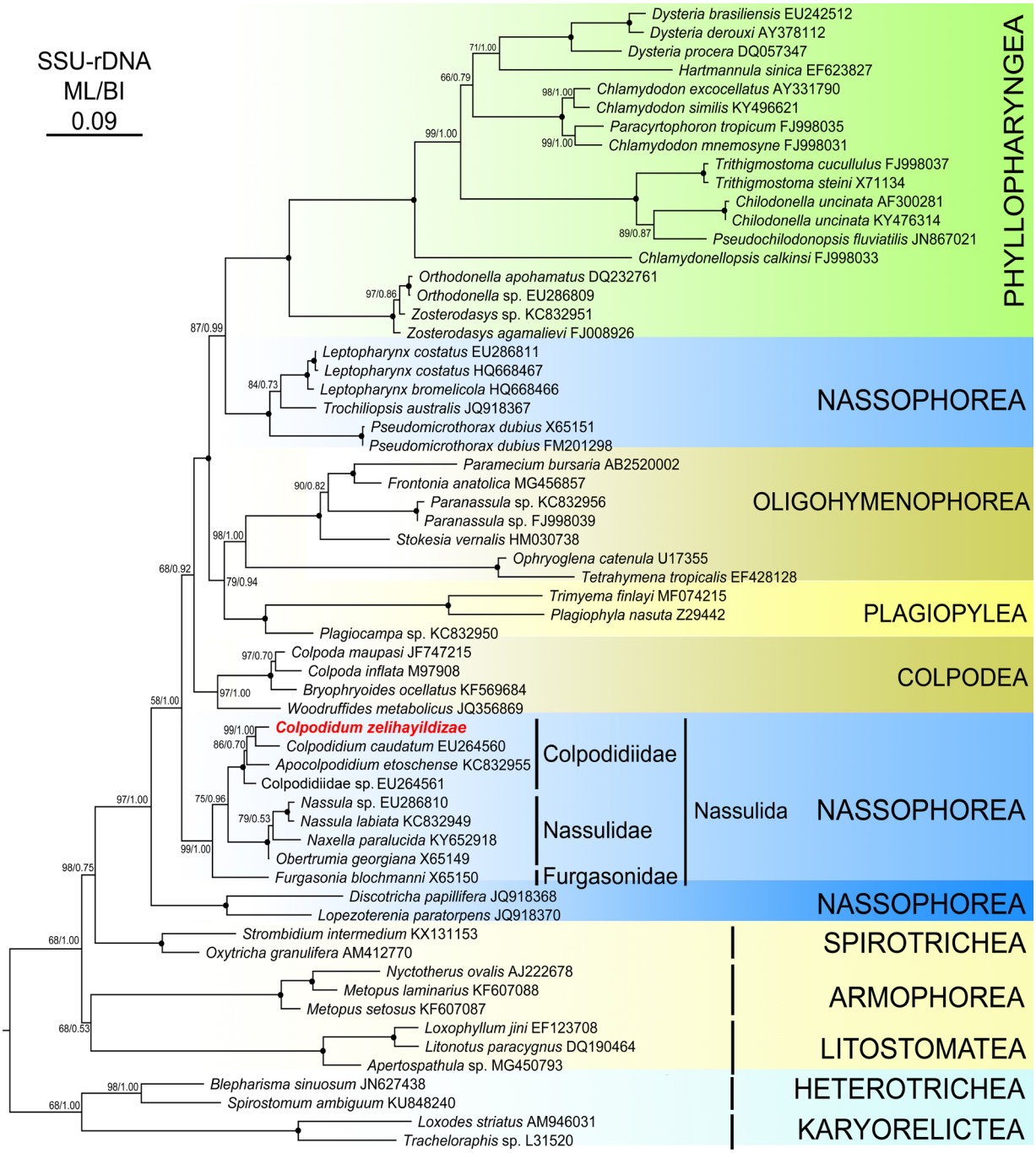

Figure 5. Phylogenetic tree obtained from SSU rRNA gene sequences analyses using ML and BI methods to show the position of *C. zelihayildizae* (in red and bold). The numbers on the nodes represent the bootstrap values of the ML analysis and posterior probabilities of BI analysis, respectively. Full support in both analyses (100% ML and 1.00 BI) is marked with a bold circle. The scale bar indicates 9 substitutions per 100 nucleotide positions.

Pseudocolpodidium. However, the genus *Colpodidium* is represented by a small number of species, and the use of a subspecies category in ciliate taxonomy is uncommon; thus, it was preferred to not use the subgenus category in the present study.

4.1. Comparison of *C. zelihayildizae* n. sp. its relatives

Since the establishment of the genus *Colpodidium* by Wilbert (1982), 5 nominal species, namely *C. caudatum*, *C. horribile*, *C. trichocystiferum*, *C. microstoma*, and *C. bradburyarum*, have been assigned to this genus. *C.*

zelihayildizae n. sp. can be easily distinguished from *C. bradburyarum* by its anteriorly directed horn-shaped buccal cavity, more dikinetids in the paroral membrane (20 vs. 8–10), and a single macronucleus. The present species should be compared with its other 4 relatives.

C. zelihayildizae n. sp. is much similar to the type species described by Wilbert (1982) in terms of its general body shape, location of the macronucleus, and the position of NO1, according to the information obtained from the photomicrographs and illustrations. However, since the author evaluated *C. caudatum* as a member of the class Colpodea, there have been serious errors and misinterpretations in the descriptions of the somatic and oral ciliature (Foissner, 1990; Foissner, 1995; Foissner et al., 2002). However, it can be understood from the drawings that the 2 kinetosome pairs located on the left edge of the paroral membrane, which is located at a short distance from it, may be NO1. However, *C. zelihayildizae* n. sp. can be distinguished from the Wilbert (1982) population by its higher number of paroral dikinetids (20 vs. 17) and the organization of NO2 and NO3. It was not possible to compare the other characters because the original type population did not contain further details. Foissner (1995) and Foissner et al. (2002) redescribed the *C. caudatum* populations obtained from soil samples collected from various geographical regions (Costa-Rica, Namibia, and China). *C. zelihayildizae* n. sp. can be distinguished from the *C. caudatum* populations by the location of its buccal cavity (slightly below the equatorial plane vs. slightly above the equatorial plane in the populations from Costa-Rica and Namibia, while it is on the equatorial plane in the population from China), location of NO1 (distal end of paroral membrane and very close to it vs. behind the distal end of paroral membrane in the population from China), and number of kinetosome pairs in the paroral membrane (consistently 20 vs. 12–14, 12–13, and 14–19 in the populations from Costa-Rica, Namibia and China, respectively).

In terms of its general body shape, *C. zelihayildizae* n. sp. resembles *C. horribile*. However, the present species differs from it by the absence of extrusomes, body size (50 × 20 μm vs. 60 × 30 μm), number of somatic kineties (19–22 vs. 23–26), the position of NO1 (close to the distal end of the paroral dikinetids vs. behind the paroral membrane and slightly right of it), location of the macronucleus (last third of the cell vs. mid-body), and number of dikinetid paroral membranes (consistently 20 vs. 19–33) (Foissner et al., 2002).

C. zelihayildizae n. sp. can be easily distinguished from *C. trichocystiferum* by the absence of extrusomes, its body size (40–60 μm vs. 35–50 μm), position of the macronucleus (last third vs. anterior half of the body), number of somatic kineties (19–22 vs. 17–18), number of paroral dikinetids (20 vs. 15–19), and position of NO1 (the distal end of the paroral membrane vs. behind the paroral membrane) (Foissner et al., 2002).

C. zelihayildizae n. sp. can be distinguished from *C. microstoma* by its body size 40–60 μm vs. 55–80 μm), the location of NO1 (distal end of paroral membrane vs. behind the paroral membrane), position of the buccal cavity (slightly below the equatorial plane vs. in the anterior half), number of the somatic kineties (19–22 vs. 23–26), and number of paroral dikinetids (20 vs. 8–10) (Foissner et al., 2002).

4.2. Phylogenetic analyses

To date, the genus *Colpodidium* comprises 6 species, namely *C. caudatum*, *C. horribile*, *C. trichocystiferum*, *C. microstoma*, *C. barburyarum*, and *C. zelihayildizae* n. sp. Gene sequence data of *C. horribile*, *C. trichocystiferum*, *C. microstoma*, and *C. barburyarum* are currently unavailable, and the remaining 2 species and an undescribed Colpodidiidae sp. were included in the present phylogenetic tree. Both the ML and BI methods used for the phylogenetic analysis suggested that the genus *Colpodidium* is monophyletic. *C. zelihayildizae* n. sp. was clustered with *C. caudatum*, and then strongly supported as a sister to *Apocolpodidium etoschense*, while *Colpodidiidae* sp. was fully supported in the basal position of the family Colpodiidae (Figure 5). These results were in agreement with those of previous studies (Breiner et al., 2008; Fan et al., 2014; Zhang et al., 2014).

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