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First report of *Navicula jakovljevicii* Hustedt (Bacillariophyta) from Hungary: distribution, comparative morphology and a related species

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Abstract – In Hungary *Navicula jakovljevicii* was firstly recorded in biofilm of *Elodea nuttallii* in 2005 in an oxbow of the catchment area of the River Danube. Subsequently, in 2006, *N. jakovljevicii* was also found in the same oxbow on reed stems as well. In the following years it appeared in another oxbow, suggesting an expanding distribution in the tributaries of the Danube in Hungary. The Hungarian population can be characterised as having mixed morphological features in comparison with other known *N. jakovljevicii* populations of Europe. When the morphological study was expanded, a similar, but 'giant form' was detected in fossil material. We found similarities and a possible connection between *N. jakovljevicii* and *Navicula lucida*, a diatom taxon described from a Neogene deposit in the Carpathian Basin. Despite the morphological similarities in the shape, apices, striae pattern and raphe structure of these two species, there are significant differences in valve dimensions: the valves of *N. lucida* are larger and more heavily silicified than *N. jakovljevicii*.

Keywords: biofilm, Danube, *Elodea nuttalli*, *Navicula jakovljevicii*, *Navicula lucida*, oxbow, Pantocsek collection

Introduction

Navicula jakovljevicii Hustedt (1945: 931) was described from the Balkan Peninsula in 1945 by Friedrich Hustedt (Hustedt 1945). For a long time it seemed that the distribution of this diatom species was restricted to South Europe (Plenković-Moraj 1995). The taxo-

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nomical position of the species is uncertain. According to the earlier published morphological details (Reichardt 1992, Lange-Bertalot 2001, Levkov et al. 2005) neither the raphe structure (raphe fissures weakly lateral, slightly curved) nor the striae pattern (the striae consist of poroids that are elongated externally but alveolate internally) are the same as the members of *Navicula* s. str. Furthermore a hole can be detected on the valve apex, which is an unusual feature.

Lange-Bertalot (2001) indicated in the Monograph of diatoms of Europe, that some fossil species from the upper Tertiary period bear the same structures as *Navicula jakovljevicii*. He mentioned Köpecz (Căpeni, Romania today) material, and published two pictures (Plate 62 figs 1–2) as *Navicula* (?nov.) spec. from this locality. The legends of these figures are the following: Köpecz, southern Carpatians. The structural pattern conforms to the recent *Navicula jakovljevicii*, and appears like a 'giant form' of this species (Lange-Bertalot 2001 p. 360). The Hungarian Natural History Museum holds the József Pantocsek diatom collection, where – among many others – diatom samples from the Carpathians are well represented, including samples form Köpecz (Pantocsek 1892, 1905, Buczkó 2012). An obvious endeavour was to compare the recent *Navicula jakovljevicii* with other ancient *Navicula* taxa from the Köpecz material and try to find this 'giant form'.

Due to the hydrological impacts of the Bős/Gabčikovo dam, a low water flow condition was formed both in the main river channel of the Danube and in the smaller arms (Othelova and Valachovic 2002, Ráth et al. 2003, Hajósy 2012). Based on these facts, a macro- and microflora monitoring project (including diatom monitoring also) was conducted between 1994 and 2009 on the River Danube, on its river branches and on the oxbows. The aim of that study was to follow up the effects of the Bős/Gabčikovo dam on the macro- and microflora assemblages of the so-called Szigetköz region (Hajósy 2012). It was observed, that the changing water discharge and flow conditions allowed appearances and distribution of adventive and invasive macrophytes, as *Elodea canadensis* Michaux (1803: 20) and *Elodea nuttallii* (Planchon 1848: 86) H. St John (1920: 29) (Ráth et al. 2003, Othelova and Valachovic 2003, Király et al. 2007, Kočić et al. 2014). Although macrophytes could serve as 'spreading transport substrate' for invasive species, diatoms among them (Horváth and Lamberti 1997), the first Hungarian observation of a characteristic diatom taxa during the counting procedure in 2005 was surprising. This diatom taxa was identified as *Navicula jakovljevicii*.

The main aims of the present study were (i) to report for the first time observation of *Navicula jakovljevicii* in Hungary; (ii) to compare the morphological features of the Hungarian *N. jakovljevicii* population with other known populations of the species; (iii) to report the observation of fossil analogues of *Navicula jakovljevicii* in Miocene material and (iv) to give a possible explanation of the recent observation and spread of *N. jakovljevicii* in Hungary.

Material and methods

Sampling localities

Szigetköz is a major island of the Danube, harbouring a complex system of habitats. It is situated in northwest Hungary, where the river enters the Carpathian Basin (Fig. 1). Here, the fast flowing river slows down, deposits the gravel carried from the Alps, divides into many smaller arms turning into a braided channel. Diatom samples were collected between

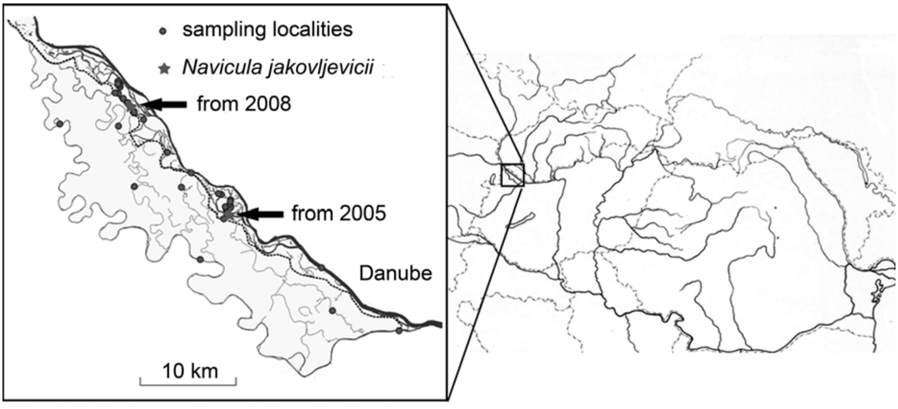


Fig. 1. The sample locations of hydrobiological monitoring and the occurrences of *Navicula jakovljevicii* (arrows) in the Szigetköz section of the River Danube. Ásványráró (EOV X: 535520 EOV Y: 279130; and EOV X: 535570 EOV Y: 278990); Cikolasziget (EOV X: 525150 EOV Y: 290300).

1994 and 2009 on the River Danube and in its catchment area twice a year (Fig. 1). Samples were collected from different macrophytes of sixteen sampling locations (Fig. 1). During the sixteen years, more than 1300 samples were collected and analysed. Nearly 300,000 valves were identified from the samples.

Sample preparation

Diatom samples were prepared using standard digestion procedures (Battarbee 1986). Aliquot-evaporated suspensions were embedded in Zrax. At least 400 valves were counted from each sample using a light microscope (LM) (LEICA DM LB2 with 100 HCX PLAN APO objective). Light microscopic pictures were taken by Fujifilm Digital Camera FinePix S2 Pro and later by VSI–3.OM(H) digital camera. For scanning electron microscopic (SEM) analyses, cleaned samples were air–dried on an aluminium stub. Coating with gold–palladium was accomplished using a XC7620 Mini Sputter Coater for 120 s at 16 mA. A Hitachi S–2600N SEM operated at 20 kV and 5–8 mm distance was used. Morphological terminology follows Barber and Haworth (1981) and Round et al. (1990). Valve measurements were made from digital images using the camera software.

The following materials were examined and presented in this paper: raw materials and permanent slides of the Hungarian Natural History Museum BP 2007/72; BP 2007/73; Pantocsek diatom collection Köpecz material (new permanent slides and stubes for SEM were made from dried diatom sample no BP-88, and Bibarczfalva no BP-22).

Results

In Hungary *Navicula jakovljevicii* was first recorded in autumn of 2005 in an oxbow at Ásványráró (Fig. 1). The substrate of the biofilm was the waterweed *Elodea nuttallii*. *Elodea nuttallii* was present also in the following three years in this oxbow en masse. In 2006 *N. jakovljevicii* was also found on reed stems in the same oxbow, as well as on the *Elodea* species. The diatom appeared in another oxbow in Szigetköz on *E. nuttallii* in 2008. Although the diatom species had moved upwards in the catchment area of Danube, its relative abundance was low in every sample, never reaching 3%. Altogether *N. jakovljevicii* was found only at three sampling sites from the sixteen sites, it was recorded continuously from 2005 until the end of the monitoring in 2009. The recent distribution of the species is shown on On-line Supplement Fig. 1. A detailed morphological description about this characteristic species for comparison with other known populations (Hustedt 1945, Reichardt 1992, Levkov et al. 2005) is given below.

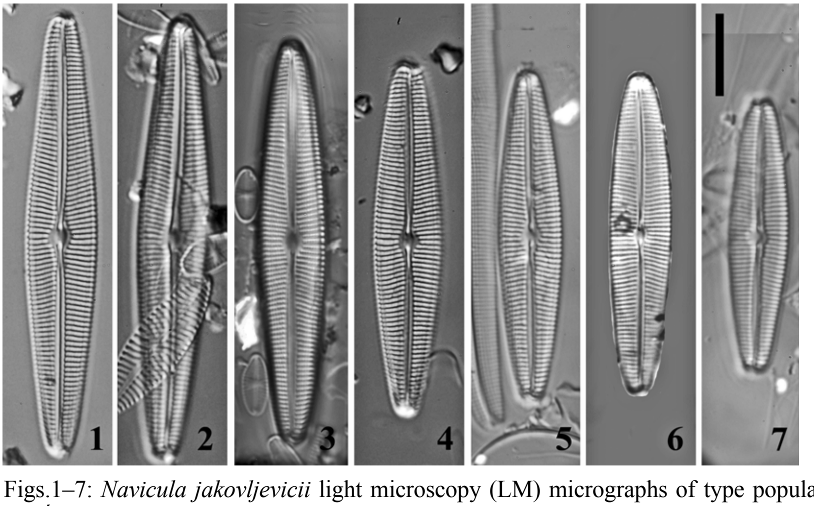
Morphological observations of *Navicula jakovljevicii*

Light microscopy

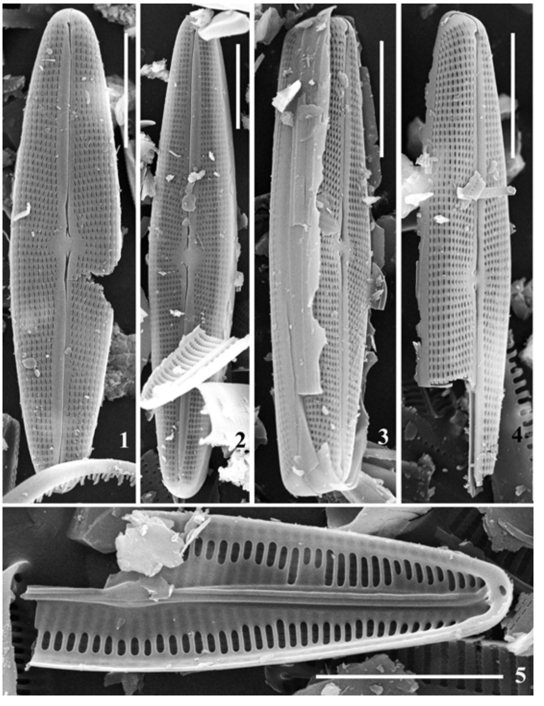
Valves are lanceolate to elliptic-lanceolate with obtusely to broadly rounded ends, 32–53 µm long, 7–8.7 µm broad (Pl. 1, Figs. 1–7). Raphe fissures are weakly lateral. Axial area is narrow, linear, central area is small, weakly asymmetrically rounded. Striae are moderately lateral, parallel to weakly convergent at the ends; there are 14–17 striae in 10 µm (Pl. 1, Figs. 1–7).

Scanning electron microscopy

Raphe fissures are distinctly lateral, narrower to the secondary side of the valve, slightly expanded at the proximal part. The proximal raphe endings externally are forked (Pl. 2, Figs. 1–4; Pl. 3, Figs. 1, 3), a silicate tongue divides the raphe terminal. Lineolae are narrow, elongated, 29–32 in 10 µm. The alveoli are partly occluded on the valve interior (Pl. 2, Fig. 5). The distal raphe ends straight (Pl. 2, Figs. 1–2) or bent to the secondary side (Pl. 2, Fig. 4) and has a well close to the valve ends (Pl. 2, Figs. 1–4; Pl. 3, Fig. 4). Internally, a hole can be detected on the valve apex (Pl. 2, Fig. 5; Pl. 3, Fig. 2). Internally, the distal raphe ends are lateral, close to each other (Pl. 3, Fig. 5). The mantle is narrow (Pl. 3, Fig. 6).



Pl. 1. Figs.1–7: *Navicula jakovljevicii* light microscopy (LM) micrographs of type population in the Ásványráró section of the Danube, collected from *Elodea nuttallii* (sample BP 2007/73). Scale bar represents 10 µm.



Pl. 2. Figs. 1–5: *Navicula jakovljevicii* scanning electron micrographs (SEM) of the population from *Elodea nuttallii* (sample 2007/73); Figs. 1–2: external view of entire valves; Fig. 3: oblique position showing the girdle bands; Fig. 4: broken valve; Fig. 5: internal detail of a broken valve. Scale bars represent 10 µm.

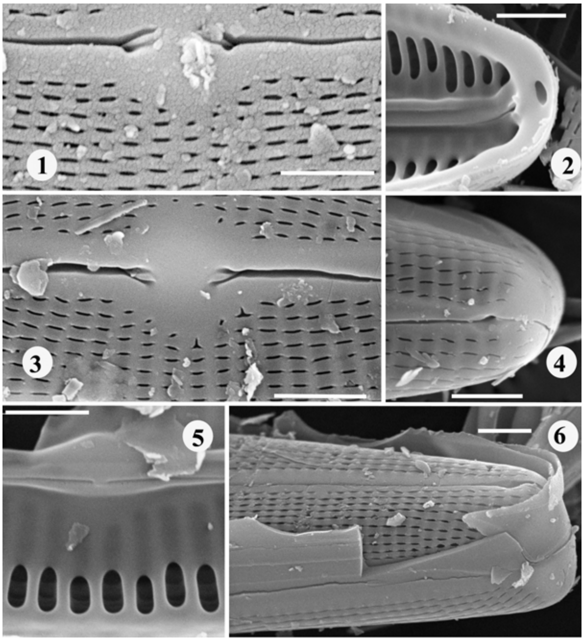
A related *Navicula* species from Tertiary diatom deposits (*Navicula lucida* Pantocsek 1892: tab. 18, fig. 264)

Based on the indication of Lange-Bertalot (2001) the Köpecz and Bibarczfalva materials were reinvestigated and some valves of a characteristic, long diatom were found in the samples that are reminiscent of the published pictures in the Monograph of diatoms of Europe (Lange-Bertalot 2001 Plate 62 figs 1, 2). We identified this diatom species as *Navicula lucida* Pantocsek (1892, 1905: 73; Plate 4, fig.1). This species was very rare, altogether twelve valves having been documented. The Latin diagnosis (Pantocsek 1905) refers to only one valve that is 192 µm long and 19 µm wide. The striae number is 15–16 in 10 µm; they are slightly radial in the middle but almost through parallel along the valves. It was found in the Tertiary, in the Miocene, in fresh water, at localities close to Köpecz and Bodos by Pantocsek.

Morphological observations of *Navicula lucida*

Light microscopy

Valves are lanceolate, gradually tapering to the apex. The ends of these valves are obtusely rounded. The lengths are 110–200 µm (n = 10), the breadths are 15–22 µm. Raphe is



Pl. 3. Figs: 1–6: *Navicula jakovljevicii* scanning electron micrographs (SEM) of the population from *Elodea nuttallii* (sample 2007/73); Figs. 1, 3: External detail of the central area with the proximal raphe endings and the typical ornamentation, note the forked central raphe ends; Fig. 2: internal detail of a valve apex showing a hole; Fig. 4: external detail of a valve apex showing the turning raphe end; Fig. 5: internal detail of the central area; Fig. 6: external detail of a frustulum apex in oblique view showing the mantle elements. Scale bars represent 2 µm.

distinctly lateral, outer fissure curved distinctly towards the central pores. Axial area is moderately narrow and linear, central area is slightly elliptic. Striae are almost parallel, 14–16 in 10 µm; lineolae hardly distinct in LM (Pl. 4, Fig. 2).

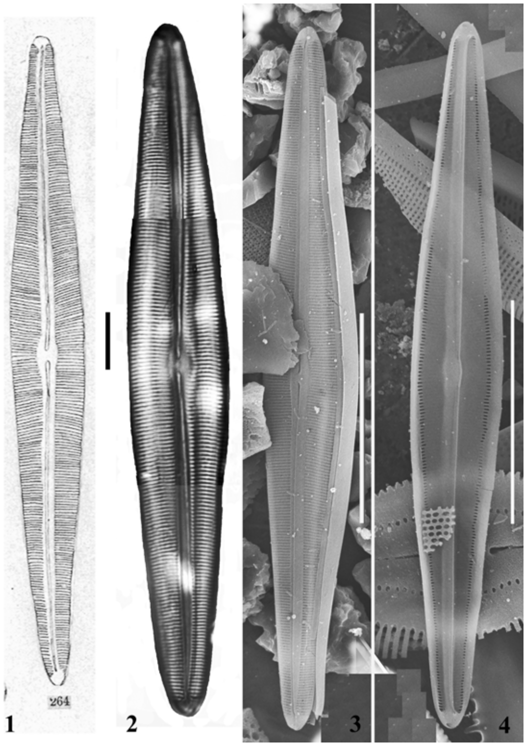
Scanning electron microscopy

Raphe is lateral; externally the central raphe endings are distinctly forked (Pl. 4, Fig. 2; Pl. 5, Fig. 1) just as in *N. jakovljevicii* (Pl. 2, Figs. 1–4; Pl. 3, Figs. 1, 3). Lineolae are narrow, elongated, 34–38 in 10 µm. The alveoli are partly occluded on the valve interior (Pl. 4, Fig. 4). Externally, the distal raphe ends turn to the girdle (Pl. 4, Fig. 3; Pl. 5, Fig. 3). Internally, the proximal raphe endings are close to each other (Pl. 5, Fig. 2). Internally, there is an expansion on the proximal part of the raphe fissure (Pl. 5, Fig. 2). There is a hole on the apex close to the valve end (Pl. 5, Fig. 4).

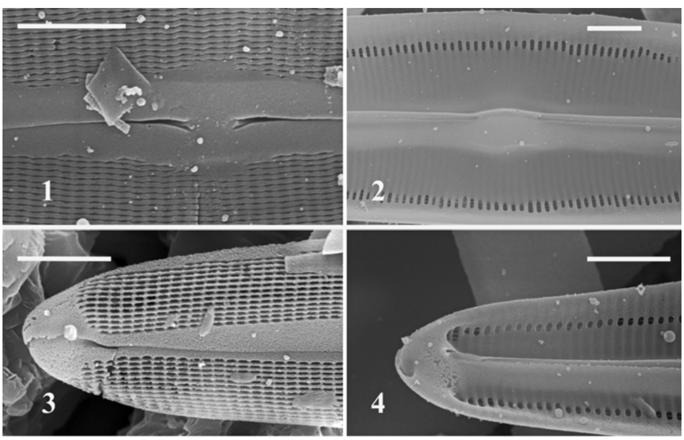
Discussion

Taxonomical remarks on *Navicula jakovljevicii* and its distribution

In the original description by HUSTEDT (1945), *N. jakovljevicii* was characterized by 32–60 µm in length, 8–11 µm in width and 16–18 striae in 10 µm. REICHARDT (1992) found



Pl. 4. Figs. 1–4: *Navicula lucida*; Fig. 1: iconotype, reprint from Pantocsek 1892; Fig. 2: light microscopy (LM) micrograph from Köpecz (Romania), Miocene; Figs. 3–4: scanning electron micrographs (SEM), Fig. 3: external but oblique view of a whole frustule, Fig. 4: internal view. Scale bar for LM is 10 µm; on SEM bars are 50 µm.



Pl. 5. Figs. 1–4: Details of *Navicula lucida* valves. Fig. 1: external view of central area with the characteristic forked central raphe endings; Fig. 2: internal details of central area. Fig. 3: external view of a valve apex. Fig. 4: internal view of a valve apex with hole. Scale bars represent 5 µm.

three, slightly different, populations in Lake Zug, in the River Krka and in the Plitvice Lakes. The population found in Lake Zug was slightly thinner (8.4–9.7 µm in width) and smaller (32.9–53.0 µm in length), moreover there were fewer striae (14–15 in 10 µm) than in the form described by HUSTEDT. In contrast, valves from the River Krka were characterized by being 39–85 µm in length and 8.8–11.7 µm in width. The characteristics of the valves from populations originating in the the Plitvice Lakes were completely in the middle range. Subsequently, LEVKOV et al. (2005) published a detailed morphometric analysis of *N. jakovljevicii* populations from Nidze Mountain and Lake Ohrid. They found that at least two different populations could be distinguished: the first, *N. jakovljevicii* morphotype 1, is similar to HUSTEDT's (1945) description as regards the number of striae in 10 µm (16–18) and the presence of a longitudinal band, but has a greater length (50–80 µm). The second, *N. jakovljevicii* morphotype 2, has smaller valves (30–50 µm), an indistinct longitudinal band and considerably fewer striae in 10 µm (12–14).

The lengths of the valves of the Hungarian populations were partly similar to the transitional forms described by HUSTEDT (1945), and partly to the populations from Lake Zug (REICHARDT 1992). The numbers of striae of individuals from Hungary were the closest to the original description (HUSTEDT 1945).

Navicula jakovljevicii was primarily known from southern, south-east, and central Europe (Lake Ohrid – JURILJ 1954, Lake Zug, River Krka and Plitvice Lakes – REICHARDT 1992, France – COSTE and ECTOR 2000, Transylvania in fossil deposit – LANGE-BERTALOT 2001, Nidze Mountain and Lake Ohrid – LEVKOV et al. 2003, Macedonia – SMITH and SMITH 2003, River Trnovcica – LEVKOV et al. 2005, Apennine Mountains – TORRISI and DELL'UOMO 2008, River Raška – VIDAKOVIC et al. 2014; On-line Supplement Fig. 1). Recently *N. jakovljevicii* was reported from the northern region of Europe (The Netherlands, 2010, On-line Supplement Fig. 1). Only few data are available regarding to the substrate of this diatom taxon. Samples containing *N. jakovljevicii* were collected from limestone (Lake Zug – REICHARDT 1992, Apennine Mountains – TORRISI and DELL'UOMO 2008), branches (River Krka – REICHARDT 1992) and encrusted moss (Plitvice Lakes – REICHARDT 1992).

A related species: *Navicula lucida*

We proved the presence of the 'giant form' of *Navicula jakovljevicii* in Tertiary deposits that was mentioned by LANGE-BERTALOT (2001). This large diatom was described by József Pantocsek as *Navicula lucida* (PANTOCSEK 1982). All of the main valve features (shape, apices, striae pattern, raphe) of these two species are the same, but there are significant differences in valve dimensions. In our observation the valves belonging to *N. lucida* are large and heavily silicified. This is not surprising, since most diatomite in Central Europe is connected to volcanisms (e.g. HAJÓS 1986). High silicate concentration in the lake water, related to volcanic activity, probably facilitated the development of strong diatom cell walls during the Miocene (WITKOWSKI et al. 2011). All characteristic features are in agreement with *N. jakovljevicii* and *N. lucida*, but probably the forked raphe terminals are the most remarkable. We can only speculate about the connection between the extinct and the extant *Navicula* species. It might be possible that these two species are close relatives. *N. lucida* could be the ancestor of *N. jakovljevicii*, the large form gradually diminishing in parallel with the restricted silica supply because of the reduced volcanic activity. But maybe they developed independently from each other. Due to the sporadic occurrences of diatom deposits, the lack of a continuous profile makes the answer to this question difficult.

Navicula lucida was found in Neogene fossil deposits – Köpecz (Căpeni), Bodos (Bodoş) and Bibarczfalva (Baraolt) – in Romania. Furthermore, the presence of this taxon was detected in the Kichevo Basin Macedonia (Ognjanova-Rumenova and Dumurdzhanov 2008 as *Brachysira* sp. on Plate 2 fig. 6).

Perspectives

Notwithstanding the intensive studies on the biogeography of diatoms, our knowledge about their distribution pattern is very limited. Detailed taxonomical studies have often revealed several new taxa (Potapova and Hamilton 2007, Novais et al. 2009), but changes in species' distribution are poorly known. Recently, attention has been paid to invasive and expansive diatom taxa (Coste and Ector 2000, Blanco and Ector 2009, Kaštovský et al. 2010, Jellyman et al. 2011, Beltrami et al. 2012, T-Krasznai et al. 2014), but there are only a few taxa with sufficient available data. The species mostly in the focus of attention are those that have caused drastic ecological and/or economic events (Blanco and Ector 2009). The incorrect identification of certain taxa (especially closely related-taxa, or centric diatoms) could create difficulties in the interpretation of the appropriate biogeographical data in some cases (Kaštovský et al. 2010).

Navicula jakovljevicii has even been regarded as a rare species (Coste and Ector 2000, Vidakovic et al. 2014). Its occurrence in the Hungarian part of the Danube catchment area might be related to the invasive *Elodea nuttallii*, which may refer to the changes of the habitat. Affected by the Bős/Gabčikovo dam, water flow conditions have changed and water discharge has decreased in the Danube and its catchment area (Ráth et al. 2003). These changes in hydrological circumstances primarily led to the spread of adventive and/or invasive species, like the waterweeds *Elodea canadensis* and *E. nuttallii* (Ráth et al. 2003). A lot of abiotic and biotic factors control the appearance of non-native species in new habitats (B-Béres et al. 2012, Porter et al. 2013, T-Krasznai et al. 2014). We speculated that the new observations of *N. jakovljevicii* in the Hungarian flora were primarily due to the spread of *E. nuttallii*. These macrophytes could be regarded as the transport substrate of *N. jakovljevicii*. But it is pertinent to emphasize that this 'transport substrate assumption' requires a lot of further studies. The improving taxonomical skills of diatomologists and the available flora books (e.g. Diatoms of Europe) could also contribute new data of the presence and ecological demands of *N. jakovljevicii*.

Conclusion

Appearance of new taxa in diatom-flora, especially a rare species with little information about its ecological relevancy, requires a lot of care. Even if the abundance of the given taxa is low, its effects on the local microflora are unknown and unpredictable. Our results were the first report about the distribution of *Navicula jakovljevicii* in Hungary. There are only a few data about its distribution in Europe. In order to predict the effects and the directions of the current expansion of the taxon, every single item of data has a major importance. Our result suggests that *N. jakovljevicii* might appear in the further Danubian catchment area, not only in Hungary but in adjacent countries as well.

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