KSÜ Tarım ve Doğa Derg 28 (4), 914-929, 2025
KSU J. Agric Nat 28 (4), 914-929, 2025
DOI:10.18016/ksutarimdoga.vi.1590418

Morphology and Molecular Phylogeny of *Blepharisma hyalinum* Perty, 1849 (Ciliophora, Heterotrichida) Isolated from Soil

Naciye Gülkız ŞENLER[1], İsmail YILDIZ[2]✉, Hilal URAL[3], Rıfat BİRCAN[4]
[1,3,4]Tekirdağ Namık Kemal University, Department of Biology, Tekirdağ, [2]Van Yüzüncü Yıl University, Department of Biology, Tuşba, Van
[1]https://orcid.org/0000-0002-5035-0880, [2]https://orcid.org/0000-0001-9986-0358, [3]https://orcid.org/0009-0004-9928-7626
[4]https://orcid.org/0000-0001-5291-8620
✉: iyildiz@yyu.edu.tr

ABSTRACT

In this study, two successive populations of *Blepharisma* were observed in cultures prepared from soil samples collected from Tekirdağ (Türkiye). The cells and cysts belonging to these populations were examined morphologically and morphometrically, and their phylogenetic positions were determined based on SSU rDNA gene sequences. Both populations were morphometrically very similar to each other, but population 1, the initial population observed in soil cultures, had a pyriform body that was inflexible and rigid while population 2, observed subsequent to the disappearance of population 1, exhibited a flexible, fragile, lanceolate body. In addition, the cell width/length ratios of both populations were found to be statistically different from each other. However, since the SSU rDNA nucleotide sequences of both populations are very similar to each other and to other *B. hyalinum* populations described previously, it is concluded that the observed *Blepharisma* populations represent different morphotypes of *B. hyalinum*. It has been hypothesised that the observed morphological differences may result from diet, environmental factors, and variations in the life stages of the cultured cells. Comparison of SSU rDNA nucleotide sequences of *Blepharisma* populations and phylogenetic analyses showed that the nucleotide sequences of some different species are identical or very similar. To clarify this chaotic situation, extensive molecular data based on detailed morphological studies on *Blepharisma* populations are necessary.

Microbiology

Research Article

Article History
Received : 25.11.2024
Accepted : 21.05.2025

Keywords
Blepharisma hyalinum
Morphology
Resting cyst
Phylogeny
Türkiye

Topraktan İzole Edilen *Blepharisma hyalinum* Perty, 1849 (Ciliophora, Heterotrichida) Türünün Morfolojisi ve Moleküler Filogenisi

ÖZET

Bu çalışmada Tekirdağ (Türkiye) topraklarından hazırlanan kültürlerde birbiri ardınca ortaya çıkan iki *Blepharisma* popülasyonu gözlendi. Bu popülasyonlara ait hücre ve kistler morfolojik ve morfometrik olarak incelenmiş ve SSU rDNA gen dizisine dayanarak filogenetik konumları belirlenmiştir. Her iki popülasyon da morfometrik olarak birbirine çok benzerdi, ancak toprak kültürlerinde gözlenen ilk popülasyon olan popülasyon 1, esnek olmayan ve sert olan piriform biçime sahipken, popülasyon 1'in kaybolmasının ardından gözlenen popülasyon 2 ise, esnek, kırılgan, mızrak biçimindedir. Ayrıca, her iki popülasyonun hücre genişliği/uzunluğu oranlarının istatistiksel olarak birbirinden farklı olduğu bulunmuştur. Bununla birlikte, her iki popülasyonun SSU rDNA nükleotid dizileri birbirlerine ve daha önce tanımlanan diğer *B. hyalinum* popülasyonlarına çok benzediğinden, bu çalışmada gözlemlenen *Blepharisma* popülasyonlarının *B. hyalinum'un* morfotipleri olduğu sonucuna varıldı. Gözlenen morfolojik farkların çevresel faktörlere ve kültürlerdeki hücrelerin farklı yaşam evrelerinden kaynaklanabileceği varsayılmıştır. *Blepharisma* popülasyonlarının SSU rDNA nükleotid dizilerinin karşılaştırılması

Mikrobiyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 25.11.2024
Kabul Tarihi : 21.05.2025

Anahtar Kelimeler
Blepharisma hyalinum
Morfoloji
Dinlenme kisti
Filogeni
Türkiye

ve filogenetik analizler bazı farklı türlere ait nükleotid dizilerinin aynı ya da çok benzer olduklarını göstermiştir. Bu kaotik durumun açıklığa kavuşturulması için *Blepharisma* popülasyonları üzerinde ayrıntılı morfolojik incelemelere dayalı çok sayıda moleküler veriye ihtiyaç duyulmaktadır.

Atıf İçin : Şenler, N G, Yıldız, İ, Ural H & Bircan, R (2025). Topraktan İzole Edilen *Blepharisma hyalinum* Perty, 1849 (Ciliophora, Heterotrichida) Türünün Morfolojisi ve Moleküler Filogenisi. *KSÜ Tarım ve Doğa Derg 28* (4), 914-929. DOI: 10.18016/ ksutarimdoga.vi.1590418

To Cite: Şenler, N G, Yıldız, İ, Ural H & Bircan, R (2025). Morphology and Molecular Phylogeny of *Blepharisma hyalinum* Perty, 1849 (Ciliophora, Heterotrichida) Isolated from Soil. *KSU J. Agric Nat 28* (4), 914-929. DOI: 10.18016/ ksutarimdoga.vi.1590418.

INTRODUCTION

Ciliates (Ciliophora) are heterokaryotic protists. Within one cell, there are macro- and micronuclei of different sizes and functions (both somatic and germline). By regulating specific metabolic pathways, soil ciliates increase nutrient flow for the benefit of other microorganisms, plants, and animals and contribute to soil fertility (Kuikman et al., 1989; Foissner, 1999; Li et al., 2010; Ceja-Navarro et al., 2021). They also have higher susceptibility, reproduction rate, and trophic niche diversity (bacterivore, carnivore, omnivore) and thus respond faster to environmental contamination (Madoni, 2005; Li et al., 2010; Lara & Acosta-Mercado, 2012).

Ciliate species belonging to the genus *Blepharisma* (class Heterotrichea, order Heterotrichida) are cosmopolitan organisms. In many parts of the world, they are found in many habitats, including soil, lichen, freshwater (lakes, ponds, streams and wastewater treatment ponds), brackish and seawater (Kahl, 1932; Bhandary, 1962; Isquith et al., 1965; Dragesco, 1970; Giese, 1973; Dragesco & Dragesco-Kernéis, 1986; Aescht & Foissner, 1998; Lee & Shin, 2009; Fernandes et al., 2013; Yan et al., 2016; Hao et al., 2022) and even in extreme habitats (Post et al., 1983; Al-Rasheid et al., 2001; Pan & Stoeck, 2017). Most, but not all, *Blepharisma* spp. have a reddish or pink colouration due to granules of the light-sensitive pigment "blepharismin" (formerly zoopurpurin) located just below the plasma membrane (Giese, 1973; Lee & Shin, 2009; Gupta et al., 2015; Pan & Stoeck, 2017). Colourful blepharismids show a photophobic response, avoiding bright light and tending to accumulate in shady or dark areas (Giese, 1973; Fabczak et al., 2001).

Blepharisma was first established by Perty (1849), and its diagnostic properties were described. There are many confusions, renaming and reorganisations in the taxonomic history of the genus (Suzuki, 1954; Bhandary, 1962; Hirshfield et al., 1965; Aescht & Foissner, 1998). The most important reason for this situation is that the morphological and morphometric characteristics of *Blepharisma* species are related to environmental factors such as nutrients, temperature, and pH (Hirshfield et al., 1965; Giese, 1973; Lee & Shin, 2009; Pan & Stoeck, 2017). In feeding experiments, extreme changes in macronuclear configuration and cell size were observed in some species depending on the nutrient (Hirshfield et al., 1965). Nevertheless, in early studies on the taxonomy of the genus, the macronuclear configuration was widely used as the main criterion for species distinction (Stein, 1867; Suzuki, 1954; Bhandary, 1962; Hirshfield et al., 1965; Isquith et al., 1965). Furthermore, the available ontogenetic data do not support the identification of subspecies and subgenera based on nuclear configuration (Aescht & Foissner, 1998).

As a result of the development and application of modern taxonomic techniques (e.g. silver staining, electron microscopy, molecular biology), some less-known *Blepharisma* species and new species have been described (Yan et al., 2016; Hao et al., 2022), while some species are reported to be synonyms (Fernandes et al., 2013). About 75 nominal *Blepharisma* species have been reported so far (Hao et al., 2022). Phylogenetic relationships among ciliates are based primarily on structural features of the cortex, infraciliature, extrusomes and information from ontogeny (Greenwood et al., 1991). The fine structure of the somatic and oral ciliature is an important feature for phylogeny because they are not variable. However, the importance of integrative approaches that combine morphology, morphogenesis, and molecular phylogeny to understand ciliate systematics and ecosystem function is also clear (Liu et al., 2017).

Some molecular studies on *Blepharisma* species show that they do not form a monophyletic clade, and it is noteworthy that different populations of the same species cluster far from each other, while populations belonging to different species cluster closely. Since there is very little molecular data on *Blepharisma* species, more generally on ciliates, the phylogenetic relationships of these organisms have not been fully clarified and contain contradictory situations. In order to resolve this problem, a large amount of molecular data from different geographical regions combined with detailed morphological methods is needed. Apart from the record of Çapar (2007) and the oral presentation produced from a PhD thesis in progress (Ural et al., 2023), no taxonomic study on the species belonging to the genus *Blepharisma* in Tekirdağ province or even in Türkiye. In the present study, two populations

of ciliates belonging to the genus *Blepharisma* isolated from soil in Tekirdağ were examined morphologically and morphometrically using live and silver-staining methods (Vďačný & Foissner, 2012; Foissner, 2014), and their phylogenetic positions were evaluated by comparing their SSU rDNA gene sequences with the gene sequences of the genus *Blepharisma* obtained from NCBI (https://www.ncbi.nlm.nih.gov/nucleotide). This study is the first comprehensive study of *Blepharisma* isolated from the soils of Türkiye. Some notes on the cyst and division morphology of *Blepharisma* populations are also presented in this study.

MATERIAL and METHOD

Study area, sampling, preparation of cultures (Adobe Inc, 2014)

Soil samples were taken from pine and meadow areas (40°59'58.0" N, 27°34'53.9" E) on the campus of Tekirdağ Namık Kemal University between 2019 and 2022. The samples were taken from near the surface (to a depth of 10 cm), together with vegetative debris, and dried in the shade for one month in order to stimulate encystment. Soil analyses were carried out at the soil analysis laboratory of Tekirdağ Namık Kemal University. Physicochemical analysis of the soil samples indicated a clay loam texture, slightly acidic reaction (pH 6.82), moderate calcareous content (lime content 6.84%), organic matter 7.23%, electrical conductivity 520 µS/cm, phosphorus 6.1 ppm, total nitrogen 0.36%, and potassium 93.17 ppm. The soil is a slightly acidic reaction, salt-free class, with very high organic matter content, and moderately calcareous. Potassium and phosphorus contents are low or very low, nitrogen content is high.

Non-flooding Petri dish cultures were prepared to reactivate resting cysts of ciliates found in dried soil samples and incubated at room temperature out of direct sunlight (Foissner et al., 2002; Foissner, 2014). *Blepharisma* populations were observed in the cultures from day 5–9 until the end of one month. At the end of this period, the cultures were renewed due to microbiotas (Foissner, 1987; 1997). For resting cyst morphology, cells isolated from cultures were kept in sterilized ambient water for a week. To observe the morphology of resting cysts, vegetative individuals were kept in sterile, nutrient-free media for one week.

Morphological methods

About 2-5 mL of culture liquid taken from the Petri cultures was transferred to a watch glass and examined under the stereo microscope (Euromex), at 20X-40X magnification, blepharismid ciliates were observed live, and their movement and morphological characteristics were recorded. For more detailed live observations, ciliate cells collected with capillary micropipettes from a watch glass were examined at 100-1000X magnification with the CX41 Olympus light research microscope, and images of morphological features of cells were recorded. Morphometric data were obtained based on intact naturally structured specimens during the live observation process. Various silver staining methods (protargol, silver nitrate, silver carbonate) were used to determine diagnostic features such as nuclear pattern, infraciliature (Foissner et al., 2002; Foissner, 2014). Resting cysts were examined either directly or by supravital staining (methyl green-pyronin). Measurements, counts, and photomicrographs were done with the SC30 Olympus digital camera, compatible with the CX41 light microscope, and the Cell Software micro imaging and measurement system. Illustrations of live cells were based on freehand sketches, micrographs, and video recordings; those of silver-impregnated cells were drawn from micrographs and preparations. The processing of the photographs was carried out on the computer (Adobe Inc, 2014), taking into account the original dimensions and proportions. Taxonomy and terminology were according to Lynn & Small (2000), Lynn (2008).

Biostatistical analyses

The data obtained from measurements and counting of various morphological characters of the *Blepharisma hyalinum* cells were analyzed using SPSS 15.0 (SPSS Inc, 2006) and Minitab 16 (Minitab LLC, 2016) statistical programs, and the results of descriptive statistics were summarized in tables.

Molecular phylogenetic methods

Cells isolated from soil culture were first transferred to sterile (filtered with a 0.22 µm syringe filter and autoclaved) ambient water and washed three times with sterile distilled water to prevent possible contamination. Usually 1-3, rarely more, cells were transferred to 0.2 mL microcentrifuge tubes with 1-2 µL of purified water and stored in a deep freezer (-20°C) for PCR (polymerase chain reaction).

Frozen samples were subjected to 5-10 freeze-thaw cycles to disintegrate the cell pellicle and nuclear membrane. After that, cells were directly subjected to PCR without further genomic DNA extraction, and 18S rDNA gene regions were amplified using universal eukaryotic primers (Medlin et al., 1988; Ural et al., 2020; Yıldız, 2021). The presence and size of the PCR product were checked by running 1.5% agarose gel electrophoresis in the presence of

marker DNA.

Sequencing was performed in the NABILTEM laboratory of Tekirdag Namık Kemal University using a Bekcman Coulter GenomeLab sequencer. PCR products were sequenced with PCR primers and the internal primer (reverse BR2-5'-AAG AAC GGC CAT GCA CCA-3'). The sequences obtained in SCF format were checked in CodonCode Aligner ver. 9.0.1 (www.codoncode.com). Consensus sequences were obtained by aligning good-quality and reliable sequence regions. The nucleotide sequences of the present populations were aligned with the gene sequences of *Blepharisma* species obtained from GenBank (NCBI) using the Clustal W algorithm in the Mega 11 software (Tamura et al., 2021). Nucleotide similarity and dissimilarity matrices between *Blepharisma* species were derived with BioEdit 7.2 software (Hall, 1999).

The best substitution model (TIM2 I+G) for Bayesian phylogenetic analyses was selected according to AIC with JModelTest 2.1.10.2 software (Darriba et al., 2012). Bayesian analysis (BI) was performed using MrBayes v.3.2.7a (Ronquist et al., 2012), sampling 3.000,000 generations and one tree every 100 generations (Ayres et al., 2012; Ronquist et al., 2012). The course of the analyses was monitored with the Tracer software (Rambaut et al., 2018). It was checked whether the sample size was sufficient (ESS>800) and whether the sample size was saturated. The first 25% of the phylogenetic trees were burned, and the consensus tree was constructed from the remaining trees. Maximum likelihood (ML) analysis was done with the Iqtree2 for Mac (Minh et al., 2020). Model selection for ML analysis (TIM+F+I+R2) was performed using the same software. The ML analysis was carried out as 1000 bootstrap runs, and the phylogenetic tree was constructed. Both phylogenetic trees rooted and edited the position of the taxa in FigTree v. 1.4.4 software (http://tree.bio.ed.ac.uk/software/figtree).

RESULTS

In this study in soil cultures, two *Blepharisma* populations (population 1 and population 2) differing in some morphological characteristics were observed. Morphological and morphometrical data were collected from both populations based on live observations and silver impregnated preparations. Although there are some differences in the shape and movement of the living cells of the two populations, morphometry and DNA sequence comparisons indicate that the two populations belong to *Blepharisma hyalinum*. The morphological characteristics of both populations are given below.

Blepharisma hyalinum Perty, 1849 (Syn: *B. lateritium* f. *minima* Roux, 1902)

Morphological descriptions

Population 1 (P1). Since it was the first blepharismid ciliate population observed in soil cultures in this study, it was named population 1. Body shape is elongated pyriform, anterior end distinctly pointed, posterior broadly rounded; ventral side slightly depressed (concave) along the adoral region (peristome), dorsal side slightly bulbous (convex) (Figs. 1a, 2a). Cells are rigid, not flexible, and move slowly between soil particles, mainly at the bottom. Cell size was approximately 66–126 µm in length and 18–36 µm in width in vivo, with a mean value of 96 × 28 µm, whereas the cells were shrunken to 75×21 µm in silver-impregnated preparations. The width is approximately 28% of the length, and this value is 30% in living cells (Table 1, 2). The macronucleus is usually in the center of the cell, closer to the anterior body end; elongate, egg-shaped (oblong); contains many small spherical nucleoli (Figs. 1a, 2d). Size is variable, approximately 10-24 µm in length and 3-7 µm in width. Its distance to the anterior tip is approximately 25 µm in silver impregnated specimens and about 30 µm in live cells (Table 1, 2). In some individuals, more macronuclear fragments of smaller size (17×6 µm, 14×5 µm, 17×9 µm, 13×6 µm, 13×7 µm) were observed, which are thought to be associated with the various life cycle stages. Micronucleus numbers vary from 1 to 7; individual micronuclei are globular in shape, 0.90-1.45 µm across (Table 1), adjacent to or close to the macronucleus at various positions (Figs. 1a, c, 2d). Single contractile vacuole globular and conspicuous, located at the posterior body end, 6.5-9.0 µm across in vivo (Figs. 1a, 2a). The cortex is distinctly corrugated along the ciliary rows (kineties), contains innumerable colorless granules. Cortical granules are arranged in a band of 5-6 granules rows between the ciliary rows (Fig. 2c). The Cytoplasm is colorless and contains many food vacuoles, mainly in the posterior body part.

Somatic and oral ciliature as in congeners. Somatic ciliature entirely composed of dikinetids; number of ciliary rows fairly variable, 14-19 somatic kineties (including 2 postoral kineties) run longitudinally along the cortical ridges (Figs. 1c, 3a, b; Table 1). Adoral zone membranelles (AZM), conspicuous, J-shaped, starting about 4 µm (2-4 µm; N=20) behind the anterior end, proximal part distinctly bent into a semicircle, ends at cytostome (Figs. 1a, c, 2a). AZM extends almost to the middle of the cell, up to about 46% of the cell in protargol-impregnated samples (Table 1) and up to 48% in live samples (Table 2), composed of 26-32 membranelles, each adoral membranelle consisting of two long and one short row of kinetosomes (Fig. 1e), base of longest adoral membranelle length up to 7 µm (Table 1). The paroral membrane is located to the right of the peristom, ends posteriorly at the cytostome; length approximately 29 µm (22-35 µm) (Table 1), differentiated into two distinct parts (PMa, PMp); anterior part

(PMa) 26-29% of paroral membranal length, composed of monokinetids in a straight line, while the posterior consists of dikinetids arranged in a "zig zag" pattern (Figs. 1e, 4a-d).

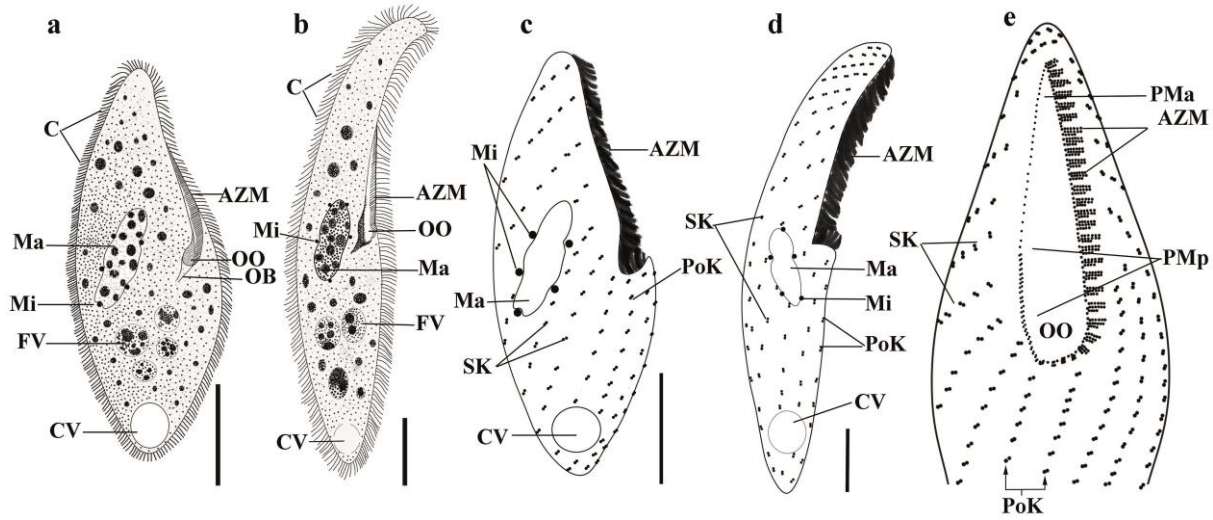

Figure 1. Morphology and ciliary pattern of *B. hyalinum* from life (a,b) and after silver impregnation (c-e). Right lateral views of representative individuals from population 1 (a) and population 2 (b). Infraciliature of the right lateral of population 1(c) and population 2 (d). e: Ventral infraciliature and details of oral region. AZM: adoral zone of membranelles, C: cilia, CV: contractile vacuole, FV: food vacuoles, Ma: macronucleus, Mi: micronucleus, OO: oral opening, PMa: anterior portion of paroral membrane, PMp: posterior portion of paroral membrane, PoK: postoral kineties, SK: somatic kineties. Scale bars 20 µm (a, c), 50 µm (d, e).

Şekil 1. B. hyalinum'un canlı (a, b) ve gümüş boyanmış örneklerinin (c-e) morfolojisi ve siliyatürü.

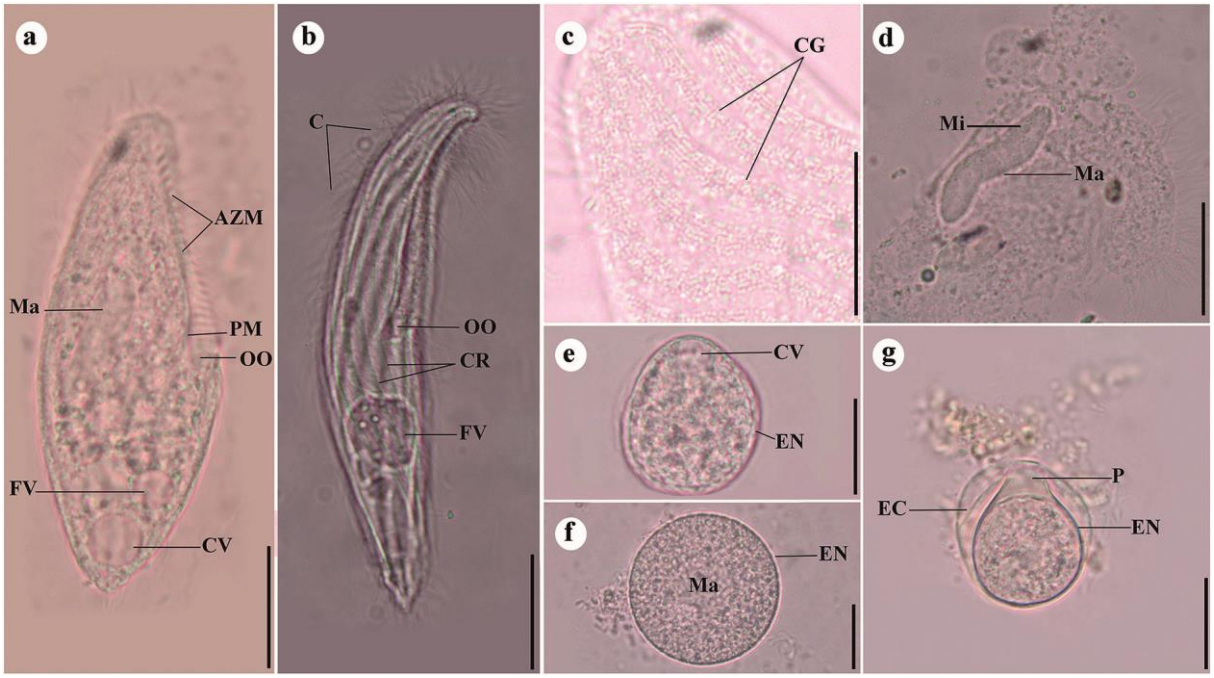

Figure 2. Photomicrographs of *B. hyalinum* from life. a, b: General views of typical individuals from population 1 (a) and population 2 (b). c: Cortical granules between somatic kineties. d: A squashed individual, showing the nuclear apparey. e, f, g: Some stages of resting cyst in vivo. In the early phase of encystment, the contractile vacuole and endocyst membrane are observed (e, f). Mature resting cyst with an endocyst, an ectocyst, and a conical plug (g). AZM: adoral zone of membranelles, C: cilia, CV: contractile vacuole, CR: cortical ridges, CG: cortical granules, EN: endocyst, EC: ectocyst, FV: food vacuole, Ma: macronucleus, Mi: micronucleus, OO: oral opening, P: plug, PM: paroral membrane. Scale bars 20 µm.

Şekil 2. B. hyalinum'un canlı fotomikrografları.

Table 1. Morphometric characteristics of fixed specimens of *B. hyalinum* P1 (upper line) and P2 (lower line). Measurements are in µm*.
*Çizelge 1. B. hyalinum P1 (üst satır) ve P2 (alt satır) tespitli örneklerinin morfometrik özellikleri. Ölçümler µm olarak verildi**.

Characteristics	x̄	M	SD	SE	CV	Min	Max	n
Body, length (P)	74.68	75.40	8.60	1.92	11.52	60.00	87.47	20
	74.51	74.76	13.79	3.08	18.51	48.50	93.30	20
Body, maximum width (P)	20.67	20.02	3.53	0.79	17.08	15.80	28.30	20
	16.31	16.55	2.80	0.63	17.17	10.80	22.90	20
Body width/length, ratio (%)	27.74	27.09	3.88	0.87	13.99	23.10	36.85	20
	22.09	22.32	2.45	0.55	11.09	18.49	26.24	20
AZM, total length (P)	31.83	31.90	3.88	0.87	12.19	24.15	38.80	20
	30.93	31.10	4.42	0.99	14.29	18.60	39.30	20
Anterior body end to proximal point of AZM, distance (P)	33.82	33.54	4.07	0.91	12.03	25.61	40.35	20
	33.37	33.75	4.14	0.93	12.41	22.00	39.78	20
Distance from the proximal of the AZM to the anterior body end/body length, ratio (%)	45.47	45.06	4.42	0.99	9.72	38.59	53.52	20
	45.80	44.10	7.83	1.75	17.10	33.59	64.74	20
Adoral membranelles, number (SC)	29.37	29.50	1.59	0.29	5.41	26.00	32.00	30
	29.00	29.00	1.93	0.35	6.66	24.00	32.00	30
Base of longest adoral membranelle, length (SC)	4.58	4.50	0.74	0.14	16.16	3.10	6.60	30
	4.87	4.90	0.69	0.13	14.17	3.50	6.40	30
Paroral membrane, length (P)	28.72	28.50	3.72	0.83	12.95	22.24	35.20	20
	27.92	28.28	3.87	0.86	13.86	17.90	33.50	20
Macronucleus, number (P)	1.00	1.00	0.00	0.00	0.00	1.00	1.00	20
	1.00	1.00	0.00	0.00	0.00	1.00	1.00	20
Anterior body end to macronucleus, distance (P)	25.03	24.22	4.52	1.01	18.06	16.40	36.60	20
	24.03	24.05	4.11	0.92	17.10	16.60	33.10	20
Macronucleus, length (P)	16.39	16.30	3.97	0.89	24.22	9.60	23.89	20
	15.27	15.05	2.51	0.56	16.44	11.20	19.30	20
Macronucleus width (P)	5.21	5.46	1.01	0.23	19.39	3.10	6.90	20
	5.05	4.60	1.35	0.30	26.73	3.10	8.20	20
Micronucleus, number (P)	3.35	3.00	1.79	0.40	53.43	1.00	7.00	20
	3.22	3.00	1.31	0.31	40.68	1.00	6.00	18
Micronucleus, largest diameter (P)	1.20	1.20	0.15	0.03	12.50	0.90	1.45	20
	1.27	1.22	0.25	0.06	19.69	0.93	2.00	18
Somatic kineties, postoral number (SC)	16.63	16.00	1.38	0.25	8.30	14.00	19.00	30
	16.63	16.00	1.71	0.31	10.28	14.00	20.00	30
Postoral kineties, number (SC)	2.00	2.00	0.00	0.00	0.00	2.00	2.00	30
	2.00	2.00	0.00	0.00	0.00	2.00	2.00	30

*Data based on randomly selected protargol-impregnated (P) and silver carbonate-impregnated (SC) specimens from soil cultures. AZM: adoral zone of membranelles, CV: coefficient of variation in %, M: median, Max: maximum, Min: minimum, N: number of individuals investigated, SD: standard deviation, SE: standard error of the mean, x̄: arithmetic mean.

Table 2. Morphometric characteristics of living specimens of *B. hyalinum* P1 (upper line) and P2 (lower line). Measurements are in µm*.
*Çizelge 2. B. hyalinum P1 (üst satır) ve P2 (alt satır) canlı örneklerinin morfometrik özellikleri. Ölçümler µm olarak verildi**

Characteristics	x̄	M	SD	SE	CV	Min	Max	n
Body, length	95.82	91.80	17.10	3.42	17.85	65.50	126.60	25
	100.78	98.40	11.37	2.27	11.28	81.80	128.80	25
Body, width	27.85	27.70	4.55	0.91	16.34	17.80	35.50	25
	21.56	20.40	3.72	0.74	17.25	16.10	30.00	25
Body width/length, ratio (%)	21.48	22.37	3.29	0.66	15.32	15.16	27.34	25
	43.40	42.90	7.47	1.59	17.21	28.90	55.60	22
AZM, total length	43.40	42.90	7.47	1.59	17.21	28.90	55.60	22
	41.80	40.93	3.92	0.88	9.38	36.60	50.00	20
Anterior body end to proximal point of AZM to apical, distance	49.42	49.15	7.36	2.13	14.89	35.80	60.02	12
	46.92	45.95	3.68	0.92	7.84	40.60	53.00	16
Distance between the proximal point of AZM and anterior end of the body /body length, ratio (%)	48.44	47.39	6.30	1.82	13.01	40.31	65.47	25
	46.08	45.80	3.99	1.00	8.66	39.83	52.90	16
Anterior body end to macronucleus, distance	30.28	30.68	6.78	2.77	22.39	20.00	38.80	6
	38.57	40.80	8.37	3.16	21.70	23.26	49.50	7
Macronucleus, length	20.81	21.49	4.75	1.37	22.83	11.70	29.30	12
	16.82	15.20	4.06	1.23	24.14	12.00	24.00	11
Macronucleus, width	9.37	9.15	2.84	0.82	30.31	4.53	13.70	12
	6.51	7.00	1.21	0.37	18.59	4.40	8.00	11

*Data based on randomly selected specimens from soil cultures. AZM: adoral zone of membranelles, CV: coefficient of variation in %, M: median, Max: maximum, Min: minimum, N: number of individuals investigated, SD: standard deviation, SE: standard error of the mean, x̄: arithmetic mean.

Population 2 (P2). This population was named population 2 because it appeared after population 1 in Petri cultures. The overall body shape is spindle-shaped and different from the previous population (Figs. 1b, 2b). Slender with tapered posterior end, anterior pole curved ventrally; flexible, not contractile. In the liquid phase, it glides slowly by swinging its rear around the long axis. Except for body width, morphometry is similar to the previous population (Table 1). In vivo length approximately 101 µm (82-129 µm), width 22 µm (16.10 -30.00 µm). The body width is approximately 22% of the length (15.16-27.34 µm) (Table 2). Cortical ridges are more prominent than in the previous population, giving the cell a wavy appearance (Fig. 2b). The AZM runs to midbody, 46% of body length (Table 2). The cytoplasmic appearance, nuclear apparatus, nutrient vacuoles, and contractile vacuole are similar to the previous population (Figs. 1b, 2b). The macronucleus is compact, ellipsoidal (Fig. 2b), with numerous nucleoli (Fig. 3g). The somatic and oral ciliature is identical in general organization to the previous population (Figs. 1d, e, 3c-h). The posterior part of the paroral membrane is composed of tightly packed dikinetids. Near the front, the distance between the kinetids is greater (Fig. 1e).

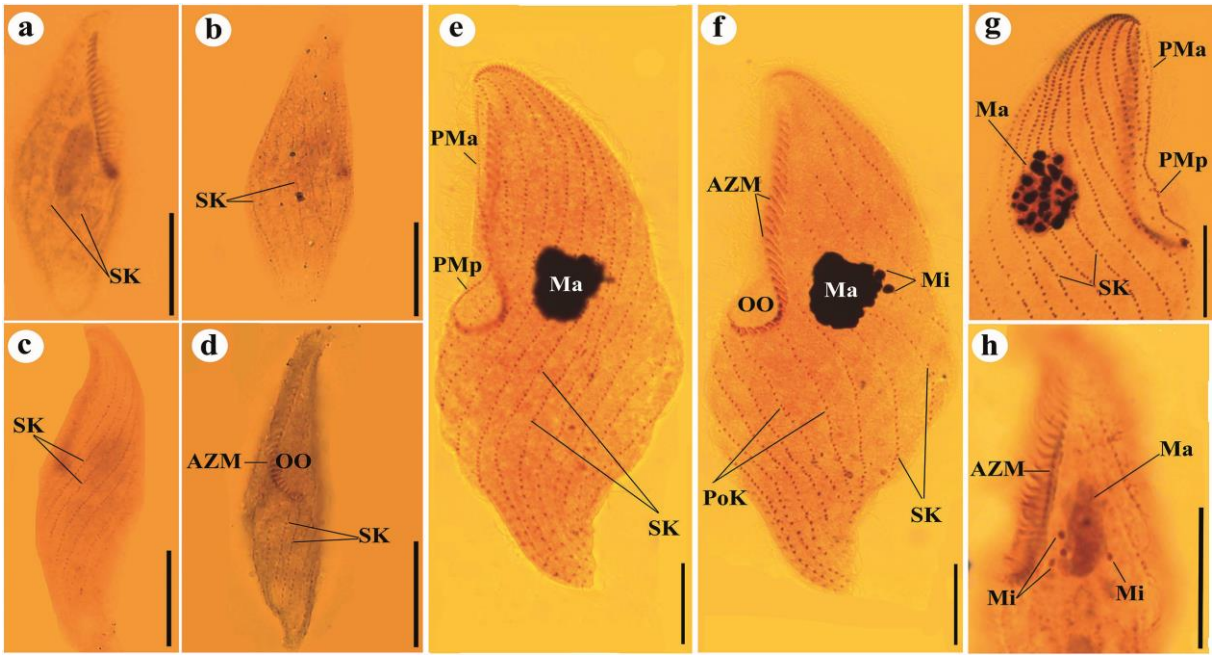

Figure 3. Photomicrographs of *B. hyalinum* after staining with protargol (a-d, h) and silver carbonate (e, f, g), showing somatic and oral infraciliature. AZM: adoral zone of membranelles, Ma: macronucleus, Mi: micronuclei, OO: oral opening, PMa: anterior portion of paroral membrane, PMp: posterior portion of paroral membrane, PoK: postoral kineties, SK: somatic kineties. Scale bars 20 µm.

Şekil 3. B. hyalinum'un protargol (a-d, h) ve gümüş karbonat (e-g) ile boyanmış somatik ve oral infrasiliyatürü gösteren fotomikrografları.

Notes on the divisional morphogenesis. Stomatogenesis begins with kinetosomes proliferating within and to the left of the middle portion of a postoral ciliary row (Fig. 4a). The irregular groups of kinetosomes formed at the end of replication clump together to form a single oral anlage. With the continuous proliferation of kinetosomes, the oral anlage gradually enlarges (Figs. 4b, 4c). The oral primordium is unequally longitudinally divided, and two kinetosomal areas are formed. The narrow kinetosomal area on the right side forms the paroral membrane anlage and the wide kinetosomal area on the left side forms the adoral membrane anlage (Fig. 4d). Until this stage, the oral anlage of the opisth (posterior cell) is formed, whereas no reorganization of the oral structure in the proter (anterior cell, parental) is observed. In the late stage of stomatogenesis, the oral structures (AZM and PM) of the opistine become fully differentiated. Following stomatogenesis, transverse division (cytogenesis) occurs (Figs. 1e, 2f). The division of the macronucleus is simple: the elongated macronucleus divides into two parts before the two daughter cells separate from each other. The behavior of the micronuclei in the division process is not observed. The daughter cells are almost the same size as each other.

Notes on the resting cyst morphology. It was possible to obtain a small number of cysts in this study. Therefore, detailed analysis could not be performed in the encystment and excystment process. The formation of cysts in this ciliate is a long process that takes 4-5 days. The morphology of the cysts is characteristic of the genus *Blepharisma*, it has three morphological structures: an inner wall (endocyst), an outer wall (ectocyst), and an emergency pore (plug).

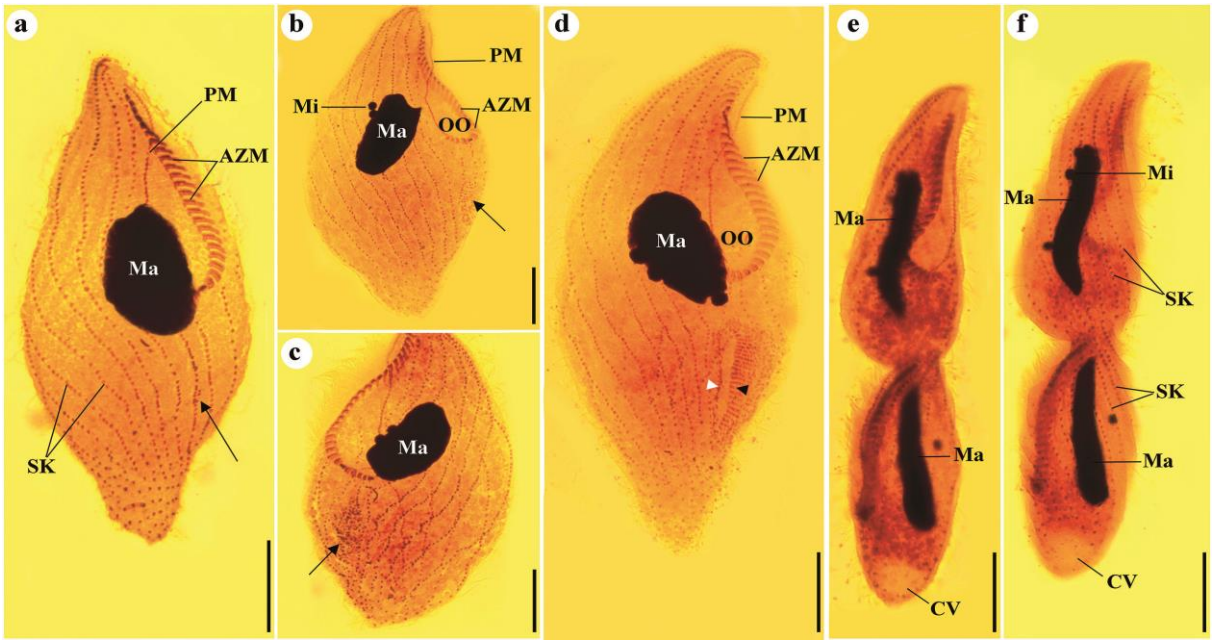

Figure 4. Morphogenesis in *B. hyalinum* after silver carbonate. Formation of the oral primordium of the opisthe (a-d). a: Ventral view of an early divider, showing proliferation of kinetosomes (arrow). b, c: The oral primordium (arrow) has enlarged by continued proliferation of kinetosomes. d: Ventral view of a middle divider, showing paroral membrane (white arrowhead) and adoral membranelles anlage (black arrowhead). e, f: Late divisional stage, showing ventro-lateral and dorso-lateral views of a cell before cytokinesis. Proter and opisthe are about to separate. AZM: adoral zone of membranelles, CV: contractile vacuole, Ma: macronucleus, Mi: micronuclei, OO: oral opening, PM: paroral membrane, SK: somatic kineties. Scale bars 20 µm.

Şekil 4. B. hyalinum'da gümüş karbonat sonrası morfogenez.

At the end of the 4th or 5th day, the general body shape of starved vegetative individuals begins to change, with cell volume decreasing, followed by the formation of cyst walls. Individuals that will form cysts will begin to rotate rapidly in a clockwise direction around themselves, gradually becoming smaller and rounder. Cyst is surrounded by a cyst wall (endocyst) with a width of 0.93 µm (n=4) (early stage of cyst formation). At the first stage, contractile vacuoles are observed, and there is motility in the cytoplasm (Fig. 2e). Cysts are spherical, 40-50 µm in diameter, in the early stage. After a few hours, the contractile vacuole disappears, and the cytoplasm becomes homogeneous (Fig. 2f). At 7-9. days, an outer wall (ectocyst) (6.21 µm, n= 4) and a conical plug (emergence pore) (length: 8.07 µm, n= 4) independent of the ectocyst are recognized around the cyst (Fig 2g). The mature cysts measure approximately 36.29 µm in length and 31.74 µm in width (n=4).

Inter-population variation. One-way analysis of variance (ANOVA) showed no significant difference between the two populations in most morphometric traits (Table 3). Although there was no significant difference between cell lengths ($p > 0.05$), the difference between cell widths was significant ($p < 0.001$), and the difference between the ratio of body width to body length (%) was very significant ($p < 0.001$).

Molecular results

The 18s rDNA gene region, with a length of 1289 base pairs (bp) for the P1 population and 1658 bp for the P2 population, was sequenced. The SSU rDNA nucleotide sequences of *Blepharisma hyalinum* (P1) and *Blepharisma hyalinum* (P2) were deposited in GenBank with accession numbers PV489986 and PV489987, and G+C ratios were calculated to be 45.7% and 46.8%, respectively.

The nucleotide sequences of the present populations were aligned with the gene sequences of other *Blepharisma* species obtained from the GenBank using the ClustalW multiple alignment algorithm, and 123 of the 1289 bp were found to differ by at least one base (Fig. 5). Similarity and dissimilarity matrices obtained using aligned nucleotide sequences constructed only from sites showing polymorphism showed that the current populations (P1 and P2) differed from the other populations by only two nucleotide sites, showing a similarity of 98.2% (Table 3).

It is noteworthy that there are no nucleotide differences between populations belonging to the same or different species such as *B. americanum* (AM713182, MT175503), *B. undulans* (KP970231, KY855549, KKP790233,

AM131183) *B. sinosum* (MK354013, KP970229) *B. musculus* KJ651813 and the similarity is complete (100%) (Table 3).

Table 3. Nucleotide similarity and dissimilarity matrix based on alignment of base positions of Blepharisma species.

Çizelge 3. Blepharisma türlerinin baz pozisyonlarının hizalanmasına dayalı nükleotid benzerlik ve farklılık matrisi.

Difference (count) / Similarity (%)	1. *B. hyalinum* (P1)	2. *B. hyalinum* (P2)	3. *B. hyalinum* (KP970225)	4. *B. hyalinum* (AM713184)	5. *B. elongatum* (AM713186)	6. *B. americanum* (MF002400)	7. *B. americanum* (AM713182)	8. *B. americanum* (MF002385)	9. *B. americanum* (MF002395)	10. *B. americanum* (M97909)	11. *B. americanum* (MT175503)	12. *B. bimicronucleatum* (KX119522)	13. *B. undulans* (KP970231)	14. *B. undulans* (KY855549)	15. *B. ndulans* (KP970233)	16. *B. undulans* (AM713183)	17. *B. sinuosum* (MK354013)	18. *B. sinuosum* (KP970229)	19. *B. sinuosum* (JN627438)	20. *B. steini* (AM713187)	21. *B. japonicum* (KP970226)	22. *B. japonicum* (KP970228)	23. *B. halophilum* (MF437020)	24. *B. musculus* (KJ651813)	25. *B. penardi* (MT175501)	26. *Blepharisma sp.* (KF206427)
1	—	0.982	0.982	0.982	0.879	0.756	0.791	0.747	0.747	0.721	0.791	0.807	0.791	0.791	0.791	0.791	0.791	0.791	0.765	0.736	0.762	0.782	0.264	0.791	0.815	0.798
2	2	—	0.982	0.982	0.879	0.756	0.791	0.747	0.747	0.721	0.791	0.807	0.791	0.791	0.791	0.791	0.791	0.791	0.765	0.736	0.762	0.782	0.264	0.791	0.815	0.798
3	2	2	—	1.000	0.896	0.773	0.808	0.765	0.765	0.739	0.808	0.824	0.808	0.808	0.808	0.808	0.808	0.808	0.782	0.754	0.779	0.800	0.282	0.808	0.833	0.815
4	2	2	0	—	0.896	0.773	0.808	0.765	0.765	0.739	0.808	0.824	0.808	0.808	0.808	0.808	0.808	0.808	0.782	0.754	0.779	0.800	0.282	0.808	0.833	0.815
5	14	14	12	12	—	0.794	0.829	0.786	0.786	0.760	0.829	0.827	0.829	0.829	0.829	0.829	0.829	0.829	0.803	0.706	0.800	0.820	0.277	0.829	0.818	0.801
6	28	28	26	26	24	—	0.965	0.991	0.991	0.913	0.965	0.800	0.965	0.965	0.965	0.965	0.965	0.965	0.939	0.721	0.949	0.956	0.305	0.965	0.826	0.808
7	24	24	22	22	20	4	—	0.956	0.956	0.930	1.000	0.834	1.000	1.000	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843
8	29	29	27	27	25	1	5	—	1.000	0.904	0.956	0.791	0.956	0.956	0.956	0.956	0.956	0.956	0.930	0.713	0.940	0.947	0.296	0.956	0.817	0.800
9	29	29	27	27	25	1	5	0	—	0.904	0.956	0.791	0.956	0.956	0.956	0.956	0.956	0.956	0.930	0.713	0.940	0.947	0.296	0.956	0.817	0.800
10	32	32	30	30	28	10	8	11	11	—	0.930	0.765	0.930	0.930	0.930	0.930	0.930	0.930	0.904	0.678	0.915	0.921	0.254	0.930	0.791	0.773
11	24	24	22	22	20	4	0	5	5	8	—	0.834	1.000	1.000	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843
12	22	22	20	20	20	23	19	24	24	27	19	—	0.834	0.834	0.834	0.834	0.834	0.834	0.808	0.824	0.805	0.826	0.350	0.834	0.956	0.938
13	24	24	22	22	20	4	0	5	5	8	0	19	—	1.000	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843
14	24	24	22	22	20	4	0	5	5	8	0	19	0	—	1.000	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843
15	24	24	22	22	20	4	0	5	5	8	0	19	0	0	—	1.000	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843
16	24	24	22	22	20	4	0	5	5	8	0	19	0	0	0	—	1.000	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843
17	24	24	22	22	20	4	0	5	5	8	0	19	0	0	0	0	—	1.000	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843
18	24	24	22	22	20	4	0	5	5	8	0	19	0	0	0	0	0	—	0.973	0.747	0.966	0.991	0.313	1.000	0.860	0.843
19	27	27	25	25	23	7	3	8	8	11	3	22	3	3	3	3	3	3	—	0.721	0.940	0.965	0.288	0.973	0.834	0.817
20	30	30	28	28	34	32	29	33	33	37	29	20	29	29	29	29	29	29	32	—	0.720	0.739	0.299	0.747	0.833	0.815
21	28	28	26	26	24	6	4	7	7	10	4	23	4	4	4	4	4	4	7	33	—	0.957	0.305	0.966	0.830	0.813
22	25	25	23	23	21	5	1	6	6	9	1	20	1	1	1	1	1	1	4	30	5	—	0.305	0.991	0.852	0.834
23	86	86	84	84	86	82	81	83	83	88	81	76	81	81	81	81	81	81	84	82	84	82	—	0.313	0.341	0.324
24	24	24	22	22	20	4	0	5	5	8	0	19	0	0	0	0	0	0	3	29	4	1	81	—	0.860	0.843
25	21	21	19	19	21	20	16	21	21	24	16	5	16	16	16	16	16	16	19	19	20	17	77	16	—	0.982
26	23	23	21	21	23	22	18	23	23	26	18	7	18	18	18	18	18	18	21	21	22	19	79	18	2	—

Since the phylogenetic trees produced by Bayesian inference (BI) and maximum likelihood (ML) are topologically very similar, the results of the phylogenetic analysis are presented by showing the BI posterior probability values (pp>0.50) on the ML tree (Fig. 6).

Although the vast majority of *Blepharisma* populations form a monophyletic clade derived from a common ancestor, *B. halophilum* populations are more closely related to Follicolinidae than their congeners (Fig. 6).

The distinction between the major clade Blepharismidae and the clade containing Folliculinidae, Fabreidae, and Maristentoridae, in which *B. halophilum* is clustered, was strongly supported by Bayesian phylogenetic analysis (BI=0.96), while maximum likelihood analysis moderately supported this distinction (ML=54). In both phylogenetic analyses, this populations (PV489986, PV489987) were placed together as sister taxa with strong support (BI/ML=0.99/85). Other populations of *B. hyalinum*, AM713184 and KP970225, were positioned at the base of this populations by both algorithms with full support. At the basal position of the *B. hyalinum* cluster, *B. elongatum* (AM713186) appears as a closely related population.

DISCUSSION

Studies on the species belonging to the genus *Blepharisma* go back a long way. *B. hyalinum* was first isolated and named by Perty (1849) from freshwater containing duckweed (*Lemna*) and filamentous green algae. However, the description was inadequate as the type of population consisted of only a small number of individuals. In the present study, 2 blepharismid populations, which were morphologically and mode of movement distinguishable from each other, were successively observed in soil cultures. As a result of one-way ANOVA, cell width and the ratio of cell width to length (%) of both populations were found to be significantly different (P<0.001). However, when the 18S rDNA gene region was compared, the nucleotide sequences were very similar, and it was concluded that these two populations were two different morphotypes of *B. hyalinum*. It is widely believed that intra-population variations in species belonging to the genus *Blepharisma* are to a large extent influenced by environmental factors, such as diet and stage of the life cycle (Giese, 1973; Larsen & Nilsson, 1983). Such morphological variations therefore make no taxonomic sense (Yan et al., 2016), but tend to confuse those trying to identify the species (Gupta et al., 2015). These variations also cause many morphological features to overlap between *Blepharisma* species, as shown in

previous studies (Stein, 1867; Kahl, 1932; Larsen, 1982; Al-Rasheid et al., 2001; Lee & Shin, 2009; Fernandes et al., 2013; Yan et al., 2016; Pan & Stoeck, 2017; Hao et al., 2022). Among its congeners, *B. hyalinum* is the only one that closely resembles *B. elongatum*. *B. hyalinum*, as well as *B. elongatum*, has colourless cortical granules and a single compact macronucleus (Larsen & Nilsson, 1983; Larsen & Nilsson, 1988).

```
                                    10        20        30        40        50        60        70        80
B.hyalinum(P1)                      AATCTACAAA TCG--TAGAT ATT-GGCCGC GATACTGGTT GGTCT-TTGT TTCG-ACGAT CCACCATTCG CGGC-GGG-T
B.hyalinum (P2)                     AATCTACAAA TCG--TAGAT ATT-GGCCGC GATACTGGTT GGTCT-TTGT TTCG-ACGAT CCACCATTCG CGGC-GGG-T
B.hyalinum(KP970225)                AATCTAAAAA TCG--TAGAT ATT-GGCCGC GATACTGGTT GGTCT-TTGT TTCG-ACGAT CCACCATTCG CGGC-GGG-T
B.hyalinum(AM713184)                AATCTAAAAA TCG--TAGAT ATT-GGCCGC GATACTGGTT GGTCT-TTGT TTCG-ACGAT CCACCATTCG CGGC-GGG-T
B.elongatum(AM713186)               AATCTAAAAA TCG--TAGAT ATTGGGCCGC GATTCCGGCT GGTCT-TGAT TTCGCACGAT CCACCATTCG CGGC-GGG-T
B.americanum(MF002400)              AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.americanum(AM713182)              AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.americanum(MF002385)              AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCTTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.americanum(MF002395)              AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCTTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.americanum(M97909)                AATCTGAAAA TCG--TAGAT AT---GCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.americanum(MT175503)              AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.bimicronucleatum(KX119522)        ATTCTAAGAA TCG--TAGAC ATT-GGCCGC GGCCCCGGCT GGTCT-CAGC TTCG-ATGGT CCACCGCTCG CGGC-GGG-T
B.undulans(KP970231)                AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.undulans(KY855549)                AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.undulans(KP970233)                AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.undulans(AM713183)                AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.sinuosum(MK354013)                AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.sinuosum(KP970229)                AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.sinuosum(JN627438)                AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.steini(AM713187)                  AATCTAAAAA TCG--TAGAC ATT-GGCCGC GGCGCTAGTT GGCTG-CAGC TTCG-ATGGT TGACATCTCG CGGC-GGG-T
B.japonicum(KP970226)               AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGCGGGGGT
B.japonicum(KP970228)               AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.halophilum(MF437020)              TTGTTAAAGT CTCTTAGCGC GCT-GATTAG ACAGCTCGAA TAGCTACTAG CCTA-GTGGA CTCGGTCCGT TCCA-CTA-C
B.musculus(KJ651813)                AATCTGAAAA TCG--TAGAT ATT-GGCCGC GGTCCTGGCT GGTCT-TGAC TTCG-ATAGT CCACCTCTCG TGGC-GGG-T
B.penardi(MT175501)                 AATCTGAAAA TCG--TAGAC ATT-GGCCGC GGCCCTGACT GGTCT-CAGC TTCG-ATGGT CCACCGCTCG CGGC-GGG-T
Blepharisma sp.(KF206427)           AATCCGAAAA TCG--TAGAC ATT-GGCCGC GGCCCTGACT GGTCT-CAGC TTCG-ATGGT CCACCGCTCG CGGC-GGG-T
                                    90        100       110       120
B.hyalinum(P1)                      ATCATCGT-A TTCAG-TCAG CCTGAGTCTT CTCTCAGAGT CAC
B.hyalinum (P2)                     ATCATCGT-A TTCAG-TCAG CCTGAATATT CTCTCAGAGT CAC
B.hyalinum(KP970225)                ATCATCGT-A TTCAG-TCAG CCTGAATCTT CTCTCAGAGT CAC
B.hyalinum(AM713184)                ATCATCGT-A TTCAG-TCAG CCTGAATCTT CTCTCAGAGT CAC
B.elongatum(AM713186)               ATCATCGT-A CCAAG-TCAG CCTGAATCTT CTTTCAAAGT CAC
B.americanum(MF002400)              ATCATCGT-C TAACAGTCAG CCTGAATCCT CCTCCAAGGT CGC
B.americanum(AM713182)              ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.americanum(MF002385)              ATCATCGT-C TAACAGTCAG CCTGAATCCT CCTCCAAGGT CGC
B.americanum(MF002395)              ATCATCGT-C TAACAGTCAG CCTGAATCCT CCTCCAAGGT CGC
B.americanum(M97909)                ATCATCGT-C TAAAAGTCCA TG-GAATCCT CTTCCAAAGT CGC
B.americanum(MT175503)              ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.bimicronucleatum(KX119522)        ATCATCGT-A CCAAG-TCAG CCTGAATCCT CTCTCAGAGT CGC
B.undulans(KP970231)                ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.undulans(KY855549)                ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.undulans(KP970233)                ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.undulans(AM713183)                ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.sinuosum(MK354013)                ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.sinuosum(KP970229)                ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.sinuosum(JN627438)                ATYATMST-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.steini(AM713187)                  ATCATCGT-A C------CAG CCTG-AGCCT CTCTCAGAGT CGC
B.japonicum(KP970226)               ATCATCGTTC TAAAAGTCAG CCTGAATCCT CTTCCAAAGT CGC
B.japonicum(KP970228)               ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGT
B.halophilum(MF437020)              GCCGCCGT-A CCAAA-TGAG CCTAAATCCC AGCAAGGCTG TGC
B.musculus(KJ651813)                ATCATCGT-C TAAAAGTCAG CCTGAATCCT CTTTCAAAGT CGC
B.penardi(MT175501)                 ATCATCGT-A CCAAG-TCAG CCTGAATCCT CTCTCAGAGT CGC
Blepharisma sp.(KF206427)           ATCATCGC-A CCAAG-TCAG CCTGAATCCT CTCTCAGAGT CGC
```

Figure 5. Nucleotide divergence sequence of Blepharisma species based on alignment of 18S rDNA base positions.
Şekil 5. 18S rDNA baz pozisyonlarının hizalanmasına dayalı Blepharisma türlerinin nükleotid farklılık dizisi.

The geographical distribution of *B. hyalinum* is very wide; it has been reported at low densities but in all geographical regions (Holarctic, Neotropical, Palaeotropical, Austral, Antarctic). It has been isolated from the eutrophic pond on the island of Funen in Denmark (Larsen & Nilsson, 1983), the Ivory Coast in West Africa (Dragesco & Dragesco-Kernéis, 1986), Austrian soil samples (Foissner, 1989), the Jubail Marine Wildlife Sanctuary on the Persian Gulf coast of Saudi Arabia (Al-Rasheid, 1999), the Anzali Wetland of the Caspian Sea (Asadullayeva & Alekperov, 2007), and terrestrial Antarctica (Thompson et al., 2019). Despite being one of the most common species within the genus *Blepharisma*, detailed morphometric data on *B. hyalinum* remain scarce, except for the studies by Larsen & Nilsson (1983), Foissner (1989), and Aescht & Foissner (1998). The populations under study here are consistent with the original and other previous definitions in terms of general morphology and infraciliature. However, morphometrics differ slightly (Table 3). Compared to previous descriptions of *B. hyalinum*, this populations have a larger body size (population 1: about 96 x 28 µm; population 2: about 101 x 22 µm). The cell size of this populations is closer to aquatic populations (100-130 µm, 100 µm, 80-160 µm) (Kahl, 1932; Larsen & Nilsson, 1983; Dragesco & Dragesco-Kernéis, 1986) than to soil isolated populations (about 70 µ) (Foissner, 1989; Aescht & Foissner, 1998), contrary to what Foissner (1989) previously stated. Foissner (1989) noted that populations in freshwater tend to be larger than those in terrestrial environments.

This observations agree well with Aescht & Foissner (1998) on stomatogenesis. The divisional morphogenesis of *B. hyalinum* is parakinetal stomatogenesis, typical of heterotrich ciliates, in which the oral primordia are derived from postoral somatic kineties (Lynn, 2008). In contrast to some other *Blepharisma* species (e.g., *B. americanum*, *B. undulans*; (Aescht & Foissner, 1998), the proliferation of the kinetosomes for the oral primordium of the opisthe occurs only one post-oral ciliary row (monoparakinetal) and no reorganization of the ancestral oral apparatus in the proter is seen.

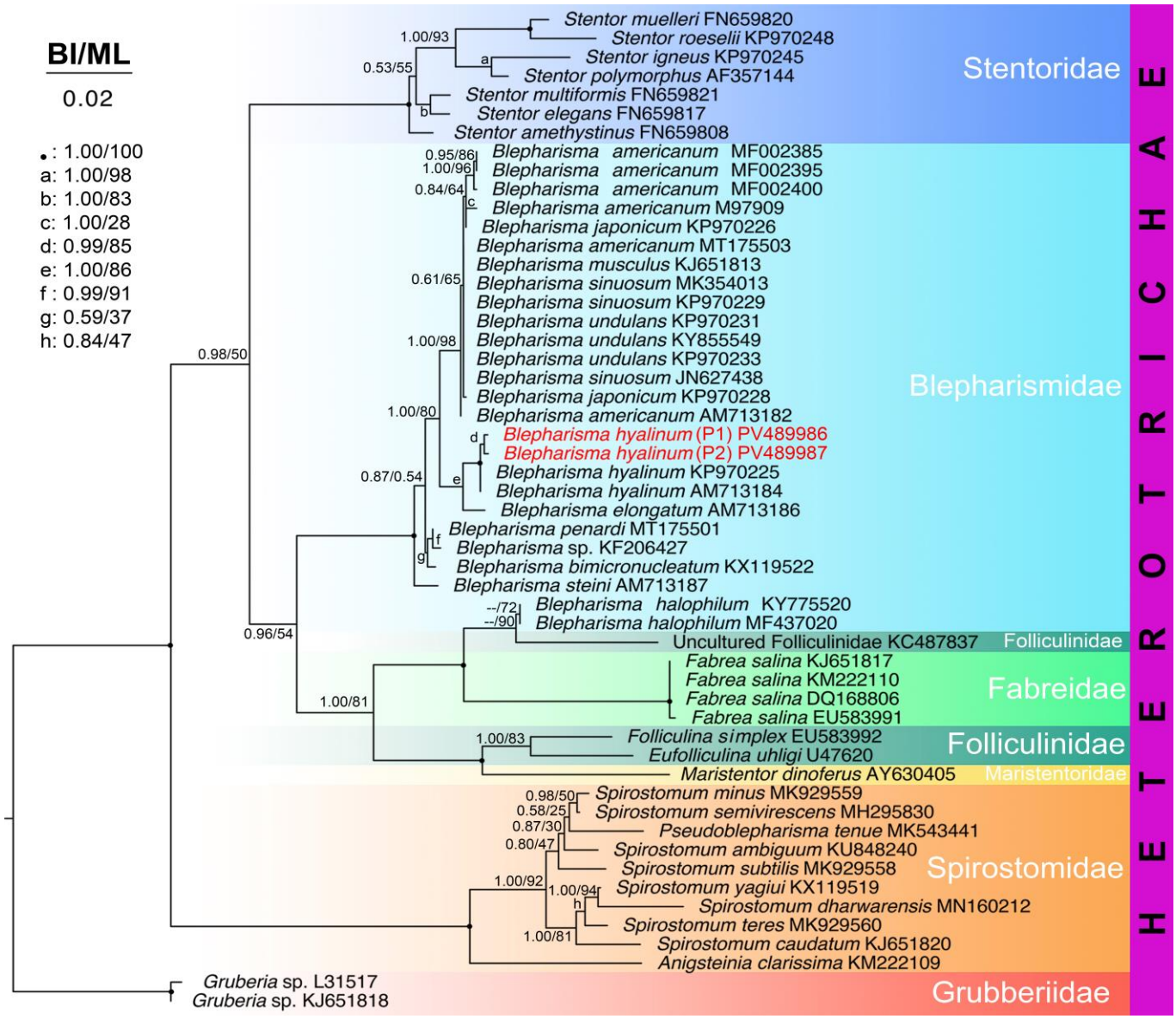

Figure 6. Phylogenetic tree of BI (Bayesian inference) and ML (maximum likelihood) based on the sequence of the 18S rDNA gene. The populations shown in red on the tree are those isolated in this study. Numbers at node indicate Bayesian posterior probability (BI) and maximum likelihood bootstrap (ML) values. Solid circles on the nodes indicate full support by both algorithms. The scale bar corresponds to 2 substitutions per 100 nucleotide positions.

Şekil 6. 18S rDNA gen dizisine dayalı BI (Bayesian cıkarsaması) ve ML (maksimum olabilirlik) filogenetik ağacı.

The formation of cysts is a survival strategy of the ciliates in unfavorable conditions, and it also plays an important role in their geographical dispersal (Cavaleiro et al., 2018). The cyst morphology of *B. hyalinum* is the same as that of its congeners (Isquith et al., 1965; Repak, 1968; Larsen, 1982; Larsen & Nilsson, 1988; Cavaleiro et al., 2018; Li et al., 2022). Within the genus *Blepharisma*, the frequency of cyst formation varies between species (Isquith et al., 1965; Larsen, 1982). *Blepharisma hyalinum*, which is widely distributed, is expected to have a high encystment capacity and consequently a high incidence of cyst formation. However, despite repeated attempts under laboratory conditions, cyst formation was observed less frequently than anticipated. The process of cyst formation also takes a longer time (in 4–5 days).

The positions of *Blepharisma* populations on the phylogenetic tree (Fig. 6) and the similarities and differences of nucleotide sequences (Table 4) are compatible with each other. The two populations obtained in the present study were concluded to represent two distinct morphotypes of the same species, as they showed high similarity to previously reported *B. hyalinum* populations (AM713184 and KP970225) (Schmidt et al., 2007; Fernandes et al., 2016), in terms of nucleotide sequences. This populations differ from others by only two nucleotides.

The first molecular phylogenetic studies on *Blepharisma* spp. have shown that this genus is a monophyletic taxon (Miao et al., 2009; Thamm et al., 2010; Fernandes et al., 2013; Shazib et al., 2014; Fernandes et al., 2016; Yan et al., 2016). Recent studies, including gene sequences of *B. halophilum* reveal that the genus *Blepharisma* is paraphyletic (Pan & Stoeck, 2017; Jin et al., 2021; Ye et al., 2021; Hao et al., 2022). In this study, populations of *B. halophilum* (KY775520 and MF437020) were found more closely related to members of the families Folliculinidae, Fabreidae, and Maristentoridae than their other congeners, supporting that the genus is a paraphyletic taxon. In previous studies (Pan & Stoeck, 2017; Hao et al., 2022), it was stated that *B. halophilum* was included in the genus *Blepharisma* due to its morphological features, such as a contractile body, unfragmented and two-part paroral membrane, but should be considered in a separate genus because of its extremely saline habitat, as in the karyorelicted ciliates *Loxodes* and *Remanella*. The nucleotide similarities and differences determined in this study and the location of *B. halophilum* populations (KY775520 and MF437020) on phylogenetic trees support the suggestion of Hao et al. (2022).

Table 4. Comparison of the main characteristics of *B.hyalinum* populations (P1: population 1, P2: population 2).
Çizelge 4. B. hyalinum popülasyonlarındaki ana karakteristiklerin karşılaştırılması (P1: popülasyon 1, P2: popuşasyon 2).

Characteristics	Denmark, Funen (Larsen & Nilsson, 1983)	Austria, Pfennigberg (Foissner, 1989)	Austria, Pfennigberg (Aescht & Foissner, 1998)	P1	P2
				Present work	
Body length, µm	80-160	47-78	61-79	66-127	82-129
Body width, µm	24-48	12-25	16-24	18-36	16-30
Macronucleus length, µm	25	9-20	11-19	12-29	12-24
Macronucleus width, µm	12	4-7	5-8	5-14	4-8
Number of micronuclei	4-7	2-5	1-2	1-7	1-6
Diameter of micronucleus, µm	2	1.2-1.7	1-2	1-1.45	1-2
Number of somatic kineties	18	11-14	12-14	14-19	14-20
AZM length, µm	-	-	32-43	26-40	22-40
Number of adoral membranelles	30	22-30	26-30	26-32	24-32
Habitat	eutrophic lake	forest soil	forest soil	pine and meadow soil	

In the similarity and dissimilarity matrices, *B. americanum* (MT175503), *B. musculus* (KJ651813), *B. sinuosum* (JN627438, MK354013, KP970229), *B. undulans* (KP970231, KY855549, KP970233), and *B. japonicum* (KP970228) populations have no or very low nucleotide differences, while nucleotide similarity is complete or quite high. This similarity is clearly reflected in the phylogenetic trees obtained with both algorithms (BI and ML), where populations from morphologically distinct species are closely positioned and exhibit polyatomic relationships within the same cluster. Similar results were found in the phylogenetic trees reported in previous studies (Luo et al., 2019; Chi et al., 2021; Hao et al., 2022). We think that this taxonomic uncertainty may be caused by nucleotide sequences obtained from morphologically misidentified populations. In morphological studies in soil cultures, variations in cell shape and size, macronucleus shape, and number were observed in populations in different culture dishes or different periods of culture.

In conclusion, it is clear that the genus *Blepharisma* needs to be revised using modern taxonomic techniques, as previously suggested by (Foissner et al., 2002). Therefore, study provides valuable morphological and molecular data that may serve as a basis for future taxonomic revisions within the genus *Blepharisma*.

-ACKNOWLEDGMENTS

This study was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK, Project No: 221Z208) Türkiye.

Contribution Rate Statement Summary of ResearchersContribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of InterestConflict of Interest

The authors of the article declare that there is no conflict of interest between them.

REFERENCES

Adobe Inc. (2014). *Adobe Photoshop* (Version CC 2014). Retrieved from https://www.adobe.com/products/photoshop.html

Aescht, E. & Foissner, W. (1998). Divisional morphogenesis in *Blepharisma americanum*, *B. undulans*, and *B.*

hyalinum (Ciliophora: Heterotrichida). *Acta Protozoolica, 37*(2), 71-92.

Al-Rasheid, K. A. (1999). Records of marine interstitial hymenostomatid ciliates from the Jubail Marine Wildlife Sanctuary, Saudi Arabia. *Qatar Univ. Sci. J., 18*, 123-130.

Al-Rasheid, K. A. S., Nilsson, J. R. & Larsen, H. F. (2001). *Blepharisma intermedium* Padmavathi, 1959 (Ciliophora: Heterotrichida) from Al-Hassa inland hypersaline oassis Saudi Arabia. *Acta Parasitologica, 40*, 63-69.

Asadullayeva, E. & Alekperov, İ. (2007). Free-living ciliates of the Anzali Wetland of the Caspian Sea. *Turkish Journal of Zoology, 31*(2), 143-149.

Ayres, D. L., Darling, A., Zwickl, D. J., Beerli, P., Holder, M. T., Lewis, P. O., Huelsenbeck, J. P., Ronquist, F., Swofford, D. L., Cummings, M. P., Rambaut, A. & Suchard, M. A. (2012). BEAGLE: an application programming interface and high-performance computing library for statistical phylogenetics. *Systematic Biology, 61*(1), 170-173. https//doi.org/10.1093/sysbio/syr100

Bhandary, A. V. (1962). Taxonomy of genus *Blepharisma* with special reference to *Blepharisma undulans*. *Journal of Protozoology, 9*, 435-442. https//doi.org/10.1111/j.1550-7408.1962.tb02649.x

Çapar, S. (2007). Checklist for ciliate species (Protozoa, Ciliophora) living in Turkish inland waters and flooded zones. *E.Ü. Journal of Fisheries & Aquatic Sciences, 24*, 207-212.

Cavaleiro, J., Fernandes, N. M., da Silva-Neto, I. D. & Soares, C. A. G. (2018). Resting cysts of the pigmented ciliate *Blepharisma sinuosum* Sawaya, 1940 (Ciliophora: Heterotrichea). *Journal of Eukaryot Microbiology, 65*(3), 422-426. https//doi.org/10.1111/jeu.12483

Ceja-Navarro, J. A., Wang, Y., Ning, D., Arellano, A., Ramanculova, L., Yuan, M. M., Byer, A., Craven, K. D., Saha, M. C., Brodie, E. L., Pett-Ridge, J. & Firestone, M. K. (2021). Protist diversity and community complexity in the rhizosphere of switchgrass are dynamic as plants develop. *Microbiome, 9*, 96. https//doi.org/10.1186/s40168-021-01042-9

Chi, Y., Chen, X., Li, Y., Wang, C., Zhang, T., Ayoub, A., Warren, A., Song, W. & Wang, Y. (2021). New contributions to the phylogeny of the ciliate class Heterotrichea (Protista, Ciliophora): analyses at family-genus level and new evolutionary hypotheses. *Science China-Life Sciences, 64*(4), 606-620. https//doi.org/10.1007/s11427-020-1817-5

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods, 9*(8), 772. https//doi.org/10.1038/nmeth.2109

Dragesco, J. (1970). *Ciliés libres de Cameroun*. Ann. Fac. Sci., Yaounde, Cameroun.

Dragesco, J. & Dragesco-Kernéis, A. (1986). Ciliés Libres de l'Afrique Intertropicale. *Faune Tropicale, 26*, 1-559.

Fabczak, H., Groszyńska, B. & Fabczak, S. (2001). Light regulation of protein phosphorylation in *Blepharisma japonicum*. *Acta Parasitologica, 40*, 311-315.

Fernandes, N. M., Dias, R. J., Senra, M. V., Soares, C. A. & da Silva Neto, I. D. (2013). Morphology and 18S rDNA gene sequence of *Blepharisma sinuosum* Sawaya, 1940 (Ciliophora: Heterotrichea) from Brazil. *European Journal of Protistology, 49*(4), 623-633. https//doi.org/10.1016/j.ejop.2013.04.003

Fernandes, N. M., Paiva Tda, S., da Silva-Neto, I. D., Schlegel, M. & Schrago, C. G. (2016). Expanded phylogenetic analyses of the class Heterotrichea (Ciliophora, Postciliodesmatophora) using five molecular markers and morphological data. *Molecular Phylogenetics and Evolution, 95*, 229-246. https//doi.org/10.1016/j.ympev.2015.10.030

Foissner, W. (1987). Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. *Progresss in Protozoology, 2*, 69-212.

Foissner, W. (1989). Morphologie und Infraciliatur einiger neuer und wenig bekannter terrestrischer und limnischer Ciliaten (Protozoa, Ciliophora). *Sber. Akad. Wiss. Wien, 196*, 173-247.

Foissner, W. (1997). Global soil ciliate (Protozoa, Ciliophora) diversity: a probability-based approach using large sample collections from Africa, Australia and Antarctica. *Biodiversity and Conservation, 6*(12), 1627-1638. https//doi.org/10.1023/A:1018378822687

Foissner, W. (1999). Soil protozoa as bioindikators: pros and cons, methods, diversity, representative examples. *Agriculture, Ecosystems and Environment, 74*, 95-112.

Foissner, W. (2014). An update of basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *International Journal of Systematic and Evolutionary Microbiology, 64*(Pt 1), 271-292. https//doi.org/10.1099/ijs.0.057893-0

Foissner, W., Agatha, S. & Berger, H. (2002). Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha Region and the Namib Desert. *Denisia, 5*, 1-1459.

Giese, A. C. (1973). *Blepharisma. The biology of a light-sensitive protozoa*. Stanford University Press, U.S.A.

Greenwood, S. J., Schlegel, M., Sogin, M. L. & Lynn, D. H. (1991). Phylogenetic relationships of *Blepharisma americanum* and *Colpoda inflata* within the phylum ciliophora inferred from complete small subunit rRNA gene sequences. *Journal of Protozoology, 38*(1), 1-6. https//doi.org/10.1111/j.1550-7408.1991.tb04783.x

Gupta, R., Kumari, B., Ojha, D., Lohani, S. & Bhujabal, R. (2015). Comparative analysis of various media and food source on culturing of the eukaryotic microbe *Blepharisma*. *DU Journal of Undergraduate Research and Innovation, 1*(3), 144-152.

Hall, T. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95-98.

Hao, T., Li, B., Song, Y., Warren, A. & Pan, X. (2022). Taxonomy and molecular phylogeny of two new *Blepharisma* species (Ciliophora, Heterotrichea) from northeastern China. *European Journal of Protistology, 85*, 125908. https//doi.org/10.1016/j.ejop.2022.125908

Hirshfield, H. I., Isquith, I. R. & Bhandary, A. V. (1965). A proposed organization of the genus *Blepharisma* Perty and description of four new species. *Journal of Protozoology, 12*(1), 136-144. https//doi.org/10.1111/j.1550-7408.1965.tb01826.x

Isquith, I. R., Repak, A. J. & Hirshfield, H. I. (1965). *Blepharisma seculum*, sp. nov., a member of the subgenus (*Compactum*). *The Journal of Protozoology, 12*(4), 615-618. https//doi.org/10.1111/j.1550-7408.1965.tb03264.x

Jin, D., Zhao, X., Ye, T., Huang, J., Warren, A., Al-Farraj, S. A. & Chen, X. (2021). Taxonomy and SSU rDNA-based phylogeny of two heterotrich ciliates (Ciliophora, Heterotrichea) collected from subtropical wetlands of China, including the description of a new species, *Linostomella pseudovorticella* n. sp. *Front. Microbiol., 12*, 719360. https//doi.org/10.3389/fmicb.2021.719360

Kahl, A. (1932). Urtiere Oder Protozoa I: Wimpertiere Order Ciliata (Infusoria). 3. Spirotricha. *Tierwelt Dtl, 25*, 399-650.

Kuikman, P. J., Van Vuure, M. M. I. & Van Veen, J. A. (1989). Effect of soil moisture regime on predation by protozoa of bacterial biomass and the release of bacterial nitrogen. *Agriculture, Ecosystems & Environment, 27*(1-4), 271-279. https//doi.org/10.1016/0167-8809(89)90091-1

Lara, E. & Acosta-Mercado, D. (2012). A molecular perspective on ciliates as soil bioindicators. *European Journal of Soil Biology, 49*, 107-111. https//doi.org/10.1016/j.ejsobi.2011.11.001

Larsen, H. F. (1982). Light microscopic observations and remarks on the ecology of *Blepharisma persicinum* Perty, 1849. *Archiv Fur Protistenkunde, 125*, 63-72. https//doi.org/10.1016/s0003-9365(82)80005-5

Larsen, H. F. & Nilsson, J. R. (1983). Is *Blepharisma hyalinum* truly unpigmented? *Journal of Protozoology, 30*(1), 90-97. https//doi.org/10.1111/j.1550-7408.1983.tb01039.x

Larsen, H. F. & Nilsson, J. R. (1988). Observations on the ecology and morphology of *Blepharisma elongatum* (Stokes, 1884), Kahl, 1926). *Archiv Fur Protistenkunde, 136*(1), 51-63. https//doi.org/10.1016/s0003-9365(88)80035-6

Lee, E. S. & Shin, M. K. (2009). New record of ciliates, *Blepharisma undulans* and *B. steini* (Ciliophora: Heterotrichida: Blepharismidae) from Korea. *Korean J. Syst. Zool., 25*(1), 41-47. https//doi.org/10.5635/kjsz.2009.25.1.041

Li, J., Li, M. G., Yang, J., Ai, Y. & Xu, R. L. (2010). Community characteristics of soil ciliates at Baiyun Mountain, Guangzhou, China. *Zoological Studies, 49*(6), 713-723.

Li, Y., Wang, Y., Zhang, S., Maurer-Alcala, X. X. & Yan, Y. (2022). How ciliated protists survive by cysts: some key points during encystment and excystment. *Frontiers in Microbiology, 13*, 785502. https//doi.org/10.3389/fmicb.2022.785502

Liu, W., Jiang, J., Xu, Y., Pan, X., Qu, Z., Luo, X., El-Serehy, H. A., Warren, A., Ma, H. & Pan, H. (2017). Diversity of free-living marine ciliates (Alveolata, Ciliophora): Faunal studies in coastal waters of China during the years 2011-2016. *European Journal of Protistology, 61*, 424-438. https//doi.org/10.1016/j.ejop.2017.04.007

Luo, J., Ma, M., Lu, B., Li, X., Warren, A., Shi, Y. & Chen, X. (2019). The taxonomy and phylogeny of the poorly known heterotrich ciliate *Ampullofolliculina lageniformis* Hadzi, 1951 (Ciliophora: Heterotrichea). *Journal of Eukaryotic Microbiology, 66*(6), 925-936. https//doi.org/10.1111/jeu.12743

Lynn, D. H. (2008). *The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature*. Springer, Dordrecht.

Lynn, D. H. & Small, E. B., (2000). *Phylum Ciliophora Doflein, 1901*. In J. J. Lee, G. F. Leedale & P. Bradbury (Eds.), *The Illustrated Guide to the Protozoa* (pp. 371-656). Allen Press Inc.

Madoni, P. (2005). Ciliated protozoan communities and saprobic evaluation of water quality in the hilly zone of

some tributaries of the Po River (northern Italy). *Hydrobiologia, 541*(1), 55-69. https//doi.org/10.1007/s10750-004-4667-8

Medlin, L., Elwood, H. J., Stickel, S. & Sogin, M. L. (1988). The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene, 71*, 491-499. https//doi.org/10.1016/0378-1119(88)90066-2

Miao, M., Song, W., Clamp, J. C., Al-Rasheid, K. A., Al-Khedhairy, A. A. & Al-Arifi, S. (2009). Further consideration of the phylogeny of some "traditional" heterotrichs (Protista, Ciliophora) of uncertain affinities, based on new sequences of the small subunit rRNA gene. *Journal of Eukaryotic Microbiology, 56*(3), 244-250. https//doi.org/10.1111/j.1550-7408.2009.00391.x

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A. & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol., 37*(5), 1530-1534. https//doi.org/10.1093/molbev/msaa015

Minitab LLC. (2016). *Minitab* (Version 16.0). Retrieved from https://www.minitab.com

Pan, H. & Stoeck, T. (2017). Redescription of the halophile ciliate, *Blepharisma halophilum* Ruinen, 1938 (Ciliophora, Heterotrichea, Heterotrichida) shows that the genus *Blepharisma* is non-monophyletic. *European Journal of Protistology, 61*(Pt A), 20-28. https//doi.org/10.1016/j.ejop.2017.07.004

Perty, M. (1849). *Mikroskopische organismen der Al-pen u.d. Italienischen Schweiz*. Mitt. Natureforsch, Ges, Bern.

Post, F. J., Borowitzka, L. J., Borowitzka, M. A., Mackay, B. & Moulton, T. (1983). The protozoa of a Western Australian hypersaline lagoon. *Hydrobiologia, 105*(1), 95-113. https//doi.org/10.1007/bf00025180

Rambaut, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using tracer 1.7. *Syst Biol, 67*(5), 901-904. https//doi.org/10.1093/sysbio/syy032

Repak, A. J. (1968). Encystment and excystment of the heterotrichous ciliate *Blepharisma stoltei* Isquith. *Journal of Protozoology, 15*(3), 407-412. https//doi.org/10.1111/j.1550-7408.1968.tb02148.x

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology, 61*(3), 539-542. https//doi.org/10.1093/sysbio/sys029

Schmidt, S. L., Foissner, W., Schlegel, M. & Bernhard, D. (2007). Molecular phylogeny of the Heterotrichea (Ciliophora, Postciliodesmatophora) based on small subunit rRNA gene sequences. *Journal of Eukaryotic Microbiology, 54*(4), 358-363. https//doi.org/10.1111/j.1550-7408.2007.00269.x

Shazib, S. U., Vďačný, P., Kim, J. H., Jang, S. W. & Shin, M. K. (2014). Phylogenetic relationships of the ciliate class Heterotrichea (Protista, Ciliophora, Postciliodesmatophora) inferred from multiple molecular markers and multifaceted analysis strategy. *Mol. Phylogenet. Evol., 78*, 118-135. https//doi.org/10.1016/j.ympev.2014.05.012

SPSS Inc. (2006). *SPSS for Windows* (Version 15.0). Retrieved from https://www.spss.com

Stein, F. (1867). *Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bear- beitet*. Engelmann, Leipzig.

Suzuki, S. (1954). Taxonomic studies on *Blepharisma undulans* Stein with special reference to the macronuclear variation. *Journal of Science of the Hiroshima University Series B15*, 204-220.

Tamura, K., Stecher, G. & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol Biol Evol, 38*(7), 3022-3027. https//doi.org/10.1093/molbev/msab120

Thamm, M., Schmidt, S. L. & Bernhard, D. (2010). Insights into the phylogeny of the genus *Stentor* (Heterotrichea, Ciliophora) with special emphasis on the evolution of the macronucleus based on SSU rDNA data. *Acta Protozoologica, 49*(3), 149-157.

Thompson, A. R., Powell, G. S. & Adams, B. J. (2019). Provisional checklist of terrestrial heterotrophic protists from Antarctica. *Antarctic Science, 31*(6), 287-303. https//doi.org/10.1017/s0954102019000361

Ural, H., Bircan, R., Aral, C., Yıldız, İ. & Şenler, N. G. (2020). A new approach to DNA isolation from dileptid ciliates (Ciliophora: Litostomatea: Rhynchostomatia). *Acta Zoologica Bulgarica, 72*(2), 199-205.

Ural, H., Şenler, N. G. & Yıldız, İ. (2023). Morphological and molecular characterization of species of genus *Blepharisma* Perty, 1849 and genus *Frontonia* ehrenberg, 1838 isolated from İğneada Longoz Forests (floodplain) National Park (Longoz of Lake Mert) (Sözlü bildiri). V. International Agricultural, Biological & Life Science Conference, Edirne, Türkiye, 18-20 September 2023, pp. 308.

Vďačný, P. & Foissner, W. (2012). Monograph of the dileptids (Protista, Ciliophora, Rhynchostomatia). *Denisia, 31*, 1-529.

Yan, Y., Fan, Y. B., Chen, X. R., Li, L. F., Warren, A., Al-Farraj, S. A. & Song, W. B. (2016). Taxonomy and phylogeny of three heterotrich ciliates (Protozoa, Ciliophora), with description of a new *Blepharisma* species. *Zoological Journal of the Linnean Society, 177*(2), 320-334. https//doi.org/10.1111/zoj.12369

Ye, T. T., Zhao, X. T., Chi, Y., Zheng, B. H., Zhang, H., Huang, J., Warren, A. & Chen, X. R. (2021). Morphology and molecular phylogeny of two marine folliculinid ciliates found in China (Ciliophora, Heterotrichea). *Journal of Ocean University of China, 20*(4), 959-972. https//doi.org/10.1007/s11802-021-4965-z

Yıldız, İ. (2021). Morphology and phylogeny of a new soil ciliate, *Colpodidium zelihayildizae* n. sp. (Ciliophora, Nassophorea, Colpodidiidae), from Van, Turkey. *Turkish Journal of Zoology, 45*, 304-313. https//doi.org/10.3906/zoo-2101-20