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A new small-celled naviculoid diatom species, *Mayamaea pannonica* sp. nov. (Bacillariophyceae) from soda pans in Serbia

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ABSTRACT

Combining morphological and molecular approaches, a new diatom species is described in the genus *Mayamaea* from a Special Nature Reserve, Okanj bara in Serbia, an alkaline and subsaline soda pan with pronounced seasonal drying. *Mayamaea pannonica* sp. nov. possesses typical characteristics of the genus *Mayamaea*, such as uniseriate striae and hymenes that cover the areolae from the outside. It can easily be distinguished from similar species by the valve outline and shape of the central area. Although presently no culture of the species is available, we identified in an amplicon data set from the same sample a partial 18S rRNA gene sequence that is thought to represent the new species, with high probability. A comparison of our sequence to those present in the Diat.barcode and NCBI databases showed that the sequence is closely related to, but significantly different from, that of *Mayamaea terrestris*. Natural saline habitats in Serbia and neighbouring countries are under anthropogenic threat and climate change, so knowing which species live here is the first step in preserving these unique habitats.

ARTICLE HISTORY

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KEYWORDS

18S rRNA gene marker;
Diatoms; NCBI; Pannonian Plain

INTRODUCTION

Small cell size and boat-shaped (naviculoid) valve outline are features of several genera often referred to as ‘small-celled naviculoid diatom species’, such as *Mayamaea* Lange-Bertalot, *Sellaphora* Mereschkowski, *Adlafia* Gerd Moser, Lange-Bertalot & Metzeltin, etc. Hustedt (1962) was one of the first to provide a detailed account of small naviculoid species and provided drawings of several taxa based on type material. Difficulties of identification by light microscopy (LM), lack of scanning electron microscopic (SEM) images and frequent changes in taxonomic concepts have led to uncertainty about the identities of many species of this group. Recently, a few studies have provided more detailed information by revising type material by LM and SEM observations (e.g. Potapova 2013; Wetzel *et al.* 2015), resulting in nomenclatural changes and transfer of species from *Navicula sensu lato* to *Mayamaea*, *Sellaphora*, etc.

Lange-Bertalot (1997) described the genus *Mayamaea* and initially included 13 taxa. Currently, there are 29 species names, one variety and one form listed in AlgaeBase (Guiry & Guiry 2023). The main generic characters indicate small, solitary cells with two chromatophores and more or less elliptic to lanceolate-elliptic valves with broadly rounded ends, filiform raphe, and rounded areolae with external hymenes (Lange-Bertalot 1997, 2001). The genus is distributed worldwide, and usually prefers

wet and dry soil habitats, but was also found in freshwater ecosystems (Lange-Bertalot 2001; Wetzel *et al.* 2015; Barragán *et al.* 2017; Kezlya *et al.* 2020). Soil is a challenging habitat for aquatic organisms due to the non-permanent presence of water. According to Wetzel *et al.* (2015), *Mayamaea* species are specifically aerophilic diatoms and are among the most abundant taxa in this type of habitat.

Up to now, DNA sequence information is only available for a few taxa. *Mayamaea terrestris* N. Abarca & R. Jahn was described by linking sequences derived from cultures to a type specimen (Zimmermann *et al.* 2014), while the description of *M. ectorii* Ács, K.T. Kiss & C.E. Wetzel was supported by a sequence obtained from a metabarcoding data set (Bíró *et al.* 2022). Metabarcoding using next-generation sequencing is a cultivation-independent molecular technique for community composition assessment; it is increasingly being used routinely for diatom analyses (Zimmermann *et al.* 2014; Bíró *et al.* 2022). Sequences obtained in such studies are assigned taxonomic names through comparison with publicly available sequences obtained from cultured strains. Diat.barcode (Rimet *et al.* 2019) is one of the best-curated diatom-specific sequence databases for such taxonomic annotations.

In this study, based on LM, SEM and molecular information, we describe a new species of the genus *Mayamaea* from extreme environments (soda pans) in the northern part of Serbia. In addition to salinity, soda pans are very shallow

water habitats subject to seasonal drying (Boros *et al.* 2013; Vidaković *et al.* 2019). These saline habitats have high conservation priority according to the EU Habitats Directive (92/43/EC) and are part of the Natura 2000 Network in the EU. Besides morphological information, similarly to Bíró *et al.* (2022) we present a partial 18S rRNA gene sequence from an amplicon (metabarcoding) library obtained from the same sample, a marker sequence that, with high probability, belongs to this new species.

MATERIAL AND METHODS

Sample collection and microscopy analysis

Samples used in this study were collected in two pristine (in natural status) saline ponds located in the Vojvodina Region, Serbia (Fig. 1). Okanj bara is a Special Nature Reserve, while Bela bara is part of the National Ecological Network.

Diatom samples and samples for water chemical analyses were collected in February 2020 from Bela bara, and in March 2021 from Okanj bara. Diatoms were collected in two different microhabitats, submerged reed stems [*Phragmites australis* (Cavanilles) Trinius *ex* Steudel] and mud. Samples from Okanj bara were divided into those for morphological analysis and 18S rDNA metabarcoding analysis. Samples for metabarcoding analysis were placed in 50-ml falcon tubes, with 99% ethanol added to maintain a 1:3 sample:ethanol ratio. The remaining samples were placed in 50-ml plastic vials for morphological analysis.

To remove organic matter, diatom samples were treated with hot concentrated HCl and a supersaturated solution of KMnO₄

following the procedure described by Taylor *et al.* (2007). Permanent slides of the cleaned material were mounted with Naphrax (Brunel Microscopes Ltd, Chippenham, UK). Light microscope observations and micrographs were obtained with a Zeiss AxioImagerM.1 microscope with DIC optics and AxioVision 4.9 software (Zeiss, Oberkochen, Germany). The relative abundance of the new species was estimated by counting 400 valves per slide. Part of the material was dried onto SEM stubs, coated with 5 nm Au and Pd, and observed with a Zeiss Gemini Ultra plus (operated at 3–5 kV, with 3–12 mm working distance; the Natural History Museum, London, Imaging and Analysis Centre). Additionally, after identical preparation, the material was observed with an Apreo S LoVac SEM (Thermo Fisher Scientific, Cambridge, UK) at the University of Duisburg-Essen in Essen, Germany.

The holotype slide is deposited in the Diatom Collection of Serbia (DCSR) at the University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia. An isotype slide is deposited in the Institute of Biology, Faculty of Natural Sciences, Skopje, Republic of North Macedonia.

Water analysis

The following physicochemical parameters were measured during sampling using a Water Multimetric 18.52.01 (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands): water temperature (T), pH, and conductivity (COND). The determination of metal cations (Na⁺, K⁺, Ca²⁺ and Mg²⁺) was done by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Thermo Scientific iCAP 6500 Duo



Fig. 1. Location of the investigated saline ponds in the Vojvodina Region, Serbia. 1, Bela bara; 2, Okanj bara.

ICP (Thermo Fisher Scientific). Ammonium (NH_4^+ , SRPS EN ISO 14911:2009), nitrite (NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-} , US EPA 1997) concentration, total phosphorus (TP, SRPS EN ISO 6878:2008), total nitrogen (TN, SRPS EN 12260:2008), carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) (APHA, AWWA and WPCF 1995a), chloride (Cl^- , APHA, AWWA and WPCF 1995b) and sulfate (SO_4^{2-} , APHA, AWWA and WPCF 1995c) were measured in the laboratory using standard quantification methods.

Metabarcoding and sequence data processing

Total DNA was extracted from a mud sample from Okanj bara using the power Soil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA). DNA concentrations were quantified using a Qubit 4 Fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA), used for the amplification of the V4 region of the gene encoding the 18S nuclear ribosomal DNA (SSU rDNA) and the cleaned PCR product was sent for sequencing to the Novogene Sequencing Service (Cambridge, UK). Primer sequences, a description of polymerase chain reactions (PCR), library preparation, and sequencing are provided in Vidaković *et al.* (2022).

Paired DNA reads were merged and amplicon sequence variants (ASVs) were delimited using the DADA2 pipeline (Callahan *et al.* 2016). Taxonomy assignment was performed using the library 2021-06-25-Diat.barcode-release-version 10.1.xlsx (Rimet *et al.* 2019). Taxon assignment was performed using the IDTAXA algorithm of the DECIPHER package for R (Murali *et al.* 2018) with default options used for classification up to genus level. Species-level annotation was obtained using the species assignment set generated from the above-mentioned Diat barcode 10.1. An ASV closely related to, but different from *Mayamaea* sequences in public databases was aligned with other *Mayamaea* sequences using the MUSCLE option in the MEGA X software (Kumar *et al.* 2018). A phylogenetic tree was obtained using the maximum likelihood method with the General Time Reversible substitution model (Nei & Kumar 2000) and discrete

Gamma distribution to model evolutionary rate differences among sites (5 rate categories, parameter = 0.1752); branch support was assessed in 100 bootstrap replicates. The tree was drawn to scale, with branch lengths proportional to the number of substitutions per site. This analysis involved 40 nucleotide sequences, and there was a total of 283 alignment positions in the final dataset. The sequence probably representing the new species *M. pannonica* has been deposited in the NCBI Gen-Bank database under the accession number OP868963.

RESULTS

Mayamaea pannonica Vidaković, Krizmanić & Levkov *sp. nov.*

Figs 2–31 (LM), 32–42 (SEM)

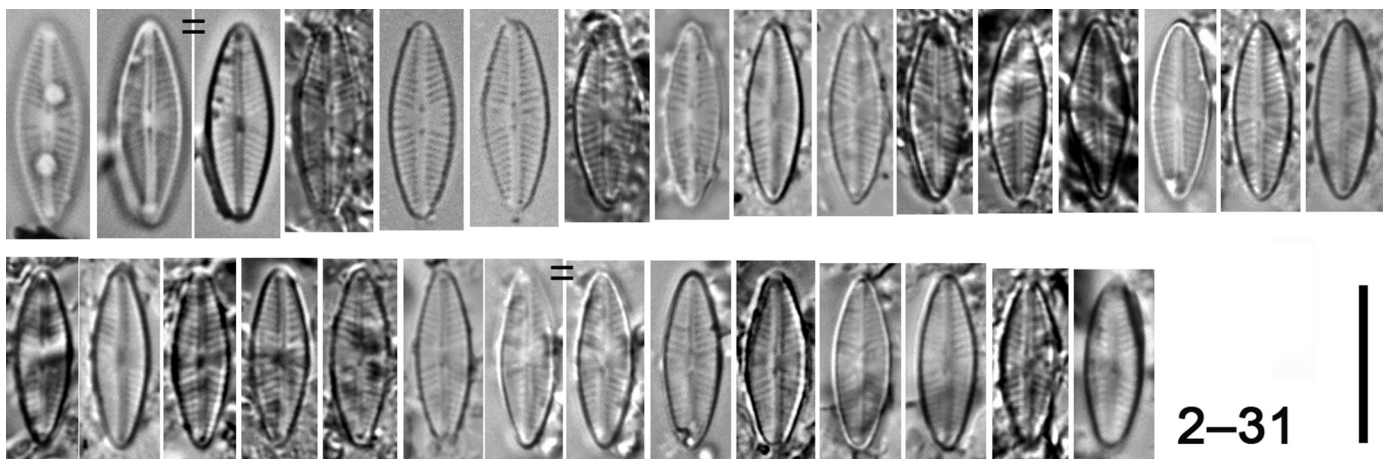
DESCRIPTION, LM: Valves small, lanceolate-elliptic with wedge-shaped apices. Valve length 10.5–13.0 μm , width 3.4–4.1 μm ($n = 30$). Axial area very narrow, linear, central area wide, bow-tie-shaped, sometimes asymmetrical. Striae radiate throughout, 2–3 shorter irregular striae in the center area, 21–23 in 10 μm . Areolae not discernable with LM.

DESCRIPTION, SEM: External view – striae uniseriate composed of rounded areolae, different in size, and covered by hymenes, 50–60 in 10 μm (Figs 32–38). Areolae next to raphe usually larger than others (Figs 32, 34, 35). Axial area linear over most of its length (Figs 32, 34, 40). Proximal raphe endings straight, drop-shaped (Figs 34, 35, 37). Distal external raphe fissures bending strongly to the same side of the valve (Figs 36, 38). Internal view – areolae circular, not covered by hymenes (Figs 39–42). Proximal raphe endings slightly curved (Fig. 41). Small helictoglossa present at the distal raphe endings (Fig. 42).

HOLOTYPE: Slide DCSR 000267/A (illustrated in Fig. 16), collected 31 March 2021 by D. Vidaković and M. Ćirić from the Okanj bara saline pond, Republic of Serbia; deposited in the Diatom Collection of Serbia (DCSR) at the University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia (Accession No. DCSR 000267).

ISOTYPE: slide MKNDC 014241, deposited in the Institute of Biology, Faculty of Natural Sciences, Skopje, Republic of North Macedonia.

TYPE LOCALITY: 45°27.60'N, 20°17.18'E, Okanj bara, Serbia.



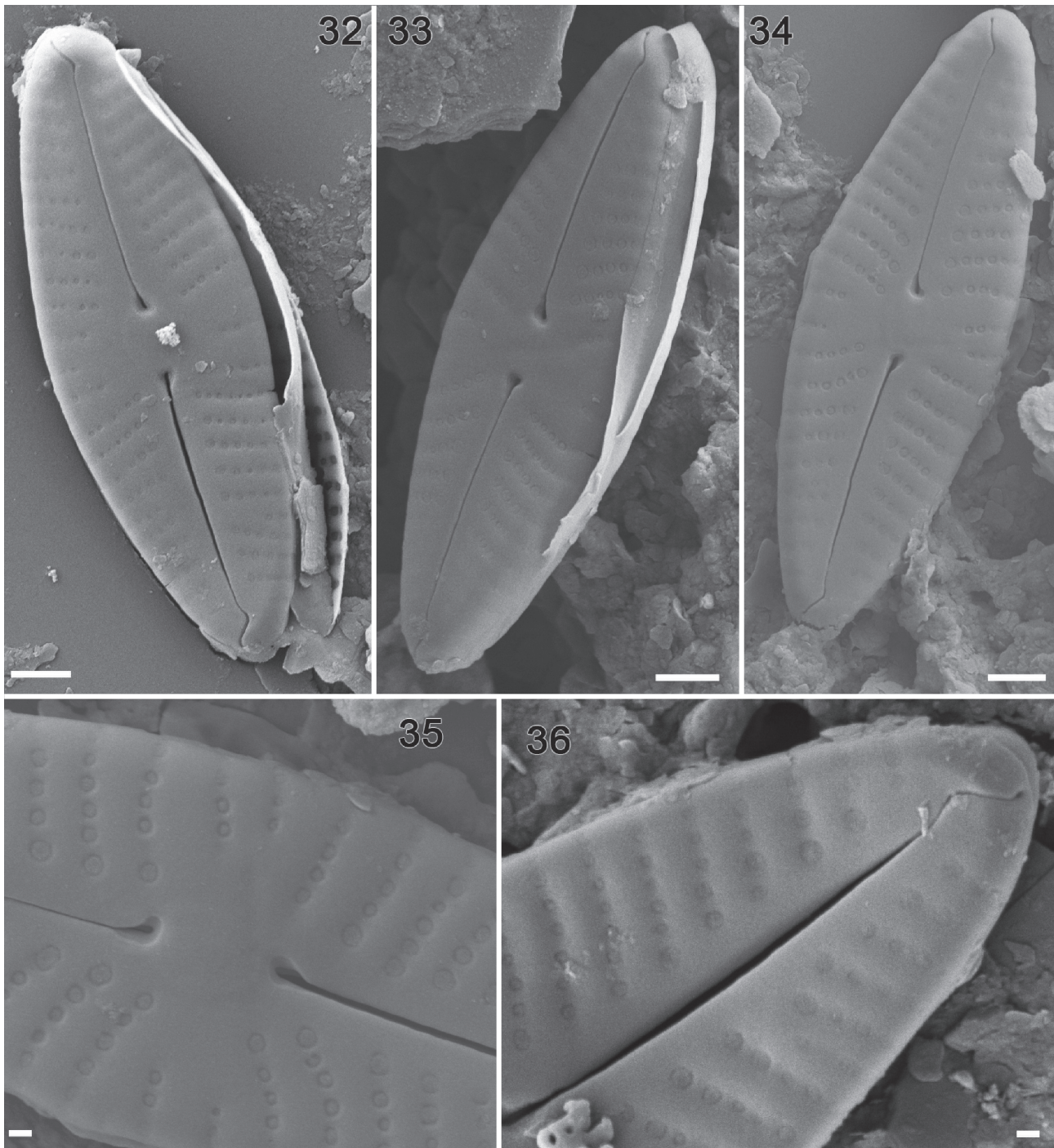
Figs 2–31. *Mayamaea pannonica* *sp. nov.*, LM. Images connected by the sign '=' were taken from the same specimen. Scale bar = 10 μm .

Figs 2–4. Specimens from Bela bara.

Fig. 5. Specimen from Okanj bara.

Figs 6, 7. Specimens from Bela bara.

Figs 8–31. Specimens from Okanj bara.



Figs 32–36. *Mayamaea pannonica* sp. nov., SEM (external view).

Figs 32–34. Whole valve. Scale bars = 1 μ m.

Fig. 35. Central area. Scale bar = 500 nm.

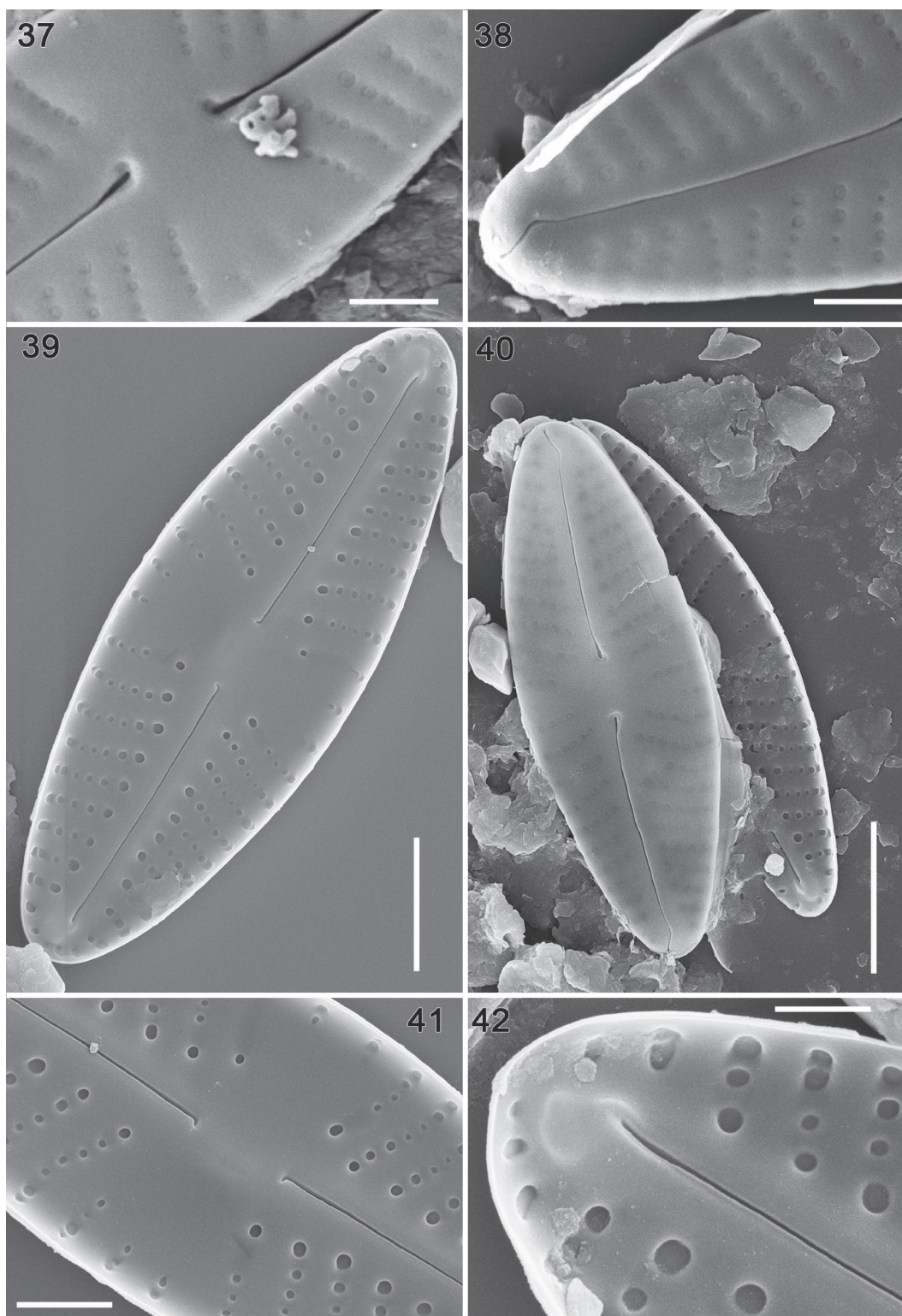
Fig. 36. Valve end. Scale bar = 500 nm.

ETYMOLOGY: The specific epithet *pannonica* refers to the geographical area where the type locality is found, the Pannonian Plain.

Distribution and ecology

Mayamaea pannonica was recorded in two saline ponds in Serbia (Fig. 1). The species was found both in epipellic (Okanj bara and Bela bara) and epiphytic communities (Bela bara). The highest relative abundance of this species was found on

mud (epipellic material), at the type locality of Okanj bara (7.46%). In Bela bara, the species was infrequent in an epipellic community but occurred with a relative abundance of 6% in the epiphytic community. Species richness was higher in the Okanj bara (epipellic community, 17) than in Bela bara (epiphytic community, 11), and the most dominant taxon was *Nitzschia austriaca* Hustedt (50% in both samples). As subdominant taxa in the epipellic community in the Okanj bara were recorded *Craticula molestiformis* (Hustedt) Mayama



Figs 37–42. *Mayamaea pannonica* sp. nov., SEM.

Fig. 37. External view of central area. Scale bar = 1 μ m.

Fig. 38. External view of valve end. Scale bar = 1 μ m.

Fig. 39. Internal view of whole valve. Scale bar = 2 μ m.

Fig. 40. External and partial internal view of valves. Scale bar = 3 μ m.

Fig. 41. Internal view of central area. Scale bar = 1 μ m.

Fig. 42. Internal view of valve end. Scale bar = 500 nm.

(10.45%) and *Cylindrotheca gracilis* (Kützing) Grunow (9.95%), while in the Bela bara were observed *Halamphora veneta* (Kützing) Levkov (27%) and *C. molestiformis* (9%). In

the community, together with the new species, the presence of *Mayamaea permitis* (Hustedt) Bruder & Medlin was also noticed. The relative abundance of *M. permitis* was lower

compared with *M. pannonica*, 4.5% in Bela bara, and 1.99% in Okanj bara.

Both saline ponds are shallow water bodies with a pH above 8 (Table 1). According to Hammer's (1986) salinity classification, Okanj bara and Bela bara can be characterized as subsaline, sodic base type ($\text{Na} - \text{HCO}_3^-$) water bodies.

Molecular analysis

Molecular analysis of a mud sample from Okanj bara showed the presence of two ASVs assignable to the genus *Mayamaea* that differed from each other at 5 nucleotide positions (0.0177 p distance). One of them was identical with several Genbank sequences annotated as *M. permitis* (LC648454, LC648455, KM084891), its older synonym *Navicula atomus* var. *permitis* (Hustedt) Lange-Bertalot (AJ867024) and *Navicula pelliculosa* (Kützing) Hilse (AJ544657; possibly a misidentification?). The other ASV had no identical matches in either Diat.barcode or NCBI; the closest NCBI matches (KM084897, KM084898 and LC648452) were mutually identical, annotated as *M. terrestris*, and showed a 0.016 p-distance (3 basepairs) from our ASV. The relative abundance of reads for this unknown *Mayamaea* species was 16.12%, while for the ASV matching *M. permitis* it was only 0.12%. Due to inconsequent nomenclature and lack of voucher documentation of GenBank sequences, it is difficult to draw precise conclusions about any possible intraspecific variability in the sequenced gene region, but when taking taxonomic annotations at face value, there are pairs of publicly available *M. permitis* sequences differing from each other in up to two alignment positions over the V4 region (p distance 0.0035–0.007; a distance matrix of *Mayamaea* sequences is shown in Table S1). The closest matches to our 'unknown' ASV (*M. terrestris*) show three nucleotide differences (p distance 0.00106). Based on the fact that during microscopy the two *Mayamaea* species (*M. permitis* and *M. pannonica* sp. nov.) were observed, and taking into account the amplicon data with two *Mayamaea* ASVs detected, the ASV without identical GenBank matches is interpreted here

as having originated from our *M. pannonica* sp. nov. with a high probability. Its differences (three basepairs or more) from all so far known *Mayamaea* sequences in the V4 region of 18S rDNA further support our conclusion that this species has not been encountered and described previously. The ASV assigned to *M. pannonica* sp. nov. was positioned on a separate branch of a phylogeny calculated from known *Mayamaea* sequences, as a sister group to lineages of *M. terrestris*, *M. permitis*, *M. atomus* (Kützing) Lange-Bertalot and *M. pseudoterrestris* Tuji & Haruyo Yamaguchi (Fig. 43).

DISCUSSION

Mayamaea pannonica sp. nov. was not easy to distinguish from other small-celled naviculoid diatom species under the light microscope. A more detailed morphological analysis using SEM has shown that the new species possesses typical characters of the genus *Mayamaea*, such as uniseriate striae and hymenes that cover the areolae from the outside (Lange-Bertalot 1997; Barragán et al. 2017). In contrast, the genus *Sellaphora* has uniseriate or biseriata striae and small round poroids occluded near their internal apertures by hymenes (Barragán et al. 2017). Typical small-sized *Navicula* species possess uniseriate striae composed of apically elongated areolae called 'lineolae' (Round et al. 1990).

Mayamaea pannonica sp. nov. is morphologically similar to some known *Mayamaea* species, but it differs from them by some features (see Table 2). In terms of valve size, the most similar species to *M. pannonica* sp. nov. is *M. petersenii* Barragán, Ector & C.E. Wetzel. The main difference can be noticed in valve shape: elliptical to oval with broadly rounded apices in *M. petersenii* (Barragán et al. 2017), and lanceolate-elliptical with wedge-shaped apices in *M. pannonica*. Also, *M. petersenii* has lower striae density (13–21 in 10 μm) and a symmetric central area in contrast to the new species. *Mayamaea cahabaensis* E. Morales & Manoylov (2009) has elliptical valves with narrowly rounded apices, but the absence of a central area and parallel to slightly radiate striae distinguish it from *M. pannonica*. *Mayamaea agrestis* (Hustedt) Lange-Bertalot (Lange-Bertalot 2001) can be distinguished from *M. pannonica* by its more obtusely rounded ends, smaller central area, and higher striae density (24–28 in 10 μm). *Mayamaea terrestris* is clearly different from *M. pannonica* in outline and in the absence of a central area (Zimmermann et al. 2014) even though the two species are genetically closely related, which will be discussed below. Other *Mayamaea* species with elliptical valve shape and with a very small or absent central area, such as *M. atomus* (Kützing) Lange-Bertalot (Lange-Bertalot 1997), *M. vietnamica* Glushchenko, Kezlya, Kulikovskiy & Kociolek (Kezlya et al. 2020), *M. crassistriata* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito (Lange-Bertalot et al. 2003), *M. fossalis* (Krasske) Lange-Bertalot (Lange-Bertalot 2001), *M. alcimonica* (E. Reichardt) C.E. Wetzel, Barragán & Ector (Barragán et al. 2017) will not be compared and further discussed because the distinction of the newly described species was clearly visible even under a light microscope.

Table 1. Physical and chemical characteristics of water in two Serbian saline ponds.

Parameters/Ponds	Bela Bara	Okanj bara
Temperature (°C)	9.2	24.8
pH	8.57	8.39
Conductivity ($\mu\text{s cm}^{-1}$)	1028	1603
Potassium (mg l^{-1})	3.39	8.95
Sodium (mg l^{-1})	252.3	321.3
Calcium (mg l^{-1})	25.70	15.11
Magnesium (mg l^{-1})	5.43	22.86
Carbonate (mg l^{-1})	750	164.4
Bicarbonate (mg l^{-1})	1122.4	1052.9
Ammonia (mg l^{-1})	1.4	2.44
Nitrite (mg l^{-1})	0.09	0.09
Nitrate (mg l^{-1})	0.90	<0.5
Phosphate (mg l^{-1})	0.038	0.66
Total phosphorus (P mg l^{-1})	0.039	1.280
Chloride (mg l^{-1})	25.3	164.8
Sulphate (mg l^{-1})	20.0	127.5

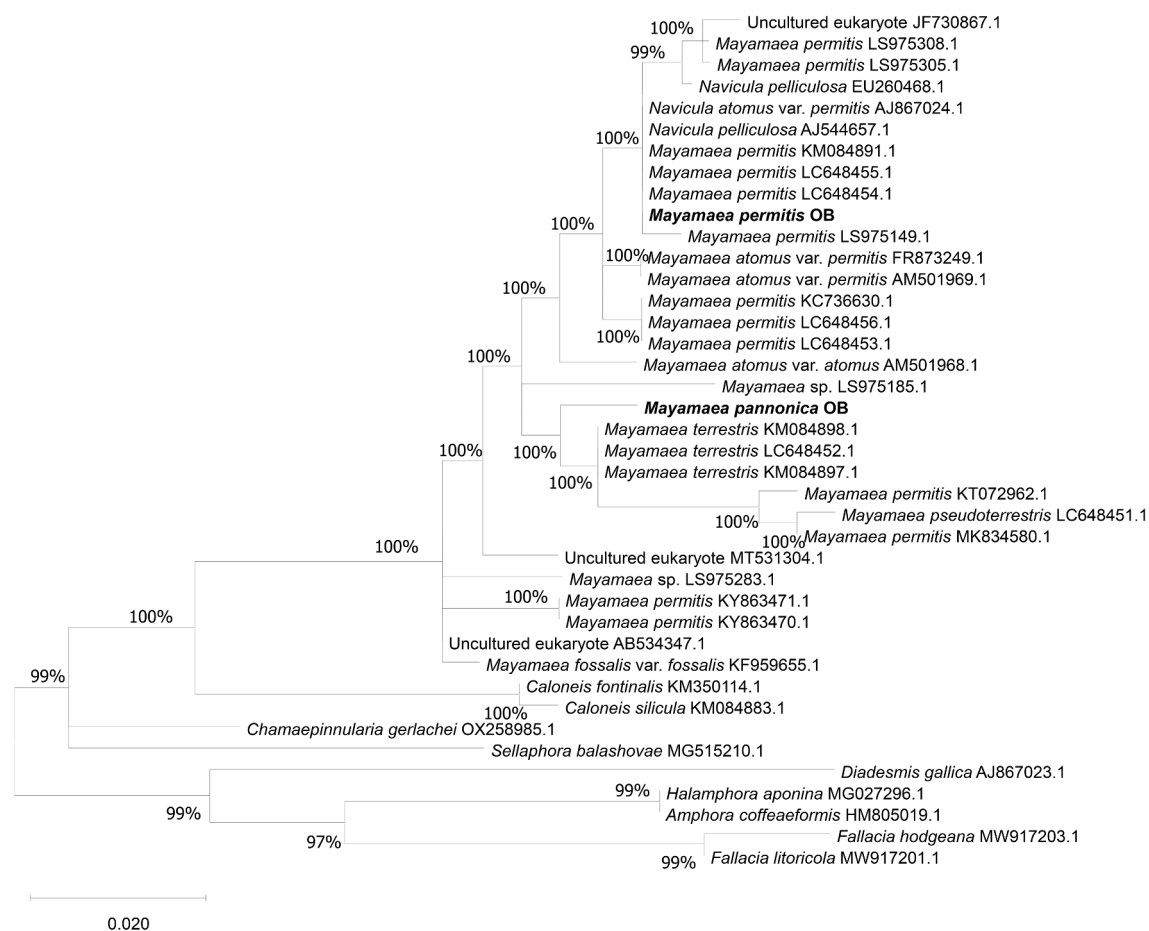


Fig. 43. Phylogenetic tree of the two *Mayamaea* ASVs from this study (bold; OB refers to Okanj bara) and similar publicly available sequences, plus outgroups. The tree with the highest log likelihood (−935.15) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths proportional to the number of substitutions per site.

Table 2. Comparison of morphological features of *Mayamaea pannonica* sp. nov. and related *Mayamaea* species. ND indicates no data available.

	<i>M. pannonica</i> sp. nov.	<i>M. petersenii</i>	<i>M. cahabaensis</i>	<i>M. agrestis</i>	<i>M. terrestris</i>
Outline	lanceolate-elliptic with wedge-shaped ends	elliptical to oval with broadly rounded ends	elliptical with narrowly rounded ends	linear-elliptic to elliptic-lanceolate with cuneate and obtusely ends	narrow linear-elliptic
Central area	wide, bow-tied, sometimes asymmetrical	wide, symmetrical, bow-tied or rectangularly shaped	absence of a central area	absence of a central area	absence of a central area
Valve length (µm)	10.5–13.0	7.7–9.9	5–14	9–11	7.0–8.7
Valve width (µm)	3.4–4.1	2.6–3.4	1.5–3.7	(2.5–)3–3.8	3.0–4.5
Striae in 10 µm	21–23	13–21	18–28	24–28	22–24(–26)
Areolae in 10 µm	50–60	ND	ND	ND	50
References	This study	Barragán <i>et al.</i> (2017)	Morales & Manoylov (2009)	Lange-Bertalot (2001)	Zimmermann <i>et al.</i> (2014)

We identified a novel 18S rDNA V4 sequence most probably originating from the new *Mayamaea* species, differing at three or more base positions from all currently known homologous *Mayamaea* sequences. For most of the above-listed species, which are morphologically closest to *M. pannonica* sp. nov., no sequence information for comparison is currently available (Kezlya *et al.* 2020; Bíró *et al.* 2022), but since none

of those species were observed in our samples, our conclusion that the novel ASV belongs to the new species is relatively robust. In the publicly available sequence data for the genus, *M. permitis* is represented by the highest number of sequence variants, as also observed by Kezlya *et al.* (2020), Bagmen *et al.* (2021) and Bíró *et al.* (2022). In a phylogenetic analysis, *M. pannonica* sp. nov. clustered close to *M. terrestris* but was

located on a distinct branch (Fig. 43), which is also in accordance with the hypothesis that it is a different species.

According to the available literature, *Mayamaea* species mostly occur in freshwater or aerophilic habitats, mainly in wet and moist or temporarily dry places (Barragán et al. 2017; Bíró et al. 2022). The newly described species, *M. pannonica* sp. nov., was found in a unique and extreme environment of soda pans. Although the physicochemical conditions are quite different from those in fresh water (higher conductivity and salinity), seasonal drying might be the trigger for the colonization of this species. A similar type of soda pans can be found in the 'Hungarian lowlands' ecoregion. There, the only recorded *Mayamaea* species so far has been *M. permitis* (Stenger-Kovács et al. 2014; Stenger-Kovács & Lengyel 2015).

Comparing the available data on the distribution of saline habitats in the Pannonian plain (Vojvodina province, Serbia) from the last century until today shows that their number has decreased significantly (Ćirić et al. 2021). The same situation is recorded in the neighbouring country, Hungary (Boros et al. 2014; Stenger-Kovács et al. 2014). The most significant negative factor affecting these habitats in Hungary and Serbia is intensive agriculture, which can reduce salinity and disturb the natural hydrological regime of soda pans. Results of a study made by Williams (2002) predicted that the number of these saline habitats will decrease even more drastically by 2025 due to climate change caused by human activity.

The results of this research enhance our knowledge about the biodiversity of soda pans, increase species diversity within the genus *Mayamaea*, provide morphological, ecological, and molecular data of the newly described species, and contributes to supplementing the sequences database. All this information together with the appropriate legal protection is the first step in preserving these unique habitats.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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