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Research Article

Metabarcoding as an effective complement of microscopic studies in revealing the composition of the diatom community – a case study of an oxbow lake of Tisza River (Hungary) with the description of a new *Mayamaea* species

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Abstract

Diatoms are valuable bioindicators and their traditional classification and identification are mainly based on the morphological characteristics of their frustules. However, in recent years, DNA-based methods have been proposed and are rapidly growing in the scientific literature as a complementary tool to assess the ecological status of freshwaters. Diatom-based ecological status assessment uses indices calculated from sensitivity and tolerance values as well as relative abundance of species. Correct assessment requires an accurate identification of species. In the present study, diatom assemblages of an oxbow lake were investigated using light and scanning electron microscopy as well as metabarcoding using *rbc*L marker, and the identification results were compared, intending to match barcode sequences of species that are currently missing in the diatom reference database. The investigated oxbow is an important wetland for bird conservation, although it is impacted by land use. Taxon lists based on morphology and metabarcoding considerably differed when bioinformatics analysis involved DADA2 pipeline with Diat.barcode database. Previously unknown sequence variants of four pennate species were found with additional BLAST search. Using phylogeny and p-distance calculations sequences could be matched to three small-celled naviculoid species that were found under a microscope. One of them was found to be a new species of the genus *Mayamaea* and was described as a new species, *Mayamaea ectorii*. Additionally, spatial distribution maps for several small-celled naviculoid species are provided for the Hungarian territory.

Key Words

Mayamaea, metabarcoding, new diatom species, oxbow

Introduction

River floodplains are some of the most valuable ecosystems on Earth; they play an important role in maintaining biodiversity and are essential for ecosystem services, but they are vulnerable (Vörösmarty et al. 2010). They are threatened not only by global warming (faster evaporation, less water recharge, changed physico-chemical conditions in warmer waters, etc. (Prakash 2021)), but also by the increasingly long-term loss of lateral connectivity, with the causes of this loss including the significant role of bed subsidence. The loss of lateral hydrological connectivity has been identified as a major cause of biodiversity loss and eco-

system degradation in such habitats (e.g. Amoros and Bornette 2002; Wang et al. 2016).

Diatoms frequently constitute a dominant group in benthic aquatic habitats. These algae have a significant role as primary producers and also as bioindicators in ecological status assessments. For surface water monitoring, a light microscope (LM) is the primary tool available to investigators, and the EU WFD (Water Framework Directive) itself still currently requires its use (European Commission 2000). However, for accurate identification of small diatom species, a scanning electron microscope (SEM) is essential, although not mandatory.

Because traditional morphology-based identification of species is labour intensive and requires deep taxonomic knowledge (Rimet and Bouchez 2012), the application of DNA-based methods has been proposed. Metabarcoding is a process in which short, so-called barcode DNA sequences (Hebert et al. 2003) are acquired with high-throughput sequencing (HTS) and used for the identification of species (Taberlet et al. 2012). In case of diatoms, various DNA regions was suggested as barcode markers such as V4 and V9 subregions of the nuclear 18S rDNA (Zimmermann et al. 2011; Guo et al. 2016), a 312 bp region of the plastid gene, *rbc*L (Vasselon et al. 2017), for certain groups internal transcribed spacer (ITS) and cytochrome-c oxidase subunit 1 gene (COI, Guo et al. 2015). Vasselon et al. (2017, 2018) elaborated a diatom metabarcoding process based on a short barcode region of the plastid gene *rbc*L, that was tested by several authors in recent years (Mortágua et al. 2019; Borrego-Ramos et al. 2021; Duleba et al. 2021). As reference for taxonomic assignment of sequences the method by these authors uses Diat.barcode, a curated database that was developed for the taxonomic assignment of the sequences (Rimet et al. 2019) and is under continuous development and frequently updated.

Within the framework of a national scale project associated with the EU WFD, a total of 242 sampling points were selected to cover all water types in Hungary according to the typology. During this project, in an oxbow of Tisza River (Körtvélyesi Holt-Tisza near Szeged), we have found several small-celled naviculoid diatom species. Some of them were dominant; moreover one species (with a relative abundance of 10.2%) among them was unidentifiable on the basis of present literature. Small naviculoid diatoms frequently dominate in freshwaters, and when their relative abundance exceeds 5%, their ecological importance is unquestionable (Wetzel et al. 2015).

This work aimed to compare microscopy and metabarcoding in the view of the performance in detecting diatom species in the community. We intended to identify species as precisely as possible. On one hand, during morphological investigation, scanning electron microscopy (SEM) was used in addition to light microscopy (LM), especially for small naviculoids. On the other hand, during metabarcoding, the first bioinformatics analysis (DADA2-based pipeline and taxonomic assignment using Diat.barcode) was supplemented with Basic Local Alignment Search Tool (Altschul et al. 1990) in NCBI GenBank database. Furthermore, we could identify the sequences of some naviculoid species that were found with microscopy. These included a new *Mayamaea* species that is described in the present paper.

Methods

Site description

The Körtvélyesi Holt-Tisza oxbow (46.423980°N, 20.230290°E) is located near Hódmezővásárhely city in the Tisza River floodplain, in the Mártély Landscape Protection Area. It was created when the Tisza was regulated in 1887. Its area is 69 ha, has a length of 4.7 km, an average width of 128 m, an average depth of 3 m and a water volume of 1.8 million m^3. It is an important wetland for bird conservation, a so-called sanctuary-like oxbow, although the impact of land use is reflected in its water quality. In recent decades, a significant amount of nutrients has entered the water body from the surrounding canals in the catchment area due to intensive agriculture, use of fertilisers, pesticides and herbicides (http://users.atw.hu/kettoef/csszabolcs/Hmvhely/kortvelyesi-holtag.htm).

Sampling and sample preparation

Epiphyton samples were collected from *Typha angustifolia* stems in 5 replicates on 22 June and 9 September 2019. Stems were chosen randomly, and 20 cm sections were cut with clippers starting at 10 cm below the water surface. Stem sections were placed in plastic bags and transported to the laboratory, where epiphyton was scraped into tap water using a toothbrush. The slurry acquired was homogenised and divided into two parts. For DNA analysis, 2–3 ml was pipetted into a 15 ml sterile plastic tube, then filled with absolute ethanol resulting in a final ethanol concentration ≥ 70%. DNA samples were stored at 4 °C until processing. The rest of the slurry was fixed with buffered formaldehyde for microscopic studies (European Committee for Standardization 2002).

Environmental variables

The following environmental variables were measured: Secchi-transparency, pH, dissolved oxygen content (DO), conductivity (cond.), total nitrogen (TN), total phosphorous (TP), chlorophyll *a* concentration (Chl *a*), chemical oxygen demand (COD), biological oxygen demand (BOD), silica, hydrogen carbonate, chloride, sulphate, ammonium, nitrite, nitrate. *In situ*, the pH, dissolved oxygen content and conductivity were measured with a portable multiparameter digital meter (Multi 350i-WTW, Germany). Other variables were measured in samples carried to the laboratory according to the following national standards: MSZ EN 12260:2004 (Hungarian Standards Institution 2004) for TN; MSZ EN ISO 11885:2009

(Hungarian Standards Institution 2009) for TP; MSZ ISO 10260:1993 (Hungarian Standards Institution 1993) for Chl *a*; ISO 15705:2002 (International Organization for Standardization 2002) for COD; and MSZ ISO 6060:1991 (Hungarian Standards Institution 1992) for BOD. The other measured parameters were determined according to APHA (1995).

Microscopy

For microscopic observations, the frustules were cleaned with hydrochloric acid and hydrogen peroxide, washed in distilled water and mounted with Naphrax mounting medium (CEN 2014). Diatoms were investigated using a Zeiss Axio Imager Z2 microscope equipped with differential interference contrast (DIC) optics (numerical aperture: 1.4) at a 1500× magnification. At least 500 valves were identified to species or genus level. For the identification of diatoms we used Lange-Bertalot et al. (2017) as the basis. Accurate identification of small species was carried out in SEM; in LM they were only separated by OTUs based on their main characteristics seen in LM.

For SEM studies, part of the cleaned and washed samples was filtered through a 3 µm Isopore polycarbonate membrane filter (Merck Millipore), which was then fixed onto an aluminium stub using double-sided carbon tape and coated with gold using a rotary-pumped spatter coater Quorum Q150R S. Diatom ultrastructures were observed with Zeiss EVO MA 10 SEM operated at 10 kV and around 10 mm distance.

Metabarcoding

DNA was extracted from the samples using NucleoSpin Soil Kit (Macherey-Nagel), mainly following Vautier et al. (2020)'s protocol. The only modification was applying a mixture of two kinds of lysis buffer from the kit in a 1:1 ratio.

A 312 base pair (bp) region of the *rbc*L gene was amplified and sequenced on the Illumina MiSeq platform. Primer sequences, description of polymerase chain reactions (PCR), library preparation and sequencing are provided in Duleba et al. (2021). First, the target was amplified using gene-specific primers developed by Vasselon et al. (2017) and supplemented with Illumina overhang P5/P7 adapters. Then the PCR products were purified using 1.0× AMPure XP magnetic beads (Beckman Coulter) and then diluted to equimolar concentrations. Concentrations were quantified by using a Qubit 4 Fluorometer (Invitrogen) with the Qubit dsDNA HS Assay Kit. Cleaned PCR products were provided with Nextera DNA CD Indexes with P5/P7 adapters and P7/P5 tags attached in index reactions. The products were cleaned, then diluted to similar concentration and pooled properly. Quality and concentration control of the samples were performed with a Qubit 4 Fluorometer using the Qubit dsDNA HS Assay Kit reagents (concentration) and with an Agilent TapeStation System 4150 (Agilent) using the Agilent High Sensitivity D1000 ScreenTape Assay Reagents. The final library pool was checked for size and concentration and diluted to a 4 nM starting concentration for the run on the Illumina MiSeq system. After denaturation and dilution, sequencing was performed using the Illumina MiSeq V2(500) Reagent Kit and a 2×250 bp read length.

Polymerase chain reactions, library preparation and sequencing were performed by Biomi Ltd.

Raw sequence data are available at https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/MEQY4Q.

Data analysis

For sequence data analysis, following the workflow of Keck et al. (2019), a version of the DADA2 pipeline (Callahan et al. 2016) was modified according to the specific needs of diatom metabarcoding with *rbc*L (Keck et al. 2019). This includes the following main steps: removing primer sequences, trimming and filtering according to read quality, dereplicating, filtering with the core sample inference algorithm of DADA2, aligning and merging paired forward and reverse reads into one contig sequence, removing chimaeras and taxonomic assignment. The taxonomic assignment step was done using Diat.barcode version 9.2 (Rimet et al. 2019) as the reference database. Read numbers were corrected according to cell biovolume using the correction factor developed by Vasselon et al. (2018). For some taxa correction factor was zero or not available, for these taxa, correction factor of species in similar size was applied. The following replacements were applied: *Nitzschia paleacea* (Grunow) Grunow in Van Heurck for *Nitzschia bulnheimiana* (Rabenhorst) H.L.Smith, *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot for *Planothidium victorii* PP.M Novis, J. Braidwood & C. Kilroy, *Mayamaea permitis* (Hustedt) Bruder & Medlin for the new *Mayamaea* species. More details can be found in Duleba et al. (2021).

Sequences that could not be assigned with the DADA2 pipeline were aligned to sequences in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Tool (BLAST, Altschul et al. 1990). The search was performed in the Nucleotide collection database (nr/nt) using the Standard Nucleotide BLAST program, megablast (highly similar sequences) algorithm with the default parameter settings. If a sequence showed similarity higher than 99% with a record in the database, it was accepted as being the species the record belonged to. Sequences from algae other than diatoms, as well as centric diatoms, were removed from the comparison with morphology-based results because only pennate diatoms were identified under a microscope following the Hungarian protocol (Ács et al. 2021).

Sequences for comparison with naviculoid sequences from our samples were acquired from Diat.barcode 9.2 (Rimet et al. 2019). The *rbc*L sequences were aligned by codon to sequences downloaded from GenBank using Clustal W (Thompson et al. 1994) implemented in

MEGA X (Kumar et al. 2018). This software was applied also for p-distance calculations and phylogenetic analyses. Uncorrected p-distance was calculated between sequences within the genus *Mayamaea* as well as within *Craticula*, in addition, mean p-distance within these genera was also computed.

Maximum likelihood analyses were performed using *Mayamaea*, *Sellaphora* and *Craticula* sequences. The most appropriate substitution model for DNA sequence evolution identified by MEGA X (Kumar et al. 2018) were the followings: for *Mayamaea* phylogenetic tree Tamura-Nei model with gamma distribution, for *Craticula* tree Tamura 3-parameter model with gamma distribution, for *Sellaphora* tree Tamura-Nei model with gamma distribution and invariant sites were used. Bootstrap test was performed in 500 replicates. For *Mayamaea* tree *Fallacia*, *Rossia*, *Sellaphora* species, for *Craticula* tree *Stauroneis* species, for *Sellaphora* tree *Mayamaea*, *Fallacia*, *Rossia* species were applied as outgroups.

For ecological status assessment, IBD (Indice Biologique Diatomées (Lenoir and Coste 1996; Coste and Prygiel 1998; Prygiel 2000)), EPI-D (Eutrophication Pollution Index Daitoms (Dell'Uomo 1996)) and TDIL (Trophic Diatom Index for Lakes (Stenger-Kovács et al. 2007)) indices were calculated using OMNIDIA 6.0.8. (Lecointe et al. 1993). Averaging these indices, we calculated the Multimetric Index for Lakes (MIL), which is an intercalibrated standing water quality index used in Hungary (Ács et al. 2015).

Results

Using light and scanning electron microscope, a total of 50 taxa from 15 diatom genera were identified in the samples. The Shannon diversity was 4.23 in the summer and 3.50 in the autumn sample. Dominant species (relative abundance of at least 5% or more in one sample) were *Brevilinea kevei* Ács & Ector in Ács, C.E. Wetzel (3.5%, 11.9%), *Craticula subminuscula* (Manguin) C.E. Wetzel & Ector (7.6%, 10.5%), *Mayamaea permitis* (14.9%, 0.2%), *Mayamaea* sp. (10.2%, 0.2%), *Navicula cryptotenella* Lange-Bertalot (11.9%, 0%), *N. microrhombus* (Cholnoky) Schoeman & Archibald (0.4%, 13.6%), *N. veneta* Kützing (5.1%, 0%), *Nitzschia archibaldii* Lange-Bertalot (2.0%, 10.7%), *N. inconspicua* Grunow (4.7%, 7.0%), *N. lacuum* Lange-Bertalot (1.8%, 22.0%), *N. paleacea* (5.5%, 8.4%), *N. palea* (Kützing) W. Smith (6.3%, 0%), *N. supralitorea* Lange-Bertalot (5.1%, 1.4%). The numbers in brackets are relative abundances, the first always refers to the sample from June, the second from September (for the whole taxon list with relative abundances see Suppl. material 1: Table S1).

In addition to the dominant species, the following small naviculoid diatom species were also found in the sample: *Craticula importuna* (Hustedt) Lange-Bertalot (3.3%, 0%), *Sellaphora archibaldii* (J.C. Taylor and Lange-Bert.) Ács, C.E. Wetzel & Ector (0%, 1.8%) and *S. nigri* (De Not.) C.E. Wetzel & Ector (0.6%, 2.3%). Exact identification of these small naviculoid species was

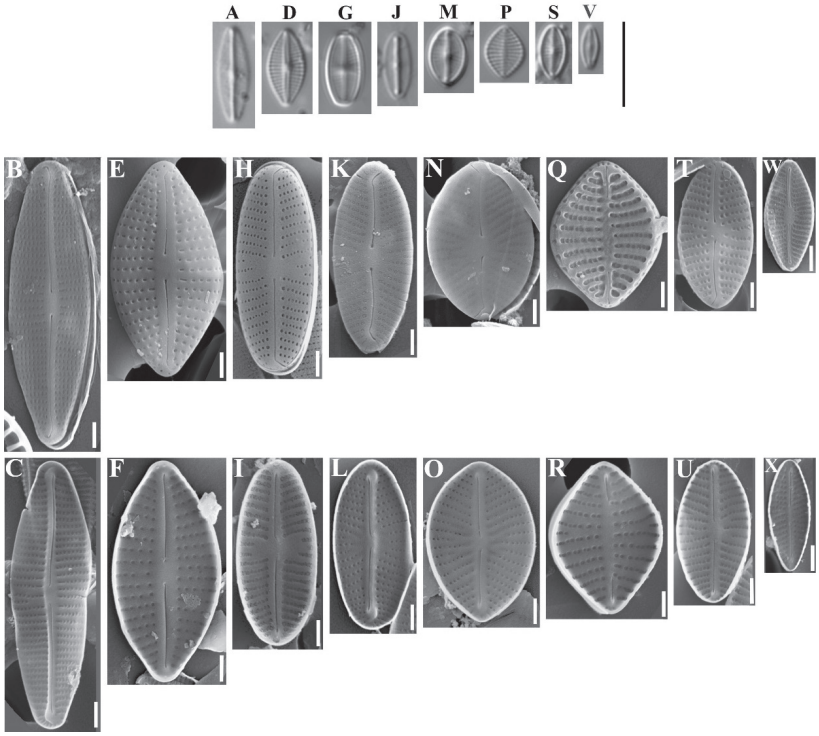

Figure 1. Images of small naviculoid diatoms from the Körtvélyesi Holt-Tisza. **A–C**. *Craticula importuna*; **D–F**. *Craticula subminuscula;* **G–I**. *Sellaphora nigri*; **J–L**. *Mayamaea permitis*; **M–O**. *Mayamaea ectorii*; **P–R**. *Navicula microrhombus*; **S–U**. *Sellaphora archibaldii*; **V–X**. *Brevilinea kevei*. LM (**A, D, G, J, M, P, S, V**; scale bar: 10 µm) and SEM (external view: **B, E, H, K, N, Q, T, W** and internal view: **C, F, I, L, O, R, U, X**). Scale bars: 1 µm.

only possible by electron microscopy (Fig. 1), which was important, because the accuracy of species-level identification can influence the classification results, especially if the given species is dominant in the sample.

Based on metabarcoding in both the summer and the autumn samples, 58–58 diatom amplicon sequence variants (ASVs) were identified at species level with the DADA2 pipeline. After the final step of the bioinformatics processing of the HTS data, there were 226,469 and 124,259 reads in the June and the September sample, respectively. These constituted 88 and 110 ASVs, respectively. Of these, 19 and 26 ASVs did not belong to Bacillariophyta. Seven ASVs were additionally identified using BLAST search, these belonged to *Cyclostephanos dubius* (Hustedt) Round, *Pantocsekiella ocellata* (Pantocsek) K.T. Kiss & Ács, *Nitzschia paleacea*, *Gomphonema gracile* Ehrenberg, *Amphora indistincta* Levkov, and *Sellaphora pupula* (Kützing) Mereschkovsky.

In the case of 24 ASVs that could not be identified at species level with the DADA2 pipeline, BLAST search found 98% or lower similarity with the most similar record in the GenBank. Most of them related to the genera *Nitzschia* and *Gomphonema*. Identifying the sequences of *Brevilinea kevei* and *Navicula microrhombus* was not possible. We did not have information on the lineage of their genera. All sequences that belonged to the genus *Navicula* could be assigned to *N. cryptotenella* and *N. veneta*. The presence of these *Navicula* species in the samples was proved by microscopy.

Furthermore, we used the taxon lists obtained with microscopy to help identify more sequences that could not be assigned based on the reference DNA databases (Diat. barcode, GenBank).

One ASV showed 95% similarity with *Mayamaea permitis* and it was dominant in the June sample but occurred only in a few read numbers in the September sample. This abundance distribution in the samples was similar to that shown by a presumably undescribed *Mayamaea* species (listed as *Mayamaea* sp.) under microscope. A variant of this ASV (1 nucleotide difference) was also detected, however, in low abundance (94 reads) and only in the June sample. Based on the *Mayamaea* sequences in Diat. barcode, the mean p-distance of this region of *rbc*L in this genus is 0.04, the minimum distance between two taxons [*M. atomus* (Kützing Lange-Bertalot and a *M. permitis* variant] was 0.008, the maximum distance [between *M. alcimonica* (E. Reichardt) C.E. Wetzel, Barragán & Ector and *M. permitis*] was 0.13. This ASV showed 0.053–0.068 p-distance with variants of *M. permitis* (Suppl. material 2: Table S2). Therefore, these ASVs were accepted as the sequences of the undescribed *Mayamaea* species. Using this sequence in phylogenetic analysis, this species was positioned between *Mayamaea* species but on distinct lineage as a sister to a group containing lineages of *M. permitis*, *M. atomus*, *Mayamaea terrestris* N. Abarca & R. Jahn, *Mayamaea pseudoterrestris* Tuji et H.Yamaguchi and *Mayamaea vietnamica* Glushchenko, Kezlya, Kulikovskiy & Kociolek (Fig. 2, the new species referred as *Mayamaea ectorii* KHT; see description and naming of this species below; the abbreviation KHT refers to Körtvélyesi Holt-Tisza). This separation was supported by a relatively high bootstrap value. Various *M. permitis* sequences were identified from Körtvélyesi Holt-Tisza (*M. permitis* KHT1-6) by DADA2-based analysis and these were located in a group of *M. permitis* that supported their identification. *Mayamaea alcimonica* represented a distinct lineage formed by a sequence from the database and an ASV from Körtvélyesi Holt-Tisza. This environmental sequence occurred in low abundance. It was identified by DADA2-based analysis that was confirmed by phylogenetic analysis. *Mayamaea* sequences grouped separately from *Rossia*, *Fallacia* and *Sellaphora*.

Based on the morphological investigations, a dominant *Mayamaea* species showed a distinct morphology, different from all other known *Mayamaea* species, and this was also confirmed by the metabarcoding results of the *rbc*L amplicon. So we described it as new as follows:

***Mayamaea ectorii* Ács, Kiss & C.E. Wetzel, sp. nov.**

Fig. 3A–M

Morphology. Valves are small, oval with slightly pointed apices. The observed range of valve dimensions (n = 35): length 6.2–7.9 µm, width 4.2–5.3 µm, 32–36 striae in 10 µm.

In light microscope the "three dots" can be detected as spots, which is characteristic for the genus *Mayamaea* (Fig. 3A–I). Striae are uniseriate, radiate throughout; two to three shorter striae present in the very middle of the valve (Fig. 3J–M). Striae composed of 8 to 11 areolae located on the valve face, extending down onto an extremely shallow mantle, bearing a single row of areolae (Fig. 3K). Small silica depositions can be observed in some specimens on the valve mantle/face junction (Fig. 3J).

The axial area is narrow, linear over most of its length. Central area is relatively wide, symmetrical, marked by the "three dots" corresponding to a thickened siliceous area. Proximal raphe endings are slightly curved towards the same side, with teardrop proximal pores (Fig. 3J). Distal external raphe fissures with different lengths, bending strongly to the same side of the valve, continuing onto the mantle (Fig. 3K).

Internally, proximal raphe endings slightly deflected to the same side and distal raphe endings terminating on helictoglossae (Fig. 3L). Areolae showing a wide diameter and rounded shape (Fig. 3M) are covered by hymens located towards the outer opening of the areolae (Fig. 3J, K). The species was found in hypertrophyic conditions (Suppl. material 3: Table S3).

Holotype. (Fig. 3K, M) designated here. Type material: CER-14/13 and CER-14/14, deposited at the Centre for Ecological Research, Institute of Aquatic Ecology, Budapest, Hungary, epiphyton, collected by B. Csányi 22/06/2019. HNHM-ALG-D002301 and HNHM-ALG-D002302 deposited at the Hungarian Natural History Museum, Budapest, Hungary.

Type locality. Körtvélyesi Holt-Tisza, Hungary (46.423980°N, 20.230290°E).

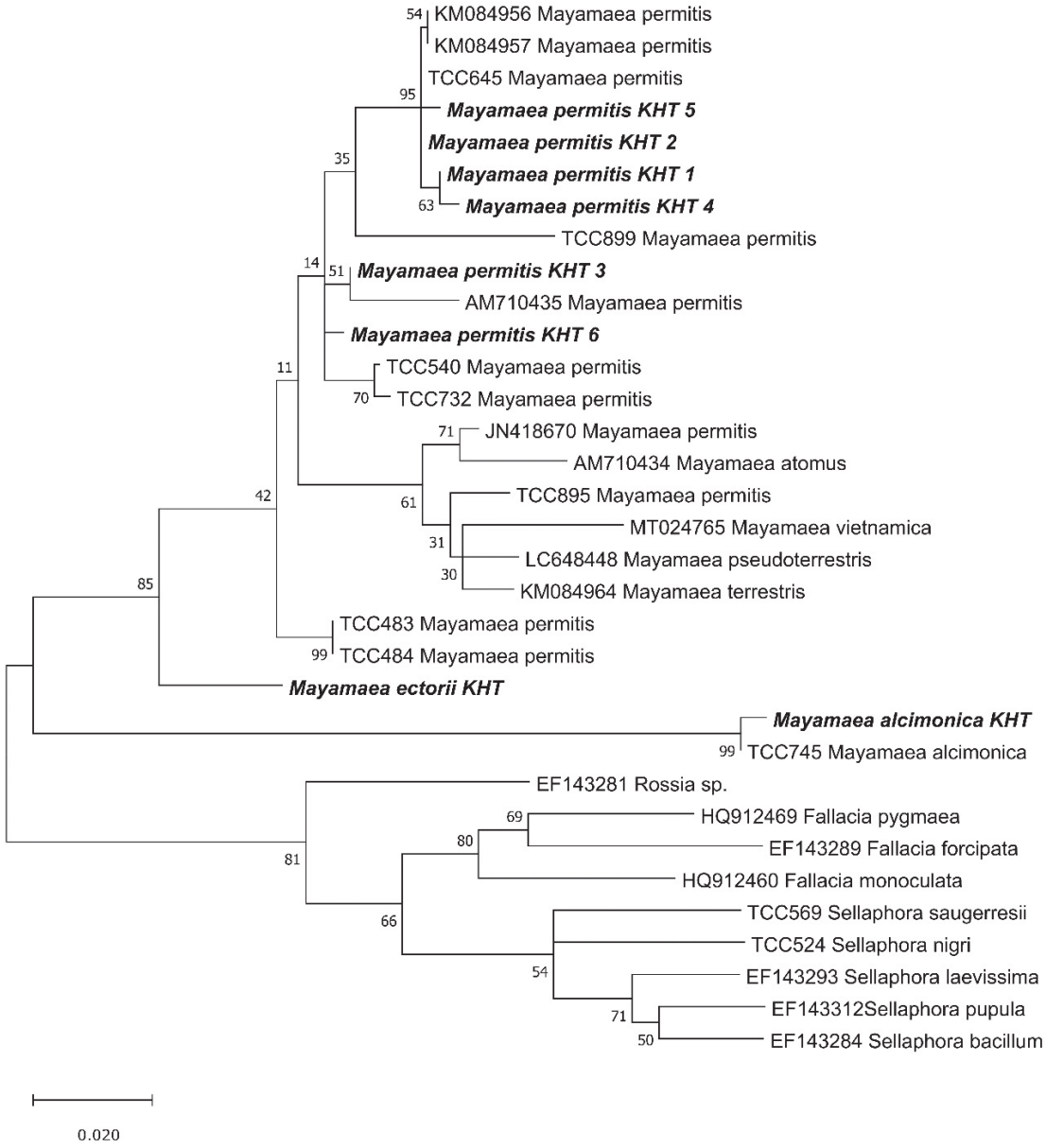

Figure 2. Maximum likelihood phylogenetic tree of *Mayamaea* species using the *rbc*L fragment. *Rossia*, *Fallacia* and *Sellaphora* taxa were used as outgroups. Bootstrap values are indicated at nodes. Sequences acquired in present study are in bold and indicated with "KHT" (referring to Körtvélyesi Holt-Tisza). Sequences from database are provided with NCBI GenBank accession number (if available) or culture ID of Thonon Culture Collection.

Isotypes. Fig. 5J, L designed here.

Etymology. The name is chosen in honour of our dear friend, the famous Belgian diatomologist Luc Ector (1962–2022†).

Similar logic to that presented for *Mayamea* species led to finding the sequence of two *Craticula* species. One ASV was dominant in samples and showed 95% similarity with *Craticula* species [*Craticula cuspidata* (Kützing) D.G. Mann, *C. ambigua* (Ehrenberg) D.G. Mann]. Within this genus, the mean p-distance was 0.08 in the studied region of *rbc*L. The minimum pairwise distance was 0.004 between *C. ambigua* and *C. cuspidata*, the maximum was 0.14 between *C. accomoda* (Hustedt) D.G. Mann and *C. importuna* (Hustedt) K. Bruder & Hinz. Thus, this ASV belonged to the *Craticula* genus. Moreover, *C. subminuscula* was dominant in both samples based on microscopy. The ASV showed the lowest p-distance (0.046) with *C. subminuscula* (Suppl. material 4: Table S4). Therefore, it was accepted as the sequence of *C. subminuscula*. Another ASV occurred only in the June sample and showed 0.015 p-distance (4 nt differences) with *C. importuna* AT-70Gel14a (accession number AM710444) that was regarded as *C. molestiformis* in its original publication (Bruder and Medlin 2007) and later was transferred to *C. importuna* by Bruder and Medlin (2008). High similarity (99%) between sequences may suggest conspecifity of our taxon with *C. importuna* AT-70Gel14a (accession number AM710444).

Phylogenetic analysis (Fig. 4) showed that the two *Craticula* species that occurred in our samples (marked

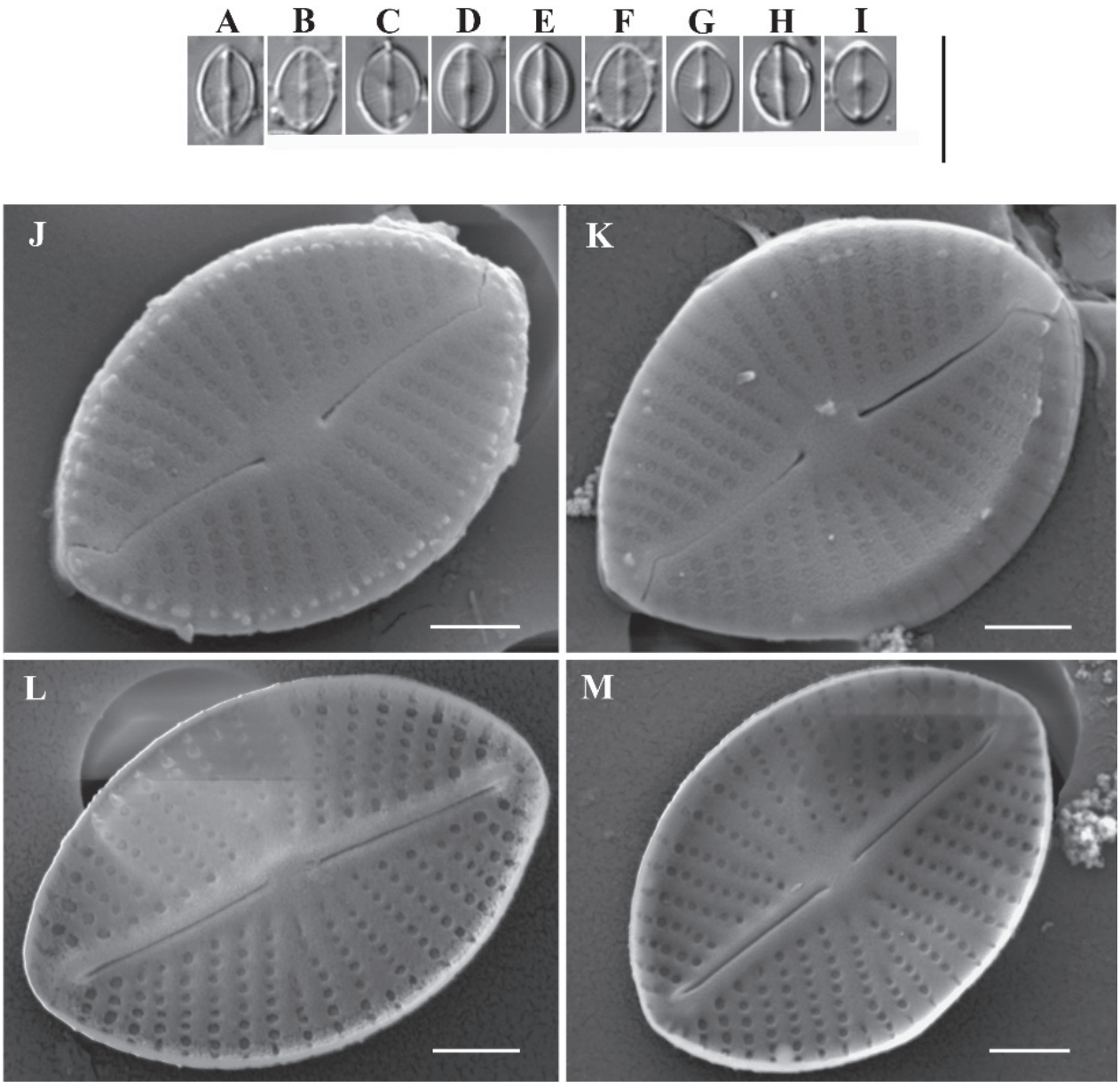

Figure 3. *Mayamaea ectorii* sp. nov. LM (**A–I**; scale bar: 10 µm) and SEM (external view: **J, K** and internal view: **L, M).** Scale bars: 1 µm.

with "KHT") were located within *Craticula* species that formed several lineages. *Craticula subminuscula* from Körtvélyesi Holt-Tisza (KHT) was located most closely to two *C. subminuscula* strains. Our *C. importuna* was the closest relative of *C. importuna* AT-70Gel14a (AM710444), forming a lineage distinct from *C. molestiformis* sequences (Fig. 4). Separation of *C. subminuscula* as well as *C. importuna* from its closest relatives was supported by medium bootsrap values.

Sequences of *M. ectorii*, *C. subminuscula* and *C. importuna* have been deposited in the NCBI GenBank database under accession numbers OP354489–OP354492.

The sequence of *Sellaphora archibaldii* could not be identified either. *Sellaphora nigri* and *S. saugerresii* sequences were identified by DADA2-based pipeline, BLAST search the sequence of *S. pupula*. Almost all *Sellaphora* sequences were *S. nigri*, one of them was *S. saugerresii* (Desm.) C.E. Wetzel & D.G. Mann in Wetzel et al., and the other one was *S. pupula*. On the phylogenetic tree (Fig. 5) *S. nigri* sequences (KHT1–4) grouped with *S.nigri* sequences from the database, *S. saugerresii* KHT formed a group with other *S. saugerresii* sequences. This confirmed their original identification. *Sellaphora pupula* KHT located in the group of taxa of *S. pupula* complex. The presence of *S. nigri* was verified under microscope in both samples. The other two *Sellaphora* sequences were present only in small numbers (*S. saugerresii* had merely 18, *S. pupula* had 15 reads).

In the summer sample (June 2019), *Mayamaea permitis*, *Navicula veneta*, *Nitzschia palea* and *Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot reached relative abundance of more than 5% based on metabarcoding using the DADA2 pipeline

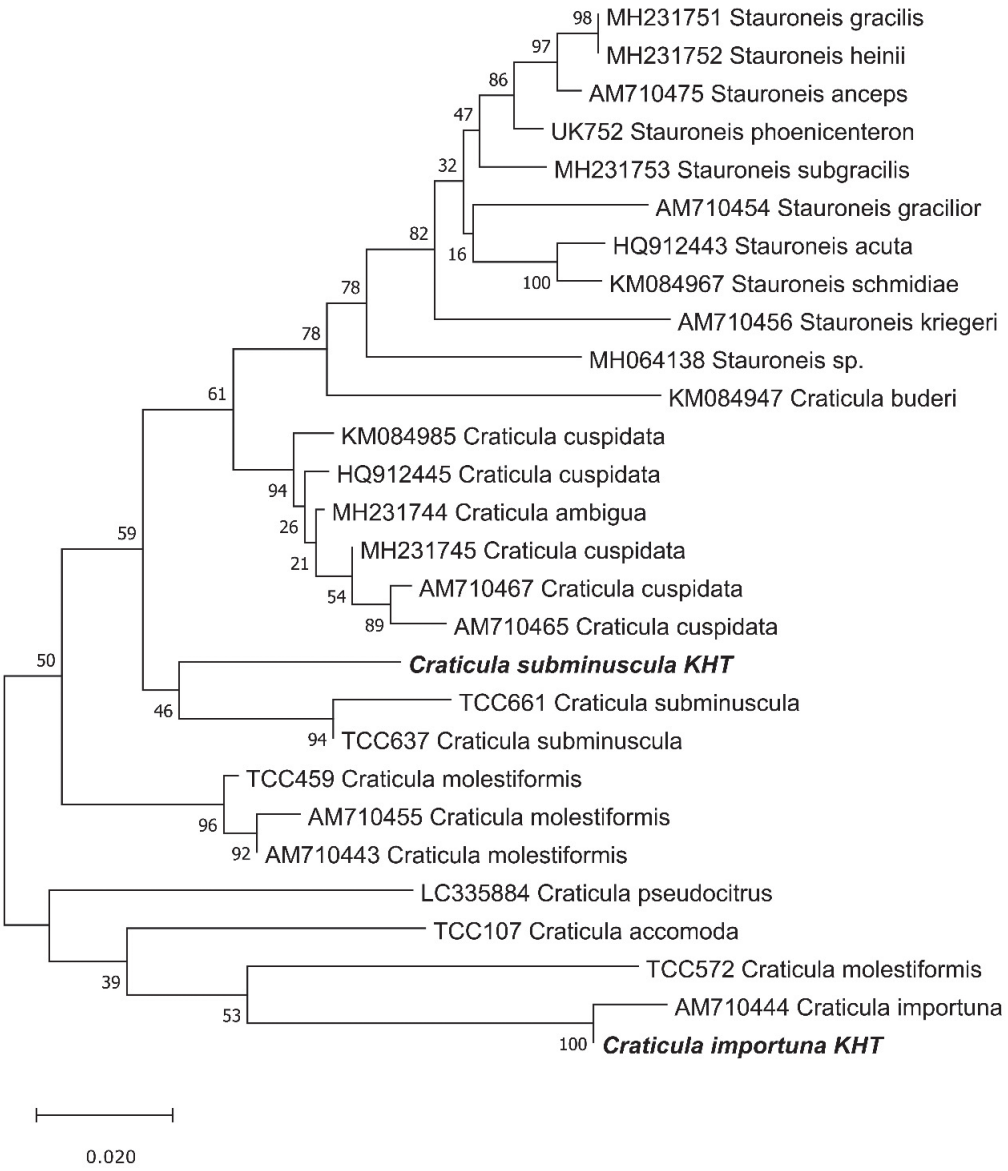

Figure 4. Maximum likelihood phylogenetic tree of *Craticula* species using the *rbc*L fragment. *Stauroneis* taxa were used as outgroups. Bootstrap values are indicated at nodes. Sequences acquired in present study are in bold and indicated with "KHT" (referring to Körtvélyesi Holt-Tisza). Sequences from database are provided with NCBI GenBank accession number (if available) or culture ID of Thonon Culture Collection.

and supplemented with a BLAST search. Based on microscopy, all of these species were dominant in the sample except for *Fistulifera saprophila* that was not detected. After comparison with morphology, the sequences of *Craticula subminuscula* and the presumably new *Mayamaea* species were identified and seemed to be dominant instead of *Navicula veneta*.

In the autumn sample (September 2019), *Nitzschia filiformis* (W.M. Smith) Van Heurck, *N. inconspicua*, *N. palea* and *N. supralitorea* were dominant. After BLAST search, *N. paleacea* also became dominant. Under microscope, *N. filiformis* and *N. palea* were not detected, *N. supralitorea* was present in 1.36%, *N. inconspicua* and *N. paleacea* were dominant. After comparison with morphology, *Craticula subminuscula* was also recognised as a dominant species based on DNA (Suppl. material 1: Table S1).

In the summer sample, eight dominant species were found based on light microscopy, of which six were detected also based on metabarcoding (using both DADA2 and BLAST), however, the identification of the sequence of two other species (*Craticula subminuscula* and *Mayamaea* sp.) became possible after the comparison with microscopy results. In the autumn sample, microscopy showed seven dominant species, however, only two (*Nitzschia inconspicua* and *N. paleacea*) were detected based on metabarcoding (with DADA2 and BLAST). The comparison of the morphology- and the DNA-based method added one further identification (*Craticula subminuscula*).

The MIL index calculated from the results of microscopy and metabarcoding are presented in Table 2. The MIL index calculated based on metabarcoding results (using any of the analyses) was lower than the morphology-based ones.

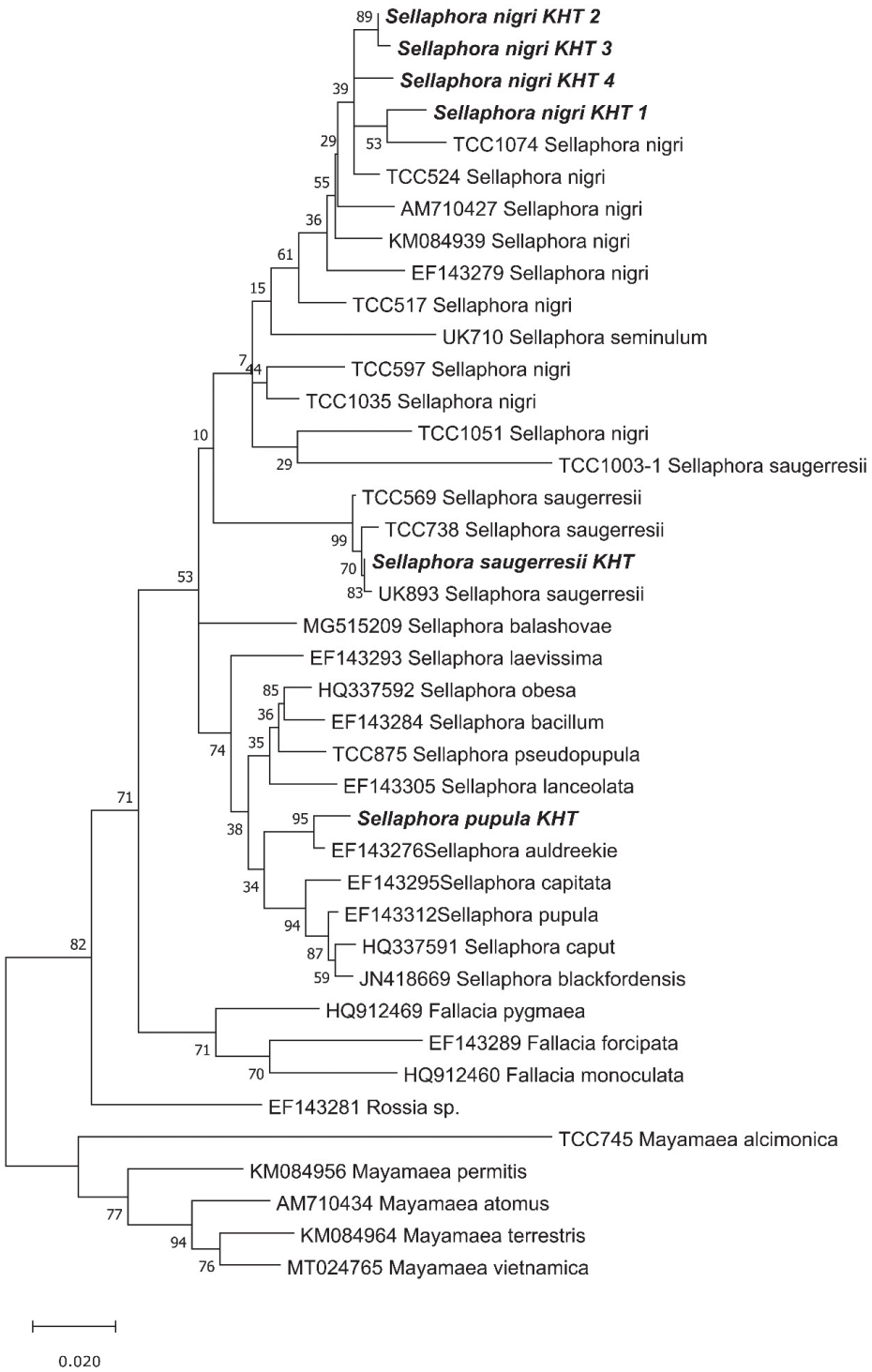

Figure 5. Maximum likelihood phylogenetic tree of *Sellaphora* species using the *rbc*L fragment. *Mayamaea*, *Rossia* and *Fallacia* taxa were used as outgroups. Bootstrap values are indicated at nodes. Sequences acquired in present study are in bold and indicated with "KHT" (referring to Körtvélyesi Holt-Tisza). Sequences from database are provided with NCBI GenBank accession number (if available) or culture ID of Thonon Culture Collection.

When microscopy results were also used for the taxonomic assignment of the sequences, *Craticula submminuscula* and *C. importuna* could influence the value of the index. *Mayamaea ectorii* could be added as *Mayamaea* sp. (specified only at genus level) to OMNIDIA. The genus has species with different index values, so in such form sensitivity and tolerance values were not available for *M. ectorii* and it could not be involved in index calculation. Further research on samples from various environments will be required to assign index values to this species. Comparison with morphology did not help reduce the difference between microscopy- and metabarcoding-based indices. In the case of the June sample, this may be due to the lack of

ecological parameters of the newly described species. In the case of the September sample, the reason may be that sequences of dominant species e.g. *B. kevei* and *N. microrhombus* could not be identified.

Discussion

The dynamics of the main branch-sub-branch relationship play a fundamental role in the life of rivers. The diversity and abundance of biota in tributaries fundamentally depend on the dynamics of water flow (Bayley 1991; Nilsson and Svedmark 2002). The issue of the vulnerability of river floodplains caused by changes in water flow due to river regulation is a priority and this is particularly true for sanctuary-like oxbows, which can maintain high and unique biodiversity. Comparing the benthic diatom species richness of different-sized lakes in Hungary, Bolgovics et al. (2016, 2019) found that lakes in the 10^5 size range were the most diverse. This size range includes Körtvélyesi Holt-Tisza. TN and TP concentrations and Chl *a* values indicate the high nutrient content of the Körtvélyesi Holt-Tisza: this is a common phenomenon in wetlands which are important for bird conservation (Boros et al. 2008), moreover the land use also has significant effects on the water quality of this oxbow.

R-selected, small-celled species often develop in these oxbows, which are highly exposed to land use or natural eutrophication (Ács et al. 2016, 2017).

Among the small-sized naviculoid diatoms from the oxbow, *Craticula importuna*, C. *subminuscula*, *Mayamaea permitis* and *Sellaphora nigri* were also common in the other waters we studied (Suppl. material 6: Fig. S1). These diatoms are cosmopolitan species, and were also very common in studies including those from e.g. France (Bey and Ector 2013; Peeters and Ector 2019), or Reunion (Gassiole et al. 2019). *Brevilinea kevei*, *Navicula microrhombus* and *Sellaphora archibaldii* were rarely found in our surveys (their frequency was 2.9%, 1.4% and 4.5% respectively). *Brevilinea kevei* and *Navicula microrhombus* were not found, e.g. in the above-mentioned surveys in France or Reunion.

Brevilinea kevei is a relatively recently described species (Ács et al. 2016), but since then it has been found in Sweden (Sundberg 2022). *Brevilinea* genus contains only two species and so far, DNA sequence information is not available for either of them. *Navicula microrhombus* was described in 1970 by Cholnoky (Cholnoky 1970) from a fishpond in South Africa. Its first occurrence outside South Africa was reported from Slovakia in 2000 (Hindáková 2000), and it was first recorded in Hungary during a sampling campaign in 2001 (Szabó et al. 2004). The uncertain taxonomic position of this species has been reported by Hindáková (Hindáková 2001), and has not been clarified so far. Morphology (e.g. the raphe characteristics) of *N. microrhombus* suggests that it belongs to another genus other than *Navicula* sensu stricto. Though we could not identify the sequence of *N. microrhombus*, all sequences belonging to the *Navicula* genus were assigned to other species whose presence was proved under microscope, suggesting that *N. microrhombus* is not a *Navicula* species.

The applicability and comparison of HTS data and microscopy observations for finding barcode sequences of diatom species were first shown by Rimet et al. (2018). One of the identified sequences was the sequence of *Gomphonema clavatuloides* Rimet, D.G. Mann, Trobajo & N. Abarca that they described as a new species. This method has the advantage that the reference database can be enriched both with taxa and sequence variants of a given taxon complementing the labour intensive and often unsuccessful culturing (Rimet et al. 2018; Mora et al. 2019). This method helped us to identify sequence variants of species already recorded in the database and the sequence corresponding to a new species.

Our new species has the main characteristics of the genus *Mayamaea*, but no exact match could be found with any described *Mayamaea* species.

The genus *Mayamaea* was described by Lange-Bertalot in 1997 (Lange-Bertalot 1997); main characteristics: cells are small, with a maximum length of 16 µm and a maximum width of 7 µm, but mostly less than 10 µm long and 5 µm wide. Valves are always elliptical with broadly rounded poles. The axial area is thickened and contains a straight, filiform raphe. Distal raphe ends are curved in the same direction. Proximal ends are also slightly curved to the same side, but to the opposite side to the distal ends, and terminating in well-developed pores on the outside. Internally, the proximal ends of the raphe terminate in a central nodule and the distal ends in a medium-sized helictoglossa. These three nodules are clearly visible under a light microscope. Each stria is formed by a single row of areolae. *Mayamaea* species are found in ephemeral habitats, waters with high nutrient content and soils (Barragán et al. 2017).

Currently, 30 freshwater or terrestrial valid *Mayamaea* taxa are listed in the AlgaBase (Guiry and Guiry 2022, https://www.algaebase.org/) and/or the DiatomBase (Kociolek et al. 2022, https://www.diatombase.org/aphia.php?p=-searh). Of these, four have overlapping striae number with *Mayamaea ectorii*, and these are *M. arida*, *M. lacunolaciniata*, *M. nolensoides* and *M. permitis* (Table 1). *Mayamaea arida* has wide, well-developed axial area and the striae are parallel in the central part, while *M. ectorii* has a narrow, linear axial area and striae strongly radiate along the whole length of the valve. *Mayamaea lacunolaciniata* is narrower (width 3.5–4 µm) and its axial area is different (its sternum expanded to broadly rhombic-lanceolate, structureless or with some irregularly dispersed areolae). *Mayamaea nolensoides* differs from *M. ectorii* in its distal raphe ends and completely hyaline area with short and higher striae density (impossible to solve even with DIC). The terminal nodules are in more proximal position, whereas in *M. ectorii* the distal raphe end continues on the mantle. *Mayamaea permitis* is narrower (width 3–4 µm) and it has broadly rounded apices, while *M. ectorii* has slightly pointed ones. In appearance, *Mayamaea ectorii* most resembles *M. alcimonica*, but it is wider and has a higher number of striae.

Molecular information on the genus *Mayamaea* is scarce (Kezlya et al. 2020); sequence data are available

Table 1. Main morphological characters of freshwater and/or terrestrial *Mayamaea* species.

Taxon	Length (µm)	Width (µm)	No. of stria in 10 µm	Ends of valves	Axial area	Central area	Striae	Distal raphe end	Proximal raphe end	Environment	Reference
Mayamaea agrestis (Hustedt) Lange-Bertalot, 2001	9.0–11.0	(2.5)3–3.8	24–28	rather cuneate and obtusely rounded	narrow, linear	not developed	uniseriate, moderately radiate, visible in LM	bend strongly to the same side, continuing onto the mantle	drop-shaped, slightly deviated in one direction	freshwater	Lange-Bertalot (2001), p. 134
Mayamaea alcimonica (E. Reichardt) C.E. Wetzel, Barragán & Ector, 2017	9.0–12.0	4.0–6.0	24–26(28)	pointed	narrow, linear	reduced or absent	uniseriate, strongly radiate, visible in LM	bend strongly to the same side, continuing onto the mantle	slightly curved towards the same side, with tear-drop pores	freshwater	Reichardt (1984), p. 39
Mayamaea arida (Bock) Lange-Bertalot, 1997	4.8–9	3.3–4.7	24–32	widely rounded	wide, well developed	absent	uniseriate, parallel in the central part, and radial in the ends, visible in LM	strongly deflected to one side	drop-shaped, slightly deviated in one direction	freshwater, terrestrial	Lange-Bertalot (1997), p. 72
Mayamaea asellus (Weinhold) Lange-Bertalot, 1997	12.0–16.0	5.0–6.0	15–20	broadly rounded	variable from moderately narrow to very broad	rectangular to wedge-shaped, bordered by 2–3 shorter striae	uniseriate, strongly radiate, visible in LM	no information	no information	freshwater, terrestrial	Lange-Bertalot (1997), p. 72
Mayamaea atomus (Kützing) Lange-Bertalot, 1997	8.5–13	4–5.5	19–22(24)	broadly rounded apices	narrow, linear	reduced, sometimes stretching into an irregular shape; or absent.	uniseriate, strongly radiate, visible in LM	bend strongly to the same side, continuing onto the mantle	slightly curved towards the same side, with tear-drop pores	freshwater, terrestrial	Lange-Bertalot (1997), p. 72
Mayamaea cavernicola B.Van de Vijver & E.J. Cox, 2013	6. 5–12	2. 8–3. 2	26–28	broadly rostrate	narrow, linear	rectangular to wedge-shaped, bordered by 2–3 shorter striae	uniseriate, weakly radiate, interrupted by ahyaline line at the valve face/ mantle junction, hardly visible in LM	hooked external distal raphe fissures	weakly deflected, hardly expanded	freshwater	Van de Vijver & Cox (2013), p. 40
Mayamaea crassistriata H. Lange-Bertalot, P. Cavacini, N. Tagliaventi & S. Alfinito, 2003	7.5–10.5	3.3–4	18–20	obtusely rounded	neither narrow nor broad widening lanceolate to the middle of the valve	lacking	uniseriate, moderately radiate, visible in LM	distinctly short-deflected, not continuing onto the valve mantle	gently arcuate with distinct central pores	freshwater	Lange-Bertalot et al. (2003), p. 75
Mayamaea desticta (Hustedt) Lange-Bertalot, 1997	11.0–12.0	4–4.5	26	obtusely to broadly rounded	narrow, linear	not developed	less distinctly radiate in proximal parts, and hardly becoming convergent but parallel at the ends, visible in LM	no information	no information	freshwater	Lange-Bertalot (1997), p. 72
Mayamaea disjuncta (Hustedt) J.Y. Li & Y.Z. Qi, 2018	14–16	3–3.5	24–28	subcapitate	narrow, linear	rectangular	radiate	no information	no information	freshwater	Li & Qi (2018), p. 56
Mayamaea disjuncta f. *anglica* (Hustedt) J.Y. Li & Y.Z. Qi, 2018	14.5–17	4.4–5	24–28	subcapitate	narrow, linear	rectangular	radiate	no information	no information	freshwater	Li & Qi (2018), p. 56
Mayamaea ectorii Ács, Duleba, K.T. Kiss & C.E. Wetzel	6.2–7.9	4.2–5.3	32–36	slightly pointed	narrow, linear	relatively wide, symmetrical	uniseriate, weakly radiate, hardly visible in LM	bend strongly to the same side, continuing onto the mantle	slightly curved towards the same side, with tear-drop pores	freshwater	present study
Mayamaea elongata H. Lange-Bertalot, P. Cavacini, N. Tagliaventi & S. Alfinito, 2003	12.0–14.0	3–3.5	16–18	cuneately rounded	moderately narrow	not separated	uniseriate, subparallel to parallel, visible in LM	distinct terminal pores, terminal fissures absent	distinct central pores	freshwater	Lange-Bertalot et al. (2003), p. 76
Mayamaea excelsa (Krasske) Lange-Bertalot, 1997	12.0–16.0	5.0–7.0	16–18	broadly rounded apices	moderately narrow, linear	not developed (absent)	uniseriate, strongly radiate, visible in LM	bend strongly to the same side, continuing onto the mantle	slightly curved towards the same side, with tear-drop pores	freshwater, terrestrial	Lange-Bertalot (1997), p. 72
Mayamaea fossalis var. *fossalis* (Krasske) Lange-Bertalot, 1997	(9)10–12	(3–3.5)4–5(5.5)	16–21	broadly rounded apices	slightly broad, widening lanceolately towards the middle of the valve	Elliptical, more or less extensive depending on the length of the shorter central striae.	uniseriate, strongly radiate, visible in LM	bend strongly to the same side, continuing onto the mantle	almost straight, or slightly curved towards the same side, with tear-drop pores	freshwater, terrestrial	Lange-Bertalot (1997), p. 72

Taxon	Length (µm)	Width (µm)	No. of striа in 10 µm	Ends of valves	Axial area	Central area	Striae	Distal raphe end	Proximal raphe end	Environment	Reference
Mayamaea fossalis var. *obsidialis* (Hustedt) Lange-Bertalot, 1997	10.0–13.0	5, but not up to 6	16–20	broadly rounded apices	wide, well developed	linear-elliptic, larger due to more shortened striae than in *M. fossalis* var. *fossalis*	uniseriate, strongly radiate, visible in LM	no information	no information	freshwater, terrestrial	Lange-Bertalot (1997), p. 72
Mayamaea fossaloides (Hustedt) Lange-Bertalot, 1997	11.0–12.0	4–4.5	23.5–24.5	obtusely to broadly rounded	narrow, linear	medium-sized with irregular outline due to alternately shorter and longer striae around this area	uniseriate, radiate becoming distinctly convergent at the ends, visible in LM 7–8.5	no information	no information	freshwater	Lange-Bertalot (1997), p. 72
Mayamaea fukiensis (Skvortsov) J.Y. Lin & Y.Z. Qi, 2018	30.6–35	8.5–12	10–12	broadly rounded	linear or lanceolate	rectangular, running from one edge of the valve to the other	slightly radiate	no information	no information	freshwater	Li & Qi (2018), p. 56
Mayamaea ingenua (Hustedt) Lange-Bertalot & G. Hofmann, 2011	7.1–9.5	2.9–4.0	20–24	moderately acutely rounded	narrow, linear	distinctly asymmetrical and running from one edge of the valve to the other	uniseriate, strongly radiate, visible in LM	bend strongly to the same side, continuing onto the mantle	almost straight with small pores	freshwater	Hofmann et al. (2013), p. 356
Mayamaea josefelsteri K. Kopalová, L. Nedbalová & B. van de Vijver, 2013	10.5–11.5	2.9–3.4	25–27	bluntly rounded apices	narrow, linear	rectangular to almost rounded, bordered by several (2–5) shorter striae	uniseriate, slightly radiate, central area, becoming straight and almost parallel near the apices	bent, not continuing onto the valve mantle	expanded	freshwater	Van de Vijver et al. (2013), p. 195
Mayamaea lacunolaciniata (Lange-Bertalot & K. Bonik) Lange-Bertalot, 1997	7–8.5	3.5–4	30–35	broadly rounded	No defined, sternum expanded to broadly rhombic-lanceolate, structureless or with some irregularly dispersed areolae	not defined	not visible in LM	bend strongly to the same side, continuing onto the mantle	slightly curved towards the same side, with tear-drop pores	freshwater	Lange-Bertalot (1997), p. 72
Mayamaea mediterranea H. Lange-Bertalot, P. Cavacini, N. Tagliaventi & S. Alfinito, 2003	9–11.5	3.3–3.6	20–21	obtusely rounded	narrow, linear	inconspicuous, lacking or slightly expanded because of some shortened striae occasionally	uniseriate, strongly radiate, visible in LM	deflected, not continuing onto the valve mantle	somewhat expanded, distinctly deflected	freshwater	Lange-Bertalot et al. (2003), p. 76
Mayamaea muraliformis (Hustedt) Lange-Bertalot, 1997	10.0–12.0	3.5–5	22–24	broadly rounded	narrow, linear	indistinct	parallel	no information	no information	freshwater	Lange-Bertalot (1997), p. 72
Mayamaea nolensoides (Bock) Lange-Bertalot, 2001	7.5–9	3.5–5	35	broadly rounded	axial area not noted	not noted	uniseriate, radiate, not visible in LM	terminal nodules are in more proximal position	no information	freshwater, terrestrial	Lange-Bertalot (2001), p. 140
Mayamaea permitis (Hustedt) K. Bruder & L.K. Medlin, 2008	6.0–9.0	3.0–4,0	(25)30–36	broadly rounded apices	narrow, linear	Reduced and rounded	uniseriate, radiate, not visible in LM	deflected, with terminal fissures	slightly deflected with small pores	freshwater	Bruder & Medlin (2008), p. 327
Mayamaea petersenii Barragán, Ector & C.E. Wetzel, 2017	7.5–10	2.5–3.5	13–21	broadly rounded apices	narrow, linear	wide, symmetrical, bowtied or rectangularly shaped	uniseriate, radiate, visible in LM	bend strongly to the same side of valve mantle	straight with tear-drop proximal pores	terrestrial	Barragán, et al. (2017), p. 77
Mayamaea pseudopermitis H. Lange-Bertalot, P. Cavacini, N. Tagliaventi & S. Alfinito, 2003	8.0–10.0	2–2.3	29–31	obtusely rounded	very narrow	indistinct	uniseriate, radiate, not visible in LM	straight, distinct terminal pores, terminal fissures lacking completely	almost straight with small pores	freshwater	Lange-Bertalot et al. (2003), p. 78
Mayamaea recondita (Hustedt) Lange-Bertalot, 1997	(8)9–9.5	3.5–4.5	20–24	broadly rounded	variable, never narrow, widened to lanceolate	not differentiated	uniseriate, tending to biseriate arrangement of the areolae, radiate, visible in LM	slightly bent, not continuing onto the valve mantle	drop-like, shortly and sharply bent	freshwater	Lange-Bertalot (1997), p. 72

Taxon	Length (µm)	Width (µm)	No. of striа in 10 µm	Ends of valves	Axial area	Central area	Striae	Distal raphe end	Proximal raphe end	Environment	Reference
Mayamaea sweetloveana Zidarova, Kopalová & C. Van de Vijver, 2016	6.0–7.0	3–3.5	25–30	broadly rounded apices	narrow, linear	almoſt entirely lacking, central ſtriae alternatingly shortened	uniseriate, ſtrongly radiate, visible in LM; areolae becoming more elongated near the valve mantle	very short, deflected opposite to the proximal raphe endings, never continuing onto the mantle but terminating near the laſt ſtriae at the apices but terminating near the laſt ſtriae at the apices	almoſt not deflected	freshwater	Zidarova et al. (2016), p. 43
Mayamaea terreſtris N. Abarca & R. Jahn, 2014	7.1–8	3–4.3	24–26	obtusely rounded	slightly broad, widening lanceolately towards the middle of the valve	almoſt entirely lacking, central ſtriae alternatingly shortened	uniseriate, radiate	deflected to the opposite side, never continuing onto the mantle	expanded by depressions around the central pores and deflected	freshwater, terreſtrial	Zimmermann et al. (2014), p. 16
Mayamaea tytgatiana Zidarova, Kopalová & Van de Vijver in Zidarova et al., 2016	12.0–15.0	2.5–3	19–22	slightly protracted, rounded to almoſt subroſtrate apices	narrow, linear	forming a bow-tie shaped, asymmetrical fascia, widening toward the margins	uniseriate, moderately radiate in the middle, becoming parallel and even weakly convergent. Areolae gradually becoming smaller on the valve mantle, usually rounded, rarely slit-like near the mantle edge toward the apices	hooked, continuing onto the mantle	drop-like enlarged	freshwater	Zidarova et al. (2016), p. 43
Mayamaea vietnamica Glushchenko, Kezlya, Kulikovskiy & Kociolek, 2020	9.1–10.5	3.9–4.8	19–22	broadly rounded	tapers from the central area, becoming narrower towards the ends	more or less expressed, rounded to asymmetrical, rarely transversally elongated, and bordered on each margin by 3 shortened ſtriae and / or 3 isolated areolae	uniseriate, radiate, visible in LM	bend ſtrongly to the same side of valve mantle	ſtraight, dropshaped	terreſtrial	Kezlya et al. (2020), p. 330

Table 2. The Multimetric Index for Lakes (MIL) in the two samples calculated from different analyses.

Analysis	June	September
microscopy	6.6	7.4
metabarcoding with DADA2	5.0	5.4
metabarcoding with DADA2 and BLAST	4.9	7.3
metabarcoding with DADA2 and BLAST supplemented with species from microscopy	4.9	5.0

only for a few (seven) *Mayamaea* species. We used only six species because the sequence of *M. fossalis* was renamed to *M. permitis* in the Diat.barcode database. Moreover, in these data, *M. permitis* is overrepresented, and most of the other species are represented by one sequence. We also found several *M. permitis* sequence variants. A large diversity within *M. permitis* was observed by Kezlya et al. (2020) and Bagmet et al. (2021), raising the possibility of a cryptic species. *Mayamaea ectorii* was close to *M. permitis* variants, however, it was located on a distinct lineage suggesting that it can be accepted as an independent species.

The ecological requirements of *Mayamaea ectorii* and also the codominant species are nutrient rich freshwaters.

Our phylogenetic analysis showed the genus *Craticula* was not monophyletic which could be an artefact maybe due to short sequences. But deciding this is beyond the scope of this investigation. Nevertheless, phylogeny along with p-distance calculations confirmed that these two environmental sequences belonged to *C. subminuscula* and *C. importuna* detected under microscope.

Craticula importuna was identified for the first time in Hungary. It was probably previously misidentified as either *C. minusculoides* or *C. molestiformis*. The main distinguishing characteristics of *C. minusculoides* are the following: the areolae are not occluded with hymens externally and the distal raphe fissures are curved and do not continue on the valve mantle, while the areolae of *C. molestiformis* are covered by hymens externally and the distal raphe endings are long, strongly hooked and continuing onto the valve mantle (Levkov et al. 2016). We have not any information about the hymens in the case of *C. importuna*, but its distal raphe fissures are curved and do not continue on the valve mantle (Bruder and Medlin 2008). Based on the p-distance and the known morphological characteristics, we accepted our ASV as the sequence

of *C. importuna*. However, as the studied gene sequence is short, the question requires further investigation.

Among the *Sellaphora* species, we identified *S. archibaldii*, *S. nigri*, *S. pupula* and *S. saugerresii*. *Sellaphora archibaldii* was described as an endemic species of South Africa for a long time (Taylor and Lange-Bertalot 2006). However, Ács et al. (2017) showed that it has been found in Hungary, France and Germany and more recently found on the island of Reunion as well (Gassiole et al. 2019). This species was not dominant in our studied samples and various explanations can be given for failing to find its sequence: e.g. its cell wall was more resistant, dead cells were observed under microscope or the primers did not match its sequence (Duleba et al. 2021). Identification of *S. nigri* and *S. saugeresii* sequences with DADA2 pipeline was confirmed by phylogeny. *Sellaphora pupula* is a species complex involving several cryptic species (Evans et al. 2008), our environmental sequence (identified after BLAST search) grouped with the taxa of this complex so it could be accepted as a member of the complex. *Sellaphora nigri* was also detected by microscopy. *Sellaphora saugerresii* and *S. pupula* occurred in very low read numbers so it was not surprising that they could not be found among 500 valves counted under microscope.

Conclusions

In this study, we investigated the diatom assemblages of an oxbow using two approaches, metabarcoding as well as microscopy (LM and SEM) in comparison. Both methods revealed high diversity, however, significant differences were found in the taxon lists. These discrepancies were mitigated when some sequences being unassigned by DADA2 pipeline were assigned using BLAST. Identifying sequences of three species (including two dominant species) was possible only after the comparison with the results of morphological analyses. Moreover, one of these taxa could be described as a new species of *Mayamaea* genus. Our results also demonstrate that metabarcoding comparing with microscopy can contribute to the complementing of the reference database.

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Supplementary material 1
Table S1

Author: Tibor Bíró, Mónika Duleba, Angéla Földi, Keve T. Kiss, Péter Orgoványi, Zsuzsa Trábert, Edit Vadkerti, Carlos E. Wetzel, Éva Ács

Data type: Excel file.

Explanation note: **Table S1.** Taxa with relative abundances (%) found in samples from Körtvélyesi Holt-Tisza in June and September 2019 based on microscopy and metabarcoding with different analyses as well as with and without applying correction factor. Omnidia codes are also provided. As a new species, *Mayamaea ectorii* Acs, Kiss & C.E.Wetzel sp.nov does not have Omnidia code yet, therefore, the code of *Mayamaea* sp. was assigned to it.

Link: https://doi.org/10.3897/mbmg.6.87497.suppl1

Supplementary material 2
Table S2

Author: Tibor Bíró, Mónika Duleba, Angéla Földi, Keve T. Kiss, Péter Orgoványi, Zsuzsa Trábert, Edit Vadkerti, Carlos E. Wetzel, Éva Ács

Data type: Excel file.

Explanation note: **Table S2.** Uncorrected p-distance values between selected sequences belonging to the genus *Mayamaea* downloaded from Diat.barcode database and the *Mayamaea* amplicon sequence variant found in the Körtvélyesi Holt-Tisza samples. Sequences from database are provided with NCBI GenBank accession number (if available) or culture ID of Thonon Culture Collection. Sequences were trimmed to the same length, therefore a total of 265 nucleotide positions were in the dataset.

Link: https://doi.org/10.3897/mbmg.6.87497.suppl2

Supplementary material 3
Table S3

Author: Tibor Bíró, Mónika Duleba, Angéla Földi, Keve T. Kiss, Péter Orgoványi, Zsuzsa Trábert, Edit Vadkerti, Carlos E. Wetzel, Éva Ács

Data type: Word file.

Explanation note: **Table S3**. The measured environmental variables.

Link: https://doi.org/10.3897/mbmg.6.87497.suppl3

Supplementary material 4
Table S4

Author: Tibor Bíró, Mónika Duleba, Angéla Földi, Keve T. Kiss, Péter Orgoványi, Zsuzsa Trábert, Edit Vadkerti, Carlos E. Wetzel, Éva Ács

Data type: Excel file.

Explanation note: **Table S4.** Uncorrected p-distance values between selected sequences belonging to the genus *Craticula* downloaded from Diat.barcode database and two *Craticula* amplicon sequence variants found in the Körtvélyesi Holt-Tisza samples. Sequences from database are provided with NCBI GenBank accession number (if available) or culture ID of Thonon Culture Collection. Sequences were trimmed to the same length, therefore a total of 265 nucleotide positions were in the dataset.

Link: https://doi.org/10.3897/mbmg.6.87497.suppl4

Supplementary material 5
Table S5

Author: Tibor Bíró, Mónika Duleba, Angéla Földi, Keve T. Kiss, Péter Orgoványi, Zsuzsa Trábert, Edit Vadkerti, Carlos E. Wetzel, Éva Ács

Data type: Excel file.

Explanation note: **Table S5.** Description of sampling sites illustrated on Suppl. material 6: Fig. S1.

Link: https://doi.org/10.3897/mbmg.6.87497.suppl5

Supplementary material 6
Figure S1
Author: Tibor Bíró, Mónika Duleba, Angéla Földi, Keve T. Kiss, Péter Orgoványi, Zsuzsa Trábert, Edit Vadkerti, Carlos E. Wetzel, Éva Ács
Data type: Image.
Explanation note: **Figure S1.** Hungarian distribution records of *Brevilinea kevei* (A), *Craticula importuna* (B), *C. subminuscula* (C), *Mayamaea permitis* (D), *Navicula microrhombus* (E), *Sellaphora archibaldii* (F) and *S. nigri* (G) in this study.

Link: https://doi.org/10.3897/mbmg.6.87497.suppl6

Supplementary material 7
Alignment S1
Author: Tibor Bíró, Mónika Duleba, Angéla Földi, Keve T. Kiss, Péter Orgoványi, Zsuzsa Trábert, Edit Vadkerti, Carlos E. Wetzel, Éva Ács
Data type: Fas file.
Explanation note: Alignment for maximum likelihood phylogenetic tree of *Mayamaea* species using the *rbc*L fragment (Fig. 2.). *Rossia*, *Fallacia* and *Sellaphora* taxa were used as outgroups.

Link: https://doi.org/10.3897/mbmg.6.87497.suppl7

Supplementary material 8
Alignment S2
Author: Tibor Bíró, Mónika Duleba, Angéla Földi, Keve T. Kiss, Péter Orgoványi, Zsuzsa Trábert, Edit Vadkerti, Carlos E. Wetzel, Éva Ács
Data type: Fas file.
Explanation note: Alignment for maximum likelihood phylogenetic tree of *Craticula* species using the *rbc*L fragment (Fig. 4.). *Stauroneis* taxa were used as outgroups.

Link: https://doi.org/10.3897/mbmg.6.87497.suppl8

Supplementary material 9
Alignment S3
Author: Tibor Bíró, Mónika Duleba, Angéla Földi, Keve T. Kiss, Péter Orgoványi, Zsuzsa Trábert, Edit Vadkerti, Carlos E. Wetzel, Éva Ács
Data type: Fas file.
Explanation note: Alignment for maximum likelihood phylogenetic tree of *Sellaphora* species using the *rbc*L fragment (Fig.5.). *Mayamaea*, *Rossia* and *Fallacia* taxa were used as outgroups.

Link: https://doi.org/10.3897/mbmg.6.87497.suppl9